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# Biotechnological solutions for improving the quality and environmental friendliness of sugar in the food industry

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**Abstract.** Sugar production has a significant impact on the environment, which makes it necessary to introduce more efficient and environmentally friendly technologies. One such alternative is the use of biotechnological approaches to improve the quality of sugar syrups and the production of organic sugar without the use of chemicals. The purpose of this study was to evaluate the efficiency of enzymatic methods of syrup purification using pectinesis and amyloglucosidase and determine their impact on the quality of the final product, as well as to investigate the possibilities of processing sugar by-products into useful substrates for other food industries. Enzymatic purification and ultrafiltration methods were used to achieve this goal. During the experiments, a syrup with a residual pectin content of  $0.02\% \pm 0.005$  and starch content of no more than 0.01% was obtained, which indicates a high level of purification. The combined use of enzymatic and membrane methods allowed improving the stability of the process and increasing productivity to 120 litres of syrup per hour, which also helped to reduce energy costs by 8% and reduce the duration

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of technological stages by 10%. The results showed that enzymatic purification of the syrup reduced the level of impurities in molasses and contributed to an increase in the yield of bioethanol to 88%  $\pm$  2, which is 9% higher than in the control group. It was found that the feed obtained from pulp after biotechnological treatment had high quality indicators: a decrease in moisture content to 10%  $\pm$  0.5, a decrease in microbial activity to 5  $\times$  103 CFU/g and increase the mechanical strength of pellets to 92%  $\pm$  4. The results showed significant benefits of enzymatic technologies, in particular, reducing the cost of chemical reagents and waste disposal. The practical significance of the study lies in the fact that the use of biotechnological methods can significantly increase the efficiency of organic sugar and feed production, helping to reduce the environmental burden

**Keywords:** chemical impurities; enzymatic purification; membrane purification; molasses; byproducts

#### Introduction

Biotechnologies are increasingly influencing food production, especially in the sugar industry, opening up new opportunities to improve the quality of the final product and reduce the negative environmental impact. Sugar production faces many challenges, such as the need to reduce impurities in the final product as well as to process significant amounts of by-products. In this context, research on the impact of innovative biotechnologies is critical. Biotechnological methods allow producing pure organic sugar without the use of chemicals, while contributing to sustainable development through ecological waste processing. For example, the study by G. Kovalenko (2019) showed that the introduction of biotechnological solutions can significantly optimise waste processing costs and increase the environmental compliance of production processes. Similar ideas were reflected in the paper by A. Bezpala et al. (2024), who focused on the rational use of sugar by-products, such as pulp, for the production of feed or substrates for other industries.

Improving the quality of the final product in biotechnological production is made possible by the use of advanced microbial cultures and enzymes (Nabiyev *et al.*, 2024). For example, V. Nikulshyn *et al.* (2022) considered energy-saving options to optimise production processes that simultaneously reduce the negative impact on the environment. M. Steinwand & P. Ronald (2020) noted that the use of genetically modified sugar beet crops can not only reduce the number of pests and chemical treatments, but also provide a product with improved organoleptic qualities. Processing of sugar by-products opens up significant opportunities for ensuring sustainable industrial development (Panasiuk & Myskovets, 2023). M. Meghana & Y. Shastri (2020) explored ways to use waste efficiently, such as converting it into biofuels or biopolymers. This increases the value of waste and helps to reduce environmental pollution. Such innovations correspond to global trends in sustainable development and allow creating circular economic models that integrate several branches of the food industry. The relevance of this problem was emphasised by M. Rajaeifar et al. (2019), who analysed the transition to a waste-to-energy conversion strategy as an efficient way to reduce the negative impact on the environment, which promotes more sustainable use of resources and reduces the environmental footprint. P. Sukphun et al. (2023) considered the double stages of processing sugar industry waste to produce biogas. This approach allows not only reducing the amount of waste, but also obtaining renewable energy sources, which can affect the sustainable development of the industry as a whole.

In the context of the development of biotechnology in the food industry, it is also important to consider the economic and political factors that influence the introduction of innovations (Kolisnik & Polishchuk, 2023). K. Alekseieva et al. (2023) highlighted the importance of government support for businesses, especially in crisis situations, to ensure economic sustainability and food security. These researchers analysed state support programmes in Ukraine, in particular, in the agro-industrial sector, which directly affects the production of sugar and other food products. They emphasised the need to create a favourable environment for the functioning and reproduction of businesses in the new economic environment, which is especially relevant for innovative biotechnological enterprises in the food industry. As noted by K. Alekseieva et al. (2023), the effectiveness of government support programmes and clear criteria for selecting viable enterprises are key factors for stimulating the development of promising technologies, including biotechnological innovations in the sugar industry.

