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Simulation of hydrodynamic phenomena in valve feeders of adaptronic modules for dosing liquid products

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Abstract

Introduction. Rational geometric and hydrodynamic parameters of valve feeders of adaptron modules for dosing liquid food products in automatic packaging machines are determined.

Materials and methods. The study of hydrodynamic phenomena based on the simulation modeling of the feeder operation with liquid media, the physical and mechanical characteristics of which are close to Newtonian liquids, was conducted. A feeder with a conical valve and purified drinking water were used in the study. The feeder throughput was 500 cm^3/s; the internal diameter of the drain nozzle was 20 mm.

Results and discussion. To ensure the continuous flow, minimum overall dimensions of the feeder, and the possibility of regulation by changing the throughput of the feeder nozzle according to a given law, the angle at the base of the cone should be within 50–60°, and the length of the saddle base 20–25 mm.

During the movement of the liquid in the valve feeder, three negative factors affecting the parallel laminar movement of the liquid were found: (a)reverse movement of the liquid when it comes into contact with the surface of the base of the valve cone; (b) turbulence cells at the entrance of the liquid into the valve channel, and (c) the tubular form of the liquid flow in the nozzle. These negative factors can be eliminated by using a ball-conical valve with a truncated top.

To eliminate turbulence cells in the valve feeder, counter-current movement of liquid, and tubular flow of liquid in the nozzle, it was proposed to make the valve in the form of a conical-spherical shape with a cut-off cone top, and also to extend the inner surface of the seat to the inner surface of the measuring cylinder of the feeder.

Under such conditions, a parallel flow of liquid is ensured, which contributes to the accuracy of dose formation and the duration of product storage.

Conclusions. The design of the valve in the form of a conically spherical figure with a cut-off cone top according to the provided recommendations allows eliminating the centers of turbulence in the movement of liquid products in the valve feeder.

Introduction

Automatic machines for packaging of food products in consumer packages are characterized by a large number of indicators of technical excellence, which determine their competitiveness. One of these indicators is functional accuracy (Gavva et al., 2023). In machines in which dose formation is provided before packaging, the indicators of functional accuracy are the accuracy of product dose formation. According to international standards, the tolerance field for dosing accuracy is in the plus field. It is possible to ensure such requirements and taking into account, the efficiency of the machine by developing and implementing the latest technological systems based on elements of mechatronics, adaptronics, micro-drives, and micro-sensors.

In the presence of modern computer technologies for managing technological processes, technical means, the best results can be achieved by applying the product weighing method. For products characterized as a solid medium, dynamic weighing is typical (Gavva et al., 2023). Dynamic weighing is affected by a significant number of factors that can be taken into account when implementing adaptronic modules.

The implementation of adaptronic modules for dosing of liquid products in a weighted way involves establishing functional dependencies between the flow of products through the feeder nozzle and the structural and kinematic parameters of the valve, which changes the amount of product flow. Therefore, the search for rational parameters of adaptronic dosing modules is an urgent task that requires modeling and research of hydrodynamic phenomena in feeders, which, for the most part, are based on the application of product flow separation valves.

The formation of a dose of a light-flowing bulk product, which in terms of structural and mechanical properties is close to a Newtonian liquid, was investigated by weight method (Badiru et al., 2023). The dynamic component of the weighing force is significantly affected by the mode of product power flow. In order to optimize the dosing and packaging operation in terms of its duration and accuracy of dose formation, it is important to ensure the appropriate law of changing the throughput of the feeder (Rangappa et al., 2020). This task can be solved in different ways: by using valve system, pneumatic, shut-off valves (Bauer, 2019; Gavva et al., 2023).

In functional modules with a valveless power supply system, it is quite difficult to implement the required product flow modes. Each type of liquid products has certain properties that have a corresponding effect on the geometry of the feeder and the pressure control system (Ma, 2017). Existing packaging machine designs widely use a valve system with an individual actuator on the valve and a microprocessor control system for valve movement relative to the nozzle seat (Brody, 2000). The effectiveness of controlling valve movement depends on its geometric shape and nozzle seat (Gavva et al., 2023).

It was established that the cone valve makes it possible to most effectively implement the law of its movement and change the throughput of the feeder.

Much attention is paid to the modeling of hydrodynamic phenomena in dispenser feeders (Petrenko, 2021). Thus, electrical methods are still widely used today (Vazguez-Santacruz, 2023), but they cannot ensure the completeness of the feeder calculation, including the change in flow characteristics under different operating modes (da Silva, 2019; Lammerink, 1993). The accuracy of product dosing is considered in works (Vavrik et al., 2023; Furmann, 2017), where the authors confirm that dosing systems with weighing equipment are more accurate and reliable.

Hydrodynamic phenomena in faucets, valve feeders are also investigated using computational fluid dynamics (CFD) (Zic et al., 2020). Computational hydrodynamics is

used to obtain important information based on the analysis of liquid flow in faucets and feeders through simulation modeling.

Thus, with the help of the ANSYS program (Jia et al., 2021), four different shapes of the V-sector valve core hole were studied. In the work (Song et al., 2009), the authors used the finite element method to study the design of ball valves in order to optimize mass and dimensions. The particle flow tracking method (PTFV) is also used for research. The phenomena that occur during the closing and opening of ball valves were studied (Cui et al., 2017). The obtained results can contribute to increasing the productivity of cranes by stabilizing the dynamics of the liquid flow. Experimental studies of throttle valves (Dumitrache et al., 2018) made it possible to compare the effectiveness of their functions.

