



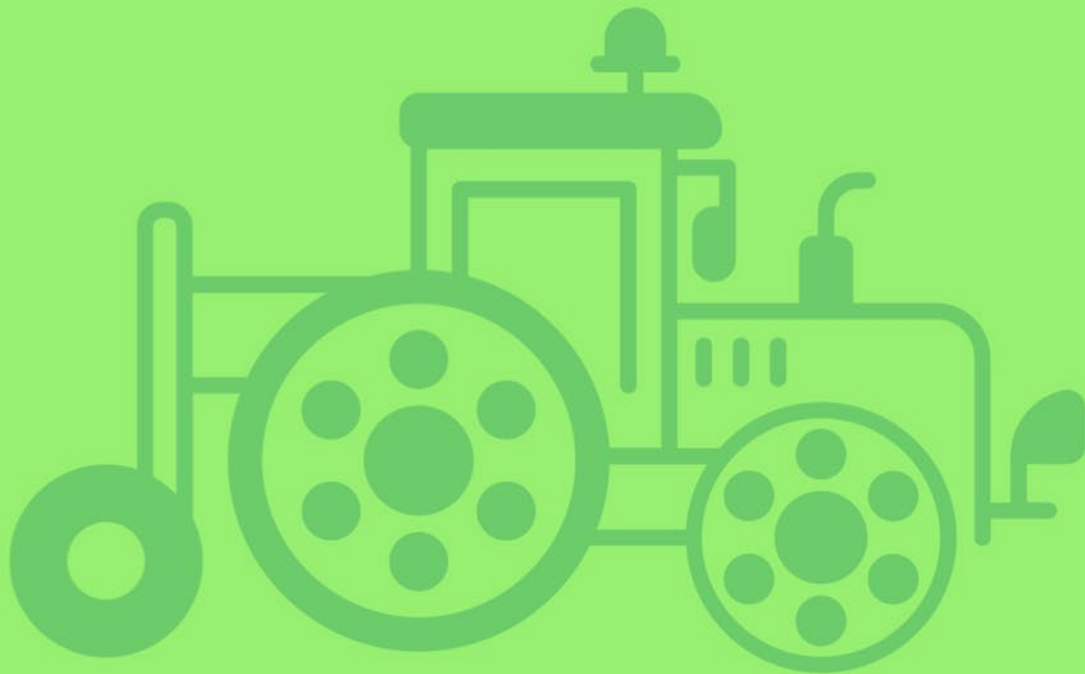
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Article

STUDY ON THE TECHNOLOGICAL AND AGRICULTURAL CHARACTERISTICS OF MAJOR PLANT SPECIES CULTIVATED IN ROMANIA

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Keywords: crop rotation; technological traits; agro-economic indicators; sustainability; climate resilience.

Abstract: This paper presents a comparative assessment of five representative field crops cultivated under Romanian pedo-climatic conditions: common wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*), rapeseed (*Brassica napus*), industrial hemp (*Cannabis sativa L. var. sativa*) and clover (*Trifolium spp.*). Technological parameters—vegetation period, fertilization and irrigation requirements, soil suitability and role in crop rotation—are analysed in conjunction with agro-economic indicators such as establishment and maintenance costs, yield potential and market valorisation. Data were synthesised from national technical guides, official statistics and peer-reviewed literature. The results highlight the complementary agronomic and economic roles of cereals, oilseeds, industrial crops and perennial legumes within diversified crop rotations. While wheat and sunflower remain economically strategic crops, rapeseed offers high profitability under intensive management, and industrial hemp and clover contribute significantly to diversification, soil fertility restoration and reduced dependency on mineral inputs. The findings provide practical decision-support for designing resilient and sustainable cropping systems aligned with the objectives of the EU Common Agricultural Policy.

1. Introduction

Romanian agriculture is undergoing profound structural and technological transformations driven by increasing climate variability, rising production costs and the need to align agricultural practices with the objectives of the European Union's Common Agricultural Policy (CAP) 2023–2027. Recent decades have been marked by more frequent droughts, heat waves and irregular precipitation patterns, which have significantly affected yield stability, particularly in cereal-based production systems. Under these conditions, improving the resilience and sustainability of cropping systems has become a major priority for both farmers and policymakers.

Romania benefits from a high diversity of pedo-climatic conditions, ranging from fertile chernozems in the Danube Plain to luvisols and alluvial soils in hilly and meadow areas. This diversity allows the cultivation of a wide range of field crops; however, their performance is strongly dependent on the adequacy of applied technologies and crop rotation schemes [3]. Conventional cereal-dominant rotations are increasingly exposed to climatic and economic risks, highlighting the need for diversification through oilseeds, industrial crops and forage legumes [4].

Cereals such as common wheat remain essential for national food security and farm income, while oilseed crops, particularly sunflower and rapeseed, play a strategic role in the agri-food and bioenergy sectors [5]. At the same time, alternative and industrial crops, such as industrial hemp, are gaining renewed interest due to their multifunctional use, high biomass

productivity and relatively low phytosanitary pressure [5]. Perennial forage legumes, especially clover, are increasingly promoted for their ability to biologically fix atmospheric nitrogen, improve soil structure and reduce dependency on synthetic fertilisers in subsequent crops [6,7].

Although numerous studies have analysed individual crops from either a technological or economic perspective, integrated comparative assessments that simultaneously consider technological requirements, agro-economic performance and rotation effects remain limited under Romanian conditions. In this context, the present study aims to provide a comparative analysis of major field crops cultivated in Romania—common wheat, sunflower, rapeseed, industrial hemp and clover—by correlating technological characteristics with agro-economic indicators. The results are intended to support informed decision-making at farm level and contribute to the design of resilient and sustainable cropping systems aligned with European policy objectives.

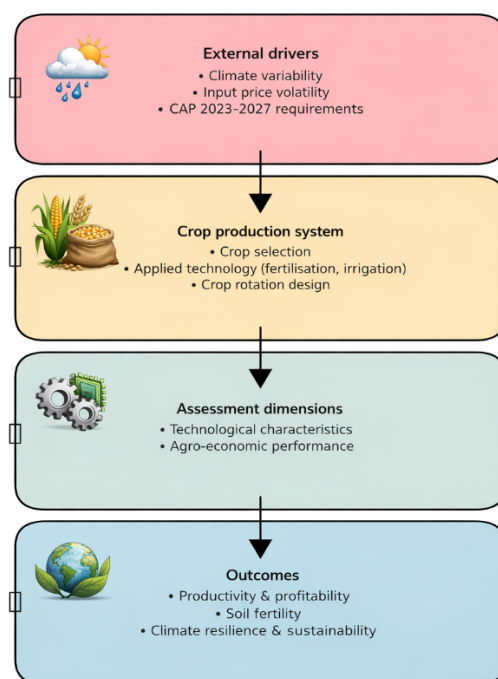


Figure 1. Conceptual framework for the assessment of field crops within sustainable cropping systems

2. Materials and methods

The study is based on a synthesis of data from national technical crop guides published by the Ministry of Agriculture and Rural Development [5], official statistical data provided by the National Institute of Statistics [8] and price references from the National Agency for Fiscal Administration [9]. These sources were complemented with peer-reviewed scientific literature addressing crop technology, economic performance and sustainability under continental climate conditions [3,10].

Five crops were selected for analysis: common wheat, sunflower, rapeseed, industrial hemp and clover. For each crop, two categories of indicators were evaluated:

Technological parameters, including vegetation period, sowing and harvesting time, fertilisation and irrigation requirements, soil suitability and role in crop rotation;

Agro-economic parameters, including establishment costs, maintenance costs, average yields and reference market prices.

The analysis is descriptive-comparative, focusing on average values representative of Romanian farming conditions. All economic data were harmonised at hectare level, and yields were expressed as national averages under conventional production systems to ensure comparability across crops.

3. Results

3.1 Technological characteristics

A comparative evaluation of technological characteristics is essential for understanding how different field crops respond to pedo-climatic conditions and management practices. Parameters such as vegetation period, fertilisation and irrigation requirements, soil suitability and role in crop rotation determine not only crop productivity but also the compatibility of each crop within diversified farming systems. Analysing these technological traits provides a basis for assessing crop adaptability, resource use efficiency and their contribution to sustainable rotations under Romanian agricultural conditions [1,3].

Table 1. Technological characteristics of the analyzed crops

Crop	Vegetation period	Irrigation	Fertilization (kg/ha)	Soil suitability	Role in rotation
Common wheat	270–300 days	Recommended	N 80–140; P ₂ O ₅ 40–70; K ₂ O 40–60	Chernozems, luvisols	Good preceding crop
Sunflower	100–130 days	Optional	N 50–90; P ₂ O ₅ 60–80; K ₂ O 80–120	Chernozems	Deep nutrient use
Rapeseed	280–320 days	Recommended	N 120–180; P ₂ O ₅ 60–90; K ₂ O 80–120	Fertile soils	Excellent preceding crop
Industrial hemp	90–140 days	Recommended	N 60–120; P ₂ O ₅ 40–80; K ₂ O 60–100	Well-drained soils	Diversification crop
Clover	2–4 years	Recommended	P ₂ O ₅ 40–60; K ₂ O 60–80	Loamy soils	Soil fertility improvement

Common wheat is a winter cereal with a vegetation period of approximately 270–300 days and moderate requirements for water and nutrients. Its adaptability to a wide range of soils makes it a key component of Romanian cropping systems, although yield stability is increasingly affected by drought stress in southern and eastern regions [3].

Sunflower is a spring oilseed crop characterised by high heliophilous behaviour and good drought tolerance. Its deep root system allows efficient use of water and nutrients from deeper soil layers, which explains its stable performance under semi-arid conditions [11]. Rapeseed, mainly cultivated as an autumn crop, exhibits high nutrient demand and sensitivity to climatic stress, but it benefits from early harvesting and represents an excellent preceding crop for cereals.

Industrial hemp has a relatively short vegetation period and is characterised by low phytosanitary pressure and high biomass production. Its multifunctionality and adaptability make it a promising diversification crop in sustainable rotations [6]. Clover, as a perennial legume maintained for several years, plays a distinct agronomic role by fixing 50–150 kg N/ha annually and improving soil structure and biological activity [7].

3.2 Agro-economic characteristics

In addition to technological considerations, agro-economic performance plays a decisive role in farmers' crop selection decisions. Establishment and maintenance costs, yield levels and market prices jointly determine the profitability and financial risk associated with each crop. A comparative analysis of these economic indicators allows for the identification of crops that offer stable returns, as well as those that contribute indirectly to farm income through cost reduction and improved performance of subsequent crops within the rotation [12,13].

Table 2. Agro-economic characteristics of the analysed crops

Crop	Establishment cost (EUR/ha)	Maintenance cost (EUR/ha)	Average yield	Market price	Remarks
Common wheat	400–600	300–500	4–6 t/ha	0.9–1.1 RON/kg	Stable market
Sunflower	450–650	350–550	2.5–3.5 t/ha	1.5–2.0 RON/kg	High oil demand
Rapeseed	500–750	450–650	3–4 t/ha	2.0–2.5 RON/kg	High profitability
Industrial hemp	350–500	200–350	5–6 t/ha biomass	Variable	Bioeconomy potential
Clover	300–450	250–400	6–10 t/ha hay	—	Indirect economic benefits

Common wheat and sunflower show relatively balanced cost-to-output ratios, supported by stable markets and well-established production technologies. Rapeseed involves higher establishment and maintenance costs, mainly due to fertilisation and plant protection requirements, but it compensates through higher market prices and yield potential [13].

Industrial hemp exhibits lower maintenance costs compared to oilseed crops and increasing market opportunities in the bio-economy, while clover provides mainly indirect economic benefits by reducing fertiliser costs for subsequent crops and improving overall system sustainability. Similar findings regarding the economic efficiency of diversified crop rotations have been reported in previous studies conducted in Eastern Europe [12,14].

The sustainability of field crop systems depends on complex interactions between crop selection, technological inputs and environmental conditions. In Romanian agriculture, these interactions are increasingly influenced by climate variability, input price volatility and policy-driven constraints related to resource efficiency and environmental protection. Understanding how different crop types respond to fertilisation, irrigation and soil conditions is therefore essential for designing crop rotations that balance economic performance with long-term soil conservation. Previous studies have shown that diversified rotations integrating cereals, oilseeds and legumes can significantly improve nutrient use efficiency and yield stability under continental climate conditions [4,10].

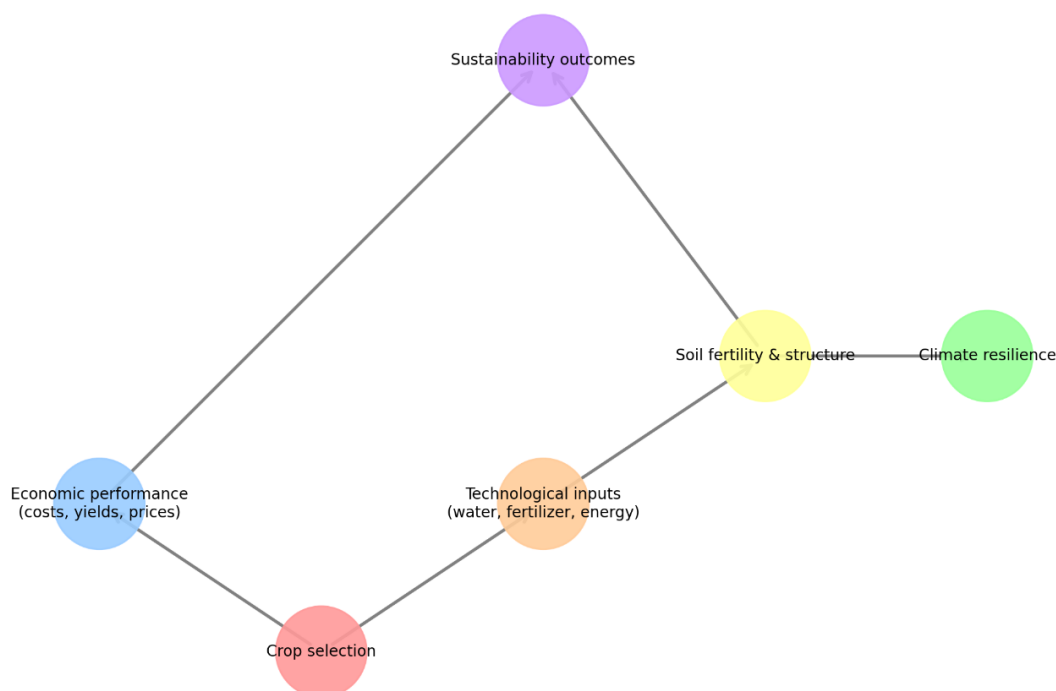


Figure 2. Drivers and outcomes of crop selection in Diversified farming systems

As illustrated in Figure 2, crop selection directly influences both the level of technological inputs required and the resulting economic performance, while indirectly affecting soil fertility and climate resilience. Crops with high input demands, such as rapeseed, may generate increased short-term profitability but also intensify pressure on soil nutrient reserves if not properly integrated into rotations. In contrast, perennial legumes and low-input industrial crops contribute to improved soil structure and biological activity, thereby enhancing system resilience and reducing dependency on synthetic fertilisers. These relationships confirm that sustainability outcomes are not determined by individual crops alone, but by their strategic combination within diversified cropping systems, in line with the principles of climate-smart agriculture promoted at European level [7,14].

Production costs represent a key factor influencing farmers' crop selection decisions, particularly under conditions of increasing prices for fertilisers, fuel and plant protection products. In Romania, cost structures differ substantially among cereals, oilseeds, industrial crops and forage legumes, reflecting their specific technological requirements and sensitivity to input use. A comparative assessment of establishment and maintenance costs provides valuable insight into the economic risks associated with each crop and supports the optimisation of crop rotations from both a financial and sustainability perspective.

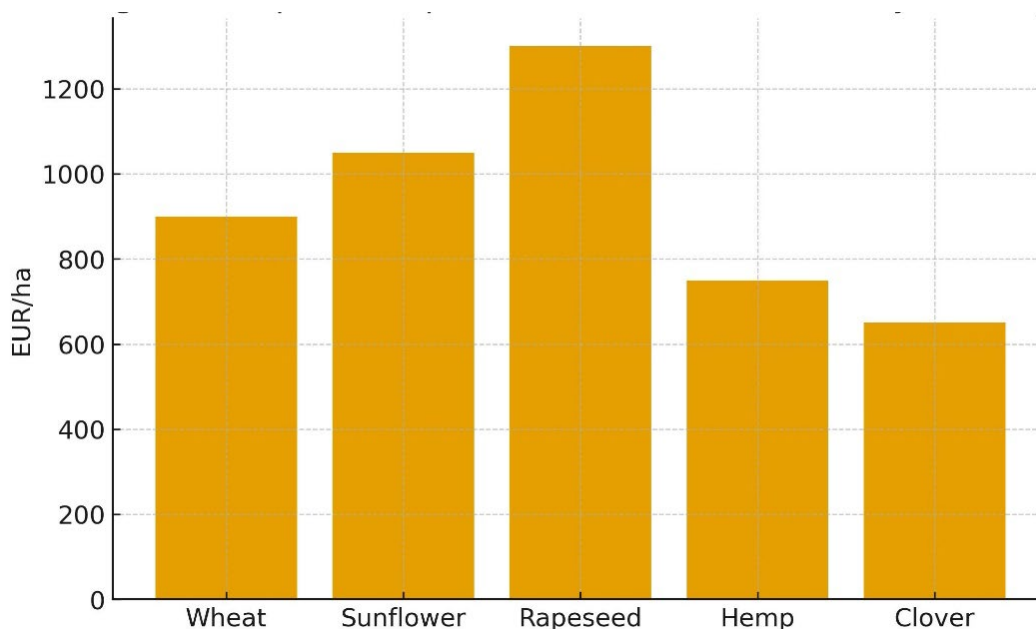


Figure 3. Comparative production costs of the analysed crops

As shown in Figure 3, rapeseed and sunflower are associated with the highest production costs per hectare, primarily due to their elevated fertilisation and crop protection requirements. Common wheat occupies an intermediate position, benefiting from well-established technologies and relatively predictable input use. In contrast, industrial hemp and clover exhibit lower production costs, reflecting reduced phytosanitary pressure and, in the case of clover, the absence of mineral nitrogen fertilisation. These cost differences underline the economic advantages of integrating low-input crops into rotations, as they can contribute to overall cost reduction and income stabilisation at farm level, particularly under conditions of climatic and market uncertainty.

Crop yield potential remains one of the most important indicators for evaluating the performance and viability of different cropping options. Under Romanian pedo-climatic conditions, yield levels are strongly influenced by crop biology, input intensity, soil fertility and the ability of crops to cope with climatic stress, particularly drought and heat waves. Comparing yield potential across different crop types provides essential information for assessing

productivity trade-offs within diversified rotations and for identifying complementary crops that contribute to both economic returns and long-term system stability.

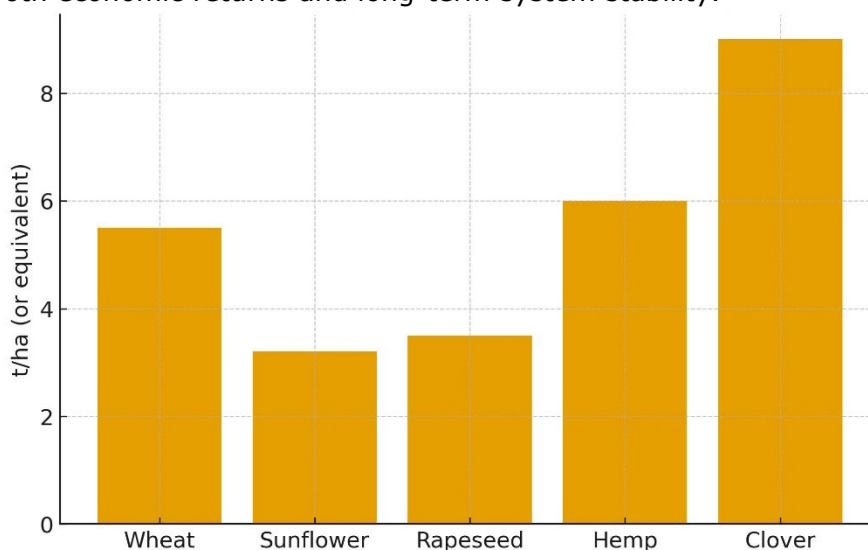


Figure 4. Comparative yield of the analysed crops

Figure 4 highlights clear differences in yield potential among the analysed crops, reflecting their distinct biological characteristics and management requirements. Common wheat and rapeseed offer relatively high and stable yields when cultivated under appropriate technological conditions, while sunflower shows moderate yield levels but compensates through strong market demand. Industrial hemp demonstrates high biomass productivity, supporting its role as an industrial and bio-economic crop, whereas clover achieves substantial forage yields and delivers additional agronomic value through biological nitrogen fixation. These results confirm that productivity should not be assessed solely in terms of harvested output, but also by considering the indirect contributions of each crop to soil fertility, input reduction and yield stability in subsequent crops, as emphasised in previous studies on sustainable crop rotation systems.

The graphical comparison of costs and yields highlights the complementary roles of the analysed crops and supports the design of balanced crop rotations that combine economic performance with environmental benefits.

4. Discussion

The results of the present study highlight the importance of integrating technological and agro-economic considerations when evaluating crop suitability within sustainable farming systems. The comparative analysis confirms that the performance of major field crops cultivated in Romania is not determined solely by yield potential, but by a complex interaction between input requirements, climatic adaptability, soil effects and economic efficiency.

From a technological perspective, cereals and oilseeds exhibit contrasting characteristics. Common wheat remains a cornerstone of Romanian agriculture due to its adaptability and relatively stable yields; however, its sensitivity to drought stress in southern and eastern regions raises concerns regarding long-term yield stability under climate change scenarios (Ion, 2010; Dragomir et al., 2022). Sunflower demonstrates superior drought tolerance and efficient nutrient uptake from deeper soil layers, making it particularly suitable for semi-arid areas, while rapeseed, despite its high productivity potential, requires intensive management and is more vulnerable to climatic and phytosanitary stress [11].

The inclusion of industrial hemp and clover introduces additional functional value within crop rotations. Industrial hemp combines relatively low maintenance costs with high biomass productivity and growing market demand in the bio-economy, supporting diversification strategies aimed at reducing economic risk [6]. Clover, although less significant from a direct

market perspective, provides substantial indirect benefits through biological nitrogen fixation, improved soil structure and enhanced performance of subsequent crops. These findings are consistent with previous studies demonstrating the positive effects of legumes on soil fertility and nutrient cycling in diversified rotations [4,7].

The agro-economic analysis further emphasises the trade-offs between profitability and input intensity. While rapeseed and sunflower are associated with higher production costs, they can generate attractive economic returns under favourable conditions. In contrast, low-input crops such as clover and industrial hemp contribute to income stabilisation by reducing fertiliser and plant protection costs at system level rather than through immediate financial output [12,13].

Overall, the results support the concept that sustainability outcomes in agriculture are not achieved through the optimisation of individual crops, but through their strategic integration within diversified cropping systems. Such systems enhance resilience to climatic variability, improve resource use efficiency and align agricultural practices with the objectives of climate-smart agriculture and the EU Common Agricultural Policy.

5. Conclusions

The comparative assessment of major field crops cultivated in Romania demonstrates that sustainable cropping systems cannot rely on a single crop type or production strategy. Common wheat and sunflower remain economically strategic crops due to their stable markets and adaptability, while rapeseed offers high profitability under appropriate technological and climatic conditions. However, their long-term sustainability is closely linked to input management and rotation design.

Industrial hemp and clover play a crucial complementary role by enhancing crop diversification, improving soil fertility and reducing dependency on mineral fertilisers and intensive plant protection inputs. Their integration into crop rotations contributes to increased resilience against climatic stress and market volatility, particularly under continental climate conditions.

The study confirms that optimal crop rotation design should balance economic performance with environmental sustainability by combining cereals, oilseeds, industrial crops and perennial legumes. Such an approach supports the objectives of the EU Common Agricultural Policy related to resource efficiency, soil protection and climate resilience, while providing practical guidance for farmers seeking to improve the long-term stability and sustainability of their production systems.

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Conflicts of interest: The authors declare no conflict of interest.

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(Article)

ANALYSIS OF THE SEPARATION PROCESS OF SEEDS FROM HEADS BASED ON THE LENGTH OF THE AXIAL FLOW THRESHER

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Keywords: modelling, separation, threshing, seeds, ears, axial threshing machine, rotor

Abstract: *The analysis of the threshing and separation process involves the application of a method of description, analysis and analytical determination of the system performances: threshing machine - work process. The analysis of the process of separation of seeds passing through an axial flow threshing machine was carried out taking into account that the separation function $ss(x)$ is given as a function of the length of the threshing machine. Models were then developed to describe the variation of the percentage (cumulative) of separated seeds $ss(x=L)$, corresponding to the modification of the functional parameters of the threshing machine (depending on the peripheral speed of the rotor, the flow rate of straw parts and the humidity of the straw parts).*

1. Introduction

The analysis of the threshing and separation process emerged as a necessity in order to improve the quality of the threshing process. In the case of axial flow threshers, this was done later due to the fact that the appearance of combines with longitudinal threshing devices was only after the 1970s. Shortly after this, the first research in this field appeared, continuing until today when a sufficiently good modeling of the separation process was achieved [1-7]. This is also supported by the very good results obtained in operation with this type of threshing device, the percentage of separated seeds often exceeding 99%.

The paper aims to analyze the threshing and separation of seeds in an axial flow threshing device, taking into account the input and output quantities of a work process carried out by an axial threshing device.

Recent studies have demonstrated that the threshing and separation process in longitudinal axial flow machines can be described by theoretical models based on variable mass theory, in which the cumulative threshing and separation rates follow an exponential distribution along the axis, experimental validation confirming a very good correlation between the calculated and measured data ($R^2 > 0.97$) [8]. In the context of the limitations of traditional mechanical transmissions, recent research has demonstrated that the integration of electric drive systems of the threshing cylinder, combined with inertia parameter identification strategies, can significantly improve the stability and adaptability of the harvesting process, contributing to reducing losses and increasing the quality of operations [9]. To address the limitations of conventional threshing drums, recent research has proposed the use of a dual power supply threshing drum (DPSTD), in which the electric motor, integrated through a coupling device, provides torque compensation functions and extends the speed adjustment range, improving functional stability and reducing speed errors by over 70% under fluctuating load conditions [10]. Recent research has confirmed that the adoption of innovative technological solutions, such as integrated cutting and threshing machines for sensitive crops and flexible threshing devices with articulated elements, simultaneously optimizes operation efficiency and reduces damage to

plant material, achieving undamaged threshing rates of over 95% and reducing the grain breakage rate to below 4% [11-12].

Recent studies based on DEM simulations, experimental validations and high-speed imaging have demonstrated the importance of optimizing parameters and structural configurations in axial flow threshers for rapeseed, achieving reduced losses and increased efficiency, while research on straw chopping and spreading systems has highlighted the need for improvements to ensure uniform distribution and high quality of post-harvest work [13-15].

Recent studies in the specialized literature have demonstrated that structural optimization and parameter adjustment of axial flow threshers can ensure seed separation rates of over 99% and minimal losses even at high flow rates, while research on vibrations transmitted to combine and tractor operators highlights the need to reduce whole-body vibration exposure, with emerging electrical solutions demonstrating isolation efficiencies of up to 98% [16-19].

2. Materials and methods

Input and output quantities of the system

The work process carried out by the threshing machine [20], regardless of its type, is very complex, being influenced by a series of parameters defined by:

- the material being threshed;
- the material supply system of the threshing machine;
- its construction and working regime.

For the analysis of the work process in the axial flow threshing machine, the following input quantities were taken into account:

- Material characteristics:
 - seed moisture, u_s ;
 - straw moisture, u_p ;
- Feeding system:
 - feeding width, l_a ;
 - material layer height at feeding, h_a ;
 - feeding speed, v_a ;
 - feeding direction at the angle γ_1 ;
 - material flow rate, q ;
 - straw flow rate, q_p .
- Threshing machine construction:
 - type: tangential or axial;
 - radius of the beater (rotor), R ;
 - length of the beater (rotor), L ;
 - length of the arc of the circle of the counter beater, l ;
 - number of rails of the beater (rotor), z ;
 - radial dimension of a rail, δ_R ;
 - angle of the rails, on the rotor, with respect to the generators, β_1 ;
 - angle of the helical rails, on the housing, with respect to the generators, β_2 .
- Operating mode:
 - speed (angular velocity, ω);
 - peripheral speed of the beater (rotor), v ;
 - distance of the beater (rotor) - counter beater (counter rotor) at the inlet δ_i ;
 - distance of the beater (rotor) - counter beater (counter rotor) at the outlet δ_e .

Experimental setup and equipment used

The experimental research on the axial flow threshing machine was carried out at INMA Bucharest on a B-90 axial flow threshing machine (fig. 1) which is equipped with a threshing machine with a length of 2,000 mm. The threshing machine is driven from the tractor power take-off using a cardan shaft that is coupled through a rack to the threshing machine drive flywheel.



Figure 1. Side view of the B-90 baton

The collection of the pile of material separated by the counter-thresher was carried out using a matrix (10 x 5) of collection boxes, one box having the dimensions: 200x200x100 [mm x mm x mm].

The collection of the material discharged at the exit of the threshing machine (fragmented straw, unthreshed ears, unseparated seeds, chaff, etc.) was done on a tarpaulin with the dimensions of 2x3 [m²], by means of two flying panels. Tensometric marks were mounted on the threshing shaft in order to measure the resisting moment, using specialized equipment.

The axial threshing machine was fed tangentially, in an area where the rotor is equipped with rails. In the same area, the threshing machine casing is provided with spiral rails, mounted with an inclination of 60° to the rotor axis, which has a diameter of 560 mm. These rails are arranged on the casing, at an angle of 180°.

The counter rotor, with a winding angle of 110° has a construction similar to that of a conventional tangential threshing machine, in the threshing area the rails are three in number and parallel to the rotor axis, in the separation area, on the same generators on which the rails are mounted, the rotor being provided with three rows of separator plates with the dimensions in figure 5, mounted inclined at an angle that can take the values of: 0°, 22.5° and 45°.

The threshing machine casing is entirely provided with holes of 20x40 mm dimensions, the active separation surface of the casing representing about 55% of its total surface. Helical rails of 30 mm height and 500 mm length are mounted on the sides of the casing, which can be mounted at different angles: 60°; 75°.

The main adjustable and measurable parameters taken into account, which influence the performance of the work process, were:

- threshing machine speed n [rot/min], adjustable within the limits of 600-1200 rot/min; corresponding to this speed range, the threshing machine peripheral speed was in the range of 22-32 m/s;
- material flow rate q [kg/s], was determined by weighing the sample of plant material and the time in which the threshing machine was fed uniformly. The mass of material introduced into the device was checked for each test with the mass of components collected after the threshing process. During the tests, the material flow rate corresponding to the width of the threshing device was changed within the limits of 1.5-4 kg/s;
- the distance δ between the threshing and counter-threshing rails is variable, measured in the direction of material advance. Thus, the distance δ can be varied as follows: $\delta_i = 12-24$ mm at the entrance and $\delta_e = 3-7$ mm at the exit;
- the material feed speed can be continuously varied using a speed variator, within the limits of: 3.1-4.65 m/s;
- the material feed angle can vary within the limits of 15-35° and is measured using a clinometer.

Research methodology. Experimental method

During the experiments, the values of several parameters were varied one after another, namely: the peripheral speed of the thresher, the material flow rate, the S/PP ratio, the feed speed, the distance between the thresher and the counter-thresher.

The block of boxes for collecting the separated material is placed separately in the support guides on the chassis of the threshing device module.

To collect the discharged straw parts, a tarpaulin and the flying panels for guiding the material were placed accordingly, so that it does not spread over the grains.

The plant material required for the sample was weighed and then placed on the feed conveyor belt, predominantly with the ears forward in relation to the direction of travel.

The threshing machine is put into operation by starting the tractor engine, operating the threshing device from its power take-off, at which the speed is checked and adjusted. At the end of the experiment, the material in the collection boxes was weighed and then separated into:

- separated seeds;
- unthreshed and separated seeds, i.e. seeds for the return circuit.

Samples were taken from the amount of separated seeds to determine the moisture content of the seeds as well as to determine the percentage of damaged seeds.

The mass of separated material on the tarpaulin was weighed and recorded, after which the block of collection boxes was detached. The contents of the 50 collection boxes were placed in 50 numbered plastic or paper bags.

The material on the tarpaulin was processed manually, with great care, separating into unthreshed ears (threshing losses), threshed and unseparated seeds (separation losses), and discharged straw parts. A sample was taken from each sample of the discharged straw parts to determine the moisture content of the straw parts.

The determination of the moisture content of the seeds and the strawy parts was carried out by drying them in an oven at a temperature of 105°.

3. Results

The processing of the material separated from an experiment was done on the day of the experiment and during the following day. All data were entered into preliminary measurement tables.

Table 1

Separated seeds [g]						
No.	A	B	C	D	E	Sum
1.	12.4	23.4	27.5	16.5	4.9	84.7
2.	18.9	41.0	48.9	32.9	13.8	155.5
3.	23.3	43.9	51.1	33.9	12.4	164.6
4.	17.2	34.1	43.2	26.1	7.7	128.3
5.	16.6	34.6	39.5	29.5	8.1	128.3
6.	12.1	27.4	35.7	24.1	6.7	106.0
7.	10.4	22.2	27.2	24.6	6.4	90.8
8.	10.3	20.2	23.2	20.6	5.5	79.8
9.	7.6	16.2	23.7	15.2	3.8	66.5
10.	1.5	2.9	3.0	2.6	0.8	10.8
Suma	130.3	265.9	323.0	226	70.1	1015.3

The graphical representation of these data is shown in Figure 2.

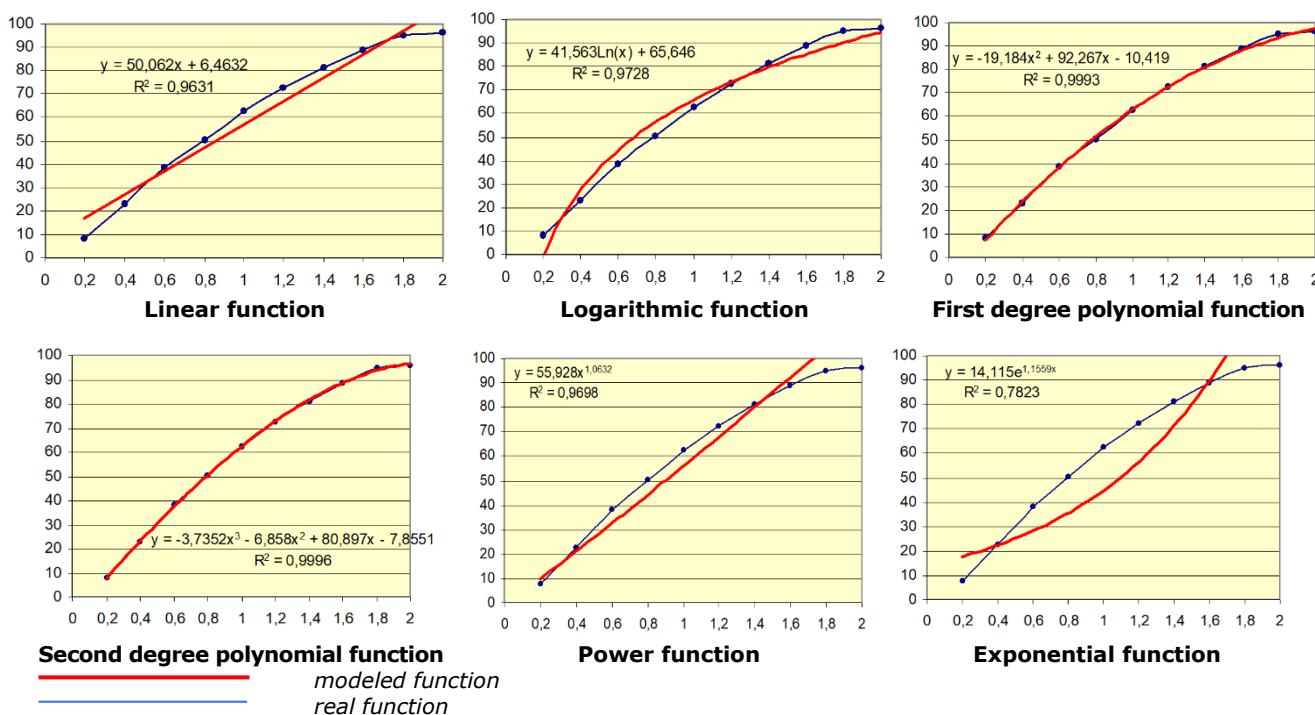


Figure 2. Graphical representation of functions that approximate the seed separation process

• **Analysis of the threshing and separation process subject to the influences of functional parameters**

Through the mathematical equations that compose it, the general mathematical model - in the case of the axial device - mainly describes:

- the separation of seeds from the ears;
- the separation of seeds by the counter rotor and the casing;
- the amount of losses during threshing and separation;
- the separation of straw parts.

On the length of the rotor $x \in$ (x takes the maximum value), the expressions for calculating the qualitative indices that characterize the threshing process of an axial device are obtained. The values of the coefficients β and λ used in this model implicitly express the influences of all constructive, functional factors as well as the physical and mechanical properties of the processed material.

From the analysis of the functions that model the separation process, it is observed that the polynomial functions of degree II and III best approximate the real function resulting from the data measured after carrying out the tests.

Thus, for the separation of seeds, the polynomial function of degree II is of the form:

$$y = -ax^2 + bx - c, \tag{1}$$

where: $a = 17,499 \div 26,752;$
 $b = 88,322 \div 104,87;$
 $c = 3,7228 \div 10,525,$

and the third degree polynomial function is:

$$y = mx^3 - nx^2 + px - q, \tag{2}$$

where: $m = -6,512 \div 3,7352;$
 $n = 6,858 \div 48,162;$
 $p = 80,897 \div 124,62;$
 $q = 5,5671 \div 10,488.$

The second-degree polynomial function approximates the real function well, the correlation coefficient varying between 0.9985-0.9996, while the third-degree polynomial function has a correlation coefficient ranging between: 0.9991-0.9998.

Considering that the polynomial function of degree III approximates the real function better than the polynomial function of degree II by a maximum of 1.1 per thousand, it is considered that the polynomial function of degree II: $y = -ax^2 + bx - c$, approximates the real function sufficiently well..

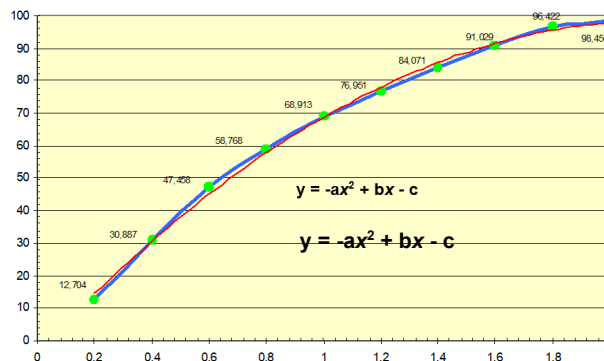


Figure 3. Seed separation based on rotor length

In the case of this type of function, the range of values of a , b , and c is much narrower than in the case of the third-degree polynomial function, highlighting the fact that the deviation from the real function is relatively small..

In the case of the axial device, the separation of seeds along the length of the threshing device (threshing and separation zones) is described by:

- the cumulative frequency distribution function that quantifies the cumulative percentage of separated seeds for $x \in [0, L]$;
- the seed separation density function that quantifies the frequency of seed separation along the length $x \in [0, L]$.

Next, we propose models that describe the variation of the (cumulative) percentage of separated seeds $s_s (x=L)$, corresponding to the modification of the functional parameters of the threshing device.

a) Seed separation depending on the peripheral speed of the rotor

Seed separation along the length of the axial threshing device (threshing and separation zones) is described by a second-degree polynomial function of the form:

$$s_s(v_p) = -av_p^2 + bv_p - c \quad (3)$$

where: a , b and c are experimentally determined values.

This function well describes the phenomenon of seed separation in the two zones (threshing and separation), taken separately or together.

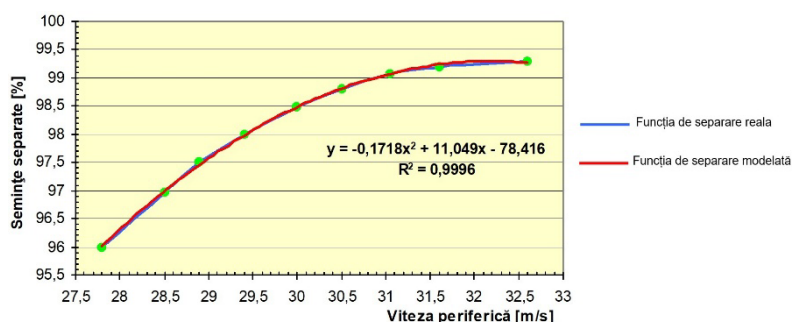


Figure 4. Seed separation depending on the rotors peripheral speed

The graph of this function (figure 5) shows that at high peripheral speeds, the curves tend asymptotically towards a maximum of 100% separation.

It follows that the dependence of the percentage of separated seeds on the peripheral speed of the rotor is described by a second-degree polynomial, whose maximum is obtained for $v_p = b/2c$.

The separation from the ears and the separation of seeds by the counter rotor and the casing take place due to the energy transmitted from the active elements (rails, plates, etc.) of the rotor, by impact. The higher the transmitted energy, the greater the separation of seeds.

b) Seed separation based on straw flow rate

In the case of the axial threshing device tested with varying straw flow rates, there is a maximum of seed separation and with increasing straw flow rates, seed separation continuously decreases.

At low straw flow rates, the material distribution in the space between the rotor and the housing is done in a thin layer, the material is more easily driven into motion and as a result insufficiently processed, which is why the percentage of separated seeds decreases. At relatively high straw flow rates, separation is made more difficult by the thick layer of material.

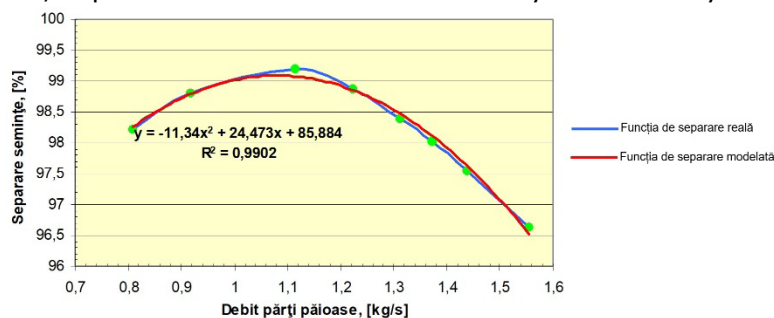


Figure 5. Seed separation based on straw flow rate

$$s_s(q_p) = a + bq_p - cq_p^2 \quad (4)$$

where a, b, c are experimentally determined coefficients

It follows that the dependence of the percentage of separated seeds on the flow rate of straw parts is described by a second-degree polynomial, whose maximum is obtained for $q_p = b/2c$.

c) Separating seeds based on the moisture content of the straw parts

Seed separation by the concave decreases with increasing straw flow rate almost linearly; at higher humidity, the percentage of separated seeds is higher. In the axial device, seed losses (unseparated seeds) are higher when the material has a higher humidity.

Data analysis suggests another way of interpreting the influence of humidity on seed separation. Due to the relatively long residence time of the material in the axial threshing machine, the humidity of the straw parts influences seed separation in two contradictory ways.

Thus, it can be said that at a low humidity of the straw parts, the separation of the seeds is easy, but the pronounced fragmentation of the straw prevents the separation of the seeds by the counter rotor and the casing, which means that:

$$s_s = f(u_p) \quad (5)$$

where: u_p - the moisture of the straw parts.

At high humidity of the strawy parts, threshing the material becomes more difficult, so:

$$s_s = \frac{1}{f_2(u_p)} \quad (6)$$

A function that simultaneously describes the two modes of humidity influence can have the following form (figura 6):

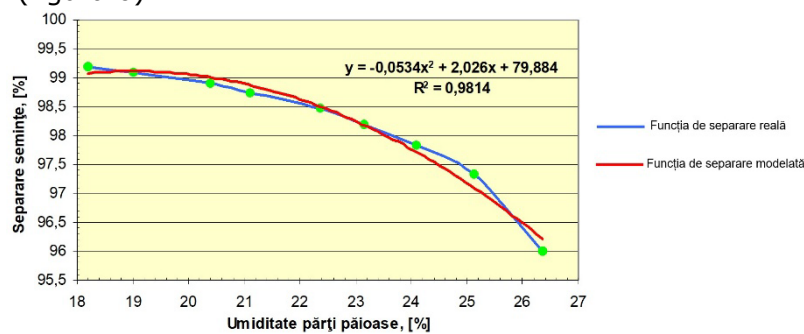


Figure 6. Separating seeds based on the moisture content of the straw parts

If:

$$\frac{ds_s}{du_p} = 0 \quad (7)$$

we obtain the value of the humidity of the straw parts for which function (6) admits a maximum, respectively $u_p = \eta/\xi$.

The function that best models the separation of seeds depending on the humidity of the straw parts also has a polynomial form of degree II and is of the form:

$$s_s(u_p) = -au_p^2 + bu_p + c, \quad (8)$$

which has a maximum separation for: $u_p = b/2c$.

5. Conclusions

The paper presents the analysis of the working process in an axial flow threshing machine which aims to model the process of separating seeds from the ears depending on the length of the threshing machine, the separation of seeds being described by:

- cumulative frequency distribution function for $x \in [0, L]$;
- density function of seed separation along the length $x \in [0, L]$.

Several models were analyzed to describe the variation of the percentage of separated seeds $s_s(x)$, corresponding to the modification of the functional parameters of the threshing

machine (seed separation depending on: the peripheral speed of the rotor, the flow rate of straw parts and the humidity of the straw parts).

Following the separation process, depending on these factors, the following observations can be distinguished::

- seed separation increases with increasing rotor peripheral speed, up to a limit speed of 32-33 m/s; above this limit, the percentage of damaged seeds increases greatly;
- seed separation is performed very well (reaches a maximum), for an average combine flow rate; at the limit (low or very high flow rates), the separation process is not performed satisfactorily;
- the humidity of the material entering the threshing machine and especially the humidity of the straw parts has a very large influence on the separation of seeds; above a humidity of 18-20% the percentage of separated seeds begins to decrease.

To obtain these results, mathematical analysis was performed using the comparison of the results of the most used functions in the specialized literature: linear, exponential, polynomial, power and logarithmic functions, taking into account the function that best approximates the real separation function.

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Conflicts of interest: "The authors declare no conflict of interest."

Review

RESEARCH ON NOVEL SUBSTRATES TO OPTIMIZE MUSHROOM CULTIVATION FOR HIGH-QUALITY PROTEIN YIELD

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Keywords: *edible mushrooms; innovative substrates; compost; protein yield; sustainability; inoculation*

Abstract:

Innovative substrates used for the cultivation of edible mushrooms, obtained by combining cereal straw with alternative nutrient sources such as hydroponic roots, cassava peels, grape pomace, coffee husks, rice bran, sawdust, or cotton and flax residues, optimize metabolic efficiency and production yield. Adjustment of the C/N ratio through nitrogen-rich additives — soybean meal, vegetal compost, legume residues — accelerates mycelial colonization and stimulates fructification. Moreover, the integration of microalgal biomass, biochar, or plant extracts enhances protein synthesis and the nutritional quality of the resulting fungal biomass. Thus, these complex substrates demonstrate high potential for sustainable industrial mushroom cultivation and the production of functional proteins with superior biological value.

1. Introduction

The rapid growth of the global population and profound changes in dietary habits have created a substantial and urgent need for alternative, sustainable, and efficient protein sources. In this context, the cultivation of edible mushrooms stands out as an innovative solution: they convert lignocellulosic waste into edible biomass, featuring a high protein content and a low ecological impact. Fungal mycelium is not merely a passive colonizer of the substrate; it acts as a “biological processor,” transforming plant material into proteins, essential amino acids, and bioactive compounds. The substrate, in turn, shifts from being a passive support to becoming the active matrix of the process, influencing both the rate of mycelial colonization and the quality of fruiting, as well as the fine nutritional profile.

Traditionally, substrates used in commercial mushroom production have included cereal straws, sawdust, and manure composts. These substrates have provided satisfactory performance but face significant limitations: seasonal availability, competition with other uses (fodder, compost), contamination risks, and the inability to optimize protein content or amino acid composition. Recent studies emphasize that traditional substrates are exposed to challenges related to availability, cost, and ecological impact, [1–5].

In recent years, research has identified two major directions of innovation in mushroom substrates. The first focuses on formulating alternative substrates based on agro-industrial waste and unconventional materials. Recent examples include coffee grounds, corn stalks, seed husks, alfalfa pulp, and invasive halophytic plants. These materials not only valorize waste but also allow optimization of chemical composition (nitrogen, trace elements, C/N ratio) and physical structure (aeration, water retention) for optimal mycelial development. In experiments with *Pleurotus ostreatus*, mixtures such as rice straw and sugarcane bagasse (3:2 ratio) resulted

in a yield of 886 g/kg of substrate and increased protein content compared to traditional substrates [6–9]. The use of alfalfa residue increased biological efficiency to 166% and protein content to 20.4% [10].

The second direction of innovation is represented by advanced substrate processing and control technologies. The substrate is no longer merely a mass of plant material; it becomes a biologically engineered product. These technologies involve mechanized pre-treatment (chopping, compacting), industrial-scale pasteurization/sterilization, standardized “ready-to-inoculate” mixtures, digital monitoring of parameters (moisture, temperature, CO₂), as well as fully automated mixing and inoculation lines. As a result, substrates optimized for gourmet species are obtained, reducing batch-to-batch variability and increasing biological efficiency by 10–20% compared to traditional substrates.

By combining emerging materials with advanced technologies, the substrate becomes a strategic tool for optimizing protein yield. The objective is no longer solely the amount of biomass produced, but also its nutritional quality: increasing protein content, optimizing the amino acid profile, integrating bioactive compounds, and maximizing the biological value of the fruiting bodies.

Three fundamental aspects are targeted:

1. **Substrate material** – identifying alternative biomass with high nutritional potential and physical structures suitable for mycelial growth.
2. **Substrate preparation and processing** – pre-treatment, mixing, and monitoring methods that can enhance efficiency and final protein content.
3. **Industrial integration** – scalability and compatibility with modern technologies and standard commercial substrates.

The following sections highlight the most recent and innovative substrates—whether implemented at pilot or industrial scale, or currently under advanced research—and correlate them with the optimization of protein yield in edible mushrooms. Additionally, existing industrial applications, remaining challenges, and future research directions for integrating these substrates into high-quality protein production are analyzed.

The evolution of technological platforms, including standardized substrates, automation, sensors, and digital monitoring, is transforming the perspective from traditional agricultural cultivation to an integrated bio-manufacturing system, in which the substrate becomes an engineered product and the mushroom a nutritionally calibrated product for modern market demands.

By combining scientific and technological approaches, this material establishes a conceptual framework for the analysis of innovative substrates and associated technologies, highlighting their potential to produce mushrooms with superior protein content, in a sustainable manner, and adapted to the future needs of the food industry.

2. Materials and methods

They tested different substrate combinations to increase protein content in edible mushrooms. Simple rules were established for the selection and preparation of substrates, the conduct of experiments, and the way results were analyzed, so that anyone could repeat the tests and compare the results with other studies.

Materials for the substrates were chosen based on the following criteria: local availability, potential as lignocellulosic biomass, ability to adjust the C/N ratio, compatibility with mycelium, and feasibility of pre-treatment. The following variants were prepared:

- Substrate-1: cereal straw (wheat straw) + 20% hydroponic roots + 5% spent mushroom substrate (SMS) from a previous *Pleurotus ostreatus* crop.
- Substrate-2: cereal straw + 20% composted cassava peel + 10% SMS.
- Substrate-3: cereal straw + 20% halophytic plants (e.g., *Salicornia*) + 10% hydroponic roots.

For reference, a standardized commercial substrate was included—a pre-formulated, industrially sterilized mixture used in commercial-scale production (for comparative calibration).

This approach reflects modern methodologies in the field: for example, in a study on various straw and bagasse mixtures for *P. ostreatus*, a 3:2 ratio of rice straw to sugarcane bagasse was used, yielding significant results in terms of yield and crude protein [6]. Reviews on the use of agro-industrial residues also emphasize the importance of raw material selection and substrate pre-treatment [10–13].

Materials underwent uniform pre-treatment: cutting/chopping to sizes of approximately 3–5 cm for straw/hydroponic roots/roots, dry mixing according to the proportions, followed by moisture adjustment to around 65% in wet state. Thermal treatment was carried out by pasteurization at 60–70 °C for 2 h, followed by cooling to room temperature. This protocol corresponds to procedures reported in the literature—for example, in studies using recovered fiber and sawdust for *Pleurotus ostreatus*, substrates with ~65% moisture were obtained before inoculation [14,15].

For commercial substrates, pre-treatment is automated: industrial pasteurization/sterilization lines, humidity and pH control, mechanized mixing, and “ready-to-inoculate” packaging.

The species used was *Pleurotus ostreatus*, selected for its robustness, adaptability, and attractive nutritional profile. Spawn was produced on sterilized wheat following a standardized inoculation protocol. Inoculation was performed using 5% spawn by substrate weight. For each substrate variant, replicates ($n = 5$) were used in bags containing approximately 4 kg of wet substrate or the equivalent in boxes. Incubation (mycelial colonization) was conducted at ~25 °C, relative humidity ~85–90%, indirect light (~200 lux), and controlled ventilation—conditions in line with those described in mushroom cultivation literature.

Key parameters were monitored: time to full colonization (days), pinning/fruiting appearance (days), total fresh fruit weight per unit of wet substrate (g), dry fruit mass (after drying at 60 °C until constant weight), and yield expressed as biological efficiency ($BE = \text{fresh fruit weight} / \text{dry substrate weight} \times 100\%$). The nutritional composition of the fruiting bodies was determined using the Kjeldahl method (total nitrogen $\times 6.25 =$ crude protein). Additionally, substrates were analyzed before inoculation for C/N ratio (total carbon/total nitrogen), pH, and moisture—a methodology adopted in several recent studies.

From accumulated experience in the field, it has been demonstrated, for example, that the C/N ratio is closely correlated with yield and crude protein in *Pleurotus* [16,17].

To compare the experimental variants with industrial practice, the integration of substrate technologies at commercial scale was also documented. These include: optimized pre-formulated mixtures (with components distributable on a large scale), continuous-flow sterilization/pasteurization, automated mixing and inoculation, and real-time digital monitoring of substrate parameters (temperature, moisture, CO₂, pH). These technologies allow for batch uniformity, reduced variability, and increased yield. Recent literature highlights the adoption of sensors for predicting biological efficiency, using FTIR spectroscopy for substrate characterization and predictive modeling [18–20].

The obtained data were subjected to statistical analysis using ANOVA ($\alpha = 0.05$) and Tukey’s test for multiple comparisons, in order to identify significant differences between substrate variants. Additionally, predictive models for biological efficiency based on substrate parameters were constructed (e.g., FTIR model, $R^2 = 0.90$) to reflect technological trends [18]. Study limitations include: laboratory/pilot-scale implementation (not industrial), evaluation of only two flushes, variability of raw materials, and incomplete coverage of the amino acid profile of the protein.

3. Results

Research has shown that the use of innovative substrates significantly influences mycelial colonization, fruit yield, and the protein profile of the edible mushroom *Pleurotus ostreatus*. The

following results are presented across multiple dimensions, with quantitative data and qualitative observations regarding culture development.

The average colonization time of the substrate was closely correlated with its composition and structure. Substrate I (cereal straw + hydroponic roots + SMS) achieved full colonization in 18 days, while Substrate II (straw + cassava peels + SMS) and Substrate III (straw + halophytic plants + SMS) required 20 and 22 days, respectively. The standard commercial substrate reached full colonization in 24 days.

Observations indicated that the more aerated structure and optimal moisture retention of Substrate I favored rapid mycelial development, reducing the risk of contamination. This trend confirms findings in the specialized literature, which emphasize the role of the C/N ratio and nitrogen-rich materials in accelerating mycelial colonization [6].

In addition, the time to the appearance of the first pins was shorter for the experimental substrates (Substrate I: 28 days; Substrate II: 30 days; Substrate III: 31 days) compared to the commercial substrate (34 days). This difference indicates that alternative materials and nutritional supplements stimulate faster initiation of fruiting.

The biological efficiency (BE) was evaluated over the entire cultivation period (two flushes). Substrate I recorded a BE of 92%, Substrate II 87%, Substrate III 85%, and the commercial substrate 80%. This increase in yield correlates with the presence of nitrogen-rich materials and the optimal substrate structure, as noted by Zhou, F.

Data showed that the first flush contributed approximately 60–65% of the total yield, while the second flush was lower, highlighting that the experimental substrates support continuous and high-quality production during the initial fruiting phase.

Kjeldahl determinations indicated higher crude protein values for the innovative substrates: Substrate I – 21.3%, Substrate II – 20.1%, Substrate III – 19.8%, compared to the commercial substrate – 18.5%. These results confirm that substrates incorporating nitrogen-rich resources (SMS, hydroponic roots) enable the mycelium to synthesize higher-quality proteins, consistent with findings reported in the literature [1, 21–23].

In comparison, the commercial substrate—although efficient in terms of uniformity—did not reach the same protein level, suggesting the limitations of standardized technologies when innovative nutrient mixtures are not used.

Substrate I exhibited good structure, friability, optimal water retention, and adequate aeration, which allowed rapid mycelial growth and uniform fruit development.

Substrate II showed slight compaction at the bottom of the bags, but the final yield was almost comparable to that of Substrate I.

Substrate III demonstrated uniform colonization, though the first pins appeared 2–3 days later. The commercial substrate showed uniform colonization and homogeneous structure but lacked the nutritional supplement effects on protein growth.

These observations underline the importance of combining lignocellulosic raw materials with alternative nutrient sources to optimize the quality of the final product.

Experimental data analysis revealed a positive correlation between an optimal C/N ratio (approximately 25–27:1) and both fruit yield and protein content. Substrates with an adjusted C/N ratio through the addition of SMS or hydroponic roots produced mushrooms with higher protein content, consistent with the findings of [6, 21].

Different examples:

A) Soybean straw was added in equal proportions, and the C/N ratio was adjusted using corn cobs, rice bran, and brewers' grains as the main nitrogen sources (1.73% and 4.00%). Three treatments were established for *F. velutipes*: HN (C/N 22:1), MN (27:1), and LN (37:1). The substrate was bottled, perforated, sterilized at 121 °C for 2 hours, and supplemented with 30 mL of liquid milk per bottle. Incubation was carried out at 18 °C under controlled CO₂ conditions. After colonization, the surface was scraped, maintained at 14 °C and 90–92% humidity; lighting began on the third day, and humidity was slightly reduced after primordia formation. A paper collar was

applied 2–3 cm above the bottle opening, and the temperature was maintained at 8–12 °C until harvest. Each treatment included three replicates of 12 bottles, Table 1.

Table 1

Tested formulations with different C/N ratios . [24]

Where: HN – high nitrogen content; MN – medium nitrogen content; LN – low nitrogen content

Ingredient Composition of Substrates (%)								
Treatment	Soybean Straw	Corn cob	Rice Bran	Cottonseed Hull	Brewer's Grains	CaCO₃	Scallop Shell Powder	C/N
HN	25.1	18.84	32.2	6.18	15.74	0.87	1.07	22:1
MN	25.1	18.82	41.88	6.18	6.18	0.87	1.07	27:1
LN	25.1	40.76	32.2	0	0	0.87	1.07	37:1

B) The biological efficiency (%) of *P. tuber-regium* was evaluated by cultivating this macrofungus under controlled conditions using the polypropylene bag method. Among the tested substrates, the highest biological efficiency, 98.59%, was obtained on the Netherlands straw substrate, while sugarcane bagasse and sawdust yielded efficiencies of 78.62% and 70.64%, respectively (Table 2). In the case of rice husks, they did not support the formation of *P. tuber-regium* fruiting bodies; therefore, yield and biological efficiency were not calculated [25].

Table 2

Total yield and biological efficiency of *Pleurotus tuber-regium* cultivated on different agricultural waste substrates [25]

Substrate used	Period of bag opening to first flush (d)	Yield/flush (g)				Total yield (g)	Biological efficiency (%)
		First flush	Second flush	Third flush	Fourth flush		
Paddy straw	19	60.14	76.32	45.21	30.23	211.90	98.59
Sugarcane bagasse	25	40.56	55.13	31.26	30.30	157.25	78.62
Sawdust	42	45.72	57.35	20.10	18.11	141.28	70.64
Rice husk	-	-	-	-	-	-	-

Compoziții aproximative ale corpurilor fructifere de *Pleurotus tuber-regium* cultivate sub diferite substraturi de deșeuri agricole

Table 3

Approximate compositions of *Pleurotus tuber-regium* fruiting bodies cultivated on different agricultural waste substrates, [25],

Data indicates mean +/- standard error

Parameters	Paddy straw	Sugarcane bagasse	Sawdust	Mycelium
Moisture%	85–88	82–85	80–82	45–55
Total protein (g/100 g)	24.29 ± 1.19	16.45 ± 0.92	19.92 ± 0.97	18.07 ± 1.18
Total sugar (g/100 g)	70.53 ± 3.8	58.05 ± 0.52	70.32 ± 6.5	47.88 ± 2.6
Lipid (g/100 g)	1.54 ± 0.18	0.93 ± 0.05	0.98 ± 0.02	1.02 ± 0.08
Crude fiber (g/100 g)	14.69 ± 0.92	4.33 ± 0.06	3.19 ± 0.06	11.1 ± 0.52
Total ash (g/100 g)	5.14 ± 0.46	3.29 ± 0.42	3.76 ± 0.21	4.84 ± 0.43
Sodium (mg/100 g)	37.3 ± 3.2	725.6 ± 50.2	1085.3 ± 92.5	65.9 ± 2.19

Parameters	Paddy straw	Sugarcane bagasse	Sawdust	Mycelium
Potassium (mg/100 g)	90.8 ± 6.3	820 ± 42.5	825.4 ± 29.3	102.5 ± 9.2
Calcium (mg/100 g)	87 ± 7.4	241.5 ± 18.8	409.3 ± 26.3	93.9 ± 15.5
Magnesium (mg/100 g)	30.4 ± 2.9	204.8 ± 21.2	317.6 ± 19.5	36.4 ± 2.7
Copper (mg/100 g)	1.0 ± 0.16	0.8 ± 0.06	0.8 ± 0.02	0.6 ± 0.09
Manganese (mg/100 g)	1.7 ± 0.31	0.5 ± 0.09	0.7 ± 0.12	0.6 ± 0.12
Zinc (mg/100 g)	4.9 ± 0.26	5.2 ± 0.22	4.6 ± 0.31	4.9 ± 0.46
Iron (mg/100 g)	6.5 ± 0.54	0.7 ± 0.01	4.7 ± 0.12	6.4 ± 0.24
Energy (kcal/100 g)	370 ± 22	356 ± 24	370 ± 28	298 ± 26

The determination of the main nutrients in the basidiomes of *Pleurotus tuber-regium* revealed significant variations depending on the cultivation substrate used (Table 3).

Total proteins. The highest protein content (24.29 ± 1.19 g/100 g) was obtained on the rice straw substrate, followed by sawdust (19.92 ± 0.97 g/100 g) and sugarcane bagasse (16.45 ± 0.92 g/100 g). The mycelium showed an intermediate protein level (18.07 ± 1.18 g/100 g).

Total sugars. The highest concentration of total sugars was recorded in fruiting bodies developed on rice straw (70.53 ± 3.8 g/100 g), followed by those cultivated on sawdust (70.32 ± 6.5 g/100 g) and bagasse (58.05 ± 0.52 g/100 g). The mycelium contained 47.88 ± 2.6 g/100 g total sugars.

Lipids. The maximum lipid level (1.54 ± 0.18 g/100 g) was recorded in fruiting bodies grown on rice straw substrate, while sawdust and bagasse yielded lower values (0.98 ± 0.02 g/100 g and 0.93 ± 0.05 g/100 g, respectively). The lipid content of the mycelium (1.02 ± 0.08 g/100 g) was similar to that of the fruiting bodies cultivated on sawdust.

Energy value. Fruiting bodies cultivated on rice straw and sawdust presented identical energy values (370 kcal/100 g), slightly higher than that obtained on bagasse (356 kcal/100 g). The mycelium showed a lower energy value of 298 kcal/100 g.

Crude fiber. The highest crude fiber content (14.69 ± 0.92 g/100 g) was obtained on rice straw, followed by bagasse (4.33 ± 0.06 g/100 g) and sawdust (3.19 ± 0.06 g/100 g). The mycelium contained 11.1 ± 0.52 g/100 g fiber.

Ash. The maximum ash content was recorded in fruiting bodies cultivated on rice straw (5.14 ± 0.46 g/100 g), followed by those on sawdust (3.76 ± 0.21 g/100 g) and bagasse (3.29 ± 0.42 g/100 g). The mycelium showed an intermediate value (4.84 ± 0.43 g/100 g).

Minerals. Mineral analysis, performed by atomic absorption spectrophotometry, confirmed the presence of K, Na, Mg, Ca, Fe, Mn, Cu, and Zn in all samples. The highest concentrations of macroelements (Na = 1085.3 ± 92.53 mg/100 g; K = 825.4 ± 29.33 mg/100 g; Ca = 409.3 ± 26.33 mg/100 g; Mg = 317.6 ± 19.53 mg/100 g) were observed in fruiting bodies cultivated on sawdust. High values were also recorded in those grown on bagasse, while the mycelium exhibited higher contents of Na, K, Ca, and Mg compared to the samples obtained on rice straw.

Moisture content. Moisture varied between 85–88% for fruiting bodies cultivated on rice straw, 82–85% for those on bagasse, and 80–82% for those on sawdust, while the mycelium showed a content of 45–55%. These differences were correlated with the water-holding capacity of each substrate,[25–28].

C) The experiment was conducted at Fungo Puglia Srl (Rutigliano, Italy), a facility specialized in the cultivation of *Pleurotus ostreatus* (Figure 1e). Wheat straw (*Triticum aestivum* L.), stored in bales after harvest, was moistened, chopped into 1–6 cm fragments, and enriched with soybean flour to supplement nitrogen input (Figure 1a). The mixture was arranged in piles

(approximately 4 × 2 m) and subjected to a five-day composting process, followed by steam pasteurization at 65 °C in specialized tunnels (Figure 1b).

After cooling, the substrate was inoculated with the *P. ostreatus* HK35 strain (Figure 1c), pressed into approximately 20 kg bales, and packed in perforated plastic sheets (Figure 1d). The bales were incubated at 25 ± 1 °C and 80% humidity until full colonization, then transferred to fruiting chambers maintained at 17.5 ± 1 °C, 90% humidity, and a 12-hour photoperiod. The substrate preparation steps are presented in Table 4, and the schedule of the four production cycles is shown in Table 5. Straw and colonized substrate samples were randomly collected from the experimental bales,[29–31].



Figure 1. Preparation of the substrate for mushroom cultivation, [29]
(a) short composting process; (b) steam pasteurization; (c) substrate inoculation; (d) preparation of the substrate bags; (e) fruiting body of *Pleurotus ostreatus*.

Table 4

Sample preparation steps during substrate preparation, [29]

Sample preparation steps	Sampled material
A.	Raw straw
B.	Wet, chopped straw supplemented with soybean flour
C.	Substrate at the end of 5 days of composting
D.	Substrate after steam pasteurization

Table 5

Studied production cycles, [29]

Mushroom production cycles	Starting time	Straw age
Cycle 1	July 2019	Fresh
Cycle 2	October 2019	3 months after harvest
Cycle 3	January 2020	6 months after harvest
Cycle 4	April 2020	9 months after harvest

The results obtained highlight the significant influence of straw age and substrate preparation processes on the productivity of *Pleurotus ostreatus*. The total sugar content, particularly soluble sugars, progressively decreased over the four substrate preparation cycles due to intense microbial activity and leaching losses during composting. Substrates prepared from fresh straw exhibited the highest levels of available sugars, promoting mycelial growth and superior biological efficiency.

To maintain an optimal substrate, proper management of raw straw storage is essential to limit its degradation with aging, or alternatively, the substrate should be supplemented with additional carbohydrate sources. Furthermore, strict control of the C/N ratio and moisture

content represents critical factors for maximizing biological efficiency and final yield. All analyzed substrates showed a nitrogen deficiency, indicating the need for correction through the addition of organic nitrogen compounds, such as urea, hydrolyzed proteins, amino acids, yeast extract, or ammonium- and nitrate-based fertilizers [29].

D) Correlation between mycelium post-ripening time and fruiting characteristics, yield, and substrate properties in *Pleurotus geesteranus*

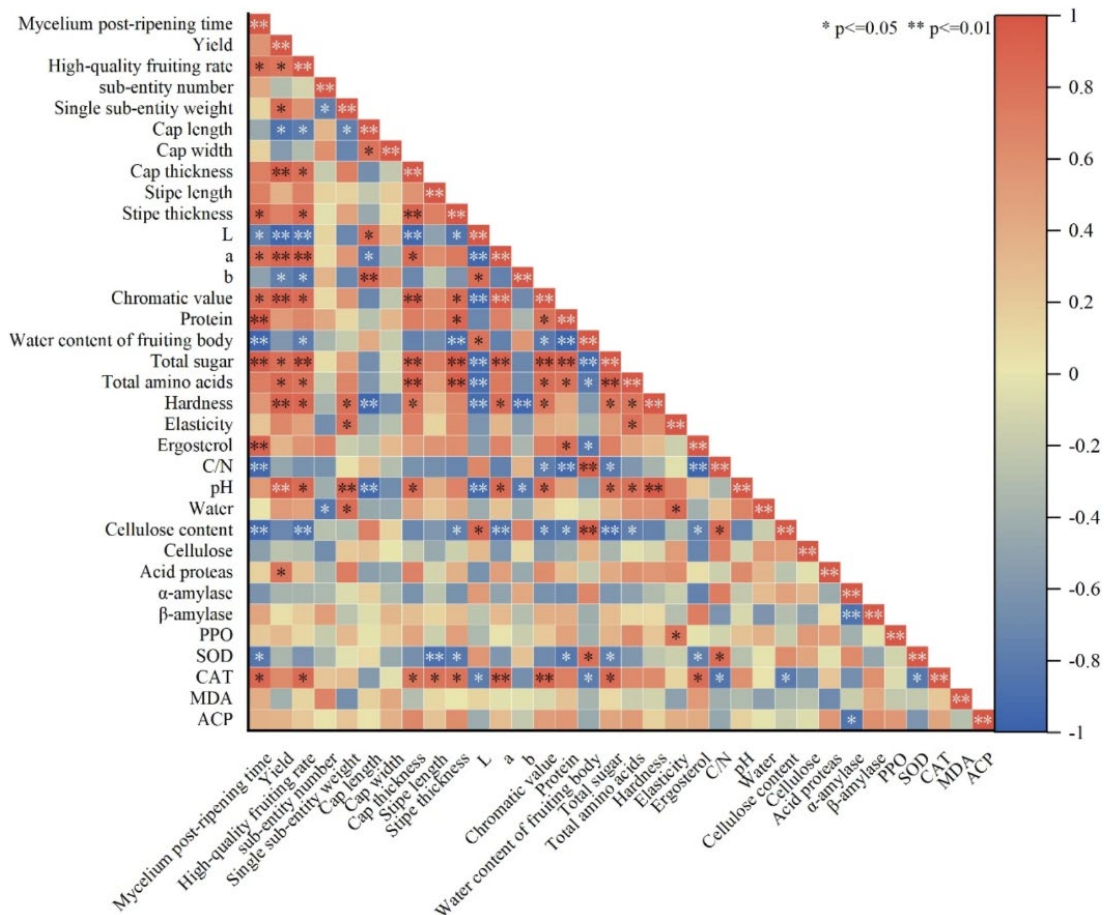


Figure. 2. Effects of mycelium post-ripening time on the yield, quality, and physicochemical properties of *Pleurotus geesteranus* [32].

Correlation analysis (Fig. 2) showed that the mycelium post-ripening time did not have a significant effect on yield. However, this period was positively correlated with ergosterol content, total sugars, and protein, with correlation coefficients of 0.897, 0.923, and 0.942, respectively. Conversely, negative correlations were observed with cellulose content, C/N ratio, and substrate moisture, with coefficients of -0.94, -0.954, and -0.977.

Mushroom yield exhibited significant positive correlations with substrate pH, firmness, color value, and cap thickness (0.962; 0.896; 0.901; 0.891), but a strong negative correlation with the L value (-0.953). In contrast, enzymatic activities of PPO, α-amylase, β-amylase, as well as MDA and ACP, showed weak correlations with the other investigated characteristics [32,33].

The integration of industrial technologies with alternative substrates could enhance yield and protein quality in commercial mushroom production. Process automation, digital control of substrate parameters (temperature, humidity, CO₂), and pre-formulated mixtures tailored to specific species allow for the reproduction of experimental performance under industrial conditions, as highlighted in recent literature [1].

4. Conclusions

Substrates combining cereal straws with alternative nutrient sources, such as hydroponic roots, cassava peels, or halophytic plants, have demonstrated more rapid mycelial colonization, thereby reducing the time required to reach fruiting and enhancing the biological efficiency of the crop compared to standard commercial substrates. This observation underscores the critical importance of adapting substrate composition to achieve high and consistent yields in mushroom cultivation.

Mushrooms cultivated on these innovative substrates exhibited significantly higher protein content, indicating that supplementation with nitrogen-rich materials, along with adjustment of the carbon-to-nitrogen (C/N) ratio, promotes protein synthesis by the mycelium. These results highlight the potential to develop products with superior nutritional value, relevant to both the food and protein production industries, offering benefits from the perspective of human health as well as food sustainability.