The studies by B. Biyashev et al. (2023), E. Shahini et al. (2024), and D. Breus et al. (2024) highlighted a critical issue of the environmental impacts of war, which can be long-term and irreversible for ecosystems and human health. The researchers emphasised the importance of understanding the extent of the impact of such events on natural resources and developing effective measures to restore them. These aspects are also directly related to the sugar industry, since a violation of the ecological balance can significantly affect the quality of raw materials and production conditions. As noted by E. Shahini et al. (2024), planning for the recovery of affected areas, including ecosystem restoration, is an important component of ensuring the sustainable development of various food industries, including sugar production, which highlights the need to integrate environmental considerations into the development of biotechnological innovations for this sector.

M. Nawaz et al. (2021) considered the concept of zero waste through bio-wastewater treatment, which allows the conversion of contaminated water typical of the sugar industry into useful resources and contributes to the production of valuable products. This is another example of a strategy for converting industrial waste into useful resources, contributing not only to the sustainable development of the industry, but also to the preservation of the natural environment. It is also worth noting the study by K. Bueno-Zabala et al. (2020), which focused on the optimised production of glucose syrup using membrane reactors, which simultaneously allows the production of valuable by-products. This approach increased the efficiency of raw material use and reduced production costs, which is important for improving environmental sustainability and economic benefits.

Special attention should also be paid to the latest technologies for processing sugar beet, the development of which was considered by B. Muir (2022), highlighting innovative approaches designed to improve the efficiency of final product production and reduce the impact on the environment. These technologies are essential for optimising production processes and conserving resources, ensuring increased environmental sustainability of the sugar industry. Thus, the main areas of scientific research aimed at innovation in the sugar industry are waste processing, reducing the ecological footprint of production and improving the efficiency of resource use, which ensures the sustainable development of the industry and contributes to the preservation of ecological balance. The purpose of this study was to identify key biotechnologies that can improve the quality of both the final product and by-products of production, namely, molasses and pulp, and to minimise the environmental impact by recycling waste.

#### **Materials and Methods**

Samples of sugar beet (*Beta vulgaris*) were provided by LLC Agrofirma im. Dovzhenka

(Astarta-Kyiv Group, Poltava Oblast). The inclusion criteria were conditions under which the samples met maturity standards (beet sugar content  $\geq$  16%, was declared by suppliers), had no defects or visible signs of disease. The sample did not include specimens with damage, infested with pests or fungal diseases, such objects were excluded after visual inspection and microbiological analysis. Microorganisms used at the stage of disposal of by-products (bacteria Zymomonas mobilis, strain ATCC 31821, providing a high level of ethanol fermentation) were provided by D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The study was conducted from March to September 2024 at the Institute of Agricultural Microbiology and Agro-Industrial Production of the National Academy of Sciences of Ukraine (Chernihiv, Ukraine). The experimental part included working with control and experimental samples, where the former characterised the usual chemical approach to syrup processing, and the latter – testing the proposed biotechnological methods. The study focused on evaluating enzymatic methods for syrup purification to reduce the impurity content without using chemical reagents.

Sugar beet samples of the control and experimental groups were cleaned of dirt and ground to a uniform consistency (particle size – 2-3 mm). To obtain the syrup, the raw material was extracted with hot water (temperature 80°C, mass/volume ratio 1:2), and thorough mixing was ensured during extraction to maximise the release of sugars from the cell mass. Control samples were prepared according to conventional technology, which involves the use of chemical reagents such as limestone milk and sulphur anhydride to purify the syrup. In the test samples, an enzyme complex was added to the resulting syrup, including: pectinase for the breakdown of pectin compounds (dosage 20 U/mL, activity 100 PE); amyloglucosidase for the conversion of starch residues to glucose (dosage 15 U/mL, activity 120 AO).

Fermentation was carried out in a Biostat B bioreactor (Sartorius, Germany) at a temperature of 50°C and a pH of 5.5 for 6 hours. This temperature was chosen because used enzymes have optimal activity in the range of 45-55°C, which ensured maximum efficiency in the breakdown of pectins and starch residues and increased the purity of the final product. The temperature of 50°C was also favourable for enzymatic activity, but too high for the development of unwanted bacteria. The pH value of 5.5 maintained the stability of enzymes, microbial activity, and prevented the development of contaminants.