The results of experimental studies of ball valves at different volume opening angles are given in work (Chernet al., 2007). The analysis of research results showed the impossibility of smoothly regulating the fluid flow with ball valves.

Takase et al (2022) considered the tasks of multi-component dosing of liquids. To solve such problems, it is necessary to consider the method of simultaneous portioned dosing of components of multicomponent products with a given content of components. The results of these studies are not aimed at the formation of a flow of liquid products of a given capacity, and they do not have final recommendations regarding the design of the feeder, methods of regulating product flows. The analysis of the results of the performed studies confirmed their imperfection for the creation of the latest samples of adaptronic modules for dosing liquid products by weight method.

The aim of study is simulation modeling of hydrodynamic phenomena and substantiation of rational values of geometric and kinematic parameters of the feeder of the adaptronic dosing module with a conical valve for dividing the flow of liquid products.

The tasks of the research are:

- to develop a simulation model of fluid movement in the intervalve channel of the feeder with a variable value of the angle β at the base of the valve cone and to evaluate characteristic changes in hydrodynamic parameters;
- to develop a simulation model of fluid movement in the intervalve channel of the feeder with a variable base of the base l_0 of the saddle of the feeder nozzle and evaluate characteristic changes in hydrodynamic parameters;
- to develop a simulation model of fluid movement in the intervalve channel of the feeder at different surfaces of the cone valve base and evaluate characteristic changes in hydrodynamic parameters;
- check the adequacy of simulation models of fluid movement in the intervalve channels of the feeder with a real process.

Materials and methods

Adaptronic module for dosing liquid products by weight method

Adaptronic modules of different structure and composition are used to implement the weighing method of dosing liquid products. Figure 1 shows the improved structural diagram of the adaptronic dosing module, which provides for control of the current values of product weight, the flow rate of products in the feeder, and the level of products in the container.

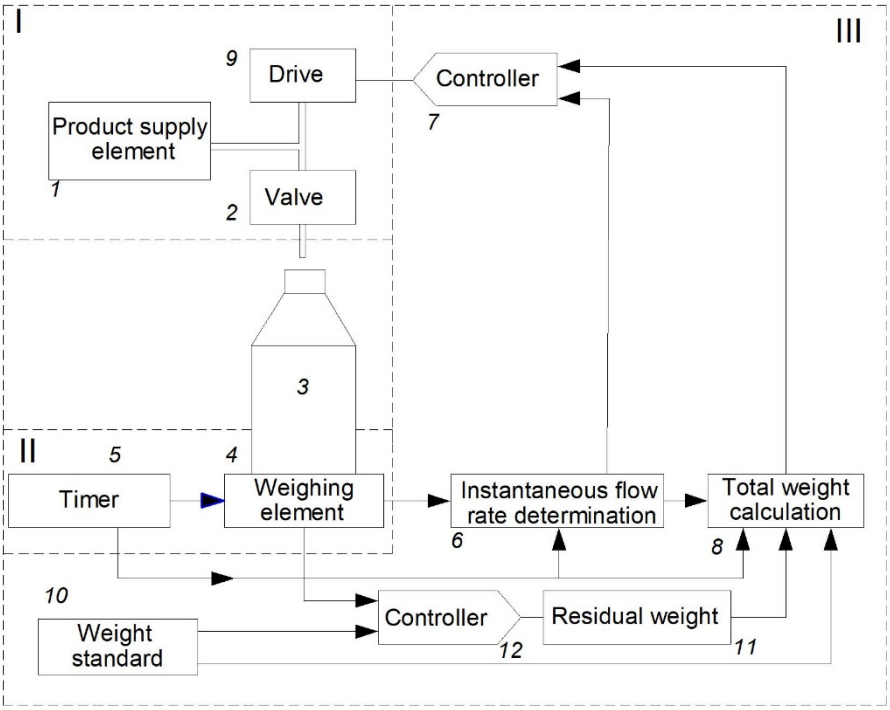


Figure 1. Structural diagram of the adaptronic module for dosing liquid products by weight method

According to this scheme, the product enters through the supply element 1, which is connected to the valve 2 and the device 6 for equalizing the instantaneous flow rate of the liquid in the feeder. For the most part, the vertical movement of the valve provides the change in the capacity of the feeder. The valve movement gradient is realized by sensor drives (pneumatic, electric). To ensure laminar modes of fluid movement in the valve channels of the feeder, at the moment of valve opening and closing, a pneumatic product pressure regulator is installed in the product supply element. A consumer packaging 3 is located under the nozzle of the feeder with valve 2. Consumer packaging 3 is installed on the platform of the weighing element 4. The adaptronic module also includes a timer 5, which provides a common temporal reference base for all elements of the structure that implement the process of product formation and dosing.

For full control of product dosing, the weighing element 4 is connected to the device 6 for measuring the instantaneous rate of product flow in the feeder, and this device is connected to the control element 7 and the element for calculating the total weight of products and consumer packaging 8. To measure the values of instantaneous product consumption, the signal from weighing element 4 is received at regular time intervals. The obtained instantaneous value of the product flow rate is first transmitted to the control element 7, which forms a command for the position of the valve 2 relative to the nozzle seat, and then to the element for calculating the total weight 8 (product in the consumer packaging).

The control element 7 of the valve position 2 also receives the value of the product flow rate 9, with which the measured flow rates are compared to adjust the position of the valve 2. Along with this, the product total weight calculation element 8 receives the signal

from the weight element 10 and the added product weight determination signal 11. The total weight calculation element 8 compares the calculated total product net weight with the indicators of the signals 10 and 11 and forms the signal of the element 7 to reduce or stop the product flow when the two values are the same.