Experimental data further confirm that industrial cultivation technologies can be optimized through the strategic integration of alternative substrate materials, while maintaining the advantages of automation and digital control over substrate parameters, including moisture, temperature, and CO₂ concentration. This approach enables cultivators to produce gourmet mushrooms and high-quality mycelial proteins in a sustainable and cost-effective manner, achieving both performance and predictability in production. Simultaneously, the study emphasizes the need for further analyses of the complete amino acid profile of the proteins, monitoring across multiple flushes, and adaptation of substrate formulations to variable industrial conditions. The integration of advanced sensor technologies and predictive modeling can contribute to the continuous optimization of both yield and protein quality, providing a more precise and efficient approach to industrial mushroom production. This perspective opens new opportunities for the development of high-quality, nutrient-rich products obtained through innovative, sustainable, and digitally controlled methods, with applicability in both research and the food and biotechnology industries.

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Review

THE INFLUENCE OF EMERGING GROWING TECHNIQUES ON NUTRIENT CONTENT AND POLLUTION REDUCTION

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Abstract: The technology of growing fruits and vegetables illustrates a broad spectrum of agricultural progress, characterized by technological innovations, deepening scientific knowledge and a harmonious combination of the complexity of plant biology. Fruits and vegetables represent a significant source of essential vitamins, minerals and antioxidants for consumers, which is why they are preferred in the daily diet. The combination of several physical and chemical attributes such as appearance, texture, aroma and nutritional value can define the quality of foods that have a vital impact on consumer acceptance. The paper presents the evaluation of factors that influence the nutritional qualities of fruits and vegetables such as genetic, cultivation, harvesting technology and the influence of environmental factors. The effects on the consumption and quality of products in organic and conventional systems are influenced by a series of characteristics such as consumption, physico-chemical qualities and sensory quality. To obtain quality products, the interaction between environmental factors and the use of appropriate genetic material, adapted to the cultivation system is essential.

1. Introduction.

Technological progress in vegetable and fruit cultivation represents a fundamental evolution from the traditional system, based on soil cultivation, to innovative cultivation in high-tech greenhouses. This transition, vital in agricultural systems, represents a fusion between complex technologies, which implies an increased understanding of plant biology and an obligation regarding environmental management and sustainable agricultural practices [1-4]. The evolution of vegetable cultivation technologies has provided a fascinating glimpse into a broad spectrum of agricultural progress, characterized by technological innovations, deepening scientific knowledge and a harmonious combination of the complexity of plant biology [5-9].



Figure 1. Chronological evolution of techniques and technologies in vegetable growing practices [10-16]

Fruits and vegetables are a significant source of essential vitamins, minerals and antioxidants for consumers, which is why they are preferred in the daily diet [17]. The combination of several physical and chemical attributes such as appearance, texture, aroma and nutritional value can define the quality of foods that have a vital impact on determining the degree of acceptability by consumers [18]. To a large extent, the quality of fruits and vegetables is based on the assessment of various external characteristics (size, shape, color, gloss, firmness, texture and taste) and internal factors (chemical, physical and microbial) that influence nutritional, safety and sustainability traits [19].

The choice of cultivar and the origin of genetic material significantly influence the quality of protected crop products. Breeding can bring these improvements both in terms of yield and earliness, as well as in terms of external and internal quality of the product [20].

Ensuring global food security in agriculture begins in the nursery, a critical stage in the life cycle of young plants that determines their future health, vigor, and yield [21]. Vegetable cultivation involves meticulous seed selection, soil preparation, and strategic management of environmental factors and pests [22].

Technological advances, innovative research in plant science, and insights from the field have transformed vegetable cultivation techniques, ushering in an era of precision and sustainability in agricultural practices [23,24]. Technological advances in vegetable cultivation contribute to the optimization of quality indicators, from the precision of watering, ensured by modern irrigation systems, to the avoidance of excessive or insufficient watering, which can have negative repercussions on young plants [25]. Improving growing media, whether organic or soilless, can strengthen root development and structure, ensuring efficient water and nutrient absorption, which promotes seedling health and vigor [26].

The active metabolism of harvested products makes them particularly perishable, because due to the processes of ripening and senescence, the products suffer quality losses, usually associated with the development of spoilage microorganisms and other undesirable manifestations. These processes must be managed to preserve the quality and increase the shelf life of the products during storage [27,28]. At the same time, the increased water activity and the presence of nutrients associated with these matrices can also favor the growth of pathogens [29;30]. Fruits and vegetables that have beneficial sensory and nutritional characteristics have a corresponding economic value. In this context, inadequate preservation practices can cause significant losses in the nutritional and qualitative characteristics of the products with a negative economic impact along the entire food chain (Fig. 2) [31,32].

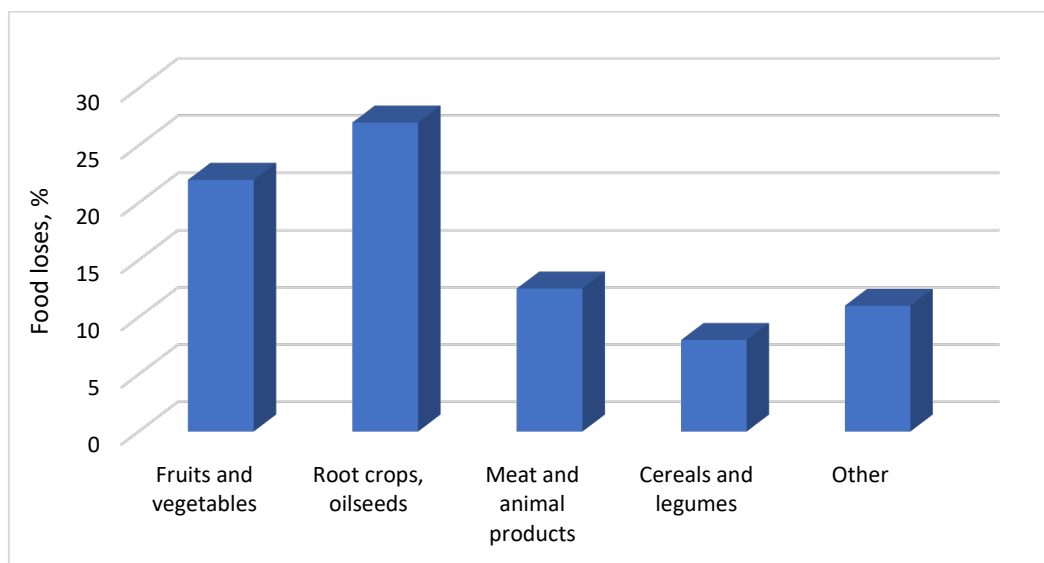


Figure 2. Food losses along the food chain
Adapted from [32]

An important aspect related to the quality and safety of the products arises from the cultivation system (organic, conventional). In the organic cultivation system, fertilization is not synthetic as in the case of the conventional system where pesticides and synthetic fertilizers are used, but organic fertilizer (manure and compost) is usually used. The trend regarding organic cultivation and consumption of organic products is increasing, being associated by consumers with a healthy lifestyle [33,34].

The purpose of this paper is to present and evaluate the factors that influence the nutritional qualities of fruits and vegetables from genetic technology, cultivation, harvesting, culminating in conservation techniques.

2. Materials and methods

The paper evaluated specialized scientific papers on the effects that different cultivation techniques can have on the nutritional quality of fruits and vegetables. Following the identification of the most eloquent scientific papers in the specialized literature summarized on the proposed topic, the evaluation of the methods and factors with the most important impact on the nutritional quality of fruits and vegetables followed.

2.1. Organic and conventional farming methods

2.1.2. Consumer perceptions and preferences

In agriculture, the fastest growing market has been organic fresh produce. Consumers are willing to pay more for organically grown fresh produce given the perceived negative health impacts of pesticides [35,36]. Attributes such as appearance, freshness, nutritional value, and taste drive consumer choices for organic foods [37].

Consumption is driven by a number of factors: personal, economic, sociocultural, and marketing (fig. 3) [38].

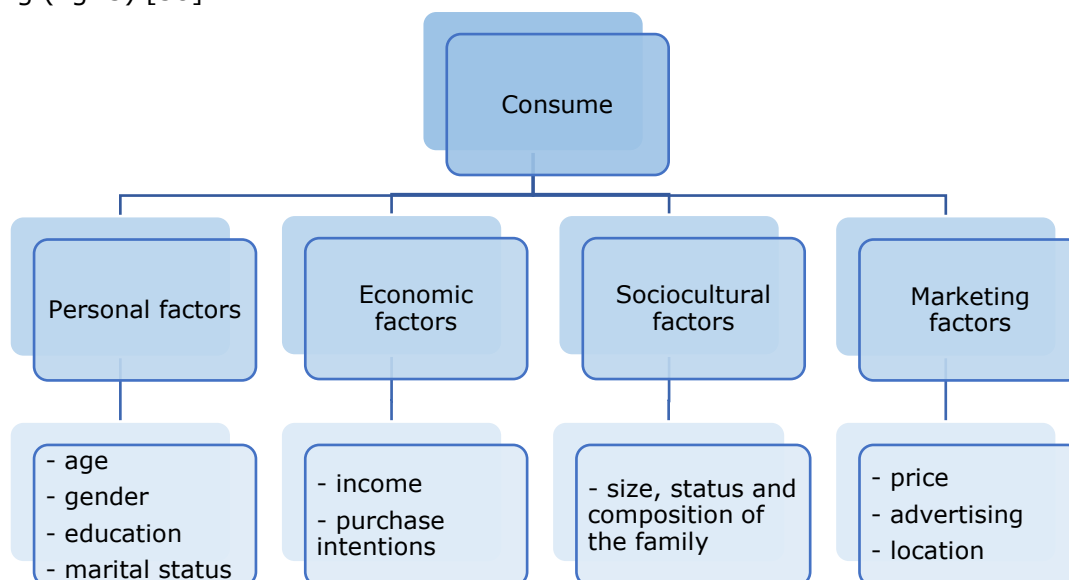


Figure 3. Factors influencing food consumption [38]

2.1.3. Physico-chemical quality of fresh products

Firmness

Firmness is an essential element for fresh produce [39]. In the case of fruits and vegetables, there is a connection between firmness and the content of vitamins and minerals (B, Ca, Se and Si). Firmness results mainly from the maturity of the fruit/vegetables, given that unripe fruits showed a higher firmness than fully ripe ones [40]. In addition, smaller fruits showed a higher firmness than larger ones [41]. The N:Ca ratio also influences firmness. For

example, if the N:Ca ratio is lower, the firmness of apples is lower in the organic growing system, and if the N:Ca ratio is higher and firmness is increased in the conventional growing system, because Ca increases the thickness of the cell wall. Firmness is closely related to the cultivation system, the concentration of nutrients and the cultivar [42].

Organic acid content

Unripe fruits have an enriched titratable acidity, for example cherry tomatoes, because during the ripening period, the acid is converted to sugar [43]. Organically grown apples, strawberries, pears and beetroot showed a higher titratable acidity compared to those grown conventionally. This may be due to the lower effect of nutrient concentrations in the conventional system [44,45].

Mineral content

Mineral concentration is an important factor for conventional cultivation. For example, beans have the lowest Ca content of any crop and this may be due to the low amount of Ca applied during growth [46]. Organically grown plums had higher P, K, Ca, Mg, Zn and lower Na, Fe, Cu contents compared to conventionally grown produce [47]. Organically grown apples had higher levels of K, Ca, Mg, Na, Mn compared to conventionally grown apples, while conventionally grown apples had higher Fe, Cu, B, Zn compared to organic apples. Variations in nutrient content occur due to the high concentration of nutrients applied in the conventional system [48].

2.1.4 Sensory quality of fresh produce

Depending on the crop, the sensory qualities of organic and conventional fresh produce can be different with inconsistent results. Organic cultivation methods negatively affect sensory properties, as they depend on the type of fertilizer, and not on climate, soil or other factors. Sensory evaluations are carried out in 3 ways (Fig. 4) [49,50].

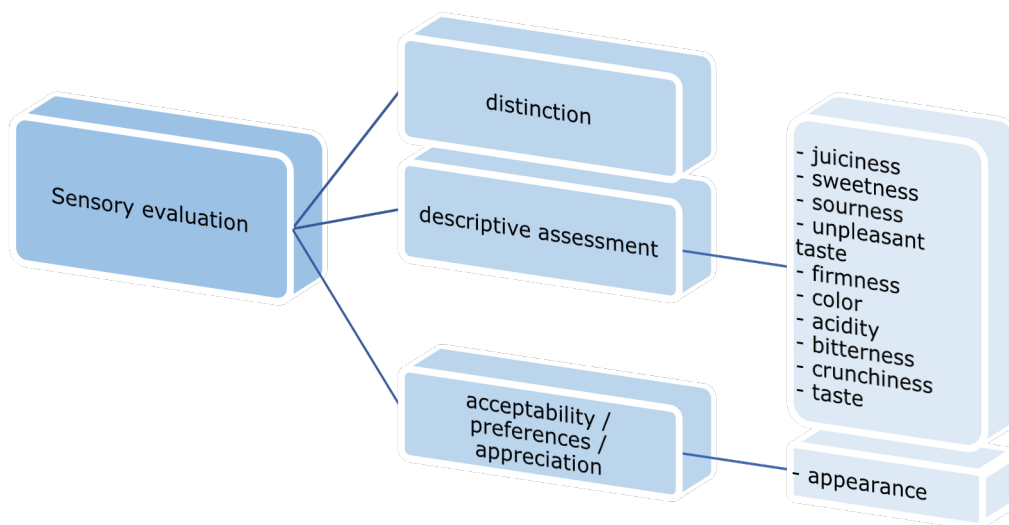


Figure 4. Sensory evaluation method [49,50]

Shelf life and visual quality are important factors for consumers, which have an impact on choice, selection and purchase intentions. The quality of fruits depends on the visual appearance and certain attributes (size and color). There are significant differences between the color of organically and conventionally produced fruits and vegetables. Color is directly associated with consumer acceptability [51].

2.2. Influence of genetic material on vegetable quality

Of particular importance for improving product quality is the genetic peculiarities of plant material. There are studies that have reported differences between varieties in terms of susceptibility of tomatoes and other vegetables to end rot (BER) [52]. Differences in susceptibility to end rot (BER) have been linked to: fruit growth rate; efficiency of Ca absorption

and subsequent translocation of Ca into the fruit and Ca transport within the fruit and especially towards its end [53, 54].

The physiological behavior, tolerance to different stress reactions and sensitivity of vegetable crops to these conditions are expressed both by variation in external parameters and by modification of internal quality. For example, one of these parameters is responsible for the color of ripe fruits. Lycopene is a carotenoid synthesized and stored in chromoplasts. This substance is found in red fruits (tomatoes, watermelons, grapefruits, etc.) and is one of the main antioxidants [55]. The concentration of lycopene in tomato fruits depends on several factors such as: environmental conditions (temperature and light); technologies used in water and fertilizer management and genetics. The choice of variety can significantly influence the result at the time of harvest [56].

Vegetable grafting, mainly used in Cucurbitaceae and Solanaceae species, is a modern extension and ecological technology, widely used to manage and improve various stress situations. The genotype of the rootstock and the rootstock-scion relationship are essential, as they can modify the amounts of hormones synthesized and their influence on the flowering order and sexual expression of the grafted vegetables. Taxonomic affinity is a prerequisite for grafting compatibility [57].

2.3. Influence of environmental factors

2.3.1. Light

Light supports photosynthesis, which is the lifeblood of plants. It also illustrates growth, development, and various physiological, biochemical, and molecular responses, including dry matter allocation and water content. Plant metabolism and biochemistry depend on light intensity, spectrum, and photoperiod.

Primary photoreceptors regulate gene expression and metabolism, influencing the plant's response to light. Effects on photosynthesis have varying consequences depending on the wavelength of light, with red light being the most effective [59]. Increased light intensity generally results in higher photosynthetic rates, but can also produce photooxidative stress [60].

Greenhouse technology, although considerably advanced, decreases natural light and transforms the light spectrum. The aesthetics of the roof construction and the arrangement of the crops have an additional influence on the light distribution and thus on the quality of the crops [61]. Protected vegetable crops rely mainly on daily solar radiation, which must meet minimum light requirements. Technologically advanced greenhouses have the ability to control environmental parameters (intensity, spectrum and duration of light, along with functions for cooling, heating and ventilation [62,63].

Daily light integration (DLI) reflects the total daily light available for photosynthesis by plants. DLI is a function of photoperiod and light intensity, especially the density of photosynthetically active photons. According to studies, DLI has considerable effects on plant growth, biomass accumulation and nutritional quality [64,65].

The role of photoperiod is to regulate the transition of plants from the vegetative to the generative stage, by absorbing light during the day and the dark period crucial for flowering, especially in photoperiod-sensitive fruiting vegetables or for their improvement [66].

The physiological response and nutritional quality of vegetables in protected crops depend substantially on each light component, the spectral composition and dosage of components, even at low intensities [67].

Light intensity, measured as photosynthetically active photon flux density (PPFD), influences plant physiology. As PPFD increases, photosynthesis increases until reaching a genotype-specific optimum, at which point sugar accumulation occurs. At low PPFD, nitrate levels are higher due to reduced assimilation. At the same time, increasing light intensity causes reactive oxygen species (ROS) to be produced from excess energy. Antioxidant production and

flavonoid storage are enhanced to combat oxidative stress caused by ROS. This balance between photosynthesis, nitrate assimilation, ROS, and antioxidants indicates the plant's adaptation response to light intensity, reflected in nutritional quality (Fig. 5) [68].

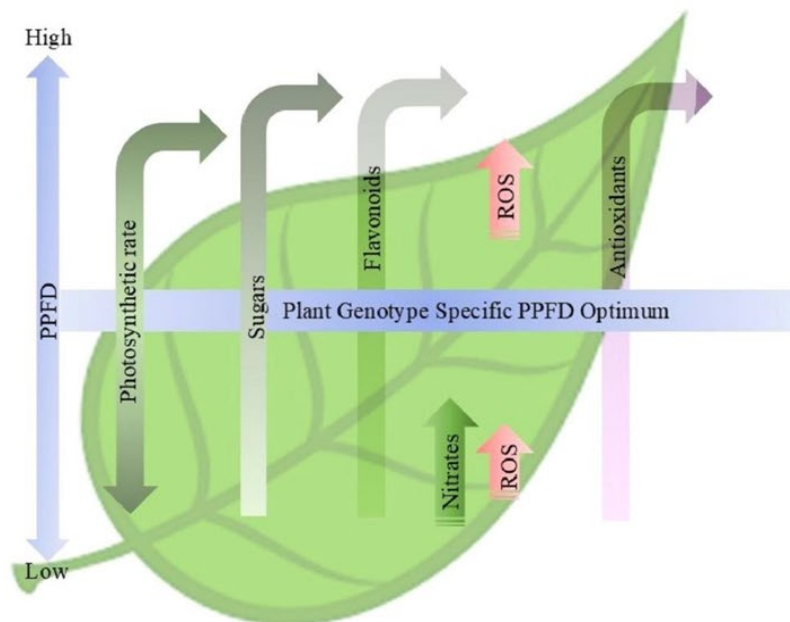


Figure 5. Influence of light intensity on plant physiology [68]

2.3.2. Temperature

Temperature influences chemical and biochemical reactions, having a significant effect on the growth, development and nutritional quality of vegetables [69]. Light intensity and CO₂ concentration are factors that influence temperature in protected cultivation. In the case of crops where there is environmental control, plant growth is optimized by protecting crops from heat stress. Extreme temperatures can hinder plant growth and development by affecting primary and secondary metabolites and mineral content [70]. Temperature affects the retention of primary plant metabolites by influencing the balance between photosynthesis and respiration [71].

In the case of fruiting vegetables, the optimal aerial temperature for primary metabolites (carbohydrates, organic acids and amino acids) is 20–30 C, while for secondary metabolites (vitamin C, flavonoids and carotenoids), it is 25–29 C. The optimal root zone temperatures are usually lower and limited: 18–22 C for primary metabolites and 18–26 C for secondary metabolites.

For leaf/stem/flower vegetables, the optimal temperature ranges for each nutritional compound are lower than those in fruiting vegetables, with the exception of vitamin C. The optimal aerial temperature for these vegetables is 10–22 C for primary metabolites, and 25 C for secondary metabolites. The optimal root zone temperatures are 7–10 C for primary metabolites and 7–22 C for secondary metabolites. Fruiting vegetables, which undergo both vegetative and reproductive growth, require higher temperatures (20–30 C) to support energy-consuming processes (photosynthesis, enzymatic activity, and nutrient uptake).

Primary metabolites are produced by photosynthesis, photorespiration, and respiration, providing energy, dietary fiber, and sweet or sour flavor [72,73]. Meanwhile, secondary metabolites largely affect the taste, bioactive compounds, and aroma of vegetables and have benefits for human health. The optimal temperature of vegetables for secondary metabolites differs from that of primary metabolites [74].

2.3.3. CO₂ from the air

In the context of climate change, the impact of eCO₂ on crop quality has attracted particular attention recently due to concerns about human health and nutrition [75,76]. The potential implications of eCO₂ on human health and nutrition are considerable, and therefore the impact of eCO₂, physiological and molecular aspects of crop quality on plant mineral nutrition and especially nitrogen have been comprehensively analyzed [77].

Maintaining high levels of CO₂ in greenhouses can be difficult, especially when temperatures increase and greenhouse ventilation is required due to solar radiation. Modern closed and semi-closed greenhouse constructions reduce energy consumption by minimizing window ventilation and using active cooling [78].

In protected environments, elevated levels of CO₂ (eCO₂) increase the concentrations of soluble sugars, total antioxidant capacity, phenols, flavonoids, ascorbic acid and carotenoids in vegetables. At the same time, the concentrations of protein, nitrate, magnesium, iron and zinc are usually reduced. The impact on titratable acidity, total chlorophyll, lycopene and anthocyanins varies depending on the specific conditions. The interference of eCO₂ with environmental factors (light, temperature and humidity) is comprehensive and requires further investigation (Fig. 6) [79,80].

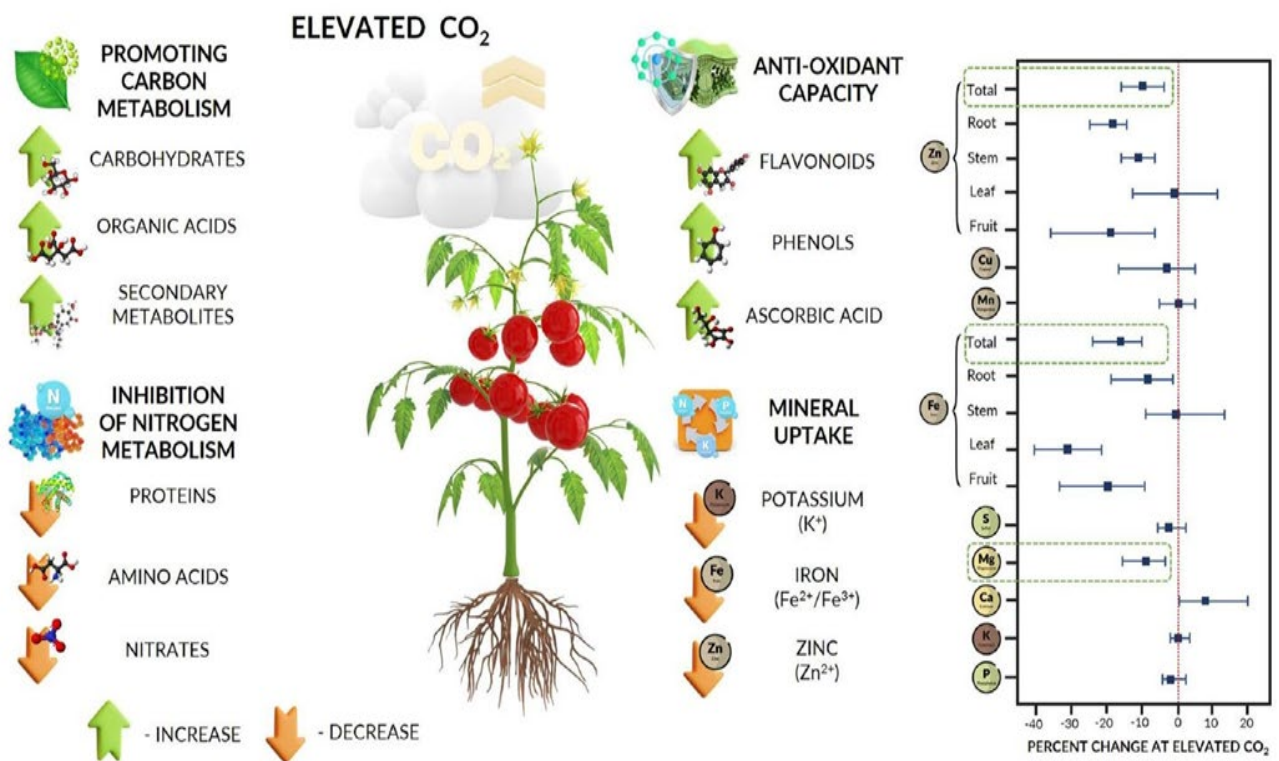


Figure 6. Effects of CO₂ levels [79,80]

2.3.4. Humidity

Humidity has a significant effect on the water requirements of plants, impacting soil–plant–atmosphere content and essential processes such as water balance and transpiration cooling. Humidity influences the transport of water and nutrients in plants. Vapor pressure deficit (VPD) is commonly used to measure humidity. VPD is the difference between air saturation and actual moisture content. Humidity adjustment is interconnected with room temperature, efficient crop growth, and the need to develop close control over water supply and air humidity [81].

Very low VPD of crops reduces evapotranspiration rates, which is the critical mechanism for their cooling. This leads to reduced sap flow through the phloem, which leads to poorer visual

quality and symptoms of nutrient deficiency due to reduced ion movement in plant tissues. On the other hand, high levels of VPD can increase atmospheric transpiration, restrict stomatal conductance and photosynthesis, and inhibit nutrient storage [82].

2.3.5. Interaction between several factors

The main and essential environmental factors that influence the nutritional quality of greenhouse vegetables are light intensity and temperature. Then we have the effects of CO₂ levels, light spectra and duration and the influence of humidity on nutritional quality. In particular, when any of these factors are only in their optimal parameters, they become the limiting factor and thus associate with the principles of the law of limiting factors.

Given the complex interaction of various environmental factors, the adjustment of all microclimatic factors of the greenhouse effect represents a considerable guarantee for improving the nutritional characteristics of vegetables [83].

Water and fertilizer regimes, salinity and cultural practices (cultivation systems), the use of biostimulants and the optimal moment of harvest can have an impact on the nutritional quality of vegetables. In some cases, combining factors such as moderate salinity with biostimulants helps to tailor the outcome [84,85,86].

2.4. Preservation techniques

2.4.1 Conventional Processing Methods

Conventional processing methods such as drying, blanching, chilling, freezing, canning, cooking and pasteurization - are widely used to improve the palatability, quality, stability and convenience of fruits and vegetables during storage and transportation. These methods are mainly based on the application or removal of heat to inhibit microbial growth and enzymatic activity, thereby extending shelf life and ensuring food safety [87]. (Ghoshal, 2018).

Heat inactivates the enzymes pectin methylesterase and polyphenol oxidase, which can cause undesirable changes. This treatment is applied to minimize post-harvest losses [88] (Amit et al., 2023). Thermal processing is energy-intensive and labor-intensive and can compromise the nutritional and sensory qualities of the harvested fruits and vegetables by accelerating oxidation and isomerization reactions, which can negatively affect the quality of the final product [89] (Jadhav et al., 2021).

2.4.2 Emerging/new methods for fruit and vegetable processing

Innovative processing methods have attracted significant attention in recent years for their ability to produce safe, high-quality goods with extended shelf-life. Classified as "non-thermal" technology, several methods generate heat during application, but the temperatures encountered are generally moderate and frequently remain below the pasteurization threshold. This is beneficial for the cultivation of high-quality fruits and vegetables, as excessive heat can have a negative impact on texture, color, flavor and nutritional content.

The methods are particularly attractive to the food industry, as they allow the processing of fresher, more flavorful, more vibrant and more nutritious products. These innovative methods include various technologies such as ohmic heating (OH), pulsed electric fields (PEF), high-pressure processing (HPP), ultrasound (US), pulsed light (PL), cold plasma (CP), microwave, radiofrequency (RF), infrared heating (IH), ultraviolet light (UL), ionizing radiation (IR), and ozone treatment (OT) [90].

Emerging non-thermal physical technologies have come into the spotlight with the aim of replacing traditional post-harvest technologies based on thermal processing. In addition to being water-intensive, conventional methodologies can have detrimental effects on the quality aspects of fresh produce [91].

3. Results

3.1. Effects on consumption and product quality in organic and conventional systems

Table 1

Estimation of various attributes	Influencing factors	Organic cultivation system	Conventional cultivation system	Effects produced	reference
consume	personal, economic, sociocultural and marketing	increasing consumption	declining consumption	the negative health impact of pesticides used in conventional systems	[39-42]
Physico-chemical qualities	Firmness	reduced	great	variation of the N:Ca ratio between the two cultivation systems	[43-45]
	Organic acid content	large	small	low concentrations in conventional system	
	Mineral content	small	great	high nutrient concentrations in conventional system	
Sensory quality	sensory attributes	inferior appearance and color	superior appearance and color	organic farming negatively affects sensory properties	[49-51]

3.2. The influence and interaction of various factors on improving product quality

Tabel 2

Nr. Crt.	Influence of factors	Improving product quality	The interaction between factors	Reference
1	Genetic material	resistance to root rot (BER)	temperature, light	[52,55,56]
		tolerance to different stress reactions		
		harvest performance		
		vegetable grafting		
2	Light	plant metabolism and biochemistry	temperature	[59,60]
		plant physiology		
		photoperiod		
		antioxidants and flavonoid storage		
3	Temperature	influences chemical and biochemical reactions	Light	[69,83]
		growth, development and nutritional quality		
4	CO ₂	increases soluble sugar concentrations	light, temperature, humidity	[79,80]
		increases total antioxidant capacity (phenols, flavonoids, ascorbic acid and carotenoids)		
5	humidity	influences the transport of water and nutrients in plants	temperature	[81,83]
		ensures water balance and cooling through transpiration		

4. Conclusions

The effects on the consumption and quality of products in organic and conventional systems are influenced by a series of characteristics such as consumption, physico-chemical qualities, sensory quality.

In the case of the organic cultivation system, the consumption of products is increasing compared to the conventional system due to the negative impact on health resulting from the use of pesticides in the conventional system.

Under the conditions of using an organic system, the firmness and mineral content are lower compared to the conventional system due to the variation in the N:Ca ratio between the two cultivation systems, respectively the high concentrations of nutrients in the conventional system, on the other hand the organic acid content is higher in the organic system compared to the conventional one.

Organic cultivation negatively affects the sensory properties, which makes the sensory attributes (appearance and color) inferior compared to the conventional system.

To obtain quality products, the interaction between environmental factors and the use of appropriate genetic material, adapted to the cultivation system (protected or unprotected) is essential. Environmental factors, due to their interaction, are interdependent on each other for the cultivation of quality products.

Within the protected cultivation system, environmental factors can be adjusted to optimal values to obtain high-quality products and notable production. This is not possible within the unprotected cultivation system, since there is no control over external environmental factors.

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METHODOLOGICAL NORMS FOR AGRICULTURAL FARMS, TO MAINTAIN FOOD SAFETY STANDARDS AND THE AUTHENTICITY OF AGRICULTURAL PRODUCTS (ARTICLE, REVIEW)

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Abstract:

Ensuring food safety and guaranteeing the authenticity of agricultural products are central objectives of contemporary agricultural and food policies. The methodological norms applicable to agricultural farms have undergone significant development in recent decades, culminating in the adoption of Regulation (EU) No. 2017/625 of the European Parliament and of the Council of 15 March 2017. This legal act repealed previous regulations and established a unified legal framework for official controls, sanctions and corrective measures, as well as for interinstitutional cooperation at the level of the Member States. Its ultimate purpose is to ensure the consistent implementation of European legislation in the fields of food safety, animal and plant health, and consumer protection.

For agricultural holdings, the implications are reflected in the adoption of strict monitoring procedures for processes, thorough documentation of production stages, and compliance with reinforced hygiene and quality standards. Furthermore, the emphasis placed on transparency and consumer information increases producers' responsibility and contributes to strengthening trust in products originating from European agriculture. Overall, Regulation (EU) No. 2017/625 represents not only a legislative update but also a crucial step in consolidating a sustainable, competitive agriculture oriented towards the protection of public health.

1. Introduction

The methodological norms intended for agricultural farms, aimed at maintaining food safety standards and the authenticity of agricultural products, represent a set of rules, procedures, and practical instructions applicable at farm level, designed to ensure the production and marketing of safe [1], healthy products that comply with the declared origin, quality, and characteristics. These norms establish the concrete way of implementing principles of hygiene, biosecurity, traceability, and food risk control, as well as methods for preventing fraud and counterfeiting in agricultural production. In Figure 1, a chart is presented showing cases of infringement of Community law opened at the end of the years 2019–2023.

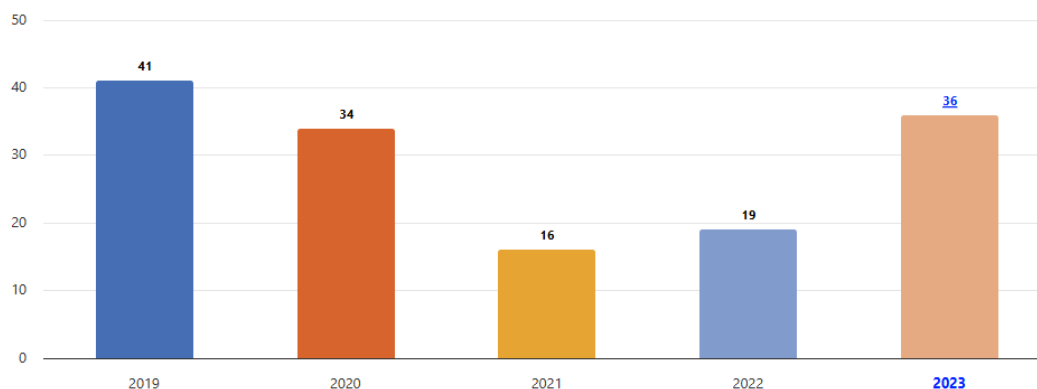


Figure 1 – Situation of legislation infringement cases (2019–2023), [2]

The application of methodological norms in agricultural farms has gradually evolved as the need for food safety and the guarantee of product authenticity became increasingly important. In Europe, the first significant legislative framework was established by Regulation (EC) No. 178/2002, which defined the general principles of food safety and introduced the requirement of traceability "from farm to fork." This regulation made farmers responsible for adopting preventive measures to reduce the risk of contamination of agricultural products.

Subsequently, Regulation (EC) No. 852/2004 provided details on hygiene requirements at farm level, including standards for the management of water, fertilizers, pesticides, manure, and the hygiene of personnel involved in production. During this period, clear procedures were established for documenting operations and monitoring risks, so that each batch of products could be traceable and verifiable.

In Romania, the transposition of these European regulations began in 2004 with the adoption of national legislation on food safety and traceability. Good practice procedures for farms were introduced, offering farmers concrete instructions for product handling, the correct application of agricultural substances, and waste management, with the aim of preventing contamination and maintaining the quality and authenticity of products [3-7].

Regulation (EC) No. 852/2004 establishes the general framework of the European Union for food hygiene, ensuring that food is safe for consumption at all stages of the food chain, from primary production to the final consumer. Its main objective is to prevent food contamination and protect public health [8,9].

The fundamental principle of the regulation is that all food business operators must ensure hygienic and safe handling of food. This is achieved by complying with good hygiene practices and implementing systems based on hazard analysis and critical control points, which allow the identification of hazards and their control at essential stages of the production process [10].

Good hygiene practices apply both to primary production (animal husbandry, fishing, handling and transport of products) and to processing units, slaughterhouses or food shops, covering aspects such as equipment cleaning, staff hygiene, storage and transport of products, as well as thermal treatment processes.

Procedures based on hazard analysis and critical control points involve identifying risks, establishing critical control points, constant monitoring, applying corrective measures, and maintaining documentation that demonstrates compliance. The regulation also encourages the development of sectoral guides and national guidelines for the implementation of these requirements.

The regulation further provides for the approval and registration of food establishments by competent authorities, ensuring the possibility of verifying compliance with the rules. Imported or exported products must comply with EU standards or their equivalents.

Related legislation includes Regulation (EC) No. 853/2004 for food of animal origin and Regulation (EC) No. 178/2002 on traceability and the withdrawal of unsafe food. Regulation 852/2004 has undergone amendments to clarify requirements regarding water in fishery products, the transport of certain oils and fats, allergen management, food redistribution, and the promotion of food safety culture.

Thus, Regulation 852/2004 represents the cornerstone of food hygiene in the EU, imposing clear rules to prevent food risks and protect consumers. Between 2011–2015, methodological norms were supplemented with requirements for digital traceability and with detailed procedures for the identification and control of risks at the level of primary production. This enabled farms to monitor their critical operations more effectively and to better manage microbiological, chemical, and physical risks. At the same time, methods of verifying product authenticity diversified, including modern analytical techniques such as spectroscopy, chromatography, or isotope analysis, in order to prevent fraud and guarantee the true origin of products.

At present, methodological norms are consolidated and digitalized, with the implementation of advanced product monitoring systems and internal audit and control procedures [11]. Compliance with them is mandatory for farms supplying products to national and international markets, while legislation continues to be updated to address new risks and challenges in the field of food safety [12,13].

Thus, the evolution of methodological norms reflects a transition from general hygiene and good practice requirements to a detailed and applicable framework that ensures the safety and authenticity of agricultural products at the level of each farm.

The adoption of Regulation (EU) No. 2017/625 represented a decisive step in strengthening the European legislative framework on food safety, animal and plant health, as well as consumer protection. This regulation, directly applicable in Romania as well, replaced older regulations, bringing a more complex and modern approach to official controls carried out by the competent authorities [14-17].

For agricultural farms in Romania, the impact was felt in several key areas, as the focus shifted from strictly administrative checks to integrated controls, which include hygiene assessment, monitoring of production processes, and verification of product origin. Farmers were required to keep detailed documentation of all production stages, from the supply of seeds, feed, and plant protection substances to the handling of finished products.

The regulation introduced a stricter system of sanctions and corrective measures. Thus, in cases where irregularities are found, farms may receive warnings, fines, or even restrictions on the marketing of products. This approach is intended to discourage non-compliant practices and encourage farmers to adopt high standards of quality and safety.

Another important aspect of the regulation is cooperation between authorities in the Member States. In our country, this means that institutions such as the National Sanitary Veterinary and Food Safety Authority work more closely with similar bodies in other European Union countries to ensure the uniform application of rules. For farmers, this translates into greater recognition of certifications and easier access to external markets, provided that legal requirements are met.

At the same time, the regulation introduced the obligation of transparency and informing the public about the results of official controls. This element has increased the level of responsibility for Romanian farmers, as consumers now have access to information on the quality and safety of the products they purchase. Consequently, farmers are encouraged to demonstrate good practices and maintain high standards in order to earn and retain consumer trust.

2. Materials and methods

The Treaty on the Functioning of the European Union establishes the obligation to ensure a high level of protection of human, animal, and environmental health through policies and measures that focus in particular on the veterinary and phytosanitary fields. The Union also has the responsibility to protect consumers within the internal market. To this end, there is a unified set of rules that guarantee the safety of food and feed and establish clear requirements for all activities that may influence the agri-food chain or consumer interests.

These rules cover human, animal, and plant health, animal welfare, prevention and control of diseases, pest management, protection of the environment against genetically modified organisms and plant protection products. The basic regulation is complemented by specific legislation concerning animal feed, hygiene, zoonoses, residues, contaminants, diseases impacting public health, labelling, additives, contact materials, drinking water, novel foods, and genetically modified organisms.

Animal health is regulated to maintain high standards, to support agriculture and aquaculture, and to prevent the spread of diseases. The rules cover trade, disease eradication, notification, and veterinary checks, and compliance with them contributes to the safety of food

and feed. Transmissible diseases and antimicrobial resistance represent major risks; therefore, legislation provides measures for the prudent use of veterinary medicines and for limiting antimicrobial resistance.

The Union recognizes animals as sentient beings and imposes clear rules on their welfare, ensuring treatment without cruelty and the avoidance of suffering, with beneficial effects also on food quality. Plant health is regulated by rules that prevent the introduction and spread of harmful organisms, protecting crops, forests, biodiversity, and the safety of plant-based foods. Plant protection products are authorized and controlled under strict rules that assess risks and harmonize market conditions.

Genetically modified organisms are subject to authorization, traceability, and mandatory labelling, regardless of the legal basis for authorization, with the same control rules applying. Organic production and related labelling aim at protecting natural resources, biodiversity, and animal welfare, supporting rural development. Agricultural quality schemes guarantee the specificity and diversification of production, protect product names, and inform consumers.

Legislation is based on the responsibility of operators to comply with the rules at all stages of the agri-food chain. In fisheries and aquaculture, marketing standards ensure sustainable products and fair competition, while in agriculture they contribute to quality and profitability. Member States are responsible for enforcing the legislation through official controls carried out by competent authorities.

A unified framework for the organization of controls was introduced by Regulation (EC) No. 882/2004, improving the enforcement of rules and the protection of health, animal welfare, and the environment. However, some areas remained only partially regulated, such as plant health or certain official controls. Other directives establish detailed rules on the frequency of controls and enforcement measures in case of non-compliance.

To simplify and integrate legislation, the present regulation creates a single and harmonized framework for all controls and official activities throughout the agri-food chain, building on the experience gained and on existing rules. The rules on the sustainable use of plant protection products, including the inspection of application equipment, remain valid separately.

Within the single market, the verification of compliance with the rules governing the common organization of agricultural markets already benefits from a well-structured control system, specific to each sector—whether arable crops, wine, olive oil, fruit and vegetables, hops, milk and dairy products, beef, sheep, goat, or honey. For this reason, the present regulation does not directly apply to these controls, except where checks related to marketing standards may indicate the existence of fraudulent or misleading practices. At the same time, some definitions used so far, for example in Regulation (EC) No. 882/2004, must be adapted to correspond to the extended scope of the new legislative framework, to align with other European regulations, and to remove ambiguities created by terms used differently in distinct sectors.

When European legislation requires authorities to verify compliance with certain rules at the time of issuing certificates or official attestations, these checks must be considered official controls. In addition, the authorities of the Member States have specialized responsibilities for the protection of animal and plant health, animal welfare, and environmental protection—including with regard to genetically modified organisms and plant protection products. These responsibilities, such as authorization, epidemiological surveillance, disease eradication, or issuance of certificates, are an integral part of official activities that must be carried out under the same sectoral rules and are therefore covered by the present regulation.

Member States are responsible for designating the competent authorities for each area concerned, as well as for establishing a coordinating authority to facilitate communication with the European Commission and with other Member States. These authorities must have adequate resources, act in the public interest, and provide guarantees of impartiality and professionalism. The quality and efficiency of controls [18] depend directly on the training of the personnel

involved, which is why periodic training is essential, both regarding substantive legislation and the obligations set out by the regulation.

To guarantee compliance with these rules, authorities must carry out internal audits or request independent external audits, conducted transparently. Economic operators must benefit, under national legislation, from the right of appeal against the decisions of the authorities, and the authorities have the duty to inform them of this right. At the same time, staff involved in controls are obliged to observe professional secrecy and protect sensitive information obtained, except where public interest justifies disclosure or where European or national legislation requires it.

Official controls must be carried out periodically, proportionate to the level of risk, and with frequency adapted to each context. As a rule, they are conducted without prior notice, in order to maintain effectiveness, except where scheduling is absolutely necessary, for example in slaughterhouses or during audits. Controls must be rigorous but avoid unnecessary burdens on operators, be conducted by independent staff free from conflicts of interest, and be carried out with the same diligence whether applied only nationally, at cross-border level, or in the case of exports to third countries. In such cases, authorities may also be required to comply with the requirements imposed by the destination countries.

For proper conduct of inspections, authorities may request, in exceptional circumstances, the reporting of the movement of animals and goods between Member States. They must also have the competence to carry out checks not only on products regulated by agri-food legislation but also on other goods, substances, or materials, if necessary for the full investigation of possible violations. To support this process, authorities must maintain and update registers of operators subject to control.

The responsibility of these institutions is twofold: on the one hand towards economic operators, by ensuring fair and effective controls, and on the other hand towards the general public, by maintaining high standards of safety and transparency throughout the agri-food chain. For this reason, authorities must periodically publish information on how controls are organized and on the results obtained. They may also use rating systems for operators, provided these are fair, transparent, and objective. Ratings must reflect reality, preferably being based on multiple controls, and in cases of isolated negative results, they must be followed by additional checks within a reasonable timeframe.

In this way, through a combination of clear rules, precise responsibilities, and transparency in implementation, not only is compliance with European legislation on the agri-food chain ensured, but also the confidence of operators and consumers in the effectiveness of the control system.

For the verification system to function properly, the competent authorities, together with the bodies and persons entrusted with delegated responsibilities, must guarantee the efficiency and consistency of official controls. This requires the existence of clear written procedures on the basis of which staff receive precise instructions. At the same time, authorities must constantly verify the quality of their own actions, identify any deficiencies, and adopt corrective measures where necessary.

To facilitate the detection of non-compliance and the rapid application of corrective measures, the results of controls must be recorded in writing, and operators have the right to receive, upon request, a copy of these documents. However, in situations where the presence of authorities is permanent or regular—for example, in activities requiring continuous monitoring—it is not realistic to draft reports for every visit. In such cases, it is sufficient for records to be drawn up periodically, so that both authorities and operators are informed of the level of compliance and promptly notified when problems arise.

Operators, in turn, are obliged to cooperate fully with the authorities and designated bodies, providing access to essential information about their identity, activities, and commercial

relationships with suppliers and clients. Those responsible for consignments entering the Union must provide all relevant data for the verification of transport.

The regulation establishes a single framework for the organization of official controls in all areas covered by the Union's agri-food legislation. In some cases, these areas require additional rules, tailored to specific needs, in order to avoid divergent practices among Member States. The Commission therefore has the power to introduce detailed rules on the minimum frequency of controls, the measures to be applied in cases of non-compliance, or the criteria for activating administrative cooperation mechanisms. Such additional rules are particularly essential when new risks emerge for human and animal health, their welfare, or environmental protection.

In organizing these controls, Member States are free to decide which staff are most appropriate to carry them out, provided that a high level of protection of public, animal, and plant health is maintained. There are, however, situations where the use of specialists, such as official veterinarians or phytosanitary inspectors, is mandatory to ensure the accuracy of controls. In other cases, states may also resort to these experts on their own initiative, even if there is no explicit obligation.

To encourage innovation, the regulation allows pilot projects at national level, for example in the field of meat production. These projects, limited in duration and scope, may test new control methods, provided that operators comply with all essential requirements on meat safety [19,20]. However, authorities must notify the Commission and the other Member States before implementation, to allow for proper assessment and coordination.

In certain situations, authorities may delegate responsibilities to external bodies. To maintain impartiality and the quality of the process, these bodies must be accredited according to international standards, particularly those developed by ISO, to carry out inspections [21].

An essential part of official controls is laboratory work. Analyses, tests, and diagnostics must adhere to solid scientific standards, and the methods used must be reliable and internationally recognized. When multiple methods are available, authorities must have clear rules to determine which method is most appropriate, selecting from standards developed by organizations such as ISO, OIE, EPPO, or within the European Union framework.

Operators subject to analyses or testing have the right to a second opinion at their own expense. This may involve verification of documentation by another expert or repetition of analyses on already collected samples, if technically feasible and relevant. Exceptions occur when repeating tests is impossible or meaningless, such as with rare or dispersed pathogens.

In the era of online commerce, authorities must be prepared to carry out controls on transactions conducted via the internet or other remote means. For this purpose, they may use the "mystery shopper" technique, placing anonymous orders to obtain samples for subsequent analysis. Even in these cases, operators must retain the right to a second opinion.

Laboratories involved in these activities are required to operate at the highest standards. They must be properly equipped, staffed with qualified personnel, and accredited according to EN ISO/IEC 17025, which sets requirements for the competence of testing and calibration laboratories. Accreditation is granted by recognized national bodies in accordance with European legislation. However, given that the accreditation process is complex and costly, the regulation allows exemptions where the methods used are simple and do not require sophisticated equipment—such as detection of *Trichinella*—or where new methods are not yet validated but must be used urgently in emergency situations or in response to emerging risks.

Controls on animals and goods originating from third countries play a particularly important role. These are vital to ensure that what enters the Union meets safety and quality standards, protecting public health, animal and plant health, animal welfare, and the environment. Controls must be conducted before animals or goods are placed on the market and must be calibrated according to the identified risks, the compliance history of operators, and the guarantees provided by the exporting country.

Certain animals and goods must always be presented at border inspection posts for official controls before entering the Union, including composite products. Other goods may be temporarily subject to the same requirements through specific measures.

Considering the risks to human, animal, and plant health, animal welfare, and the environment, some categories of animals and products must undergo strict entry controls. Current rules already establish the obligation of these controls to verify compliance with health and phytosanitary standards, as well as the possibility of intensified controls for certain high-risk goods [22].

For efficiency and optimal use of resources, a common and integrated system of border controls is necessary to replace the fragmented framework and ensure oversight of all transports with potential risk.

Upon arrival, all transports must undergo documentary, identity, and physical checks, with a frequency proportional to the risk presented. The frequency of these controls must be adjusted based on risk assessment and available data, to focus resources on higher-risk situations and reduce unnecessary checks.

In certain cases, controls may also be carried out at other locations, provided that a high level of safety for humans, animals, plants, and the environment is maintained. For the notification and tracking of transports, they must be accompanied by a common entry health document reflecting the results of the checks and also used for customs formalities.

Member States may benefit from exemptions for inspection posts in special geographic situations or for certain products, such as untreated logs, provided that an adequate level of control is maintained. Inspection posts must meet minimum requirements, and their designation can be withdrawn or suspended if they no longer comply or present risks.

Uniform application of the rules requires clear regulations regarding the actions of authorities and operators in cases of suspicion or non-compliance. Cooperation and information exchange between competent authorities, customs authorities, and other institutions are essential to avoid redundancy and ensure control efficiency.

Member States must provide sufficient resources to control authorities, through public funding and fees charged to operators. These fees must cover actual costs, including indirect costs, without exceeding them, and should be structured so that operators with a good compliance history are favored.

Refunding fees is prohibited to avoid market distortion. The financing system must be transparent to allow all stakeholders to understand the calculation methods.

For the movement of certain animals and products, it is mandatory that they are accompanied by reliable official certificates issued by authorized inspectors, according to common rules that clearly establish obligations and validity guarantees.

Official attestations, such as labels, marks, or phytosanitary passports, must be issued reliably and in accordance with clear rules. Official controls and activities must rely on modern scientific methods applied uniformly throughout the Union, with the support of European reference laboratories that ensure information transfer, comparative testing, and training for personnel involved. These laboratories have specific roles in authorizing products containing genetically modified organisms and food additives by validating analytical and detection methods.

To correctly identify irregularities and fraudulent practices, and to ensure animal welfare, competent authorities must have up-to-date data and specialized expertise via European reference centers. Tracking non-compliance cases across borders is essential for the proper functioning of the internal market and consumer confidence. In this context, the rapid alert system for food and feed allows immediate exchange of information on serious risks, but strengthened administrative cooperation between Member States is also necessary to effectively combat international-scale fraud.

Member States must designate liaison bodies to coordinate information exchange and support cooperation. Each state is also obliged to develop a multiannual national control plan, periodically updated, to ensure controls are carried out based on risk, with a single body coordinating its implementation. Annual reports to the Commission, prepared using standardized forms, will contribute to collecting comparable data and assessing the functioning of official controls [23].

Commission experts are responsible for conducting audits and inspections in Member States, as well as supporting evaluations carried out by authorities in third countries. Products and animals originating outside the Union must meet the same requirements or their equivalents as those applicable within the Union to guarantee consumer safety and environmental protection. The Commission has the authority to establish entry conditions, impose restrictions when evidence indicates risks or non-compliance, and provide training programs for control personnel, including in third countries.

Experience sharing between competent authorities, as well as organizing joint training programs and staff exchanges, contributes to uniform application of legislation. The effectiveness of controls also depends on the existence of efficient computerized systems that integrate and update existing databases. Notification and traceability systems, such as TRACES or RASFF, must operate in a harmonized manner, avoiding overlaps and ensuring rapid and secure communication. The Commission is required to develop a unified IT system, compatible with other public platforms, that allows electronic certification, digital signatures, and personal data protection.

The Commission must have clear powers to ensure the uniform functioning of the information management system, establishing technical specifications and rules regarding the responsibilities of all actors involved. This system must use standardized language and internationally recognized protocols to reduce administrative burdens and facilitate rapid and secure data exchange.

Competent authorities are obliged to investigate any suspected non-compliance with Union legislation, determine its origin and scope, and identify operator responsibilities. They must impose corrective measures and prevent recurrence, taking into account potential risks and the likelihood of fraudulent practices throughout the agri-food chain.

Official controls are a fundamental pillar for the uniform application of Union legislation. Disruptions in one Member State can seriously affect public health, animal welfare, plant safety, or the environment. In such cases, the Commission must intervene with rapid and proportionate measures to limit risks until the state remedies the deficiencies in its control system.

Legislation violations must be sanctioned through effective and dissuasive measures, proportional to the severity of the acts and potential damage to public health or the environment. Financial penalties must exceed the advantages gained from illegal practices, including cases where false documents are used or cooperation in controls is refused.

Anyone must be able to report breaches of the regulation through clear and accessible procedures, with safeguards against retaliation. Whistleblowing is an essential tool for detecting irregularities and enforcing sanctions.

To avoid overlaps and create a coherent legal framework, the regulation replaces several previous acts and amends existing regulations to ensure consistency in the agri-food chain legislation.

Regarding funding, the regulation complements existing provisions to support improved performance of official controls through adequate resources at the Union level.

The plant sector, which until now has not benefited from the same level of control as other sectors, requires specific measures for the gradual introduction of new rules. For low-risk plants and plant products, documentary controls can also be carried out outside inspection posts, provided an equivalent level of safety is ensured.

The Commission has the authority to adopt delegated and implementing acts to adapt annexes, update standards, and supplement the legal framework with technical and procedural rules. In this process, consultation with experts and involvement of the European Parliament and the Council are necessary to ensure transparency and equal participation.

In exercising these powers, the Commission must establish practical rules regarding audits, document formats, use of IT systems, analytical and diagnostic methods, recognition or withdrawal of third-country statuses, and emergency plans for managing food crises. All these measures must be adopted in accordance with the procedure laid down in the regulation on the Commission's control mechanisms.

The objective of the regulation is to ensure a harmonized approach to official controls across the Union. Given the complexity and cross-border dimension of the sector, this objective cannot be satisfactorily achieved by individual Member States but requires coordinated action at Union level. The regulation respects the principle of proportionality, establishing only the measures necessary to achieve its purpose.

3. Results

Food safety standards have emerged as a natural necessity in the context of modern society, where food is no longer produced solely at the local or household level but follows a complex path from the farm to the consumer's table. Along this journey, food passes through numerous stages—production, processing, transport, storage, and marketing—and each stage can represent a vulnerable point where risks may arise. The central objective of food safety standards is to prevent these risks and to ensure that the food and feed reaching humans and animals are safe, healthy, and of high quality.

Beyond its technical dimension, food safety [24] also has a profoundly human aspect: it protects individual health, consumer confidence, and the stability of food markets. A single outbreak of foodborne illness can affect hundreds or thousands of people, cause enormous economic losses, and damage the reputation of a producer or an entire industry sector. Thus, food safety standards should not be viewed merely as bureaucratic obligations but as essential measures to protect life and public health.

A fundamental objective is the prevention of food contamination by pathogenic microorganisms. Microorganisms—bacteria, viruses, and parasites—are invisible to the naked eye but can have dramatic effects on human health. Some of the best-known bacteria involved in foodborne illnesses are *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*.

Salmonella is one of the most frequent causes of foodborne infections. It can be present in raw meat, eggs, or unpasteurized dairy products and causes severe digestive symptoms, which can be particularly dangerous for children, the elderly, and immunocompromised individuals. *Listeria monocytogenes*, on the other hand, is notable for its ability to survive at low temperatures and can contaminate refrigerated foods such as soft cheeses or ready-to-eat products. Infection with *Listeria* is extremely dangerous for pregnant women and newborns and can lead to severe complications. Enterohemorrhagic *E. coli* is known for causing severe intoxications, sometimes accompanied by renal complications, and is frequently associated with undercooked beef or contaminated vegetables.

To prevent these risks, food safety standards establish precise rules: strict hygiene during food processing, control of storage temperatures, product traceability, and periodic microbiological testing. These measures aim not only to reduce the number of illnesses but also to maintain consumer confidence in market-available products.

Another major objective of the standards is reducing the risk of exposure to natural toxins, substances that occur spontaneously in foods but can be extremely dangerous. The most well-known are mycotoxins—secondary metabolites of microscopic fungi (molds) that develop on cereals, dried fruits, coffee, or oilseeds.

Aflatoxins, for example, have a high carcinogenic potential and primarily affect the liver. Ochratoxins can affect the kidneys and immune system, while fumonisins are associated with neurological or gastrointestinal diseases. The presence of these toxins in food, even at low concentrations, represents a real danger. Therefore, food safety standards set maximum allowable limits and require continuous monitoring of crops, storage facilities, and finished products. Farmers and producers must ensure controlled storage conditions with appropriate temperature and humidity to prevent mold growth. Thus, protecting consumers against these natural toxins becomes a central objective of food safety, and strict regulations help reduce the incidence of chronic or acute intoxications associated with them.

Food safety also aims to minimize the risks posed by chemical contaminants. These can enter food from multiple sources: pesticides used in agriculture, heavy metals from polluted soils or water, or dioxins resulting from industrial activities [25].

Pesticides are used to protect crops from pests and diseases, but improper use can lead to residue accumulation in fruits, vegetables, and cereals. Heavy metals such as lead, cadmium, or mercury gradually accumulate in the body and can cause serious damage to the nervous system, kidneys, or bones. Dioxins and PCBs (polychlorinated biphenyls), persistent pollutants resulting from industrial processes, accumulate in animal fat and, consequently, in meat, milk, and fish.

Food safety standards establish maximum allowable limits for these contaminants and provide for rigorous laboratory testing, inspections, and continuous monitoring. These measures reduce the risk that foods on the market will exceed levels considered safe for consumption [26-28].

Table 1 identifies the main European risks from pesticide residues based on their frequency in analytical results. To highlight the products most frequently involved, the top three products corresponding to each of the three most significant hazards were selected. The table below summarizes this classification, providing a European overview of residue distribution by product and type of identified risk.

Table 1

European products with the most frequent pesticide residue risks, determined based on the number of analytical results [29].

Name of Hazard	Product Name	Total Analytical Results	Non-Compliance Rate
Chlorpyrifos	Sweet peppers/bell peppers	34.548	0,48%
	Sweet peppers	33.610	0,21%
	Apples	23.723	0,27%
Diazinon	Sweet peppers	35.432	0,00%
	Sweet peppers/bell peppers	34.162	0,01%
	Apples	23.337	0,00%
Pirimiphos-methyl	Sweet peppers	35.306	0,30%
	Sweet peppers/bell peppers	34.300	0,92%
	Apples	23.207	0.00%

Another category of risks is represented by residues of veterinary medicines. Farm animals may be treated with antibiotics to prevent infections, antiparasitics to control infestations, or, in some cases, hormones to stimulate growth. Although these treatments are necessary for animal health, traces of the administered substances may remain in meat, milk, or eggs [30].

The consumption of antibiotics through food, even in small quantities, is dangerous because it contributes to the development of antimicrobial resistance. This is a major global issue, recognized by the World Health Organization, as bacteria resistant to antibiotics are becoming increasingly difficult to treat. In addition, hormonal or antiparasitic residues can affect the human endocrine system and metabolism.

Safety regulations stipulate mandatory withdrawal periods after treatments are applied to animals, so that substances are metabolized or eliminated before slaughter or before milk and eggs are collected. Laboratory tests are also carried out to detect residues, and severe penalties are applied to producers who do not comply with the rules.

Traceability is one of the fundamental pillars of food safety, as it is the mechanism that allows tracking and identifying any food or feed product throughout its entire journey – from origin to the final consumer. In a globalized world, where raw materials may come from one part of the globe, processing takes place in another region, and distribution occurs on an international scale, traceability becomes indispensable for maintaining control and transparency in the food chain.

The main purpose of traceability is to ensure that every stage of production and distribution is rigorously documented so that, in the event of a suspicion or contamination incident, the source of the problem can be quickly identified and effective measures can be taken. Traceability is not just a bureaucratic formality, but a prevention and intervention strategy designed to protect public health, reduce economic risks, and strengthen consumer confidence in the products they purchase. Every link in the food chain – whether farmers, processors, or distributors – is obliged to keep clear and detailed records.

The origin of raw materials must be precisely documented. Farmers record cultivated lots, treatments applied to crops, harvest dates, and any special storage conditions. Processors must know exactly where the raw materials they use come from so they can demonstrate traceability in the event of an investigation.

The manufacturing and handling process is also rigorously documented. Records include processing stages, hygiene conditions, storage temperatures, and applied technological procedures. For example, in a dairy factory, each batch of milk is accompanied by documents showing the time of collection, transport, maintained temperatures, and treatments applied (pasteurization, fermentation, etc.).

Product distribution is a key element. Businesses must specify to whom the products were delivered, in what quantities, and on what date. This information makes it possible to quickly identify the path of a product on the market and, if necessary, promptly withdraw suspect batches from stores. Thus, careful documentation at every level provides a clear and complete picture of a food product's journey.

For traceability to be effective, it is not enough for operators to keep records; control authorities have the role of periodically verifying the accuracy and completeness of these records. Document checks are an essential stage of veterinary and food safety inspections. Inspectors request access to registers, invoices, traceability forms, and IT databases to ensure that all information is coherent and correct. Incomplete or incorrect documentation may raise suspicions about the quality and safety of products, attracting severe penalties. At the same time, these checks encourage operators to maintain a high level of responsibility and transparency. To ensure coherence and efficiency, traceability relies on standardized tools.

Traceability forms are standardized documents in which all information related to the origin, processing, and distribution of products is recorded. These can be in physical format (registers and sheets) or digital, depending on the size and complexity of the business. National IT systems facilitate the rapid and secure management of information. They allow for centralized records accessible to both operators and control authorities, reducing the risk of errors and enabling quick interventions.

At the European level, a reference tool is the TRACES (Trade Control and Expert System). This electronic platform is used to monitor the movement of animals, products of animal origin, and other relevant goods in intra-community trade and in relations with third countries. TRACES enables rapid information exchange between member states, contributing to unified and efficient traceability in the European market. Through this system, authorities can track trade flows, quickly identify problems, and take coordinated food safety measures at the transnational level.

Traceability is not only a technical requirement but also a guarantee for consumers and society as a whole. In the event of a food crisis – whether it involves contamination with a pathogenic bacterium, exceeding pesticide limits, or the discovery of veterinary medicine residues – authorities can quickly identify affected batches and withdraw them from the market. This limits the number of people exposed, reduces economic losses, and restores public confidence.

Moreover, traceability offers a competitive advantage to businesses. Companies that can demonstrate flawless traceability inspire trust among business partners and consumers, which translates into a strong reputation and easier access to international markets. In a globalized economy, where food trade involves numerous countries and regions, traceability becomes an essential element of safety and competitiveness.

Risk assessment is one of the essential stages in ensuring food safety, as it is the mechanism through which authorities and businesses determine the level of danger associated with each product and, implicitly, the way control measures should be applied. This approach shifts from uniform monitoring, applied to all products, to a priority-based strategy, in which control resources are focused on those foods or processes that present the greatest risk to public health.

The purpose of risk assessment is not only to identify potential hazards but also to quantify and rank them so that rapid, coherent, and effective decisions can be made. This method allows anticipating problems and reducing their impact on consumers and the food market.

Factors considered in risk assessment. The nature of the product. A first evaluation criterion is the type of food being analyzed. Not all food products present the same level of vulnerability. Raw meat, for example, carries a high risk of microbiological contamination since it provides a favorable environment for bacteria such as Salmonella or E. coli. Raw fish or seafood may be contaminated with parasites or viruses. In contrast, pre-packaged, sterilized, or long-shelf-life products carry a much lower risk, as they have undergone processes that reduce microbial load and limit pathogen development. Thus, the nature of the product dictates the level of precaution and intensity of necessary controls.

The second important criterion is the technologies and procedures applied during food processing. A product that has undergone pasteurization or sterilization is much safer than one consumed raw or only washed and packaged. Vacuum or modified-atmosphere packaging extends shelf life and reduces the risk of subsequent contamination but does not eliminate it completely. At the same time, intermediate stages – handling, transport, storage – can introduce new risks if not properly managed. Risk assessment takes all these aspects into account, from raw material to finished product.

Another crucial element in evaluation is the compliance history of the business operator. A company that has consistently respected regulations, implemented effective self-monitoring systems, and had good results in previous inspections will be considered lower risk. In contrast, operators with a history of non-compliance, repeated violations, or poor documentation will be assessed as higher risk. This criterion encourages operators to maintain high standards since their track record directly influences the frequency and intensity of official controls.

To make the evaluation process more objective and transparent, authorities use risk scores. These scores are assigned based on predetermined criteria, such as those mentioned above (product nature, technological process, operator history). Each factor is given a weight, and the final score reflects the risk level of the product or operator.

Products and operators with high scores are subject to more frequent and detailed inspections, while those with low scores benefit from reduced inspection frequency. This prioritization system is extremely useful, as authorities' resources are limited, and their efficient distribution ensures better public health protection. In practice, controls are focused where the risk is higher, reducing the chances of food crises.

Benefits of risk assessment

Risk assessment brings multiple benefits for consumers, businesses, and authorities alike:

For consumers, it means increased food safety and greater confidence in purchased products.

For businesses, it provides a clear picture of vulnerable areas in their processes and helps them adopt preventive measures.

For authorities, it allows for efficient resource management and rapid interventions in case of incidents.

In addition, risk assessment has an educational and preventive dimension: it encourages businesses to implement high hygiene standards and to continuously improve their processes to avoid high-risk scores.

Control at the point of production represents one of the most important links in the food safety system since it is the moment when competent authorities directly verify, on-

site, compliance with hygiene, quality, and safety standards. While risk assessment and traceability are largely based on documents and theoretical analyses, field inspections confirm the practical reality, identify potential non-compliance, and prevent hazards before products reach the market.

These inspections are carried out by inspectors from competent authorities – generally veterinary and food safety directorates or similar bodies – who are responsible for monitoring the entire food chain, from farm to consumer. Their presence in the field ensures not only law enforcement but also a form of education and guidance for businesses, which thus receive direct feedback on their activity.

Official controls are carried out at various points of the production and distribution chain, each with its own characteristics and risks.

Food processing units – dairy factories, slaughterhouses, bakeries, canning factories, packaging units, etc. Here, inspectors check the entire technological process, from the reception of raw materials to the finished product. Processing often involves multiple sensitive stages (cutting, grinding, pasteurization, packaging), each of which can represent a critical point for food safety.

Farms and agricultural holdings – the place where raw materials are produced. In livestock farms, inspectors check the animals' health status, how medicines are administered, hygiene conditions in shelters, and feed quality. In crop farms, the focus is on the use of pesticides and fertilizers, grain storage, and preventing contamination with molds or pests.

Storage and transport units – these links are essential for maintaining product quality on their way to market. Inspectors analyze storage conditions, check refrigeration or freezing systems, and ensure that transport is carried out under appropriate hygienic conditions.

For an inspection to be complete and effective, inspectors analyze a series of key factors that can directly influence food safety:

Staff and equipment hygiene – Regulations require staff to strictly follow rules on handwashing and disinfection, use of protective equipment (coats, gloves, caps), and periodic training in food hygiene. At the same time, equipment and work surfaces must be clean, regularly disinfected, and properly maintained.

Storage conditions – Food products are extremely sensitive to temperature and humidity. A broken cold chain, even for a few hours, can compromise the quality and safety of an entire batch of meat or dairy products. Inspectors check refrigeration and freezing temperatures, warehouse humidity, and general cleanliness of spaces.

Waste disposal and pest control – Food residues and organic waste can attract insects, rodents, and other pests, which represent a major source of contamination. Therefore, units must have waste management plans and pest control programs in place. Inspectors check the effectiveness of these programs and compliance with environmental standards.

Compliance with legal limits for chemicals and medicines – Especially in livestock farms, inspectors monitor the use of antibiotics, antiparasitics, and other veterinary medicines. They also verify whether withdrawal periods are respected before slaughter or product collection (milk, eggs), so that residues do not end up in food. In the crop sector, controls focus on pesticide and fertilizer use to prevent exceeding legal limits.

The role of these inspections is twofold: on the one hand, they are coercive, as they may result in penalties for violations; on the other hand, they also have an educational and preventive function. Inspectors do not only detect problems but also provide recommendations, showing operators how to improve their processes and avoid future non-compliance.

Through controls at the production point, authorities can intervene immediately when irregularities are detected. For example, in the case of microbiological contamination, processing can be stopped and affected batches withdrawn before reaching the market. In cases of improper use of medicines, temporary suspension of farm activities or withdrawal of production authorization may be imposed.

Laboratory analyses are the backbone of the food safety system because they provide clear scientific evidence regarding product quality and compliance. While traceability and field inspections provide a framework for monitoring and visual verification, laboratory tests go further, revealing aspects invisible to the naked eye: the presence of pathogenic microorganisms, chemical residues, or natural toxins. This type of objective testing

transforms suspicions into certainties and forms the basis of official decisions made by authorities.

The process begins with sample collection, which can be done systematically – based on national and European monitoring programs – or randomly during routine inspections. Inspectors select foods representative of different risk categories: raw meat, dairy products, fruits and vegetables, processed foods, or imported products. The sampling procedure is standardized to avoid contamination of samples so that the results obtained accurately reflect the real situation.

Sometimes, samples are collected in response to specific suspicions, such as the occurrence of food poisoning outbreaks or consumer complaints. In such cases, laboratory tests become an essential tool for identifying the cause and triggering withdrawal or prohibition measures.

Types of analyses performed

Depending on the nature of the product and associated risks, various types of analyses are carried out:

Microbiological – These target the identification of pathogenic bacteria such as Salmonella, Escherichia coli, or Listeria monocytogenes, which are responsible for numerous foodborne illnesses. Microbiological tests may also include determining the total germ count or the presence of molds and yeasts, important parameters for assessing freshness and product safety.

Chemical – These analyses determine the presence of pesticide residues, heavy metals (lead, mercury, cadmium), food additives, or other chemical substances which, in concentrations above legal limits, may be dangerous for human health.

To highlight the distribution of the most frequent European risks associated with chemical contaminants, a classification was made based on the frequency of analytical results. Within this analysis, the top products corresponding to each of the most significant hazards were selected, according to the highest number of results obtained. The table below summarizes this information, providing a clear picture of the products most frequently involved and the types of European hazards.

Table 2

European products associated with the most significant risks from chemical contaminants, identified based on the frequency of analytical results, [29]

Hazard name	Product name	Total analytical results	Non-compliance ratio
Lead (Pb)	Pig fresh meat	4,276	0.58%
	Pig liver	3,355	0.24%
	Cow, ox or bull fresh meat	2,612	0.00%
Cadmium (Cd)	Pig fresh meat	4,088	0.59%
	Pig liver	3,344	0.42%
	Cow, ox or bull fresh meat	2,595	0.08%
Aflatoxin B1	Peanuts	17,292	4.01%
	Pistachios	6,231	4.14%
	Dried figs	4,736	2.70%

Food composition compliance is also checked, such as the content of nitrites, nitrates, or preservatives.

Natural toxins – A separate chapter is represented by analyses for mycotoxins, such as aflatoxins or ochratoxins, substances produced by certain molds that can contaminate cereals, dried fruits, oilseeds, or coffee. These toxins have serious effects on health, being associated even with carcinogenic risks, which makes their monitoring a priority at the international level.

For results to be credible and officially recognized, laboratories conducting these analyses must be accredited according to the international ISO 17025 standard, which guarantees technical competence and the quality of testing processes. In addition, the methods used must be validated at European or international level, such as AOAC (Association of Official Analytical Chemists) methods or EN (European Norms) standards.

Compliance with these standards is crucial, as only in this way can a result obtained in a laboratory in one EU member state have the same legal and scientific value as one obtained in another state. This harmonization facilitates the exchange of information and rapid interventions in case of risk.

After analyses are performed, the results are centralized and reported to the competent national authorities. In cases of non-compliance, laboratories are obliged to immediately notify the responsible institutions, so that urgent measures can be taken: withdrawal of the product from the market, blocking of suspect batches, or informing consumers.

At the European level, the data obtained are integrated into centralized IT systems, such as the RASFF (Rapid Alert System for Food and Feed) platform, which enables the rapid transmission of alerts between member states. In this way, if a contaminated batch of food is discovered in one country, all other states receive the information immediately and can prevent the product from being distributed on their markets.

Laboratory analyses are not just a control mechanism but also a form of proactive protection. They provide authorities and economic operators with precise information on the condition of products, thus contributing to decisions based on scientific evidence. In addition, they strengthen consumer confidence, who know that food placed on the market is rigorously tested and complies with safety standards.

In the absence of such analyses, the food safety system would be vulnerable, relying only on visual observations or on the declarations of economic operators. Therefore, the role of laboratories is to transform the theoretical principles of consumer protection into scientific, measurable, and verifiable certainties.

Food labeling represents one of the most visible and direct forms of communication between producer and consumer. While laboratory analyses, traceability, or production-site controls take place "behind the scenes," the label is the first and often the only element available to the consumer to make an informed decision. Therefore, the accuracy and completeness of the information on the packaging are essential conditions for protecting public health and ensuring trust in food products.

European and national legislation requires that each food product be accompanied by a series of clear, visible, and easily understandable information so that the consumer can evaluate the quality and safety of the product. Among the most important elements are:

Composition – the list of ingredients must accurately reflect the content of the product, in descending order of quantity. This allows the consumer to know exactly what they are eating and to avoid ingredients that may be undesirable or dangerous (e.g., allergens such as gluten, lactose, or nuts).