In the control group, impurities were measured after chemical treatment, and in the experimental group - after enzymatic purification. Analysis of the pectin content was performed by gravimetry: the selected syrup sample was subjected to pectin precipitation using alcohol (96% ethanol) in a ratio of 1:2. The resulting precipitate was centrifuged in an Eppendorf 5804 R centrifuge (Germany) (10 minutes at 5,000 rpm) to remove liquid residues, dried in a Memmert UF 260 drying cabinet (Germany) at a temperature of 105°C to a constant mass. The residual pectin content was calculated as a percentage of the total syrup mass. Analysis of the residual starch concentration by the enzymatic method was based on hydrolysis of the residual starch to glucose, followed by quantitative determination of glucose. The syrup sample was incubated with alpha-amylase with an activity of 50 U/mL at 37°C for 30 minutes. The resulting glucose was determined using the glucose oxidase method, which included colorimetric measurement with a BIO-TEK instruments Synergy H1 colorimeter (USA) absorption at 540 Nm. The concentration of residual starch was calculated considering the conversion factor. In the control and experimental groups, these processes were performed in the same way. After purification, the samples of the control and experimental groups were further purified by ultrafiltration. This process involved passing the syrup through a Millipore Pellicon

2.0.2 µm membrane (USA) with a pore size of 0.2 microns, which helped to remove macro-molecules, cell mass residues, and impurities.

To ensure the suitability of the syrup for further use, it was concentrated by vacuum distillation. The pressure was reduced to 50-100 mm Hg in the LabTech LT 3000 vacuum unit (China), which allowed water to evaporate at a temperature of 50-60°C. This low-temperature process prevented thermal degradation of sugars, preserving their natural composition and organoleptic properties. The syrup concentration reached 65-70% of dry substances, which provided optimal viscosity for further production stages. Determination of the colour characteristics of the syrup corresponding to its quality was carried out on the ICUMSA scale with a Lovibond PFX 8800 meter (Germany). The presence of undesirable impurities resulting from caramelisation was assessed by optical microscopy using an Olympus BX41 microscope (Japan). The experimental and experimental groups used the same technological parameters, but considered the effect of the absence of chemical reagents on the quality of the final product. In both groups, the stability of the syrup's organoleptic characteristics, such as smell, colour, and taste, was assessed by sensory analysis, and the indicators were evaluated on a 10-point scale.

Processing of by-products. The process of fermentation of molasses was carried out using microorganisms *Zymomonas mobilis*. The prepared molasses solution was inoculated at pH 4.5-5 in a Biostat B bioreactor (Sartorius, Germany). The fermentation temperature was 30°C and the duration was 24-48 hours, during which the ethanol yield was monitored by analysis on an Agilent 8890 gas chromatograph (USA). The resulting ethanol was distilled in a LabTech LT 3000 vacuum distillation unit (Italy), which made it possible to effectively separate ethanol while preserving its properties.

A Flottweg Z Series dewatering press (Germany) was used to process the pulp. Further drying was carried out in an Alvan Blanch DR 800 drum drying unit (Great Britain) at 60-90°C to achieve a residual moisture content of 10%. The dried material was ground in a Buhler MultimpactMax hammer mill (Bühler Group, Switzerland) to a particle size of 2-3 mm. Granulation was carried out on a CPM Europe Century Series granulator (CPM Europe, Netherlands), which provided the formation of feed pellets with a diameter of 8-12 mm with high mechanical strength. The quality of granular feed was evaluated by several indicators. The moisture content was determined by drying in a Binder FD115 thermostat (Germany) at 105°C, and microbiological purity was assessed by seeding on TSA agar for aerobic bacteria and SDA agar for fungi with the number of colony-forming units (CFU/g). The mechanical strength of the pellets was measured on the PDT 1100 Pellet Durability Tester (Luxembourg), while fractional analysis of particle size uniformity was performed on the RETSCH AS 200 laboratory sieve analyser (Germany).

#### Results

The enzymatic approach to syrup purification showed significantly better results compared to conventional chemical methods. The study showed that the content of residual pectin in control samples treated with chemical reagents was 0.08% ±0.01, while in samples, purified with pectinase and amyloglucosidase, this indicator decreased to 0.02% ±0.005 (Fig. 1). In parallel, the residual starch in the syrup after enzymatic purification was significantly lower –  $\leq 0.01\%$  compared to  $0.04\% \pm 0.007$  in the control samples. These results clearly demonstrate a high degree of impurity removal due to enzymatic purification. The mechanism of pectinase was to break down pectin chains, which greatly facilitated the removal of residual macromolecules. Amyloglucosidase, in turn, effectively broke down the residual starch, ensuring its minimum concentrations in the final product.



#### Indicators of syrup purification using chemical and biotechnological approaches



One of the most important advantages of biotechnological purification is the absence of chemical by-products in the final product. Chemical purification methods such as lime and sulphur compounds, despite their efficiency, lead to the development of undesirable precipitates, residues of chemical agents

in the syrup, and harmful by-products such as calcium salts (Muir, 2022). In comparison, the enzymatic approach provided not only a high degree of purification, but also the preservation of the natural composition of the syrup, which is critical for organic production. The study also revealed the economic benefits of biotechnological purification. The use of enzymes has reduced the duration of the purification process to 6 hours, while the chemical approach often requires additional stages of precipitation, filtration, and regeneration of reagents, which extends the technological process to 10-12 hours and increases costs.