The weighing element 8 signals to the element 12 to calculate the average rate of consumption of the product stream, after which the resulting signal is transmitted to the comparator 13, which also receives signal about the relative average rate of consumption of the product stream 14. The real weight of the products in the consumer packaging (net) is calculated by subtracting the weight of the consumer packaging from the total weight (gross). The signal corresponding to the net weight of the products is sent to the comparator 15, which compares the real weight of the products with the reference value 10.

Determining of weighing process components

The weighing process includes the static component P_{st} – the weight of the consumer packaging and the dynamic component P_d – the movement of products into the consumer packaging:

$$P=P_{st}+P_d$$

The static component of weighing is fixed before the product is packaged. Assuming that the amount of deformation of the tension beam of the weighing system is very small, the dynamic component of the movement of the container during product filling is less than the error of the measuring elements.

The dynamic component of weighing for liquid products with a small value of dynamic viscosity can be represented by a combination of two elements. The first element is the weight of products moved into the container during time t. The second element is the load perceived by the weighing system from the flow of products.

$$P_d = \rho \cdot Q_f \cdot t_i \cdot g + \rho \cdot Q_f \left(\vartheta_2 + \varepsilon \sqrt{2g\left(h + \frac{\Delta P}{\rho \cdot g} \right)} \right),$$

where Q_f is the throughput capacity of the feeder nozzle is determined $Q_f = f_{ef} \cdot \vartheta_2$;

f_{ef} – effective cross-sectional area of the flow of products moving from the nozzle;

ϑ_2 – the average speed of movement of the flow of products from the nozzle;

ρ – volumetric mass of products;

G – gravitational acceleration;

ε – coefficient of aerodynamic resistance of moving products into the consumer packaging, usually accepted ε=1;

H – the height of the liquid flow from the end of the nozzle to the liquid level in the consumer packaging. This value is variable depending on the state of filling the consumer packaging;

$\Delta P=P_1-P_2$ – pressure difference inside of consumer packaging and in the environment.

Construction of feeder of adaptronic dosing module. Data for modelling

The main factors affecting the accuracy of product dose formation by other weighing methods are the parameters of the element of dynamic component weighing and the speed of the shut-off valve.

To manipulate the influence of the second element of the dynamic component of weighing on the accuracy of dose formation while ensuring the specified performance of the adaptronic module, it is necessary, with the given parameters of the nozzle, to establish a change in the throughput of the feeder from the beginning of formation to the completion of dose formation. For the most part, in the existing designs of liquid product dosing modules, valves provide the change in throughput capacity of the nozzle. The geometric shape and the law of movement of the valve relative to the seat of the nozzle significantly affect the nature of the change in the throughput capacity of the nozzle. It was determined, that a conical shape is the effective shape of the valve, from the point of view of realizing the necessary law of change of throughput. (Gavva et al., 2023). The cone valve provides enough of its stroke to implement its law of movement by servo drives.

The feeder with a valve element for liquid products of the adaptronic dosing module consists of measuring container 1, nozzle 2 and valve 3 (Figure 2).

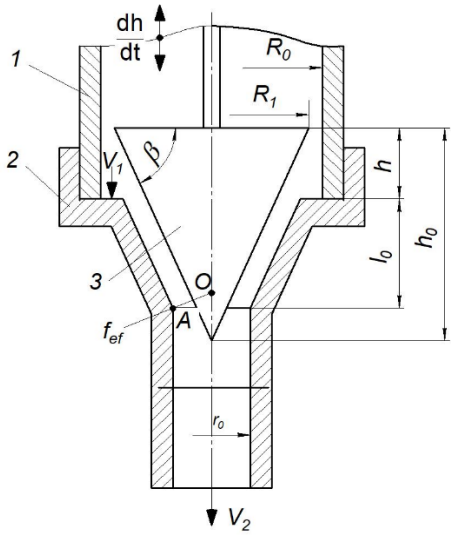


Figure 2. Calculation scheme of the feeder of the adaptronic dosing module with a conical valve for regulating the flow of liquid products:
1 – measuring container; 2 – nozzle; 3 – cone valve

Valve 3 is kinematically connected to a short-stroke servo drive through a rod. The upper inner part of the nozzle serves as a saddle. When designing such a feeder, the basic parameters are the inner diameter of the nozzle d_0; angle β at the base of the cone valve and length l_0 of the seat base. The choice of nozzle diameter d_0 depends on the inner diameter of the neck of the consumer packaging (bottle) and the required capacity of the feeder.

Other geometric parameters of the nozzle valve are functionally related and are determined by the formulas:

$$R_1 = r_0 + \frac{l_0}{tg\beta} \quad ,(1)$$

where R_1 is the base radius of the conical valve;

r_0 – the radius of the internal cone of the nozzle;

$$h_0 = r_0 \cdot tg\,\beta + l_0\,, \quad (2)$$

where h_o – valve height.

$$R_{0min} = \left(r_0^2 + R_1^2\right)^{0,5}, \quad (3)$$

where R_{0min} – the minimum value of the radius of the internal volume of the measuring container, determined from the condition of equality of the throughput capacity of the nozzle and the channel between the valve and the inner surface of the measuring container;

$$h_{max} = r_0\left(tg\beta - sin\beta\right), \quad (4)$$

where h_{max} – the maximum necessary movement of the cone valve, which ensures the equality of the throughput of all channels of the feeder.

To change the throughput of the feeder nozzle, h varies from 0 to h_{max}.