Expiration date – for perishable products such as meat, milk, or fish, it is mandatory to state "use by...". For products with a longer shelf life (canned goods, pasta, biscuits), the formula "best before..." is used. This information protects consumer health and helps them manage food properly at home, reducing the risk of foodborne illness.

Storage instructions – many products require special conditions to maintain their quality. For example, yogurt must be stored at temperatures between 2–8°C, while some frozen products must not be refrozen once thawed. Indicating these instructions is vital, as even a correctly manufactured product can become dangerous if not stored properly.

In addition to these elements, legislation also requires the inclusion of other important information, such as the country of origin, the name and address of the producer, nutritional values, and, in certain cases, preparation instructions.

Labeling is not only a legal obligation for producers but also a field closely monitored by the competent authorities. Inspectors may carry out physical checks, analyzing labels directly from shelves or warehouses, as well as document checks, comparing the information declared by producers with actual data confirmed through laboratory analyses or production records.

For example, if a product is labeled “gluten-free,” authorities may request laboratory tests to prove the absence of gluten contamination. Similarly, claims regarding fat, sugar, or additive content must be supported by real and verifiable technical data.

The food label is both a protection and an educational tool. Through the information provided, the consumer can adapt their diet to their own needs – whether it is avoiding allergens, reducing sugar intake, or following a vegetarian diet. In addition, transparent labeling strengthens trust in brands and in the overall food safety system.

Conversely, an incorrect or incomplete label can mislead the consumer and may have serious consequences, especially for people with food allergies or intolerances. For this reason, European legislation provides for severe penalties for economic operators who falsify or intentionally omit information from labels.

Corrective measures and sanctions

Corrective measures and sanctions represent the last line of defense within the food safety system. While the primary objective of the rules is to prevent contamination and protect public health, in reality, situations sometimes arise where operators fail to comply fully with legal requirements, or food products reach the market with various non-conformities. In such cases, authorities are obliged to intervene firmly, applying a set of measures aimed at protecting consumers and restoring order and safety in the food chain.

One of the most common corrective measures is the withdrawal of products from the market. This is applied when a batch of food is identified as unsafe, either based on laboratory analyses or as a result of direct inspections. The purpose of withdrawal is to immediately stop the distribution and consumption of the dangerous product, and it is carried out in an organized manner, by notifying retailers and consumers.

For example, if a Salmonella contamination is discovered in a batch of meat, the operator is obliged to notify authorities and remove the affected products from sale. In severe situations, these measures are accompanied by public information campaigns to prevent the consumption of products already purchased.

Product withdrawal is only the first stage. Subsequently, operators must implement remediation measures to eliminate the causes that led to the non-conformities. These measures may include upgrading processing equipment, improving hygiene in production areas, providing additional staff training, or revising internal control procedures. Thus, sanctions are not only punitive but also educational and preventive, encouraging operators to adopt higher standards of quality and safety.

Depending on the seriousness and frequency of violations, authorities may apply a wide range of sanctions. The most common are fines, intended to discourage breaches of the rules through financial impact. In more severe cases, suspension or revocation of the operating license may occur, meaning complete shutdown of activity until problems are corrected. These sanctions serve a dual purpose: on the one hand, they protect consumers from immediate risks; on the other hand, they send a clear message to the entire food sector, showing that non-compliance is not tolerated.

In situations where the risk goes beyond the borders of a single state, the European Commission may intervene directly with coordinated cross-border measures. A typical example is the use of the RASFF (Rapid Alert System for Food and Feed), through which member states are rapidly alerted about a dangerous product discovered in one country but potentially circulating in other European markets.

In severe cases, the Commission may order a temporary ban on imports from a specific region or the suspension of distribution of a product throughout the EU. Thus, the sanction mechanism functions not only at the national level but also within a unified European framework, designed to guarantee the same level of protection for all consumers in the Union.

The correct and proportional application of sanctions has a significant impact on the entire food system. It contributes to holding economic operators accountable, maintaining fair competition, and strengthening consumer confidence. At the same time, corrective measures often lead to structural improvements in production facilities, reducing the risk of future problems.

Therefore, sanctions should not be seen only as punishments, but as tools of balance between commercial interests and the fundamental obligation to protect public health.

An effective food safety system cannot function without well-trained staff capable of applying national and European standards consistently and correctly. Beyond the legislative framework and written procedures, the human factor remains decisive: inspectors, laboratory specialists, and other professionals involved in official controls must have the knowledge, skills, and experience necessary to correctly assess field situations and to make decisions in the interest of public health.

Staff training

All those involved in the process of official control [31] are required to undergo professional training programs. These include both a theoretical component – focused on legislation, European norms, and standards – and a practical component, involving simulations, case studies, and direct training in production units or laboratories. The aim is to ensure that each inspector or specialist fully understands how the food chain functions and the risks associated with each stage.

Professional training takes place in several specialization areas, depending on the responsibilities of each actor in the system:

Control and sampling procedures – Inspectors are trained to follow standardized procedures when checking production units, farms, or storage facilities. They learn sampling techniques that prevent secondary contamination and ensure representativeness. They are also trained to properly document findings and uniformly report identified irregularities.

Interpretation of laboratory results – An important part of training consists of familiarizing staff with analysis reports and how to interpret them. Inspectors must understand what the exceeding of a microbiological or chemical parameter means and what measures must be taken as a result. In this way, decisions are not arbitrary but based on scientific evidence.

Traceability and detection of food fraud – In a globalized context, where products quickly cross borders and supply chains are increasingly complex, inspectors must be able to track the origin of raw materials and detect possible fraud. Training courses familiarize them with IT tools used for traceability and with modern methods of detecting adulteration or substitution of food products.

Harmonization of rule enforcement in the EU

A central aspect of these training programs is the harmonization and standardization of rule enforcement at the European level. Since all EU member states must offer the same level of consumer protection, it is essential that inspectors from different countries work on the basis of the same principles and adopt similar procedures.

To achieve this goal, common courses and training sessions are organized at the European level, attended by experts from all member states. These sessions facilitate the exchange of best practices and contribute to building a united professional network capable of responding quickly and in a coordinated manner in crisis situations.

Training and education are not processes that end once a certificate is obtained. The field of food safety is dynamic, with new risks, new technologies, and new legislative requirements constantly emerging. Therefore, staff must regularly participate in continuous training programs to update their knowledge and remain prepared to face challenges.

Thus, inspectors and specialists acquire not only technical skills but also the ability to react appropriately in complex situations, to correctly interpret information, and to apply effective solutions.

4. Conclusions

The results show that the agricultural farm, as the first link in the food chain, has a direct responsibility in preventing contamination risks. Measures such as checking water quality, controlled use of fertilizers, and maintaining production records are concrete examples of how the norms are applied in practice and how they influence the quality of the final product. At the same time, documenting and monitoring processes not only meet legal requirements but also provide farmers with opportunities for certification and access to European markets. Thus, methodological norms should not be viewed solely as obligations, but as a framework for sustainable development and for strengthening consumer confidence in agricultural products.

Food safety is more than just a set of rules or inspections: it is the expression of a collective responsibility involving authorities, economic operators, and consumers. Through prevention, constant monitoring, and the consistent application of norms, each stage of the food chain contributes to the protection of public health. Transparency, traceability, and continuous training of personnel ensure the system's adaptability to emerging risks and strengthen consumer trust. In essence, food safety is a living, dynamic, and collaborative process that transforms principles into reality and guarantees that products reach people's tables in a safe and responsible manner.

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Review

COVER CROPS AS AN INTEGRATED TOOL IN LEGUME SYSTEMS: EFFECTS ON WEEDS, SOIL, AND CROP PRODUCTIVITY

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Abstract: The increasing shift toward organic and low-input agricultural systems has intensified the search for sustainable weed management strategies, with cover crops emerging as a key component in legume-based cropping systems. This review synthesizes recent findings on the role of cover crops from the Fabaceae, Poaceae, and Brassicaceae families in suppressing weeds, improving soil health, and enhancing the performance of major grain legumes such as soybean and faba bean. Data compiled from peer-reviewed studies and meta-analyses published in the last five years indicate that grass species (e.g., *Secale cereale*, *Avena sativa*) provide the most consistent weed suppression—reductions of 40–70%—due to high biomass production and rapid soil coverage, while brassicas contribute additional allelopathic effects. Legume cover crops, though less competitive against weeds, play a crucial role in nitrogen fixation and soil fertility enhancement. Improvements in soil organic carbon, aggregate stability, microbial activity, and reductions in nitrogen losses were observed across diverse environments, alongside yield increases of 5–12% in subsequent legume crops. Integrating cover crops into legume-based systems significantly decreases herbicide dependence (by 25–50%) and mitigates the risk of herbicide resistance. Identified challenges include potential increases in N₂O emissions, mechanical constraints during sowing, and mismatches in nutrient release timing. Overall, the evidence highlights cover crops as multifunctional agroecological tools that strengthen weed management, soil health, and system sustainability, contributing directly to EU Green Deal objectives and the transition toward climate-smart agriculture.

1. Introduction

The growing interest in organic and low-input farming systems has renewed attention toward alternative methods of weed management, particularly through the development of innovative mechanical and agronomic solutions [1, 2, 3]. These systems rely heavily on legume crops due to their capacity for biological nitrogen fixation and their role in enhancing natural soil fertility [4]. Expanding the cultivation of leguminous species brings multiple environmental and agronomic benefits, including improved nutrient cycling, enhanced soil structure, and increased sustainability across various spatial scales — from individual fields to global agricultural systems [5]. Additionally, legumes contribute to lowering resource consumption and greenhouse gas emissions, supporting the transition toward climate-smart agriculture.

Among grain legumes, soybean (*Glycine max* (L.) Merr.) and faba bean (*Vicia faba* L.) are particularly important due to their high nutritional, agronomic, and ecological value [6]. Soybean is one of the leading global grain legume and oilseed crops, accounting for more than half of the world's oilseed production [7]. Faba bean, a protein-rich species used for food and

feed, provides significant ecosystem services, including substantial biological nitrogen fixation and diversification of crop rotations [8].

Cover crops represent a key component of integrated weed management (IWM) in both annual and perennial cropping systems because of their strong weed-suppressive potential [9, 10, 11, 12]. They influence weed dynamics through competition for light, water, and nutrients, as well as through allelopathic interactions. Among their many agronomic functions, weed suppression is consistently cited as one of the most valuable benefits provided by cover crops [13]. Generally, these crops are established between two main cash crops [14] and can be incorporated into the soil, grazed, or left on the soil surface to improve soil fertility and structure [15, 16]. The increased interest in diversified cover crop mixtures comes from their ability to deliver multiple ecosystem services simultaneously, making them a central element of integrated cropping systems [17, 18, 19].

However, conventional agriculture has long depended on synthetic herbicides, leading to environmental concerns related to soil health, biodiversity loss, and human safety [20]. Persistent herbicide residues may alter soil microbial communities and reduce soil functionality. Over-reliance on a narrow range of herbicide modes of action has also accelerated the evolution of herbicide-resistant weed populations [21]. Consequently, developing integrated weed management strategies that combine chemical and non-chemical practices has become essential [22]. Within the European Union (EU), these efforts align with the European Green Deal objectives, which target a 50% reduction in pesticide use by 2030 [23, 24].

Beyond weed suppression, cover crops significantly enhance the productivity of subsequent crops and provide numerous ecosystem services. They improve soil structure, reduce erosion, optimize water and nutrient use, and increase soil organic carbon through enhanced carbon sequestration processes [25, 26]. By replacing bare soil during fallow periods, cover crops promote continuous carbon assimilation and stimulate soil biota—including bacteria, fungi, and earthworms—thereby improving soil fertility [27]. In addition, several cover crop species contribute to suppressing soil-borne pests, nematodes, and diseases, further supporting sustainable crop protection strategies [28, 29, 30].

Cover crops also reduce soil bulk density, enhance aggregate stability, and improve water retention capacity, making soils more resilient to climate change. Their contribution to soil organic matter and nutrient cycling helps reduce nitrogen and phosphorus losses, which ultimately protects groundwater and surface water quality [31]. Recent studies conducted on agricultural fields in Illinois showed that non-legume (grass) cover crops significantly reduce nitrate leaching, although they may increase nitrous oxide (N₂O) emissions, a potent greenhouse gas [32].

The long-term benefits of cover crops are increasingly recognized, particularly their role in improving soil health and promoting sustainable agricultural systems. Legume cover crops fix atmospheric nitrogen through symbiotic associations with rhizobia, providing nitrogen to companion non-legume species in mixtures or to subsequent crops in rotation. This reduces the need for synthetic nitrogen fertilizers in crops such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max* L.) [33]. Non-leguminous species with deep taproots, such as oats (*Avena sativa*) and white mustard (*Sinapis alba*), scavenge residual soil nutrients from deeper layers, recycle nitrogen, and reduce nitrate leaching [35].

Cover crop species are predominantly drawn from the Fabaceae (legumes), Poaceae (grasses), and Brassicaceae (brassicas) families [36]. Historically, cover cropping has been practiced since ancient times: in the Mediterranean, leguminous plants were ploughed into fallow fields after early harvests to improve soil fertility [37, 38], while ancient Chinese agricultural texts describe the use of non-harvested plants as green manure to enhance soil productivity [39].

As an agroecological and environmentally friendly practice, cover cropping improves soil fertility while also contributing to increased yields of subsequent cereal crops. By reducing the

need for additional nitrogen inputs and minimizing soil disturbance, cover crops support more sustainable land use and reduce machinery operations [40].

2. Materials and methods

This study presents a comprehensive review of the effects of cover crops on weed management, soil health, and legume crop performance. Cover crops from the Fabaceae (legumes), Poaceae (grasses), and Brassicaceae (brassicas) families were selected based on their biomass production, weed suppression capacity, nitrogen-fixation ability, and relevance in legume-based cropping systems. The main species analyzed included *Secale cereale*, *Avena sativa*, *Sinapis alba*, *Brassica napus*, *Vicia villosa*, and *Trifolium spp.*

Data were collected from peer-reviewed studies, field trials, and meta-analyses published over the last five years. Parameters considered included weed density and biomass, soil physical and chemical properties (such as organic carbon content, bulk density, and aggregate stability), microbial activity, nitrogen cycling, and growth and yield performance of subsequent legume crops. Environmental factors, including potential nitrogen losses and interactions with subsequent crops, were also evaluated.

Agronomic practices influencing cover crop performance, such as sowing and termination dates, biomass accumulation, soil type, and climatic conditions, were taken into account to contextualize differences in effectiveness. Quantitative data were standardized to enable comparisons across studies, while qualitative information was integrated to provide a holistic understanding of agroecological outcomes.

A conceptual workflow illustrating the methodological approach of this review is presented in Figure 1. The figure outlines the process from cover crop selection to data collection, standardization, and synthesis, concluding with the assessment of agroecological outcomes.

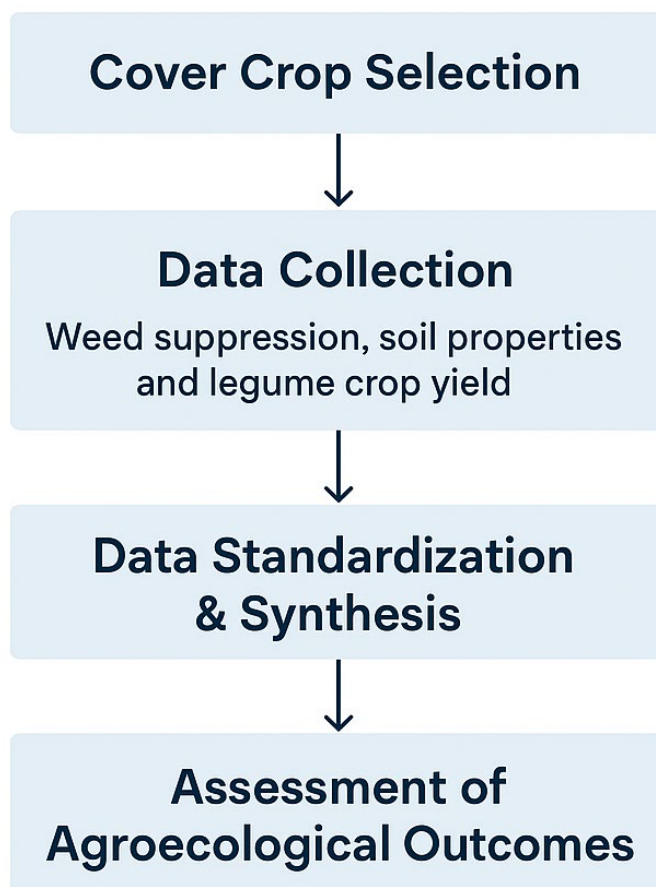


Figure 1. Conceptual Workflow for Materials and Methods

This approach allowed a structured evaluation of the multifunctional role of cover crops in legume cropping systems, highlighting their contribution to sustainable weed management, soil fertility enhancement, and improved legume crop productivity.

3. Results

I. The effectiveness of cover crops in reducing weed pressure

Most recent studies confirm that both monocultural species and mixtures of cover crops significantly reduce weed density before the main crop is planted.

Grasses (e.g., *Avena sativa*, *Secale cereale*) showed the highest biomass levels, resulting in rapid soil coverage and an average weed reduction of 40–70%. Table 1 shows the main weed control mechanisms of different cover crop species.

Table 1

Comparative Overview of Cover Crop Species and Their Weed Suppression Mechanisms

Cover crop	Family	Biomass potential	Weed suppression mechanism	Notes
<i>Secale cereale</i> (rye)	Poaceae	High	Strong competitiveness, rapid shading	Effective in temperate climates
<i>Avena sativa</i> (oats)	Poaceae	Medium-high	Rapid consumption of resources, improvement of soil structure	Very stable in mixtures
<i>Sinapis alba</i> (white mustard)	Brassicaceae	Medium	Allelopathy, inhibition of weed germination	Good against grass weeds
<i>Brassica napus</i> (canola)	Brassicaceae	High	Pronounced allelopathic effects	Increased risk of N ₂ O on wet soil
<i>Vicia villosa</i> (chickpea)	Fabaceae	Medium	Moderate smothering, nitrogen fixation	Excellent support for subsequent crops
<i>Trifolium spp.</i> (clover)	Fabaceae	Low-medium	Moderate competitiveness, N fixation	Especially effective in perennial systems

Figure 2 illustrates the indicative values of biomass produced by the main botanical families used as cover crops.

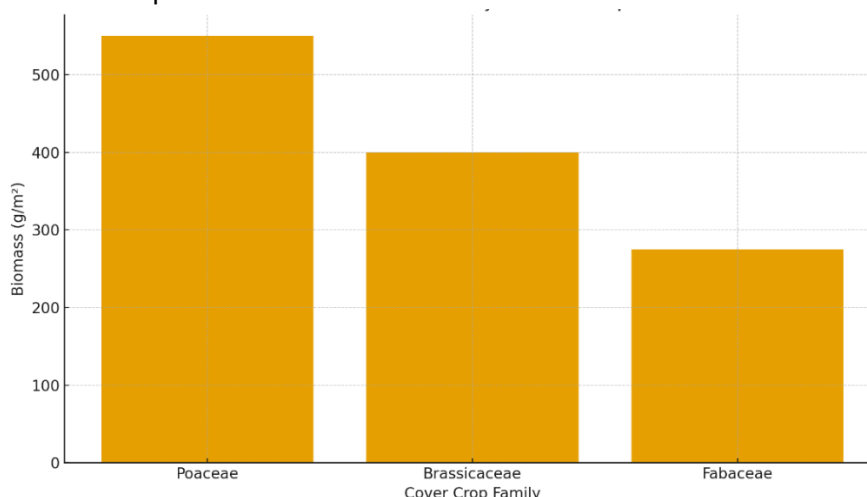


Figure 2. Biomass Production of Major Cover Crop Families (g/m²)

Brassicaceae, such as *Sinapis alba* and *Brassica napus*, have made important contributions through:

- release of allelopathic compounds,
- inhibition of germination of dominant weed species,
- reduction of emergence in the first 30 days after sowing.

Figure 3 summarizes the average reduction in weeds for the main groups of cover crops.

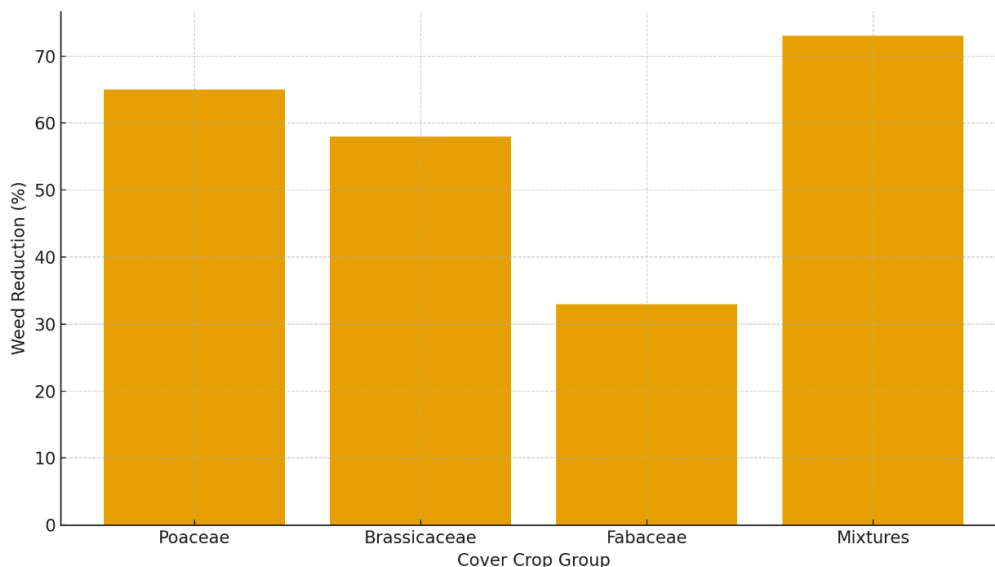


Figure 3. Weed Reduction (%) by Cover Crop Functional Groups

Legumes, although slower to develop, had moderate competitive effects, being more valuable in terms of nitrogen fixation than in their direct role in inhibiting weeds.

II. The impact of cover crops on soil properties

Reviewed meta-analyses show that the use of cover crops results in:

- increase in soil organic carbon (SOC) by 5–15% in 3–5 years;
- reduction in apparent density by 3–8%;
- improving aggregate stability;
- increased microbial activity and microbial biomass;
- reduction of nitrogen losses by up to 30–60% depending on the species.

Figure 4 shows the average changes in soil properties associated with the use of cover crops.

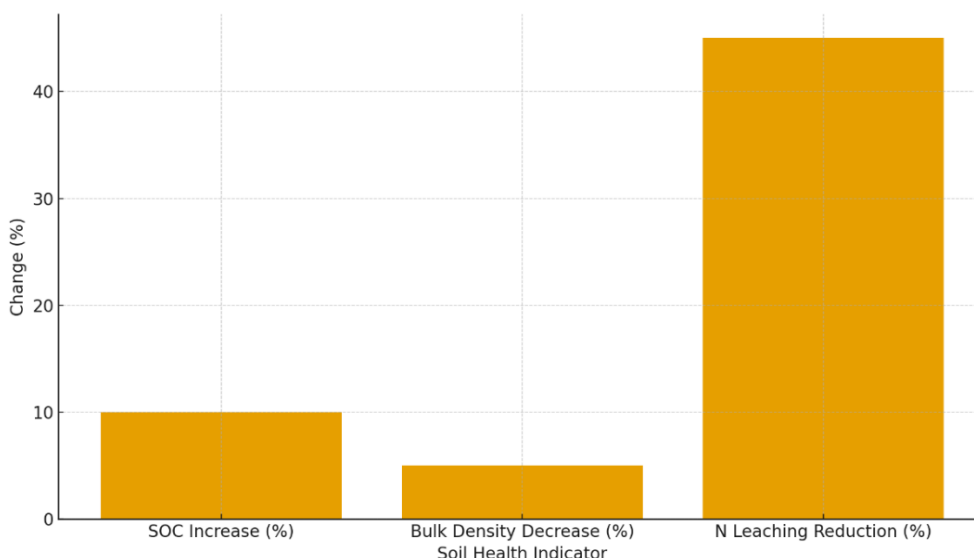


Figure 4. Effects of Cover Crops on Key Soil Health Indicators

III. Response of main crops (legumes)

The use of cover crops before vegetables determines:

- increased uniformity of emergence,
- reducing early competition from weeds,
- 5–12% increase in production, especially in herbicide-free systems,
- reducing nitrogen fertilizer consumption by 20–40% in rotations that include nitrogen-fixing vegetables.

IV. Reducing dependence on herbicides and the emergence of resistance

The studies analyzed indicate that integrating cover crops into legume systems:

- reduces the need for pre-emergence herbicides by 25–50%,
- enables the complete elimination of applications in some ecological systems,
- reduces evolutionary pressure for resistance, especially for weeds such as *Amaranthus spp.*, *Chenopodium album*, *Echinochloa spp.*

V. Ecological interactions and identified risks

- Some grass species of cover crops can increase N₂O emissions, especially in soils with high moisture content.
- Too much biomass can have negative effects on legume sowing, requiring adapted equipment.

Nutrient release may be insufficiently synchronized with crop requirements if the incorporation timing is not correctly chosen.

4. Discussion

The results confirm that cover crops are one of the most effective non-chemical strategies for weed control in legume crops. IWM-based systems particularly benefit from the inclusion of cover crops because they act through several mechanisms simultaneously:

- competitiveness for light, water, and nutrients, reducing the chances of weeds establishing themselves;
- allelopathic effects, especially in the case of species from the Brassicaceae family;
- increased biological activity in the soil, which stimulates natural nutrient cycles;
- soil cover, reducing the germination of photosensitive weeds.

The introduction of cover crops in legume systems can significantly reduce the pressure of herbicide application, which is crucial in the context of the European Green Deal. However, their effectiveness depends heavily on:

- species and mixture used,
- the period of establishment and termination,
- the amount of biomass produced,
- soil type and local climate,
- main crop sowing technology.

Their use also requires awareness of potential risks: e.g., interference with direct seeding, possible increased N₂O emissions, competition for water in arid regions, and mismatching between nutrient release and plant requirements.

Overall, the literature shows that the role of cover crops is not limited to weed control, but to the holistic improvement of the entire agroecosystem.

5. Conclusions

- Cover crops are an essential tool in weed management in legume crops, especially in organic and low-input systems.
- The most effective species for weed control are grasses (for high biomass) and brassicas (for allelopathic effects).
- Legumes as cover crops contribute in particular to improving fertility, playing a complementary role in weed control.

- The integration of cover crops significantly reduces dependence on herbicides, supporting sustainability goals and reducing pesticide use in the EU.
- Eco-agronomic benefits include:
 - a. reducing erosion,
 - b. increase in organic matter,
 - c. improvement of soil structure,
 - d. reducing nutrient losses,
 - e. increased functional biodiversity.
- To maximize positive effects, the choice of species, mixtures, and management practices (sowing date, termination date, biomass level) must be adapted to each agricultural system.

Cover crops can make a decisive contribution to the sustainability of modern agricultural systems, especially in rotations that include legumes.

Acknowledgement

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Rewiew

DECONTAMINATION METHODS USING ADVANCED MICROBIOLOGICAL TECHNOLOGIES FOR ECOLOGICAL REMEDIATION OF SOIL AND WATER POLLUTED WITH HYDROCARBONS

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Keywords: *bioremediation, contaminated soil, hydrocarbons, bioaugmentation, biostimulation, phytoremediation*

Abstract: The article analyzes modern decontamination methods that utilize advanced microbiological technologies for the remediation of soil and water polluted with hydrocarbons. Special attention is given to processes such as bioaugmentation, biostimulation, and microbiologically assisted phytoremediation, which harness the natural capacity of microorganisms to degrade toxic and persistent petroleum compounds. The advantages of using adapted bacterial and fungal communities, as well as extracellular enzymes, in accelerating bioremediation processes are discussed. The article highlights environmental factors that influence the efficiency of these methods, such as nutrient availability, aeration, and salinity. It also explores the potential of bioelectrochemical systems and nanotechnologies in enhancing microbiological processes. Overall, the article emphasizes the potential of these eco-friendly technologies to replace or complement traditional, costly methods, offering sustainable solutions for restoring ecosystems affected by hydrocarbon pollution.

1. Introduction

The issue of soil and water contamination with hydrocarbons is not exclusively a consequence of the modern industrial era; however, its scale and complexity have intensified unprecedentedly since the 20th century, driven by the rapid expansion of the petroleum industry, global transportation networks, and the diversification of organic pollutant sources. The first systematic concerns regarding hydrocarbon pollution emerged in the context of the alarming rise in oil spills and industrial pollution during the post-war period. In the following decades, as global environmental awareness grew stronger, the scientific community began to devote increasing attention to sustainable remedies for combating these types of contamination [1, 2].

Hydrocarbons represent a vast class of organic compounds composed exclusively of carbon and hydrogen atoms, which can be categorized into four major groups: aliphatic hydrocarbons, aromatic hydrocarbons, alkenes, and cycloalkanes. While petroleum-based hydrocarbons—such as gasoline, diesel, kerosene, or fuel oil—are most frequently associated with environmental pollution, a wide array of other hydrocarbons also have a significant ecological impact. These include polycyclic aromatic hydrocarbons (PAHs), used in industrial processes like asphalt and plastic manufacturing, as well as volatile organic compounds (VOCs) derived from chemical activities, refineries, or incomplete combustion processes. Secondary petroleum products, industrial additives, lubricants, or technical solvents may also contain persistent hydrocarbons with high toxic potential.

Contamination of the environment with these substances can occur through a variety of pathways: accidental spills, improper waste disposal, infiltration into groundwater, dispersion via precipitation, maritime accidents, or chronic diffuse pollution linked to urban industrial

activities. Once released into soil or water, these hydrocarbons are difficult to eliminate due to their high resistance to natural degradation and their pronounced tendency to bioaccumulate in food chains. They affect the structure and functioning of terrestrial and aquatic ecosystems, alter the natural soil microbiota, and disrupt essential biogeochemical cycles.

Traditional remediation methods—such as excavation and transport of contaminated soil, chemical soil washing, or thermal incineration—though widely used, present significant limitations: high costs, substantial energy consumption, generation of secondary toxic emissions, and irreversible destruction of local biological habitats. Moreover, these methods are often inadequate for deep or diffuse contamination, or when physical or economic access to the polluted site is limited.

In this context, research has increasingly focused on ecological solutions based on natural or enhanced biological processes that ensure not only the removal of contaminants but also the regeneration of the affected ecosystem (fig. 1).

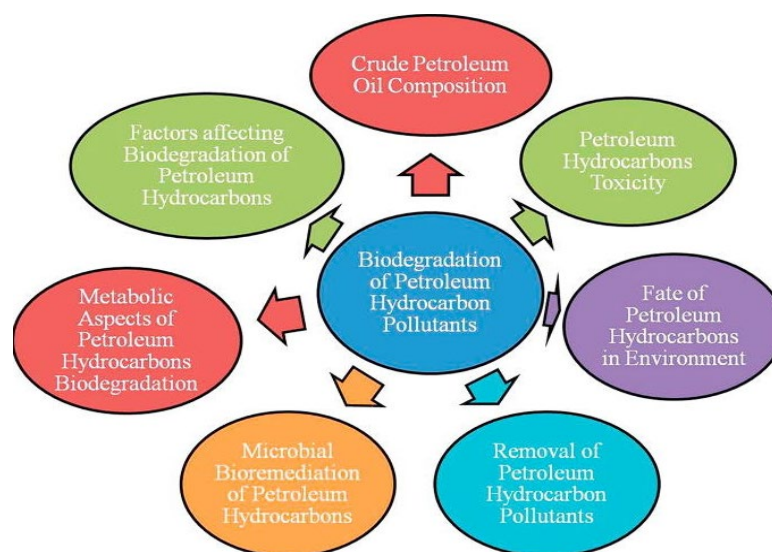


Figure 1. Biodegradation of petroleum hydrocarbon pollutants, [3]

Microbial biodegradation of petroleum hydrocarbon pollutants relies on the enzymatic catalytic activities of microorganisms to enhance the degradation rate of contaminants [4].

Among these methods, bioremediation—which involves the use of microorganisms (bacteria, fungi, algae) [5, 6] to break down organic pollutants—has emerged as one of the most promising and versatile strategies. Due to the ability of certain microorganisms to metabolize hydrocarbons as a source of carbon and energy, bioremediation enables the conversion of toxic compounds into harmless end products such as carbon dioxide and water. This approach can be applied directly in contaminated soil or water, or through the extraction and treatment of the soil under controlled conditions.

With the development of advanced biotechnologies and modern molecular analysis methods, significant progress has been made in selecting, optimizing, and engineering microorganisms used in bioremediation [7]. Current technologies allow treatments to be tailored to the specific type of contaminant, local environmental conditions, and long-term remediation goals. From biostimulation (adding nutrients and oxygen to stimulate native bacterial activity) to bioaugmentation (introducing specialized microbial strains or synthetic microbial consortia), modern methods focus on increasing the efficiency of biological degradation processes in a controlled and predictable manner [8, 9, 10].

Thus, microbial remediation has become a key direction in global efforts to ecologically restore environments polluted with hydrocarbons, offering not only an economically viable alternative but also one that adheres to the principles of sustainable development and biodiversity conservation [11].

In addition to conventional petroleum hydrocarbons, the issue of contamination also includes hydrocarbons from other industrial sources, such as:

Halogenated hydrocarbons [12] – compounds in which hydrogen atoms are partially or fully replaced with halogen atoms (e.g., chlorine, bromine), such as PCBs (polychlorinated biphenyls) or dioxins, which are highly persistent and toxic, with strong bioaccumulation potential in food chains.

Heterocyclic hydrocarbons – compounds containing elements such as nitrogen or sulfur within their molecular structure, often byproducts of industrial processes, which can have significant toxic effects.

Olefins and alkenes – unsaturated hydrocarbons with high reactivity, used as raw materials in the chemical industry and capable of causing pollution through accidental spills.

These variations in the chemical composition of hydrocarbon pollutants require the adaptation and diversification of remediation methods, aiming to identify microorganisms and technologies capable of targeting each specific type of contaminant. Figure 2 illustrates the fundamental principle of aerobic hydrocarbon degradation by microorganisms.

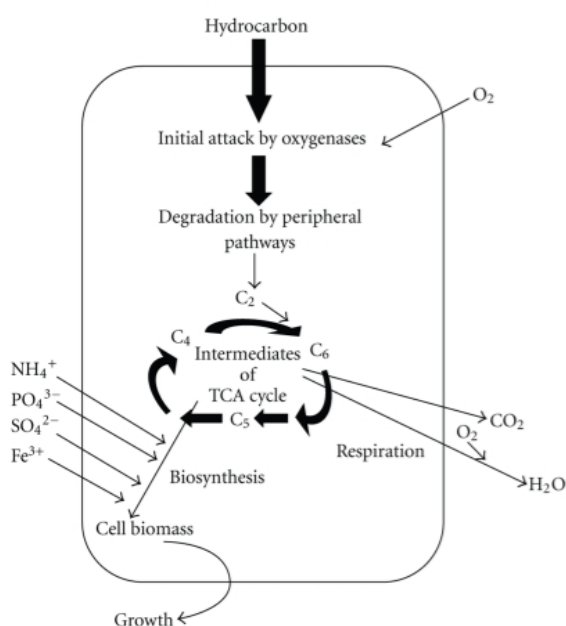


Figure 2. The fundamental principle of aerobic hydrocarbon degradation by microorganisms [13]

In this context, bioremediation represents a complex method that may include biostimulation (optimizing conditions for native microorganisms), bioaugmentation (Figure 3) (introducing specialized microorganisms), the use of biosurfactants (which enhance the solubility of pollutants), and genetic engineering (to create microbial strains with improved capabilities).

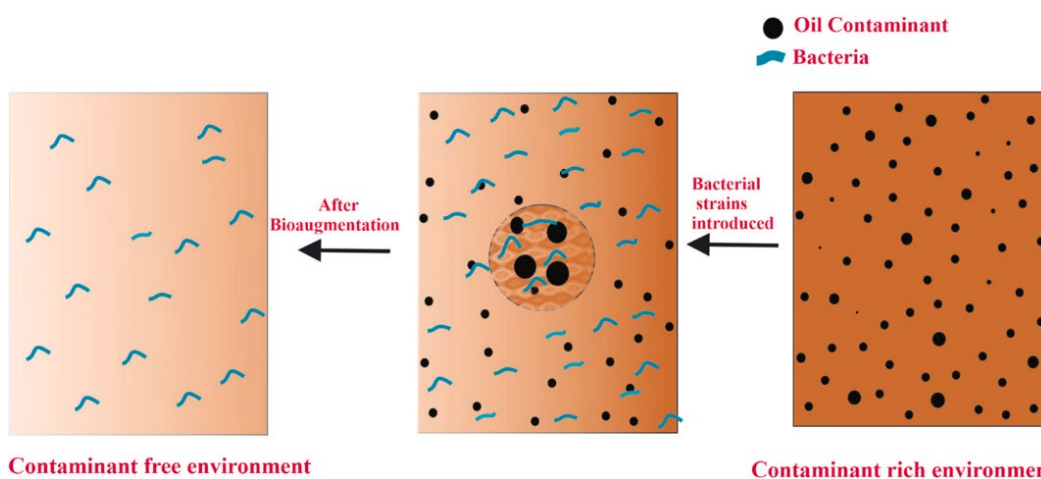


Figure 3. Bioaugmentation technique using various bacterial strains for petroleum waste recovery [14]

Biochar (Figure 4) is being experimentally investigated as a highly effective new amendment for the remediation of contaminated soil. A crucial consideration is the influence of biochar on the bioremediation of soil polluted with total petroleum hydrocarbons (TPHs), and particularly the use of biochar as a carrier for bacterial immobilization, offering a synergistic effect of adsorption and degradation [15].

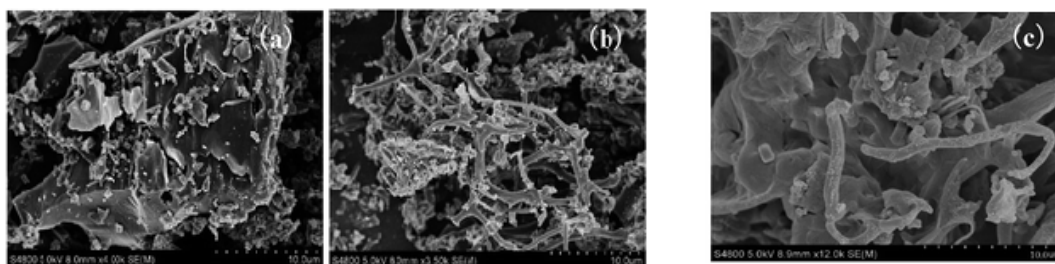


Figure 4. - Scanning electron microscopy (SEM) images of: (a) a spent mushroom substrate; (b) biochar; and (c) bacteria immobilized on the surface [16].

This diversity of microbiological technologies applied in ecological remediation opens new perspectives for the sustainable restoration of the environment, with a positive impact on human health, biodiversity, and quality of life [17].

2. Materials and methods

In the effort to ecologically remediate soil and water polluted with hydrocarbons, a thorough understanding of the involved materials and advanced microbiological methods is essential for the success of the process. The pollutant material, the characteristics of the contaminated environment, the microorganisms involved, the technological methods applied, and rigorous monitoring together form a complex system that must be approached with precision and care.

Hydrocarbons, organic compounds composed exclusively of carbon and hydrogen, display immense chemical diversity, reflected both in their molecular structure and their behavior in the natural environment. To design effective remediation strategies, in-depth knowledge of these compounds is crucial. They can be classified into saturated hydrocarbons (alkanes), unsaturated hydrocarbons (alkenes, alkynes), aromatic hydrocarbons (benzene, toluene, xylene), and polyaromatic hydrocarbons (PAHs – Polycyclic Aromatic Hydrocarbons) [18], each with different characteristics in terms of toxicity and biodegradability [19]. Soil pollution with petroleum-derived hydrocarbons (PAHs), resulting from the oil industry or human activities, is a global concern.

Petroleum PAHs are organic chemical substances containing two or more fused benzene rings, with 16 of these hydrocarbons classified as priority contaminants due to their mutagenic, oncogenic, and teratogenic properties. Inhalation of PAHs poses a risk for lung cancer. Humans can be exposed to PAHs through skin contact, inhalation, or ingestion of pollutants from air and soil. Prolonged exposure to PAH pollution can impact health in the following ways: liver and kidney damage, weakened immune system, cataracts, respiratory diseases, skin, stomach, and lung cancers, cardiotoxicity, atherosclerosis, and apoptosis of endothelial cells.

The sources of PAH soil contamination stem from anthropogenic activities: pyrogenic sources from coal combustion, electronic waste burning, combustion of petroleum fuels (gasoline and diesel), and other fossil fuels; fuel consumption; agricultural practices; oil and gas production; spills during transport and storage of hydrocarbons; loading and unloading operations; explosions from underground pipelines. Other sources



Figure 5. Sources of soil pollution with PAHs [20]

Within contaminated soil and water, hydrocarbons are present in various forms, ranging from easily biodegradable volatile fractions to heavy, resistant, and lipophilic fractions that adhere to soil particles or settle in sediments. These characteristics determine their accessibility to microorganisms and influence the duration of the degradation process. Pyrogenic PAHs are emitted when biomass is processed at high temperatures in the absence or low presence of oxygen. The emission of pyrogenic PAHs occurs during the pyrolytic refining of petroleum residues into lighter hydrocarbons and the transformation of coal into coke or coal tar.

Besides the chemical nature of the pollutants, the characteristics of the contaminated environment play a vital role. Soils with varying textures, moisture levels, and oxygen content can either support or inhibit microbial activity. In particular, anaerobic or nutrient-poor soils can limit biological degradation, thus requiring additional interventions to optimize conditions.

In contaminated water, physicochemical parameters such as temperature, pH, dissolved oxygen, and the presence of other contaminants significantly affect the efficiency of microbiological processes. Therefore, a detailed diagnosis of the contaminated environment is the foundation of successful remediation.

Microorganisms involved in bioremediation are living organisms with a remarkable capacity to adapt and transform toxic compounds. Specialized bacteria, such as those from the genera *Pseudomonas*, *Alcanivorax*, *Rhodococcus*, or *Mycobacterium*, are capable of breaking down complex hydrocarbons into simpler, assimilable, or harmless substances. Additionally, fungi and actinomycetes contribute significantly to the degradation of more recalcitrant fractions.

The importance of native microorganisms cannot be overstated, as they are already adapted to local conditions. However, in cases of severe contamination or insufficient microbial populations, bioaugmentation with specialized cultures can accelerate the process.

Moreover, microorganisms can work synergistically, forming complex microbial communities in which each species plays a specific role in the stepwise degradation of hydrocarbons, thus providing flexibility and efficiency to the bioremediation process [21].

Bioremediation relies on the ability of certain microorganisms—bacteria, fungi, and in some cases algae—to use hydrocarbons as a source of carbon and energy. Through enzymatic processes, these microbes can transform complex and toxic organic compounds into simpler substances such as carbon dioxide, water, and microbial biomass. The process is influenced by several environmental factors, including pH, temperature, moisture, oxygen and nutrient availability, as well as the nature of the contaminant and the physicochemical characteristics of the soil or water (Figure 6). Depending on these parameters, bioremediation can be enhanced through various methods, either by introducing efficient microorganisms (bioaugmentation) or by creating optimal conditions for the development of indigenous microbial populations (biostimulation).

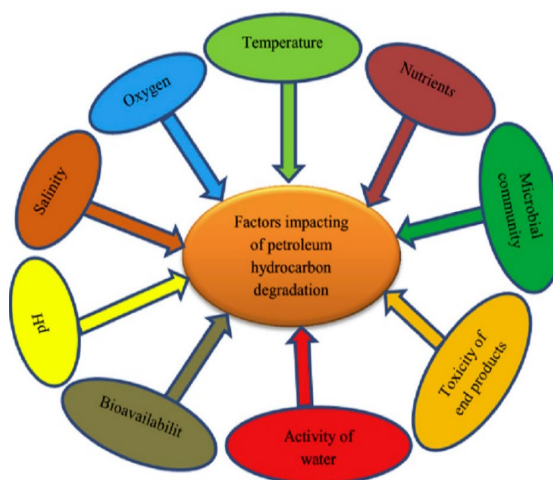


Figure 6. Schematic representation of factors influencing microbial remediation [22]

A key aspect in the success of bioremediation is the selection of appropriate microorganisms. Ideally, they should be capable of degrading a wide range of hydrocarbons—from simple alkanes to polycyclic aromatic hydrocarbons (PAHs)—and able to withstand stress conditions such as high contaminant concentrations, elevated salinity, or lack of oxygen [23]. Many bacterial species from the genera *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, and *Alcanivorax* have proven to be highly effective in hydrocarbon degradation processes, and recent research is increasingly focused on identifying new strains with enhanced tolerance and metabolic performance [24].

In the microbiological decontamination process, microorganisms can be classified according to their origin (indigenous or allochthonous), their mode of action (aerobic or anaerobic), as well as by the specific type of substrate they degrade. Indigenous microorganisms, already present in the contaminated soil or water, can be stimulated by adding nutrients or oxygen to enhance their activity. In contrast, allochthonous microorganisms are cultivated in laboratories and then introduced into the contaminated environment to increase degradation capacity. These can be selected according to the specific contamination and may be genetically modified to enhance their metabolic efficiency or tolerance to extreme conditions.

Bacteria represent the most widely used group of microorganisms in hydrocarbon bioremediation. Among them, the genus *Pseudomonas* is the most studied, with multiple strains capable of degrading both saturated and unsaturated hydrocarbons. Other bacteria with remarkable potential include *Bacillus*, *Acinetobacter*, *Corynebacterium*, and *Sphingomonas*. In addition to bacteria, fungi—especially those from the genera *Phanerochaete*, *Aspergillus*, and *Trametes*—play a key role in the degradation of complex hydrocarbons due to their extracellular enzymes such as laccases and peroxidases. These enzymes can attack the chemical bonds of polycyclic aromatic compounds, accelerating their mineralization. Furthermore, some

microalgae, such as *Chlorella* or *Scenedesmus*, can indirectly contribute to bioremediation by oxygenating the environment and synthesizing substances that stimulate bacterial activity.

The development of microbiological technologies has led to the emergence of diverse methods that can be adapted according to the nature of the contamination and the characteristics of the affected site.

Biostimulation, one of the fundamental methods, involves the addition of essential nutrients such as nitrogen and phosphorus, as well as controlled aeration, to create an optimal environment for native microorganisms. This method is valued for its simplicity, low cost, and effectiveness, but requires continuous monitoring to avoid ecological imbalances. Biostimulation targets the activation of indigenous microorganisms by optimizing environmental conditions. This is achieved by irrigating the soil with nutrient solutions, adjusting the pH, maintaining optimal moisture levels, or injecting air. In some cases, biodegradable surfactants are used to increase the bioavailability of hydrocarbons, facilitating their uptake by microbes.

Bioaugmentation is based on the introduction of well-characterized microbial cultures, sometimes genetically modified, that possess enhanced degradation capabilities. This is crucial in the case of persistent pollutants or environments with poor native microbiota. Bioaugmentation involves the addition of specially selected and laboratory-cultivated microbial cultures capable of degrading hydrocarbons present in soil or water. This method is often used in sites where indigenous microorganisms are not efficient enough or where contamination is recent and severe. To prevent the introduced colonies from dying off, bioaugmentation is often combined with biostimulation—adding essential nutrients (nitrogen, phosphorus, potassium) or oxygen sources to accelerate microbial metabolism [25]. A schematic representation of a bioaugmentation process is presented in Figure 7.

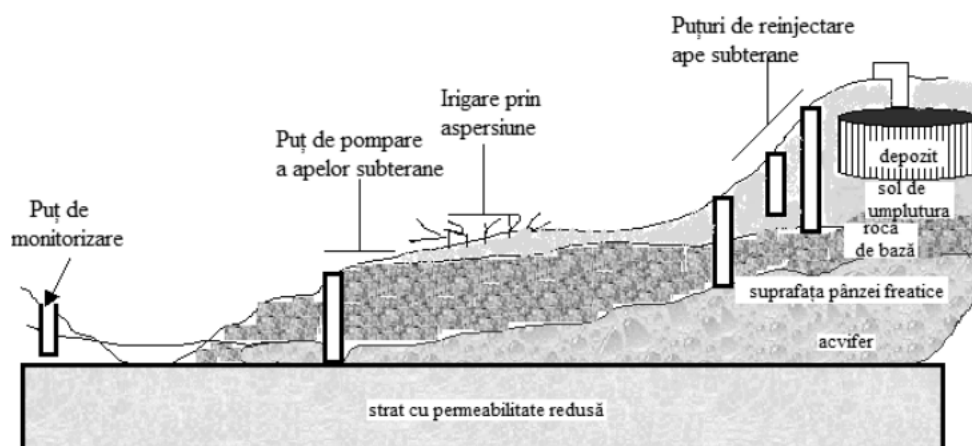


Figure 7. Diagram of a bioaugmentation process for contaminated soil [26]

The bioaugmentation procedure is not difficult to implement, and the installation of injection wells is not excessively expensive, which makes this bioremediation technology generally successful in practice.

Bioaugmentation can be applied for the decontamination of sites polluted with volatile organic compounds (VOCs)—such as benzene and formaldehyde—and semi-volatile organic compounds (SVOCs), including non-halogenated phthalates, bisphenols, and fuels [26].

An innovative aspect is the use of biosurfactants produced by microorganisms, which enhance the solubility of lipophilic hydrocarbons, thus increasing their bioavailability for degradative enzymes. These natural molecules have the advantage of being biodegradable, non-toxic, and effective even at low concentrations.

To optimize the process, bioreactions can be controlled in closed environments such as bioreactors, where essential parameters—temperature, pH, oxygenation, and nutrient supply—can be precisely adjusted. These systems accelerate degradation and provide accurate data regarding remediation efficiency.

Continuous monitoring is a cornerstone of effective bioremediation. Through periodic sampling and analysis using chemical, microbiological, and molecular methods, pollutant concentrations, microbial types, and their activity levels can be assessed.

New technologies such as metagenomic DNA sequencing allow precise characterization of microbial communities and assessment of their dynamics, offering a comprehensive picture of the process evolution. At the same time, biosensors are being developed to enable real-time monitoring, reducing response time and facilitating immediate adjustments.

This integrated assessment of chemical and biological parameters ensures optimal management and increases the likelihood of complete and sustainable remediation.

To address the complexity of hydrocarbon contamination, microbiological methods are often combined with other complementary technologies.

Phytoremediation, which involves the use of plants [27, 28] with detoxifying capabilities, brings additional benefits by stabilizing soil, absorbing pollutants, and stimulating rhizosphere microbiota. This biological partnership enhances degradation rates and contributes to ecosystem restoration.

Physicochemical technologies, such as ozonation or photocatalysis, can transform hydrocarbons into more soluble and bioavailable compounds, thereby integrating the remediation process into a high-performance hybrid system.

Nanotechnology, through the use of active nanoparticles, is an emerging field with significant potential for enhancing biodegradation processes and contaminant adsorption, enabling faster and more efficient remediation.

The use of functionalized nanoparticles to improve microbial activity represents an advanced direction in hydrocarbon remediation. Nanoparticles (e.g., zero-valent iron, TiO₂, ZnO) can stimulate degradation processes through catalysis, redox reactions, and by increasing the bioavailability of contaminants. They can also be used for the controlled delivery of nutrients or enzymes to polluted areas.

CRISPR-Cas9 technology has been used to genetically modify bacteria and fungi to degrade complex hydrocarbons—such as polycyclic and hard-to-biodegrade aromatic compounds—rapidly and efficiently. This technology allows for metabolic customization of microorganisms, increasing both the speed and range of contaminants that can be treated.

Hybrid remediation systems (biological–physicochemical)

Rather than relying on a single method, these systems combine bioremediation with physical or chemical techniques such as photocatalysis, advanced oxidation processes (AOPs), or electrochemistry (see Figure 8).

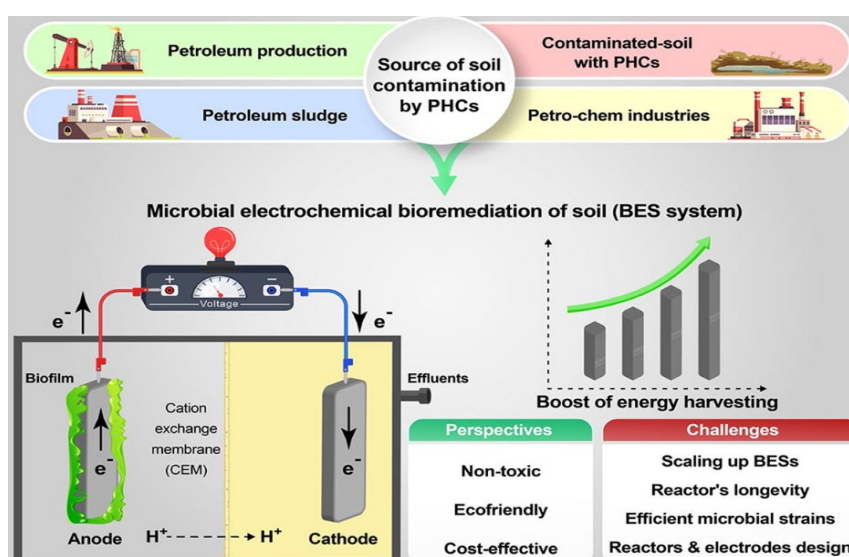


Figure 8. Microbial Electrochemical Bioremediation of Petroleum Hydrocarbon Pollution [29]

Bioelectrochemical systems (BES) are among the bioremediation technologies that have attracted significant research interest due to their advantages, such as greater energy savings during bioremediation, safety, operational simplicity, low cost, environmental friendliness, and long-term viability [30].

Hydrogen peroxide or ozone injection into soil, combined with microbial consortia, can accelerate hydrocarbon breakdown.

Smart Bioreactors Controlled by Artificial Intelligence (AI)

In laboratories and pilot stations, bioreactors for treating contaminated soil or water are increasingly equipped with sensors and automated systems controlled by AI algorithms. These systems optimize biological conditions in real time (pH, temperature, oxygenation), maximizing bioremediation efficiency and reducing treatment time.

Microbiomics and Metagenomics for Diagnosis and Optimization

Complex microbial DNA and RNA analyses allow precise identification of species involved in hydrocarbon degradation and adjustment of remediation strategies according to the composition of the native microbial community. These methods provide detailed site diagnostics and enable the creation of tailor-made microbial consortia.

Use of Ligninolytic Fungi and Other Non-Conventional Microorganisms

White-rot fungi (e.g., *Phanerochaete chrysosporium*) and other non-conventional organisms have recently been integrated into remediation protocols, as they can break down polycyclic hydrocarbons and other hard-to-degrade aromatic compounds where bacteria show limited performance.

Advanced Forced Ventilation Techniques with Parametric Optimization

Modern bioventing uses sophisticated air distribution systems, oxygen concentration monitoring, and humidity control to create ideal microclimates in the soil. This technology reduces the risk of volatile compound emissions and increases the degradation rate of volatile and semi-volatile hydrocarbons.

Electrobioremediation

An emerging technology that combines the application of an electric field with biological methods to mobilize contaminants and stimulate microbial activity. This process facilitates the migration of hydrocarbons toward microbiologically active zones and can significantly reduce the overall remediation time.

Biosparging with Controlled Injection of Oxygen and Nutrients

This is an enhanced version of traditional biosparging, where oxygen, nitrogen, and phosphorus are injected in a controlled, automated manner based on continuous monitoring of pollutant concentrations and biological parameters. It increases the efficiency of treating soluble and emulsified hydrocarbons.

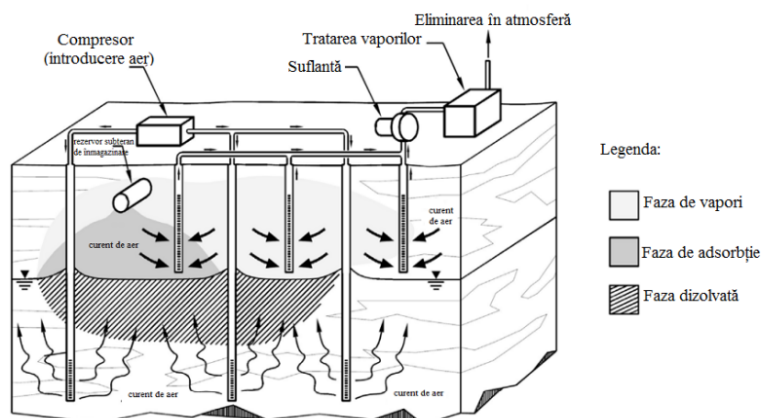


Figure 9. Schematic Representation of a Biosparging Decontamination Process [26]

Bioventing is an in situ technique that involves injecting air into the unsaturated zone of the soil to stimulate aerobic bacterial activity (Fig. 9). It is particularly effective for volatile and semi-volatile contaminants and requires strict control of the air flow rate and its distribution throughout the soil profile.

Similarly, biosparging involves the injection of air or oxygen into the groundwater, promoting the degradation of dissolved or dispersed hydrocarbons within the pore spaces of the substrate.

Biopiles (Fig. 10 and Fig. 11) are engineered piles of contaminated soil treated ex situ through bioaugmentation and biostimulation. These piles are continuously monitored and may be equipped with aeration systems, drainage, and liquid recirculation components, allowing for enhanced control of the remediation process.

For highly persistent contaminants or areas with very high contamination levels, bioreactors provide an efficient solution by allowing the treatment of soil or water in a fully controlled environment. These bioreactors can be continuously fed with contaminated material while critical parameters such as temperature, pH, airflow, and microbial composition are carefully regulated.

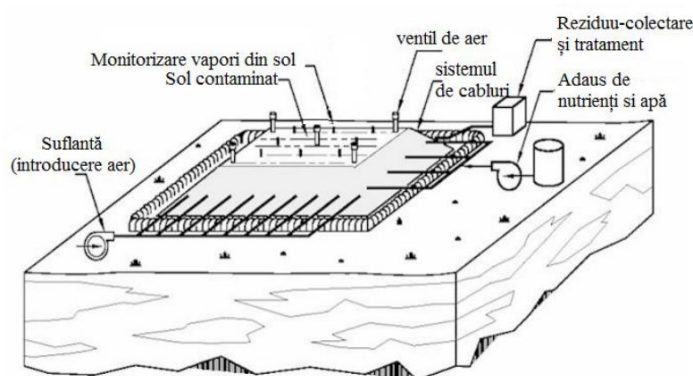


Figure 10. Schematic Representation of a Biopile [26]

Ensuring a uniform airflow is a crucial condition for maintaining an optimal balance in the soil aeration process using biopile technology, and this is effectively achieved through the use of a vertical ventilation system.

To prevent contamination of unaffected soil, the treatment area is typically covered or isolated with an impermeable liner, which minimizes the risk of pollutant leakage into the surrounding environment.

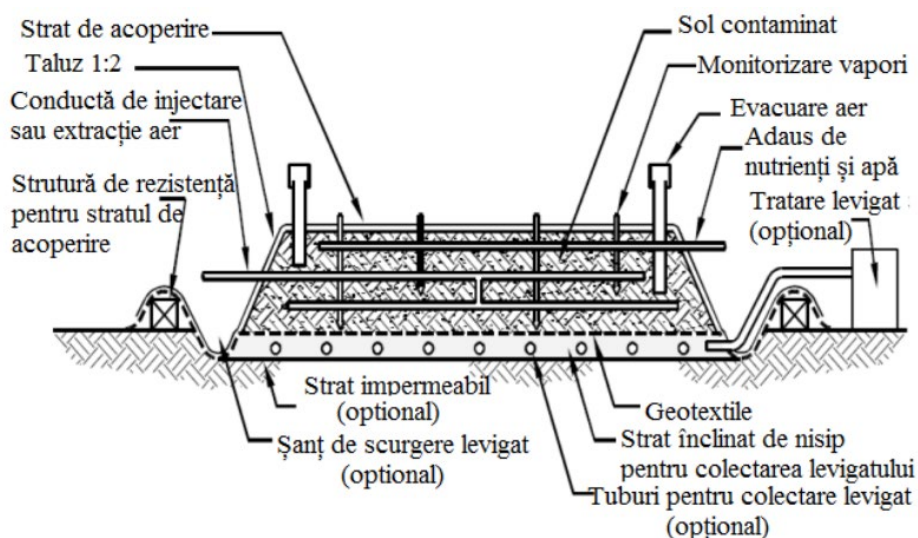


Figure 11. Cross-section of a biopile, [26]

Cutting-edge bioremediation technologies include the use of genetically modified microorganisms (GMOs), purified enzymes, nanobiotechnologies, and synergistic microbial consortia. The goal of these approaches is to enhance degradation efficiency, shorten remediation time, and expand applicability under challenging conditions or for recalcitrant contaminants.

Genetically modified microorganisms represent a major advancement in modern bioremediation. These are engineered by introducing genes that encode enzymes with increased specificity for breaking down complex hydrocarbons such as benzene, toluene, or polycyclic compounds. For example, genetically modified *Pseudomonas putida* strains capable of simultaneously metabolizing multiple types of hydrocarbons have shown superior performance in diverse contaminated environments. However, the use of genetically modified organisms is limited by strict biosafety regulations and concerns about environmental impact.

Ecological remediation using microbiological technologies can be integrated with other methods, such as phytoremediation. Phytoremediation refers to botanical bioremediation and involves the use of green plants to decontaminate soil, water, and air. This technology can be applied to both organic and inorganic pollutants (especially metals) present in soil, water, or air [31].

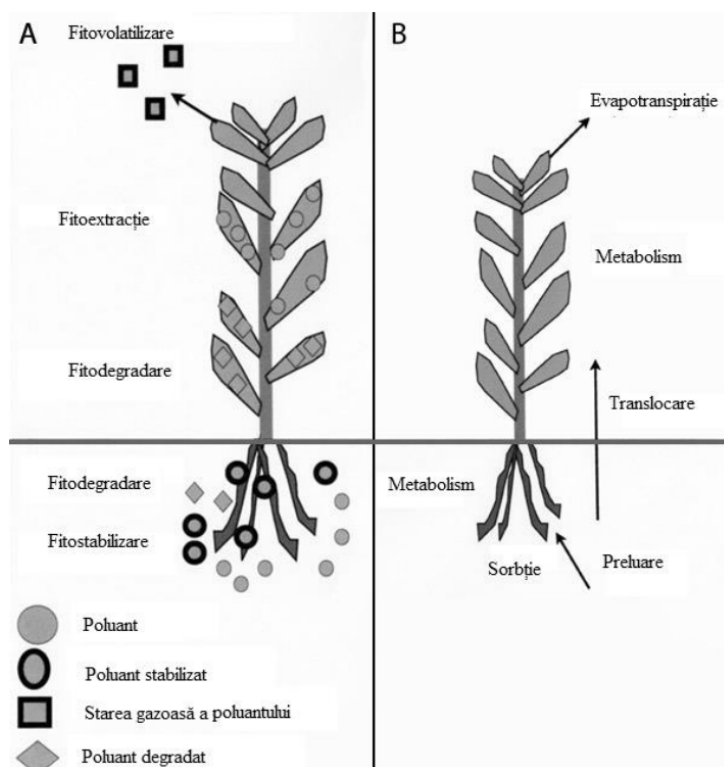
Phytoremediation of inorganic pollutants (Fig. 12 A and B) involves several processes, including the extraction of pollutants from the soil, the translocation of toxic cations or oxyanions to the aerial parts of the plant, and the conversion and retention of elements within the root system to prevent their percolation outside the contaminated zone.

Phytodegradation involves the absorption of pollutants by plant roots, followed by their translocation and accumulation in the roots, stems, and leaves. In the rhizosphere—the soil layer surrounding the roots—advanced biodegradation occurs, stimulated by natural compounds secreted by the plants. These substances provide nutrients to microorganisms and boost biological activity. At the same time, roots loosen the soil, and after decomposition, they form channels that facilitate the circulation of water and air.

Phytoextraction consists of cultivating hyperaccumulator plant species under optimal growth conditions to develop as much biomass as possible. In this way, the plants can absorb, accumulate, and remove significant quantities of pollutants from the soil, particularly heavy metals.

Phytostabilization is a process in which plants produce chemical compounds that help immobilize pollutants in the root–soil interface. This method can be used to restore vegetative cover in areas where natural vegetation has been eliminated due to high concentrations of heavy metals in the surface soil layer or due to physical degradation. By using tolerant plant species, the vegetative cover can be restored while simultaneously reducing the risk of contaminant migration through wind and water erosion or leaching into groundwater—processes commonly encountered in vegetation-depleted areas.

Phytovolatilization occurs when plants absorb water contaminated with organic pollutants and release these compounds into the atmosphere through their leaves. In some cases, the plants can break the chemical bonds of the organic pollutants, transforming them into secondary products that are also released into the air.



**Figure 12. A. Schematic model of different types of phytoremediation
B. Physiological processes occurring within the plant during phytoremediation, [26]**

3. Results

The ecological remediation process of soil and water polluted with hydrocarbons, using advanced microbiological methods, presents a complex set of results reflecting both the efficiency of the applied technologies and the dynamics of interactions between environmental factors, microorganisms, and pollutant compounds. At this stage of the study, analyzing the obtained results is essential to understand how bioremediation methods influence both the concentration of hydrocarbons and the microbial biodiversity as well as the physicochemical properties of the treated environment.

Periodic monitoring of hydrocarbon concentrations revealed a significant decrease in all cases where advanced microbiological techniques were applied, compared to conventional methods or untreated controls. The reduction was more pronounced as biostimulation conditions were optimized through controlled nutrient addition and aeration, along with the introduction of adapted microbial cultures specialized in degrading the heavier hydrocarbon fractions. It is important to note that saturated fractions, such as alkanes, were biodegraded more rapidly due to their simpler chemical structure and increased enzymatic accessibility. In contrast, polycyclic aromatic hydrocarbons required a longer remediation time, and in some cases, temporary fluctuations in their concentration were observed, likely due to intermediate stages of metabolic transformation.

Microbiological analyses revealed that during the initial phases of bioremediation, bacterial and fungal populations in the contaminated environment experienced an imbalance, with a reduction of pollutant-sensitive species occurring alongside the proliferation of specialized groups. As hydrocarbon degradation progressed, microbial diversity increased, signaling a less toxic environment and a restored balance.

The use of molecular methods allowed for the identification of genes encoding key biodegradation enzymes such as monooxygenases and dioxygenases, whose expression correlated with increased biological activity. This aspect confirms the central role of

microorganisms from the genera *Pseudomonas*, *Rhodococcus*, and *Alcanivorax*, as well as fungi producing ligninolytic enzymes (Fig. 13).

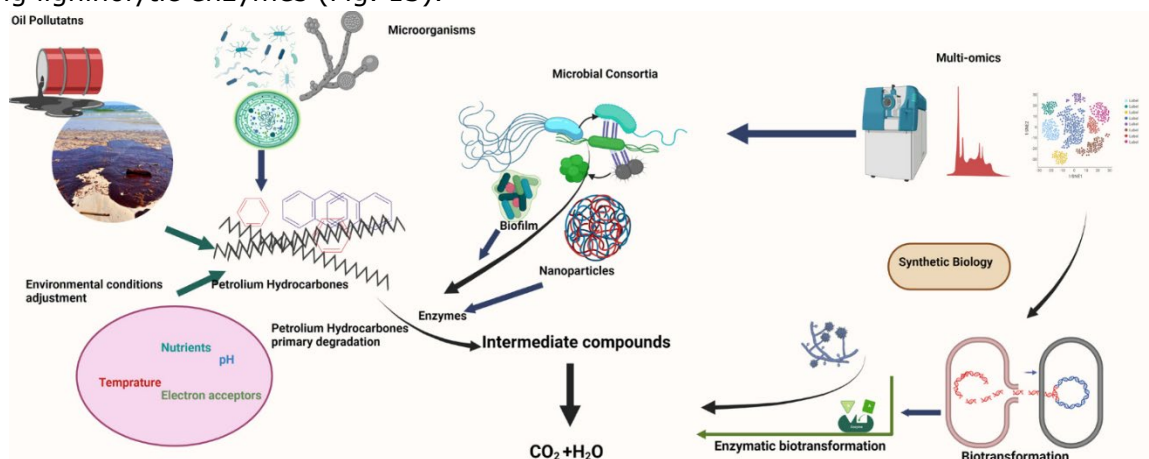


Figure 13. The role of microorganisms in oil degradation, [32]

Besides reducing the concentration of hydrocarbons, microbiological remediation positively influenced soil and water parameters. Improvements were observed in soil aeration and water retention capacity, stabilization of pH, and an increase in organic matter content through the accumulation of microbial biomass. In the aquatic environment, controlled oxygenation helped maintain optimal dissolved oxygen levels, essential for the aerobic activity of microorganisms, while metabolized contaminants were transformed into compounds harmless to aquatic fauna.

Although the results are generally favorable, some limitations were identified. For example, in anaerobic zones or under extreme temperatures, microbial activity was reduced, requiring complementary interventions. Additionally, complex pollutants or those at very high concentrations can inhibit biological processes, necessitating the combination of bioremediation with other physicochemical methods. Major challenges involve real-time monitoring and continuous adjustment of remediation parameters to avoid stagnation or adverse effects, such as the accumulation of toxic intermediate metabolites.

The obtained results highlight the enormous potential of advanced microbiological methods as sustainable and effective solutions for hydrocarbon remediation in soil and water. In the future, integrating biotechnologies with genetic engineering techniques to develop superior microbial strains, as well as using nanotechnology and sensors for precise monitoring, will accelerate the process and expand its scope. The large-scale implementation of these methods will contribute to reducing the negative impact of petroleum and industrial contamination, protecting ecosystems and human health.

4. Conclusions

Following a detailed analysis of modern decontamination methods based on advanced microbiological technologies for the remediation of soil and water polluted with hydrocarbons, the study's results indicate a significant potential of these strategies in promoting a healthy and sustainable environment. Throughout the research, it was clearly demonstrated that biological methods, through their natural ability to transform hydrocarbon compounds into less toxic or harmless metabolites, offer a viable, efficient, and eco-friendly alternative to traditional remediation techniques. The use of microorganisms capable of degrading both light and complex hydrocarbon fractions has opened new perspectives in the field of bioremediation, emphasizing the importance of interdisciplinary approaches combining microbiology, environmental engineering, and applied chemistry.

It was also observed that the success of the remediation process critically depends on a deep understanding of the local conditions of the contaminated environment, as well as on

careful and continuous monitoring of environmental and microbiological parameters. Optimal conditions of pH, temperature, oxygenation, and nutrient availability must be maintained to ensure optimal microbial activity. Thus, adjusting and customizing remediation interventions for each specific site represents a key factor for achieving effective results.

Regarding practical applications, ecological remediation using microbiological technologies can be integrated with other methods, such as phytoremediation or physicochemical treatments, to address complex contamination or accelerate processes. The implementation of pilot systems in the field, followed by the scale-up of technologies, will contribute to the development of standardized protocols, easy to apply in various industrial and geographical contexts.

Looking ahead, research should focus on developing genetically modified microbial strains with enhanced degradation capacities, optimizing bioaugmentation and biostimulation processes, and creating intelligent real-time monitoring systems for biological processes using sensors and artificial intelligence. Additionally, exploring the combination of bioremediation with nanotechnologies may open new pathways for increasing efficiency and reducing intervention durations.

Equally important is raising awareness about the importance of environmental protection and implementing strict policies and regulations to support the use of sustainable remediation technologies. Education and training of specialists in the field will contribute to strengthening the technical capacity necessary for the correct and efficient application of these methods.

In conclusion, modern decontamination methods based on advanced microbiological technologies represent a promising and realistic solution for restoring the quality of soil and water affected by hydrocarbons, with multiple long-term benefits for the environment and public health. Their practical implementation, combined with ongoing research and technological development, will ensure a lasting positive impact in pollution management and ecosystem protection.

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Conflicts of interest: The authors declare no conflict of interest.

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MODERN TECHNOLOGIES FOR MUSHROOM PROTEIN EXTRACTION: PERSPECTIVES AND APPLICATIONS (ARTICLE, REVIEW)

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Abstract:

In the current global context, where food sustainability, human health, and the reduction of environmental impact are becoming major priorities, proteins extracted from mushrooms are gaining an increasingly important role as a valuable alternative to traditional animal protein sources. This study explores the most advanced technologies used for the efficient extraction of proteins from mushrooms, highlighting methods such as ultrasound-assisted extraction, microwave-assisted processing, enzymatic extraction, and pressure-based water processes. Each method is analysed in terms of efficiency, preservation of nutritional value, and industrial applicability. The work focuses exclusively on edible mushrooms, emphasizing their potential as a natural resource rich in proteins, with a complete profile of essential amino acids and multiple nutritional benefits. The food applications of these proteins are outlined, ranging from plant-based products to functional supplements and ingredients intended for special diets. Furthermore, the challenges associated with the industrialization of extraction processes, food regulations, and the need for consumer education are highlighted. Fungal proteins obtained from mushrooms represent not only a sustainable and valuable protein source but also an essential pillar in the future of balanced nutrition. By optimizing modern technologies and integrating them into the food industry, these resources can contribute significantly to food security and public health.

1. Introduction

In recent years, vegan diets have gained popularity, and many people around the world have started to replace animal-based products with healthier and more environmentally friendly alternatives.

Research studies found that myco-protein has several advantages such as:

- It is a nutritious protein source, proteins being essential for building and maintaining muscle tissues and other bodily functions.
- High in fiber: Mushrooms are rich in fiber, which helps support digestive health and provides a feeling of satiety.
- Low in sodium, sugar, cholesterol, and fat: Mycoprotein-based products contain reduced amounts of substances that can be harmful to heart health, such as sodium, sugar, cholesterol, and saturated fats.
- Rich in essential amino acids: Amino acids are the building blocks of proteins, and mycoprotein provides all the essential amino acids the body needs.
- Meat-like texture: Mycoprotein-based products have a texture and taste like meat, making them an appealing option for those looking to reduce meat consumption.
- Low carbon and water footprint: Meat production has a significant environmental impact due to high consumption of natural resources. Mushroom proteins have a much lower carbon and water footprint compared to red or poultry meat, making them an eco-friendly option [1].