To test the reliability of the method, 10 repeated experiments with enzymatic purification were performed, and in all cases the values of residual pectin and starch corresponded to the established values (0.02% and ≤ 0.01%, respectively). All experiments were performed under the same conditions - temperature 50°C, pH 5.5, enzymatic treatment lasting 6 hours. Fluctuations in the values did not exceed 5%, which indicates a high reproducibility of the method (Table 1).

<b>Table 1.</b> Results of repeated experiments on enzymatic purification of sugar syrup						
No.	Pectin (%)	Pectin standard (%)	Starch (%)	Starch standard (%)		
1	0.02	0.02	0.009	0.01		
2	0.019	0.02	0.008	0.01		
3	0.021	0.02	0.01	0.01		
4	0.018	0.02	0.009	0.01		
5	0.02	0.02	0.009	0.01		
6	0.019	0.02	0.008	0.01		
7	0.02	0.02	0.009	0.01		
8	0.021	0.02	0.01	0.01		
9	0.018	0.02	0.008	0.01		
10	0.019	0.02	0.009	0.01		

Source: compiled by the authors

Another advantage of the enzymatic approach is the reduce in the environmental impact of production. Chemical treatment leads to the development of sediments that require special disposal, and to excessive water consumption during the regeneration of chemical agents (Nawaz et al., 2021). The enzymatic method

significantly reduces the need for additional resources, such as chemical reagents and energy, as it reduces the use of harsh chemicals for cleaning and stabilising products by 30-40%. In addition, the reduction of energy requirements for cleaning and stabilisation processes is up to 25%, which helps to reduce costs and minimise the amount of harmful residues. This makes the method the most successful for use in environmentally oriented production.

Membrane ultrafiltration technology has shown high efficiency in removing macromolecules and other impurities in syrups of both the control and experimental groups. However, the purification results were significantly better in the case of samples from the experimental group that underwent the enzymatic purification stage before membrane treatment. The formation of precipitation on the membranes was an important parameter that demonstrated the advantage of the combined approach. In the control samples, the level of deposits on the membrane increased after 20 minutes of system operation, which led to a gradual decrease in throughput by up to 60% from the initial level. Instead, in the experimental samples, deposits on the membranes were reduced by 45% compared to the control group, and stable operation of the plant was maintained for up to 30 minutes, which allowed a larger volume of purified syrup to be obtained in one filtration cycle.

The results of the analysis of residual pectin substances and starch in syrups after ultrafiltration demonstrate a higher purity of the final product in the experimental group. In the control samples, the level of residual pectin was  $\leq 0.02\%$ , and starch –  $\leq 0.01\%$ . In the samples of the experimental group, these indicators were lower than the detection limit of the analytical equipment used (< 0.01%). This indicates a deeper purification of the syrup due to the synergistic effect of pre-enzymatic treatment, which significantly reduced the content of impurities before the membrane stage. The more efficient operation of the membranes in the experimental group is explained by the reduced load on the filter elements. Enzymatic treatment reduced the level of macromolecules and the viscosity of the syrup, which facilitated the passage of syrup through the membranes and reduced the risk of contamination.

In addition to the cleaning quality, the performance of the ultrafiltration unit was also better for the experimental group. On average, the volume of purified syrup per hour was 120 litres for experimental samples compared to 90 litres for control samples, which provided an additional productivity of 30%. The absence of the need for frequent membrane washing during the processing of experimental samples also significantly increased the cost-effectiveness of the process. The combination of enzymatic and membrane purification not only improved the quality of the final product, but also demonstrated environmental benefits. The use of enzymes in the initial stages allowed reducing the volume of precipitation formed by 22-25% and reducing water consumption for cleaning the system by 25-35%, which is usually necessary for the regeneration of chemical membranes in the control group. This approach also reduced the use of chemical reagents by 40%, which will help to improve the environmental friendliness of the entire technological process. Consequently, the results of membrane syrup purification confirm the effectiveness of the proposed combined approach. Based on enzymatic pretreatment, it was possible to significantly reduce the level of precipitation on the membranes, improve the quality of cleaning, and the stability of the ultrafiltration system. This indicates the prospects of using this method to ensure high quality of organic sugar and reduce the environmental impact of production.

Syrup concentration by vacuum distillation. Vacuum distillation provided a stable syrup concentration process for both the control and experimental groups. However, the results of the final product analysis clearly demonstrated the advantages of the combined enzymatic and membrane purification approach applied to the samples of the experimental group. One of the key evaluation parameters was the determination of the colour characteristics of syrup on the ICUMSA scale, which is used to determine the degree of purity and clarification of sugar products. As a result of the combined approach to syrup purification, the samples of the experimental group showed lower values in the range of 190-210 units, which indicates a much cleaner product with a natural amber colour. For the control group, this indicator was noticeably higher and ranged from 240-270 units, which indicates the presence of residual impurities and the absence of maximum transparency.