The law of displacement of the valve is defined as a function of the change in the capacity of the feeder

$$\frac{dh}{dt} = f\left(\frac{dQ_A}{d_t}\right), \quad (5)$$

Q_A – the throughput capacity of the nozzle in the feeder channel, the effective area of which is determined by the normal at point A to the surface of the cone valve.

That is,

$$Q_A = \frac{\pi}{sin^2\beta}\left(r_0^2 - \frac{\left(r_0 tg\beta - h\right)^2}{tg^2\beta}\right)\cdot \vartheta_A\,, \quad (6)$$

where ϑ_A -the average speed of fluid movement in the channel of the feeder, the effective area of which is the smallest one (point A).

The law of changing the capacity of the feeder, in order to achieve high indicators of dosing accuracy by weight method, depends on the physical and mechanical properties of the liquid product, the amount of the dose, the configuration of the consumer packaging and the performance of the adaptronic module.

The average speed of fluid movement in the feeder channel is determined based on the solution of the hydrodynamic equations.

The problems of hydrodynamics are aimed at finding functions of fluid flow rate, pressure (stress) and dynamic viscosity using systems of the Navier-Stokes equation, equations of flow continuity and additional holonomic connections that close the system. Having thus written the analytical model of fluid movement and assuming the assumption that the fluid is incompressible, moves in axisymmetric channels, we will get partial differential equations that can be solved only by numerical methods. In this case, for a wide variation of the initial data, it is appropriate to use simulation modeling (CFD) using one of the Solid-Works – Flow – Simulation-2023 program packages.

During the simulation, the following assumptions were made: the liquid is incompressible and corresponds to a Newtonian liquid in terms of physical and mechanical properties, the valve and other parts of the feeder are made of rigid elements that do not deform under the action of hydrostatic load. The fluid movement in the axisymmetric design of the feeder is described by the equations embedded in the Solid Works program (Dassault Systems Technical Reference Solid Works Flow Simulation, 2023).

$$\frac{d\rho}{dt}+\frac{d}{dxi}\left(\rho\cdot U_i\right)=S_M^P; \tag{7}$$

$$\frac{d\rho\cdot U_i}{dt}+\frac{d}{dxi}\left(\rho U_i\cdot U_j\right)+\frac{dP}{dxi}=\frac{d}{dxi}\left(\tau_{ij}+\tau_{ij}^R\right)+S_i+S_{Ii}^P, \tag{8}$$

i=1,2,3*; j*=1,2,3;

$$\frac{d\rho H}{dt}+\frac{d\rho U_i H}{dxi}=\frac{d}{dxi}\left[U_i\left(\tau_{ij}+\tau_{ij}^R\right)+q_i\right]+\frac{d\rho}{dt}+S_iU_i+S_H^P+Q_H, \tag{9}$$

$$H=h\left(P,T,y\right)+\frac{U^2}{2}+k, \tag{10}$$

where U – fluid movement speed;

ρ – volume mass of liquid;

S_i – external force distributed by mass per unit mass

$$S_i=S_i^P+S_i^g+S_i^r, \tag{11}$$

S_i^P - the resistance of the environment;

S_i^g - gravity, $S_i^g=\rho\cdot g_i$;

S_i^r – inertial force in the direction of movement *i*;

H – total enthalpy of the local frame of reference;

h(P,T,y) – thermal enthalpy is determined at given pressure P, temperature T and components of the liquid mixture;

k – kinematic energy of turbulence;

S_M^P, S_{Ii}^P і S_H^P - are additional conditions for the interphase interaction of particles (Euler-Lagrange);

Q_H - source or sink of heat per unit volume of fluid;

τ_{ij} - tensor of viscous fluid shear stress;

q_i - diffusion heat flow.

The energy equation for calculating the flow with a high Mach number can be written in the form

$$\frac{d\rho E}{dt}+\frac{d\rho U_i\left(E+\frac{P}{\rho}\right)}{dxi}=\frac{d}{dxi}\left[U_j\left(\tau_{ij}+\tau_{ij}^R\right)+q_i\right]-\tau_{ij}^R\frac{dUi}{dxi}+\rho\varepsilon+Q_H; \tag{12}$$

$$E = e(\rho, T, y) + \frac{U^2}{2}, \tag{13}$$

where $e(\rho, T, y)$ - integral energy at a given volumetric mass ρ, temperature T and concentration of the components of the liquid mixture y.

The viscous shear stress tensor for Newtonian fluids has the form

$$\tau_{ij} = \mu\left(\frac{dU_i}{dxi} + \frac{dU_j}{dxi} - \frac{2}{3}\delta_{ij}\frac{dU_k}{dxk}\right). \tag{14}$$

Accepting Boussineux's assumption, the Reynolds stress tensor has the following form

$$\tau_{ij}^R = \mu_t\left(\frac{dU_i}{dxj} + \frac{dU_j}{dxi} - \frac{2}{3}\delta_{ij}\frac{dU_k}{dxk}\right) - \frac{2}{3}\rho k\delta_{ij}, \tag{15}$$

where δ_{ij} – Kronecker's delta function;

μ – dynamic viscosity of the liquid

μ_t – turbulent viscosity.