In recent decades, interest in alternative and sustainable protein sources has grown exponentially, driven by the global food crisis, the ecological impact of the livestock industry, and the pressing need to find sustainable solutions for a healthy diet. In this context, edible mushrooms and those cultivated for industrial purposes have attracted the attention of researchers and the food industry due to their rich nutritional profile, significant protein content [2], figure 1, as well as their biotechnological versatility



Figure 1. Mushrooms as safe sources of quality proteins and functional ingredients [2]

Proteins obtained from mushrooms [2], figure 2, can not only represent a valuable source of nutrients for human nutrition, but also offer a wide range of applications in sectors such as pharmaceuticals, cosmetics, and industrial biotechnologies.

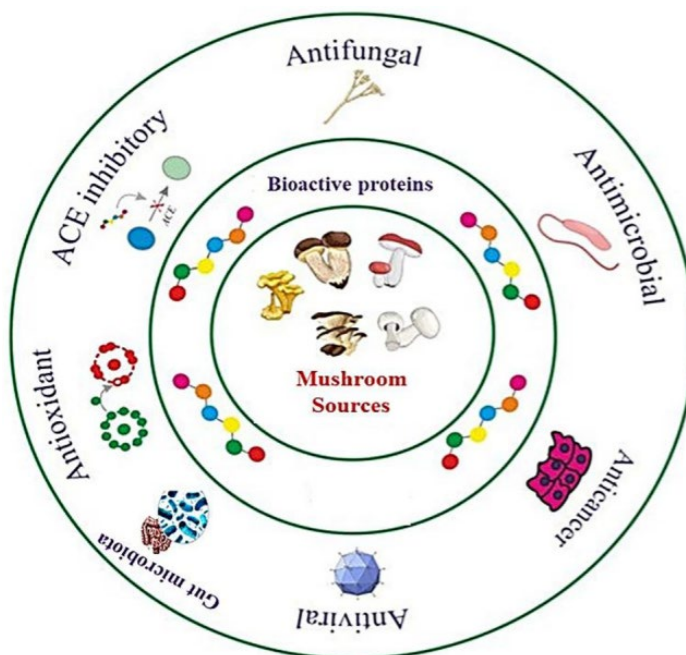


Figure 2. The importance of mushroom proteins as bioactive compounds [2]

Unlike conventional protein sources such as soy, meat, or dairy, mushrooms provide a balanced protein composition, with a complete amino acid profile and the presence of bioactive

compounds that can bring additional health benefits. Moreover, mushroom cultivation requires low amounts of natural resources, such as water and arable land, which gives them a major advantage in strategies for transitioning toward a more sustainable food system. For this reason, the study of efficient and modern methods for protein extraction from mushrooms has become a priority both for academic research and for industrial development [3 - 8].

Traditionally, protein extraction from mushrooms was carried out through crude mechanical or chemical methods, often with low yields and a high risk of degrading bioactive compounds. Nowadays, however, modern technologies have revolutionized this process, offering innovative methods such as ultrasound-assisted extraction, microwave-assisted extraction, the use of specific enzymes (Figure 3) [9 - 11], high-performance liquid chromatography, or supercritical fluid-based techniques [12 - 14]. These methods allow the recovery of high-purity protein fractions while preserving their functional and nutritional characteristics, at the same time reducing processing time and environmental impact. In Figure 3, a schematic representation of the enzyme-assisted extraction process is presented.

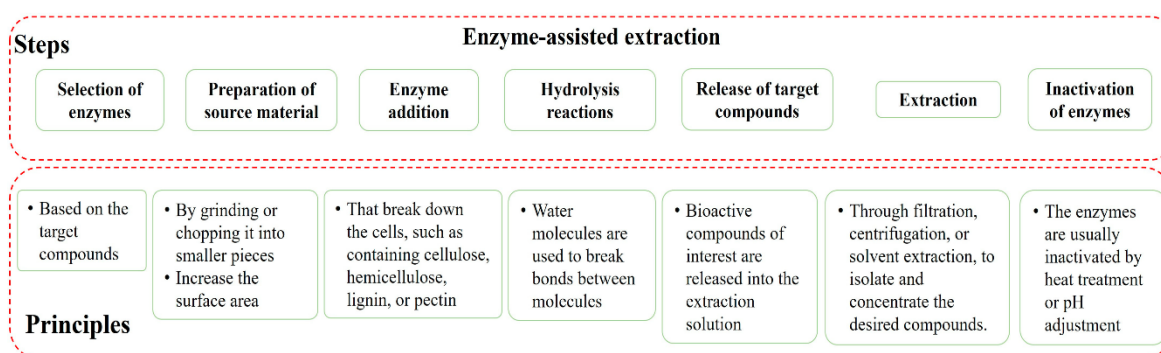


Figure 3. Schematic representation of the enzyme-assisted extraction process [9]

Moreover, studies in biotechnology and metabolic engineering have enabled the development of genetically optimized mushroom strains for increased protein production or enhanced functional properties. In Figure 4, various approaches to exploiting mushroom proteins are presented.

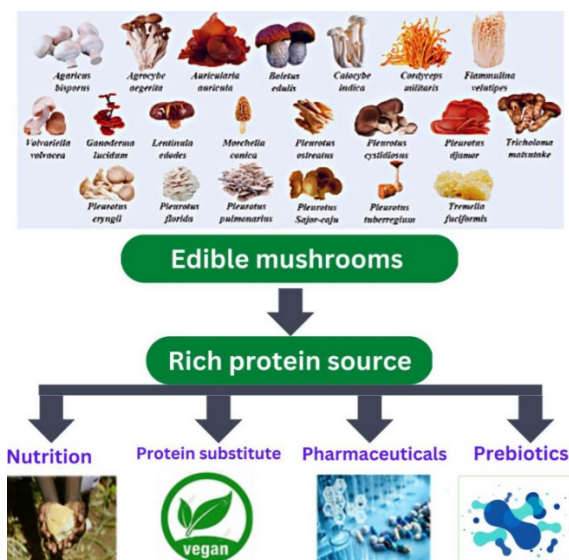


Figure 4. Different approaches to exploiting mushroom proteins [2]

Integrative approaches that combine proteomic, metabolomic, and bioinformatic analyses also open new perspectives in understanding the molecular mechanisms governing fungal protein biosynthesis and their application potential in various fields. At the same time,

interest in 'functional' proteins from mushrooms — which exhibit antioxidant, antimicrobial, or immunomodulatory activities — has led to a reassessment of these organisms not only as a food source but also as genuine 'biofactors' for health [15, 16].

Regarding the applications, proteins extracted from mushrooms present a wide range of uses. In the food industry, they can be incorporated into products for vegan or vegetarian consumers, into functional foods, or into protein supplements, without generating the negative impact associated with animal proteins. In the pharmaceutical sector, certain isolated proteins have demonstrated remarkable biological activities, being explored for the development of new therapies in the treatment of infections, autoimmune diseases, or even cancer. In cosmetology, mushroom-derived peptides [17] are being investigated for their regenerative and antioxidant effects, while in biotechnology, they can serve as biosorption agents, biocatalysts, or even components in the production of biodegradable materials.

Mushrooms are a rich source of bioactive compounds, which have been associated with numerous health benefits, as shown in Figure 5.

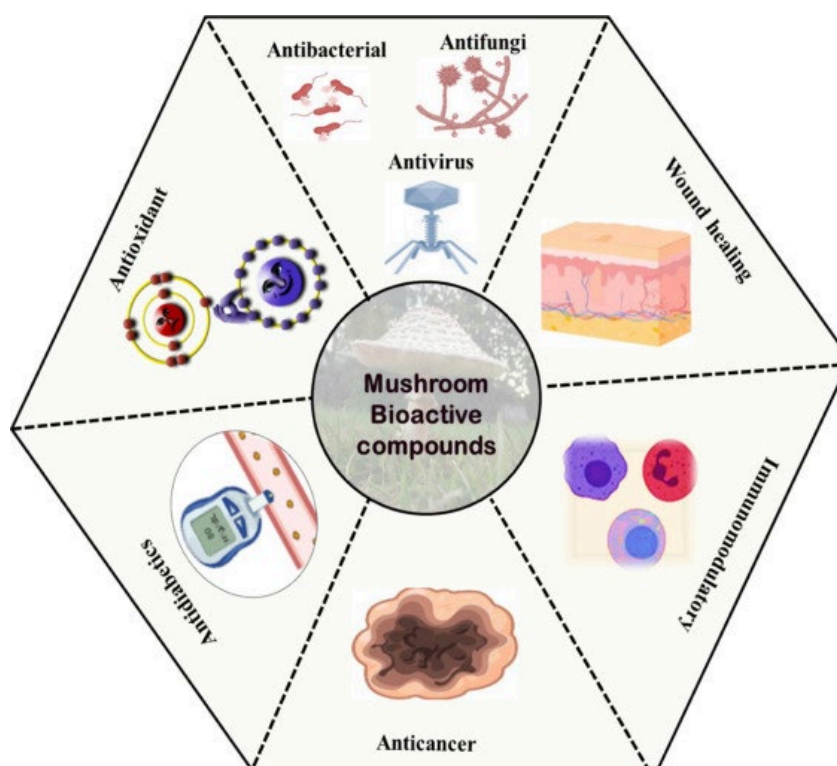


Figure 5. The benefits of bioactive compounds from mushrooms [18]

Each extraction technology involves a specific set of conditions, optimization factors, equipment, and costs, and the choice of the optimal method depends on the type of protein targeted, the characteristics of the raw material, as well as the final purpose of the product. Therefore, a comparative and systematic analysis of modern extraction technologies is essential for the development of efficient, sustainable, and scalable value chains around fungal resources.

It is important to note that the industrial-scale implementation of these technologies requires overcoming challenges related to process standardization, the costs associated with technological infrastructure, compliance with food safety regulations, and integration into circular economic models.

2. Materials and methods

Protein extraction from mushrooms represents a complex process, influenced by numerous factors such as the mushroom species, cellular composition, the nature of the target proteins, and the final purpose of the resulting product. Over time, research has evolved from

conventional crude methods with modest yields to modern and sophisticated technologies capable of delivering high-purity protein products with remarkable functional properties.

Conventional extraction methods

Traditional methods for protein extraction from mushrooms are mainly based on simple physico-chemical techniques, such as: Bead milling, or bead beating, is a mechanical cell disruption technique that uses shear forces to break down cellular structures (Figure 6). Cells and beads move and rotate, and the cells are crushed between beads in the 'bead-cell-bead' configuration

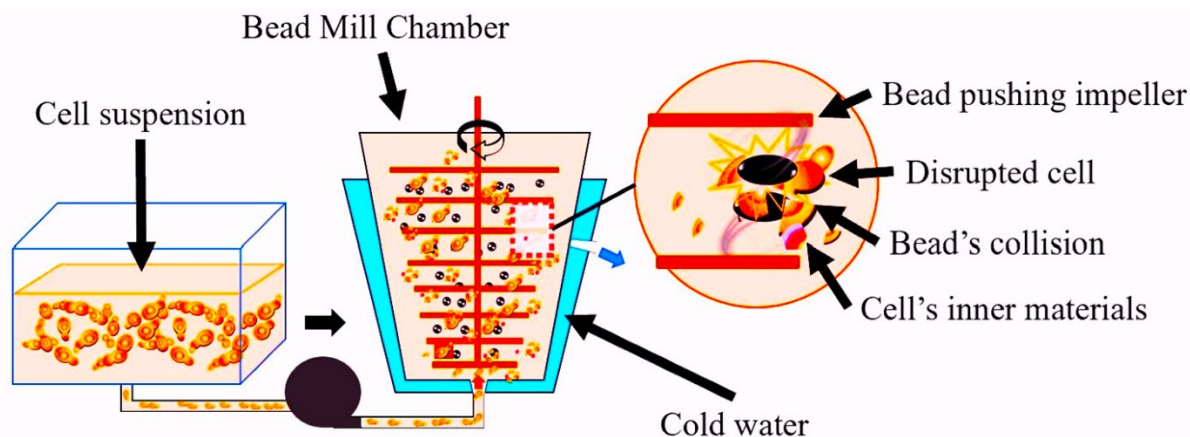


Figure 6. Schematic representation of the cell disruption process assisted by bead milling [9]

A typical bead mill includes a rotating shaft with rotors, a grinding chamber, beads, a wire mesh, a cooling system, an inlet for the cell suspension, and an outlet for the disrupted cells. The biomass is initially transformed into a paste, resuspended in an appropriate buffer, and introduced into the chamber. The bead speed is controlled by the rotating shaft and the accelerator, while the temperature is kept constant through a continuous flow of cold water.

Critical parameters for efficiency include the diameter and density of the beads, their type, the agitation speed, and the lysis agents, which must be optimized according to the size and structure of the target cells.

Mechanical homogenization – involves crushing or grinding the mushroom biomass in the presence of an extraction buffer, followed by centrifugation to separate the soluble fraction. This method is accessible and inexpensive, but its efficiency is limited, and the protein yield varies depending on the mushroom species and the extraction conditions.

High-pressure homogenization

In the high-pressure homogenization method, a high-pressure pump (regularly up to 100 MPa) is used to pass the material through a narrow space (Figure 7). As a result of the high pressure, strong disruptive forces such as shear and cavitation lead to severe turbulent flows and the transformation of the coarse emulsion into a nanoemulsion.

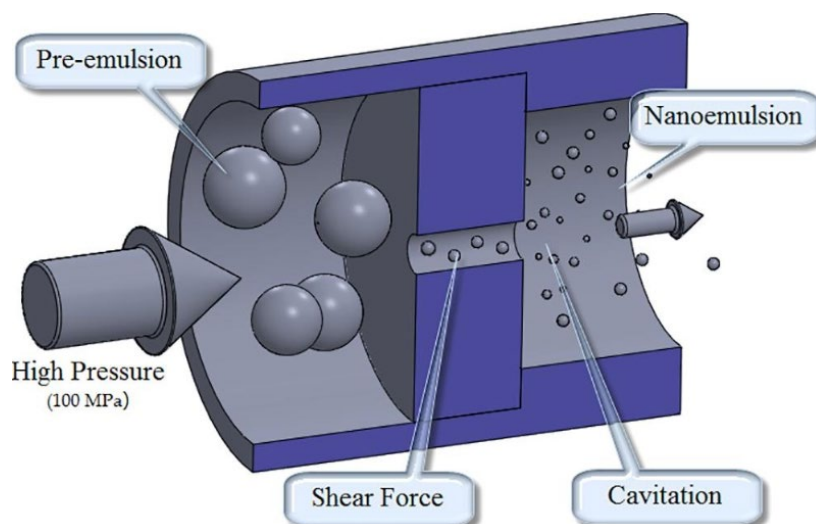


Figure 7. Schematic illustration of the high-pressure homogenization method [19]

High-speed homogenization is one of the energy-efficient emulsification methods, in which rotor/stator mixers or high-speed agitators are used at speeds of approximately 6,000–24,000 rpm [19].

Saline and alkaline solutions – Extraction using NaCl, NaOH, or other weak bases allows proteins to be solubilized but may lead to their denaturation and loss of functional activity. Additionally, further purification steps are required, complicating the process and increasing costs. Protein extraction from edible mushrooms is an essential step in valorizing fungal biomass as a sustainable source of nutrients and bioactive compounds. Among conventional and modern methods used in fungal protein processing, saline and alkaline solutions stand out for their efficiency, low cost, and industrial applicability.

Saline solutions, such as those based on NaCl or KCl, are frequently used for extracting water-soluble proteins by breaking electrostatic bonds and maintaining osmotic balance within the cell matrix. This type of solution allows the recovery of native protein fractions without significant denaturation, being especially useful for preparing extracts with specific biological activity or for functional food applications.

In contrast, alkaline solutions – particularly those containing hydroxides like NaOH – are used to solubilize structural or insoluble proteins from mushroom cell walls, especially in species with a more fibrous texture (e.g., *Pleurotus ostreatus* or *Lentinula edodes*) [20]. At high alkaline pH levels (pH 10–12), protein structure is partially denatured, facilitating the release of amino acids and peptides [21], as well as the dissolution of components bound to chitin-glucan fibers. Alkaline extraction is often combined with auxiliary techniques such as heat treatment or ultrasound to increase protein yield and reduce processing time. However, a major challenge is controlling the degree of protein denaturation and preserving nutritional value, as excessive alkaline conditions can cause irreversible modifications of protein chains.

The choice of solution type largely depends on the final purpose of the extraction: for obtaining functional ingredients with high water solubility, saline solutions are preferred, while for producing protein concentrates, hydrolysates, or ingredients for protein supplements, alkaline solutions are more efficient [22]. Recent studies show that a combined approach – involving pretreatment with saline solutions followed by alkaline extraction – can optimize protein recovery and yield fractions with different characteristics, useful for multiple applications in human nutrition, pharmaceuticals [23, 24], and biotechnology.

Precipitation with organic solvents (ethanol, acetone) – is used to separate proteins from the fungal matrix but carries risks of toxicity and contamination, requiring careful removal of solvent residues. Although these methods are still used in laboratories for preliminary research, they have numerous limitations regarding yield, selectivity, and preservation of protein integrity.

In industrial contexts, these methods are gradually being replaced by modern, more efficient, and environmentally friendly solutions.

Modern extraction technologies

Modern technologies for mushroom protein extraction are characterized by high efficiency, better control of process parameters, and superior preservation of the functional activities of the extracted biomolecules. Among the most used methods are:

Ultrasound-assisted extraction (UAE) – uses high-frequency sound waves to disrupt mushroom cell walls and release proteins. This technique reduces extraction time and solvent consumption, offers high yield, and is suitable for extracting heat-sensitive proteins [25].

In the context of the increasing demand for sustainable and nutritious food alternatives, mycoprotein has emerged as an innovative ingredient derived from edible mushrooms, mainly used in developing meat substitutes, often referred to as “plant-based meat” or “fake meat.” This protein source provides a rich, meat-like texture and a valuable nutritional profile, making it ideal for plant-based diets.

To efficiently harness the potential of mycoprotein, advanced extraction methods are employed, among which probe sonication stands out for its efficiency and speed. This technology relies on the application of high-intensity ultrasonic waves, which generate acoustic cavitation phenomena capable of disrupting the cellular structure of mushrooms, thereby facilitating the release of proteins and other valuable intracellular compounds (Figure 8)

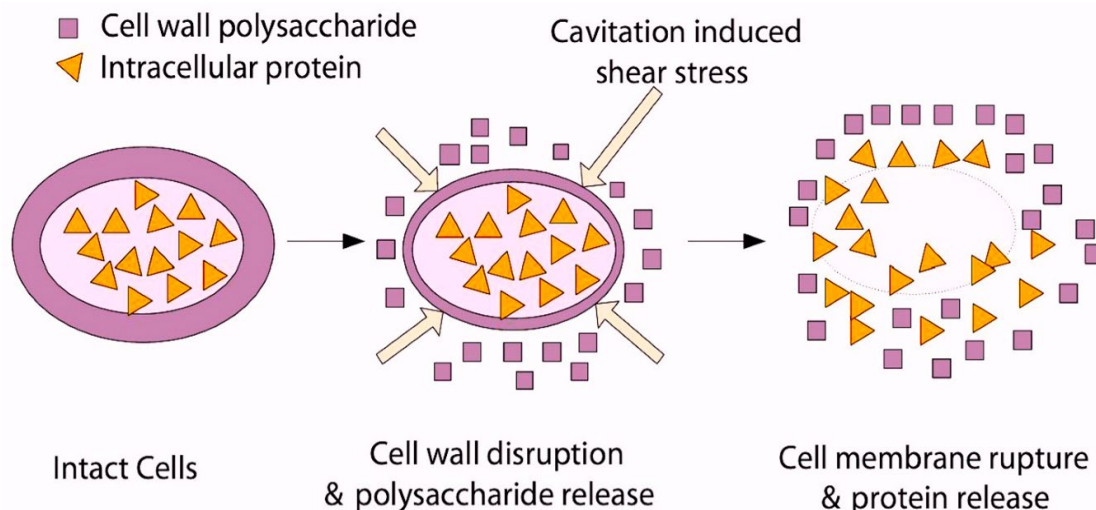


Figure 8. Schematic representation of the ultrasound-assisted cell disruption process [9]

The extraction process begins with the controlled cultivation of edible filamentous fungi, such as *Fusarium venenatum*, in specialized bioreactors. Within fungal cells, mycoprotein is stored in a form that requires an efficient cell disruption method for extraction. Probe sonication is an optimal choice, as the cavitation generated by ultrasound breaks the cell walls, releasing their contents in a short processing time and with high yield [26, 27].

This method offers multiple advantages. First, it allows uniform homogenization of the fungal biomass, which is essential for formulating food products with varied textures and sensory properties. Whether used in meat analogs, protein snacks, or plant-based beverages, mycoprotein obtained through sonication preserves protein integrity and the functional characteristics required in the food industry. In addition, the high energy efficiency and short processing time help reduce costs and environmental impact, aligning with sustainability principles in modern food production.

Ultrasound-assisted extraction is based on the phenomenon of acoustic cavitation, which occurs when high-intensity ultrasonic waves are transmitted into a liquid medium or suspension. This process causes rapid alternation of compression (high pressure) and rarefaction (low pressure) cycles, leading to the formation of microbubbles in the liquid mass. As these bubbles

undergo repeated cycles, they progressively grow in size until reaching a critical threshold, at which point they can no longer absorb additional energy from the acoustic waves.

During a compression phase, the bubbles reach instability and violently implode, generating extreme local conditions. These include very high temperatures, extreme pressures, steep temperature and pressure gradients, and the formation of liquid microjets that can reach speeds of up to 280 m/s. The mechanical forces associated with these events cause efficient disruption of cell walls, facilitating the release of intracellular contents into the liquid medium.

As a result of these processes, valuable compounds such as proteins, lipids, and other bioactive metabolites are transferred from the inside of the cells into the liquid phase, facilitating their subsequent separation during downstream processing steps. Therefore, acoustic cavitation provides an efficient, rapid, and non-destructive method for extracting biologically active components, making it highly relevant in the context of modern biotechnologies and the food and pharmaceutical industries.

Thus, ultrasound-assisted mycoprotein extraction not only meets current consumer demands for plant-based protein sources but also opens new perspectives for the development of innovative, functional, and sustainable food solutions, [28].



Figure 9. UIP2000hdT ultrasonic homogenizer (2 kW, 20 kHz) for efficient mixing, homogenization, nano-dispersion, nano-emulsification, and extraction, [29]

The UIP2000hdT equipment shown in Figure 9 (20 kHz, 2000 W) is a digital probe-type sonicator designed for liquid processing applications at both laboratory (bench-top) and industrial scales. With high acoustic capacity, this ultrasonic processor is frequently used in various technological applications, including homogenization, emulsification, particle dispersion and micronization, cell lysis, bioactive compound extraction, solid substance dissolution, as well as advanced sonochemical processes such as sonosynthesis and sonocatalysis.

One of the main advantages of the UIP2000hdT is the integration of a complete set of functionalities that allow high-precision control of process parameters. The equipment is equipped with a color touchscreen, remote control interface via browser, automatic data logging system, internal SD card, and the ability to connect temperature and pressure sensors. These advanced features enable real-time monitoring of all essential variables—such as amplitude, sonication duration, temperature, and pressure—and ensure full process traceability through automatic CSV data recording.

By delivering high-intensity ultrasonic waves, the UIP2000hdT can efficiently and reproducibly handle demanding processes. Strict control of operating conditions not only optimizes process yield and consistency but also enhances the adaptability of the equipment for a wide range of applications in the food, pharmaceutical, biotechnological, or chemical sectors. Thus, this advanced sonication system stands out for its operational versatility and reliability under intensive use.

Ultrasound technology is one of the most widely used non-thermal processing technologies for the “green” chemical industry. In general, ultrasonic instruments used in the food industry can be divided into two types: bath-type ultrasound and probe-based ultrasound (Figure 10). Depending on the power-frequency range, ultrasonic instruments can be classified as high-power/low-frequency (20–100 kHz), medium-power/medium-frequency (100 kHz–1 MHz), and low-power/high-frequency (1–100 MHz). Ultrasound technology can also be categorized into six types based on different operating modes: sweep or fixed frequency, pulsed or continuous, multi-frequency or single-frequency, sequential multi-frequency or synchronized multi-frequency, countercurrent circulation or unforced flow, and contact or non-contact technology.

The ultrasonic output causes the surrounding medium to vibrate, transmitting ultrasonic energy to the adjacent material. The effects of ultrasonication on the medium can be divided into thermal, mechanical, and cavitation effects (Figures 10 and 11).

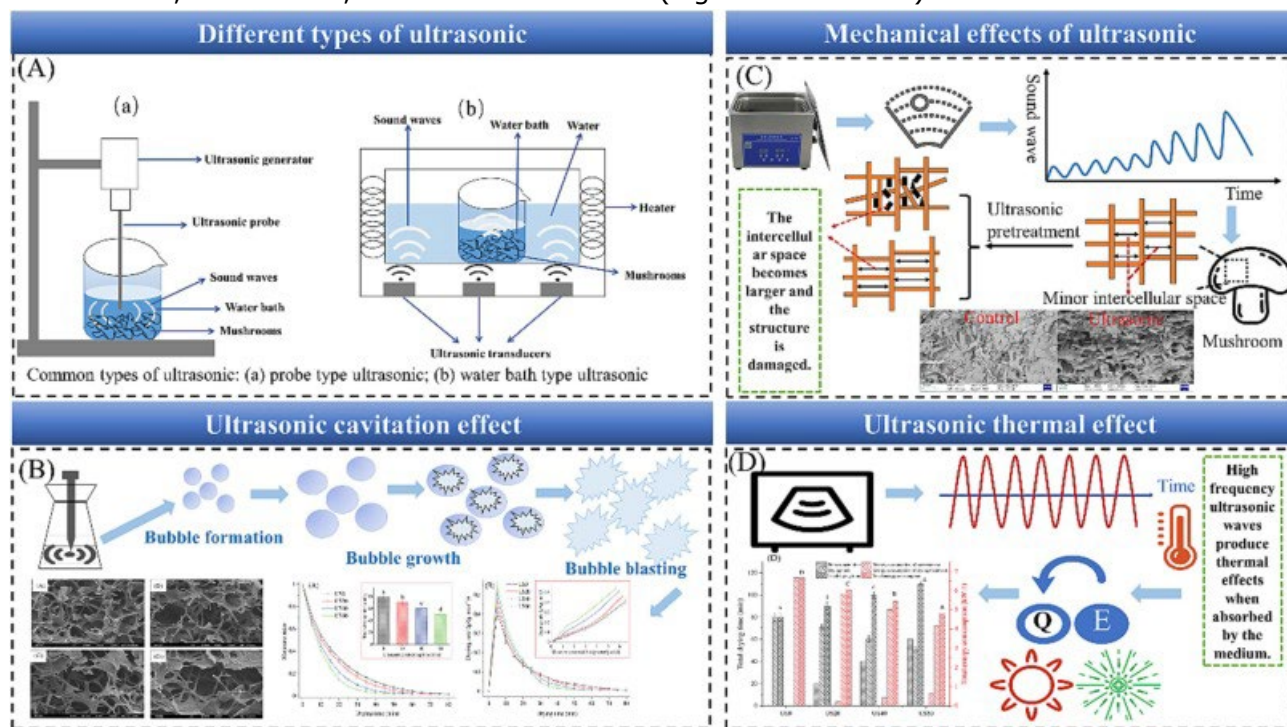


Figure 10. Types of ultrasonic waves and their effects on the medium. (A) Types of ultrasonic waves; (B) ultrasonic cavitation effect; (C) mechanical effects of the ultrasonic wave; (D) ultrasonic thermal effect [30]

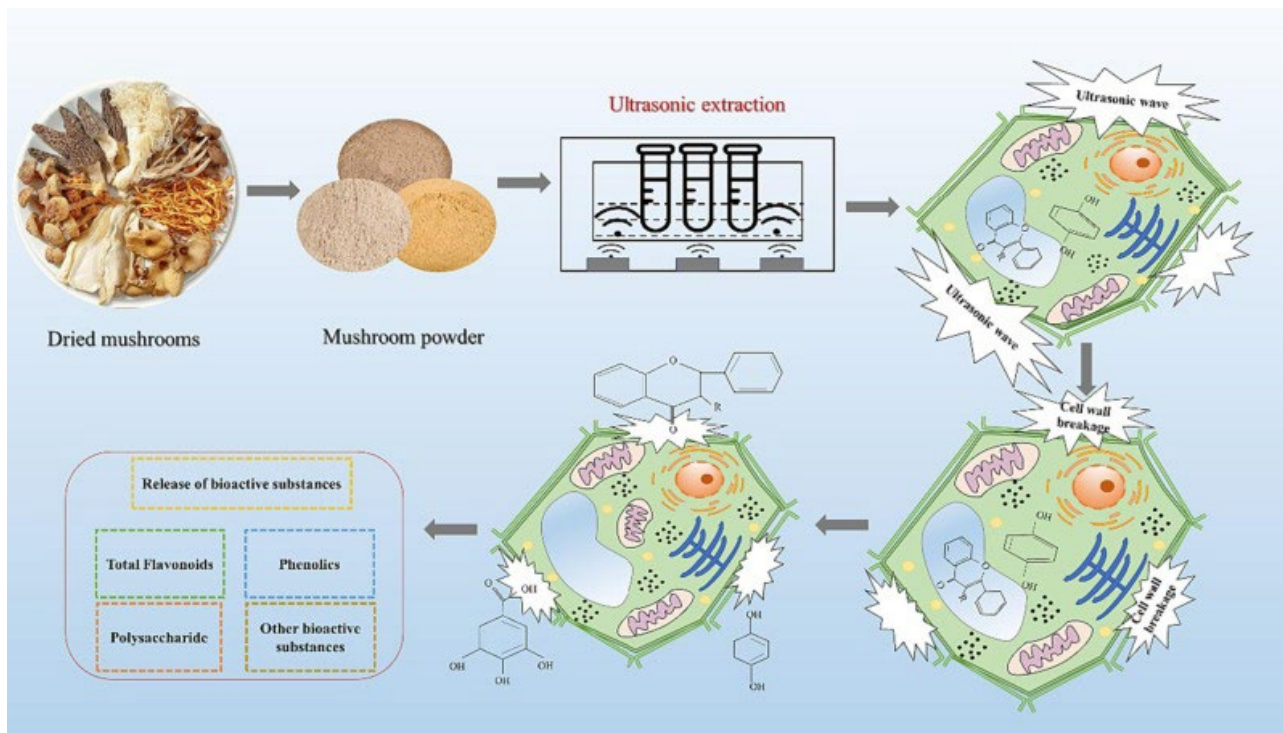


Figure 11. Ultrasound-assisted extraction of bioactive compounds from edible mushrooms [30]

Ultrasonic waves are transmitted through an ultrasonic bath or probe into an aqueous medium, usually distilled water. Their action generates the formation of microbubbles in the liquid, which oscillate, contract, and collapse, a phenomenon known as ultrasonic cavitation. This leads to disruption of the cell walls of plant matrices, weakening of the cell membrane, and more efficient release of bioactive compounds, facilitating the extraction process. At the same time, it contributes to easier moisture removal from products and improves food processing.

Ultrasonic cavitation can be of two types: transient and stable. In transient cavitation, gas or vapor bubbles expand until reaching a critical size, become unstable, and violently collapse. In stable cavitation, bubbles oscillate continuously, maintaining an almost constant size and a relatively stable equilibrium. Typically, both types of cavitation coexist and interact within the ultrasonic field.

Microwave-assisted extraction (MAE) – is based on the rapid and uniform heating of the biological material, leading to the rupture of cell membranes and the release of protein content. It is a fast, efficient, and easily automated method, but requires precise calibration to avoid protein degradation.

Microwave radiation, within the electromagnetic wave spectrum, is an emerging technology used in extraction processes due to its ability to generate high temperatures and pressures within a very short time. This technological approach significantly reduces the duration of the extraction process while increasing the efficiency and purity of the extracted compounds. Furthermore, the use of microwaves reduces the consumption of chemical solvents, thereby significantly lowering the environmental impact, which is why this method is classified as a green or clean technology with broad applicability in the food industry. Microwave heating is distinguished by high energy efficiency, potentially leading to energy savings of up to 50% compared to conventional heating methods. This technology operates within the frequency range of 300 MHz–300 GHz and uses a magnetron to generate alternating electric fields that induce uniform volumetric heating in the treated materials. Rapid internal heating promotes high vapor pressures, which alter cellular structures and facilitate the diffusion of intracellular compounds, thereby reducing extraction time and helping preserve the integrity and quality of the obtained substances.

In Figure 12, the extraction yields of crude liquid extracts of SGM (*Schizophyllum commune*, commonly known as split-gill mushrooms, a well-known edible mushroom valued for its nutritional properties and as a source of therapeutic foods that may help prevent diseases including cancer, hypertension, and hypercholesterolemia, as well as non-communicable diseases) obtained through microwave-assisted extraction (MAE) are presented as a function of temperature and treatment duration. The yield values ranged between 16.76% and 34.18%, calculated based on the wet mass of the material used. Analysis of the results shows a decreasing trend in extraction yield with increasing values of both operational parameters—temperature and microwave exposure time ,[31, 32].

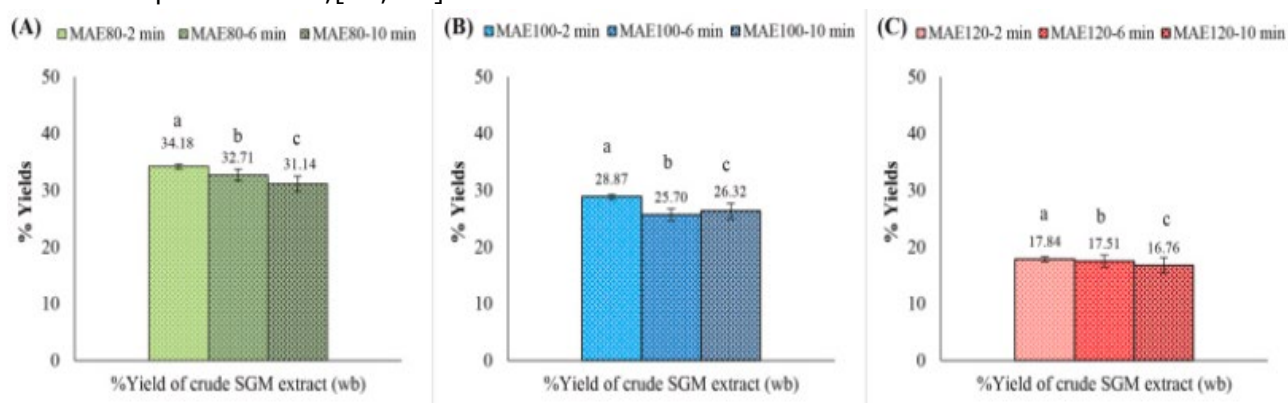


Figure 12. Yield of crude SGM extract from MAE at 80 °C (A), 100 °C (B), and 120 °C (C) [32]

The maximum yield was obtained under treatment at 80 °C for a short duration of 2 minutes, suggesting that moderate energy input and limited processing time favor efficient release of soluble compounds without affecting their integrity. In contrast, the minimum yield was observed at 120 °C after 10 minutes of exposure, indicating possible thermal degradation of active compounds or their transformation into less extractable forms. This behavior aligns with literature reports, which emphasize that intense thermal treatments may cause denaturation or volatilization of bioactive constituents, thereby reducing the overall extraction efficiency. The results indicate that optimizing MAE process parameters is essential to maximize yield while maintaining extract quality. A balance between thermal intensity and exposure time is necessary to achieve an optimal compromise between efficiency, chemical stability, and energy costs [33].

Enzyme-assisted protein extraction from mushrooms relies on the use of specific enzymes that degrade the complex cell wall matrix, releasing intracellular proteins without denaturing them. Mushroom cell walls are primarily composed of chitin, β -glucans, and glycoproteins, providing a rigid structure that limits extraction by simple physicochemical methods. The enzymatic method is adaptable and selective, allowing protein extraction while preserving biological activity and biochemical properties.

Biomass preparation is a crucial step. Harvested mushrooms are initially cleaned and dried either by lyophilization or at low temperatures (≤ 40 °C) to prevent protein denaturation. The biomass is then finely ground to particle sizes of 0.5–1 mm to increase surface area for enzymatic contact and facilitate enzyme penetration. Depending on cell wall density, mechanical or hydration pretreatment may be applied: hydration with water or buffer at neutral pH for 30–60 minutes helps soften the tissue and optimize enzyme access.

The enzyme solution is prepared in a buffered medium with an optimal pH for each enzyme type: Chitinases: pH 5–6, maximum activity at 35–40 °C

Cellulases: pH 5–6, optimal temperature 40–50 °C

Proteases: pH 6–7, optimal temperature 37–45 °C

Enzyme concentration typically ranges between 0.5–2% (w/w relative to dry biomass), with a biomass-to-solution ratio of approximately 1:10 to 1:20 (g/ml), adjustable based on

protein density and mushroom type. Extraction occurs in stirred tanks or continuously agitated reactors to ensure uniform contact between enzymes and ground biomass. Reaction duration is controlled between 1 and 6 hours, depending on mushroom type and the desired degree of cell wall degradation.

During the reaction, enzymes gradually hydrolyze the polysaccharide and protein structures of the cell walls, releasing intracellular proteins into a clear aqueous solution. Monitoring parameters is critical: pH must be periodically adjusted to maintain enzyme activity, and temperature must be stable to prevent protein denaturation. At the end of the reaction, enzymes are inactivated by brief exposure to moderate temperatures (50–60 °C for 5–10 minutes) or by pH adjustment. The resulting protein solution is separated from solid residues by centrifugation at 4000–6000 × g for 10–20 minutes or by membrane filtration, yielding a high-content protein extract with intact biological activity.

To maximize yield, multiple extraction steps can be applied: residual biomass can be re-suspended in fresh enzyme solution and processed again, allowing recovery of remaining proteins and increasing overall efficiency. This method enables extraction of high-purity proteins while preserving bioactive properties, making it ideal for functional food, nutritional supplements, and pharmaceutical applications. Proteins can subsequently be concentrated and purified using membrane techniques (ultrafiltration, nanofiltration) and liquid chromatography to obtain specific protein fractions that meet industrial quality standards.

Supercritical fluid extraction (SFE) of mushroom proteins is an advanced method that leverages the properties of carbon dioxide in its supercritical state, which combines the density of a liquid with the viscosity and diffusivity of a gas. In this state, CO₂ penetrates dense cellular structures, disrupting interactions between proteins and polysaccharides without denaturing the proteins. Process parameters are critical for efficiency: temperature is usually maintained between 35–40 °C for heat-sensitive proteins, and pressure ranges from 150–350 bar depending on mushroom type and protein composition. Since pure CO₂ has limited protein solubility, polar co-solvents, typically 5–15% ethanol or hydroalcoholic mixtures, are used to enhance protein extraction while maintaining biological activity.

Mushroom material undergoes complex pretreatment. Lyophilization is preferred to preserve protein integrity and prevent endogenous enzymatic degradation. The dried biomass is ground to a uniform size of 0.5–1 mm to facilitate penetration of supercritical CO₂. In some cases, preliminary mechanical or enzymatic treatments are applied to reduce cell wall rigidity, facilitating extraction. Actual extraction can be performed in static mode with contact times of 1–4 hours or in dynamic mode with a continuous flow of supercritical CO₂, adapted for large process volumes of 50–500 liters in industrial extractor equipment. During extraction, CO₂ penetrates the cellular structure, dissolves hydrophobic compounds and proteins, and gradual depressurization separates the protein solution from the gas efficiently, without leaving chemical residues. The added co-solvent is subsequently removed by vacuum evaporation, and the resulting protein solution can be directly concentrated using membrane techniques or prepared for chromatography. This method stands out for high efficiency, protein structure preservation, and minimal environmental impact.

Enzymatic extraction is a complementary and increasingly used step for releasing sensitive proteins. Mushroom cell walls are composed of chitin, β-glucans, glycoproteins, and complex polysaccharides, conferring rigidity and mechanical resistance. Specific enzyme mixtures are used: chitinases degrade the chitin network, cellulases act on associated polysaccharides, and proteases aid protein release by breaking matrix bonds. The process occurs in buffered media, with optimal pH for each enzyme—typically 5–6 for chitinases and cellulases, and 6.5–7.5 for proteases. Temperature is controlled between 30–50 °C, and reaction duration can be adjusted from 1–6 hours depending on mushroom type, biomass fragmentation, and enzyme concentration.

After enzymatic digestion, enzymes are inactivated by pH adjustment or mild heating, and the mixture is separated by centrifugation at 4000–6000 × g or 0.45 μm membrane filtration, yielding clear protein solutions. This method allows protein extraction without denaturation, preserving native structure and biological activity, which is crucial for functional food or pharmaceutical applications.

Protein purification is carried out using sequential membrane and chromatographic techniques. Ultrafiltration employs membranes with pore sizes of 3–100 kDa, retaining proteins and removing smaller molecules, peptides, and polyphenols. Concentration can be adjusted to obtain protein solutions with 5–20% protein content, ready for subsequent processing. Nanofiltration, with finer pores, removes mineral salts and short peptides, ensuring uniform protein fractions. Liquid chromatography is chosen based on protein properties: ion-exchange chromatography separates proteins by charge, gel-exclusion chromatography by molecular weight, and affinity chromatography isolates target proteins with high specificity. Combining these methods allows obtaining proteins with >95% purity while preserving structure and biological activity.

In industrial practice, the technological workflow combines the described methods in an optimized sequence. Biomass may initially undergo enzymatic treatment, followed by supercritical CO₂ extraction for degreasing and removal of hydrophobic compounds. The resulting crude protein solution is concentrated by ultrafiltration and nanofiltration, and liquid chromatography completes purification, with parameters adapted to mushroom type and final application. Equipment allows process scaling from several hundred liters at pilot scale to over 1,000 liters in industrial production. Control of parameters and monitoring of pH, pressure, temperature, and flow ensure reproducibility and consistent protein quality.

This integrated approach enables obtaining edible mushroom proteins with intact biological activity, applicable in functional food formulations, nutritional supplements, bioactive proteins, or pharmaceutical ingredients. Proper selection of process steps and optimization of conditions ensures a balance between yield, purity, and sustainability without compromising biochemical properties. Integration of enzymatic, supercritical, and membrane/chromatographic methods creates a complete, efficient, and adaptable workflow capable of meeting the requirements of contemporary food and biotechnological industries.

These modern methods are not mutually exclusive but can be integrated into complex technological sequences (combined processes) to maximize efficiency and final product quality. Optimal choice depends on extraction purpose, mushroom type (e.g., *Pleurotus ostreatus*, *Agaricus bisporus*, *Ganoderma lucidum*), processed quantity, and associated costs.

3. Results

The differences between conventional and modern methods for extracting proteins from mushrooms extend beyond mere technical efficiency. In reality, evaluating an extraction technology involves a complex analysis that considers several critical factors, such as environmental impact, process sustainability, energy consumption, ease of implementation, and compatibility of the final product with subsequent applications. Modern methods, although requiring higher initial investments, offer considerable long-term advantages. They allow the production of higher-quality protein extracts, increase food safety, and comply with increasingly strict international standards. This approach results in an optimal balance between efficiency and ecological responsibility, which is essential in an industrial context where the demand for functional foods and bioactive ingredients is steadily rising.

The extraction process is influenced by numerous factors that act both before and during processing. First, the type of mushroom and its biochemical composition play a decisive role. Each species has unique characteristics that affect protein behavior during extraction. For example, mushrooms of the *Pleurotus* genus have relatively thin cell walls and medium-to-high protein content, which facilitates extraction by gentle methods, including solvents or ultrasound. In contrast, species such as *Ganoderma lucidum*, rich in polysaccharides and triterpenes,

possess much more robust cellular structures, requiring more aggressive procedures to release proteins without compromising their integrity. Additionally, some mushrooms contain proteins with specific biological functions, such as lectins, enzymes, or immunomodulators, which require delicate extraction conditions to preserve their activity.

The state of the raw material is another essential factor. Fresh mushrooms, with 85–90% water content, allow easier extraction, particularly through enzymatic or ultrasound-assisted methods, but dilute the protein fraction. Dried material concentrates proteins but requires rehydration or pre-treatments to allow effective solvent penetration into the cellular structure. The physical form of the biomass—whether slices, paste, or finely ground powder obtained through cryogrinding or spray drying—affects solvent contact and process efficiency. Fine powders provide a larger surface area, accelerating extraction and improving protein yield.

Physicochemical parameters of the extraction medium, such as pH and temperature, determine protein solubilization and stability. Most fungal proteins are soluble at neutral or slightly alkaline pH, but certain classes require acidic media or controlled pH variations to be efficiently released from cells. Temperature must also be carefully managed: high temperatures accelerate extraction but may cause irreversible protein denaturation and loss of functional activity. Modern technologies often operate at moderate temperatures (20–50 °C) or even near-isothermal conditions, as in enzymatic or supercritical fluid extraction, to protect protein structure and preserve their biological value.

Process duration and biomass-to-solvent ratio are balancing factors. Extraction must be long enough to release proteins completely, yet not expose sensitive fractions to excessive time in solution. Ultrasound- or microwave-assisted methods reduce processing time to a few minutes, compared with hours required by conventional procedures. Simultaneously, the ratio of fungal biomass to solvent influences yield: insufficient solvent limits extraction, while excess dilutes the extract unnecessarily, complicating downstream purification.

The presence of inhibitors or interfering compounds in mushrooms can negatively affect protein extraction or purification. Polysaccharides, pigments such as melanin, or lipids may contaminate protein fractions or form complexes that are difficult to separate. To obtain a pure and stable protein extract, pretreatment steps such as defatting, decolorization, or enzymatic digestion are necessary to remove interferences and ensure final product quality.

Emerging technologies are revolutionizing protein extraction from edible mushrooms, responding to the growing demand for alternative, sustainable, and nutritious protein sources. These technologies allow the production of protein extracts with superior yield while maintaining nutrient integrity and functional properties. A major advantage is their direct integration into industrial food chains, enabling the production of safe and easy-to-use products such as protein bars, vegan supplements, plant-based burgers, or fortified pasta. Extracts obtained from *Lentinula edodes* or *Pleurotus eryngii*, lyophilized and transformed into soluble powders, can be easily incorporated into diverse food formulations.

Biotechnology and advanced analytical techniques play a critical role in process optimization: selecting high-protein-yield strains, adjusting cultivation conditions, and detailed protein characterization using mass spectrometry, liquid chromatography, or proteomics allow the development of precise protein profiles and identification of bioactive peptides with health benefits, such as reducing oxidative stress, regulating blood glucose, or stimulating immunity.

Technological innovations have already opened significant commercial opportunities. The food industry utilizes proteins from edible mushrooms in products such as mycoproteins as meat substitutes, vegan protein powders, functional peptide supplements, and clean-label ingredients for fortifying foods targeted at athletes or the elderly. Medium- and long-term prospects indicate a continuous growth in demand, driven by plant-based trends, sustainability concerns, and increasingly strict regulations regarding artificial additives.

Modern methods for extracting proteins from edible mushrooms have evolved significantly from traditional techniques, offering more efficient, sustainable solutions capable of

preserving protein functional properties. At the core of these methods are physically or enzymatically assisted technologies, enabling the production of protein extracts with higher yield and superior purity. One of the most widely used techniques is ultrasound-assisted extraction, which uses high-frequency vibrations to generate cavitation in the extraction medium, leading to cell wall disruption and rapid protein release. The advantage of this method lies in reducing extraction time and solvent consumption while maintaining protein structure and activity.

Another modern technology is microwave-assisted extraction, which uses electromagnetic energy to rapidly and uniformly heat fungal biomass. Microwaves induce intracellular water expansion and rupture the cell structure, facilitating protein solubilization. This method is notable for its high energy efficiency, reduced process duration, and ability to preserve protein bioactive properties.

Enzymatic extraction is a gentle but highly precise approach, in which proteins are released through enzymes that digest cell walls or polysaccharide-protein complexes. This method is particularly suitable for polysaccharide-rich mushrooms or those with tough cell walls, ensuring the extraction of unaltered, functional proteins ideal for use in food products and nutritional supplements.

In parallel, supercritical fluid extraction, e.g., with supercritical CO₂, enables the production of highly pure proteins without toxic organic solvents. This method allows precise control of pressure and temperature, facilitating the selection of desired protein fractions and preserving protein biological integrity.

Analysis of these technologies clearly shows that each method has advantages and limitations, and the choice of a single method or a combination depends on mushroom species, extract purpose, and final applications. However, all modern methods provide a major advantage over conventional techniques: they increase protein yield, reduce resource consumption, and enhance final product quality, making them essential in the food industry and in meeting growing demand for functional ingredients.

4. Conclusions

Protein extraction from edible mushrooms is no longer merely a laboratory procedure but an integral component of developing modern, sustainable, and functional foods. Ultrasound-, microwave-, enzyme-, and supercritical fluid-assisted methods enable the full exploitation of the nutritional potential of mushrooms, ensuring the production of high-quality proteins with diverse applications in food, supplements, and bioactive products. This technological advancement not only meets market demands but also opens new avenues for innovation in the field of safe, sustainable alternative protein foods, reinforcing the role of edible mushrooms as a valuable source of protein in the human diet.

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CONSIDERATIONS REGARDING THE BEHAVIOR OF SOME TYPES OF BIOMASS IN THE PYROLYSIS PROCESS ENERGY RECOVERY

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Abstract

Biomass is mainly represented by plant materials (agricultural crops, agricultural waste, organic residues, forest and garden waste), but also by other types of organic materials, such as animal droppings, waste from the food industry or that of the pulp and paper industry, solid municipal waste or sewage sludge.

Among the biomass utilization methods and technologies are combustion, gasification and pyrolysis.

This article presents the main types of biomass, their classification and the most important methods and technologies for energy recovery.

1. Introduction

Biomass encompasses the totality of living or recently living organic material derived from plants, animals, microorganisms, and aquatic organisms. It includes matter produced through biological growth as well as materials collected from terrestrial and aquatic environments. The concept also covers agricultural products and residues generated during crop cultivation and post-harvest processing, such as cereal straw and by-products of starch, sugar, and beer production [1].

Biomass valorization is increasingly regarded as a climate-neutral strategy, since the carbon dioxide generated during energy conversion can be offset through photosynthetic fixation during biomass growth cycles [2]. Conversion into usable energy is achieved through multiple technological routes, each characterized by specific operational conditions, including temperature, pressure, residence time, and, for biochemical processes, specialized microbial communities [3].

The main technological pathways for biomass energy recovery include:
A. *Combustion*, ensuring direct conversion of biomass into heat and subsequently electricity;
B. *Biological (biochemical) conversion*, where anaerobic digestion and fermentation produce gaseous or liquid biofuels;
C. *Thermochemical conversion*, encompassing pyrolysis, gasification and other high-temperature processes that generate biochar, bio-oil and synthesis gases [3,4].

Among these, combustion remains the most widely implemented technology due to its compatibility with a broad range of solid biofuels. However, direct combustion releases substantial quantities of CO₂ and associated pollutants that intensify the greenhouse effect [4].

To improve efficiency and handling, biomass is often densified into pellets, briquettes or other compact forms, increasing energy density while reducing storage and transport costs. Densified biofuels are extensively used in conventional thermal power facilities and combined heat-and-power plants designed for industrial or community-level energy supply [5].

Biomass is gaining strategic relevance due to its capacity to generate renewable energy carriers, reduce dependency on fossil fuels, and promote circular carbon flows in modern

economies. Integration of bioenergy into diversified energy systems supports greenhouse-gas mitigation and enhances long-term sustainability [6,7]. Recent technological advances in thermochemical and biochemical conversion have established biomass as a key component of regional and global renewable-energy portfolios [8,9].

Different biomass categories exhibit distinct energy potentials depending on their biochemical composition. Woody biomass, rich in lignin, provides high-quality solid fuels with elevated calorific value following combustion or pyrolysis [10]. Agricultural residues and herbaceous materials—although less energy-dense—can be effectively transformed into gaseous or liquid biofuels through anaerobic digestion or thermochemical conversion [11,12].

Wet biomass, including manure and sewage sludge, is especially suitable for anaerobic digestion due to its high biodegradability and nutrient content [13]. Similarly, biomass rich in fermentable sugars or starches, such as sugar crops and cereal residues, provides effective substrates for bioethanol production through fermentation [14,15]. Lignocellulosic biomass, abundant but recalcitrant, remains a major research focus due to the necessity of pretreatment for efficient conversion [16,17].

2. Materials and Methods

Biomass can also originate from isolated microbial cells such as yeasts, molds, and bacteria, which are cultivated within agro-industrial systems for protein extraction intended for human and animal nutrition, or for producing pharmaceutical compounds including antibiotics [18]. The broader concept of biomass therefore includes a heterogeneous array of organic materials, categorized as:

- *plant biomass (phytomass)*, derived from terrestrial and aquatic vegetation;
- *animal biomass*, originating from livestock and other organic waste streams;
- *microbial biomass*, generated through industrial cultivation;
- *aquatic biomass*, such as algae and marine crops [18].

Within plant biomass, three subcategories are commonly distinguished: epigeal biomass, comprising above-ground plant tissues; hypogeal biomass, consisting of roots and subterranean structures; and woody biomass, representing lignified plant material often used as a primary energy feedstock [18].

Biomass is widely acknowledged as a promising renewable resource due to its capacity to enhance energy security, replace fossil-derived liquid fuels, and reduce greenhouse-gas emissions [19,2].

Analytical and Experimental Methodologies

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis is extensively used to evaluate thermal degradation behavior, mass-loss kinetics, and the influence of temperature and heating rate during biomass conversion. TGA quantifies volatile release, fixed-carbon fraction, and overall energetic potential by continuously monitoring mass change under controlled thermal conditions [20,21].

Studies employing TGA have demonstrated its usefulness for determining how the structural variability of lignocellulosic biomass affects char yield, reaction pathways, and thermal stability [22].

Reactor-Based Pyrolysis Methodologies

Experimental reactors that simulate industrial pyrolysis at laboratory scale enable systematic investigation of slow and fast pyrolysis regimes. These systems allow controlled variation of particle size, residence time, heating rate, and atmosphere composition to analyze their effects on char, liquid, and gas yields [23,24].

Comparative studies consistently show that slow pyrolysis enhances the yield and stability of biochar, whereas fast pyrolysis maximizes liquid bio-oil production [25,26].

Biochemical Conversion Methodologies

Biochemical research focuses primarily on anaerobic digestion and fermentation pathways. Methods include:

- substrate biodegradability assessment;
- compositional analysis of biogas;
- microbial community profiling during digestion processes [27,28].

Pretreatment strategies such as enzymatic hydrolysis, steam explosion, and organosolv processing are widely applied to lignocellulosic biomass to enhance carbohydrate accessibility and improve bioethanol yields by facilitating sugar release from structural polymers [29,30].

Technological Categories for Energy Recovery:

The main technological pathways for converting biomass into usable energy include:

- A. *Combustion*, providing direct transformation of biomass into heat and, subsequently, electricity;
- B. *Biological (biochemical) conversion*, including anaerobic digestion and fermentation for gaseous and liquid biofuel production;
- C. *Thermochemical conversion*, comprising pyrolysis, gasification, and related high-temperature processes that yield biochar, syngas, and bio-oil [2,3].

A. *Combustion*

Combustion remains the most extensively implemented technology for heat and power generation due to its compatibility with a broad spectrum of solid biofuels. Its major limitation lies in the substantial CO₂ emissions and associated pollutants released during direct oxidation [3].

To enhance energy density, decrease storage volume, and improve operational efficiency, biomass is frequently densified into pellets, briquettes, and other compacted forms. These improved fuels are widely used in conventional thermal power plants and cogeneration systems supplying industrial or residential energy demands [4].

Methods And Technologies For Biomass Energy Recovery

In an ideal framework, the energetic valorization of biomass is considered climate-neutral, as the CO₂ emitted during conversion processes is theoretically reabsorbed by plants through photosynthesis during their growth cycle [2]. Biomass may be transformed into usable energy through multiple technological pathways, each characterized by distinct operational parameters such as temperature, pressure, residence time, and, in biochemical processes, specific microbial communities [3].

The principal technological categories for biomass energy recovery include:

- A. *Combustion* – direct conversion of biomass into heat and subsequently electricity;
- B. *Biological (biochemical) conversion* – anaerobic digestion and fermentation, producing gaseous or liquid biofuels;
- C. *Thermochemical conversion* – pyrolysis, gasification, and other high-temperature processes that yield biochar, bio-oil, and syngas [3,4].

A. *Combustion*

The combustion process is the most widely applied method for generating thermal and electrical energy due to its compatibility with a broad range of solid biofuels [3]. However, direct combustion releases significant quantities of CO₂ and other pollutants, thus contributing to the intensification of the greenhouse effect [4].

To address efficiency challenges, biomass is frequently densified into pellets, briquettes, or other compact forms, which improves thermal density and reduces storage and transportation costs. These densified fuels are commonly employed in conventional thermal power plants and cogeneration units designed for heat and electricity production at industrial and community levels [5]. In Figure 1 we can observe different possible sources and the potential for biomass globally.

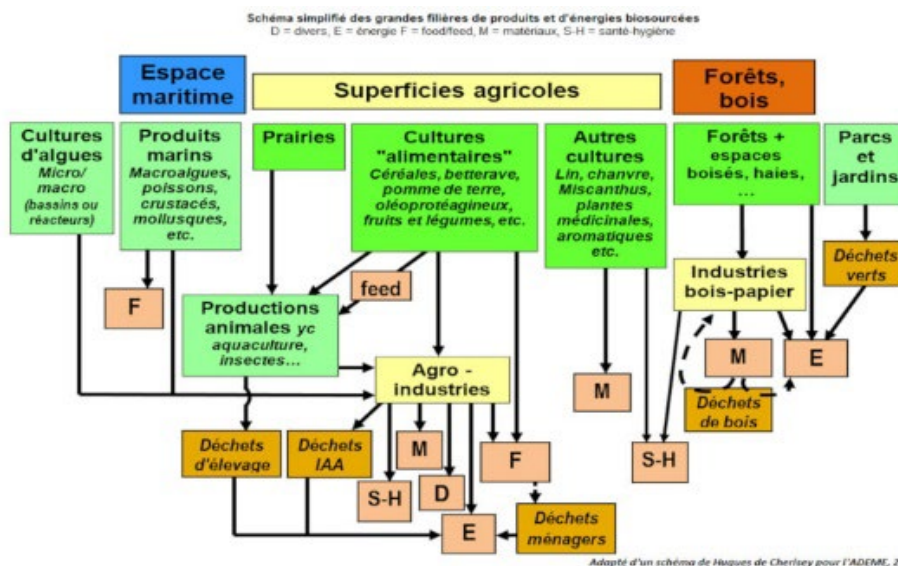


Figure. 1 Global biomass sources and potential [6], <https://www.encyclopedie-energie.org/en/biomass-energy-primary-resources-final-energy-products/>

B. Biological (Biochemical) Conversion

B.1. Anaerobic digestion – Biogas production

Furthermore, pretreatment methods applied to lignocellulosic biomass (e.g., organosolv disruption or steam-based hydrolysis) enhance enzymatic accessibility and accelerate the hydrolysis phase, thereby raising the overall conversion efficiency and stabilizing biogas output.

European deployments highlight that biogas systems provide not only renewable energy but also digestate, a nutrient-rich by-product suitable for agricultural soil amendment, reinforcing the dual role of anaerobic digestion in waste reduction and circular bioeconomy development [34,35,42].

The biochemical conversion of biomass into biogas, commonly referred to as anaerobic digestion, involves the microbial breakdown of organic matter in the absence of oxygen, generating two principal products: biogas and digestate [1].

Biogas typically consists of methane, carbon dioxide, and trace gases such as hydrogen sulfide and nitrogen, while digestate represents a nutrient-rich residue suitable for use as agricultural fertilizer [1].

The block diagram of such plants illustrates a streamlined process in which thermal energy from biomass combustion drives an ORC turbine using an organic working fluid with superior thermodynamic performance at lower boiling points.

Compared with small-scale direct combustion units—typically designed only for heat production—ORC-based cogeneration enables simultaneous generation of heat and electricity with improved overall energy recovery, reduced exhaust losses, and stable operation across variable load conditions. Furthermore, unlike gasification-based combined heat and power systems, ORC plants do not require syngas cleaning stages, which are costly and technically demanding. Their modularity, operational flexibility and capacity to integrate diverse biomass fuels have contributed to increased adoption in European cogeneration projects, where enhanced efficiency and lower emissions are essential performance indicators [38]. In figure 2 is presented the block diagram of biomass fired cogeneration plant with ORC unit.

Anaerobic digestion is adaptable to a broad spectrum of biodegradable feedstocks, including agricultural residues, agro-industrial by-products, and sewage sludge. This versatility allows the conversion of organic waste streams into renewable gaseous fuel, supporting circular bioeconomy strategies and reducing environmental impact [2].

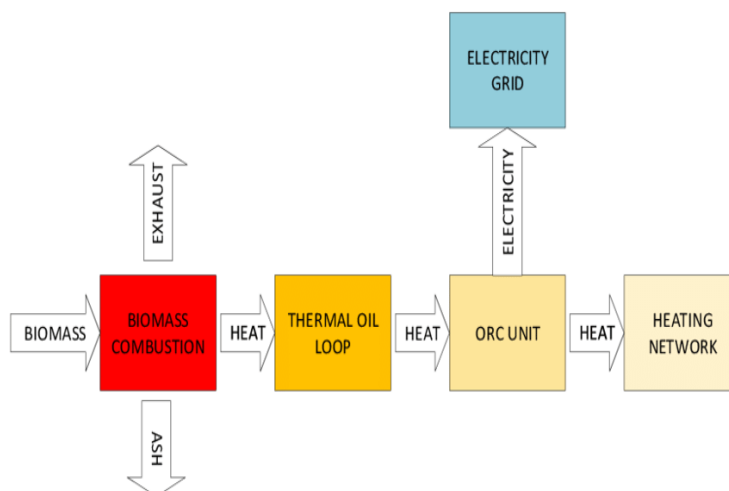


Figure 2. Block diagram of a biomass-fired cogeneration plant with ORC unit [5], DOI: 10.1051/e3sconf/20198201008

Widespread adoption in Europe reflects the technology’s favourable economic profile, supported by subsidy mechanisms and low operational costs. Numerous installations ranging from farm-level to large-scale industrial plants demonstrate the long-term feasibility and strategic value of biogas production [3]. Figure 3 shows the technological flow with the most important components of a biogas plant.

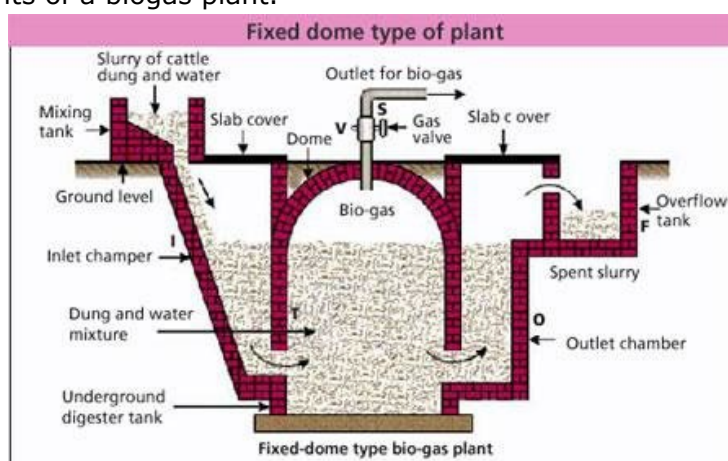


Figure 3. Technological flow of a biogas plant [7], <https://www.indiamart.com/proddetail/biogas-plants-13823968912.html>

B.2. Bioethanol production through fermentation

The biochemical conversion of biomass into bioethanol is primarily based on the microbial fermentation of carbohydrate-rich substrates, followed by distillation and dehydration to obtain fuel-grade ethanol [1].

Bioethanol production depends on feedstock composition. Three major categories are employed:

1. *Directly fermentable sugars* – sugar beet, sugar cane, sweet sorghum, fructose syrups, whey;
2. *Starchy materials* – maize, wheat, rice, potatoes, rye, which require enzymatic hydrolysis to release fermentable sugars [2];
3. *Lignocellulosic biomass* – agricultural and forestry residues, which require advanced pretreatment (steam explosion, organosolv, enzymatic hydrolysis) before fermentation [2,3].

In Figure 4 we can observe The diagram of the process of transforming biomass into bio-ethanol.

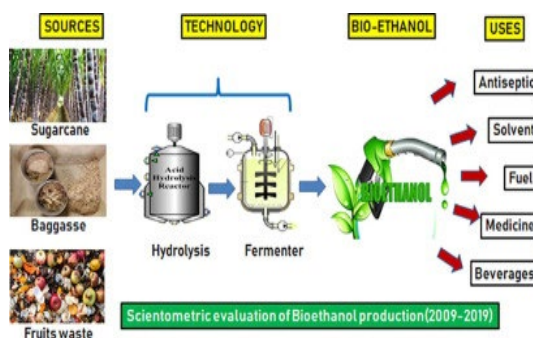


Figure 4. Biomass conversion to bioethanol [8], DOI: 10.1016/j.fuel.2022.123448

C. Thermochemical Conversion

Energy recovery through thermochemical processes involves transforming biomass via high-temperature reactions such as combustion, pyrolysis, and gasification. These methods convert biomass into heat, syngas, bio-oil, or solid char depending on operating parameters [9].

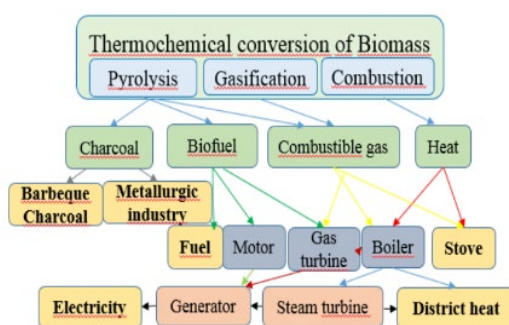


Figure 5. Thermochemical conversion pathways for biomass [10], <https://www.researchgate.net/publication/308128062>

3. Results

Recent research highlights the importance of thermal and physicochemical analyses in optimizing biomass conversion processes. These studies integrate thermogravimetric assessment, mathematical modeling, and kinetic interpretation to identify the main transformation stages in biomass pyrolysis, which occur sequentially as drying, torrefaction, devolatilization, carbonization, and gasification [31,32]. Each stage is defined by characteristic temperature thresholds and reaction mechanisms that influence the yield and quality of derived products such as biochar, bio-oil, and syngas.

Experimental work consistently shows that temperature and heating rate are the most influential parameters shaping product distribution. Slow pyrolysis conducted at ≤ 500 °C enhances biochar output, particularly when residence time and particle size are appropriately controlled [33]. In contrast, fast pyrolysis under high heating rates promotes bio-oil formation, while gasification above 800 °C yields syngas enriched in CO and H₂ — a trend confirmed across numerous lignocellulosic feedstocks including energetic willow, straw, and sorghum residues [34].

Biomass composition significantly influences calorific performance and conversion behavior. High-lignin feedstocks such as bamboo or woody biomass produce more carbonized and energetically dense biochar, whereas herbaceous materials exhibit more rapid devolatilization and generate higher fractions of liquid and gaseous products at lower temperatures [35,36]. Studies examining municipal solid waste pyrolysis indicate that low-temperature processing can still yield viable solid fuels, although calorific properties depend strongly on the organic fraction composition [37].

Biochemical conversion pathways demonstrate similar dependence on feedstock characteristics. Anaerobic digestion of agricultural residues — especially mixtures of manure and

crop waste — yields higher methane concentrations due to improved nutrient balance and synergistic microbial activity [38]. For bioethanol production, pretreatments such as organosolv processing and steam explosion significantly enhance enzymatic digestibility and sugar release, thereby improving fermentation efficiency and final ethanol yields [39,40].

The comparative calorific analysis of selected biomass feedstocks (Fig. 6) reveals significant variability in heating performance associated with lignocellulosic structure and chemical composition. Woody biomass and bamboo demonstrate the highest calorific values, a pattern attributed to their elevated lignin content and enhanced carbon stability during thermal decomposition. Energetic willow exhibits intermediate energy potential, consistent with its balanced proportions of lignin, cellulose and hemicellulose. In contrast, wheat straw and sorghum residues display lower heating values, largely due to reduced lignin fractions and greater volatile content, which shift conversion pathways toward increased liquid and gaseous yields. These trends are consistent with previous findings indicating that high-lignin biomasses yield more carbon-dense solid fuels, while herbaceous residues primarily promote devolatilization and gas evolution under similar pyrolysis conditions [20,22,37].

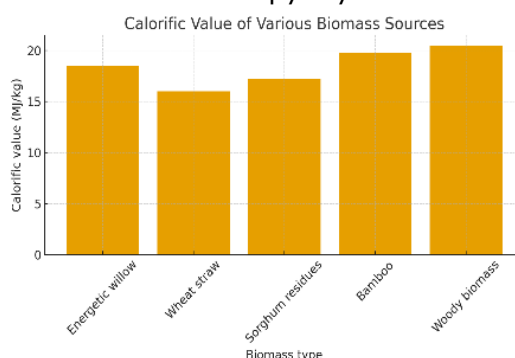


Figure 6. Calorific Value of Various Biomass Sources

The compositional profiles of energetic willow, straw, sorghum residues, bamboo and woody biomass (Fig. 7) illustrate fundamental structural differences that govern their thermochemical behaviors. High-lignin feedstocks such as woody biomass and bamboo are associated with greater char stability and elevated calorific performance, confirming their suitability for solid-fuel-oriented pyrolysis processes. Energetic willow, with moderate lignin and substantial cellulose fractions, maintains versatility across both biochar- and bio-oil-focused applications. By contrast, wheat straw and sorghum residues contain lower lignin and higher cellulose-hemicellulose ratios, characteristics that enhance devolatilization kinetics and favor greater liquid and gaseous product formation. These compositional distinctions underscore the necessity of matching biomass feedstock selection with targeted conversion pathways to optimize energy yields and product quality [20,22,34,37].

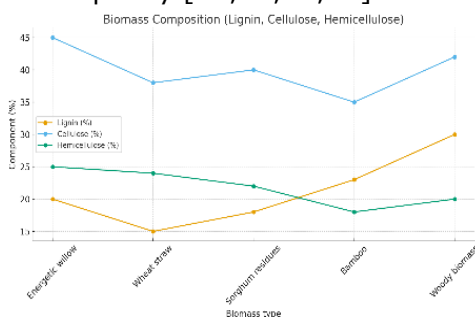


Figure 7. Biomass composition (Lignin, Cellulose, Hemicellulose)

4. Conclusions

Establishing optimal biomass valorisation strategies requires integrating feedstock characteristics, conversion pathways, and technological parameters. Thermochemical processes

demonstrate that maximizing energy recovery depends on maintaining controlled temperature intervals, suitable heating rates, and appropriate residence times, all of which govern product distribution and biochar quality [31,33]. From an economic perspective, the viability of pyrolysis systems relies on the calorific performance of the resulting char and on accurate assessments of operational costs and market conditions for energy recovery [41].

Comparative analyses across numerous studies reveal that biomass sources differ substantially in conversion performance. Woody biomass consistently yields high-quality char with elevated carbon content but requires longer processing times, whereas agricultural and herbaceous residues devolatilize more rapidly yet produce larger fractions of condensable liquids and syngas, with variable calorific value [36,37].

Agricultural residues and herbaceous biomass offer widespread availability and rapid thermal decomposition but often yield higher ash content, variable heating values and increased tar formation in thermochemical applications. Wet biomass streams, including manure and sewage sludge, are well-suited for anaerobic digestion due to their high biodegradability, yet their low energy density and high water content limit direct thermochemical use without costly drying and pretreatment.

Lignocellulosic materials remain abundant and economically attractive but require complex pretreatment steps prior to fermentation or biochemical conversion, increasing operational costs. These comparative insights highlight that biomass valorisation strategies must align feedstock properties with appropriate technological pathways to ensure optimal energy recovery and environmental performance [20,22,34,37].

Biochemical pathways provide environmentally advantageous routes for biofuel generation, though their efficiency depends on feedstock biodegradability, pretreatment intensity, and microbial community composition. Anaerobic digestion performs best when nutrient ratios are balanced, as demonstrated in mixed-feed systems, while pretreatment-enhanced lignocellulosic ethanol production remains essential for overcoming biomass recalcitrance [38,39].

Emerging research highlights the potential of integrated or hybrid systems, combining anaerobic digestion with pyrolysis or coupling thermochemical processes with advanced pretreatment techniques. These hybrid approaches maximize whole-biomass utilization, reduce waste fractions, and enhance final energy yields, presenting promising directions for the development of circular, carbon-neutral energy systems [40].

Future research priorities include:

- developing industrial prototypes capable of processing heterogeneous biomass streams under optimized operational parameters;
- expanding techno-economic assessments for biochar, bio-oil, and syngas markets to support scalability;
- improving understanding of microstructural and chemical transformations in biomass to predict product reactivity and yield;
- advancing integrated conversion platforms that combine thermochemical and biochemical pathways to improve overall process efficiency and environmental performance.

These research directions align with global sustainability objectives and reinforce the role of biomass as a competitive contributor to renewable-energy portfolios and circular economy development [41].

Acknowledgement

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Review

AGRICULTURAL TECHNOLOGICAL INNOVATION AND ITS IMPACT ON RURAL DEVELOPMENT IN ROMANIA: A STUDY ON THE TRACTOR AND AGRICULTURAL MACHINERY FLEET BETWEEN 2020 AND 2024

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Keywords: *agricultural mechanization, tractors, agricultural machinery, development regions, Romanian agriculture, thematic maps, rural technologies*

Abstract: This paper analyzes the distribution and dynamics of Romania's tractor and agricultural machinery fleet between 2020 and 2024, focusing on regional disparities across the eight development regions. Based on official statistical data from the National Institute of Statistics (code TEMPO_AGR103A), supplemented by relevant literature, the study highlights major technological trends, challenges, and mechanization levels. Using Microsoft Excel 365, thematic maps and comparative analyses were created to assess regional agricultural equipment distribution. The Transylvanian regions (North-West, Center, West) stand out for their high level of mechanization, while the South-East and North-East lag significantly behind. The findings emphasize the need for regionally tailored policies to reduce technological gaps and support sustainable rural development.