The preservation of aromatic compounds is another important parameter of the quality of the final product, since these components provide the smell and natural taste profile of the syrup. In the process of vacuum concentration under low pressure conditions (50-100 mm Hg) and the temperature range of 50-60°C, it was possible to minimise the loss of volatile substances. The samples of the experimental group showed a 15% higher level of preservation of volatile aromatic compounds compared to the control samples, which provided improved organoleptic properties of the product. Losses in the control group are explained by residues of chemical impurities, which could cause additional evaporation or degradation of aromatic components when heated.

The concentration of syrup to a sugar density of 76-78% occurred without noticeable degradation of natural sugars or the development of undesirable products (caramelisation) in both groups. However, the experimental samples had a more uniform texture without microscopic inclusions, which were sometimes recorded in the control group during microscopic analysis. This may be caused by the higher purity of the syrup before the vacuum concentration, which prevented the development of foreign crystal formations or microparticles that could remain in the control samples. Due to the high initial purity of the syrup of the experimental group, the concentration duration was reduced by 10% compared to the control samples. Reducing the level of impurities that need to be removed during distillation has reduced energy costs by 8%. This has had a positive impact on the overall performance and profitability of the production process, which is important for industrial implementation.

Tasting evaluation of concentrated syrups in both groups showed that the samples of the experimental group had a more natural taste with a pronounced beet aroma, while in the control group there was a slight bitterness, which probably arose due to the presence of residual chemicals. The average score of the organoleptic test for samples from the experimental group was  $9.2 \pm 0.3$  on a 10-point scale, while in the control group this indicator was  $7.8 \pm 0.4$  (p < 0.05). Table 2 illustrates the key differences in the concentration process parameters between the two groups, confirming the overall advantage of the technological approach used for experimental samples.

<b>Table 2.</b> Comparison of sugar syrup concentration process parameters					
Parameter	Control group	Experimental group			
Colour indicator (ICUMSA)	240-270	190-210			
Level of preservation of aromatic substances (%)	$85\pm3$	100			
Duration of concentration (min)	$60 \pm 2$	$54 \pm 1$			
Energy consumption (kWh)	25±1	$23 \pm 1$			

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Source: compiled by the authors

The use of vacuum distillation in the experimental group, which used syrup purified by enzymatic and membrane methods, made it possible to achieve significantly better

quality of the final product, including colour, aroma, and texture indicators. The high efficiency of the process, its energy saving and environmental friendliness make this

technology promising for large-scale implementation in the production of organic sugar (Bulgakov et al., 2017).

Results of by-product disposal. The fermentation process of molasses, one of the main by-products of sugar production, showed significant differences in bioethanol yield between the control and experimental groups. Molasses obtained after enzymatic purification of the syrup showed a significantly higher degree of processing of sugars into ethanol. In the control

group, where molasses was obtained after conventional chemical purification of syrup, the bioethanol yield was  $79\% \pm 3$  of the theoretical maximum (Table 3). This is caused by the high content of residual chemical impurities, which suppressed the activity of microorganisms and reduced the efficiency of fermentation. Instead, in molasses purified by biotechnological method (experimental group), impurities were minimised, which allowed achieving a bioethanol yield of  $88\% \pm 2$  (p < 0.01).

<b>Table 3.</b> Results of molasses fermentation in two groups						
Parameter	Control group	Experimental group	Difference (p-value)			
Bioethanol yield	79%±3% of the theoretical maximum	88%±2% of the theoretical maximum	p<0.01			
Time to reach maximum ethanol concentration (hours)	36 hours	30 hours	p<0.01			
Organic acid concentration (g/l)	$0.5 \pm 0.08$ g/l	0.2±0.05 g/l	p<0.01			
Y_ethanol/sugar (g ethanol/g sugar)	$0.42 \pm 0.03 \text{ g/g}$	0.48±0.02 g/g	p<0.05			

Source: compiled by the authors

Comparison of the dynamics of the fermentation process also showed a significant difference between the groups. In the control group, the maximum ethanol concentration was reached in 36 hours, while in the experimental group, this process was reduced to 30 hours. The reduction in the duration of fermentation can be explained by a favourable environment for Zymomonas mobilis in molasses purified without chemical reagents. Additional quality analysis of the obtained bioethanol showed that ethanol obtained from molasses of the experimental group had a higher degree of purity, with the content of undesirable side components 20% lower than in the control samples. In the control group, traces of organic acids (for example, acetic acid) were often found in bioethanol, which were formed due to chemical stress on microbial cells caused by residual impurities in molasses. In the bioethanol samples of the experimental group, the concentration of organic acids was  $0.2 \pm 0.05$  g/l, while in the control group  $-0.5 \pm 0.08$  g/l (p < 0.01), which

confirms the increased efficiency of the process in the absence of chemical contamination in the raw material. Reducing the level of impurities in molasses, as a result of the use of enzymatic purification of syrup, improved the viability and metabolic activity of microorganisms. The experimental group demonstrated a stable value of the Y\_ethanol/sugar parameter (efficiency of converting sugars to ethanol), which was  $0.48 \pm 0.02$  g/g, which is very close to the theoretical maximum (0.51 g/g). In the control group, this parameter reached  $0.42 \pm 0.03$  g/g, which is 12% less (p < 0.05), which leads to a lower ethanol yield.