To describe the change in turbulent and kinetic energy k and dissipation ϵ, we write down two additional transport equations

$$\frac{d\rho k}{dt} + \frac{d}{dxi}(\rho U_i k) = \frac{d}{dxi}\left[\left(\mu + \frac{\mu_t}{G_k}\right)\frac{dk}{dxi}\right] + S_k; \tag{16}$$

$$\frac{d\rho\epsilon}{dt} + \frac{d}{dxi}\left[\left(\mu + \frac{\mu_t}{G_\epsilon}\right)\frac{d\epsilon}{dxi}\right] + S_\epsilon; \tag{17}$$

where S_k, S_ϵ are defined as follows

$$S_k = \tau_{ij}^R\frac{dU_i}{dxi} - \rho\epsilon + \mu_t \cdot P_B;$$

$$S_\epsilon = C_{\varepsilon 1}\frac{\varepsilon}{k}\left(f_1\tau_{ij}^R\frac{dU_i}{dxi} + \mu_t \cdot C_B \cdot P_B\right) - C_{\varepsilon 2 f 2}\frac{\rho\varepsilon^2}{k}, \tag{18}$$

where P_B – turbulent generation from buoyancy forces

$$P_B = -\frac{g_i}{\sigma_B}\cdot\frac{1}{\rho}\cdot\frac{dP}{dxi}, \tag{19}$$

where g_i – the component of the acceleration of gravity in the direction x_i;

C_B is a constant, determined from the condition: $P_B > 0$, $C_в=1$, otherwise $C_в=0$.

$\sigma_в=0.9$ – the constant is accepted empirically.

$$f_1 = 1 + \left(\frac{0,05}{f_\mu}\right)^3,\ f_2 = 1 - exp\left(-R_T^2\right); \quad (20)$$

where $C_\mu, C_{\varepsilon 1}, C_{\varepsilon 2}, \sigma_k, \sigma_\varepsilon$ – constants are accepted by the Solid Works Flow Simulation program for drinking water. $C_\mu = 0,09$; $C_{\varepsilon 1} = 1,44$; $C_{\varepsilon 2} = 1,92$; $\sigma_k = 1$; $\sigma_\varepsilon = 1,3$.

Simulation modeling involves developing a graphic model in the Solid Works program and placing it in the Flow–Simulation subprogram. The area of calculation is limited by the contours of rigid parts of the feeder. The research consisted of three thematic approaches:

- the first approach was based on determining the hydrodynamic parameters of fluid movement in the intervalve channel under the condition that the base of the seat l0 is a constant value, and the angle β at the base of the cone varied from 30 to 60º;
- the second approach was based on determining the hydrodynamic parameters of fluid movement in the intervalve channel under the condition that the angle β at the base of the cone is a constant value, and the seat base l0 varies from 5 mm to 25 mm;
- the third approach was based on the recognition of the influence of the type of surface of the base of the cone valve and the hydrodynamic parameters of the fluid movement. At the same time, the following surfaces were considered: conical, spherical convex and concave.

The initial data for the calculations are: liquid – drinking water, volume mass ρ=1000 kg/m^3; temperature t=20 ºC; feeder throughput – 500000 mm^3/sec; the inner diameter of the feeder nozzle d_0=20 mm. Calculations were performed for different positions of the cone valve when it is moved step by step from $0 < h \le h_{max}$.

Experimental installation

The adequacy check of the results of the analytical simulation of movement in the feeder channels was performed on the created experimental installation (Figures 3 and 4).

The experimental instalation consists of a waste tank 1, which is closed by a cover 2. The cover has a built-in funnel 3, through which liquid is supplied to maintain a constant level in the waste tank 1. To control the level of liquid in the waste tank, a level sensor is installed, the signal from which is sent to the shut-off valve liquid supply. A servo-pneumatic actuator 4 is also installed on cover 2, the rod of which is connected to conical valves 13 (Figure 5).

The external geometric dimensions of the valve correspond to the dimensions of the seat 14. The tightness of the connection of the valve seat to the flow tank is ensured by installing a seal 15. The amount of movement of the valve relative to the seat is ensured by changing the air pressure in the cavities of the pneumatic cylinder using a pressure regulator with proportional control 11 (Figure 4), which is connected to the compressed air supply pipeline 12. The weight of the liquid entering the consumer packaging 7 per unit of time is measured by strain gauges 6. Strain gauges 6, a pressure regulator 11 and an electric pressure sensor transmit information through an analog-to-digital converter 8 to a computer 10, which forms a digital database using special software data.

The research was carried out with the following initial data: liquid – drinking water; water temperature -20 ºC; the height of the liquid column in the flow tank H=0.128 m; inner diameter of the nozzle d_o=20 mm; angle at the base of the cone β=60º; the length of the saddle base l_0=15 mm.