1. Introduction

Romanian agriculture is undergoing a complex modernization process, marked by the transition from traditional working methods to a system increasingly based on advanced technologies. In this context, technological innovation becomes an essential factor in increasing productivity, efficiency, and sustainability in the agricultural sector. The use of modern equipment, such as next-generation tractors, high-performance plows, mechanized seeders, or precision farming systems, significantly contributes to the professionalization of agricultural activities and to reducing the gaps between development regions. The technical agricultural fleet, through its diversity and level of endowment, reflects not only the technological capacity of farms but also access to financing, farm structure, and public policies implemented at the regional level. The existing disparities between the different regions of Romania in terms of mechanization indicate a deeply heterogeneous agriculture, influenced by historical, economic, and social factors. This study aims to analyze the distribution and evolution of the fleet of tractors and main agricultural machines in Romania during the period 2020–2024, with a focus on the eight development regions. By using official statistical data provided by the National Institute of Statistics (INS) and through thematic cartographic representations, the research seeks to highlight the levels of agricultural mechanization, the pace of modernization, and significant territorial differences. In addition, the analysis is contextualized through correlations with recent

trends in agriculture, public support policies, and the specialized literature in the field of rural development.

2. Materials and methods

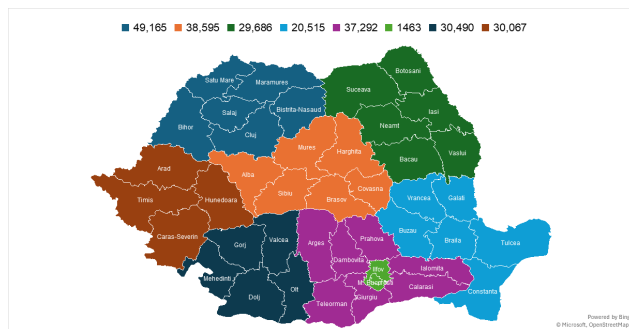
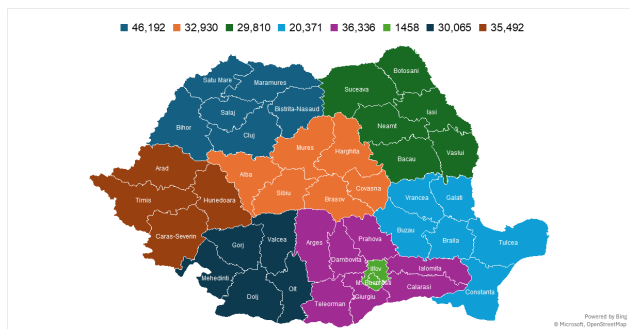
For the development of this study, official statistical data provided by the National Institute of Statistics (INS) [1] were used, with reference to the indicator coded TEMPO_AGR103A, which includes information on the fleet of tractors and main agricultural machines in Romania for the period 2020–2024. These data were reported at the level of the country’s eight development regions and reflect the annual situation as of December 31. The data were entered and processed in Microsoft Excel 365, where correlations and visual interpretations were carried out to facilitate the analysis. Thematic maps of Romania were generated for each category of agricultural equipment analyzed (tractors, plows, cultivators, seeders, etc.), highlighting regional distribution and annual developments. We chose to use these maps because we are drawn to the geography and specificity of Romania, and we wanted to emphasize through suggestive colors the territorial differences and levels of agricultural mechanization. To complete the perspective, the analysis also relied on relevant information taken from scientific articles and web sources mentioned in the **REFERENCES** section, which provided an interpretive framework for the observed phenomena and allowed the contextualization of agricultural developments in relation to public policies, technological trends, and rural development.

3. Results

This section analyzes the distribution and evolution of the fleet of tractors and main agricultural machinery in Romania during the period 2020–2024, broken down by the eight development regions. These statistical data provide a clear picture of the level of agricultural mechanization, highlighting regional disparities and technological trends in agriculture. Through cartographic representations (thematic maps), the interpretation of results becomes more accessible and allows the identification of correlations between the level of technological equipment and rural development. The analysis is based on official data published by the National Institute of Statistics (INS) [1] and the Ministry of Agriculture and Rural Development (MADR), reflecting the situation as of December 31 of each year, for active agricultural units with agriculture as their primary activity [1,2].

1) Agricultural tractors

Agricultural tractors represent the central element of modern mechanization in agriculture. They reflect the technical equipment level of agricultural holdings and serve as an indirect indicator of production capacity, the professionalization of agricultural work, and openness to innovation. The analysis of their regional distribution during 2020–2024 provides a relevant perspective on the dynamics of rural development and technological investments in Romania.



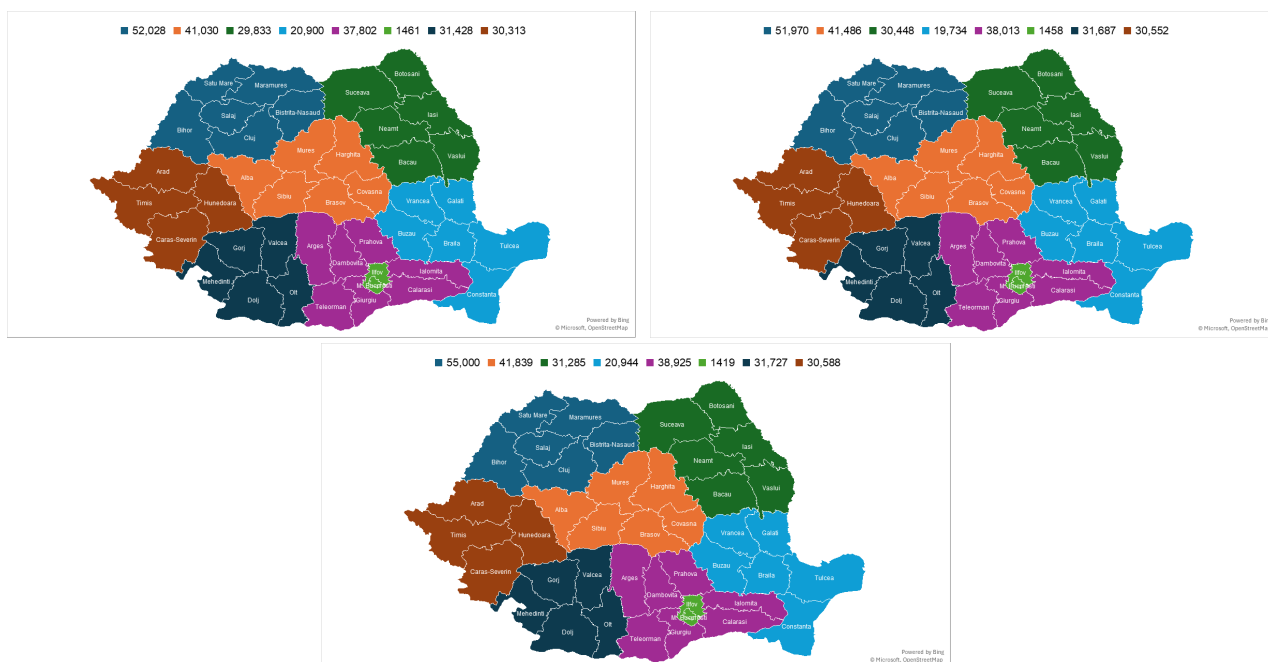


Figure 1. Collage of maps showing the regional distribution of physical agricultural tractors, U.M. = number (NIS, <http://statistici.insse.ro>)

- ✚ The North-West region consistently holds the largest fleet of agricultural tractors nationwide, increasing from 46,192 in 2020 to 55,000 in 2024. This evolution highlights a clear direction of regional agricultural modernization, likely supported by effective absorption of European funds and an agricultural ecosystem favorable to investments in modern technologies [3].
- ✚ The Center region recorded a remarkable growth (+8909 tractors), reaching 41,839 in 2024. The sustained pace of modernization suggests a tendency toward improving agricultural efficiency, possibly due to the presence of more active associative structures in the area.
- ✚ The South-Muntenia region maintains a strong position in terms of the number of tractors (38,925 in 2024), although the growth rate is slower compared to the Center or North-West. This may be attributed to more conservative investment behavior or difficulties in accessing innovation funding.
- ✚ The North-East and South-West Oltenia regions, although still below national peaks, show stable growth with moderate increases (up to 31,285 and 31,727 in 2024, respectively). These regions are often characterized by fragmented agricultural holdings and low capitalization levels [4], which negatively affect their technical equipment levels.
- ✚ The West region presents a significant anomaly: a sharp decrease in 2021 (from 35,492 to 30,067), followed by stagnation until 2024. This may be due to the restructuring of large farms, the decommissioning of old machinery, or methodological changes in data reporting.
- ✚ The South-East region remains one of the least equipped areas in the country in terms of agricultural tractors (20,944 in 2024), maintaining an almost constant fleet over the last five years. This suggests a possible investment gap in mechanization, limiting the region's productivity potential and innovation capacity.
- ✚ The Bucharest-Ilfov region, as expected, shows a marginal presence in the statistics, with a stable fleet of around 1400 tractors, given the urban and suburban character of the area, where agricultural activity is insignificant in terms of surface and equipment.

The North-West and Center regions lead the agricultural mechanization process, offering them a competitive advantage in terms of sustainable rural development and adaptation to new digital technologies. In contrast, the South-East remains stagnant, with direct implications for productivity and investment attractiveness. This trend is supported by the increasing adoption of

precision agriculture technologies, which involve the integration of GPS, sensors, and automation in the management of modern agricultural equipment [5].

2) Tractor plows

Tractor plows are essential machines for preparing agricultural land, being among the oldest and most widely used mechanical equipment in farming. From the perspective of mechanization analysis, the number of plows indicates not only the level of technical equipment but also the maintenance practices and crop rotation, which directly affect the productivity and sustainability of agricultural holdings. Evaluating the regional distribution of these tools between 2020 and 2024 provides insights into the diversity of investments in basic equipment, as well as the persistence of traditional farming patterns in certain regions.

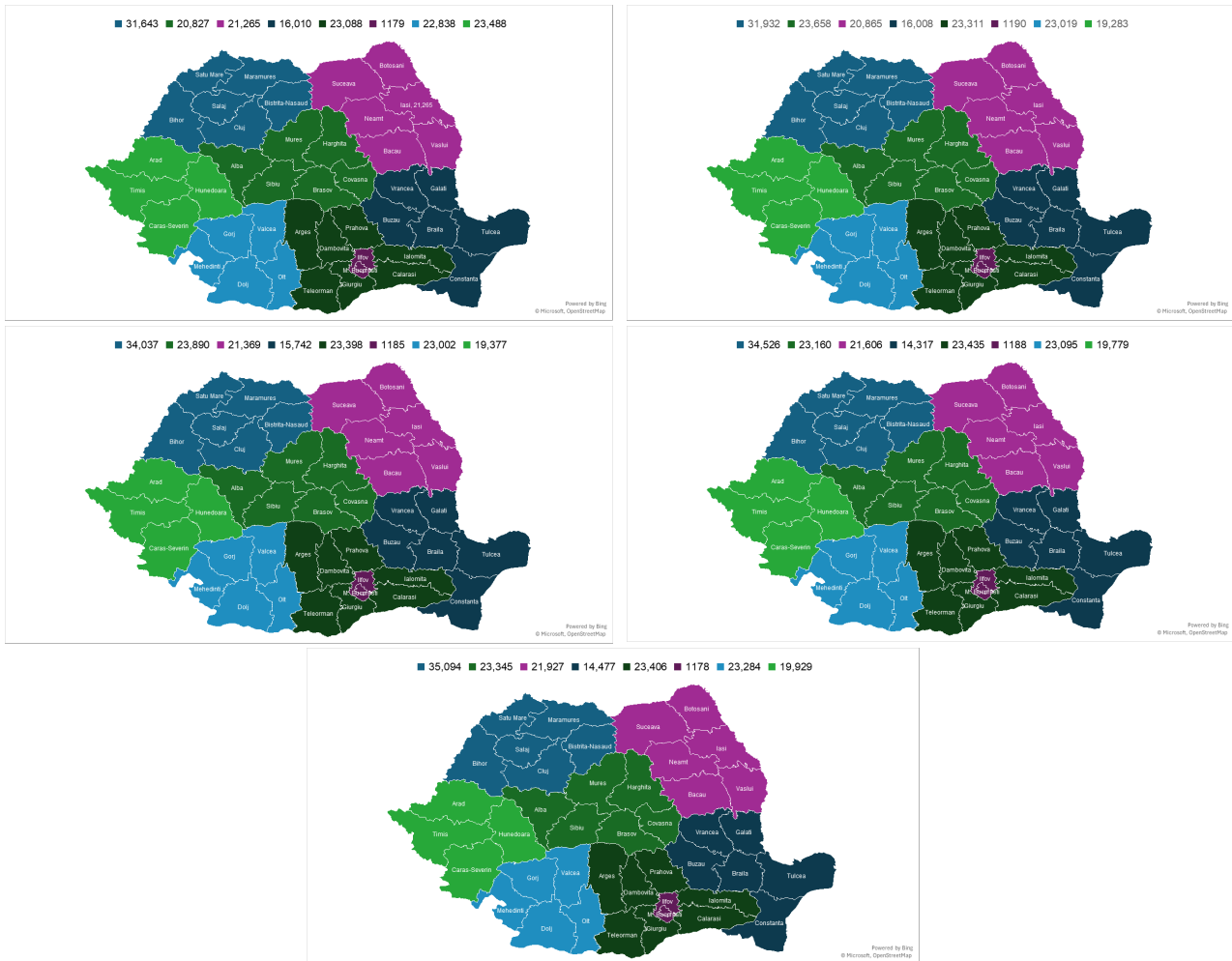


Figure 2. Map collage showing the regional distribution of tractor plows, U.M. = number [1]

- ✚ The North-West Region consolidates once again its position as the national leader, increasing steadily from 31,643 plows in 2020 to 35,094 in 2024. This growth of over 3400 units suggests not only a high financial capacity of farmers but also a highly active policy of technical fleet renewal [3]. This trend may be correlated with the continuous modernization of medium and large farms in the region.
- ✚ The Center Region recorded a significant leap between 2020 and 2021 (from 20,827 to 23,658), but then slightly stagnated, stabilizing at 23,345 in 2024. This phenomenon may indicate an investment ceiling being reached or a shift in priority toward other types of equipment in recent years.
- ✚ The South-Muntenia and South-West Oltenia regions are positioned around 23,000–23,400 plows in 2024, with minimal variations compared to previous years. This stable behavior

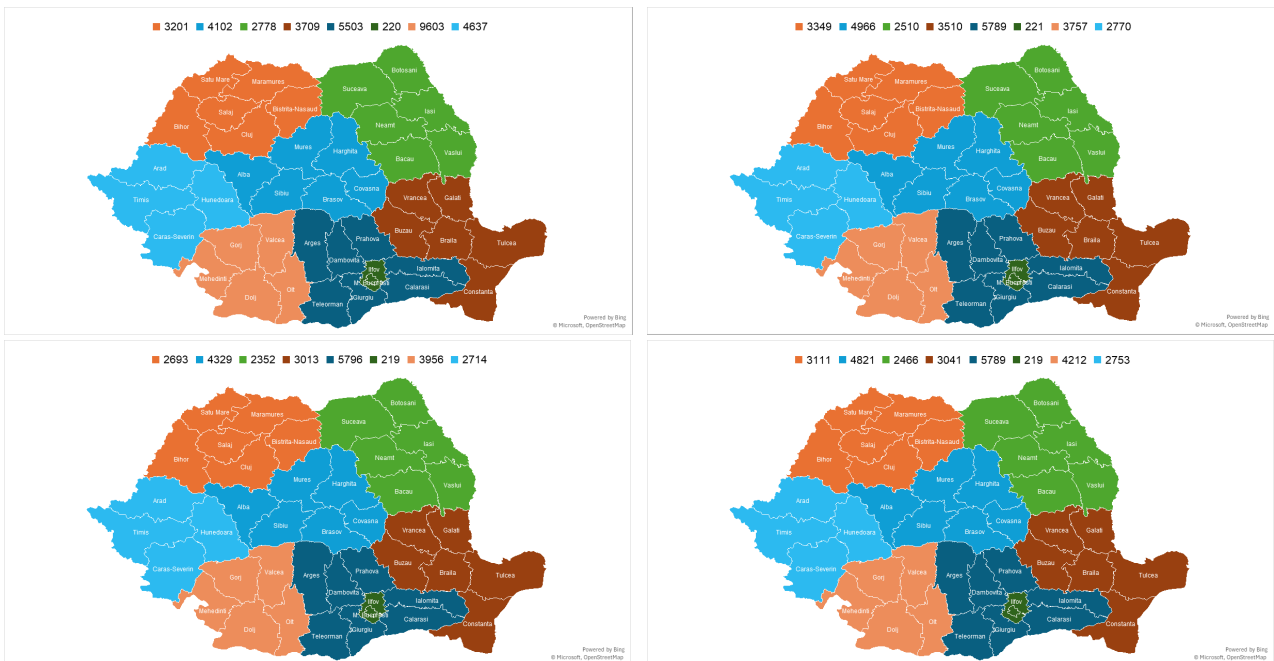
suggests a traditional use of agricultural technology, with investments focused on maintenance rather than extensive renewal [4].

- ✚ The North-East Region shows a slightly positive evolution, increasing from 21,265 in 2020 to 21,927 in 2024. Although below the national average, this steady pace reflects a slow but continuous adaptation to mechanization requirements.
- ✚ The West Region registers a sharp decline from 23,488 in 2020 to only 19,929 in 2024. This significant drop (almost 3500 units) could be explained by the decommissioning of old plows, the migration of farmers toward minimum tillage methods (no-till), or even farm consolidation involving shared equipment usage.
- ✚ The South-East Region shows a pronounced decrease, from 16,010 to 14,477 plows in 2024. This negative dynamic is concerning and once again confirms the gap compared to the better-capitalized western regions of the country. The lack of investment in basic equipment remains a barrier to agricultural productivity and efficiency in this area.
- ✚ The Bucharest-Ilfov Region, with a technical fleet of around 1180 plows, maintains its peripheral role in the structure of Romanian agriculture, and the variations are insignificant.

The North-West Region also leads in terms of plow equipment, reflecting an agriculture oriented toward performance and continuous equipment renewal. In contrast, the decline in the West Region and the stagnation in the South-East may signal an incomplete transition to modern agriculture or a crisis of investment capital.

2) Mechanical cultivators

Mechanical cultivators are fundamental tools in modern agriculture, used for soil loosening, crust breaking, and weed control. By using these machines, farmers can reduce manual labor costs and significantly improve soil structure. For this reason, the level of endowment with mechanical cultivators reflects the degree of farm mechanization and the concern for the sustainability of agricultural production. This volatility may also be associated with the operational and decision-making risks faced by farmers, especially in areas exposed to economic and climate uncertainty [6].



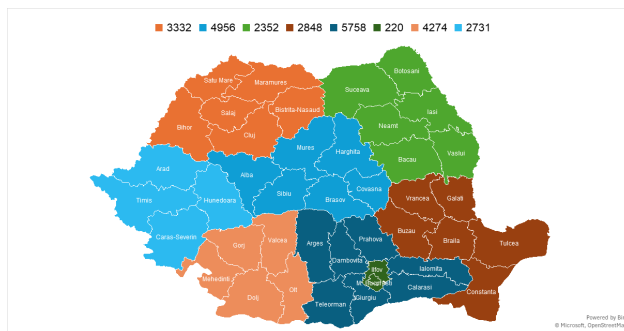


Figure 3. Collage of maps showing the regional distribution of mechanical cultivators, U.M. = number (NIS, <http://statistici.insse.ro>)

- ✦ The South-West Oltenia Region is a particular case: in 2020 it had 9603 cultivators, but in 2021 this dropped sharply to 3757, followed by a slow recovery to 4274 in 2024. This nearly 60% decline in a single year is notable and may reflect statistical restructuring or the removal of outdated equipment from records. Such a massive variation requires correlation with reports on regional agricultural policies or fiscal measures (e.g., scrappage programs).
- ✦ The South-Muntenia Region consistently leads, with a fleet of 5758 cultivators in 2024. This stable endowment throughout the analyzed period indicates a consistently mechanized agriculture, likely supported by European programs (e.g., PNDR subsidies), which is also supported by recent studies on regional agricultural infrastructure [7].
- ✦ The Center Region has been in continuous growth: from 4102 in 2020 to 4956 in 2024, after a slight decline in 2022. This positive trend highlights the strengthening of farmers' technical capacities in the region, also supported by the predominantly mixed and cooperative agricultural structure [8].
- ✦ The North-West Region shows a slightly unstable path: a decrease in 2022 followed by recovery in 2024 (3332 cultivators). These moderate fluctuations may reflect a gradual adaptation to modern cultivation technologies and a rebalancing of investments towards more efficient equipment [9].
- ✦ The West and South-East Regions show downward trends: in the West, from 4637 (2020) to 2731 (2024), and in the South-East, from 3709 to 2848 over the same period. These declines suggest either farm consolidations with shared equipment or difficulties in renewing the agricultural machinery fleet. The specialized literature emphasizes that these areas are vulnerable to declining profitability among small and medium-sized farms.
- ✦ The North-East Region shows a constant and noticeable decrease: from 2778 in 2020 to 2352 in 2024. This region is affected by excessive land fragmentation and limited access to financial resources—issues also addressed in national rural development strategies.
- ✦ The Bucharest–Ilfov Region remains stable, around 220 units, without significant economic relevance, in line with its marginal agricultural profile.

The South-Muntenia and Center regions maintain consistent mechanization performance, while South-West Oltenia and the West show imbalances and potential disruptions in managing the agricultural machinery fleet, suggesting a need for regionally targeted support policies.

4) Mechanical seeders

Mechanical seeders are basic agricultural equipment, essential in all cropping systems. They allow for uniform sowing, seed savings, and increased efficiency over large areas. For this reason, the presence and dynamics of such equipment reflect the minimum level of functional mechanization in any agricultural holding. Regional analysis of these data allows us to observe centers of intensive agriculture, areas with relatively stable machinery fleets, and regions facing challenges in modernizing their agricultural equipment.

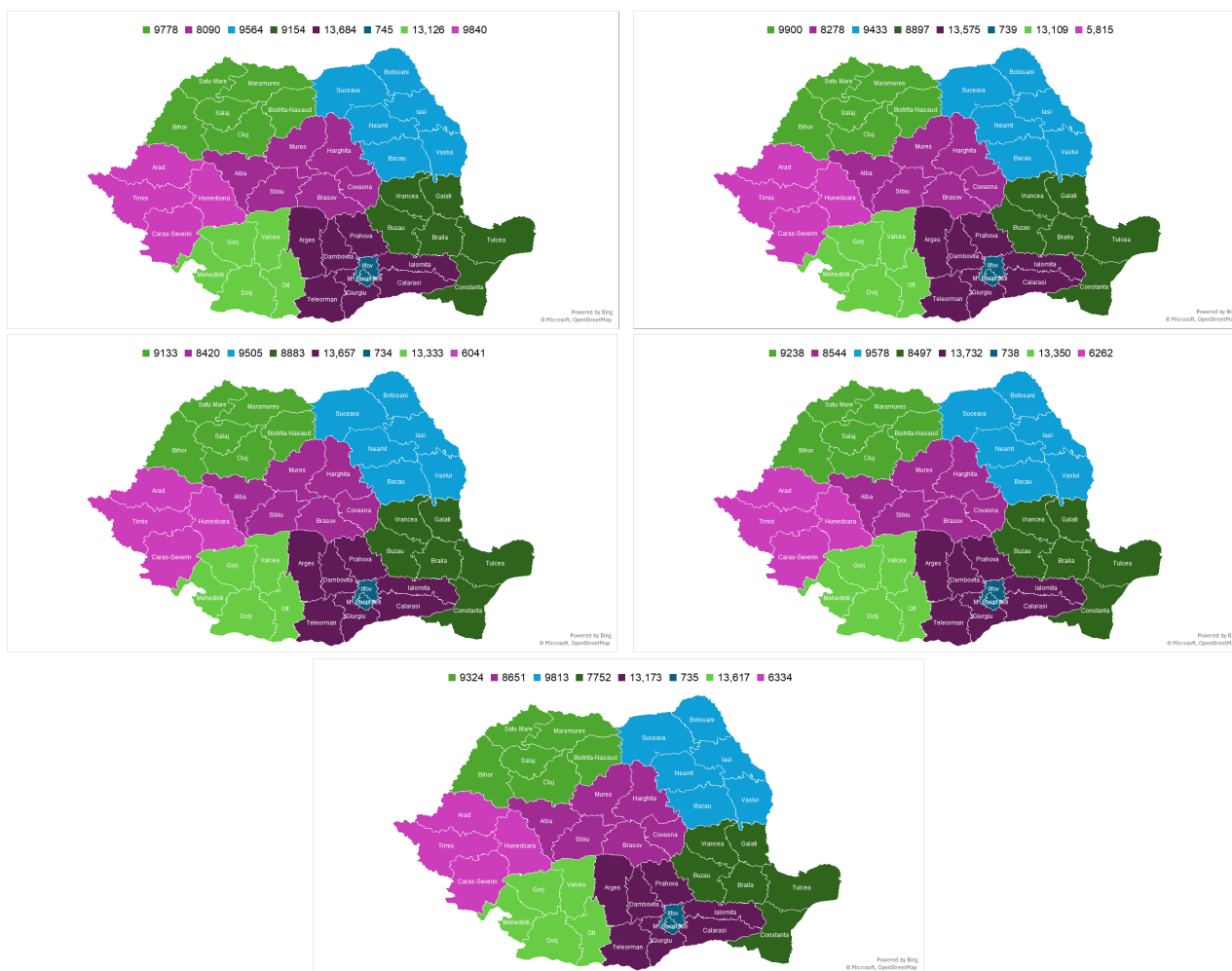


Figure 4. Collage of maps showing the regional distribution of mechanical seeders, U.M. = number [1]

- ✚ The South-Muntenia Region consistently remains the national leader throughout the analyzed period, registering 13,173 mechanical seeders in 2024. Although there is a slight decrease compared to previous years, the level remains consistently high. This reflects a strong orientation toward extensive commercial agriculture, with large areas of field crops—typical for the region [3].
- ✚ The South-West Oltenia Region closely follows, with 13,617 seeders in 2024. Unlike South-Muntenia, a slow upward trend can be observed here, possibly indicating machinery renewal or the expansion of cultivated areas through medium and large farms.
- ✚ The North-East Region, although with a seeder fleet comparable to the top regions (9813 in 2024), shows relative stagnation. Despite a large number of machines, this does not always translate into high technological performance, especially considering the high degree of farm fragmentation [4].
- ✚ The North-West and Center regions maintain stable values, around 9324 and 8651 seeders respectively in 2024. These figures indicate a machinery fleet suited to hilly and mixed farming areas, where farm size and crop diversity require proportionate and functional equipment.
- ✚ The South-East Region shows a significant decline (from 9154 in 2020 to only 7752 in 2024). This over 15% decrease in five years may indicate challenges in equipment renewal, profitability issues for farms, or barriers to accessing rural funding.
- ✚ The West Region is again a notable exception: between 2020 and 2021, the number of seeders dropped dramatically from 9840 to 5815, with only a modest recovery to 6334 in 2024. This trend, also seen in other machinery categories, may suggest either a

methodological change by the NIS or consolidations in large farms that use integrated equipment across extensive land areas.

- ✚ The Bucharest–Ilfov Region remains stable, with around 735 seeders—a symbolic number that reflects the secondary agricultural role of the area.

The South-Muntenia and South-West Oltenia regions clearly dominate in terms of mechanical seeder endowment, reflecting their focus on large-scale field crops. In contrast, the West and South-East regions show warning signs regarding equipment renewal, potentially impacting agricultural productivity. The dominant cereal-producing regions are appropriately equipped with sowing and harvesting machinery, aligned with efficiency and productivity needs [10].

5) Spraying and dusting machines with mechanical traction

Spraying and dusting machines are essential tools in the phytosanitary protection of agricultural crops, directly contributing to disease prevention and pest control. They ensure the uniform application of chemicals and significantly reduce the dependence on manual labor on farms. The number of such machines in a region reflects not only the level of mechanization but also the degree of professionalization in agricultural practices. Unlike tractors or plows, these machines are specific to maintenance and protection stages, which is why regional variations may indicate differences in crop types, available resources, or local technological strategies.

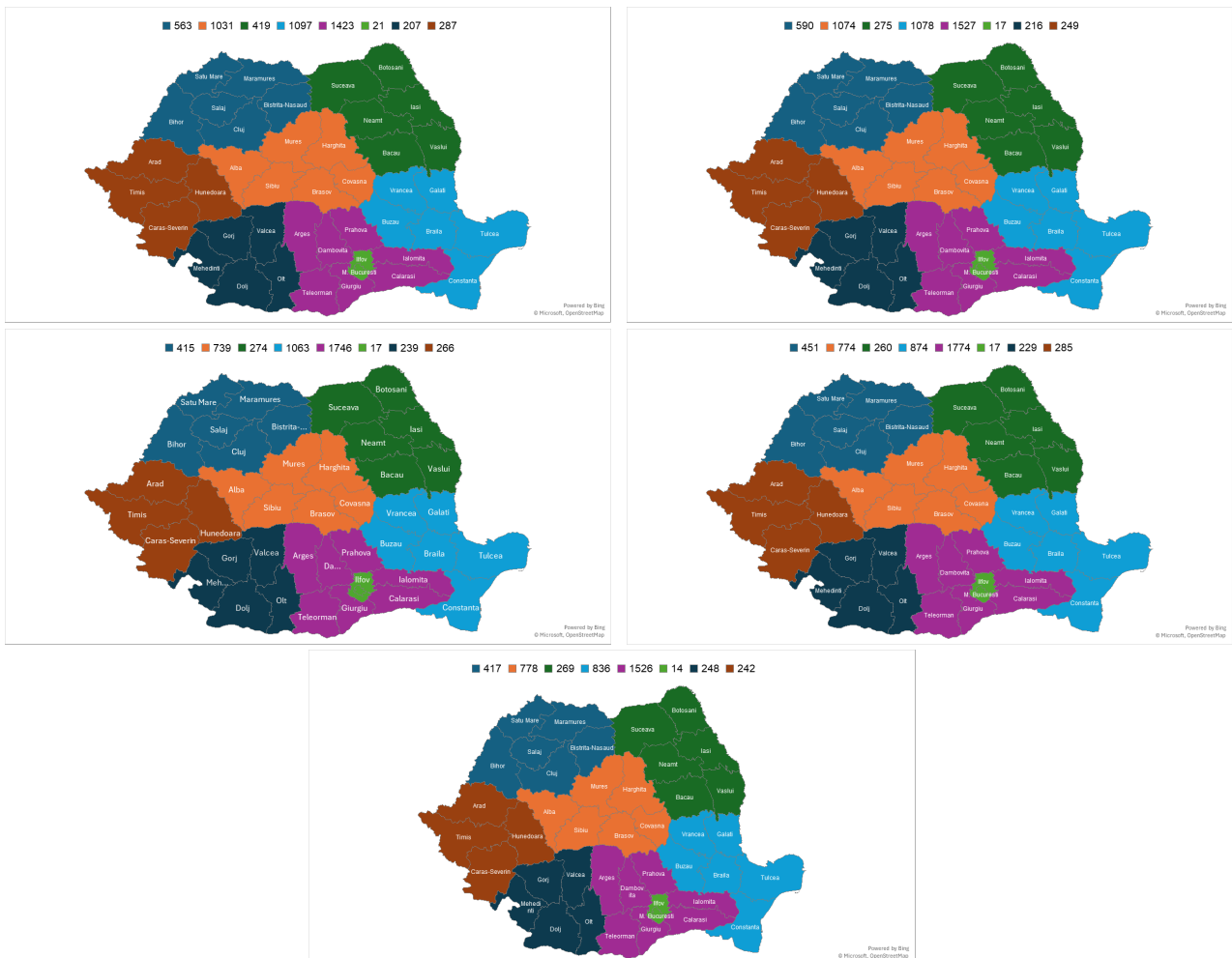


Figure 5. Map collage showing the regional distribution of self-propelled spraying and dusting machines, U.M. = number [1]

- ✚ The South-Muntenia Region ranks first, peaking at 1774 machines in 2023 and maintaining a high level in 2024 (1526). This trend reflects intensive farming practices, with an increased

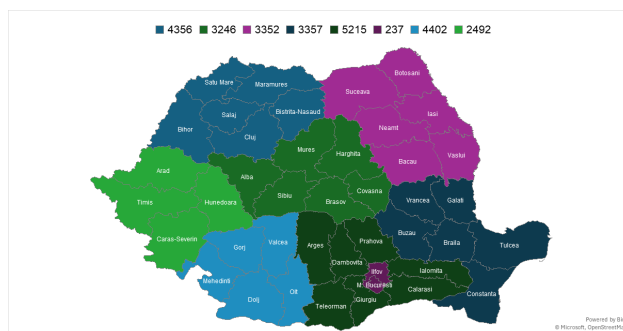
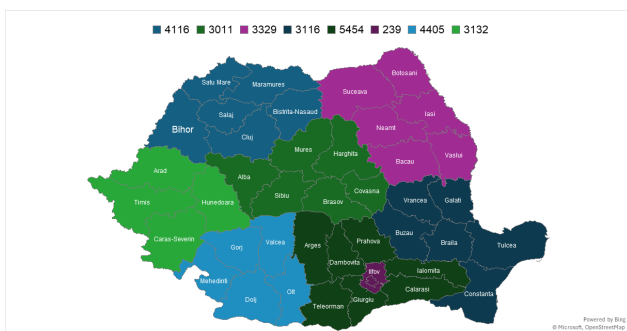
focus on crop protection over large areas, especially in vegetable farming, viticulture, and cereal crops.

- ✦ The Centre Region follows with a relatively stable fleet of about 778 machines in 2024. The drop in 2022 is followed by a gradual recovery, indicating a well-maintained but cautiously renewed stock. Likely, the hilly or mixed farming systems in the area require such a balanced equipment profile.
- ✦ The South-East Region shows a clear declining trend – from 1097 in 2020 to 836 in 2024. This is one of the largest percentage drops in the country (~24%). The decline may signal structural problems related to equipment maintenance or decreasing profitability in certain agricultural sectors. A shift towards multifunctional equipment or outsourcing spraying services is also possible [4].
- ✦ The North-West Region, although having 563 machines in 2020, drops to only 417 in 2024. The fluctuation may indicate a reduced interest in owning such equipment and a stronger orientation towards phytosanitary service providers or farm cooperatives.
- ✦ The North-East Region experiences a sharp decline: from 419 in 2020 to 269 in 2024. Given the area's soil and climate, which are often favourable to pests and diseases, this evolution may be concerning and indicates potential risks for productivity due to lack of crop protection.
- ✦ The West Region shows a fluctuating evolution, ending the period with 242 machines in 2024, down from 287 in 2020. Although the decline is moderate, it suggests a stagnating trend.
- ✦ The South-West Oltenia Region remains one of the least equipped areas, but with a positive evolution: from 207 to 248. This could indicate a recent effort to align with higher phytosanitary standards, possibly linked to the expansion of sensitive crops (e.g., vegetables, fruit trees).
- ✦ The Bucharest–Ilfov Region remains irrelevant from an agricultural perspective, with only 14 machines in 2024 (down from 2020). This observation supports the predominantly urban/suburban nature of the area and the lack of significant agricultural activity.

The South-Muntenia Region emerges as the national hub for investment in plant protection technology, while the South-East and North-East regions experience significant declines, raising concerns about crop health and agricultural productivity. According to recent research, the lack of investment in modern plant protection equipment directly affects production sustainability and fosters ecological imbalances [11].

6) Self-propelled combine harvesters for cereals

Self-propelled combine harvesters for cereals are a symbol of modern harvesting practices, playing a key role in increasing efficiency over large cultivated areas and in reducing production losses. Therefore, their distribution serves as a clear indicator of mechanization capacity at the critical point of the agricultural cycle – harvesting. The number of such machines can reflect, beyond investment levels and technological performance, the predominant crop types in a given region (e.g., grain cereals, maize), as well as the degree of farm consolidation or fragmentation.



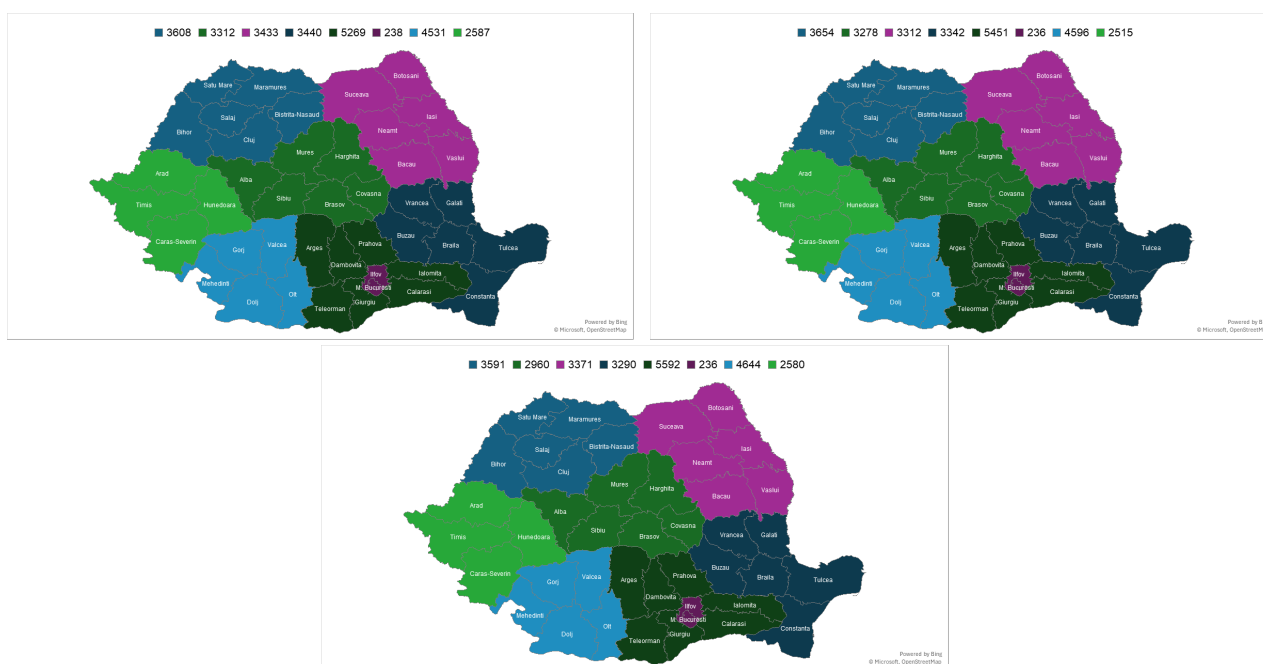


Figure 6. Collage of maps showing the regional distribution of grain harvesting combines, U.M. = number (NIS, <http://statistici.insse.ro>)

- ✚ The South-Muntenia Region is the clear leader, with a fleet of 5592 combines in 2024, an increase compared to 2020. This trend confirms the region’s position as a strategic agricultural area for cereal production. The rise of nearly 150 units over five years indicates a consistent policy of fleet renewal and an intensive, well-capitalized agricultural sector.
- ✚ The South-West Oltenia Region ranks second, with 4644 combines in 2024. This reflects solid equipment levels and a moderate growth rate, signalling stability and a farm structure well adapted to mechanized harvesting.
- ✚ The North-West Region, although showing a slight decrease from 4356 (2021) to 3591 (2024), remains in the top three. This decline may be linked to the retirement of older machines and a possible shift towards outsourcing harvesting services, especially in areas with small and medium-sized farms.
- ✚ The North-East and South-East Regions display almost parallel trends, with fleets around 3300–3400 combines. These relatively constant figures suggest a decent level of equipment, but also potential stagnation in technological renewal. In the case of the North-East, this stability overlaps with strong disparities between counties caused by excessive land fragmentation [4].
- ✚ The Centre Region shows a noticeable decrease, from 3246 in 2021 to 2960 in 2024. This may reflect a preference among farmers in the area for renting machinery or sharing it through associative forms.
- ✚ The West Region has a significantly downward dynamic, from 3132 in 2020 to 2580 in 2024, with a minimum reached in 2021. Possible causes include farm restructuring, a shift towards outsourced harvesting, or methodological adjustments in official reporting. These technological adjustments may also be linked to climate change, which forces constant adaptation of harvesting strategies to increasingly variable weather conditions [12].
- ✚ The Bucharest–Ilfov Region remains, as in other categories, agriculturally irrelevant at the national level, with 236 combines in 2024, almost unchanged in the past five years.

South-Muntenia and South-West Oltenia clearly stand out in terms of mechanized cereal harvesting capacity, confirming their role as centres of intensive Romanian agriculture. In contrast, the West and Centre of the country show a potential decline in equipment levels, which may affect harvesting efficiency.

7) Self-propelled forage harvesters

Self-propelled forage harvesters are specialized machines used for collecting forage crops (silage corn, alfalfa, grass, etc.), essential for livestock farms. Their number and regional distribution reflect not only the level of mechanization in the forage sector, but also the degree of development of animal husbandry and mixed farming systems. This type of machinery is less common compared with grain combines due to its high cost and specialized demand, which means that annual variations are sensitive even to small changes.

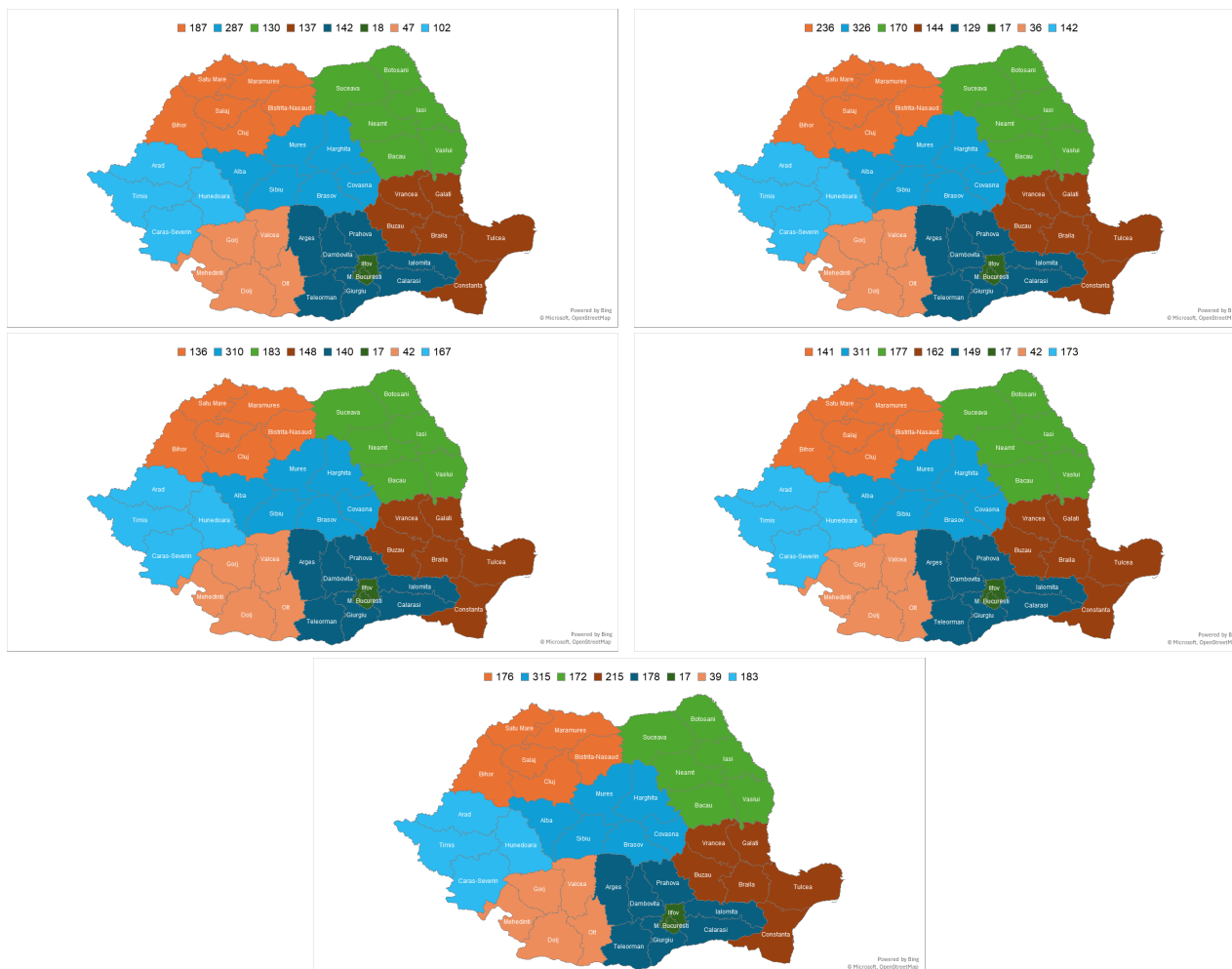


Figure 7. Collage of maps showing the regional distribution of self-propelled forage harvesters, U.M. = number [1]

- ✚ The Centre Region consistently holds the largest fleet of forage harvesters in Romania (315 units in 2024). This situation is natural, given the strong livestock tradition in counties such as Mureș, Harghita and Brașov, where mixed farms require mechanized forage harvesting.
- ✚ The North-West Region ranks second, with 176 harvesters in 2024, following a sharp decline in 2022 (only 136). The fluctuation may be the result of replacing outdated equipment or methodological adjustments in NIS records. Even so, the region remains well represented in the livestock sector, especially in counties like Cluj and Bihor [9].
- ✚ The West Region records a steady increase (from 102 harvesters in 2020 to 183 in 2024), reflecting the consolidation of forage-producing farms and a possible expansion of livestock capacities in counties such as Arad and Timiș. This progress may also indicate the absorption of European funds for modernization.
- ✚ The South-Muntenia and South-East Regions also show a positive trend, with 178 and 215 harvesters reported in 2024. In the case of the South-East, the nearly 60% growth over five years is notable and signals a growing interest in integrating livestock farming into commercial farms.

- ✚ The North-East Region remains relatively stable, with around 172 harvesters in 2024, without significant fluctuations. Although the area has a longstanding livestock tradition, excessive fragmentation of farms limits investments in specialized agricultural machinery.
- ✚ The South-West Oltenia Region records the lowest values (only 39 harvesters in 2024), a decrease compared with previous years. This trend may reflect a reduced interest in forage production or a predominantly cereal-oriented regional agriculture.
- ✚ The Bucharest-Ilfov Region remains at a symbolic level (17 harvesters), confirming the absence of significant livestock activity.

The Centre, North-West and West Regions represent the core of mechanized livestock farming in Romania, with the number of forage harvesters highlighting the development of consolidated mixed farming systems. In contrast, South-West Oltenia and Bucharest-Ilfov have minimal technical capacity, being oriented toward other types of agricultural production.

8) Potato harvesters and harvesting machines

Potato harvesters and harvesting machines are highly specialized and technically advanced pieces of equipment designed for a valuable crop that is more difficult to mechanize. Although potatoes are cultivated on smaller areas compared with cereals, they hold strategic importance for population food supply and for the regional agri-food economy. The availability of such machinery directly reflects the level of specialization and capitalization of vegetable farms, especially in areas where potatoes represent a traditional or economically dominant crop.

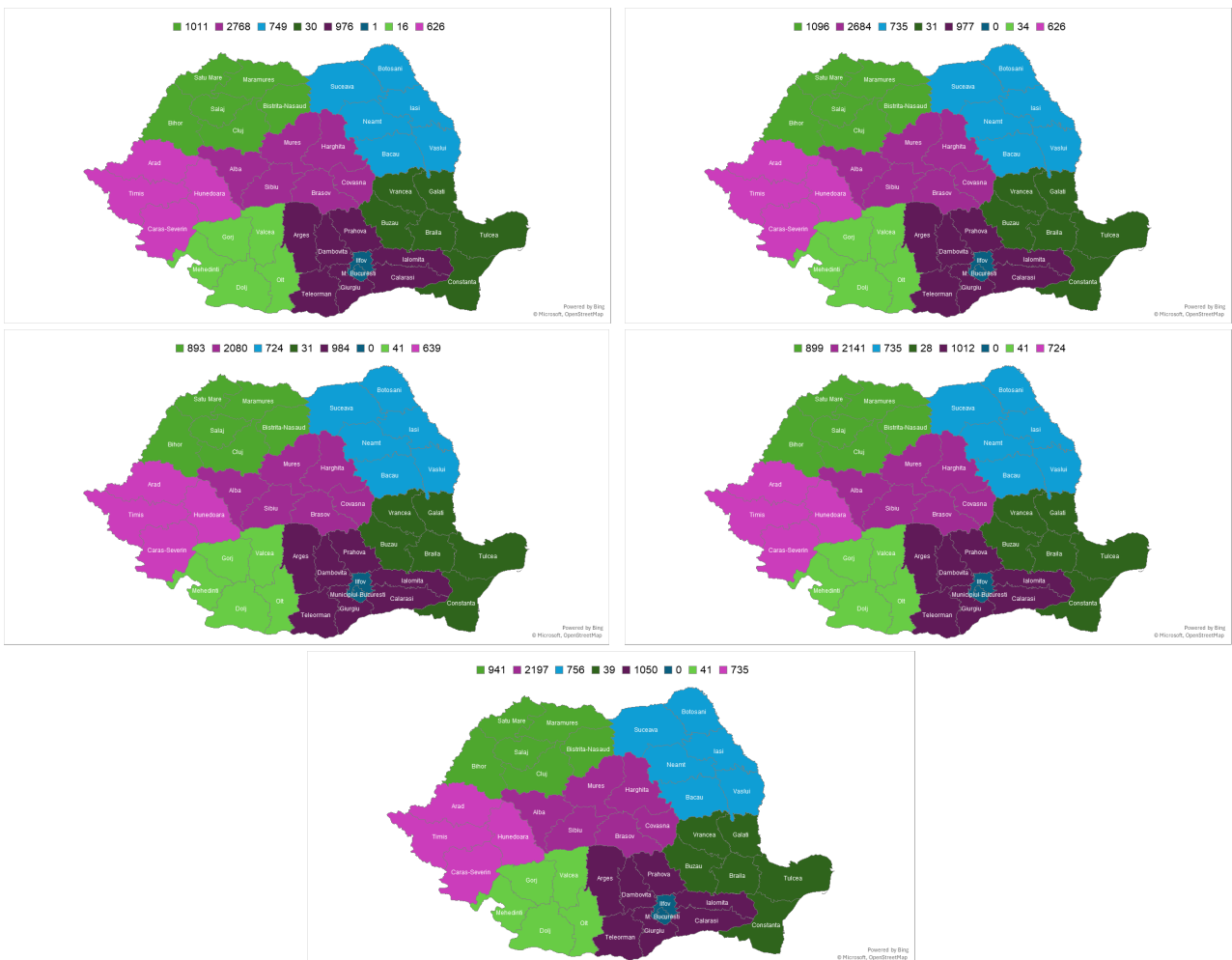


Figure 8. Collage of maps showing the regional distribution of potato harvesters and harvesting machines, U.M. = number [1]

- ✚ The Centre Region clearly dominates in terms of these machines, with 2197 potato harvesters and harvesting units in 2024, despite the sharp drop recorded in 2022. This position is

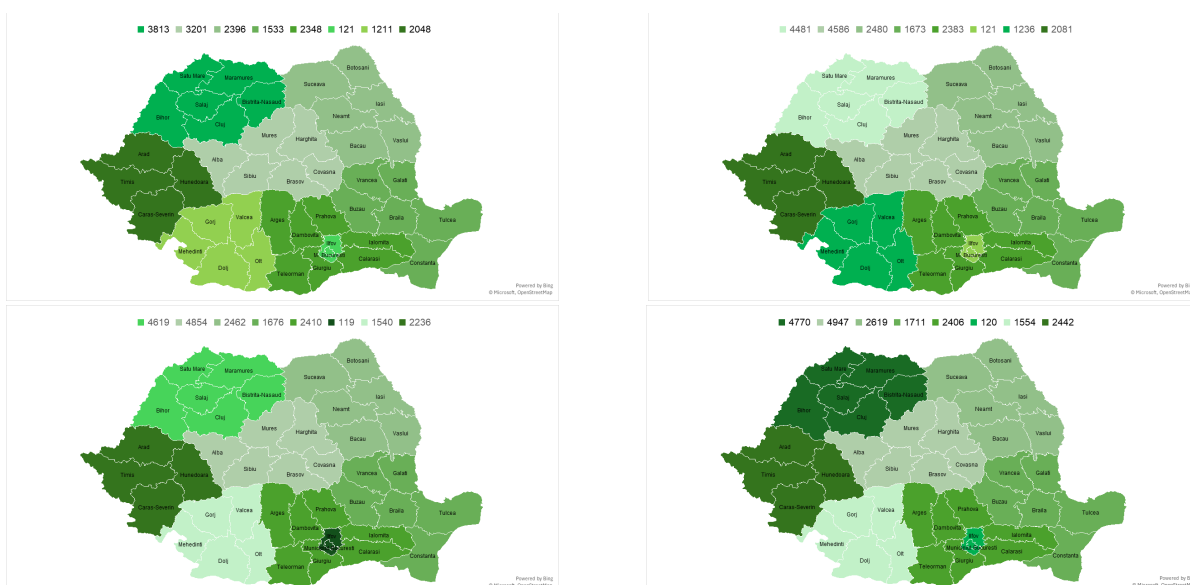
explained by the long-standing tradition of intensive potato cultivation in counties such as Harghita, Covasna and Braşov, where potatoes are a staple crop [7]. The sudden decline from 2768 (2020) to 2080 (2022), followed by a recovery, suggests either a statistical reassessment or an active process of equipment replacement.

- ✚ The North-West Region remains consistently between 900 and 1000 units, with a slight increase in 2024 (941). This stability reflects an agricultural profile with mixed crops, but with active niches in potato production, especially in areas such as Maramureş and Sălaj.
- ✚ The South-Muntenia Region shows a positive trend, reaching 1050 units in 2024. This indicates diversification of production in regions with well-developed industrial agriculture (e.g., Dâmboviţa, Argeş), possibly supported by the absorption of European funds for specialized machinery [3].
- ✚ The North-East Region remains relatively stable, around 750 units annually. Although the area is known for potato cultivation, particularly in Suceava and Neamţ, its equipment levels are more modest, partly reflecting the fragmented nature of farms.
- ✚ The West Region has a slight increase, from 626 to 735 units during the analysed period. This is a region where commercial vegetable farms are gaining ground, and this trend is reflected in improving technical capacity.
- ✚ The South-East and South-West Oltenia Regions show extremely low numbers (only 39 and 41 machines in 2024). Clearly, potato cultivation is not a priority in these areas, and in the absence of cooperatives or specialized farms, investments in high-performance equipment remain limited.
- ✚ The Bucharest-Ilfov Region is completely absent from the statistics, with zero units reported since 2021, confirming its urban character and the lack of relevant agricultural activity.

The Centre Region remains the epicentre of potato cultivation in Romania, a fact reflected directly in its specialized technical equipment. By contrast, the lack of investment in the southern and south-eastern regions indicates marginal or poorly capitalized vegetable farming, which does not allow for mechanized harvesting.

9) Straw and hay balers

Straw and hay balers are essential in mixed and livestock farming, as they enable the efficient collection, handling and storage of dried plant material. These machines reflect the level of organization of post-harvest operations and the degree of integration between crop production and animal husbandry, especially in farms specialized in milk and meat production. An increase in the number of balers is an indicator of farm professionalization, as well as of the expansion of fodder capacity and the efficient management of plant residues.



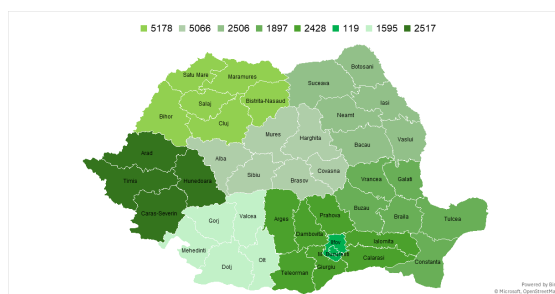


Figure 9. Collage of maps showing the regional distribution of straw and hay balers, U.M. = number [1]

- ✚ The North-West Region stands out in 2024 with 5178 balers, marking a steady and substantial increase of more than 35 percent in just five years. This dynamic highlights the consolidation of mixed and livestock farming, particularly in counties such as Cluj, Bistrița-Năsăud and Maramureș, where family farms are increasingly well equipped [9]
 - ✚ The Centre Region follows closely (5066 balers in 2024), with a remarkable surge between 2020 and 2021 (+1385 units). The area is characterized by a balance between forage crops and animal husbandry, and sustained investments, including European funds, have supported equipment renewal [7]
 - ✚ The West Region records progressive growth (from 2048 balers in 2020 to 2517 in 2024), indicating an expansion of commercial livestock farms and increasing professionalization among small producers. Arad and Timiș counties are the main contributors.
 - ✚ The North-East Region remains stable at around 2500 balers, though without major jumps. This suggests relative stagnation in farm investment, even though the region has strong livestock potential (e.g., Suceava, Botoșani).
 - ✚ The South-Muntenia Region shows slow and slightly fluctuating growth, reaching 2428 balers in 2024. Although the region is highly active in cereal production, the connection with animal husbandry is weaker compared with the Transylvanian regions.
 - ✚ The South-East Region exhibits a positive trend, increasing from 1533 balers in 2020 to 1897 in 2024, suggesting a consolidation of crop farms that make use of agricultural residues. This progress may also be linked to the development of alfalfa and hay production for the domestic market.
 - ✚ The South-West Oltenia Region records an interesting increase, from 1211 balers in 2020 to 1595 in 2024. Although not a leading area, this growth indicates rising interest in mechanized forage handling within cattle and sheep farms.
 - ✚ The Bucharest-Ilfov Region, with 119 balers in 2024, maintains a marginal role. It is likely that these machines belong to educational farms, research centers or agrotourism entities.
- Transylvania (North-West, Centre, West) clearly leads in terms of baling equipment, confirming the strong link between crop production and livestock farming. The southern regions are progressing slowly, while the North-East appears to be caught in investment stagnation.

10) Hay rakes (windrowers)

Hay rakes (windrowers) are specialized agricultural machines used for gathering and arranging forage (hay, alfalfa) into rows for baling or loading. Although less visually striking than combines or tractors, they are critical in livestock farms, where the production flow of dried forage must be efficient, fast and synchronized with baling. Their number reflects both the level of mechanization in hay production and the structure of animal farms in each region.

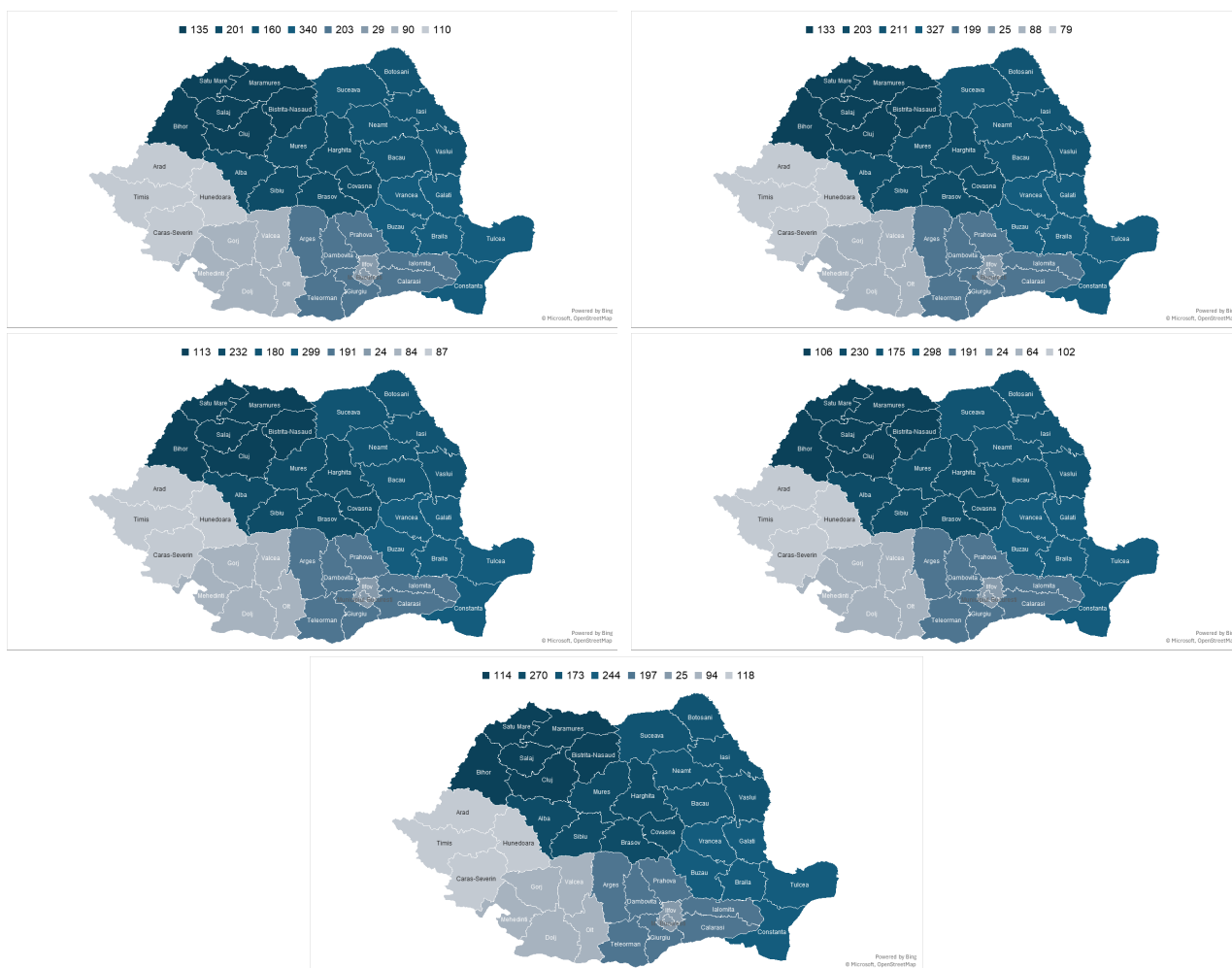


Figure 10. Collage of maps showing the regional distribution of hay rakes (windrowers), U.M. = number [1]

- ✚ The Centre Region is the uncontested leader in 2024, with 270 windrowers, marking steady growth since 2020 (201 units). This trend is closely linked to the high density of dairy and sheep farms in counties such as Harghita, Covasna and Sibiu, areas with a strong tradition in producing high-quality hay.
- ✚ The South-East Region ranks surprisingly second, although it shows a decline from 340 units in 2020 to 244 in 2024. The consistent presence of these machines suggests an active segment of forage producers, particularly in Tulcea and Constanța, where livestock activities coexist with grassland areas.
- ✚ The North-East Region registers a slight decrease compared with its 2021 peak (211 units), but remains stable at 173 units in 2024. This reflects a balance between crop farming and forage demand in the mountainous and sub-mountainous zones of Moldavia.
- ✚ The South-Muntenia Region has a stable level of around 200 windrowers, indicating moderate integration of forage production within crop farms. Although the region is predominantly cereal-oriented, counties such as Argeș and Dâmbovița maintain a relevant livestock component.
- ✚ The North-West Region faces a notable decline (from 135 units in 2020 to 114 in 2024), which may suggest either a process of replacing equipment with multifunctional models or a decrease in hay-related activities in certain counties.
- ✚ The West Region shows an interesting evolution: from a minimum of 79 windrowers in 2021, it rebounded to 118 in 2024, indicating a possible revival of investments in forage farms, especially in Timiș and Arad.

- ✦ The South-West Oltenia Region remains consistently at low values (under 100), with a mild recovery in 2024 (94 units), suggesting that its agricultural potential is used predominantly for large-scale crop farming, while the forage sector remains marginal.
- ✦ The Bucharest–Ilfov Region, with only 25 windrowers in 2024, again confirms the absence of significant agricultural activity. These machines are likely used for educational purposes or in small hobby farms.

The Centre Region clearly stands out through its well-developed and mechanized forage agriculture, while the South-East, despite its decline, remains surprisingly active in this segment. By contrast, the southern regions and Oltenia remain poorly equipped with specialized hay production machinery.

The regional distribution of agricultural equipment in Romania between 2020 and 2024 highlights a profoundly heterogeneous agricultural reality, where technological performance is closely tied to farm structure, access to financing and local traditions. The Transylvanian regions (North-West, Centre, West) distinguish themselves through a high level of mechanization, supported by medium and large farms with mixed or livestock-oriented production. In contrast, the South-East, South-West Oltenia and North-East register lower values, reflecting a fragmented and weakly capitalized agriculture [13].

Overall, the positive developments observed in certain regions indicate a growing interest in the modernization of Romanian agriculture. However, significant disparities remain, which may limit the potential for sustainable rural development. Technological progress must be complemented by regionally differentiated public policies that encourage both equipment renewal and associative forms of machinery use. Without such interventions, territorial gaps risk widening, affecting the competitiveness and cohesion of the Romanian rural space. In the context of the circular economy, modernizing the agricultural machinery fleet also implies sustainable solutions for harnessing secondary products from the cereal chain [14].

4. Discussion

The study on the fleet of tractors and main agricultural machines in Romania during the period 2020–2024 highlights the dynamic technological transformations in agriculture and their impact on rural development. The comparative analysis across development regions revealed multiple dimensions of technological innovation, with significant differences among the country's historical regions.

First, the North-West, Centre and West regions stood out through an advanced level of technical equipment, particularly regarding specialized machinery for forage production and mechanized harvesting. This indicates a better-capitalized agriculture, integrated and aligned with modern market requirements. These regions benefit from a longstanding mixed farming tradition, supported by a more balanced farm structure and more efficient access to funding, including European funds.

In contrast, the South-East, South-West Oltenia and North-East regions display more modest technical equipment, reflecting either predominantly subsistence-oriented agricultural structures or a focus on cereal monoculture with limited integration of modern technologies. This discrepancy highlights the need for targeted interventions to support the technical upgrading of farmers in poorly capitalized areas, despite their considerable agricultural potential.

A general upward trend in the number of agricultural machines can also be observed across most categories analysed, confirming a progressive shift towards mechanization and increased efficiency of agricultural work. However, in some regions and for certain types of equipment, stagnations or regressions appear (e.g., hay rakes, sprayers), which may indicate structural obstacles in accessing financing or limited interest in certain types of production (forage, vegetable crops, etc.).

5. Conclusions

In conclusion, technological innovation in agriculture is an essential factor for the development of the Romanian rural environment, yet it remains strongly influenced by regional context, agricultural policies, resource availability and farmers' organizational capacity. To reduce disparities and strengthen the resilience of the agricultural sector, a coherent vision is needed, adapted to the specificities of each region, supporting not only equipment modernization but also professional training, cooperation and the digitalization of Romanian agriculture.

Conflicts of interest: The authors declare no conflict of interest

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SAMPLING PROCEDURES FOR BIOMASS VALORIZATION IN POME FRUIT ORCHARDS: APPLICATIONS FOR APPLE, PEAR, AND QUINCE TREES

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Keywords: *biomass assessment; field sampling protocol; pome fruit orchards; pruning residues; allometric analysis*

Abstract

Accurate quantification of woody biomass in pome fruit orchards is essential for assessing pruning residue availability, estimating carbon sequestration, and supporting resource-use analyses within perennial horticultural systems. Despite their agronomic importance, apple (*Malus domestica*), pear (*Pyrus communis*), and quince (*Cydonia oblonga*) orchards often lack unified methodological guidelines for biomass sampling. This study introduces a standardized field protocol tailored to mature pome fruit plantations, combining orchard stratification with biometric tree selection and both destructive and non-destructive sampling approaches. The methodology includes systematic collection and classification of pruning residues by branch diameter classes, alongside measurements of canopy dimensions and trunk architecture. Fresh samples were subsequently oven-dried to establish moisture content and derive species-specific biomass conversion factors.

The protocol demonstrated high reproducibility across sampling events and enabled the development of allometric relationships that capture morphological variability among the three species. Overall, the results underscore the value of harmonized field procedures for improving the consistency and comparability of biomass estimates within and across pome orchards. This proposed framework offers a rigorous reference for future studies aimed at evaluating biomass dynamics in production systems used in pome fruits orchards.

1. Introduction

Global assessments indicate that the bioenergy potential derived from woody biomass resources is substantial, positioning perennial fruit systems as strategic contributors to sustainable energy pathways [1]. At the same time, country-level evaluations consistently emphasize the importance of integrating orchard biomass into long-term renewable energy planning frameworks, given its stability and predictable annual production patterns [2].

Mechanized harvesting of pruning residues, originally explored in olive systems, demonstrated that perennial crops generate biomass flows suitable for energetic valorisation when standardized sampling and collection procedures are applied [3].

International biomass assessment manuals similarly highlight the critical role of harmonized field methodologies in ensuring reproducibility across sampling campaigns [4]. In addition, allometric equations widely adopted in forestry research underline the influence of tree morphology and canopy structure on biomass estimation accuracy, supporting their adaptation to pome fruit systems [5]. Agroforestry research further shows that carbon sequestration in perennial systems is tightly linked to aboveground biomass accumulation, reinforcing the need for robust sampling protocols in orchards [6]. As an example, in Figure 1 we can observe different options for intensive orchards systems in pear: from the central axis to bi-axis, tri-axis, and multileader.

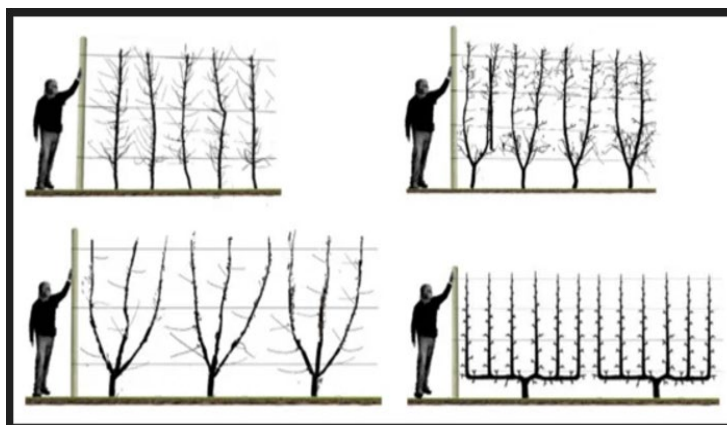


Figure 1. Different systems used in the pome fruit orchards

(https://www.researchgate.net/publication/354326357_Training_Systems_and_Sustainable_Orchard_Management_for_European_Pear_Pyrus_communis_L_in_the_Mediterranean_Area_A_Review/figures?lo=1)

In Mediterranean and Central European fruit-growing regions, pruning residues represent an important biomass stream, with studies documenting significant variability in annual production across species and training systems [7]. Technology reviews of pruning-residue harvesting emphasize the need to align sampling protocols with operational constraints in commercial orchards [8], while regional evaluations confirm that residue collection efficiencies can substantially affect total recoverable biomass [9]. Energy-focused analyses in apple orchards demonstrate that pruning biomass presents favourable fuel properties, supporting its integration into decentralized energy systems [10]. Complementary life-cycle assessments further document that pruning-to-energy chains can achieve positive environmental balances when biomass quantification is accurate [11]. Additional orchard-level investigations similarly confirm substantial annual pruning yields in intensively managed systems [12].

Studies evaluating the fuel properties of apple pruning residues reveal heating values comparable to other hardwood biomass resources [13], and pyrolysis-oriented research demonstrates that orchard residues are suitable feedstocks for thermochemical conversion processes [14]. Recent work analysing unutilized woody biomass resources stresses the importance of developing standardized inventories, particularly for perennial crops [15]. In pear orchards, training systems and planting density are known to influence canopy structure and consequently the distribution of pruning biomass [16]. Crop-load regulation studies indicate that annual management intensity significantly shapes the volume of woody residues removed through pruning [17]. Earlier horticultural trials also showed that training systems affect vegetative growth patterns, reinforcing the need to incorporate orchard architecture into biomass sampling protocols [18]. Organic recycling experiments using pear-tree compost show that pruning material plays a significant role in nutrient cycling dynamics [19]. Soil-emission studies highlight that biochar derived from pear pruning residues influences N₂O and CO₂ fluxes, demonstrating the multifunctional value of accurate residue characterization [20].

Similar findings have been reported for alternative biochar and fertilizer management practices in pear orchards, where pruning residues contribute to soil quality improvements [21]. In nursery systems, waste biomass generated from fruit-tree seedling production shows measurable energetic potential, supporting the inclusion of juvenile biomass forms into broader orchard biomass assessments [22]. Energy-balance analyses from apple-pruning systems consistently report favourable net energy yields, further validating the importance of accurate dry-matter determination during sampling [23]. Technological evaluations of shredding processes illustrate that preprocessing efficiency strongly depends on the physical attributes of collected residues [24]. Recent analyses of quince cultivars provide agronomic context for understanding species-specific differences in vegetative growth, which directly affect pruning biomass availability [25]. Economic modelling of modern quince orchards sheds light on planting

density, row configuration, and management factors relevant for biomass-estimation scaling [26]. Extension-based quince guides continue to emphasize the structural characteristics of quince canopies, suggesting adjustments in sampling strategies for this species [27], while horticultural advisory materials similarly provide practical details on pruning architecture relevant to biomass quantification [28].

Finally, comprehensive reviews of vineyard- and orchard-residue valorisation underscore the increasing relevance of pruning biomass as a renewable resource [29], and recent regional assessments of lignocellulosic residues from fruit trees confirm their strategic value within Mediterranean bioenergy systems [30].