Sugar beet pulp, which is formed as a byproduct after extraction of sugar substances, was further processed in this study to produce granular feed. Evaluation of the final product showed significant advantages of the feed obtained in the experimental group, according to the following key indicators. The experimental pellets achieved a moisture content of  $10\% \pm 0.5$ compared to  $12\% \pm 0.8$  in the control samples. The reduced moisture content provided an increased duration of feed storage without loss of quality or mould formation. Granulated feed of the experimental group contained microorganisms in the amount of  $5 \times 10^3$  CFU/g, which is significantly less than in the control group ( $2 \times 10^4$  CFU/g). Such low microbial activity was ensured by the absence of residual chemical

impurities in the pulp and careful heat treatment. The pellets obtained in the experimental group showed mechanical strength at the level of  $92\% \pm 4$ , which is significantly higher than in the control group ( $80\% \pm 5$ ). This is important to ensure the stability of the product during transportation and storage, and to prevent dust formation (Table 4).

Table 4. Comparison of the characteristics of granulated feed from pulp between groups					
Parameter	Control group	Experimental group			
Moisture content, %.	$12 \pm 0.8$	$10 \pm 0.5$			
Microbiological purity	$2 \times 10^4 \text{ CFU/g}$	$5 \times 10^3 \text{ CFU/g}$			
Mechanical strength of pellets	80±5%	92±4%			

Source: compiled by the authors

The absence of synthetic preservatives, the high quality of pellets, and their stability allows avoiding the risk of accumulation of undesirable substances in the feed. The use of the enzymatic approach at the initial stages helped to reduce the need for chemical reagents to stabilise the product, and reduce waste disposal costs due to increased drying efficiency. The long shelf life of experimental pellets without additional chemical treatment also reduced the manufacturer's costs and reduces the environmental burden. Obtained results demonstrate the superiority of biotechnological methods in all aspects of the study: syrup purification, concentration, preservation of organoleptic properties, and disposal of by-products. Comparison of control and experimental samples confirmed that the enzymatic approach allows obtaining a better final product with minimal environmental impact and better efficiency indicators.

#### Discussion

The results obtained in the course of the study show a certain similarity with the results of previous research, including individual differences, which can be explained by different approaches to processing raw materials and the variability of experimental conditions. In this regard, it is important to compare the results with studies by other researchers that focus on the environmental and technological aspects of sugar beet processing and sugar production. This study on optimising the use of sugar industry by-products has shown that beet processing and syrup production processes have significant potential to reduce waste and increase efficiency. These results correlate with the study by B. Muir & A. Anderson (2022), who noted the significance of developing sugar beet as an important area in Europe to increase yields and reduce production costs. In particular, improving the efficiency of syrup production and processing by-products can significantly improve the environmental and economic component of sugar production. However, the results obtained demonstrate that the integration of the latest technologies for preserving syrup in limited seasonality conditions can significantly increase the production period.

Such approaches are confirmed by the findings of A. Adbhai *et al.* (2022), who focused on using patent molasses left over from the sugar extraction process to create secondary products. The researchers also noted the high potential for energy production from residual molasses. This study, on the contrary, focused more on the efficiency of their processing in the context of reducing the amount of waste when using raw materials for the production

of not only sugar, but also bioenergy resources. The results of S. Singh *et al.* (2021) regarding the processing of sugar cane waste into commercial products, in particular, through the optimisation of production methods and solving environmental problems that arise in the process, are consistent with this study, since in both cases it is about the use of biotechnologies for processing sugar by-products to improve the quality of the final product and reduce environmental impact, in particular, through fermentation and reducing the need for chemical reagents, which also indicates opportunities for improving the environmental efficiency of the agricultural sector.

Similarly, the results of the studies by A. Hernández-Pérez et al. (2020) and A. Babu & S. Adeveye (2024) related to sugar extraction and sweetener production through biotechnological processes, support observations on the potential of using environmentally friendly methods to reduce waste and improve the efficiency of production processes. Although studies conducted by other researchers focus more on aspects of technological development within the one-sided use of sugar beet, the results highlight the need for multifunctional solutions to solve the problem of waste in production. The results of the development of biotechnological processes for the production of fructose-rich syrups coincide and in some aspects complement the conclusions from previous works of international researchers, in particular, the study by M. Garcia-Aguirre et al. (2009) identified significant potential for using enzymatic hydrolysis of agave fructose-oligosaccharides to produce fructose-rich syrups. This study highlighted the importance of optimising technological processes, which is directly compatible with the results obtained, which shows that modern technologies of bioconversion of raw materials can lead to an increase in the quality and efficiency of syrup production.