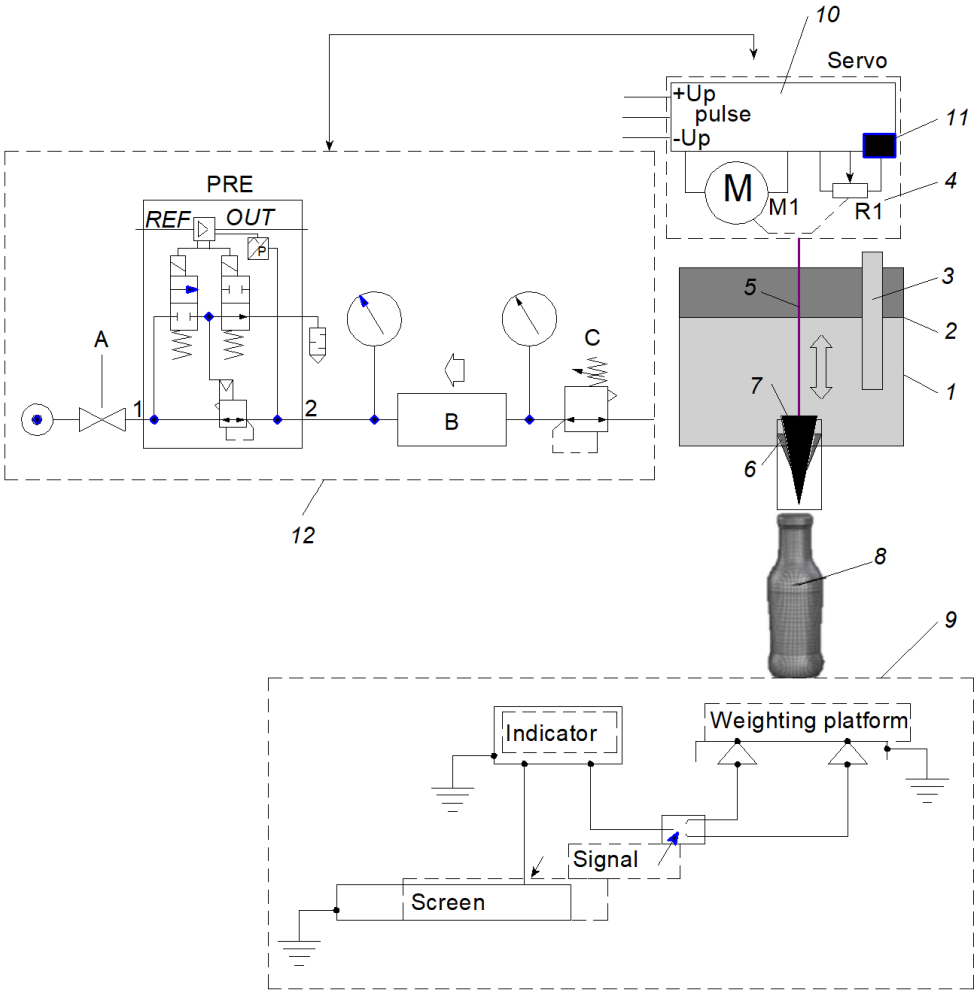


Figure 3. Schematic diagram of the experimental installation for researching the throughput capacity of the feeder of the adaptronic dosing module with a conical valve for distributing the flow of liquid:
1 – flow tank; 2 – cover, 3 – watering can;
4 servo-pneumatic drive; 5 – rod; 6 – nozzle saddle; 7 – cone valve;
8-consumer packaging; 9 – tensometric weighing system;
10 – analog-digital converter; 11 – electronic cone valve movement sensor;
12 – pressure regulator with proportional control

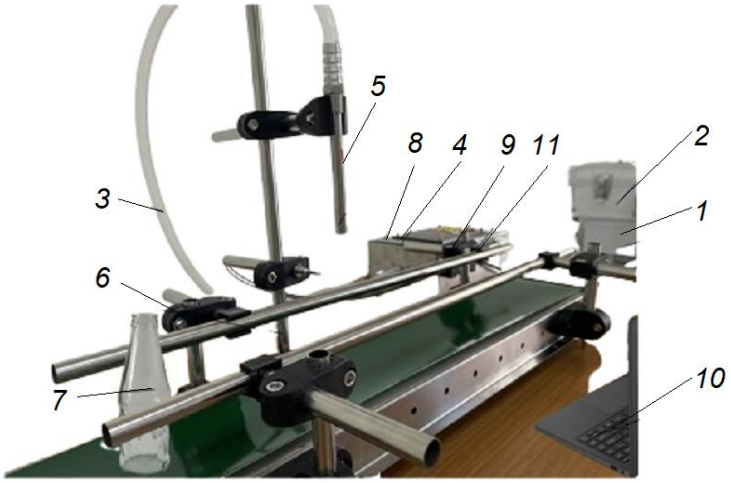


Figure 4. Experimental installation for researching the throughput capacity of the feeder of the adaptronic dosing module with a conical valve for distributing the flow of liquid:
1 – waste tank; 2 – cover; 3 – watering can,
4 – servo-pneumatic drive; 5 – nozzle; 6 – tensometric weighing system;
7 – consumer packaging; 8 – analog-digital converter; 9 – power supply unit;
10 – computer; 11 – pressure regulator with proportional control; 12 compressed air pipelines

Figure 5. Design of the feeder nozzle of the adaptronic fluid dosing module:
13 – valve of conical shape; 14 – saddle with nozzle; 15 – sealing ring

Sequence of experiment

The experiment was conducted in the following sequence. At the initial moment, the valve closes the nozzle channel, the flow tank is filled with liquid up to the level sensor, a container is installed under the nozzle on the strain gauge. The capacity is pre-weighed. Through the computer, the law of movement of the valve at a given step is set. In that case, it is 2, 4, 6, 8, and 10 mm. The power system is turned on, the valve moves to the specified

height, the liquid moves into the container. To stabilize the liquid level in the waste tank, water is supplied through a funnel and a pipeline. The liquid is moved into the container for 5 seconds, after which the system is turned off, the valve closes the nozzle channel, and the strain gauges and the computer record the weight of the gross liquid and container. The obtained value of the weight of the liquid without the container is divided by 5 to determine the throughput of the nozzle at the given position of the valve. After conducting preliminary studies, it was established according to the Student's criterion that for p=0.95 it is necessary to repeat the experiment N=4. After statistical processing of experimental data, the results are entered into a computer to construct a graphical interpretation.

Results and discussion

Figure 6 shows the interpretation of the functional dependence of the change of the effective area of the intervalve channel from the height of the lifting valve only of the nozzle seat.

Figure 6. Change in the effective cross-sectional area of the liquid channel of the feeder with a cone valve (β=60°; r_0=10 mm) from the movement of the valve

The character of the obtained dependence is close to a linear function, which makes it possible, with high reliability, to implement the desired valve motion law with a servo drive. The law of valve movement is determined from the condition of ensuring the productivity of the module, the size and accuracy of the product dose and its physical and mechanical characteristics.