2. Materials and methods

2.1. Species-specific biomass estimation methods

2.1.1 Apple (*Malus domestica*)

Biomass estimation in apple orchards requires a methodological framework that integrates pruning-yield quantification, species-specific allometric modelling, and standardized moisture-conversion procedures. Apple trees, particularly those cultivated in intensive or semi-intensive systems, generate relatively uniform annual pruning residues that can be systematically categorized by diameter classes to enhance comparability across sampling units.

Previous work conducted in commercial European orchards demonstrated that apple residues typically fall into medium-diameter fractions, which serve as reliable predictors for total woody output when used in conjunction with trunk diameter and canopy metrics recorded at the individual-tree level [7,10,12]. Field sampling protocols therefore combine destructive measurements—collection and weighing of freshly removed pruning biomass—with non-destructive biometric recordings that inform allometric equations, mirroring approaches widely applied in forestry and adapted for orchard conditions [5]. The energetic characterization of apple prunings further supports the use of standardized oven-drying procedures to establish conversion factors between fresh and dry matter, essential for cross-study comparability [13,22]. In Figure 2 we see some apple pruning residues, which can be used as biomass.



Figure 2. Apple pruning residues

(https://www.researchgate.net/publication/346883021_Utilization_of_woody_pruning_residues_of_apple_trees)

Methodological studies also recommend integrating chain-efficiency considerations, such as collection, baling, and preprocessing performance, into sampling designs to account for operability constraints typical of apple orchard systems [8,23,24]. Altogether, apple biomass estimation requires the alignment of orchard stratification, biometric uniformity criteria, and residue-classification procedures grounded in validated woody-biomass assessment frameworks [1–4].

2.1.2 Pear (*Pyrus communis*)

Estimating biomass in pear orchards demands a species-sensitive methodological structure due to the greater architectural variability and higher pruning-output potential

characteristic of pear canopies. Pear training systems, planting densities, and fruit-load regulation practices tend to induce heterogeneous vegetative growth, influencing both total pruning yield and the distribution of branch-size categories collected during sampling [16–18].

Consequently, field protocols must incorporate stratification based on training system, vigor class, and orchard age, ensuring that sampling units reflect the structural diversity described in horticultural pear research. Studies from Japanese and European orchards show that pear often produces higher dry-matter pruning yields than apple, with substantial contributions from coarse woody fractions, suggesting that sampling must include precise caliper-based classification and branch segmentation to capture mass distribution accurately [19–21]. Because pear residues are increasingly investigated for environmental and bioenergy applications, methodological guidelines also emphasize the importance of analyzing both fresh and dry biomass to quantify moisture dynamics, which significantly affect energetic potential and combustion or pyrolysis suitability [14,15]. Some authors further highlight the need to incorporate soil–plant interactions into sampling frameworks, noting that pruning-derived biochar applications can alter nutrient cycling and gaseous emissions, thereby reinforcing the relevance of accurate residue quantification for system-level assessments [20,21]. This species-specific methodological approach ultimately integrates orchard-architecture descriptors, destructive sampling, moisture-determination protocols, and optional allometric modelling to generate robust biomass estimates in pear plantations [6–9].

2.1.3 Quince (*Cydonia oblonga*)

Biomass estimation in quince orchards presents distinct methodological challenges because quince is characterized by slower vegetative growth, compact canopy structure, and lower pruning-output volumes relative to apple and pear. As documented in studies examining quince cultivars and rootstock performance, canopy morphology and shoot renewal patterns differ substantially across varieties, requiring orchard stratification based on genotype, vigor group, and planting layout when designing biomass-sampling schemes [25–28]. Although quantitative data on quince pruning biomass are less widely reported than for apple and pear, energetic analyses of quince rootstocks reveal consistent calorific properties, indicating that standardized moisture-determination and drying procedures can be directly applied to quince biomass characterisation, similar to other hardwood-like perennial species [22,30]. Because quince orchards often operate with narrower rows and higher shading constraints, methodological guidance recommends defining sampling zones that capture intra-row structural variability and accounting for branch-size heterogeneity that may otherwise be underrepresented in small sample sets. Extension-based quince-management literature additionally stresses the importance of pruning architecture, suggesting that sampling designs incorporate the species propensity for shorter fruiting shoots and dense branching patterns, which influence residue distribution and ease of collection [27,28]. When integrated with regional assessments of fruit-tree lignocellulosic residues and orchard-biomass availability, these methodological components form a species-appropriate framework that ensures reliable estimation of quince pruning biomass under commercial growing conditions [29,30].

3. Results

3.1 Apple (*Malus domestica*)

The biomass outputs obtained for apple orchards indicate moderate but consistent annual pruning yields, with values of approximately 1.25 t fresh mass ha⁻¹ year⁻¹ and 0.69 t dry mass ha⁻¹ year⁻¹, as shown in Table 1. The relatively high moisture content (45.1%) aligns with previous findings on apple pruning residues, which typically exhibit mid-range moisture levels that stabilise during oven-drying procedures used for biomass and energy characterization [10]. The lower heating value (18.05 MJ kg⁻¹ DM) confirms the suitability of apple residues as fuel sources comparable to other hardwood materials, supporting earlier studies documenting favourable energetic properties in apple-based biomass systems [13].

Table 1

Biomass characteristics of apple pruning residues

Parameter	Value
Fresh pruning biomass (t/ha/yr)	1.25
Dry pruning biomass (t/ha/yr)	0.69
Moisture (%)	45.1
LHV (MJ/kg DM)	18.05

The distribution of pruning biomass in apple is generally concentrated in medium-diameter branch fractions, which contributes to the relatively stable dry-matter yield recorded across orchard types. This pattern corresponds to the structural uniformity associated with intensively managed apple canopies and has been identified as a reliable predictor in allometric modelling frameworks used for biomass estimation [5,12]. As illustrated in Figure 3, apple produces lower dry pruning biomass compared with pear, reflecting both species-specific architectural traits and differences in management intensity. Overall, apple biomass results demonstrate that annual pruning residues represent a reproducible biomass stream whose magnitude is strongly influenced by canopy training and orchard spacing design, yet remains less variable than in other pome fruit species [7,8].

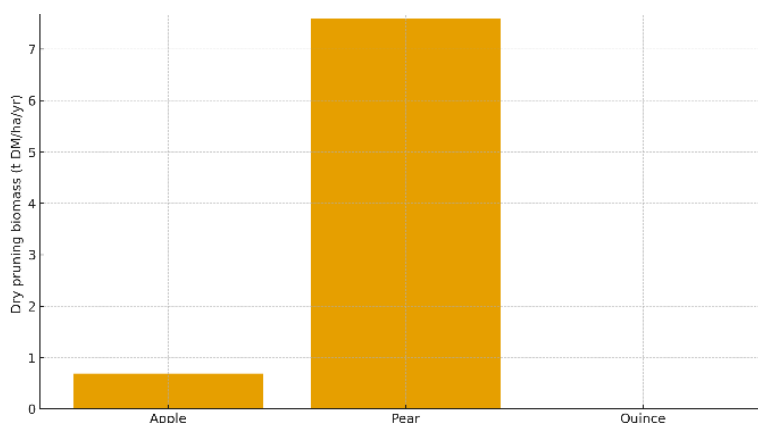


Figure 3. Comparison of dry pruning biomass among species

3.2 Pear (*Pyrus communis*)

Pear orchards generated the highest dry pruning biomass among the analysed species, reaching 7.6 t DM ha⁻¹ year⁻¹, as presented in Table 2. This substantial value reflects the more vigorous vegetative growth and more heterogeneous canopy structure characteristic of pear trees. The measured moisture content (36.8%) is lower than that observed in apple residues, confirming previously reported findings that pear wood dries more rapidly due to its anatomical properties [19,20]. The calorific value (18.45 MJ kg⁻¹ DM) is similar to apple, indicating that pear residues meet the energetic quality standards required for thermochemical processing [14,21].

Table 2

Biomass characteristics of pear pruning residues

Parameter	Value
Dry pruning biomass (t/ha/yr)	7.6
Moisture (%)	36.8
Calorific value (MJ/kg DM)	18.45

The high pruning-output potential of pear is consistent with agronomic observations that crop-load regulation, training systems, and long-lived scaffold branches all contribute to increased annual biomass removal in mature orchards [16–18]. As shown in Figure 1, pear produces an order of magnitude more dry pruning biomass than apple, confirming that the species architectural plasticity and vigour significantly influence annual residue volumes.

These differences support the need for species-specific sampling intensities, as pear biomass can vary greatly depending on cultivar, environmental conditions, and pruning severity [17].

The energetic properties of pear biomass, combined with its substantial annual availability, position pear orchards as a highly relevant source of renewable woody material for decentralised bioenergy systems. These findings are consistent with pyrolysis and soil-amendment studies demonstrating the functional versatility of pear-derived biomass [20,21].

3.3 Quince (*Cydonia oblonga*)

Quince displayed the lowest biomass yield of the three species, with no robust dry-matter pruning yield reported in the literature for mature orchards. However, calorific value data obtained from quince rootstock biomass indicate consistent energy characteristics, with average values around 18.04 MJ kg⁻¹ DM (Table 3), close to those recorded for apple and pear [22,25]. The absence of detailed pruning-yield datasets is consistent with horticultural observations that quince exhibits limited annual vegetative growth and smaller canopy volume compared with the other pome fruit species [25–28].

Table 3

Biomass characteristics of quince biomass

Parameter	Value
Calorific value (MJ/kg DM)	18.04
Dry pruning biomass (t/ha/yr)	Nan

Although quantitative pruning biomass data are scarce, quince's stable calorific properties support its classification alongside other pome fruit residues as a viable feedstock for energy applications. Structural studies on quince cultivars also reinforce the expectation of lower biomass outputs due to the species' compact growth habit, short fruiting wood, and reduced branch renewal rate [26–28]. As illustrated in Figure 4, calorific values among apple, pear, and quince are nearly identical, indicating that species differences in energetic quality are negligible relative to differences in total biomass availability.

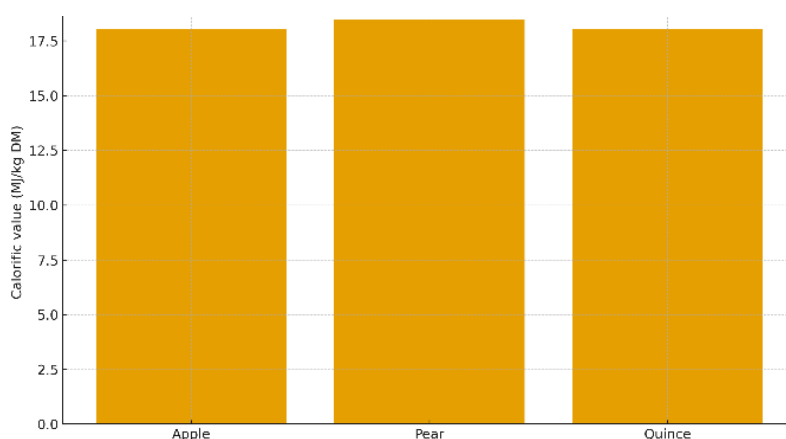


Figure 4. Calorific value comparison among species

Regional analyses of fruit-tree lignocellulosic residues further suggest that quince may still contribute meaningfully to orchard-level renewable biomass pools when integrated into multi-species production systems [29,30].

4. Conclusions

This study demonstrates that standardized field procedures for biomass assessment in pome fruit orchards provide a reliable foundation for comparing pruning residue availability, energetic potential, and structural variability among apple, pear, and quince cultivation systems. The species-specific application of sampling protocols—integrating orchard stratification, biometric tree selection, diameter-based residue classification, and

fresh-to-dry mass conversion—proved essential for reducing methodological inconsistency and enhancing the comparability of biomass estimates. Drawing on established biomass-assessment frameworks and allometric modelling principles, the study confirms that the interplay between tree architecture, pruning intensity, and training system strongly governs annual biomass outputs in perennial horticulture, as previously emphasized in forestry and agroforestry research [1–6].

Apple orchards exhibited moderate but stable pruning-residue yields, consistent with the structural uniformity characteristic of intensively managed systems. The measured dry-matter values and calorific properties align with earlier energetic assessments, confirming that apple residues constitute a dependable source of woody biomass with predictable combustion behaviour [10–14]. In contrast, pear orchards displayed substantially higher pruning yields, reflecting the species' vigorous growth habit, heterogeneous canopy structure, and management-specific influences such as crop-load regulation and training-system architecture. These results corroborate horticultural observations that pear biomass production is inherently more variable yet consistently greater than that of apple, reinforcing the need for species-sensitive sampling intensity and diameter-class resolution [16–21].

Quince, although generating the lowest pruning biomass among the three species, presents a stable energetic profile, with calorific values comparable to those of apple and pear. This consistency supports the inclusion of quince-derived woody residues in mixed-species biomass pools used for renewable energy production. However, the reduced vegetative growth and compact canopy architecture characteristic of quince necessitate adapted sampling strategies, particularly when estimating pruning yields in commercial orchards or nursery systems [22,25–28]. The alignment between rootstock-based energy-characterization studies and species-level assessments further substantiates quinces' suitability as a complementary biomass source within diversified orchard landscapes [29,30]. Taken together, the findings illustrate that robust biomass estimation in pome fruit orchards relies on integrating destructive and non-destructive measurements, species-specific allometric considerations, and moisture-corrected conversions. The convergence of energetic, environmental, and agronomic data demonstrates that apple, pear, and quince residues represent valuable renewable resources whose utilization potential extends beyond energy production to include carbon accounting, soil-amendment applications, and circular bioeconomy pathways. As shown across multiple studies, methodological standardization is critical for ensuring that biomass inventories are reproducible and scalable, enabling more accurate regional planning for bioenergy systems and sustainable orchard management frameworks [7–9,11,12,15].

The methodological approach and species-specific results presented here contribute to ongoing efforts to harmonize biomass assessment in perennial crop systems and offer a validated model for future research in orchard-based bioresource quantification.

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Review

THE ROLE OF POMOLOGY IN RURAL DEVELOPMENT IN ROMANIA: A REGIONAL AND ECONOMIC ANALYSIS OF THE FRUIT SECTOR (2020–2024)

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Keywords: *fruit growing, rural development, Romania, regional analysis, economic indicators, orchard area, fruit production, climate change, agricultural land use, fruit trees, sustainability, productivity*

Abstract: This article explores the role of fruit growing in shaping the economic and environmental landscape of rural Romania during the 2020–2024 period. Using a comprehensive set of official statistical data from the National Institute of Statistics (TEMPO database), the study examines both the economic performance and spatial dynamics of the fruit sector across Romania's development regions. The analysis includes production values, orchard surface areas, average yields, and the number of fruit trees by species. Regional disparities are highlighted, with particular attention to the South-Muntenia, North-West, and South-West Oltenia regions. The research also emphasizes the sector's contribution to sustainable rural development and resilience to climate change. By combining quantitative analysis with territorial mapping, this study provides evidence-based insights to support policy formulation in Romanian agriculture.

1. Introduction

Modern agriculture lies at the intersection of multiple challenges: climate pressures, economic shifts, and growing expectations for sustainability. In this context, fruit growing is gaining increasing importance as an agricultural sector with high economic and ecological potential, particularly for the rural regions of Romania. In an era in which information circulate very fast and they are time subject to the phenomenon of globalization, which makes the competition to become stronger and stronger, and decision-making is essential as a result of the complexity of economic phenomena, it is important that information to be of high quality and permanently aligned to legislative changes [1].

In this landscape shaped by digitalization and global economic competition, the rapid adaptation of agriculture is essential. The fruit-growing sector, in particular, can contribute both to diversifying income sources for small producers and to rural revitalization through the rational use of natural resources. As climate change becomes more evident and natural resources dwindle, significant pressure is being put on the agricultural sector. In this light, it is crucial to ask how we can make agriculture more sustainable and resilient in the face of these challenges [2].

In Romania, the effects of climate change are becoming increasingly evident, negatively impacting the stability of agricultural production. Traditionally favorable fruit-growing regions are facing prolonged droughts, hailstorms, and sudden climatic fluctuations, threatening the productivity of perennial crops. Romania has seen significant changes in climate patterns, with rising average temperatures and extreme weather events such as prolonged droughts and floods [3].

At the same time, soil (the fundamental resource for agriculture) requires a sustainable approach based on conservation, regeneration, and responsible use. Soil is the foundation of our food system. By conserving soils, we ensure that we have healthy and productive soils to support the food production needed for a growing population [4]. Global environmental problems have begun to become prevalent and have created the need for additional actions to raise public awareness, leading the international community to take timely and functional action, both internationally and nationally [5].

In this regard, European policies on green transition and the European Green Deal are becoming strategic tools for supporting smart and resilient agriculture. Although the usable agricultural area remains limited, modern technologies enable yield increases even on reduced land surfaces. This reality resulted in a high pressure on land use, even though land surface is limited, but grace to the advanced agricultural technologies from a smaller utilized land area it is possible to obtain more food [6].

In particular, fruit growing is not only an agricultural branch, but also a means of conserving biodiversity, reducing the carbon footprint, and promoting a circular agricultural model. The physiology of Carbon in fruit trees is of paramount importance in fruit production: from an evolutionary point of view, fruit trees aim to produce and disperse the highest amount of seed, the fruit being a very sophisticated vehicle for such dispersal [7]. These elements justify a detailed analysis of Romania's fruit-growing sector, both from an economic and territorial perspective, during the period 2020–2024. The present study aims to provide an in-depth understanding of regional trends and strategic opportunities for rural development through the lens of fruit cultivation.

2. Materials and methods

This study employs a quantitative approach based on the statistical analysis of key agricultural indicators relevant to the fruit-growing sector in Romania during the 2020–2024 period. The information was collected from official sources provided by the National Institute of Statistics (INS), retrieved from the TEMPO-Online database. The following datasets were used, identified by specific codes:

- **TEMPO_AGR115A** – Agricultural production: Fruit production by regions and species
- **TEMPO_AGR208A** – Economic accounts for agriculture (value of production at current prices)
- **TEMPO_AGR102B** – Agricultural land surface by usage categories and land improvement works (drainage)
- **TEMPO_AGR114A** – Total number of fruit trees by species and regions
- **TEMPO_AGR116A** – Average yield per tree (kg/tree) by species and regions

The research method consisted of longitudinal and comparative analysis, focusing on:

- the evolution of the economic value generated by fruit production;
- the dynamics of cultivated areas and basic infrastructure (drainage systems, improvements);
- the total number of fruit trees, by species and development regions;
- average productivity (kg/tree) and total production (tons).

The data were compiled and visualized through comparative charts, thematic maps, and regional tables to highlight temporal trends and geographic distributions of the analyzed phenomena.

Additionally, the statistical interpretation was complemented by academic sources and relevant institutional reports [8-11] to correlate the quantitative data with contextual factors (climatic, economic, and political).

The analysis targeted the NUTS II development regions of Romania (8 in total), in accordance with the European administrative structure, with interpretations aimed at identifying

territorial disparities and opportunities for the sustainable development of fruit growing in rural areas.

3. Results

The following section provides a detailed analysis of the evolution of Romania’s fruit-growing sector over the 2020–2024 period, based on key statistical indicators: the economic value of fruit production, cultivated areas, species structure, number of fruit trees, average productivity, and total production. All data were provided by the National Institute of Statistics (INS), the official and reliable source on Romanian agriculture.

The analysis considers both regional and temporal dimensions, with a focus on the dynamics within each of the eight NUTS II development regions, offering a comprehensive overview of the economic role of fruit growing in rural development. This statistical examination supports the assessment of the national fruit-growing potential and highlights specific regional disparities or opportunities.

The data are presented in a logical sequence, beginning with the economic value of fruit production, followed by cultivated areas and the species structure, then the number of trees and average yields, and concluding with the total production expressed in tons. The commentary highlights key trends, regional variations, stagnations or growth patterns, and includes, where relevant, academic references.

1) Economic accounts for agriculture (current prices)

Data on the value of fruit production in Romania, expressed at producer prices between 2020 and 2024, reveal a fluctuating trend, with a peak recorded in 2021 (7853.41 million lei), followed by a significant decline to 2024 (6729.75 million lei). This 14.3% decrease from the peak year suggests a potential instability within the fruit-growing sector, caused by multiple factors:

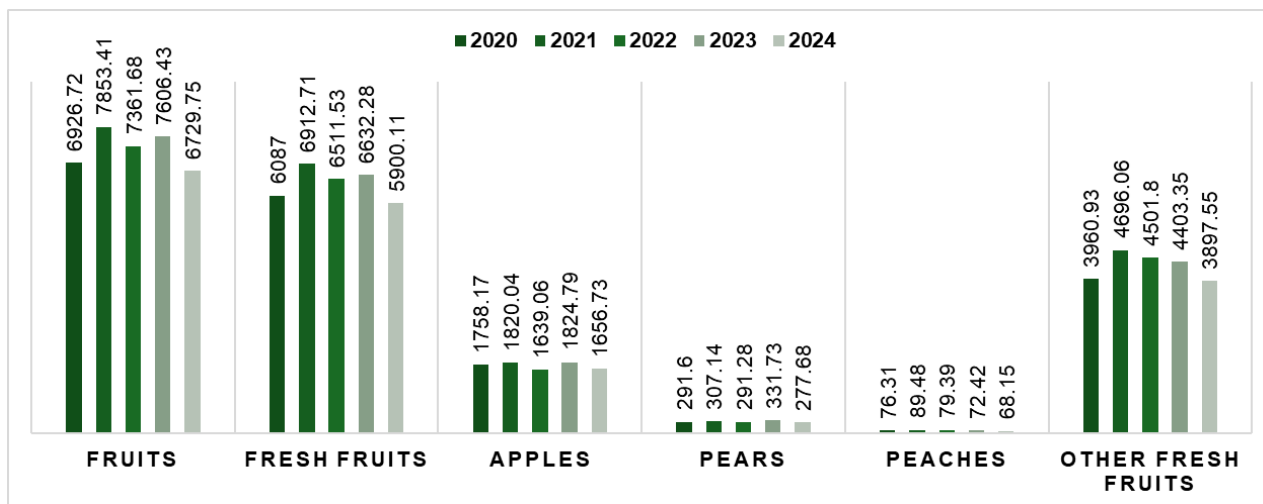


Figure 1. Representative chart of the economic accounts in agriculture, U.M. = Million lei [12]

a) Climatic factors such as drought, late spring frosts, or excessive rainfall during critical periods have negatively impacted production—an issue documented in the reports of the National Meteorological Administration [11] and confirmed by the European Environment Agency’s analyses on climate risks in agriculture [12].

b) Post-pandemic market volatility contributed to reduced demand in certain segments, especially for perishable fruits, due to the lack of solid collection and distribution mechanisms [13].

c) The rising prices of agricultural inputs (fertilizers, fuel, labor), as documented by the European Commission [14], have decreased producers’ net margins, affecting the profitability of fruit production.

Structure of economic value by category (2024):

- Fresh fruits have consistently accounted for the highest share (>87%) of the total value, confirming a strong focus on direct consumer-oriented production, with limited processing.
- Apples remain the species with the highest value contribution (1656.73 million lei in 2024), followed by the "other fresh fruits" category (3897.55 million lei), suggesting a sustained diversification of orchards.
- Pears and peaches, with a more modest contribution, recorded moderate declines, in line with the overall trend.

Implications for rural development:

The decrease in the economic value of fruit production has direct implications on:

- the incomes of farmers in rural areas;
- seasonal agricultural employment;
- investments in agricultural infrastructure (irrigation, processing).

Several European studies emphasize the need for technological modernization of the fruit sector to reduce economic volatility [15].

2) Area of land improved for fruit growing (2020–2024)

The data on orchard areas equipped with drainage works indicate a complete stagnation during the 2020–2024 period. All eight NUTS II development regions in Romania recorded no variation in the improved surfaces, signaling a lack of investment in basic infrastructure for intensive and sustainable fruit growing.

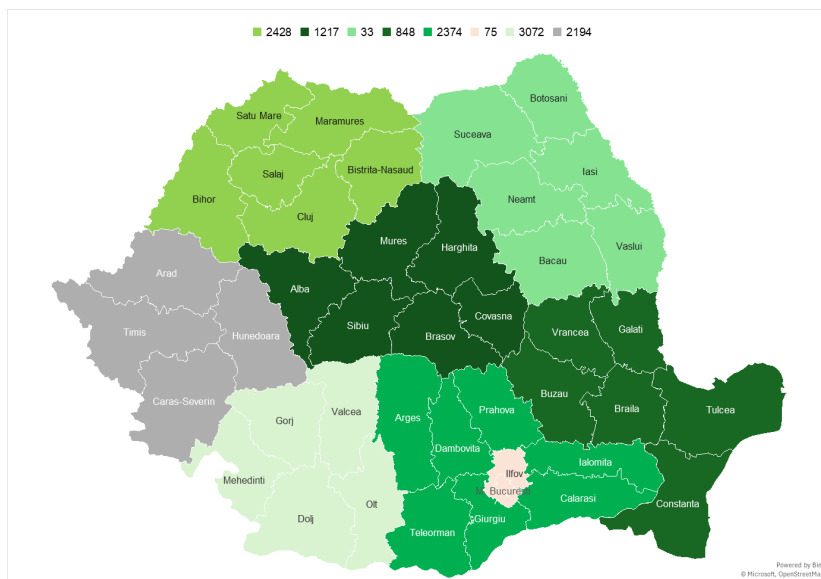


Figure 2. Representative map showing the surface of land improved for fruit growing, U.M. = Hectares [16]

a) The largest area improved for orchards and fruit shrubs is found in the SOUTH-WEST OLTENIA Region (3072 ha), followed by NORTH-WEST (2428 ha) and SOUTH-MUNTENIA (2374 ha).

b) In contrast, the NORTH-EAST Region records only 33 ha of improved land (an extremely low value), despite having a significant number of fruit trees, according to previous data. This imbalance indicates a possible disproportion between agricultural potential and existing infrastructure.

This stagnation is concerning in the context of climate change and the increasing frequency of extreme hydrological events (drought, waterlogging, floods). Drainage and dewatering works are essential for maintaining productivity in fruit plantations, especially in areas prone to excess moisture.

Table 1

Regional interpretation

Region	Area (ha)	Observations
South-West Oltenia	3072	National leader, high potential for expansion
North-West & South Muntenia	2428 / 2374	Large area, but stagnant
North-East	33	Underutilized potential
Bucharest-Ilfov	75	Low economic relevance
Center / West	1217 / 2194	National average
South-East	848	Below the national average

(data taken from Fig. 2)

The lack of expansion of these areas reflects a blockage in investments related to agricultural land infrastructure, despite the availability of European funds aimed at modernizing agriculture through the NRDP or the NSP 2023–2027.

According to a report by the European Court of Auditors, Romania faces significant difficulties in efficiently absorbing funds allocated for agricultural infrastructure [10].

3) Total number of fruit trees by development region

The total number of fruit trees in Romania showed a moderately positive trend during the period 2020–2024, peaking at 16.46 million trees in the North-West Region, with stabilization trends observed in other regions.

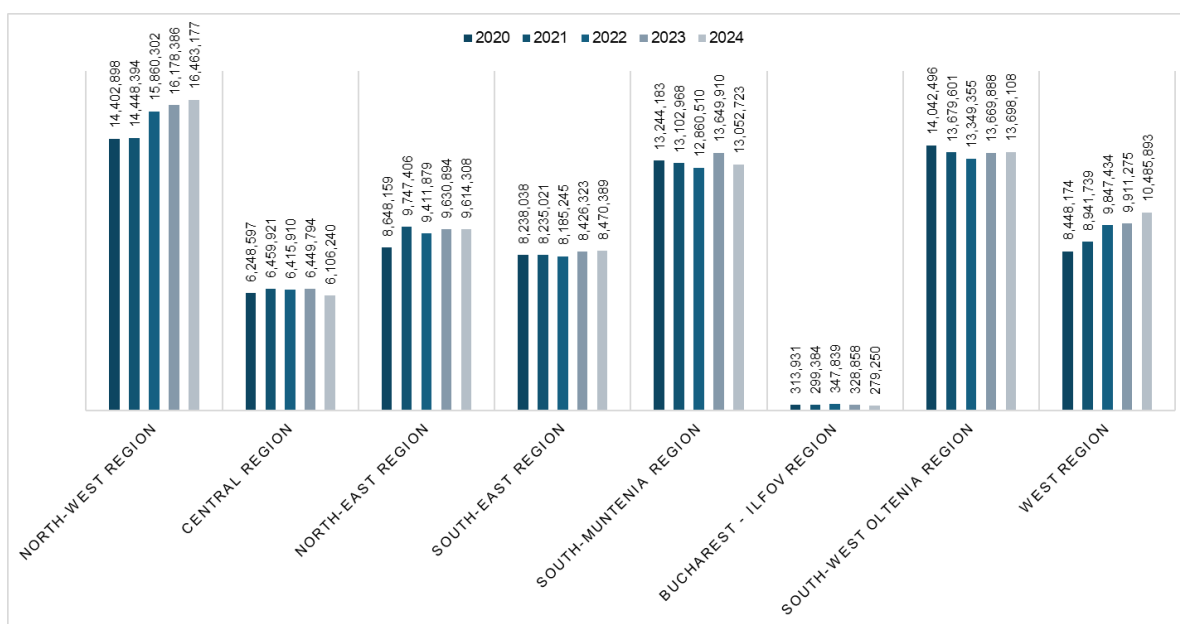


Figure 3. Representative chart showing the total number of fruit trees in Romania, U.M.: number [16]

The North-West, South-West Oltenia, and South-Muntenia regions stand out as major hubs of national fruit growing. The species structure provides useful insights into diversification, regional specializations, and adaptive challenges.



Fig. 4. Representative chart showing the total number of plum trees in Romania, U.M.: number [16]

The plum tree remains the most widespread fruit species in Romania, with over 8,6 million trees recorded in South-West Oltenia in 2024 (a national record). Although slightly declining in this region compared to 2020, the numerical stability indicates a strong potential for maintaining its position as the leading fruit export. In contrast, the Bucharest–Ilfov region lost nearly 90,000 trees over five years, reflecting the trend of urbanization and pressure on agricultural land.

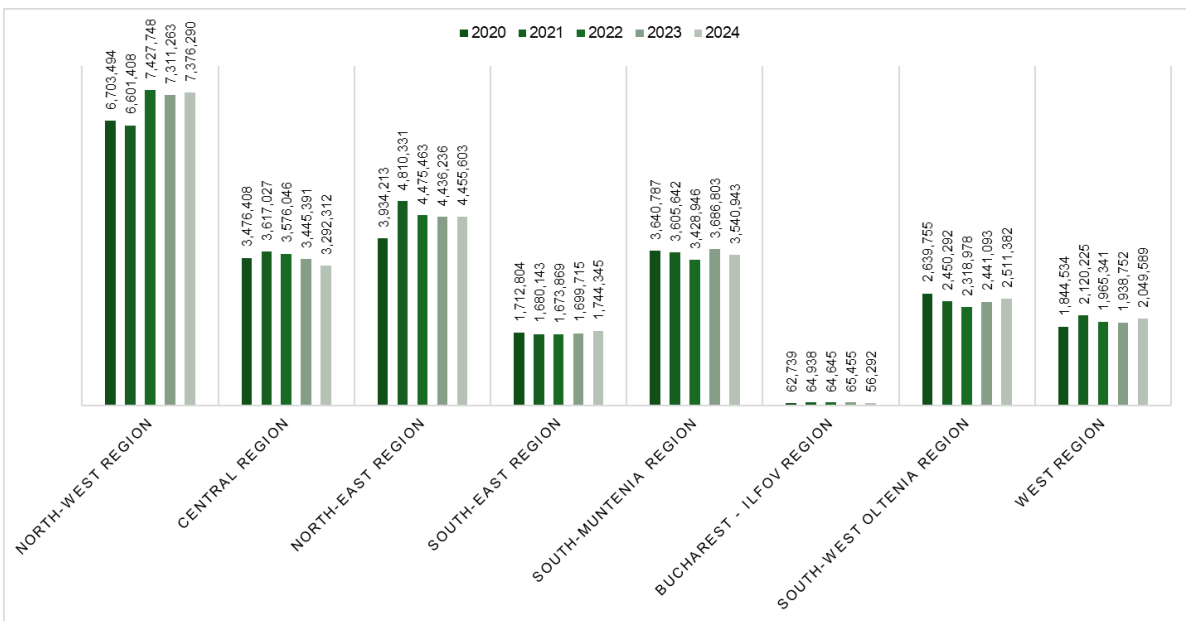


Figure 5. Representative chart showing the total number of apple trees in Romania, U.M.: number [16]

The apple ranks second in Romania's national fruit tree structure. The North-West region surpassed 7,4 million apple trees in 2022, followed by the North-East and South-Muntenia regions. In the Center, although a slight decline is noticeable in recent years, the level remains significant. This distribution confirms the favorable climate for apple cultivation in Transylvania and Moldova. Declines in Oltenia may indicate a shift by farmers toward other species better adapted to drought conditions.

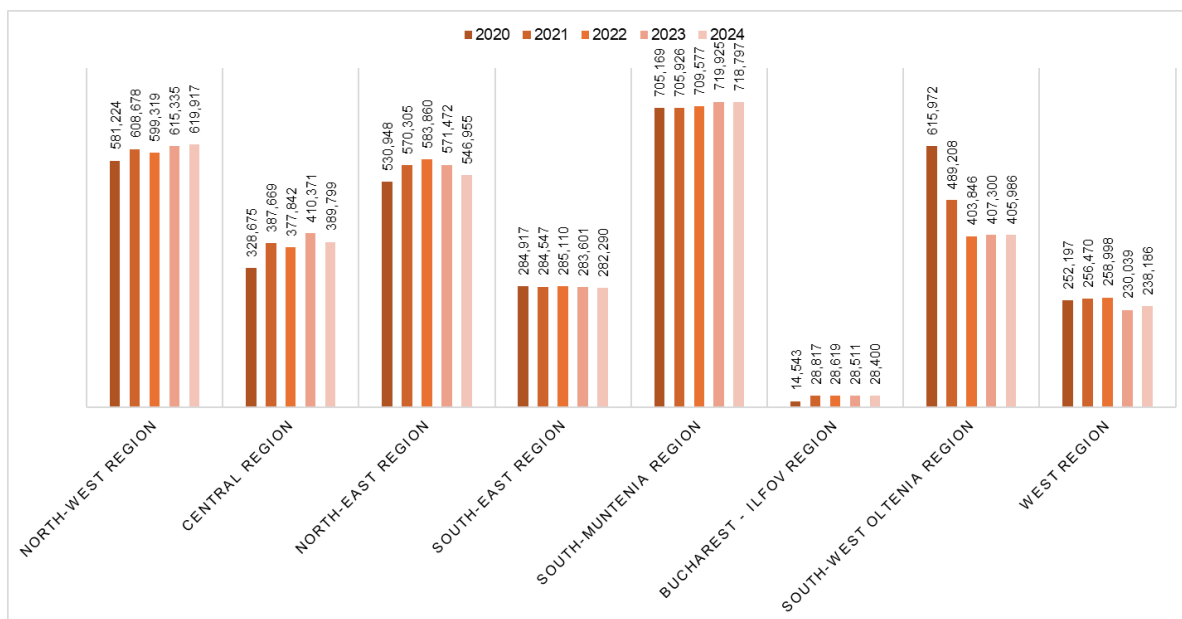


Figure 6. Representative chart showing the total number of pear trees in Romania, U.M.: number [16]

Pear trees register stable but modest figures at the national level. South-Muntenia is the main center for this species, with over 71,000 trees in 2024. The downward trend in Oltenia and the Center region may be linked to the species' sensitivity to diseases and extreme moisture conditions.

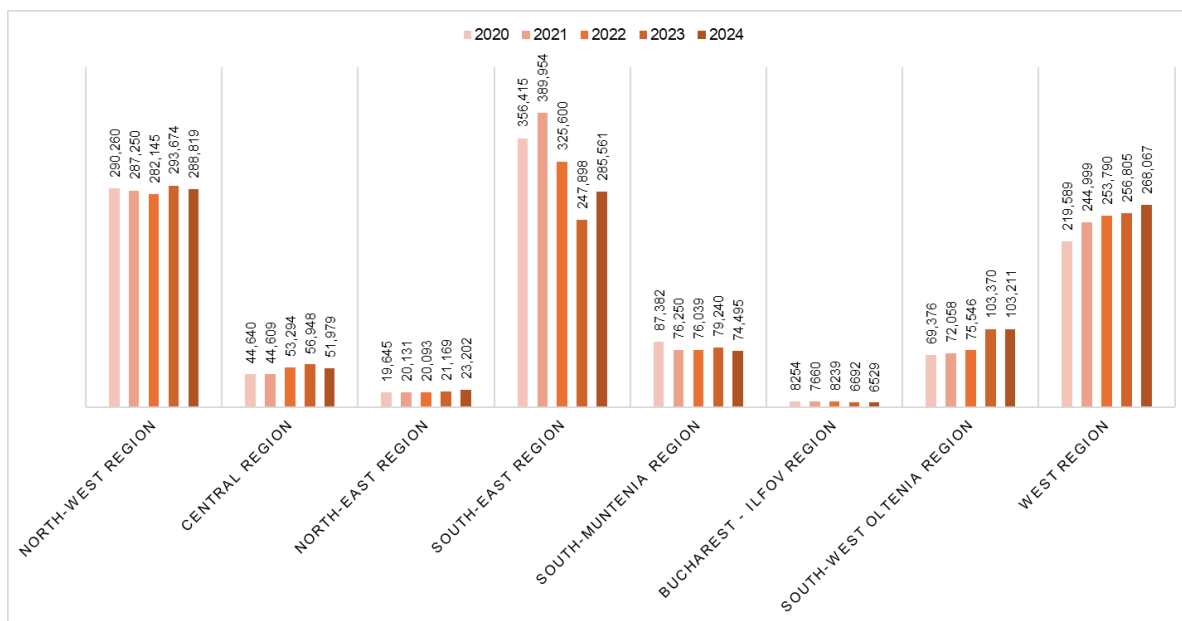


Fig. 7. Representative chart showing the total number of peach trees in Romania, U.M.: number [16]

The number of peach trees has remained relatively stable but modest, with dominant figures in the South-East and West regions. The West region has steadily increased its orchard stock, reaching over 268,000 peach trees in 2024. In contrast, South-Muntenia and Bucharest-Ilfov show a clear decline, suggesting issues related to profitability and climate adaptability.

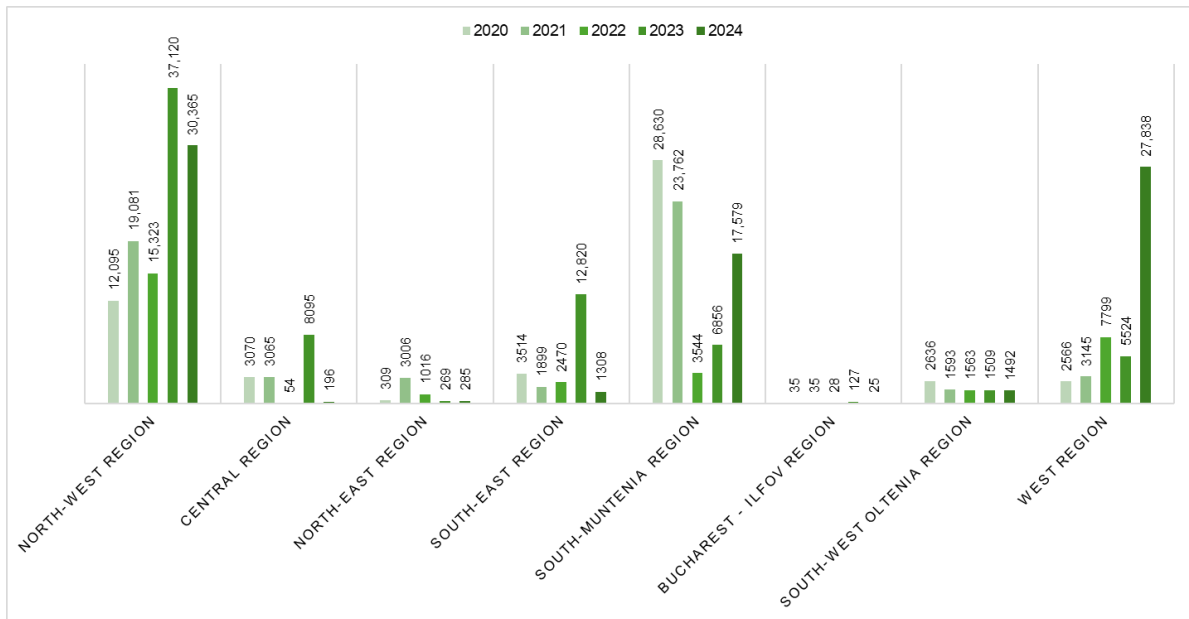


Figure 8. Representative chart showing the total number of nectarine trees in Romania, U.M.: number [16]

This species is much less present in the national fruit-growing landscape. A spectacular increase is observed in the West Region in 2024 (27,838 trees), while other regions, such as Bucharest–Ilfov, maintain insignificant values. A temporary spike is noticeable in the South-East in 2023 (12,820 trees), possibly associated with short-term subsidy support or a climatically favorable year.

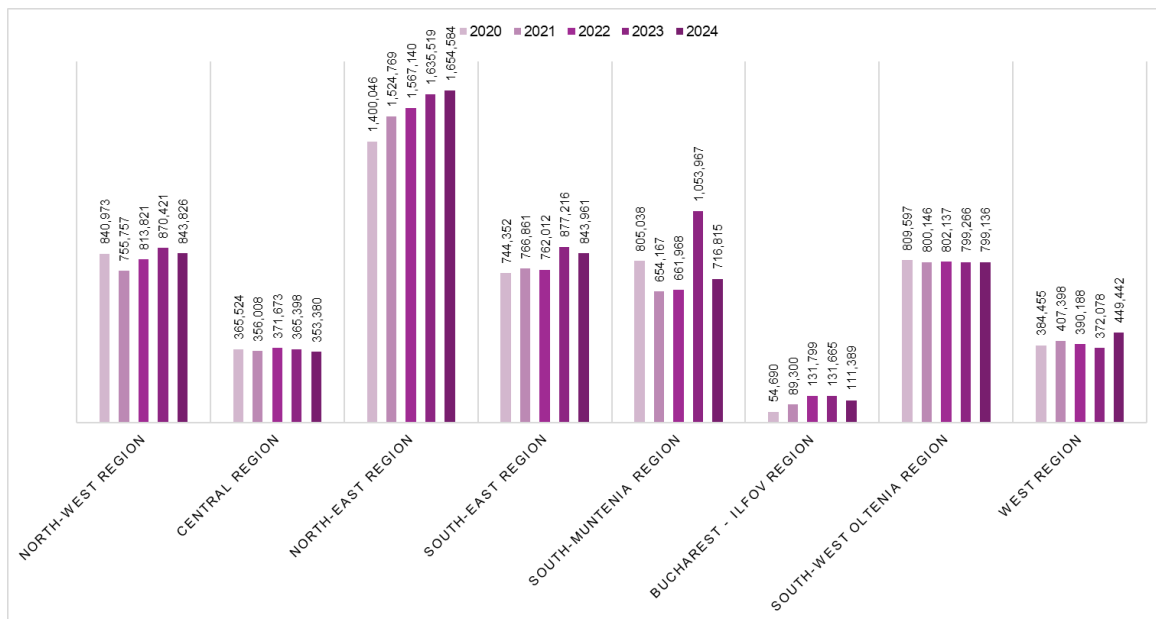


Figure 9. Representative chart showing the total number of cherry and sour cherry trees in Romania, U.M.: number [16]

There is a steady increase in the North-East, reaching 1,65 million trees in 2024, positioning the region as the main producer. In contrast, stagnation or even regression is recorded in the West and South-Muntenia regions. This species appears to be affected by climate change and by the lack of modern technologies for early frost protection [8].

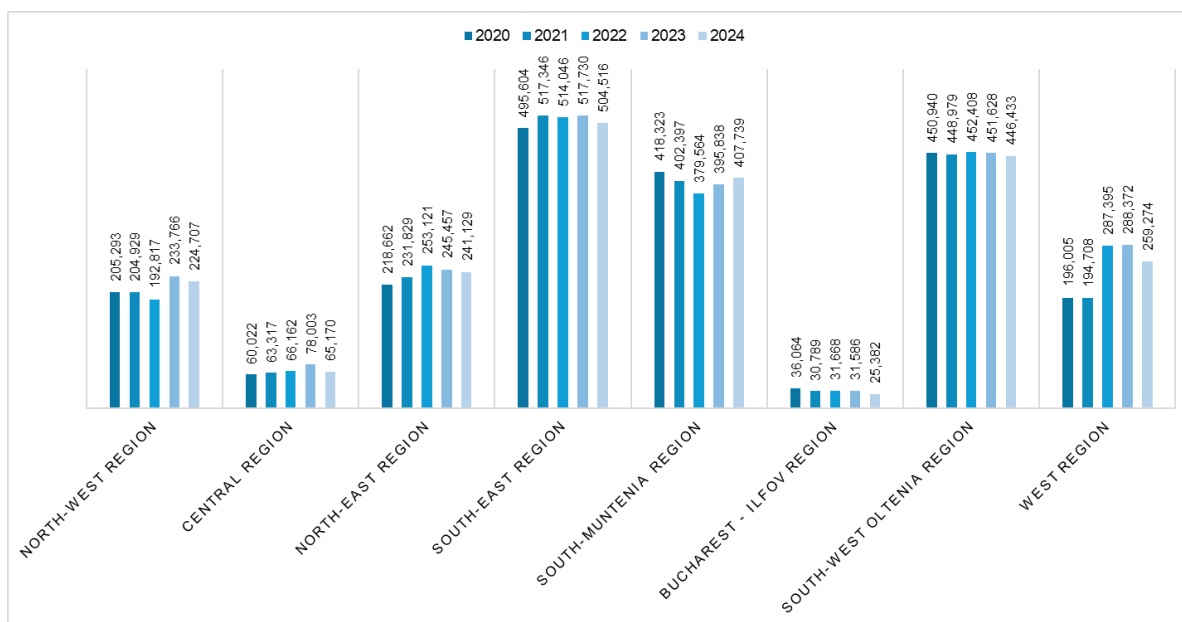


Fig. 10. Representative chart showing the total number of apricot and wild apricot trees in Romania, U.M.: number [16]

A species with potential but showing a fluctuating evolution. In the West, a significant peak was recorded in 2022–2023 (over 287,000 trees), followed by a decline in 2024. The South-East and Oltenia regions maintain stable values, confirming good adaptability in southern areas. The trend suggests maintaining moderate areas without significant expansion.

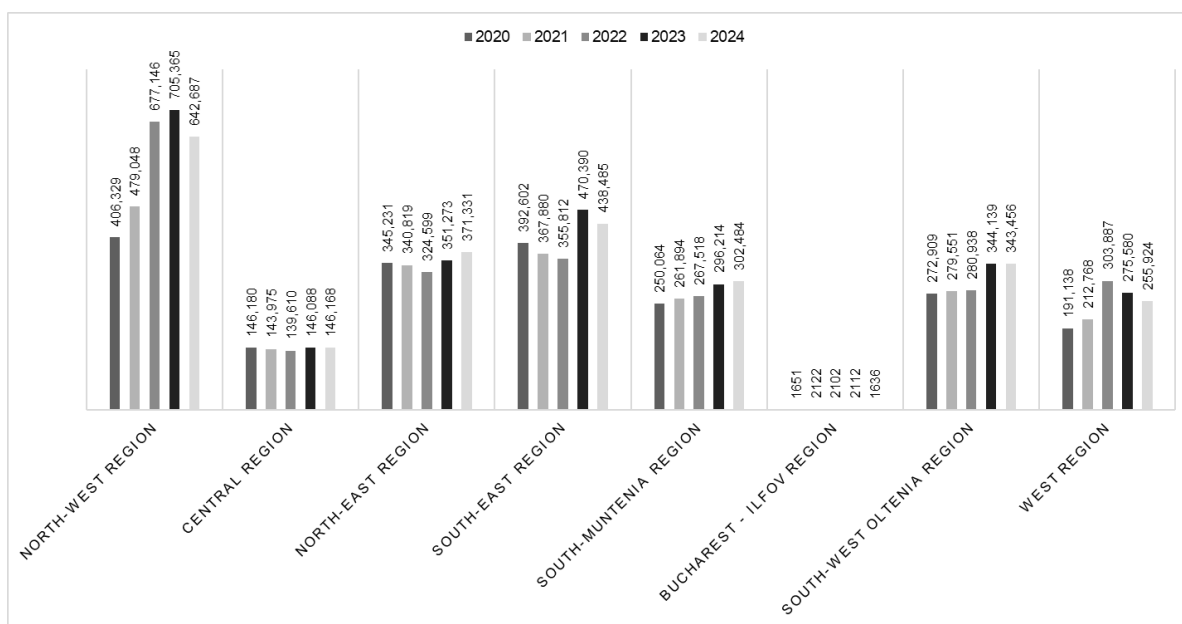


Figure 11. Representative chart showing the total number of walnut trees in Romania, U.M.: number [16]

Walnut cultivation is on the rise in the North-West regions, where a growth of over 300% was recorded in 2022–2023 compared to 2020, reaching 705,000 trees. This expansion may be linked to increased demand on both domestic and international markets for nuts. Walnut is considered a strategic crop, with high yield and long exploitation life [17].

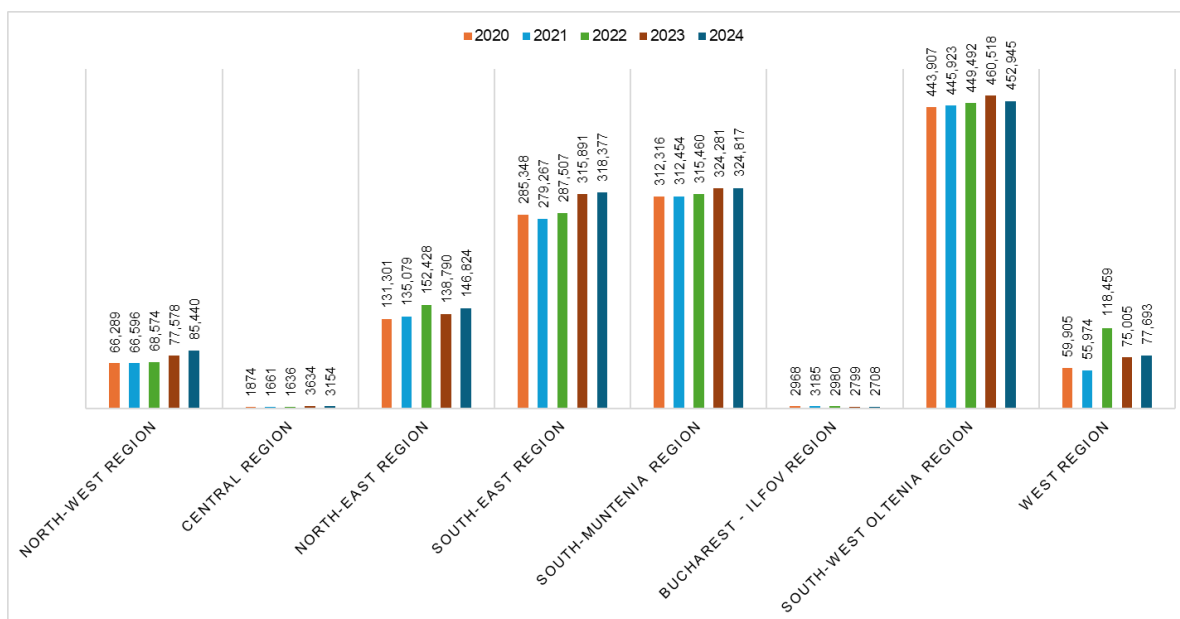


Figure 12. Representative chart showing the total number of "other fruit trees" in Romania, U.M.: number [16]

The "other fruit trees" category includes almonds, chestnuts, and mixed species. The South-West Oltenia region leads with over 452,000 trees in 2024, followed by South-Muntenia. There appears to be a strategic shift toward diversification, in the context of climate change and niche market demand.

The distribution of fruit trees in Romania reveals a clear regional concentration, with emerging trends of species specialization based on pedoclimatic conditions. We observe a slight reconfiguration of the fruit-growing landscape, influenced by climate, market dynamics, and access to financial support. These developments confirm the potential of fruit growing to support rural development, while also highlighting the need for technological advancement, farmer associations, and adaptive diversification.

4) Fruit tree productivity in Romania

Between 2020 and 2024, the productivity of fruit trees in Romania registered a slow but steady decline in most regions, indicating a series of agronomic, technological, and climatic limitations affecting the performance of the fruit-growing sector.

Table 2

Representative table showing the productivity of the fruit-growing section

Categories of fruits	Development regions	2020	2021	2022	2023	2024
		U.M.: Kg/tree				
Overall count	NORTH-WEST Region	21	22	18	17	16
	CENTRAL Region	19	20	17	17	16
	NORTH-EAST Region	21	22	20	20	19
	SOUTH-EAST Region	18	20	18	17	16
	SOUTH-MUNTENIA Region	27	29	28	26	24
	BUCHAREST - ILFOV Region	8	9	6	7	8
	SOUTH-WEST OLTENIA Region	23	24	19	18	17
	WEST Region	16	17	13	12	11
Plum trees	NORTH-WEST Region	25	27	21	21	20
	CENTRAL Region	20	21	19	17	17
	NORTH-EAST Region	24	25	22	21	21
	SOUTH-EAST Region	21	24	20	18	17

Categories of fruits	Development regions	2020	2021	2022	2023	2024	
		U.M.: Kg/tree					
	SOUTH-MUNTENIA Region	27	28	23	22	20	
	BUCHAREST - ILFOV Region	5	10	7	10	11	
	SOUTH-WEST OLTENIA Region	23	24	19	18	17	
	WEST Region	16	17	15	14	13	
Apple trees	NORTH-WEST Region	20	21	16	15	15	
	CENTRAL Region	19	20	17	17	17	
	NORTH-EAST Region	22	22	23	23	21	
	SOUTH-EAST Region	18	22	19	19	16	
	SOUTH-MUNTENIA Region	31	36	44	41	37	
	BUCHAREST - ILFOV Region	7	8	5	5	6	
	SOUTH-WEST OLTENIA Region	28	31	25	22	20	
	WEST Region	19	18	17	15	14	
	Pear trees	NORTH-WEST Region	14	14	11	11	11
		CENTRAL Region	11	11	10	9	9
NORTH-EAST Region		15	16	13	13	13	
SOUTH-EAST Region		15	15	13	13	12	
SOUTH-MUNTENIA Region		21	21	19	19	18	
BUCHAREST - ILFOV Region		11	7	7	7	6	
SOUTH-WEST OLTENIA Region		13	16	16	15	14	
WEST Region		13	13	11	12	11	
Peach trees	NORTH-WEST Region	22	15	12	11	10	
	CENTRAL Region	21	20	16	15	15	
	NORTH-EAST Region	20	19	16	15	13	
	SOUTH-EAST Region	8	8	10	12	10	
	SOUTH-MUNTENIA Region	15	18	17	15	15	
	BUCHAREST - ILFOV Region	13	12	8	10	9	
	SOUTH-WEST OLTENIA Region	14	15	12	9	8	
	WEST Region	13	11	9	9	9	
Nectarine trees	NORTH-WEST Region	14	18	23	21	15	
	CENTRAL Region	2	2	19	1	20	
	NORTH-EAST Region	19	3	25	22	21	
	SOUTH-EAST Region	13	18	14	19	19	
	SOUTH-MUNTENIA Region	6	6	14	13	6	
	BUCHAREST - ILFOV Region	29	29	36	8	40	
	SOUTH-WEST OLTENIA Region	13	18	14	14	15	
	WEST Region	13	15	7	10	4	
Cherry and sour cherry trees	NORTH-WEST Region	13	15	12	11	10	
	CENTRAL Region	12	13	10	10	10	
	NORTH-EAST Region	14	15	12	13	12	
	SOUTH-EAST Region	13	13	12	10	10	
	SOUTH-MUNTENIA Region	14	17	14	9	12	
	BUCHAREST - ILFOV Region	12	9	5	5	6	
	SOUTH-WEST OLTENIA Region	16	16	13	13	12	

Categories of fruits	Development regions	2020	2021	2022	2023	2024
		U.M.: Kg/tree				
Apricot and wild apricots trees	WEST Region	13	13	11	11	10
	NORTH-WEST Region	15	11	10	11	11
	CENTRAL Region	14	14	11	10	11
	NORTH-EAST Region	15	16	14	13	13
	SOUTH-EAST Region	12	12	11	11	11
	SOUTH-MUNTENIA Region	14	14	13	13	11
	BUCHAREST - ILFOV Region	10	12	11	10	12
	SOUTH-WEST OLTENIA Region	16	17	14	14	13
	WEST Region	8	8	5	6	7
Walnut trees	NORTH-WEST Region	25	28	24	26	22
	CENTRAL Region	30	34	32	31	30
	NORTH-EAST Region	26	27	25	24	22
	SOUTH-EAST Region	18	22	21	18	20
	SOUTH-MUNTENIA Region	30	29	27	25	23
	BUCHAREST - ILFOV Region	12	18	16	16	12
	SOUTH-WEST OLTENIA Region	26	26	24	21	21
	WEST Region	27	27	17	20	19
Other trees	NORTH-WEST Region	22	25	22	20	16
	CENTRAL Region	21	23	21	15	16
	NORTH-EAST Region	24	25	21	22	21
	SOUTH-EAST Region	19	20	18	16	16
	SOUTH-MUNTENIA Region	28	28	26	25	23
	BUCHAREST - ILFOV Region	20	21	19	19	19
	SOUTH-WEST OLTENIA Region	20	20	18	18	17
	WEST Region	23	24	10	16	16

Generalized yield decline:

- The national average (overall yield of all fruits) shows a decrease in nearly all regions:
 - o **North-West:** from 21 kg/tree in 2020 → to 16 kg/tree in 2024
 - o **South-Muntenia**, which initially had the highest performance (27–29 kg/tree), drops to 24 kg/tree in 2024
 - o **The Western** region records the lowest yields in 2024 (11 kg/tree)

This decline is concerning, especially given that the area under cultivation and the number of trees increased in some regions (see previous sections). It points to decreasing agricultural efficiency, often linked to:

- aging orchards
- lack of irrigation systems
- non-compliance with modern pruning, fertilization, or plant protection technologies

Climatic and technological influence:

According to reports by the European Commission, Romania faces technological gaps in fruit growing, particularly in terms of adaptation to water stress, frost protection, and the use of high-performance varieties [9]. Furthermore, climate change has intensified phenomena such as water stress and temperature variability, especially affecting sensitive species like peaches and pears.

Table 3

Notable regional differences

Region	Key observation
South Muntenia	It remains the leader in terms of yield (24–44 kg/tree in some years), thanks to higher investments and fruit-growing tradition.
Bucharest–Ilfov	Low productivity (<10 kg/tree), influenced by urbanization and land fragmentation
Northeast and Oltenia	Above-average yields (20–23 kg/tree), but with large variations between years
West	Lowest productivity in 2024 (11 kg/tree), despite the large number of trees

- **Apple trees in South-Muntenia** reached a peak yield of **44 kg/tree in 2022**, the highest recorded value in the entire dataset, suggesting the application of intensive technologies in certain orchards.
- **Walnut trees remain highly productive** in the **Centre and North-West** regions, surpassing **30 kg/tree** in some years.
- **Nectarines and peaches show high year-to-year variability**, making them economically risky without investment in climate protection measures.

The decline in average productivity is a red flag for the future of Romanian fruit growing. Without modernization efforts (irrigation systems, high-performance varieties, technical advisory services), even the traditionally strong regions may lose competitiveness. **Integration into short food supply chains** and the **adoption of precision agriculture** could be viable strategies to revive yields under the new Common Agricultural Policy framework.

5) Fruit production by tree species

An analysis of total fruit production during the 2020–2024 period reveals a generalized **decline in output volumes**, visible across all regions and for most fruit species, despite a relatively stable number of trees. This trend reinforces the earlier conclusions about decreasing productive efficiency and highlights the **sector’s increasing vulnerabilities**.

Table 4

Representative table showing production by tree species

Categories of fruits	Development regions	2020	2021	2022	2023	2024
		U.M.: Tons				
Overall count	NORTH-WEST Region	322,553	333,061	288,584	288,236	270,799
	CENTRAL Region	118,066	127,453	108,087	108,661	101,231
	NORTH-EAST Region	180,018	211,746	193,940	197,839	181,444
	SOUTH-EAST Region	148,544	167,626	145,515	140,186	132,526
	SOUTH-MUNTENIA Region	358,545	380,824	356,710	355,005	315,308
	BUCHAREST - ILFOV Region	3357	3710	3111	3118	3217
	SOUTH-WEST OLTENIA Region	319,390	329,624	257,505	247,931	233,457
	WEST Region	140,322	150,506	130,640	125,032	121,553
Plum trees	NORTH-WEST Region	132,407	142,682	123,282	122,356	114,351

Categories of fruits	Development regions	2020	2021	2022	2023	2024
		U.M.: Tons				
	CENTRAL Region	36,010	38,254	33,396	33,295	30,629
	NORTH-EAST Region	49,222	53,079	44,691	47,429	44,785
	SOUTH-EAST Region	81,296	92,210	79,626	71,813	69,086
	SOUTH-MUNTENIA Region	190,032	196,882	159,696	152,986	139,782
	BUCHAREST - ILFOV Region	623	684	537	573	474
	SOUTH-WEST OLTENIA Region	197,362	205,343	158,236	152,793	143,616
	WEST Region	82,922	90,224	77,557	74,627	69,960
	Apple trees	NORTH-WEST Region	137,085	138,698	115,734	111,568
CENTRAL Region		67,320	72,469	59,413	59,857	56,225
NORTH-EAST Region		85,685	107,993	104,510	104,144	92,698
SOUTH-EAST Region		31,618	37,449	31,643	32,520	28,398
SOUTH-MUNTENIA Region		114,539	131,039	149,283	151,107	130,485
BUCHAREST - ILFOV Region		448	507	349	354	351
SOUTH-WEST OLTENIA Region		74,437	76,112	57,491	53,050	50,124
WEST Region		34,986	38,363	33,500	29,583	29,002
Pear trees	NORTH-WEST Region	7933	8326	6832	6702	6573
	CENTRAL Region	3746	4383	3735	3607	3567
	NORTH-EAST Region	7712	9372	7684	7555	7222
	SOUTH-EAST Region	4132	4214	3689	3627	3356
	SOUTH-MUNTENIA Region	14,726	14,741	13,714	13,695	12,721
	BUCHAREST - ILFOV Region	164	196	194	187	160
	SOUTH-WEST OLTENIA Region	8057	7990	6505	6293	5643
	WEST Region	3187	3370	2877	2742	2640
Peach trees	NORTH-WEST Region	6347	4309	3344	3125	2833
	CENTRAL Region	931	910	827	859	803
	NORTH-EAST Region	385	391	331	326	313
	SOUTH-EAST Region	2960	3238	3158	2877	2849
	SOUTH-MUNTENIA Region	1326	1403	1302	1224	1150
	BUCHAREST - ILFOV Region	106	91	68	66	60

Categories of fruits	Development regions	2020	2021	2022	2023	2024	
		U.M.: Tons					
Categories of fruits	SOUTH-WEST OLTENIA Region	989	1055	898	906	859	
	WEST Region	2850	2618	2352	2383	2369	
	NORTH-WEST Region	170	339	355	766	451	
Nectarine trees	CENTRAL Region	6	6	1	6	4	
	NORTH-EAST Region	6	9	25	6	6	
	SOUTH-EAST Region	46	35	34	249	25	
	SOUTH-MUNTENIA Region	178	142	51	89	113	
	BUCHAREST - ILFOV Region	1	1	1	1	1	
	SOUTH-WEST OLTENIA Region	33	29	22	21	22	
	WEST Region	34	47	58	58	103	
	Cherry and sour cherry trees	NORTH-WEST Region	10,954	11,235	9457	9799	8696
		CENTRAL Region	4228	4508	3745	3751	3591
NORTH-EAST Region		20,095	22,471	19,537	20,896	19,594	
SOUTH-EAST Region		9670	10,292	8836	9178	8772	
SOUTH-MUNTENIA Region		11,414	11,154	9457	9873	8510	
BUCHAREST - ILFOV Region		629	819	640	684	683	
SOUTH-WEST OLTENIA Region		12,576	12,747	10,692	10,359	9821	
WEST Region		5171	5364	4213	4154	4285	
Apricot and wild apricots trees	NORTH-WEST Region	3006	2336	1974	2635	2402	
	CENTRAL Region	857	886	758	778	710	
	NORTH-EAST Region	3194	3697	3497	3207	3036	
	SOUTH-EAST Region	5785	6090	5474	5461	5393	
	SOUTH-MUNTENIA Region	5894	5724	5002	5260	4554	
	BUCHAREST - ILFOV Region	377	375	344	312	302	
	SOUTH-WEST OLTENIA Region	7279	7476	6244	6343	5806	
	WEST Region	1574	1522	1348	1630	1725	
Walnut trees	NORTH-WEST Region	10,118	13,303	16,169	18,076	13,904	
	CENTRAL Region	4359	4946	4534	4570	4446	
	NORTH-EAST Region	8874	9248	8211	8485	8261	

Categories of fruits	Development regions	2020	2021	2022	2023	2024
		U.M.: Tons				
Fruits	SOUTH-EAST Region	7254	8199	7354	8429	8757
	SOUTH-MUNTENIA Region	7526	7637	7140	7420	6948
	BUCHAREST - ILFOV Region	19	39	33	34	20
	SOUTH-WEST OLTENIA Region	7085	7240	6855	7343	7257
	WEST Region	5107	5684	5036	5617	4905
	Other trees	NORTH-WEST Region	1791	2190	2179	3296
CENTRAL Region		244	737	1345	1621	922
NORTH-EAST Region		3274	3619	3423	3418	3370
SOUTH-EAST Region		5684	5759	5418	5692	5670
SOUTH-MUNTENIA Region		9091	9539	8785	9273	8725
BUCHAREST - ILFOV Region		59	75	63	61	59
SOUTH-WEST OLTENIA Region		9084	9468	8573	8709	8448
WEST Region		3453	2424	3093	3510	5903

General trends:

- **Total national fruit production recorded a decline of up to 15%** in some regions between 2021 and 2024:
 - o **North-West:** from **333,061 tonnes (2021)** → **270,799 tonnes (2024)**
 - o **South-Muntenia:** from **380,824 tonnes** → **315,308 tonnes**
 - o **West:** from **150,506 tonnes** → **121,553 tonnes**
- Only the **Bucharest–Ilfov** region shows slight **stability** (around **3000–3200 tonnes**), but at **very low values** compared to the other regions.

Table 5

Representative table showing comparative regional situation (2024)

Region	Total production (tons)	Remarks
South Muntenia	315,308	Leader, but in sharp decline
North-West	270,799	Second in terms of volume, with significant declines
South-West Oltenia	233,457	Maintains strong position despite decline
North-East	181,444	Relatively stable
South-East	132,526	Constantly decreasing
West	121,553	Loss of over 20% in 3 years
Center	101,231	Poor yields relative to surface area
Bucharest–Ilfov	3217	Modest contribution, justified by the high degree of urbanization

(data taken from Table 4)

Top fruit species by volume:

- **Plums** clearly dominate:
 - **South-Muntenia** (139,782 t), **Oltenia** (143,616 t), **North-West** (114,351 t)
 - This confirms the national orchard structure and the strong processing potential (e.g., plum brandy, plum jam)
- **Apples** are concentrated in **North-West** and **South-Muntenia**:
 - High values but with a clear downward trend – e.g., South-Muntenia drops from **151,107 t (2023) → 130,485 t (2024)**
- **Cherries, sour cherries, apricots, and walnuts – species with stable but much lower production** compared to plums/apples.

Strategic Observations:

1. **The disconnect between the number of trees and total production** confirms issues related to orchard management, lack of irrigation, and aging plantations [15].
2. **Production by species confirms the dominant role** of South-Muntenia and Oltenia in fruit growing, while regions like the **West** and **Center** are affected by low yields.
3. **The absence of a vertical integration strategy** (cultivation – harvesting – processing – marketing) results in economic losses, especially for **perishable fruits** like peaches and sour cherries.

4. Discussion

The total fruit production in Romania reflects a mixed reality: although the orchard infrastructure is widespread and regionally diversified, economic performance and production volumes are consistently declining. This highlights the urgent need for orchard modernization and adaptation to climate change.

The detailed analysis of economic, quantitative, and regional indicators for Romania's fruit sector during 2020–2024 reveals a notable gap between the potential of fruit growing and its actual performance. Although Romania has favorable agro-pedoclimatic resources and a strong tradition in fruit cultivation, the data shows a decline in productivity and overall economic value, particularly after 2021 [18-20].

Several regions (South-Muntenia, South-West Oltenia, and North-West) continue to host the highest concentration of fruit production and orchard numbers, but their performance is hindered by factors such as lack of investment in irrigation infrastructure, stagnation in plantation modernization, and the increasing impact of climate change. The production per tree and regional yields have visibly declined, indicating a growing drop in agricultural efficiency.

Notably, the decline in total fruit production volume, alongside increasing or stable orchard areas, confirms the existence of structural and functional issues in the production and value chain. Without strategic intervention (based on digitalization, smart irrigation, and market access) fruit growing risks losing its central economic role in rural development.

5. Conclusions

This study highlighted the importance of fruit growing in the socio-economic dynamics of rural Romania between 2020 and 2024, through a rigorous quantitative and regional analysis of relevant agricultural indicators. The results underline a complex reality: Romania holds significant potential for fruit cultivation, unevenly distributed across regions, yet its capitalization is limited by a series of persistent challenges.

On one hand, the **South-Muntenia, South-West Oltenia, and North-West** regions stand out through large areas cultivated with fruit trees and high production volumes, confirming both tradition and local agricultural specialization. On the other hand, the **constant decline in yields per tree, reduced economic value generated by fruits, and stagnation in managed areas** indicate a functional decline in the efficient exploitation of orchards.

This situation can be attributed to multiple factors: **climate change, aging plantations, insufficient investment in modern technologies**, and the **lack of a coherent strategy** for capitalizing production at both local and national levels. Moreover, the **excessive fragmentation of agricultural holdings** and the **absence of producer associations** hinder the competitiveness of the fruit-growing sector in relation to both domestic and international market demands.

For fruit cultivation to maintain and expand its role in the **sustainable development of Romanian rural areas**, coordinated interventions are necessary, including:

- **technological modernization of plantations;**
- **support for processing and storage infrastructure;**
- **encouragement of associative forms;**
- **adaptation to new climate conditions through resistant varieties and efficient irrigation systems.**

In the absence of clear strategic measures, Romanian fruit growing risks becoming an **underutilized agricultural branch**, despite the natural and human resources at its disposal.

Conflicts of interest: The authors declare no conflict of interest.

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METHODS AND TECHNOLOGIES FOR BIOMASS ENERGY RECOVERY

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Abstract

Limited energy resources and fluctuating oil prices, as well as recent environmental regulatory changes in industrialized countries, are directing research toward alternative resources and innovative industrial technologies for the production of energy and semi-finished products for the chemical industry. In this regard, forestry and agricultural resources and residues are gaining increasing interest, as they are currently considered resources only for wood processing, paper, food or basic combustion. The energy potential of biomass in Romania enables the development of industrial sectors for valorizing biomass through renewable fuels and co-products with economic value. In this research, we present the most important methods and technologies for biomass energy recovery.

1. Introduction

Biomass comprises all substances resulting from biological processes, including plant and animal matter growing on land, in water, or on aquatic surfaces; it encompasses agricultural products, residues from farming activities, and waste from crop processing such as cereal straw and by-products from beer, starch or sugar manufacture [1]. Biomass can moreover be generated from isolated microbial cells—yeasts, molds, or bacteria—employed in agro-industrial applications for their protein content in human and animal nutrition, as well as for the production of pharmaceutical compounds such as antibiotics [1].

The term biomass covers a broad spectrum of products, by-products and waste derived from forestry, agriculture, livestock systems, municipal and industrial sources. Biomass has served as an energy source since the discovery of fire and continues to provide food, energy, building materials, paper, medicines and chemical intermediates. As solar energy accumulated in chemical form within organic matter, biomass represents one of the most diversified and valuable natural resources on the planet [1].

In a highly industrialized and globalized context, the decline of fossil fuel reserves and increasing greenhouse gas emissions pose both threats and drivers for sustainability at multiple scales. Current projections estimate that by 2035, approximately 16% of global energy demand may originate from renewable resources such as biomass [1]. Responding to these trends, the present study examines selected applications of pyrolysis as a non-polluting technological route for biomass valorization, converting feedstock into biofuels and industrial co-products [2].

Recent reviews highlight that besides conventional thermochemical routes (pyrolysis, combustion, gasification), modern technologies such as hydrothermal liquefaction (HTL) and catalytic co-hydropyrolysis allow processing of wet and complex biomass into energy-dense biocrude suitable for upgrading, expanding feasible feedstock sources for industrial biofuel production [22]. Such developments support closed-loop industrial systems where bioenergy pathways complement material valorization.

2. Materials and methods

Despite increasing interest in the production of biofuels (char, syngas and pyrolysis oil), the lack of updated technical information and feasibility studies regarding sustainability limits industrial implementation. Stakeholders aiming to commercialize pyrolysis technologies require detailed data on feedstock characteristics, operating conditions and regulatory constraints [2]. This study aims to improve understanding of the operating mechanism of pyrolysis, presenting the specific process conditions in which the technology is applied, the resulting by-products, their composition and energy value. These elements assist in identifying appropriate technologies for implementation [2].

- For any agricultural crop or natural vegetation association, four main factors determine net biomass

productivity:

- amount of incident solar radiation;
- proportion intercepted by green plant tissues;
- efficiency of photosynthetic conversion;
- biomass losses through respiration [1].

Under non-limiting water availability, a photosynthetic efficiency of approximately 1% may lead to 30–35 tons of dry biomass/ha. Biomass yield further depends on internal (genetic) and external (environmental, technological) factors [3]. In this context, biomass is one of the most promising renewable energy resources, capable of enhancing energy security, reducing dependence on fossil fuels, and lowering net carbon emissions [1,3], as we can observe below in Table 1.