The study by R. Singh *et al.* (2017) is also important. It described enzymatic methods

for the synthesis of high fructose syrups. The researchers noted the importance of using specific enzymes to improve the process of converting monosaccharides to higher fructose-rich compounds. These results support these conclusions, as it was found that the choice of specific enzymes can actually increase the yield of the product without losing quality. Similarly, research by H. Atiyeh & Z. Duvnjak (2005) on the use of ultrafiltration membranes and activated carbon for the purification of fructose syrups produced from cane molasses indicates the importance of such technologies in the syrup purification process. The results obtained indicate the effectiveness of these methods, in particular for achieving the desired purity of syrups while preserving their useful properties.

The study by J. Hubbuch & M. Kula (2007), with a focus on the isolation and purification of biotechnological products, confirms the effectiveness of the approach used in providing high-quality fructose-rich syrups. The methods recommended by these researchers can be adapted for cleaning products at the production stage, which will allow achieving the maximum level of purification with minimal energy costs. Other studies, for example by D. Lima et al. (2011), which highlighted the biotechnological potential of fructose syrup, are consistent with the results in terms of improving the use of fructose as a biologically active product. These results support the possibility of optimising the use of fructose-rich syrups for industrial purposes, especially given their wide range of applications in the food industry. The fundamental research by E. Vandamme et al. (1987) examined microbial sucrosophosphoratases in the context of fermentation and biotechnological applications that improve the process of converting sugar to fructose syrups. The results of this sucrose bioconversion study also confirmed the ability of enzymatic technologies to significantly increase production efficiency when using microbial agents, similar to what was found in the current study. The studies by J. Bicas *et al.* (2010) on the biotechnological production of bioflavours and functional sugars support conclusions about the broad potential for the production of not only basic bio-products, but also additives with high functional properties. This proves that fructose syrups can serve as a basis for the development of products with additional functional properties, which increases their value in the food and pharmaceutical industries.

The results of this study confirm the possibility of obtaining syrups from organic raw materials under a similar concept, but with a greater emphasis on maximising resource recovery and reducing costs. T. Kwan et al. (2019) focused on the possibility of using food and beverage waste for the production of syrups using biorectification technologies: a technical and economic assessment showed the high efficiency of this approach. The study by H. Olsen (1995), describing the enzymatic production of glucose syrups, was useful for understanding the mechanisms of starch and sugar hydrolysis in the manufacturing process. In particular, theoretical approaches to the use of enzymes such as amylases and glucosidases confirm their own results regarding the use of microbial enzymes for the efficient production of glucose syrups. The use of enzymatic technologies significantly improves the yield and purification of syrups, which coincides with the data on high results in optimising enzymatic hydrolase (Bekbayev et al., 2024; Osokina et al., 2024).

Conclusions on the technology of raw material preparation considering biotechnological processing also confirm the findings of A. Bušić *et al.* (2018) regarding the production of bioethanol from renewable raw materials, a similar approach to the efficient use of organic waste is shown. Although this study focuses more on syrup production, these results confirm the correctness of the chosen waste reuse strategy. Studies by P. Singh & S. Kumar (2019) on microbial enzymes in food biotechnology also support conclusions about the role of enzymes in the processes of sugar breakdown and syrup production. The researchers focused on the improvement of processes using various types of microbial enzymes. The results of the study, in particular, demonstrate significant advantages as syrups due to the use of specific microbial enzymes that activate bioconversions in the early stages. In addition, the study by E. Martínez et al. (2015), which discussed xylitol purification and crystallisation strategies, provides key guidance on such processes in the context of ongoing research. Although the work was done with fructose syrups, some of the purification principles proposed in their study may be useful for improving the characteristics of syrups. D. Peters (2006) revealed the importance of carbohydrates for fermentation, which confirmed the chosen approach to fermentation of syrups based on simple and complex sugars. These results confirm the ability of carbohydrates in syrups to optimise processes in the production of high-calorie products for the food industry, as indicated by D. Peters (2006).

The findings coincide with the conclusions of S. McKelvey & R. Murphy (2017) regarding the use of fungal enzymes in biotechnology. Their study demonstrated the effectiveness of using such enzymes to break down complex organic compounds, which was confirmed in the production of sugar syrups. However, in contrast to their approach, this study focused more on optimising conditions for more efficient fermentation, which allowed for improved yield and quality of finished products. Thus, it can be argued that the results of this study are consistent with a number of important international trends in the field of technological progress and environmental efficiency of sugar beet production. Moreover, the presence of some differences related to the conditions and methods of research suggests additional opportunities for improving the processing processes and use of raw materials at different stages of production.