Figure 7 shows the results of simulated modeling of the movement of liquid products in the intervalve channel of the feeder for different values of the angle at the base of the valve cone. The results of the calculations are a change in the values of the fluid movement speed at different points of the feeder and at different positions of the valve relative to the nozzle seat. The generalized results of changes in the speed of fluid movement at the exit from the intervalve channel are shown in the form of 3D graphs.

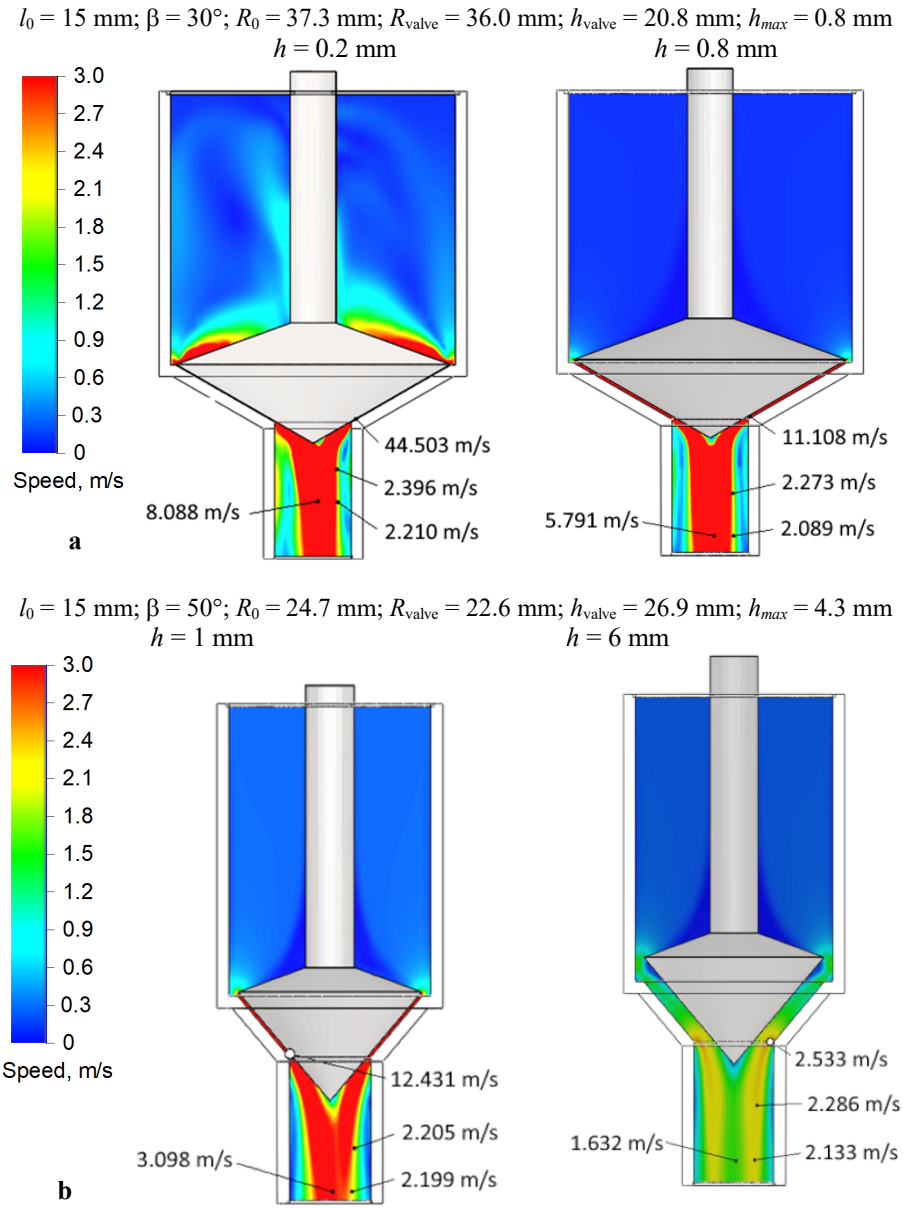


Figure 7. Change in the field of the velocity vectors of the movement of liquid products in the intervalve channel of the feeder for different values of the angle β at the base of the valve cone: a – 30°; b – 50°; c – 60°.

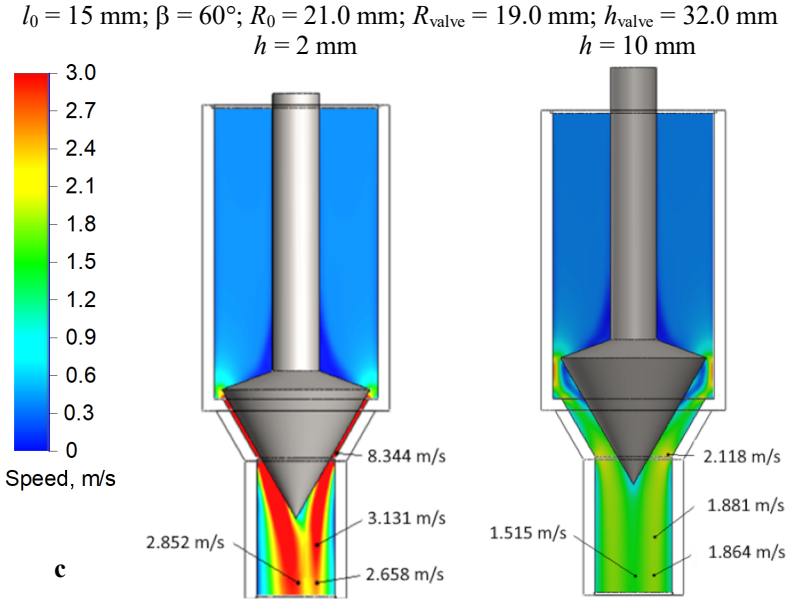


Figure 7 (*continue*). Change in the field of the velocity vectors of the movement of liquid products in the intervalve channel of the feeder for different values of the angle β at the base of the valve cone: a – 30°; b – 50°; c – 60°.