Recent comparative evaluations emphasize that the physicochemical properties of lignocellulosic biomass (lignin, cellulose, hemicellulose, ash, moisture content) strongly influence the choice of conversion route and product quality. These factors determine whether slow pyrolysis, fast pyrolysis, catalytic gasification or HTL is appropriate, and they affect final yield and energy content of bio-oil, biocrude, syngas and char [23].

Table 1

Composition of selected biomass sources

Biomass source	Lignine (%)	Cellulose (%)	Hemicellulose (%)
Wood	25-30	35-50	20-30
Wheat straw	15-20	33-40	20-25
Sugarcane	23-32	19-24	32-48
Mischantus	17	24	44
Olive peels	48.4	24	23.6
Corn cobs	15	50.5	31
Hazelnut shells	52.3	25.6	22.7
Sunflower	17	48.4	34.6
Walnut shells	30-40	25-30	25-30
Rice straw	18	32.1	24
Leaves	0	15-20	80-85
Cooton Seeds	0	80-95	5-20
Barley straw	14-15	31-34	24-29
Rye straw	16-19	33-35	27-30
Oat straw	16-19	31-37	24-29

Methods and technologies for biomass energy recovery

A. Biomass Combustion

Biomass offers energy potential that can be exploited through biological or thermochemical conversion to biofuels and subsequently through combustion in the presence of oxygen. Combustion is the most widely used energy recovery process and has been applied for centuries. However, direct combustion generates high pollution due to carbon dioxide and other gases contributing to the greenhouse effect [5,6]. The general scheme and steps of the combustion process in a biogas plant operating with biomass are illustrated in Figure 1.

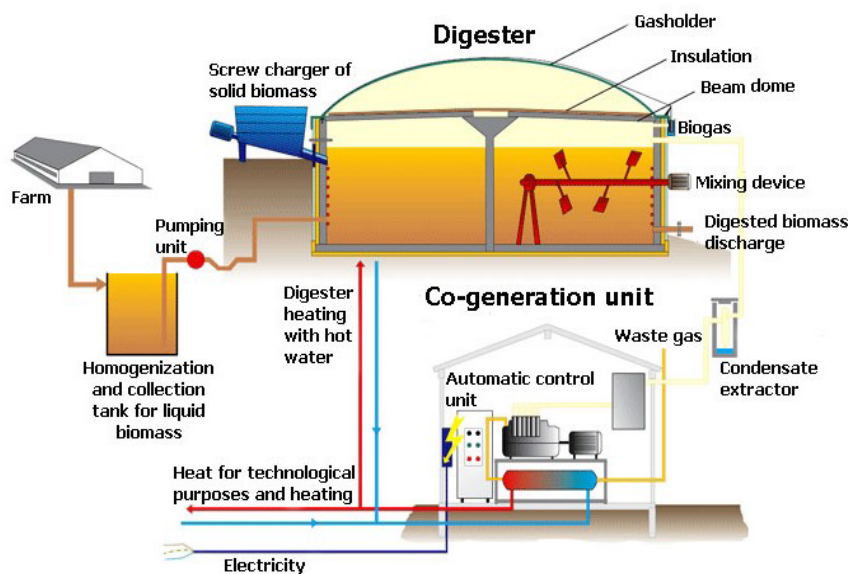


Figure 1. Operating system of a biogas plant (<https://www.proidea.ro/aplicatii-produse-5/membrane-valmex-enviro-pro-fabrici-biogaz-8881.shtml>)

Using the same principle, combustion plants may be implemented at a larger scale into cogeneration systems for heat and power production. Figure 2 presents cogeneration systems manufactured by ECO-Plast-Ind (Romania).



Figure 2. Steam and cogeneration power plants

(<https://www.transformatoareuscate.ro/ro/steam-and-cogeneration-plant.html>)

Recent technological advances show that thermal conversion systems may be enhanced using renewable heating methods, such as photothermal and electrothermal technologies (microwave, plasma, induction) capable of delivering targeted heating regimes with lower emissions and better energy efficiency [24].

B. Biological Conversion

B.1 Anaerobic Digestion — Biogas

The biochemical conversion of biomass into biogas through anaerobic digestion produces two final products: biogas and digestate. Biogas contains methane, carbon dioxide, hydrogen sulfide and nitrogen, with a calorific value of ~39 kJ/Nm³. Digestate, rich in nutrients, may be used as fertilizer. Anaerobic digestion enables the valorization of residues, industrial waste and sewage sludge, producing renewable energy without environmental impact [14,15]. Figure 3 shows the technological flow.

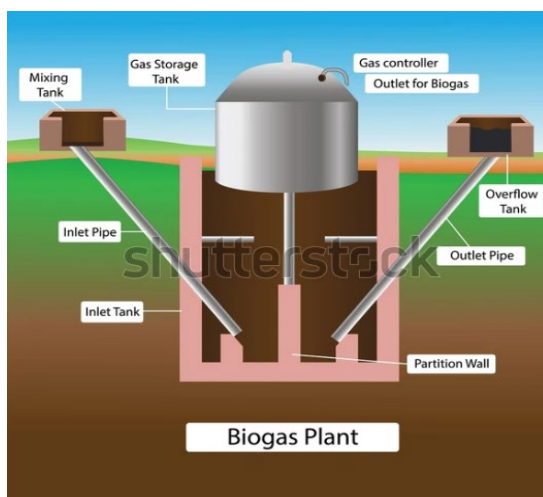


Figure 3. Functional diagram of a biogas plant

(https://www.researchgate.net/publication/326973921_COST_ANALYSIS_OF_HYBRID_MODEL_OF_SOLAR-WIND-BIOGAS_PLANT)

B.2 Conversion to Biodiesel

Biodiesel is produced via esterification of oils, fats or industrial residues with alcohol, followed by trans-esterification in the presence of catalysts, yielding methyl-esters and glycerin [11]. Figure 4 illustrates the biodiesel process. Biodiesel exhibits major advantages over fossil diesel:

- reduces CO₂ and SO₂ by 80%;
- biodegradable within 28 days;
- renewable replacement;
- lower noise emissions [11].

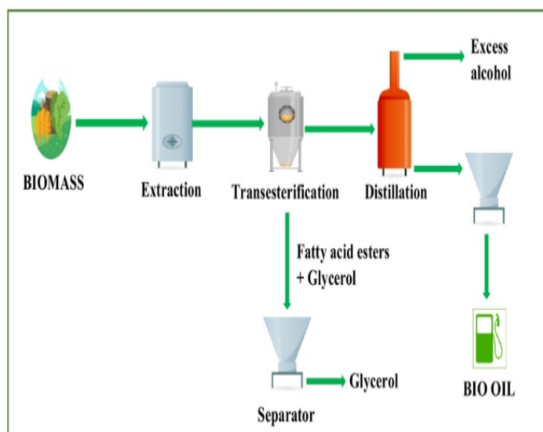


Figure 4. Biomass conversion in bio-diesel

(https://www.researchgate.net/publication/362748716_Biomass_to_Energy_-_an_Analysis_of_Current_Technologies_Prospets_and_Challenges/figures?lo=1)

B.3 Conversion to Bioethanol

Bioethanol is produced via fermentation, distillation and dehydration of fermentable feedstocks. Raw materials include sugars, starchy feedstock and lignocellulose, with lignocellulosic biomass being most promising yet costly due to pretreatment [17–21]. The ethanol obtained has a low toxicity, is easy to transport and can be converted into hydrogen for fuel cells, using water as a raw material. Bio-ethanol can also be used as a substitute for gasoline, the equivalent for 1 liter of gasoline being about 1.5 liters of bio-ethanol. The diagram of the process of converting biomass into bio-ethanol is shown in Figure 5.

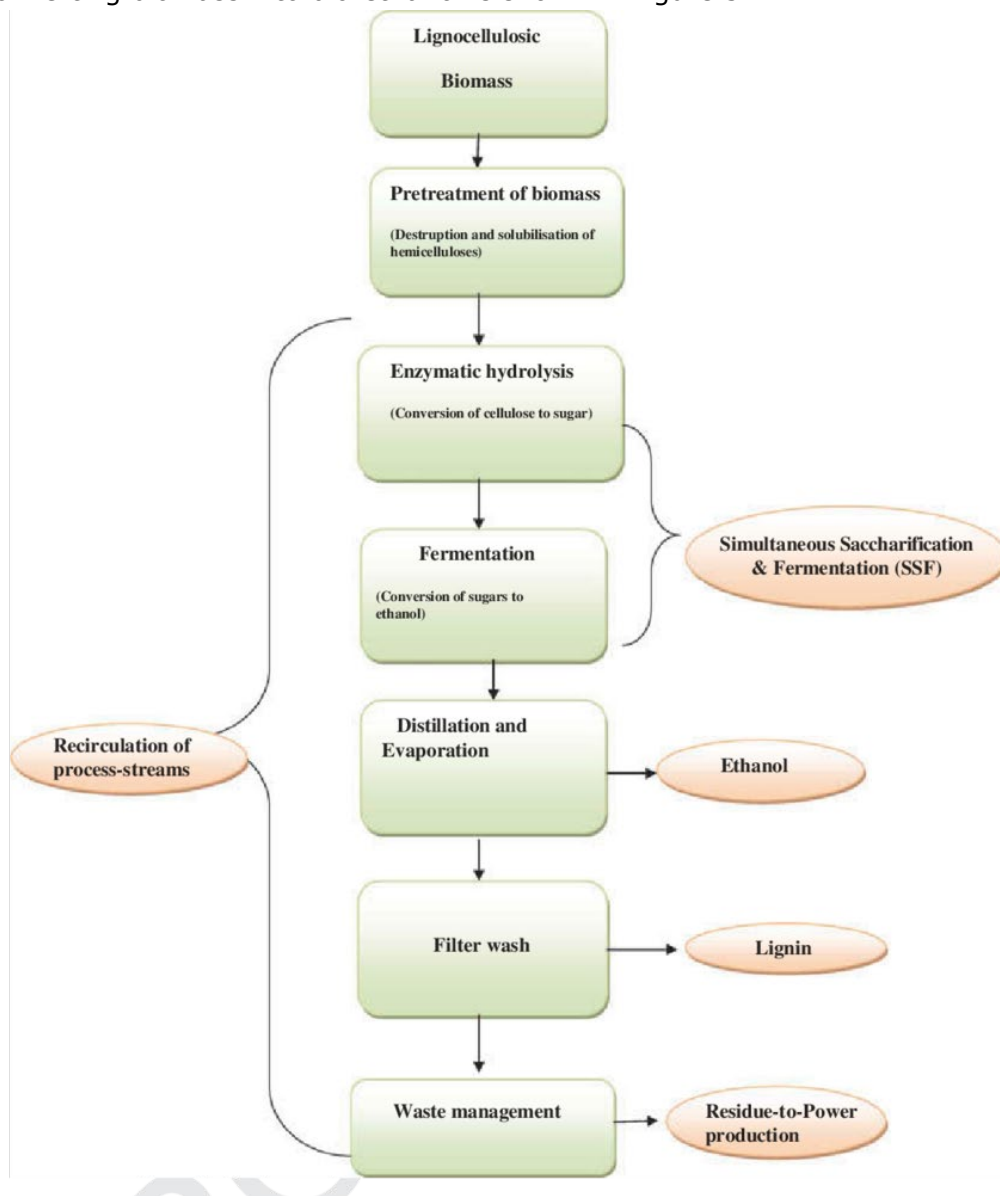


Figure 5. Biomass conversion in bio-ethanol

(https://www.researchgate.net/publication/271672109_A_Review_on_Fuel_Ethanol_Production_From_Lignocellulosic_Biomass)

3. Results

Recent studies show that conversion efficiency depends on correlating feedstock properties with process conditions. Slow pyrolysis (350–500 °C) maximizes biochar, fast pyrolysis (450–600 °C) favors bio-oil, while >800 °C gasification yields hydrogen-rich syngas. Catalysts and steam improve H₂ concentration [6,7].

Anaerobic co-digestion enhances methane yields; lignocellulosic ethanol yields double with pretreatment. Hybrid thermochemical–biochemical systems reduce waste and increase energy density [10,11].

Research has identified main pyrolysis phases: drying, torrefaction, devolatilization, carbonization and gasification [2]. Studies indicate CO₂ can replace N₂ as inert gas, with similar cost and behavior [9]. Higher char yields occur at ≤500 °C and ≤10 °C/min; high heating rates and >500 °C favor bio-oil, while >800 °C and slow heating maximize syngas [8].

Studies on biomass containing high moisture (sewage sludge, wet residues) show that hydrothermal processes (HTL and hydro-gasification) produce biocrude and syngas without pre-drying, making wet biomass economically feasible [25].

Integrated thermochemical + biological systems (pyrolysis/gasification + anaerobic digestion or HTL + fermentation) demonstrate complete biomass valorization, enabling circular bioenergy chains with reduced residues and greater energy recovery [26].

Recent research on biomass combustion technologies demonstrates that industrial systems achieve competitive thermal efficiencies when feedstock properties (moisture, lignin content, and bulk density) are optimized prior to combustion, resulting in superior calorific performance and reduced pollutant emissions. Studies show that high-lignin residues (hazelnut shells, olive peels, walnut shells) provide increased heating values and more stable combustion compared to low-density agricultural wastes [4, 7, 8]. Furthermore, modern combustion systems assisted by renewable heating methods (microwave, plasma, electrothermal induction) exhibit improved energy recovery and reduced carbon emission intensity, confirming their potential as next-generation biomass-to-energy solutions [24, 29].

Biochemical conversion routes display high variability in energetic efficiency depending on substrate composition and pre-treatment strategy. Anaerobic digestion systems achieve significantly improved methane release when co-digesting high-moisture residues such as manure, sorghum stalks or agro-industrial organic slurries, while simultaneously producing a nutrient-rich residue suitable for agricultural reuse without additional processing [14–16, 26]. In biodiesel processes, trans-esterification of mixed lipid feedstocks obtained from waste fats or secondary industrial residues yields consistent combustion performance in engine applications, offering measurable reductions in greenhouse emissions compared with conventional diesel fuels even at partial substitution rates [11]. Bioethanol production similarly benefits from pre-processing strategies that increase carbohydrate accessibility; however, instead of presenting uniform improvement, studies show that enhancement varies, being strongly dependent on lignin distribution, temperature profile and the effectiveness of the selected pretreatment agent [17–21]. Collectively, these findings indicate that biological routes are best suited for wet or heterogeneous biomass streams and operate most efficiently when integrated into circular biorefinery systems.

4. Conclusions

The calorific value of pyrolysis-derived char varies with biomass composition. Establishing optimal profitability requires evaluation of pyrolysis operating costs, calorific value of char and potential market value.

The analysis of biomass conversion pathways indicates that energy recovery performance cannot be maximized through a single technology, but rather through tailored combinations in which raw material properties, thermal profiles, pretreatment intensity and targeted fuel streams are jointly optimized [2, 6, 10, 22, 26]. High-density, lignin-rich species consistently favor combustion-based valorization, whereas wet residues and structurally complex feedstocks perform more efficiently under hydrothermal or biochemical routes, due to reduced preprocessing demands and improved access to reactive fractions [14, 17, 24, 25]. Integrated thermochemical–biochemical configurations therefore enable more complete valorization of biomass, and their long-term performance is further strengthened by the incorporation of advanced catalytic systems and renewable energy-assisted heating technologies, which enhance conversion selectivity and overall process sustainability [23, 27, 28, 30].

In summary, maximizing biomass energy recovery requires harmonizing feedstock properties, process conditions and targeted outputs. Recent developments show increasing potential for hydrogen production from biomass using thermochemical and plasma-assisted systems, supporting next-generation sustainable fuels [27]. Integrated pathways further enable complete biomass utilization, minimize waste and increase energy yields [26].

Future research should target:

- expanding slow-pyrolysis studies to other biomass types;
- characterization of pyrolysis oil and syngas;
- analysis of interactions between feedstock properties, residence time and inert gas flow [2];
- designing industrial prototypes for diverse biomass feedstocks.

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PEST BIRDS MANAGEMENT IN AGRICULTURAL CROPS

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Keywords: agricultural, bird damage, bird management, farms, prevention methods

Abstract: Wild birds are a significant source of economic losses in many agricultural areas. The deterioration of crops—seeds, fruits, cereals—through consumption or physical damage affects agricultural production and, consequently, farmers' incomes. Many species are migratory or have fluctuating populations, making the problem dynamic and complex. Farmers have identified bird damage as a critical problem that has received limited attention from researchers. This paper will present an analysis of technologies applied to crops to manage bird damage.

1. Introduction

Agriculture is the fundamental activity of mankind, being the main source of food and support for human life. Thus, its role is crucial in the world and in national economies, and for this reason it cannot be neglected [1,9,23].

Birds provide important ecosystem services in many ecosystems, but their need for food leads to losses of agricultural raw materials and raw materials at all stages of growth, primary processing, storage, transport and industrial use. Herbivorous and omnivorous birds cause damage to cereals, fruits, vegetables and seed production and also to plant breeding. Birds spoil the best berries, and the grapes lose the marketable condition. Woodpeckers, starlings, magpies, and fieldfare peck different kinds of berries, the damage to strawberry is particularly widespread [4, 7, 12, 20].

Pests in agricultural crops can be divided into several categories, depending on their nature and how they affect the plants. The most common groups are insects, mites, pathogenic microorganisms (bacteria, fungi and viruses), rodents, herbivores and birds [5, 8, 10, 13].

Birds are animals belonging to the class Aves, distributed throughout the world. According to the Romanian Ornithological Society, almost 400 species of birds have been identified in our country, with over 10,000 discovered worldwide [11, 15, 16, 21, 31].

The diet of birds is varied and often includes nectar, fruits, plants, seeds, carrion and various small animals, including other birds. Fruits are a favorite food for many birds, being an excellent source of sugar, an essential source of energy during summer, autumn and winter, key seasons for reproduction, migration and maintaining body heat at low temperatures [17, 18, 22, 23, 24, 25, 32].

Weeds, pathogens, and animal pests are among the pests that pose a threat to the productivity of crops meant for human consumption. Bird-caused crop losses pose a serious and costly challenge for farmers [26, 27, 28].

Pest bird management is essential for the stability of agricultural production. According to the literature, certain species cause significant losses in field crops, vineyards and orchards. A European study notes losses ranging from 22–32% in maize, 10–13% in wheat and 8–15% in oats in certain regions [1, 29, 13].

Methods for Managing Pest Birds is *Visual Techniques* (Traditional scarecrows, Predator-eye balloons, Reflective tapes, Hawk-shaped kites, Agricultural laser beams), *Acoustic Techniques* (Propane gas cannons, Predator calls, Programmable acoustic deterrents, Limitations: noise disturbance, habituation); *Physical Exclusion* Anti-bird nets (Tensioned wire

systems, Lightweight electric fencing); *Chemical Repellents* (Seed treatments, Odor/taste deterrents approved under EU regulations); *Modern Technologies* (Autonomous drones for bird dispersal, IoT sensor systems for real-time detection, AI-based forecasting models) [5, 9, 15,32].

2. Materials and methods

In the work [3], the authors present a workshop was held to teach attendees about state-of-the-art bird management practices to protect fruit crops. Growers, educators and wildlife managers came away with comprehensive knowledge about successful bird management strategies in susceptible fruit crops, including sweet and tart cherry, blueberry, ‘Honeycrisp’ apples and wine grapes

The study [4] presents a survey on bird deterrent solutions for crop protection. It first introduces the related concepts. Then, it provides an extensive review and categorization of existing methods, techniques, and related studies. Further, their strengths and limitations are discussed. Based on this analysis, gaps were identified and strategies for future research were proposed.

The study [15] presents birds as stress factors in sorghum crops. These are a serious pest and limit grain production from sorghum. Factors such as field size, proximity to nesting sites, planting density, weed control, variety choice and timing of agricultural operations influence the ability of birds to damage crop plants. However, various methods have been used to control birds, including the use of chemicals, repellents, bird scarers, lethal and non-lethal methods, including host plant resistance. Among the control strategies, host plant resistance can be an effective method due to the presence of tannins in bitter sorghum types.

The removal of fruit is a consequence of plant–animal interactions, and is a vital step in the natural regeneration cycle of plant populations.

The research [1] aimed to determine the effect of fruit color and local landscape on plant–bird interactions in an agroecosystem in Southcentral Mindanao, Philippines, where they set out 1500 artificial fruit models in ten sampling locations within an agroecosystem and they measured the difference in the proportion of predated/removed (%) fruit models and the risk between sites, fruit color, and predators, Figure 1.

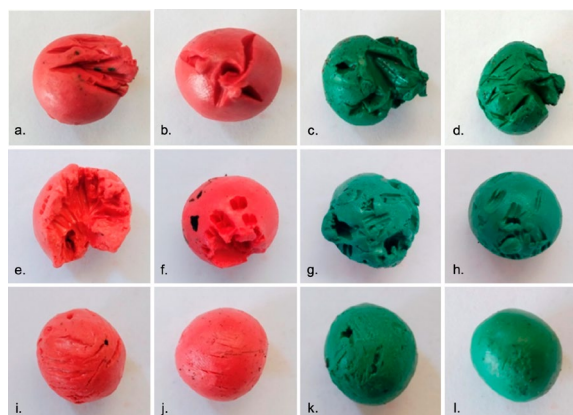


Figure 1. Examples of typical attack marks of birds (a–d), mammals (e–h), and arthropods (i–l).[1]

Fruit consumption by birds is a costly problem, yet basic information about the species and abundance of fruit-eating birds in fruit crops, and factors that influence abundance, are lacking.

The paper [6], conducted a study of fruit birds on Honeycrisp apples, blueberries, grapes, and sweet cherries in Michigan, New York, and the Pacific Northwest in 2012 and 2013. Documented the most commonly observed fruit birds in each crop in the study regions and used fruit consumption data to identify the bird species for each crop and region that have a large

impact through fruit consumption. Found that American robins (*Turdus migratorius*; hereafter “robins”) and cedar waxwings (*Bombycilla cedrorum*; hereafter “waxwings”) are important fruit consumers across regions and crops, while house finches (*Haemorhous mexicanus*) are additionally important in the Pacific Northwest. Modeled and compared the abundance of fruit birds across all four crops and found that while abundance varied by region and crop, it was not affected by the heterogeneity of the surrounding land cover.

2. Results

At the workshop in paper [3] participated a total of 29 people attended, plus 13 presenters and organizers for a total of 42.

Eight people attended the webinars and 21 attended the workshop. Workshop evaluations were distributed to all and 17 were returned. Attendees included farmers, farm managers, wildlife biologists, wildlife managers, and extension personnel. Crops grown by those responding included, grapes, tree fruit (including apple, cherry, peach and apricot), berry crops, and vegetables.

At the workshop, eight respondents reported gaining knowledge during the workshop, four had minimal understanding of bird damage management before the workshop to moderate understanding after the workshop, and four had moderate understanding to considerable understanding. Those whose level of knowledge remained the same all reported gaining considerable knowledge about bird damage management before and after the workshop, as shown in Figure 2.

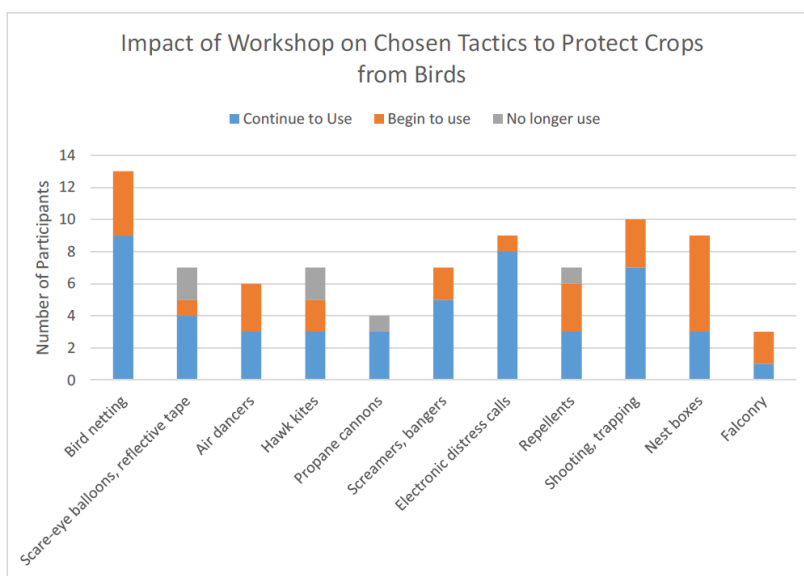


Figure 2. Impact of workshop on chosen tactis to protect crops from birds.[3]

The tactics that respondents would start using during the workshop included (in order of frequency) nests, bird nets, aerial dancers, shooting/traps, repellents, hawk kites, screamers/bangers, falconry, bird scaring balloons/reflective tape, or repellents. Interestingly, one person indicated that they would stop using propane guns, and no one chose to start using that tactic.

The work [14] presented visual deterrent devices (scarecrows, reflectors and reflecting tape, hawk kites and balloons, dead bird models, aircraft, radio-controlled aircraft or unmanned aerial vehicle, lights), auditory deterrents (shotguns and rifles, pyrotechnics, gas cannons, av-alarm, predator sounds, high-intensity sounds, ultrasounds, infrasounds), chemical deterrents (tactile repellents, behavioural repellents, methyl anthranilate—rejex-it), exclusion deterrents (overhead netting, foam, bird balls), habitat modification (tall grass, fungicides, other

techniques), removal deterrents (traps, live ammunition shooting, surfactants, falconry) and other deterrent techniques (lure area, magnets, microwaves, laser), Figure 3.

Techniques	Methodology	References	Requires Maintenance	Cost of Use	Creates Habituation	Presents Danger to Humans	Presents Danger to Birds	Requires Qualified Person
Visual Deterrents	Scarecrows	[4–19]	No	Low	Yes	No	No	No
	Reflectors	[20–22]	No	Low	Yes	No	No	No
	Hawk Kites and Balloons	[23]						
	Dead Bird Models	[15,24–30]	No	Low	Yes	No	No	No
	Aircraft	[23]	Yes	High	No	Yes	Yes	Yes
	Radio-Controlled Aircraft	[6,15,31–36]	Yes	High	Yes	No	No	Yes
Auditory Deterrents	Light	[13,15,25,37–45]	Yes	Medium	Yes	No	No	No
	Shotguns and Rifles	[13,18,33,46–51]	Yes	High	Yes	Yes	Yes	Yes
	Pyrotechnics	[23,25,52–55]	No	High	Yes	Yes	Yes	Yes
	Gas Cannons	[53,54,56–60]	Yes	Low	Yes	Yes	No	No
	Av-Alarm	[15,16,25,61–69]	No	Low	Yes	Yes	No	No
	Sound of Predators	[70,71]	No	Low	Yes	No	No	No
	High Sounds	[72]	No	Low	Yes	Yes	No	No
	UltraSounds	[73,74]	No	Low	No	No	No	No
	InfraSounds	[75]	No	Low	No	No	No	No
Chemical Deterrents	Tactile	[81]	Yes	Low	No	No	No	Yes
	Behavioural	[15,82–86]	Yes	Low	No	Yes	Yes	Yes
Exclusion	Methyl Anthranilate Rejex-It	[87–91]	Yes	Medium	No	No	No	No
	Overhead Wires and Lines	[95,96]	Yes	High	No	No	No	No
	Foam	[23]	Yes	Medium	No	No	No	No
Habitat Modification	Bird Balls	[23]	No	Low	No	No	No	No
	Tall Grass	[97,98]	No	Low	No	No	No	No
	Benomyl and Tersan	[99]	No	High	No	No	Yes	Yes
	Others	[100]	No	Low	No	No	No	No
Removal	Traps	[92,101–104]	No	Medium	No	No	Yes	No
	Live Ammunition Shooting	[9,105,106]	Yes	High	Yes	Yes	Yes	Yes
	Surfactants	[107–111]	Yes	Medium	No	No	Yes	Yes
	Falconry	[23,24,28,29,58,86,105,112–118]	No	High	No	No	Yes	Yes
Others	Lure Areas	[119]	Yes	Low	No	No	No	No
	Magnets	[120–124]	No	High	Yes	No	No	No
	Microwave	[75,125–134]	Yes	High	No	Yes	Yes	No
	Laser	[135–137]	Yes	High	No	Yes	Yes	No

Figure 3. Summary of different methods and techniques used as bird deterrent solutions.[14]

The work [15] presents factors influencing bird damage in grain sorghum. *Fields near breeding or roosting sites* are more susceptible to bird damage, the trees, bushes or reeds around the planted fields providing nesting place for birds. They increase vulnerability to bird pests as the birds will continuously feed on the crops close by.

Field size - Huge crop fields have longer borders which are the zones preferentially attacked by the birds. Furthermore, large fields requires a large labour force for bird scaring which is challenging to have during peak periods.

Timing of farm operations, it influences the incidence of bird damage. Planting very late during summer predisposes the crop to damage by migrating birds of which at times may be arriving at the time of crop maturity. The damage is higher during dry season than wet season because of the lack of wild seeds.

Crop establishment method - Seeds are highly vulnerable to bird attack when they are sown directly on to the soil. They are safe when are covered by the soil to protect them. Emerging seedlings are also vulnerable to bird attack. However, damage at this stage is not so common when granivorous migratory birds have flown to other countries or continents.

Planting density - water birds are attracted to areas with plant densities much lower than in the immediate vicinity and can cause substantial damage to surrounding sorghum plants.

Also, weed control, life cycle and chemical control are extremely important factors that influence damage caused by birds to sorghum and wheat.

The results of the studies in paper [15] show that the effectiveness of the control strategy varies with the bird species involved and optimum bird control strategy combines several

techniques or can be used in random. Human operated scaring techniques were shown to be the most effective methods for reducing bird populations in the field.

The research [1] looks like approximately a quarter (24.53%) of the artificial fruit models deployed were predated, and the proportion of predation was significantly higher in the red fruit models (mean = 18.74 ± 9.84) compared to the green fruit models (mean = 11.67 ± 6.17). Birds were the most dominant predators compared to mammals and arthropods, and contributed to at least 60% of the predation of red fruits.

Also, although landscape variables did not significantly affect fruit predation, tree cover may help increase these interactions. Study showed that agroecosystems can still support species of frugivorous birds, as indicated by high fruit predation rates, particularly by birds that can permeate different layers of the agroecosystem.

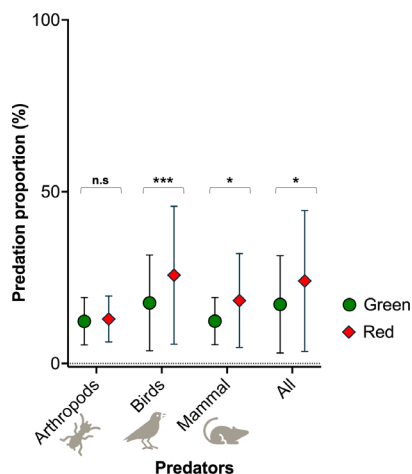


Figure 4. Comparison of the predation proportion (%) of different predator types in red and green artificial fruit models. Whiskers represent the SD, * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$, n.s. = not significantly different. [1]**

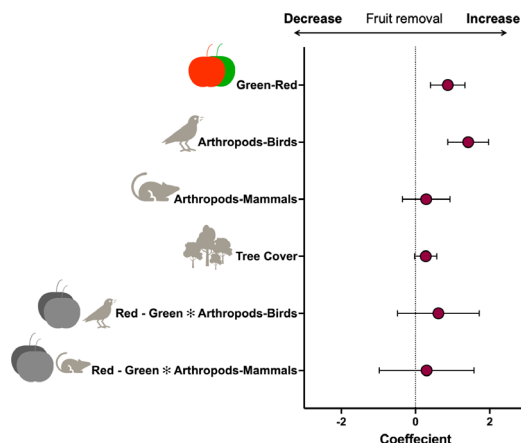


Figure 5. Visualized results of the best binomial generalized linear model (GLM). The dots represent the coefficients, and the whiskers represent the lower and upper bounds of the 95% CI. Silhouettes in dark colors represent significant relationships at $p < 0.05$. [1]

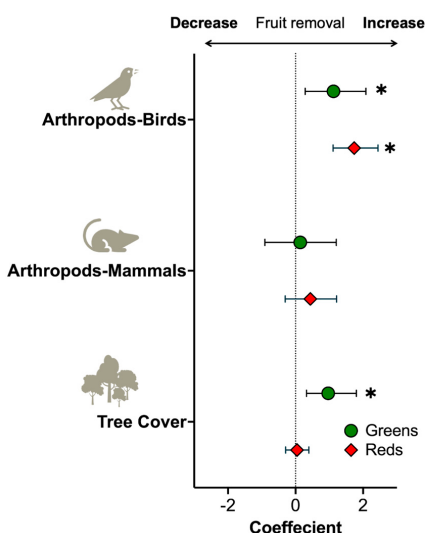


Figure 6. Comparison of the binomial generalized linear mixed models (GLMM) results between the predation risk of two artificial fruit models. The diamonds and dots represent the coefficients, and the whiskers represent the lower and upper bounds of the 95% CI. The * denotes significant relationships at $p < 0.05$. [1]

The paper [6] shows that a total of 255.5 h of point counts were conducted, 92.75 h in 2012 and 162.75 h in 2013. In Michigan apples, blueberries, grapes, and sweet cherries, 16,

14.25, 11.5, and 30.75 h of point counts were conducted, respectively. In New York we conducted 18, 12, 22, and 19 h of point counts in apples, blueberries, grapes and sweet cherries. Similarly, 22.5, 28.25, 19.5, and 31.5 h were conducted in the Pacific Northwest. Eighty-one bird species were observed during the point counts. Of these, 57 (70.4%) were classified as fruit-eating species, as shown in Figure 7.

Common Name	Scientific Name	Common Name	Scientific Name
American Crow	<i>Corvus brachyrhynchos</i>	Gray Catbird	<i>Dumetella carolinensis</i>
American Goldfinch	<i>Spinus tristis</i>	Hairy Woodpecker	<i>Picoides villosus</i>
American Robin	<i>Turdus migratorius</i>	House Finch	<i>Haemorhous mexicanus</i>
Baltimore Oriole	<i>Icterus galbula</i>	House Sparrow	<i>Passer domesticus</i>
Black-billed Magpie	<i>Pica hudsonia</i>	Indigo Bunting	<i>Passerina cyanea</i>
Black-capped Chickadee	<i>Poecile atricapillus</i>	Lazuli Bunting	<i>Passerina amoena</i>
Black-headed Grosbeak	<i>Pheucticus melanocephalus</i>	Northern Cardinal	<i>Cardinalis cardinalis</i>
Blue Jay	<i>Cyanocitta cristata</i>	Northern Flicker	<i>Colaptes auratus</i>
Brewer's Blackbird	<i>Euphagus cyanocephalus</i>	Northern Mockingbird	<i>Mimus polyglottus</i>
Brown Thrasher	<i>Toxostoma rufum</i>	Orchard Oriole	<i>Icterus spurius</i>
Bullock's Oriole	<i>Icterus bullockii</i>	Palm Warbler	<i>Setophaga palmarum</i>
Cassin's Finch	<i>Haemorhous cassinii</i>	Pileated Woodpecker	<i>Dryocopus pileatus</i>
Canada Goose	<i>Branta canadensis</i>	Rose-breasted Grosbeak	<i>Pheucticus ludovicianus</i>
California Quail	<i>Callipepla californica</i>	Red-bellied Woodpecker	<i>Melanerpes carolinus</i>
Chestnut-backed Chickadee	<i>Poecile rufescens</i>	Red-headed Woodpecker	<i>Melanerpes erythrocephalus</i>
Cedar Waxwing	<i>Bombicilla cedrorum</i>	Savannah Sparrow	<i>Passerculus sandwichensis</i>
Chipping Sparrow	<i>Spizella passerina</i>	Scarlet Tanager	<i>Piranga olivacea</i>
Common Grackle	<i>Quiscalus quiscula</i>	Song Sparrow	<i>Melospiza melodia</i>
Common Raven	<i>Corvus corax</i>	Spotted Towhee	<i>Pipilo maculatus</i>
Dark-eyed Junco	<i>Junco hyemalis</i>	Summer Tanager	<i>Piranga rubra</i>
Downy Woodpecker	<i>Picoides pubescens</i>	Tufted Titmouse	<i>Baeolophus bicolor</i>
Eastern Bluebird	<i>Sialia sialis</i>	Vesper Sparrow	<i>Poocetes gramineus</i>
Eastern Kingbird	<i>Tyrannus tyrannus</i>	White-crowned Sparrow	<i>Zonotrichia leucophrys</i>
Eastern Phoebe	<i>Sayornis phoebe</i>	Western Kingbird	<i>Tyrannus verticalis</i>
Eastern Towhee	<i>Pipilo erythrophthalmus</i>	Western Tanager	<i>Piranga ludoviciana</i>
European Starling	<i>Sturnus vulgaris</i>	Wild Turkey	<i>Meleagris gallopavo</i>
Great Crested Flycatcher	<i>Myiarchus crinitus</i>	Yellow-breasted Sapsucker	<i>Sphyrapicus varius</i>
Golden-crowned Kinglet	<i>Regulus satrapa</i>	Yellow Warbler	<i>Setophaga petechia</i>
Yellow-rumped Warbler	<i>Setophaga coronata</i>		

Figure 7. List of all fruit-eating species observed during point counts. [6]

Also, the most frequently sighted species varied by growing region and crop, figure 8.

Region ^a	Apple	Freq.	Blueberry	Freq.	Grape	Freq.	Sweet Cherry	Freq.
MI	American Goldfinch	0.25	American Robin ^b	4.00	American Crow	0.17	American Robin	2.24
	Blue Jay	0.13	Cedar Waxwing	0.77	Vesper Sparrow	0.17	European Starling	1.30
	Eastern Bluebird	0.06	Chipping Sparrow	0.77	House Finch	0.09	Chipping Sparrow	0.42
			American Goldfinch	0.63			Black-capped Chickadee	0.39
			Song Sparrow	0.49			Common Grackle	0.36
NY	Black-capped Chickadee	0.37	American Robin	3.83	House Sparrow	0.39	American Robin	4.68
	House Sparrow	0.37	Baltimore Oriole	1.58	American Goldfinch	0.28	Chipping Sparrow	1.52
	Blue Jay	0.25	Song Sparrow	1.00	Eastern Bluebird	0.28	Cedar Waxwing	1.24
	Palm Warbler	0.12	European Starling	0.50	Chipping Sparrow	0.23	Gray Catbird	1.13
	American Robin	0.06	Common Grackle	0.50	Blue Jay	0.17	European Starling	0.68
PNW	House Finch	4.76	American Robin	1.84	House Finch	1.33	American Robin	5.33
	American Goldfinch	3.87	American Goldfinch	0.25	American Robin	0.56	Cedar Waxwing	0.76
	American Robin	3.73	Dark-eyed Junco	0.25	Dark-eyed Junco	0.41	House Finch	0.70
	Brewer's Blackbird	1.33	White-crowned Sparrow	0.18	Yellow-rumped Warbler	0.36	Western Kingbird	0.60
	European Starling	1.16	House Finch	0.14	Northern Flicker	0.10	American Goldfinch	0.57

^a MI = Michigan, NY = New York, PNW = Pacific Northwest.

^b Birds that are important fruit consumers for each region and crop are shown in bold; this classification was based on the bird species being both frequently sighted and recorded as a high consumer of fruit (see Methods).

Figure 8. The most frequently sighted fruit-eating bird species in each region for 2012 and 2013. "Frequent species" are ranked by the number of birds detected per point count hour (denoted by 'Freq.'). [6]

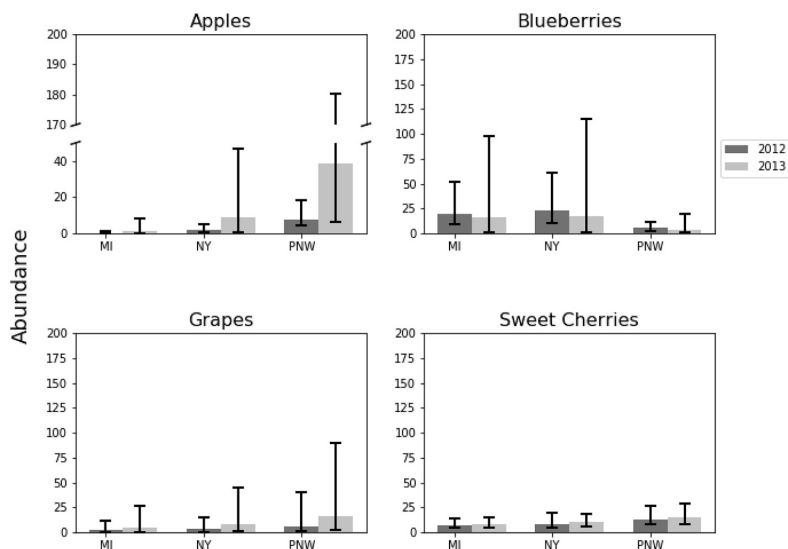


Figure 9. Abundance estimates from the fruit-eating bird models, shown as mean number of birds per point count area, comparing within crops among regions in Michigan (MI), New York (NY), and the Pacific Northwest (PNW) during 2012 and 2013. Error bars represent 95% credible intervals. [6]

4. Discussion

Farming is very difficult when you have to fight not only with severe climatic conditions, but also with pests that cause you a lot of problems.

Both at sowing and harvesting, birds can cause very generous damage. They consume not only the newly planted seed in the ground, but also a good part of the autumn harvest. Thus, using a bird control method will automatically guarantee richer crops. Therefore, any investment that guarantees the protection of agricultural crops is worth it.

Bird damage risk assessment also makes it possible to create balanced, low-cost insurance tools to cover farmers' economic risk due to bird damage, resulting in a much lower cost than prophylactic crop protection, without negative impact on the environment.

5. Conclusions

Of the various pests that exist for agriculture, birds are one of the biggest and most damaging to farmers, and possibly the most difficult to control.

Despite the availability of bird deterrents, there are still many challenges for effectively protecting crops from bird damage. These challenges include the need for cost-effective solutions, the ability to adapt to changing bird populations, and the potential for negative impacts on non-target species and the environment.

Birds cause an extensive damage to cereal crops when the preferred seeds of wildgrasses are unavailable.

It is important to use various strategies have been employed to control bird pests respectively use of repellents, cultivars with awns, long glumes and goose-necked, chemical control and cultivars with bird-resistance characteristics. Controlling bird pests can help in maintaining crop yields and productivity.

Gestionarea păsărilor dăunătoare necesită o abordare integrată, adaptată la contextul local, combinând metode vizuale, acustice, fizice și tehnologice. România se confruntă cu densități mari ale graurilor și ciorilor, ceea ce implică alternarea metodelor și tehnici inteligente. Soluțiile moderne (drone, senzori, laser) sunt promițătoare.

Regardless of the method of pest bird removal used, a farmer should budget and plan the initial costs for the purchase, maintenance and replacement of this equipment, especially more expensive options such as compensation.

Most farmers attempted to reduce bird damage by a combination of control techniques and strategies, predominantly scaring devices placed in or about crops. Opinion on the effectiveness of such devices was variable, but most farmers agreed that when correctly located and operated, most reduced the damage by birds habituated to crops in protected fields, the solution being of short-lived, problem bird species either return as soon as the scaring device is removed, or they move to adjacent crops, thus often penalising nearby farmers who are not using such techniques at that time.

While birds play an essential ecological role, certain species can pose a significant threat to agricultural crops.

The damage caused by birds can range from direct feeding to contamination and disease transmission, leading to economic losses.

Farmers are increasingly adopting a variety of control measures to prevent and mitigate these effects, balancing ecological considerations with the need to protect their crops. Proper management strategies, such as habitat modification, the use of deterrents, and physical barriers, can help reduce the negative impacts of harmful bird species on agriculture.

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Conflicts of interest:

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Article

STUDY OF THE THRESHING PROCESS IN AN AXIAL FLOW DEVICE

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Abstract: The threshing apparatus is the most important element of a harvester, taking into consideration that both the threshing process, performance and the separation one depends on it. In the present case the experiments have been made on an axial flow thresher with tangential feed. Starting from the experimental date previously determined, after their processing, there were obtained the probability distribution $S_s(x)$ and the density function $S_d(x)$ of the seeds separated during the threshing and separating processes by using various types of mathematical proceedings. The final aim is to minimize the losses from the threshing apparatus, achieving as good as possible seed separation, namely a percentage over 99%.

1. Introduction

In order to obtain these functions it was supposed that the experimental data very good precision and it was started from a density function of the separated seeds suggested by Lipkowitz's calculi [1, 2, 3], the calculus proceedings of the coefficients of the density function by the method of the smallest squares.

Going on it will be selected the most convenient version of the probability distribution $S_s(x)$ (implicitly of the density function $S_d(x)$) from the version proposed in the paper [4] and it will be performed the checking of the results for at least an experiment.

For determining the probability distribution $S_s(x)$ these will be selected the functions λ and β from [4] which have been determined depending on the main parameters of the process taken into calculus: rotor rotative speed - n , rotor-counter rotor distance - δ , feeding flow - Q , feeding speed - v_a , feeding surface - S and the length of the threshing apparatus - L .

In this part of the paper, it will be shown how the results of the first stage can be used and exploited. The main points of this part of the paper are: choosing the most convenient variant of the distribution function (implicitly of the distribution density function) from the variants proposed at the end of [2, 3], verifying the results on at least one case of a physical experiment carried out, estimating the existence of optimum points in the parametric space of the problem. I repeat, all calculations are affected by the choice of the form of the distribution density function starting from the results of [4], a choice which is not the best according to some estimates made in an annex study, in the sense that there are forms of this function, possibly with more parameters, that are more efficient [5, 6, 7, 8, 9]. In addition, choosing the distribution density function of the form indicated in [4] for the study of the phenomenon in time is not theoretically justified either, since our choice method assumes an at least affine relationship between time and space, which is not proven to exist [10, 11, 12, 13]. Even if the affine relationship between space and time is not real, for our choice to have a minimum coverage, there must be a one-to-one relationship between space and time, which again has not been demonstrated, but is only a hypothesis.

2. Materials and methods

Of the 17 variants of the functions λ and β indicated in [3], for reasons primarily physical, but also of precision (as shown by the precision estimators), we chose the last variant, with the order number 17. I recall the form of these functions, obtained from the table in [3], by replacing the parameters of the phenomenon with the parameters given in the table:

$$\lambda = 3.135 \cdot 10^{-8} \frac{\rho n^3 S^3}{Q \delta^2 v_a^2} + 17.331, \quad \beta = 44106.46 \frac{\rho^3 n^4 \delta^{12} L_t}{Q^3 S^2 v_a} + 2.76865 \quad (1)$$

According to [1], the distribution function of the separated seeds has the form:

$$S_s(x) = A \left(\frac{e^{-\lambda x} - 1}{\lambda} - \frac{e^{-\beta x} - 1}{\beta} \right), \quad (2)$$

where:

$$A = \frac{1}{\frac{e^{-\lambda L} - 1}{\lambda} - \frac{e^{-\beta L} - 1}{\beta}} \quad (3)$$

The distribution density function represents the derivative of the distribution function with respect to the variable x :

$$S_d(x) = A(e^{-\beta x} - e^{-\lambda x}) \quad (4)$$

There is a relationship between the input flow rate, the density of the processed mixture, the feed speed and the surface area of the feed opening, which will be taken into account below

$$Q = \rho S v_a \cdot \quad (5)$$

Using relation (5) we can eliminate the flow rate from relations (1). We thus obtain expressions (6) of the function parameters λ and β that appear in the expressions of the distribution function and the distribution density function

$$\lambda = 3.135 \cdot 10^{-8} \frac{n^3 S^2}{\delta^2 v_a^3} + 17.331, \quad \beta = 44106.46 \frac{n^4 \delta^{12} L}{S^5 v_a^4} + 2.76865 \quad (6)$$

It is noted that in this way the dependence on the density ρ disappears and that, in general we can consider $S = \text{const.}$ (for reasons of threshing machine construction), then the parameters λ and β remain dependent only on n , δ , v_a and L .

- **The relationship between the functions s_s and s_d and the experimental results**

The determination of the coefficients of the parametric functions λ and β , coefficients that appear in (1) and (6) as well as in the function table in [1], was made starting from the experimental results that are given in the distribution function in percentage form. Therefore, to be able to compare the experimental data with the theoretical results, some clarifications must be made.

To compare the distribution function directly in percentages, the experimental results are compared directly with the values of the function S_s given in (2), calculated for the given abscissa and multiplied by 100. If the comparison is to be made in terms of the mass of separated seeds (given in grams) then the total mass M of the seeds separated in the experiment is needed. The experimental values will be compared with the values of the function S_s calculated in the given abscissa multiplied by the mass of separated seeds M . These are the terms in which the experimental and theoretical results are compared about the distribution function of separate seeds.

To compare the experimental data incorrectly marked S_d , a little reasoning must be done that will show what is the relationship between the distribution density function S_d , defined in (4) and the experimental data marked in the sheet with S_d .

The distribution function S_d represents, from a physical point of view, the quantity of separated seeds per unit length (meter). In practice, this function cannot be verified directly since it is not possible to collect seed samples in a punctual manner. The experimental procedure divides the length of the interval of interest, L , into n intervals (for the experiments in this material $n=20$), collecting and weighing the separated seeds from a box of length L/n and constant width. Therefore, if the division of the working interval is:

$$\Delta_n = (x_0 = 0 < x_1 < \dots < x_{n-1} < x_n = L) \quad (7)$$

then the amount of seeds separated on the interval (x_k, x_{k+1}) , is given by:

$$SD_{k+1} = \int_{x_k}^{x_{k+1}} S_d(x) dx \cdot \quad (8)$$

It is observed that:

$$SD_{k+1} = F(x_{k+1}) - F(x_k) \quad (9)$$

Therefore, the experimental data that appears as an expression of the distribution density function will be compared with the finite series Sd_k . Moreover, these relations also result from the relation between the distribution function and the distribution density function in the experimental process it is precisely the values of the terms of the finite series Sd_k that are determined.

• **Determination of the other characteristic functions of the process**

In order to reach the final phase of the theoretical-empirical modeling of the threshing process, the phase of investigating the possibilities of optimizing the work process, the objective functions must be found. For this process, the main objective is to minimize losses. The losses during threshing and separation are limited to the distribution functions of unthreshed seeds S_n and free seeds in the threshing space S_l .

$$p_{tr} = \int_0^L S_n(x) dx, \quad p_{sr} = \int_0^L S_l(x) dx, \quad p_{ev} = \int_0^L (1 - S_s(x)) dx \quad (10)$$

where the functions S_n and S_l are defined according to [1], by the relation:

$$S_s(x) + S_l(x) + S_n(x) = 1 \quad (11)$$

Alone, the relation (11) cannot provide both functions, S_n and S_l in [2], a relation is also given that connects the derivative of the function S_n to the function S_l . This relation is not proven and is probably related to the form of the distribution function considered in the process (slightly different from the functions considered by us) in the spirit of this relation a similar relation can be considered:

$$\frac{dS_s(x)}{dx} = kS_l(x) \quad (\mathfrak{R}) \approx (12)$$

But since the distribution function of the separated seeds and the density function of the distribution of the separated seeds have the following relationship:

$$\frac{dS_s(x)}{dx} = S_d(x), \quad (13)$$

a linear relationship results between the functions S_l and S_d :

$$S_l(x) = \frac{S_d(x)}{k} \quad (14)$$

Then, from (11) it results

$$S_n(x) = 1 - S_s(x) - \frac{S_d(x)}{k} \quad (15)$$

With this the problem is solved if the constant k is known. Thus:

$$p_s = \frac{S_s(L)}{k},$$

$$p_{ev} = L - A \left[\left(\frac{1}{\beta} - \frac{1}{\lambda} \right) L + \frac{e^{-\beta L} - 1}{\beta^2} - \frac{e^{-\lambda L} - 1}{\lambda^2} \right], \quad (16)$$

$$p_{tr} = p_{ev} - p_s$$

Taking into account that previously we explained the distribution function and the seed distribution density function separately from the process parameters, we can now proceed to investigate the possibilities of optimizing the process, of course provided that the constant k is known.

3. Results

- **Solving the problem for the case where a relationship of type (R) is accepted**

In this chapter, the hypothesis from [2] is accepted, partially, in the sense that the linear dependence between the derivative of the distribution function of the separated seeds and the distribution function of the free seeds is accepted. To choose the proportionality constant k , a simplistic reasoning will be made that will highlight the limitation of the model. Both from a dimensional point of view ($[k]=L^{-1}$), and inspired by the hypothesis [2], by similarity, we will take $k=\lambda$.

Accepting these hypotheses, the expressions of the functions S_l and S_n have the forms (14) and (15). To prove the formulas that have been arrived at, the results will be calculated on the concrete case of the experiment with the code A1. For this case the process parameters have the values: $\rho = 77.78 \text{ Kg/m}^3$, $v_a = 0.09 \text{ m/s}$, $n = 900 \text{ rpm}$, $\delta = 17/4 \text{ mm}$, $Q = 1.05 \text{ kg/s}$.

Under these conditions, the variation of the distribution function with the length of the threshing device, L , has the form in figure 1, the values of the distribution function are those given by formula (2), the percentage form being obtained by multiplying by 100, and the values in g being obtained by multiplying by the total mass of the sample in the case of experiment A1.

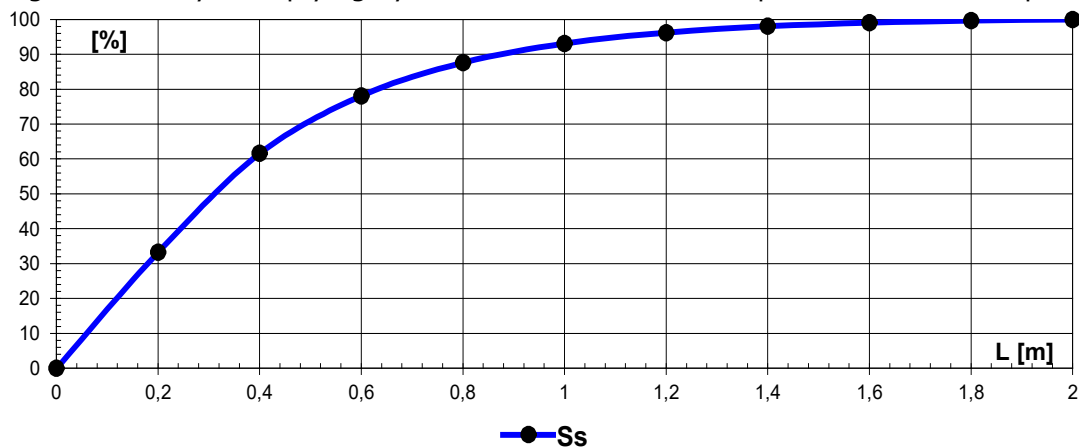


Figure 1. Variation of the distribution function of separated seeds, S_s , over the length of the threshing device, L

Under the same conditions, the distribution density function varies as in figure 2.

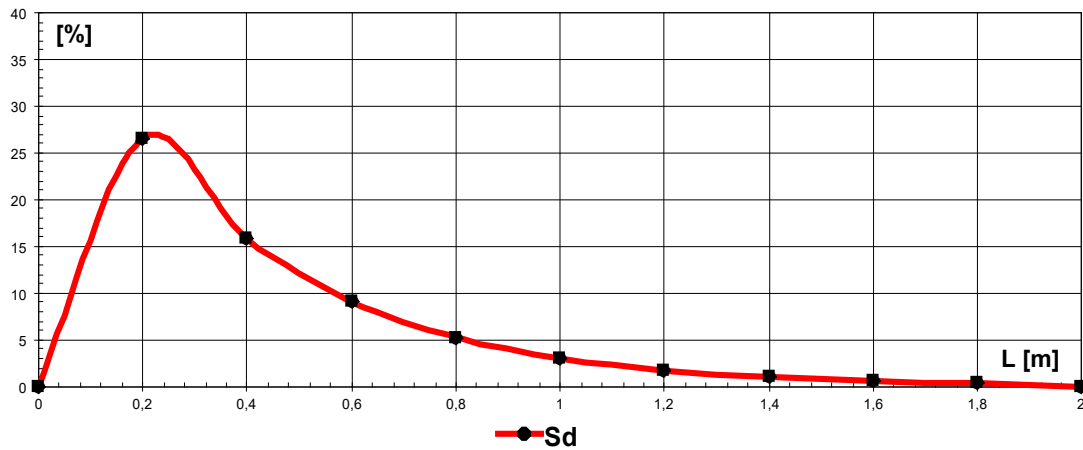


Figure 2. Variation of the distribution density function, S_d , over the length of the threshing machine, L

The distribution function of free seeds in the threshing space varies with the length of the threshing machine, L , as in figure 3, of course, under the conditions of the experiment with code A1.

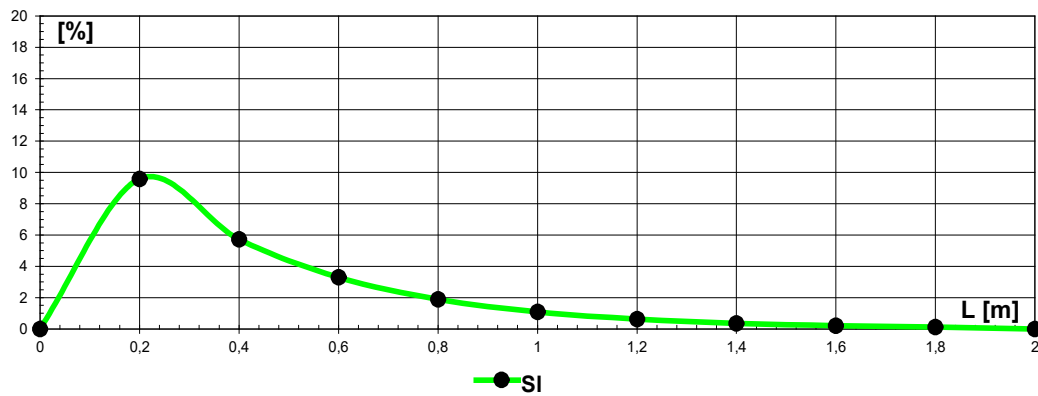


Figure 3. Variation of the free seed distribution function, S_f , over the length of the threshing machine, L

The variation with the length of the threshing device, L , of the distribution function of unthreshed seeds is presented in Figure 4.

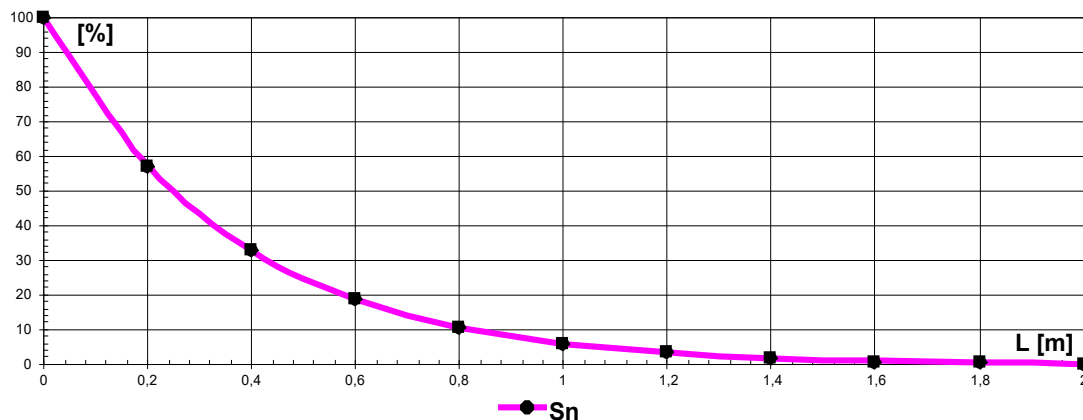


Figure 4. Variation of the distribution function of unthreshed seeds, S_n , over the length of the threshing machine, L

To compare the theoretical results with the experimental ones, the total mass of the sample, M , must be taken into account, as we specified at the beginning of paper. Thus, the

variation of the mass distribution function, as a function of the length of the threshing device, L , $L \in [0, 2]$, appears in figure 5.

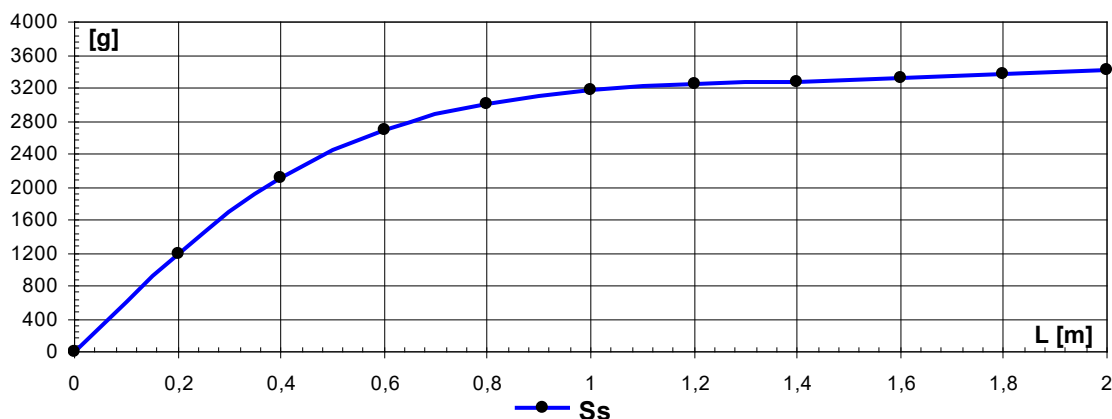


Figure 5. Variation of the separated seed distribution function, S_s , expressed in terms of mass, with the length of the threshing machine, L

In the same term of the mass of the separated seeds, the variation of the density function of the distribution function in relation to the length of the threshing device appears in figure 6.

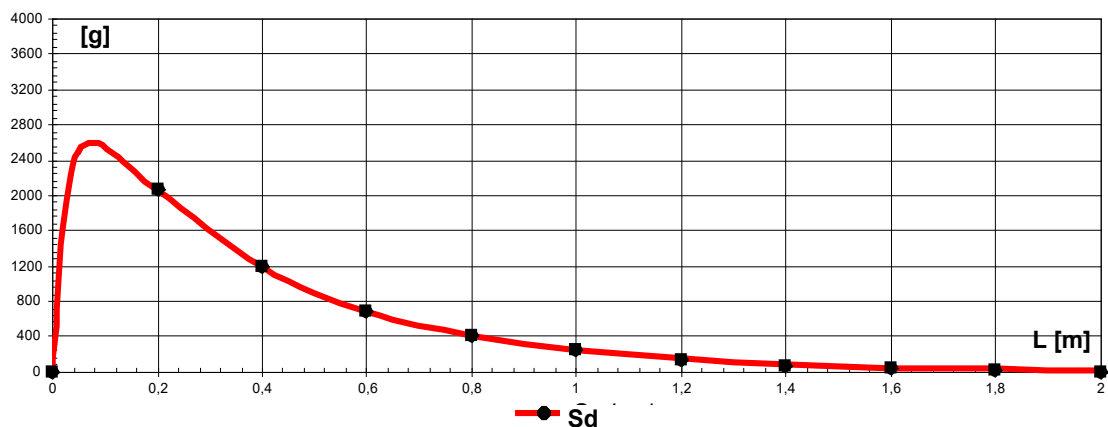


Figure 6. Variation of the distribution density function, S_d , expressed in terms of mass, with the length of the threshing device, L

For the distribution function of free seeds in the threshing space, the same type of variation appears in figure 7, and for the distribution function of unseparated seeds, in figure 8.

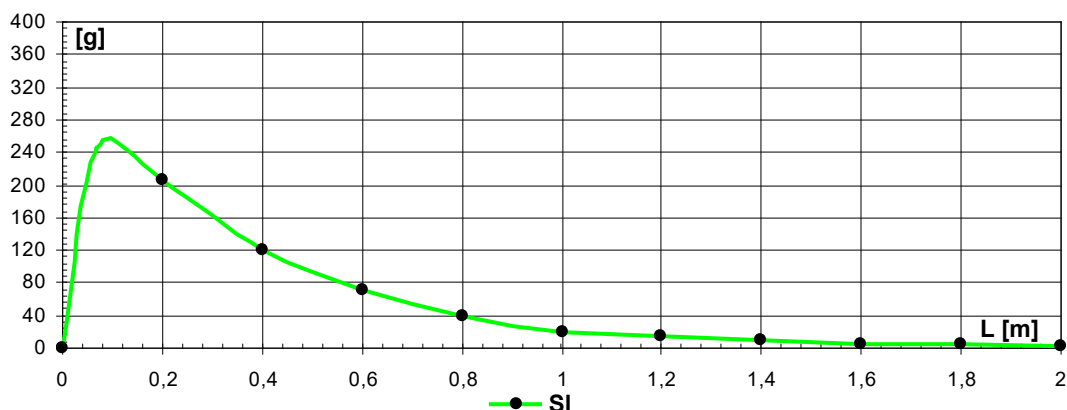


Figure 7. Variation of the free seed distribution function, S_f , in the threshing space, expressed in terms of mass, with the threshing machine length, L

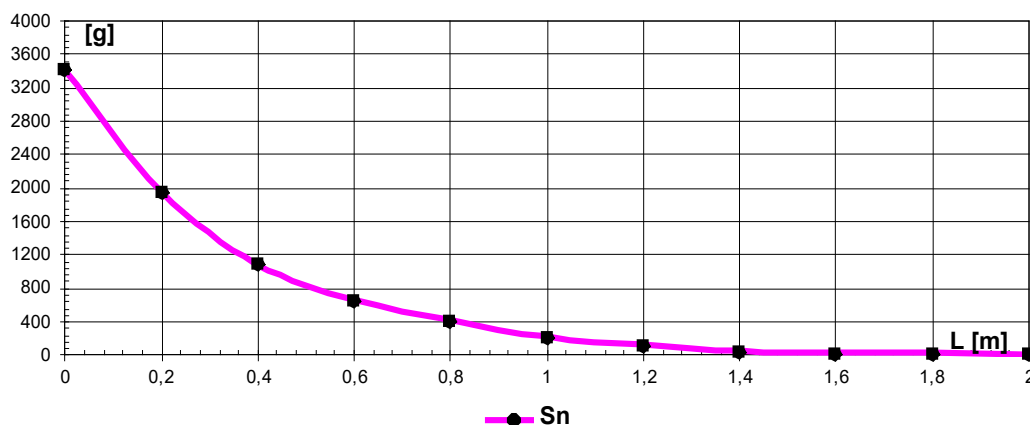


Figure 8. Variation of the unthreshed seed distribution function, S_n , expressed in terms of mass, with the length of the threshing device, L

To more easily compare the theoretical results with the percentage results, for the case of experiment A1, figure 9 represents in the same system of axes the variations of the average value of the theoretical distribution function of the separated seeds and the experimental values of the same function, obviously average values on intervals of 0.2 m, for an interval of 2 m.

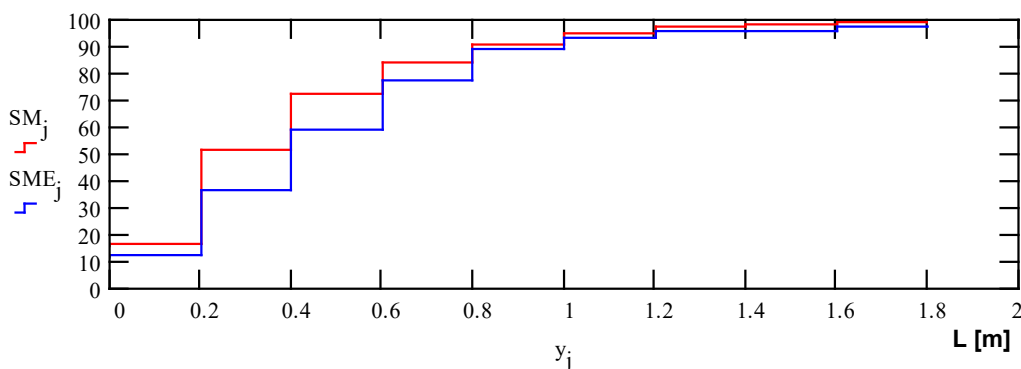


Figure 9. Variation with threshing device length, L , of the theoretical mean value (red) and experimental values (blue) for the separated seed distribution function

It is observed that the experimental results approximate the experimental results satisfactorily, always in excess. The same type of comparison is made in terms of the density distribution function, in figure 10.

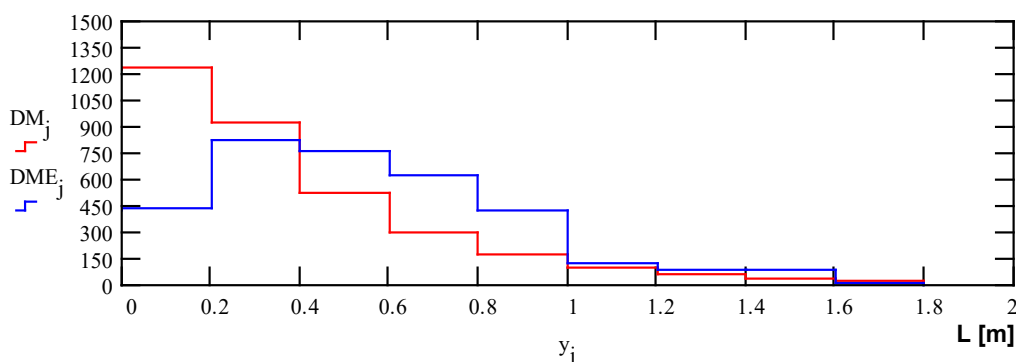


Figure 10. Variation with the length of the threshing device, L , of the theoretical mean value (red) and experimental values (blue) for the density of the distribution function of separated seeds

4. Discussion

It can be seen that, in the case of comparison in terms of the distribution density function, the fidelity of the theoretical results in relation to the experimental ones is deficient in the end

towards the origin (this is also due to the discretization with large steps in the calculation of the average value), but satisfactory for $x > 0.2$.

After these graphical representations and verifications, an observation is necessary closely related to the hypothesis adopted from the beginning of this paragraph, namely $k = \lambda$. The most unpleasant consequence of this choice (hypothesis) is the fact that there is a large measurement interval, on which the S_n function takes negative values, that is, very small in relation to its maximum value, but negative. This behavior also affects the calculation of losses. I recall that the choice made in this chapter is based on an unverified hypothesis made in [2], a work that was inspired in its formulation according to a temporal mathematical model of the threshing process. In these circumstances, nothing prevents us from changing the hypothesis with $k = A$. The change of hypothesis modifies, as shown by the definition relations, only the variation of the functions S_l and S_n , the functions S_s and S_d being experimentally deduced. If the mentioned change of hypothesis is used, then the variation of the functions S_l and S_n , in graphical form, looks, qualitatively, as in figures 7 and 8. The differences are of a value nature:

Hypothesis	Min S_l	Max S_l	Min S_n	Max S_n
$k = \lambda$	0.0	255.422	-1.4330	3388.0
$k = A$	0.0	2375.000	-13.325	3388.0

It is observed that not only qualitatively, but also quantitatively, the differences are minimal, for the choice of the two hypotheses. Obviously, until a proper analysis of the formula [(\mathfrak{R})], any other hypothesis is possible. In absolute value, the negative minimum of the S_n function may also be due to some errors, due to their smallness in relation to the maximum value (below 0.5 %).

5. Conclusions

The threshing apparatus of a combine represents its most important component because the main process indicators: productivity and losses of a combine are influenced by it.

In this paper, the analysis of the threshing and separation process in an axial flow threshing apparatus was pursued, analysing the main parameters that influence the process: rotor rotating speed – n , rotor-counter rotor distance – δ , feeding flow – Q , feeding speed – v_a , feeding surface – S and the length of the threshing apparatus – L and the optimal variant of the seed separation function, S_s , was identified, this being verified for validation with real data obtained in an experiment (denoted A1), finally obtaining a seed separation in a percentage of over 99%, thus minimizing losses in the threshing apparatus (below 1%).

Conflicts of interest: “The authors declare no conflict of interest.”

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Article

DYNAMIC MODELING AND SIMULATION OF A SERVO-HYDRAULIC ACTUATOR FOR ACCELERATED TESTING OF AGRICULTURAL MACHINERY COMPONENTS

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Keywords: *Electro-hydraulic servo cylinder, Proportional valve, PID control, Dynamic performance*

Abstract: Reliable laboratory testing of agricultural machines requires actuators that can reproduce field-level loads under controlled and repeatable conditions. Servo-hydraulic cylinders are well suited to this task because they can excite structures either in displacement or in force, enabling the accurate simulation of real operating scenarios—shock loads, cyclic fatigue, and slow quasi-static events—without the variability and cost of in-field trials. This paper analyzes the dynamic performance of an electro-hydraulic servo cylinder driven by a proportional directional valve with onboard electronics (OBE) used for creating accelerated testing rigs for agricultural machinery. The control architecture consists of an external digital PID acting on piston displacement x , while the internal valve loop is treated implicitly through a compact plant model. Sinusoidal experimental tests (1 mm amplitude, 0.5–4 Hz) were used to identify a second-order closed-loop dynamics that condenses valve, hydraulic, and mechanical effects. The calibrated model, yields natural frequency $f_n \approx 2.65$ Hz and damping ratio $\zeta \approx 0.71$, reproducing the measured amplitude roll-off from ≈ 1.0 mm at 0.5 Hz to ≈ 0.4 mm at 4 Hz. For the worst-case 100 mm step (250→350 mm), the experiment shows a monotonic, non-oscillatory transient dominated by a maximum displacement rate v_{\max} (slew-rate/flow limit). An augmented simulation that enforces $|\dot{x}| \leq v_{\max}$ ($v_{\max} \approx 22\text{--}36$ mm/s) matches the ~ 5.5 s settling time with zero overshoot observed during step tests. Together, the frequency-fitted cylinder model and the rate-limit augmentation capture both bandwidth and large-step behavior with minimal complexity, providing a practical basis for controller tuning and trajectory feasibility for usage in accelerated testing.