#### Conclusions

The enzymatic method of syrup purification using pectinase and amyloglucosidase showed significantly better results than conventional chemical approaches. The obtained indicators of the content of residual pectin  $(0.02\% \pm 0.005)$ and starch ( $\leq 0.01\%$ ) indicate a high level of purification of impurities, which confirms the feasibility of a biotechnological approach in the sugar industry. Repeated experiments showed the reliability of the enzymatic purification method, where fluctuations in the results did not exceed 5%. The stability of the results allows considering this approach as a standard method for ensuring product quality. The use of membrane ultrafiltration technology after enzymatic treatment significantly improved the quality of purification. Reducing the level of impurities before membrane filtration reduced the amount of precipitation on the membranes, improved the stability of the plant and provided an increase in productivity of up to 120 litres of syrup per hour. This approach provided a synergistic effect, significantly improving the characteristics of the final product.

Samples of syrup purified by combined methods are characterised by a lower colour index (190-210 ICUMSA units), better preservation of aromatic compounds (+ 15%) and the absence of micro-inclusions, which indicates an improved texture and organoleptic characteristics. This provided an increased consumer score  $(9.2 \pm 0.3 \text{ vs. } 7.8 \pm 0.4 \text{ in control samples})$ , while also reducing energy costs by 8% and reducing the duration of technological stages by 10% indicates the economic advantage of the combined method of syrup purification. In addition, the enzymatic approach significantly reduces the environmental impact of production, reducing

the development of harmful waste and water consumption. Vacuum distillation of the syrup after combined purification allowed achieving a stable sugar density (76-78%), minimising the loss of volatile aromatic compounds, and also ensured the preservation of the natural taste and aroma of the syrup. The duration of the concentration process was reduced due to the higher initial purity of the syrup, which reduced production costs. Molasses obtained after enzymatic purification allowed achieving a high bioethanol yield (88% ± 2), which significantly exceeds the indicators of the control group  $(79\% \pm 3)$ . Additional processing of pulp using enzymatic membrane technology helped to reduce residual impurities, improving its quality as a raw material for feed or bioenergetics use. This approach ensured more efficient use of resources and reduced the environmental burden.

One of the main limitations of the study was the dependence on specific enzymatic preparations that are used to purify the syrup. This limits the ability to scale the technology to large production volumes due to the high cost of certain enzymes and their availability. In addition, some aspects of the process, such as optimising fermentation time and membrane purification, require additional research to achieve even better results in terms of productivity and cost-effectiveness. Further research should consider integrating this technology with other biotechnological processes to provide a more integrated approach to the purification and processing of sugar syrups.

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# **Conflict of Interest**

None.

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# Біотехнологічні рішення для покращення якості та екологічності цукру в харчовій промисловості

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Анотація. Виробництво цукру має значний вплив на навколишнє середовище, що зумовлює необхідність впровадження більш ефективних та екологічно чистих технологій. Однією з таких альтернатив є використання біотехнологічних підходів для покращення якості цукрових сиропів та виробництва органічного цукру без застосування хімічних речовин. Метою даної роботи було оцінити ефективність ферментативних методів очищення сиропів з використанням пектинезу та амілоглюкозидази і визначити їх вплив на якість кінцевого продукту, а також дослідити можливості переробки побічних продуктів цукрового виробництва в корисні субстрати для інших галузей харчової промисловості. Для досягнення поставленої мети використовували методи ферментативного очищення та ультрафільтрації. В ході експериментів було отримано сироп із залишковим вмістом пектину 0,02 % ± 0,005 та вмістом крохмалю не більше 0,01 %, що свідчило про високий рівень очищення. Комбіноване використання ферментативного та мембранного методів дозволило підвищити стабільність процесу та збільшити продуктивність до 120 л сиропу на годину, що також сприяло зниженню енерговитрат на 8 % та скороченню тривалості технологічних стадій на 10 %. Результати показали, що ферментативне очищення сиропу знизило рівень домішок у мелясі та сприяло підвищенню виходу біоетанолу до 88 % ± 2, що на 9 % вище, ніж у контрольній групі. Встановлено, що корм, отриманий з жому після біотехнологічної обробки, має високі показники якості: зниження вологості до 10 % ± 0,5, зменшення мікробної активності до 5 × 10. КУО/г та підвищення механічної міцності гранул до 92 % ± 4. Отримані результати продемонстрували значні переваги ферментативних технологій, зокрема зниження витрат на хімічні реагенти та утилізацію відходів. Практичне значення роботи полягає в тому, що використання біотехнологічних методів дозволяє значно підвищити ефективність виробництва органічного цукру та кормів, сприяючи зменшенню навантаження на навколишнє середовище

Ключові слова: хімічні домішки; ферментативне очищення; мембранне очищення; меласа; побічні продукти