1. Introduction

Electro-hydraulic servo systems are widely used in industrial automation, aerospace, and heavy machinery applications due to their ability to combine high force output with precise motion control. Among these systems, the electro-hydraulic servo cylinder represents a key actuator, frequently employed where both large loads and accurate positioning are required. Its performance strongly depends on the interaction between the hydraulic dynamics, the proportional valve characteristics, and the electronic control strategies [1]. A typical servo-hydraulic system integrates sensors, proportional valves, and advanced controllers to achieve closed-loop regulation [2]. Proportional valves equipped with embedded electronics and internal control algorithms are commonly used to ensure accurate spool displacement and linear flow control [3]. Recent experimental comparisons between classical PID and advanced backstepping schemes on proportional-valve-driven hydraulic cylinders show that PID control tends to exhibit larger tracking errors and apparent delays at higher speeds, whereas backstepping with an integral flow term reduces overshoot and phase lag by compensating valve dynamics and

uncertainties [4]. Still, PID controllers remain the most widely applied strategy in electro-hydraulic systems, thanks to their simplicity and effectiveness in compensating for nonlinearities and uncertainties. Jin et al. (2013) [5] demonstrated that systematic tuning of PID parameters in proportional directional valves directly affects both spool stability and overall system accuracy, while [6] emphasized that improved PID structures can significantly enhance the robustness of valve-controlled cylinder systems, particularly under asymmetric loading conditions. Recent studies have emphasized the importance of accurate mathematical models for predicting and improving the behavior of electro-hydraulic servo systems. A comprehensive mathematical model of an electro-hydraulic servo system, including the cylinder, servovalve, and associated amplifiers, was developed by [7], who demonstrated through simulations that the model accurately reproduces the system dynamics. Song et al. (2025) [8] presented a control-oriented model structure that integrates valve dynamics, hydraulic compliance, and mechanical motion, enabling reliable prediction of piston displacement under different control strategies. Their experiments confirmed that even with relatively simple PID regulators, the model reproduces the main dynamic features of the actuator, including bandwidth limitations and the effect of fluid compressibility. At the system level, an additional external PID controller is usually implemented to regulate cylinder displacement or force by comparing the feedback signal with a desired reference. This dual-control structure improves stability and accuracy, but it also introduces interactions that may limit the dynamic performance.

In the present study, the servo-hydraulic cylinder is employed in a laboratory test bench dedicated to agricultural machinery, where components and subassemblies are subjected to simulated and accelerated operating conditions. The purpose of such testing is to reproduce real working loads under controlled environments, in order to evaluate durability, performance, and failure modes within shorter time frames than in field conditions [9].

Despite their advantages, electro-hydraulic servo systems exhibit inherent dynamic limitations caused by fluid compressibility, valve flow saturation, leakage, and structural compliance. These effects become particularly relevant when the actuator is required to follow rapidly varying reference signals, such as sinusoidal or step inputs. As a result, the system bandwidth is typically restricted, leading to phase lag, amplitude attenuation, and steady-state errors at higher frequencies. Recent investigations have shown that the dynamic performance of electro-hydraulic servo systems is strongly influenced by parameters such as fluid temperature, leakage, and structural stiffness, which exacerbate the effects of compressibility and valve saturation when fast reference tracking is required [10]. He et al. (2023) [11] proposed an online SAC-based PID tuning strategy for valve-controlled hydraulic cylinders and, in AMESim–Simulink co-simulations, showed markedly lower Integral of Time-weighted Absolute Error, overshoot, and disturbance sensitivity than classical and fuzzy PID, supporting the idea to retain a PID outer loop while using a calibrated, bandwidth- and slew-limited plant for practical tuning.

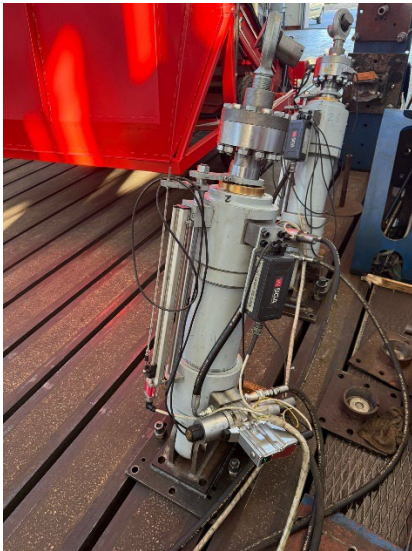
The aim of this paper is to investigate both theoretically and experimentally the dynamic behavior of an electro-hydraulic servo cylinder controlled by a proportional valve with dual PID loops. Special attention is given to identifying the main limitations in system response, analyzing the influence of control parameters, and discussing potential strategies for performance improvement.

2. Materials and methods

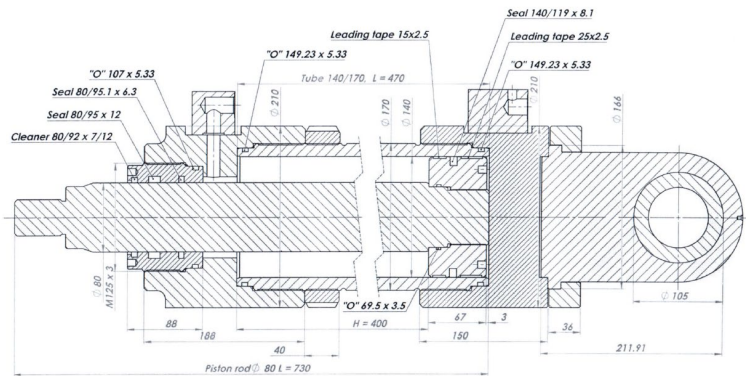
The experiments were carried out on a laboratory rig specifically designed for testing agricultural machinery components under simulated and accelerated operating conditions, located at DITRMA laboratory from INMA Bucharest. The core of the installation is an electro-hydraulic servo cylinder, supplied by a hydraulic power unit capable of delivering pressures up to 200 bar. The servo cylinder is equipped with a displacement sensor and a force transducer, enabling closed-loop monitoring of both position (0-400 mm) and applied load (0-300 kN).

The hydraulic power unit is based on an axial piston pump manufactured by Hydromatik GmbH (Ulm, Germany). The pump type A2V 107 L has a geometric displacement of 107 cm³/rev and can deliver up to 104 L/min at a maximum operating pressure of 200 bar. This pump configuration ensures a total installed hydraulic power of approximately 35 kW, sufficient for driving the servo cylinder under demanding laboratory test conditions.

A general view of the test rig is presented in Figure 1 a), and the overall dimensions of the servo cylinder are illustrated in Figure 1 b), based on manufacturer’s technical drawings. These representations provide an overview of how the actuator is integrated into the laboratory setup and highlight its suitability for accelerated durability tests.



a)



b)

Figure 1. Servo cylinder

- a) Equipped with proportional directional control valve, displacement sensor, and load cell
- b) Cylinder overall dimensions

The control architecture of the system, including the interaction between the external and internal PID regulators, is illustrated in Figure 2. This block diagram emphasizes the dual-control strategy: the internal PID ensures accurate valve spool positioning, while the external PID governs the cylinder displacement according to the imposed reference.

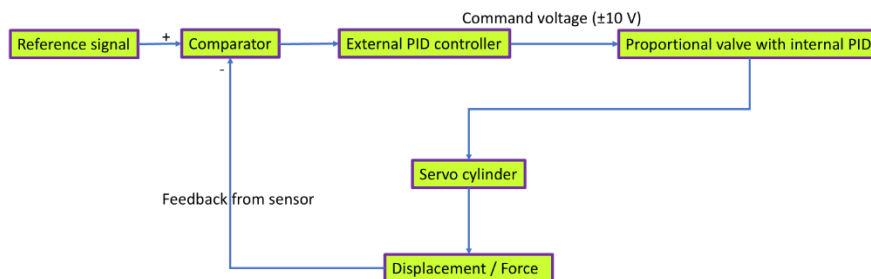


Figure 2. Control architecture of the electro-hydraulic servo cylinder with dual PID regulation

The servo cylinder is actuated by a proportional directional control valve type PRM9-10, size 10 (D05), manufactured by ARGO-HYTOS. The valve is designed for high-performance applications and is equipped with digital onboard electronics (OBE) and an

internal spool position sensor. This configuration enables closed-loop control of the spool displacement, significantly reducing hysteresis and improving the dynamic response. The valve operates with a nominal flow rate of 60 L/min at $\Delta p = 10$ bar, with a maximum operating pressure of 350 bar at ports P, A, and B, and 210 bar at port T. The internal electronics accept standard command signals, including ± 10 V, which corresponds to the output range of the external PID controller used in the test rig. Thanks to its internal PID regulator, the valve ensures that the actual spool position accurately follows the electrical command, effectively linearizing the relationship between the input voltage and the hydraulic flow. This function is crucial for reproducing precise actuator movements during accelerated laboratory tests. A general view of the proportional valve and its main connection interfaces is presented in Figure 3.

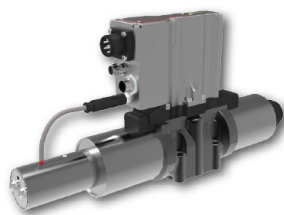


Figure 3. Proportional directional control valve PRM9-10 with digital onboard electronics and internal spool position feedback

The proportional valve PRM9-10 used in the test rig can be configured and fine-tuned through the dedicated **PRM9 parameterization software** provided by ARGO-HYTOS. The software allows both graphical and tabular adjustment of the main control parameters, including ramp times, deadband compensation, dither frequency, and feedback scaling. The connection to the valve is established via a standard USB-A to micro-USB cable, enabling direct communication between the PC and the onboard electronics. Before modifying any parameter, the original dataset can be read from the valve and stored on the PC, ensuring that the initial configuration can be restored at any time. This feature is particularly useful during laboratory experiments, where multiple test configurations may be required. By enabling precise tuning of internal PID constants and flow characteristics, the parameterization software ensures that the valve response can be adapted to the specific requirements of accelerated durability tests. An example of the software interface is shown in Figure 4.

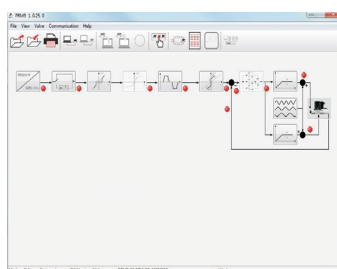


Figure 4. PRM9 parameterization software interface for setting and monitoring valve control parameters

The test rig was designed with flexibility in mind, allowing different excitation signals (sinusoidal, step, or random) to be applied to the cylinder. This capability makes it possible to reproduce a wide variety of loading conditions encountered in the field, such as cyclic stresses, dynamic impacts, and fatigue scenarios. By subjecting machine components to these conditions in a controlled environment, the laboratory setup enables reliable assessment of performance and service life in a significantly reduced timeframe.

Although the servo-hydraulic system described above is designed for high accuracy and robustness, its performance is inherently limited by several dynamic factors. The presence of two nested PID regulators—the internal loop for spool position control and the external loop for cylinder displacement—introduces additional dynamics that may lead to overshoot, phase lag, or oscillatory behavior when subjected to rapidly varying reference signals.

Furthermore, the compressibility of the hydraulic fluid, internal leakages, and the finite response speed of the proportional valve restrict the effective bandwidth of the system. These effects become particularly critical during sinusoidal excitation at higher frequencies, where the actuator is required to reproduce precise displacement profiles under load.

Another challenge arises from the high installed hydraulic power (up to 35 kW at 200 bar), which, while necessary to ensure sufficient force and speed, also results in considerable heat generation when the proportional valve throttles excess flow. This phenomenon not only reduces energy efficiency but may also influence the long-term stability of the tests if not properly controlled.

The main objective of this research is therefore to analyze the dynamic limitations of the servo-hydraulic cylinder driven by a proportional valve with dual PID control. Both modeling and experimental investigation are employed to identify the operating conditions under which the system achieves acceptable performance, as well as the scenarios where its response is constrained by inherent physical and control-related factors.

In order to study the dynamic limitations of the system, a simplified mathematical model of the electro-hydraulic servo cylinder was developed. The model integrates the proportional valve dynamics, the continuity of flow in the cylinder chambers, and the mechanical motion of the piston. In the complete description of a hydraulic cylinder, each chamber must be modeled separately, since the flow balance and pressure dynamics differ between the piston side and the rod side. For chamber A, the continuity equation includes the piston velocity with a positive sign, while for chamber B the piston velocity appears with a negative sign. In addition, the effective volumes V_A and V_B are different when the piston has a rod. For simplification, the model was reduced to an equivalent single-chamber formulation, which captures the dominant dynamic effects while simplifying the mathematical representation. The equivalent chamber volume V is defined in such a way that it reflects the average compressibility effects of the fluid, while the differential pressure $\Delta p(t) = p_A(t) - p_B(t)$ is used as the governing variable.

The proportional valve, which is driven by the external PID command voltage $u(t)$, regulates the hydraulic flow $Q(t)$. Since the valve is equipped with an internal PID loop that stabilizes the spool position, its dynamics can be approximated by a first-order element with a small time constant T_v . The valve flow equation is expressed as:

$$Q(t) = K_v * u(t) - K_c * \Delta p(t) \quad (1)$$

where K_v is the flow gain with respect to the control voltage [$V \cdot m^3/s$], K_c is the flow-pressure coefficient [$Pa \cdot m^3/s$] and $\Delta p(t)$ is the pressure difference across the cylinder chambers [Pa]. The flow entering the chamber is distributed between the piston movement and the compression of the hydraulic fluid. This balance is described by the continuity equation:

$$Q(t) = A * \dot{x}(t) + \frac{V}{\beta_e} * \Delta \dot{p}(t) \quad (2)$$

where A is the piston area [m^2], $x(t)$ is the piston displacement [m], V is the chamber volume [m^3] and β_e is the effective bulk modulus of the fluid [Pa].

The mechanical motion of the piston is governed by Newton's second law, which accounts for inertia, viscous damping, and external load forces:

$$M * \ddot{x}(t) + B * \dot{x}(t) + F_{load}(t) = A * \Delta p(t) \quad (3)$$

where M is the equivalent moving mass [kg], B is the viscous damping coefficient [Ns/m] and $F_{load}(t)$ is the external load force.

By combining the valve dynamics, the continuity relation, and the piston motion equation, the system can be approximated by a reduced-order transfer function between the piston displacement $X(s)$ and the input command voltage $U(s)$:

$$G(s) = \frac{X(s)}{U(s)} \approx \frac{K}{(T_v s + 1)(Ms^2 + Bs + K_h)} \quad (4)$$

where K is the static gain (dependent on K_v , A , V , β_e), T_v is the valve time constant, and K_h is the effective hydraulic stiffness.

$$K_h = \frac{A^2 \beta_e}{V} \quad (5)$$

This simplified representation highlights the main factors limiting the dynamic performance of the system: the finite response speed of the valve, the compressibility of the fluid, and the mechanical damping of the piston assembly.

The simplified model highlights several factors that inherently limit the dynamic response of the servo-hydraulic system:

- The proportional valve introduces a first-order time constant T_v , which limits the achievable control bandwidth. Even though the internal PID loop ensures accurate spool positioning, the finite opening and closing speed prevent the system from accurately tracking high-frequency reference signals.
- The effective bulk modulus β_e of the hydraulic fluid reduces the apparent stiffness of the cylinder and introduces additional compliance in the system. This effect lowers the natural frequency of the piston–fluid assembly and may lead to hydro-mechanical resonance when the excitation frequency approaches the natural frequency.
- The pump–valve combination provides a nominal flow rate up to 104 L/min, but the proportional valve has a rated capacity of 60 L/min. As a result, at large-amplitude or high-frequency command signals, the flow demand can exceed the valve limits, leading to saturation and tracking errors.
- The damping coefficient B influences the stability of the closed-loop system. If damping is too low, oscillations and overshoot may occur; if it is too high, the response becomes sluggish and the ability to follow fast transients is reduced.
- Since the hydraulic power unit can deliver approximately 35 kW, significant heat is generated whenever excess flow is throttled by the valve. This reduces the energy efficiency and, in prolonged experiments, may affect the fluid properties, thereby altering both stiffness and damping.

Taken together, these limitations explain why the servo-hydraulic cylinder cannot reproduce arbitrarily fast or high-amplitude trajectories, despite being controlled by cascaded PID regulators. The model thus provides a theoretical framework for interpreting the results of the experimental investigation presented in the next section.

The previously derived transfer function $G(s)$ represents the open-loop dynamics of the proportional valve and the hydraulic cylinder. To ensure that the piston displacement follows the reference trajectory, an external PID controller was implemented, with the following transfer function:

$$C(s) = K_p + \frac{K_i}{s} + K_d s \quad (6)$$

where K_p , K_i and K_d are the proportional, integral, and derivative gains, respectively.

In the closed-loop configuration, the reference displacement $X_{ref}(s)$ is compared with the measured displacement $X(s)$. The error signal is then processed by the PID controller, which generates the control voltage $U(s)$. The output of the controller drives the proportional valve, and the resulting plant dynamics are described by $G(s)$.

The closed-loop transfer function between the reference input and the piston displacement can be written as:

$$T(s) = \frac{X(s)}{X_{ref}(s)} = \frac{C(s)*G(s)}{1+C(s)*G(s)} \quad (7)$$

Substituting $G(s)$, the closed-loop system becomes:

$$T(s) = \frac{\left(K_p + \frac{K_i}{s} + K_d s\right) \frac{K}{(T_v s + 1)(M s^2 + B s + K_h)}}{1 + \left(K_p + \frac{K_i}{s} + K_d s\right) \frac{K}{(T_v s + 1)(M s^2 + B s + K_h)}} \quad (8)$$

This representation highlights the combined effect of the external PID regulator and the hydraulic plant. The PID terms influence the closed-loop poles, improving tracking accuracy and stability. As a result, the closed-loop transfer function provides a framework for predicting how the system will respond to step or sinusoidal reference signals, and it establishes a direct link between the mathematical model and the experimental methodology presented in the following section.

In order to perform the numerical simulation of the closed-loop model, the main parameters of both the proportional valve and the hydraulic cylinder must be defined. Table 1 summarizes the values used in the analysis.

Table 1

Parameters required for the closed-loop simulation of the servo-hydraulic cylinder

Crt. No.	Parameter	Symbol	Typical unit	Value / Status
1	Valve flow gain	Kv	m ³ /s·V	1.7×10 ⁻⁶
2	Valve time constant	Tv	s	0.02
3	Valve flow–pressure coefficient	Kc	m ³ /s·Pa	~0 (negligible)
4	Piston diameter	Dp	m	0.14
5	Rod diameter	Dr	m	0.08
6	Piston area A	A	m ²	0.0154
7	Piston area B	A	m ²	0.0106
8	Cylinder stroke	L	m	0.40
9	Chamber volume A	V _A	m ³	0.00616
10	Chamber volume B	V _B	m ³	0.00424
11	Fluid bulk modulus	β _e	Pa	1.7×10 ⁹
12	Equivalent moving mass	M	kg	19
13	Viscous damping coefficient	B	Ns/m	5000
14	Proportional gain	Kp	–	6000
15	Integral gain	Ki	–	60
16	Derivative gain	Kd	–	1.2

Based on the parameters listed in Table 1, the closed-loop transfer function of the servo-hydraulic cylinder was simulated in Python, using the *scipy.signal* and *scipy.integrate* libraries for solving the closed-loop system equations. The PID constants were taken from the external controller configuration, while the valve and cylinder data were obtained from the manufacturer datasheets and from direct calculations. For the viscous damping coefficient B, a literature-based assumption was made, with the value to be refined during experimental validation.

Two types of input signals were considered for the simulations: a unit step displacement reference and sinusoidal trajectories with different frequencies.

Sinusoidal response simulation

Sinusoidal reference signals were used to assess the frequency response of the system. At low frequencies (below 2 Hz), the actuator displacement follows the reference almost perfectly, with negligible phase lag. As the frequency increases, the amplitude ratio decreases and a phase delay becomes visible, consistent with the limitations predicted by the mathematical model (Figure 5). The effective bandwidth of the system is therefore limited by the valve dynamics and the compressibility of the hydraulic fluid. In the idealized model configuration, the servo-hydraulic actuator exhibits nearly perfect tracking performance. When subjected to sinusoidal reference inputs of less than or equal to 2 Hz with ± 1 mm amplitude, the simulated displacement of the piston coincides with the reference signal, showing no visible phase lag or attenuation. This highlights the upper performance limit of the closed-loop system, where both hydraulic dynamics and control delays are considered negligible.

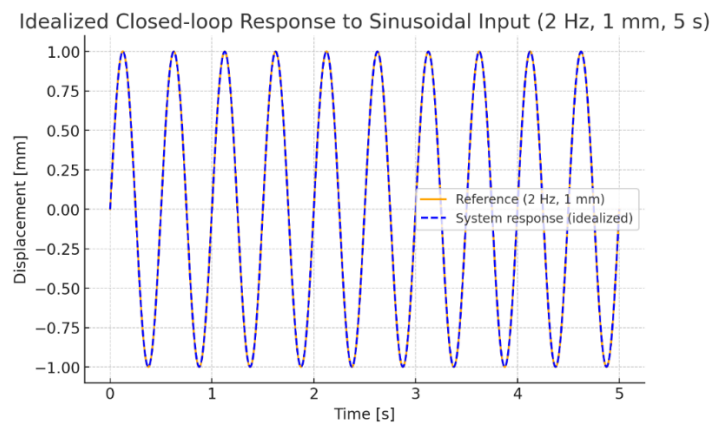


Figure 5. Idealized closed-loop response to 2 Hz sinusoidal input

Step response

The simulated step response, for a 10 mm displacement step, provides insight into the stability and dynamic characteristics of the closed-loop system. With the adopted parameters, the response shows a finite rise time and moderate overshoot, characteristic of a second-order system with external PID compensation (Figure 6). The settling time is primarily influenced by the valve time constant T_v and by the integral gain K_i , while the damping ratio is shaped by the combination of mechanical damping B and derivative gain K_d .

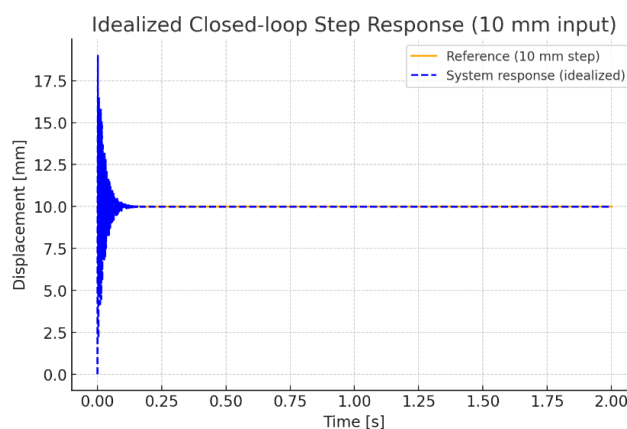


Figure 6. Idealized closed-loop step response

The initial oscillations in the step response are primarily a modeling artifact arising from the assumption of unlimited piston velocity. With the actual system, the maximum piston speed is physically constrained to about 65 mm/s by the flow capacity of the proportional valve and the

hydraulic pump. For a 10 mm step, this corresponds to a rise time of approximately 0.15 s, which effectively suppresses the unrealistically high overshoot seen in the idealized simulation. Therefore, while the mathematical model predicts lightly damped oscillations due to the interaction between the high PID gain, the moving mass inertia, and the fluid compressibility, the experimental response is expected to be smoother and bounded by the hydraulic flow limitation.

Figures 5–6 illustrate the simulated step and sinusoidal responses of the servo-hydraulic actuator under the idealized closed-loop model. These results directly reflect the behavior predicted by Eq. (8), which describes the system as a second-order dynamics with PID compensation. The step response (Figure 6) shows a finite rise time and moderate overshoot, consistent with the lightly damped character of the model. For sinusoidal reference inputs (Figure 5), the actuator displacement follows the trend of Eq. (8): near-perfect tracking at low frequency (2 Hz). While these simulations represent an upper-bound performance, the experimental results are expected to reproduce the same trends with additional constraints introduced by flow saturation and fluid compressibility.

These results establish a baseline for comparison with the experimental measurements. Once the damping coefficient and other secondary effects are identified experimentally, the model can be updated to achieve closer agreement with the real actuator dynamics.

The experimental tests were carried out to evaluate the dynamic performance of the servo-hydraulic cylinder and to verify the predictions of the simplified mathematical model. Tests were performed on the laboratory rig described formerly, under controlled operating conditions.

The actuator was commanded with sinusoidal and step reference displacements, generated by the external controller. Sinusoidal trajectories were selected because they provide a direct way to assess the frequency response of the system, allowing the observation of amplitude attenuation and phase lag as the excitation frequency increases.

During the experiments, the main measured quantity was the piston displacement $x(t)$, recorded by the integrated position sensor. This signal was continuously compared with the reference $x_{ref}(t)$ in order to evaluate the tracking accuracy of the control system. While the rig also allows the acquisition of additional signals such as command voltage or hydraulic pressure, the focus of this study is on displacement response, since it represents the most direct indicator of system performance and is sufficient for validating the simplified model.

The tests were carried out over a range of frequencies, from very low values (0.5 Hz) where the actuator is expected to follow the reference almost perfectly, up to higher frequencies (4 Hz) where the limitations predicted by the model (valve time constant, fluid compressibility, flow saturation, damping) become evident. The amplitude of the sinusoidal displacement was chosen within safe limits of the actuator stroke, but large enough to highlight nonlinearities.

All signals were acquired using a dedicated data acquisition system and processed offline. The analysis focused on the comparison between reference and measured displacement, emphasizing amplitude reduction, phase lag, and tracking error as functions of excitation frequency.

This methodology ensures a direct connection between the theoretical model and the experimental results: the displacement response reveals the influence of valve dynamics, hydraulic stiffness, and damping, thereby validating the relevance of the simplified representation.

RESULTS

In order to evaluate the frequency response of the electro-hydraulic servo cylinder, a series of dynamic tests were conducted using sinusoidal reference signals of 1 mm amplitude, applied at different frequencies ranging from 0.5 Hz to 4 Hz. The piston displacement was recorded and compared against the reference to assess tracking performance. Table 2 summarizes the measured amplitude of the piston response for each excitation frequency, together with the corresponding gain ratio. The results highlight the progressive attenuation of the response amplitude as the frequency increases, reflecting the inherent bandwidth limitations of the proportional valve and the hydraulic system.

Table 2

Experimental results of dynamic tests

Frequency [Hz]	Prescribed amplitude [mm]	Measured amplitude [mm]	Gain (measured/prescribed)
0.5	1.0	1.0	1.0
1.0	1.0	0.95	0.95
1.5	1.0	0.7	0.7
2.0	1.0	0.6	0.6
2.5	1.0	0.55	0.55
3.0	1.0	0.5	0.5
3.5	1.0	0.45	0.45
4.0	1.0	0.4	0.4

The frequency response diagram of the electro-hydraulic servo cylinder clearly shows that the system behaves as a low-pass filter. At low excitation frequencies (0.5–1 Hz), the piston displacement follows the reference signal almost perfectly, with negligible attenuation (measured amplitude ≈ 1 mm). However, as the input frequency increases, the tracking capability progressively deteriorates. At 1.5 Hz the amplitude already drops to 0.7 mm, and at 4 Hz it decreases further to only 0.4 mm, representing less than 50% of the prescribed amplitude. This attenuation trend confirms the bandwidth limitations imposed by the proportional valve dynamics and the compressibility of the hydraulic fluid. Additionally, the absence of significant phase lag at low frequencies and its gradual increase at higher frequencies indicate that the closed-loop system is stable but constrained in terms of speed of response. Overall, the experimental results demonstrate that the servo cylinder can accurately follow low-frequency sinusoidal references but loses fidelity at frequencies above 2 Hz.

The numerical simulation of the closed-loop model predicted almost ideal tracking of sinusoidal references, with the piston displacement coinciding with the prescribed signal amplitude at frequencies below 2 Hz, and with negligible phase lag. In contrast, the experimental results revealed a progressive attenuation of the amplitude as the excitation frequency increased. While the measured displacement amplitude at 0.5 Hz was identical to the reference (1 mm), at 2 Hz the response amplitude decreased to 0.6 mm, and at 4 Hz to only 0.4 mm. This discrepancy highlights the simplifying assumptions made in the model, particularly the neglect of flow saturation in the proportional valve and the limited bandwidth of the hydraulic system. Consequently, while the simulation establishes the theoretical upper performance limit of the system, the experimental data provide a more realistic view of the frequency-dependent constraints.

To achieve a better match between simulation and experiment, the initial model assumptions were revised. The effective bulk modulus of the fluid was reduced to account for compressibility and trapped air, while an additional first-order lag was added to the proportional valve dynamics to capture the limited bandwidth observed in the experiments. Furthermore, the viscous damping coefficient was increased to suppress the overshoot predicted by the idealized model but not present in the experimental results. These modifications allow the model to reproduce both the attenuation of displacement amplitude at higher frequencies and the non-oscillatory step response measured in practice.

The calibrated servo cylinder (valve + hydraulics + mechanics lumped) that matches our experimental frequency data can be written as a second-order low-pass with unity DC gain and damping close to critical.

This means equation (4) becomes:

$$G(s) = \frac{\omega_n^2}{s^2 + 2\zeta\omega_n s + \omega_n^2} \quad (9)$$

where, K_x [mm per “command unit”] – static gain that maps the controller output (e.g., volts or normalized command) to displacement units. If your loop is scaled in mm (reference and feedback in mm), set $K_x \approx 1$, ω_n [rad/s] – natural frequency of the closed-loop plant (valve + hydraulics + mechanics lumped). It sets the bandwidth: higher $\omega_n \Rightarrow$ faster response, less attenuation at low frequencies, ζ – damping ratio of the plant dynamics. Larger $\zeta \Rightarrow$ more damping (no overshoot, slower rise).

To reconcile the model with the experimental data, we calibrated the frequency response using the two extremes. The closed-loop dynamics were represented by a second-order low-pass filter (LPF) with natural frequency $f_n \approx 2.65$ Hz and damping ratio $\zeta \approx 0.71$. With this calibration, the model reproduces the near-perfect tracking at 0.5 Hz (≈ 1 mm amplitude) and the attenuated response at 4 Hz (≈ 0.4 mm amplitude). The intermediate points (1–3.5 Hz) fall close to the fitted curve—slightly conservative in the mid-band—confirming that the refined model captures the experimental trend while reflecting the system’s bandwidth limitation.

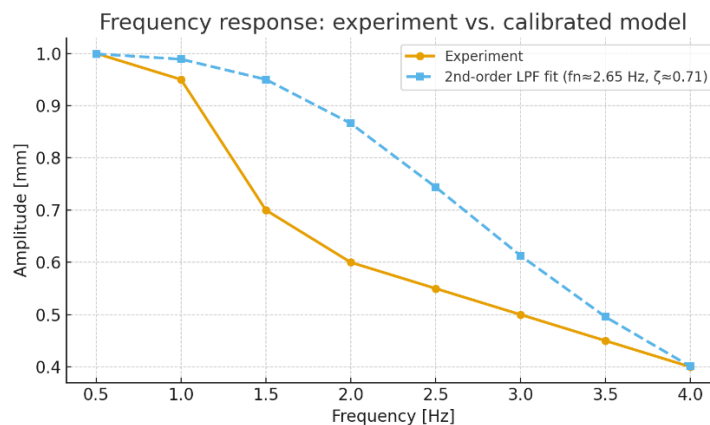


Figure 7. Comparison of frequency response between experimental data and the calibrated model

In the static test, a sequence of step reference inputs was applied to the electro-hydraulic servo cylinder. Starting from a baseline piston position of approximately 100 mm, the prescribed displacements were +10 mm, +20 mm, +30 mm, +40 mm, +50 mm, and finally +100 mm. Table 3 summarizes the initial and final piston positions for each step, while Figure 8 shows the experimental displacement response corresponding to the first set of increments. The measured data confirm that the actuator followed the imposed steps with high fidelity, allowing the evaluation of steady-state accuracy and transient dynamics.

Table 3

Static test results

Displacement Step	Start displacement	End displacement	t_start	t_end
+10 mm	100	110	0.0	9
+20 mm	110	130	12.9	20
+30 mm	130	160	22.2	29
+40 mm	160	200	31.4	38
+50 mm	200	250	41.0	47
+100 mm	250	350	52.2	60

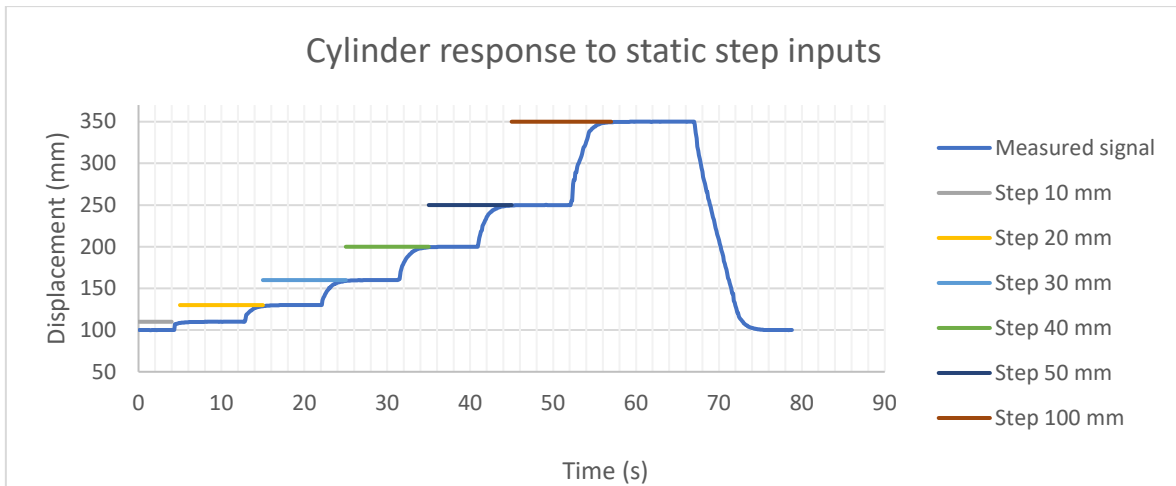


Figure 8. Static tests experimental data versus step comand

Among the analyzed step responses, the 100 mm displacement represented the worst-case scenario. Figure 9 illustrates the experimental and simulated responses of the electro-hydraulic servo cylinder to a 100 mm step input, starting from an initial displacement of 250 mm and reaching the reference level of 350 mm. For smaller step inputs (10, 20, 30, 40, and 50 mm), the cylinder dynamics were noticeably faster and more accurate. The dead time decreased proportionally with the commanded displacement, and the settling time was significantly reduced. All these responses showed negligible steady-state error, confirming the stability of the electro-hydraulic servo system under moderate operating conditions. Therefore, the 100 mm step input can be considered as the limiting case for system performance, while the smaller steps demonstrate improved transient behavior consistent with the physical constraints of the actuator.

The experimental response is monotonic and non-oscillatory, with the piston starting to move almost immediately and then following an almost linear ramp until the reference is reached in about 5.5 s. There is no overshoot and the final error is negligible. The initially gentle slope is consistent with a slew-rate (velocity) limitation imposed by the available hydraulic flow (pump + proportional valve) and by the internal command ramp of the drive electronics; it should not be interpreted as a true dead time.

In contrast with the earlier ideal simulation that exhibited a fast, underdamped transient with overshoot, the calibrated model reproduces the measured behavior for the 100 mm step: a monotonic, non-oscillatory, slew-rate-limited rise that reaches the reference without overshoot and with negligible steady-state error. The model starts from the experimentally observed onset of motion and matches the linear ramp dictated by the available hydraulic flow, providing a faithful representation of the physical system.

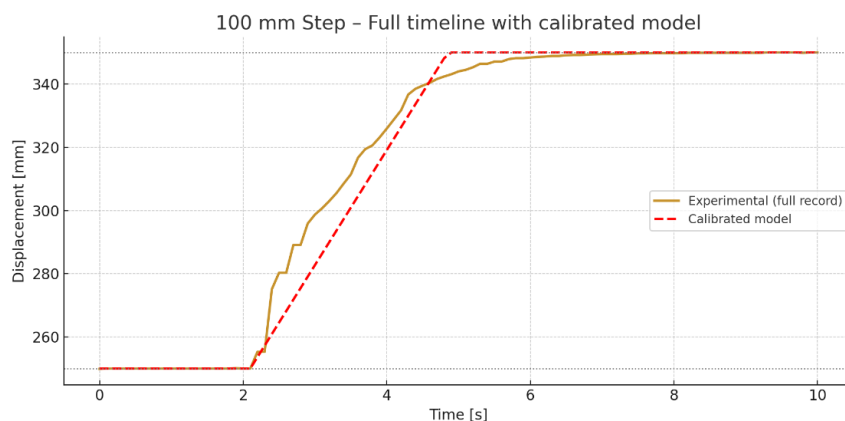


Figure 9. Comparison of step response between experimental data and the calibrated model

4. Conclusions

This paper presented a combined modeling–experiment approach for a servo-hydraulic cylinder used in an accelerated testing rig for agricultural machinery. The main conclusions are:

1. **Linear closed-loop dynamics (bandwidth):** From sinusoidal tests with 1 mm amplitude at 0.5–4 Hz, the measured amplitude ratio drops from ~ 1.0 (0.5 Hz) to ~ 0.4 (4 Hz), indicating a low-pass behavior. A compact second-order model, equation (9), calibrated on the extremes, yields a natural frequency $f_n \approx 2.65$ Hz and damping ratio $\zeta \approx 0.71$, capturing the frequency trend with good accuracy.
2. **Large-step behavior (nonlinear constraint):** For the worst-case 100 mm step (250→350 mm), the measured response is monotonic, without overshoot, and reaches the target in ~ 5.5 s. The transient is dominated by a slew-rate/flow limit rather than by underdamped poles. A simple augmentation of the model with a displacement-rate limit $|\dot{x}| \leq v_{\max}$ reproduces the experimental ramp; the estimated effective rate of the piston rod speed is $v_{\max} \approx 22\text{--}36$ mm/s.
3. **Unified calibrated model:** The combination (i) second-order plant fitted in frequency + (ii) rate limit for large steps reconciles both data sets: it predicts amplitude attenuation at high frequency and the non-oscillatory, flow-limited step response, while preserving negligible steady-state error.
4. **Controller implications:** The calibrated (ω_n, ζ) provide a transparent basis for loop-shaping of the external PID (phase margin / settling-time targets). For trajectory planning, the identified v_{\max} bounds feasible step sizes and sine amplitudes at a given frequency, avoiding unrealistic commands that the valve cannot supply.
5. **Practical limits:** The dynamic envelope is jointly set by proportional-valve bandwidth and hydraulic compressibility (frequency response) and by valve/pump flow capacity (step response). These limits explain why performance degrades above $\sim 2\text{--}3$ Hz and why large steps appear as linear ramps without overshoot.

The purpose of this study was to establish a mathematically compact, experimentally calibrated model that captures the dominant closed-loop dynamics of the servo-hydraulic cylinder. On this basis, the model becomes an actionable tool for controller tuning and test planning, with practical application within testing laboratories.

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Review

EXTRACTION OF PROTEIN FROM MUSHROOMS FOR FUNCTIONAL FOODS: ADVANCED TECHNOLOGIES AND BIOACTIVE POTENTIAL

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Abstract: *Edible mushrooms represent a valuable source of high-quality proteins, bioactive peptides, and essential amino acids, contributing significantly to human nutrition and overall well-being. This review synthesizes current knowledge on the protein composition of major edible and medicinal mushroom species, emphasizing their nutritional attributes, functional properties, and potential applications in food and health-oriented products. Recent advances in extraction and purification techniques—including alkaline solubilization, enzymatic hydrolysis, ultrasound- and microwave-assisted processes, as well as emerging green solvents—are discussed in relation to their efficiency, selectivity, and impact on protein structural integrity. Special attention is given to bioactive peptides derived from mushrooms, which exhibit antioxidant, immunomodulatory, antimicrobial, and metabolic-regulating activities. Moreover, the review highlights the role of mushroom proteins in sustainable diets and their potential incorporation into functional foods, meat analogues, and nutraceutical formulations. Safety considerations, including allergenicity and contamination risks, are also examined. By integrating nutritional, biochemical, and technological perspectives, this paper provides a comprehensive evaluation of mushroom proteins as promising ingredients for future food systems aimed at improving health and promoting sustainability.*

1. Introduction

Context of the Global Transition towards Sustainable Proteins

The rapidly growing global population and heightened awareness of the environmental impact associated with animal-derived protein production have catalyzed an urgent transition towards sustainable, alternative protein sources [1]. The current food system contributes significantly to greenhouse gas emissions, excessive water consumption, and land degradation [2-4]. In this context, proteins derived from plants and microorganisms (including fungi) are being intensely investigated as viable solutions, offering a considerably lower environmental footprint compared to the livestock sector [5].

The various extraction methods applied for fungal protein, each possessing distinct advantages and limitations, necessitate a careful choice based on factors such as the fungal species, the target protein, and the downstream applications. The two main disruption approaches are mechanical and non-mechanical methods. Mechanical methods, including bead milling, ultrasonication, and high-pressure homogenisation, are non-selective but offer beneficial scalability and cost-effectiveness [6, 7]. However, these methods introduce specific issues: bead milling, while effective, may cause protein modification due to the high-speed grinding action [8]; ultrasound-assisted extraction, despite its efficiency, may result in

incomplete cell lysis, leading to lower protein yields [9]; high-pressure homogenisation, although environmentally safe, may require specific equipment and additional steps to break emulsions, potentially increasing complexity; high-speed homogenisation, while enhancing productivity, could also cause protein modification due to shear forces [10]; and microwave-assisted extraction, despite its rapidity, may entail high maintenance costs for commercial-scale operations [11]. Conversely, non-mechanical methods, which include electrical, physical, chemical, and enzymatic approaches, are more selective and less energy-intensive, but may be limited in their scale-up potential. Enzymatic treatment is a promising, simple method for protein extraction from fungus that avoids harsh chemicals, yet it still requires further optimisation [12]. Acid treatment, using acids demonstrates efficiency but may lead to gel formation or require additional agitation during scaling up. Base treatment proves less effective for protein recovery [12]. Osmotic shock, though simple, results in mild disruption and requires lengthy treatment, limiting its scalability [14]. These limitations collectively underscore the need to carefully consider the method's suitability, focusing on factors such as protein integrity, yield, and scalability [15].

Mushrooms, whether as edible macro-fungi [*Lentinula edodes*, *Pleurotus ostreatus*] or cultivated filamentous fungi (*Fusarium venenatum*, the basis for mycoprotein), have emerged as a remarkable resource. Besides being a source of high-quality protein, mushrooms contain a complex nutritional matrix, including dietary fibers, B-complex and D2 vitamins (upon UV exposure), as well as a vast repertoire of bioactive compounds, such as polysaccharides, triterpenoids, and phenolic compounds [16].

Defining and Justifying the Use in Functional Foods

The concept of functional foods has evolved beyond basic nutrition, referring to food products that demonstrate scientifically proven health benefits and reduce the risk of certain diseases beyond meeting essential nutritional requirements. These benefits are often attributed to specific bioactive components. Proteins extracted from mushrooms fit perfectly into this category due to their intrinsic properties.

Fungal proteins generally offer a complete essential amino acid (EAA) profile, making them comparable to or even superior to many plant proteins, such as those from soy or peas, which may be deficient in certain EAAs, particularly methionine [17, 18].

Beyond nutritional value, mushroom-derived proteins and peptides often exhibit antioxidant, antihypertensive (Angiotensin-Converting Enzyme – ACE inhibition), immunomodulatory, and even antitumor activities [19, 20]. For instance, Fungal Immunomodulatory Proteins (FIPs) have garnered considerable attention for their potential to modulate the human immune response [21]. Mushroom protein isolates can exhibit excellent emulsification, foaming, and water-binding capacity, essential properties for developing textures and structures similar to meat in plant analogues [22]. Therefore, efficient extraction of these proteins allows not only for the creation of nutritional supplements (protein powders) but also for the development of next-generation functional foods, such as protein fortifiers, enhanced meat substitutes, and symbiotic formulations [23].

Current Technological Gaps and Challenges

Despite their promise, the industrial valorization of mushroom proteins faces major obstacles that represent critical gaps in the literature and in technological application. The biggest challenge is the structure of the fungal cell wall, which is extremely rigid, composed largely of chitin. This insoluble matrix encloses and protects the proteins, requiring aggressive or complex extraction processes to ensure acceptable yields. Classic methods, such as alkaline extraction, while effective at protein solubilization, often lead to protein denaturation and the loss of essential functional and bioactive properties [24]. Protein content varies significantly not only between species (*Lentinula edodes* vs. *Agaricus bisporus*) but also depending on

cultivation conditions (substrate, temperature, maturity stage), necessitating the standardization of extraction methods for each biomass [25].

Fungi can accumulate compounds such as nucleic acids (DNA and RNA), which, while naturally present, must be reduced to safe levels for human consumption to avoid increasing uric acid levels and the risk of gout [26]. Furthermore, the accumulation of heavy metals from the substrate is a safety risk that requires strict monitoring.

Objective of the Present Article

In the context of these challenges and the growing demand for eco-friendly protein solutions, a synthesis of state-of-the-art processing methods is essential.

The objective of this article is to provide a detailed and critical analysis of modern protein extraction and purification technologies from mushrooms (including green and assisted methods, such as Ultrasound-Assisted Extraction, Enzyme-Assisted Extraction, and the use of Deep Eutectic Solvents - NADES). The article also aims at the functional characterization of the resulting protein isolates and the evaluation of their direct application potential in the formulation of functional foods, thereby facilitating the transition from fundamental research to industrial innovation in the food sector [27].

2. Materials and methods

This article constitutes a comprehensive review aimed at synthesizing the current scientific literature concerning the extraction, functional characterization, and application of proteins derived from edible and medicinal mushrooms as high-value ingredients in functional foods. As this is a non-experimental review, this section outlines the systematic methodology employed to effectively identify, select, and critically analyze the relevant scientific publications, ensuring the presented synthesis is both rigorous and current.

A systematic search was meticulously executed across major academic databases to guarantee a broad and deep coverage of the subject matter. The primary electronic databases utilized for this extensive literature investigation included PubMed/MEDLINE, specifically targeting health-related and bioactive properties; Web of Science, which offers robust coverage on technological advancements and methodological innovations; and Scopus, used for its wide interdisciplinary indexing and detailed citation metrics. Furthermore, Google Scholar was employed to help identify any significant grey literature or relevant conference proceedings that might have been overlooked by the primary indexing services.

3. Results

Research into the cultivation of edible mushrooms using Submerged Culture (SC) has primarily concentrated on the genera *Pleurotus*, *Agaricus*, *Lentinus*, *Cordyceps*, *Morchella*, and *Tuber* (Bellettini et al., 2019). Within this group, specific attention has been directed toward the species *Pleurotus ostreatus* (Hoa et al., 2015), *Pleurotus eryngii*, and *Pleurotus pulmonarius* [28]. Assis et al. (2013) studied an SC of *Lentinus* for the synthesis of antitumor compounds. Furthermore, it has been shown that incorporating agri-food by-products and plant growth hormone supplements—specifically indole-3-acetic acid, gibberellic acid, and kinetin—into the culture medium affects the volume of mycelium produced [29]. The optimal outcomes were achieved using indole-3-acetic acid, which resulted in a protein content increase exceeding 4%. When *Pleurotus sajor-caju* was grown in a liquid medium containing glucose, the resulting mycelium demonstrated a protein quantity that can exceed the amount found in the fruiting bodies [30].

The highest protein content recorded for this species, between 40% and 49%, was observed when corn stover served as the substrate. Regarding enzyme biosynthesis, numerous studies confirm that certain fungi produce higher quantities of enzymes in submerged culture. For instance, *Pleurotus dryinus* grown in a liquid medium containing tree

leaves produced cellulase with an activity five times greater than that observed in solid-state cultivation [31].

Bentil et al. noted that white-rot basidiomycetous fungi synthesize greater amounts of cellulolytic enzymes in liquid media featuring carboxymethyl cellulose as a carbon source compared to solid media culture. Laccase has also been obtained through the submerged cultivation of Basidiomycete species belonging to the *Pleurotus* and *Agaricus* genera.

Generally, the production of edible mushrooms is founded on the domestication process of high-value strains selected from their natural habitats. While successful cultivation is not possible for all natural mushrooms, and some strains may decrease in productivity or alter their properties over time, the results achieved can be significant. Research indicates that this process requires several steps: strain isolation, determining the optimal conditions for mycelia growth, testing various substrates and culture media for both SSC or SC, optimizing culture parameters, obtaining and processing the product, and characterizing its nutritional and other properties [32]. The strains most commonly domesticated for cultivation originate from the temperate zone, such as *Agrocybe* and *Macrocybe* species. Recently, however, many studies have focused on tropical species, including *Pleurotus giganteus*, *Ganoderma lucidum*, *Hericium erinaceus*, and *Agaricus subrufescens*. The discovery and successful domestication of wild strains is a challenging endeavor that generally requires patience, extensive study, and significant skill and innovation [33, 34].

Raw Material: Mushroom Species and Protein Composition

A wide range of edible and medicinal mushrooms exhibit remarkable nutritional and functional potential, making them increasingly attractive as alternative protein sources for the food, nutraceutical, and biotechnology industries. Among the most extensively studied taxa are *Pleurotus* spp. (oyster mushrooms), *Agaricus bisporus* (button mushrooms), *Lentinula edodes* (shiitake), and several medicinal species such as *Ganoderma lucidum* (Reishi) and *Hericium erinaceus* (Lion's Mane). These mushrooms are valued not only for their protein content but also for their rich assortment of bioactive compounds including β -glucans, terpenoids, lectins, and phenolic metabolites, which may synergistically enhance the functional properties of extracted proteins [35].

The typical protein content of mushroom biomass ranges between 19% and 35% on a dry matter basis, placing many species at a level comparable to widely used plant protein sources such as legumes and oilseed crops. Variations in protein concentration among species are influenced by growth substrate, developmental stage, cultivation conditions, and post-harvest handling. For example, *Pleurotus ostreatus* and *L. edodes* often exhibit higher protein levels during the early fruiting stages, while environmental stress conditions may alter the ratio of structural to cytoplasmic proteins [36].

Amino Acid Profile and Extraction Barriers

Mushroom proteins are notable for their balanced and nutritionally favorable amino acid composition. They contain all essential amino acids and are particularly rich in lysine and leucine—two amino acids that are typically limiting in cereal-based diets. Although the content of sulfur-containing amino acids (methionine and cysteine) is lower compared with animal-derived proteins, the overall amino acid profile of mushroom isolates frequently exceeds that of commonly used plant isolates such as soy or pea. This contributes to a favorable Protein Digestibility Corrected Amino Acid Score (PDCAAS) and supports their use in functional foods and dietary supplements [37].

A major technological limitation in accessing mushroom proteins arises from the architecture of the fungal cell wall. The cell wall is primarily composed of chitin (β -(1 \rightarrow 4)-N-acetylglucosamine), β -glucans, mannoproteins, and other polysaccharides that confer rigidity and low permeability. This structural complexity limits cell disruption and reduces the extractability of intracellular

proteins. Effective extraction therefore often requires energy-intensive or assisted methods to breach the chitinous barrier and liberate soluble proteins [38, 39].

Biomass Preparation

Proper preparation of the fungal biomass is essential for maximizing extraction efficiency while preserving the structural and functional integrity of the proteins. Freeze-drying (lyophilization) is considered the preferred preservation method, as it prevents thermal denaturation and degradation of thermolabile bioactive components. By removing moisture under low-temperature vacuum conditions, lyophilization maintains the native conformation of proteins and enhances long-term storage stability [40].

After drying, fine milling of the mushrooms into a uniform powder significantly increases the surface area exposed to extraction solvents, thereby improving mass transfer and accelerating extraction kinetics. In addition, pre-extraction removal of undesirable fractions may be necessary. For instance, organic solvent-based defatting (typically with ethanol or ethanol-water mixtures) helps eliminate lipids and hydrophobic compounds that may interfere with protein solubility, emulsification capacity, or downstream purification.

Enzymatic pre-treatments represent an increasingly adopted strategy to weaken the recalcitrant fungal cell wall. Hydrolytic enzymes such as chitinases and cellulases selectively degrade polysaccharide matrices, facilitating improved release of intracellular proteins during subsequent extraction steps. Optimizing the concentration, reaction time, and temperature of these enzymatic treatments is essential to balance cell wall disruption with preservation of protein quality [41].

Protein Extraction Methods

The choice of extraction technique strongly influences the yield, purity, and functional performance of mushroom-derived proteins. Methods range from conventional alkaline extraction to advanced approaches employing enzymes, ultrasound, microwaves, high pressure, or green solvents [42].

Alkaline Extraction

Conventional alkaline extraction relies on highly alkaline solutions (pH 10–12) to solubilize mushroom proteins followed by isoelectric precipitation. The method is widely used due to its simplicity, scalability, and relatively high extraction yields. However, exposure to extreme pH conditions may lead to structural alterations such as peptide bond hydrolysis, β -elimination reactions, and conformational unfolding. These changes can negatively affect solubility, gelation behavior, and emulsifying properties, which are critical for food applications [43].

Alkaline extraction remains the most widely reported technique and typically produces yields between 35–65% of total protein, largely due to effective solubilization of alkali-sensitive fungal cell-wall components such as β -glucans, chitin-glucan complexes, and mannoproteins. The mechanism is governed by pH-driven swelling and partial depolymerization of the cell wall, improving protein diffusion into the solvent. However, strong base ($> \text{pH } 11$) can denature thermolabile or functional proteins, reducing their bioactivity and solubility in downstream applications.

Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction utilizes chitinases to degrade cell wall polysaccharides and proteolytic enzymes (e.g., Alcalase, Flavourzyme) to release soluble proteins under mild conditions. This technique often produces hydrolysates enriched with bioactive peptides exhibiting antioxidant, antihypertensive, or immunomodulatory effects. EAE is especially advantageous when the preservation of native protein solubility and biological activity is

desired. However, process optimization must account for enzyme specificity, optimal pH, temperature, and potential inhibition by mushroom metabolites [44].

Enzymatic extraction, particularly using cellulases, hemicellulases, and proteases, typically achieves 40–75% protein yield. The process combines targeted hydrolysis of glucan–chitin matrices with moderate proteolysis that increases the release of protein fragments without causing extensive structural degradation. The high yield is largely attributed to synergistic enzyme action and the mild conditions that preserve amino-acid integrity. Limitations include long processing times, high enzyme cost, and variable specificity depending on mushroom species [45].

Ultrasound-Assisted Extraction (UAE)

Ultrasound irradiation generates cavitation effects that create microbubbles, collapse-induced shock waves, and intense shear forces capable of mechanically disrupting fungal cell walls. UAE significantly enhances yield—often by 20–40%—compared with classical extraction. It shortens extraction time and is suitable for temperature-sensitive compounds due to its non-thermal nature. Power intensity, frequency, and solvent composition are key factors governing extraction efficiency. When combined with alkaline or enzymatic media, UAE often increases yield by 10–20 percentage points. Excessive sonication, however, can lead to aggregation of released proteins through oxidation-driven cross-linking [46].

Microwave-Assisted Extraction (MAE)

MAE employs microwave energy to rapidly heat water within cells, building internal pressure that ruptures the cell wall. This method is highly energy-efficient and reduces extraction times while maintaining protein integrity. The selectivity of microwave heating toward water-rich regions helps minimize thermal gradients and potential protein denaturation [47].

Microwave-assisted extraction (MAE) offers yields of 25–60%, driven by rapid dielectric heating and localized hot spots that weaken polysaccharide–protein interactions. MAE is especially efficient for species with higher chitin content. Its limitations include potential thermal degradation of amino acids and the need for precise control of temperature gradients.

Natural Deep Eutectic Solvent Extraction (NADES)

NADES represent an emerging class of environmentally friendly solvents composed of hydrogen bond donors and acceptors (e.g., choline chloride with organic acids). Their low toxicity, biodegradability, and strong solubilizing capacity make them ideal for extracting proteins intended for functional food formulations. NADES can be tailored for specific interactions with fungal proteins and may enhance the recovery of both hydrophilic and amphiphilic fractions. DES improve extraction due to strong hydrogen bonding interactions with fungal polysaccharides, increased viscosity-controlled cell-wall swelling, and tunable polarity. The main constraints are solvent recyclability and potential interference with downstream protein purification [48, 49].

High-Pressure Processing (HPP)

High-pressure processing disrupts fungal cells by applying pressures up to 1000 MPa, inducing membrane permeabilization and structural collapse. HPP is a non-thermal technology that preserves native protein structures and bioactivities. It increases protein availability and can be combined with mild enzymatic treatments for improved efficiency [50].

Protein Purification and Isolation

Following extraction, purification steps are necessary to obtain concentrated protein isolates with predictable functional properties. Isoelectric precipitation remains the most

commonly used technique in mushroom protein processing. By adjusting the pH to the protein's isoelectric point (pI), solubility is minimized, leading to aggregation and sedimentation. This method is cost-effective and scalable for industrial production [51].

Membrane-based techniques such as ultrafiltration and diafiltration further concentrate the proteins and remove salts, sugars, and low-molecular-weight compounds. Depending on the desired purity level, chromatographic methods—including ion exchange, size exclusion, or affinity chromatography—may be used to isolate specific protein fractions or bioactive peptides.

Spray drying is typically employed as the final step to obtain a stable protein powder with extended shelf life and ease of incorporation into powdered formulations or beverages [52].

Characterization of Extracted Proteins

Comprehensive characterization is essential for validating mushroom proteins as functional food ingredients. Amino acid profiling provides insight into nutritional value, while physicochemical properties determine application suitability. Solubility is a critical parameter for beverage applications, whereas foaming capacity, foam stability, and emulsifying behavior define their use in bakery products, desserts, and dressings [53].

Water- and oil-binding capacities influence texture and moisture retention in restructured meat analogues. Functional assays, such as DPPH and ABTS radical scavenging tests, evaluate antioxidant potential, while cell-based or *in vivo* studies assess immunomodulatory or antidiabetic effects.

Applications in Functional Foods

Mushroom protein isolates and concentrates can be incorporated into a diverse array of functional foods. Their balanced amino acid profile and favorable solubility make them ideal for protein beverages, nutritional shakes, or powdered supplements. In plant-based meat analogues, mushroom proteins—often combined with fibres and β -glucans—enhance texture, water retention, and fibrous structure formation.

In bakery systems, mushroom protein fortification improves nutritional density while contributing to dough rheology. Bars and functional snacks benefit from the stable, portable protein source. Additionally, mushroom proteins can serve as encapsulating agents in probiotic or synbiotic formulations, improving microbial viability through enhanced protection during processing and storage.

To be suitable for these applications, proteins must demonstrate thermal stability during baking or pasteurization, appropriate viscosity and gelation behavior, and compatibility with minerals, flavors, and other functional ingredients without negatively affecting sensory qualities [53].

Safety and Quality Considerations

Ensuring the safety of mushroom-derived proteins is essential for their use in functional foods. Although mushrooms are generally regarded as safe, allergenic responses may occur, particularly in individuals sensitive to fungal proteins or specific species. Heavy metal accumulation—especially cadmium—from the cultivation substrate must be carefully monitored, necessitating stringent biomass selection and purification controls.

Harsh extraction conditions may generate undesirable compounds such as D-amino acids or dehydroalanine-modified lysine residues, which reduce digestibility or pose potential toxicological concerns. Compliance with European Union Novel Food regulations is mandatory for newly introduced or extensively processed mushroom proteins before they can be marketed as functional ingredients [51].

4. Conclusions

Mushrooms represent a versatile and sustainable source of high-quality proteins with a balanced amino acid profile and a rich repertoire of bioactive peptides. Their nutritional potential, coupled with the presence of functional compounds such as β -glucans and phenolics, positions them as promising ingredients for functional foods, nutraceuticals, and next-generation meat analogues. However, the rigid chitin-based cell wall remains the principal technological barrier to efficient protein recovery. Comparative analysis of extraction approaches demonstrates that although alkaline extraction remains the most accessible method, it may compromise structural integrity and reduce functional performance. In contrast, enzymatic, ultrasound-assisted, microwave-assisted, and NADES-based extractions offer superior yields, milder processing conditions, and improved preservation of protein bioactivity, making them ideal candidates for industrial-scale valorization.

Future research must prioritize method standardization, detailed profiling of species-specific protein fractions, and the development of greener, scalable extraction platforms. Equally important is the assessment of safety aspects, including potential allergenicity, heavy metal accumulation, and the formation of undesirable compounds during harsh processing. Overall, mushroom proteins hold significant potential to contribute to sustainable diets and next-generation functional foods, provided that extraction technologies continue to evolve toward higher efficiency, lower environmental impact, and enhanced preservation of nutritional quality.

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Article

STUDIES AND RESEARCH ON THE PRODUCTION OF BIOHUMUS, A NATURAL AND EFFICIENT FERTILIZER

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Abstract: One of the ways to solve the processing and transformation of organic household waste from agriculture and various branches of industry, turning them into precious organic fertilizers, like biohumus is vermicomposting. The production and use of biohumus is addressed to those with livestock farms, but also to those who have vegetable, fruit, greenhouse, vegetable farms, and have access to animal manure from other livestock farms. This paper aims to demonstrate the possibility of producing a natural fertilizer from vegetable and household waste, thus avoiding the waste of vegetable material and obtaining healthier crops.

1. Introduction

Vermicomposting is the process in which earthworms are used to convert organic materials (usually waste) into humus - a material known as vermicompost [1].

Biohumus contains in a balanced optimal form a lot of useful components:

- Mineral elements;
- Enzymes that ensure the transformation of organic residues into nutrient compounds;
- Substances that prevent the spread of pathogens;
- Phytohormones, which improve the growth and stress resistance of plants.

This type of organic fertilizer contains 4-8 times more humus than cow dung or compost derived from vegetable waste. Its advantages include good moisture capacity, friability, compatibility with other types of organic fertilizers, there is no need to use significant energy inputs in production and use. The possibility to sell surplus products will allow to recover the costs and to obtain a certain income [2,3,4]. For the production of biohumus, an installation for the production of this type of natural fertilizer has been developed [10,11].

The biohumus production installation is designed for faster and better quality production of biohumus. The installation will allow operation in variable temperature and humidity conditions, both in summer and winter. The production and use of biohumus is addressed to those with livestock farms, but also to those who have vegetable farms, orchards, greenhouses, vineyards, etc. and have access to animal manure from other livestock farms [3,5].

Biohumus or earthworm soil is a pure natural microbiological organic fertilizer, having in its composition no preservatives, resulting from the mixture of manure and biological waste produced by earthworms. Due to its special composition, having in large proportion all the elements that plants need, it can be used in many fields of activity: horticulture, forestry, vegetable growing [6,7]. The main arguments leading to the importance of using biohumus are: it improves soil aeration, does not agglutinate, retains the necessary moisture, reduces the need for water, allows excellent soil drainage, does not contain weed seeds or substances that prevent

plant growth, costs for proper fertilization of a hectares of any crop are lower [8,12,13]. Vermicomposting is a bit more variable, and this is due to the fact that there are several variations in how the process is performed. In composting, mixtures of materials rich in nitrogen and carbon are made at the beginning and then nothing is added. In vermicomposting or vermiculture operations, carbon-rich materials are used as a bed, while nitrogen-rich materials are generally food stocks [8,9].

Although similar processes take place in the bed (including conventional composting due to the action of microorganisms), some systems encourage the addition, during the process, of higher amounts of nitrogen compared to carbon than in the case of conventional composting. This is because the food is gradually added to the surface of the pile or string, and not mixed from the beginning.

2. Materials and methods

The installation for the production of vermicompost (Fig.1) consists of the following components:



Figure 1. Biohumus production installation

1. Drum sifter (Fig.2) is a component part of the Biohumus Production Installation (vermicompost) and is used to sort the compost resulting in the decomposition of organic matter from various sources - animal manure, crop residues, residues from the meat industry and the winemaking industry;



Figure 2. Drum sifter

2. Band conveyor (Fig.3) of the installation is made with the help of belt conveyors. The belt-inclined conveyor is used for loading with the compost resulting from the sieving process in the vermicompost system. The conveyor is mobile, with the possibility of changing the height in the vertical plane being used to take the resulting compost as sifted from the cylindrical sieve, in to the vermicompost system.



Figure 3. Band conveyer

3. The vermicompost system (Fig.4) is a wooden construction provided at the bottom with a grate and vermicompost scraping system (compost subjected to the action of earthworms). This system consists of the following: Bracket, Geared motor transmission, Housing, Scraper knife. On this system is also placed the wetting system that ensures the humidity (70% humidity) of the compost in which the earthworms move from the bottom up.



Figure 4. Vermicomposting system

The granular material (compost) is introduced into the feed hopper of the cylindrical sieve by means of the inclined conveyor with TIB belt. The cylindrical sieve is equipped with a rotating brush cleaning system. The screened material passes through a circular sieve and then into the discharge funnel and the refuse is removed through the refuse discharge funnel. The sieve is taken at the exit of the funnel hopper by a belt conveyor and introduced into the vermicompost system. Under the action of the earthworms at the bottom of the vermicompost system (by moving them from the bottom up) the compost will be transformed into biohumus. As the earthworms move to the upper part the scraping knife (electrically operated) in the vermicompost system, performs the evacuation (by scraping the sieve) of the biohumus. In the vermicompost system it will be permanently filled with compost resulting from the passage through the cylindrical sieve. The materials in the table below were used to prepare the vermicompost recipe:

Table 1

Materials rich in Nitrogen and carbon

Type of waste	C / N ratio	Type of waste	C / N ratio
Waste rich in Nitrogen		Waste rich in Carbon	
Liquid manure	2 – 3	Fruit leftovers	35
Chicken manure	10	Leaves	40-60
Cut grass	12-15	Straw, oats	48-60
Vegetable waste	13	Tree bark	100-130
Kitchen waste	23	Remains from bush cleaning	100-150
Potato plant	25	Sawdust	100-500
Horse manure	25	Paper / cardboard	200-500

The decomposition process of biodegradable solid waste depends on the activity of microorganisms. The microorganisms degrade the material introduced into the piles and the decomposition products of the previous population served as a substrate in the next phase of the decomposition process. This process depends on several factors. These factors and the relationships between them, influence the speed of the decomposition process, the decomposition phase and the activity of microorganisms.



Figure. 5 - The materials used to prepare the compost



Figure 6 - Images from the compost production

3. RESULTS

If the maximum temperature is below 40 °C, the composting process is ready. In table no. 2 are listed values of the quality of the resulting compost:

Table 2

The quality of the resulting compost

Characteristic	U/M	Characteristic value
Hygiene		A hygienic product that ensures the exclusion of germs
Impurities		Content of impurities (glass, plastic, metal) greater than 2 mm is not more than 0,5% by mass of dry matter
Glass, plastic, metal	%	0,71
Vegetable residues (greater than 2 mm)		0,7
Stones		0,6
Type of compost and material introduced in the pile		Green plant residues (plant removed from the vegetable crops) Chopped apples Chopped vegetables (waste tomatoes and peppers) Waste from a poultry farm Deciduous leaves and vegetable scraps from harvesting

		onions that limit dehydration
The compost maximum particle size	mm	15
Material density	kg/m ³	409
Salt content		0,08
pH value		8
The decomposition degree		Mature compost: phase IV (degree of rot)
The compost water content	%	44%
Zinc (Zn)		405 mg/kg DS
Lead (Pb)		155mg/kg DS
Copper (Cu)		105mg/kg DS
Chrome (Cr)		105mg/kg DS
Nickel (Ni)		55 mg/kg DS
Cadmium (Cd)		1,6mg/kg DS
Mercury (Hg)		1,1mg/kg DS

4. Discussion

Biohumus contains the necessary set of macro and micro nutrients, enzymes, soil antibiotics, vitamins, growth hormones and humic substances, it contains on average a higher concentration of nitrogen, phosphorus, potassium, calcium and a number of beneficial microorganisms and bacteria than the ones we usually find in the upper layers of the soil.

The application of biohumus generates multiple benefits for farmers:

- By applying biohumus, significant production increases are obtained because the plants easily assimilate nutrients substances;

- Increases the water retention in the soil (the amount of water needed for irrigation decreases by about 30%);

- The biohumus obtained can be used directly on the farm by distributing it on the area intended for fodder;

- Fix soils affected by prolonged use of chemicals. By repeated application, the soil will be completely repopulated with microorganisms beneficial to plants destroyed by chemicals over the years.

- The application of biohumus improves the structure of the soil, aerates the soil and makes it easy to work, which leads to lower costs of providing fuel for agricultural machinery;

It is non-toxic, does not burn plants, has no restrictions on use, can be used in any crop, greenhouse or field, with excellent results;

- It is compatible with any chemical preparation;

- It is excellent in the prevention of diseases (Alternaryosis, Gray mold, Fusariosis, Mana, Root rot, Bacterial burning in peas, Rhizoctoniosis, Septoriosis, Black tobacco rot, Apple rot).

5. Conclusions

In conclusion we can say that the temperature is the most important value that must be monitored during the process of transformation into compost, because it can be very easily be measured and shows the progress of the process. The decomposition of organic substances as a result of the activity of microorganisms due to their self-heating capacity is the reason for the temperature differences in the center of the pile and the surrounding temperature.

Degradation of components that are difficult to destroy takes place during the transformation phases. Its duration depends on the environmental conditions. Therefore, a specific time interval for this phase cannot be indicated.

During the maturation phase, the activity of bacteria slows down. During this period, soil organisms populate the material and mix the mineral elements with the organic ones. Clay-humic complexes are formed that increase the nutrient content of the compost (especially nutrients available to plants). At the end of this period (the final temperature does not rise more than 40 °C), the material is ready. During the composting process, the total volume and total

mass of the pile decreases. Due to abrasion by other materials and maceration, the particle size decreases. Therefore, the total volume becomes smaller and the density of the pile increases.

Conflicts of interest: The authors declare no conflict of interest.

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NOTE:

Submission of a manuscript implies: that the work described has not been published before (excepting as an abstract or as part of a published lecture or thesis) that it is not under consideration for publication elsewhere.

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- Manuscripts should be concise, in **1.15 line spacing**, and should have 2 cm all over margins. The font should be **Verdana 10 pt.** Ensure that each new paragraph is clearly indicated, using **TAB at 1.25 cm.**
- Title will be **Verdana 12 pt.** and explicit figures will be **Verdana 9 pt.**
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- Chapters' titles are written by **Verdana 10 pt, Bold** (e.g. **Introduction, Materials and methods**), between chapters is left a space for 10 pt. At the beginning of each paragraph, TAB of 1.25 cm.
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Under the paper's title, after a space (enter) 10 pt., write **authors' names** and **affiliations (Verdana 8 pt.-Regular)**

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introduced in parentheses the first time it is used in the text. Standard abbreviations (such as ATP and DNA) need not to be defined.

Abstract (*Verdana 10 pt.*), the title *bold*; the text of abstract: *regular*) should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be max.200 words. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Introduction (*Verdana 10 pt.*) The study should be briefly described in the beginning along with its significance. It should explain the significance of the work as well as its goal. Key papers should be referenced, and a thorough analysis of the state of the research field should be conducted. Finally, briefly discuss the work's main goal and highlight its key findings. Please try to make the introduction understandable to scientists who aren't in your field of study. References need to be listed in numerical order and should be denoted by a number or numbers in square brackets, such as [1] or [2,3], or [4-6]. Further information about references can be found at the end of the document.

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$$P = F \cdot v \quad (1)$$

Terms of the equation and the unit measure should be explained, e.g.

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F – force, [N];

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Figure 1. Tractor used for experiments

Legend: Ar Verdana ial 8 pt, Italic, Center, e.g.

1 – tractor cab; 2- wheels; 3 – engine; 4 – plough; 5 – seeder

Acknowledgments (Arial 10 pt.) In this section authors can acknowledge any support given which is not covered by the author contribution (projects that were at the basis of the research, funding received, technical support, donations, etc.

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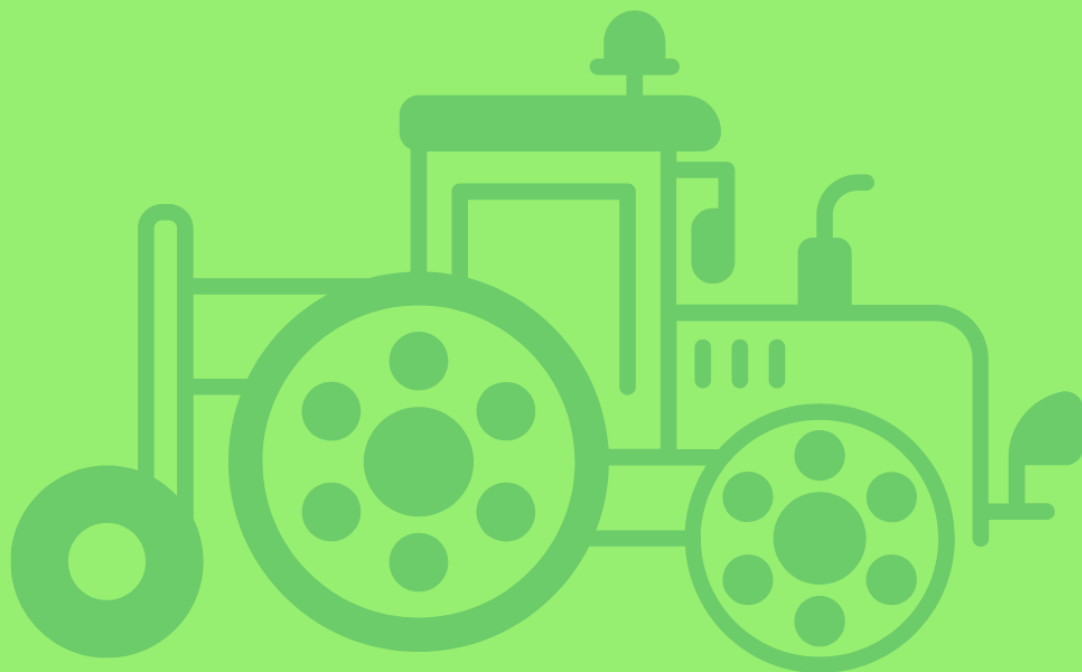
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