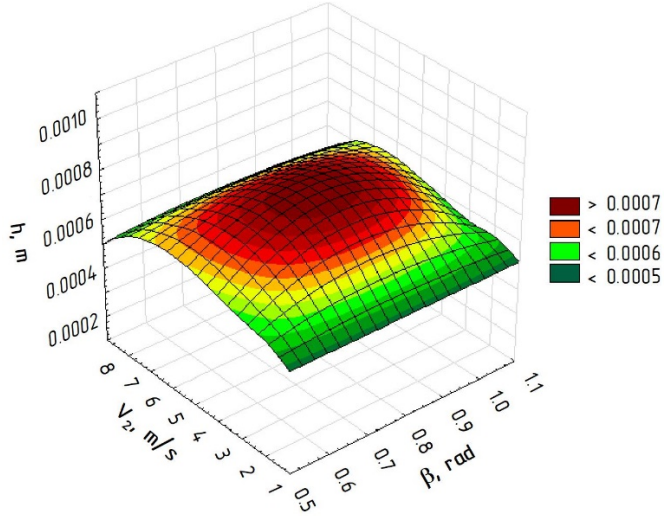
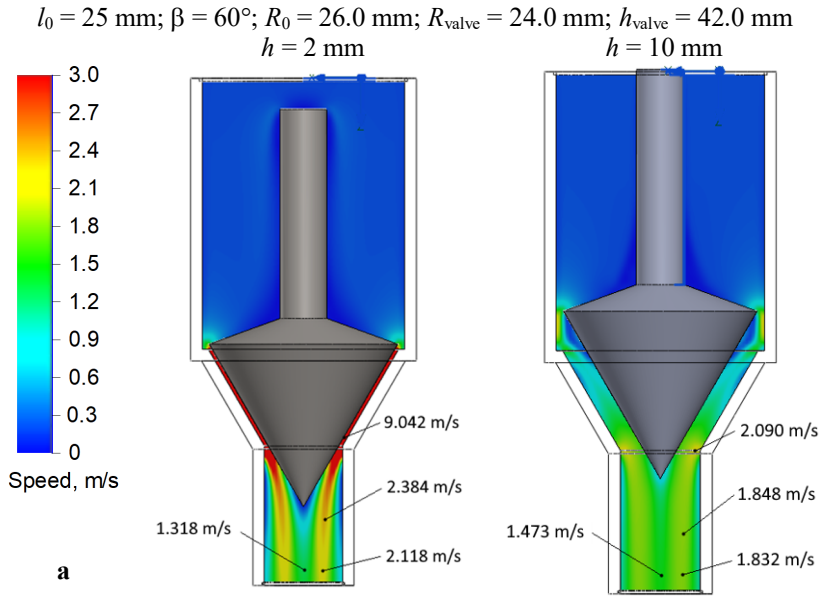


Figure 8. Change in the speed of fluid movement at the exit from the intervalve channel at different values of the angle at the base of the valve cone



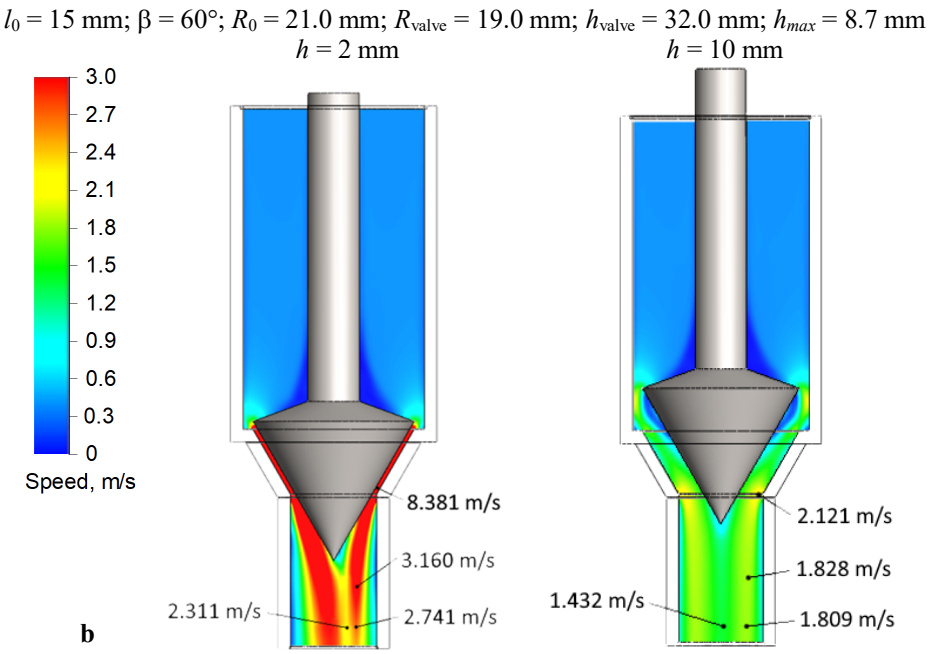
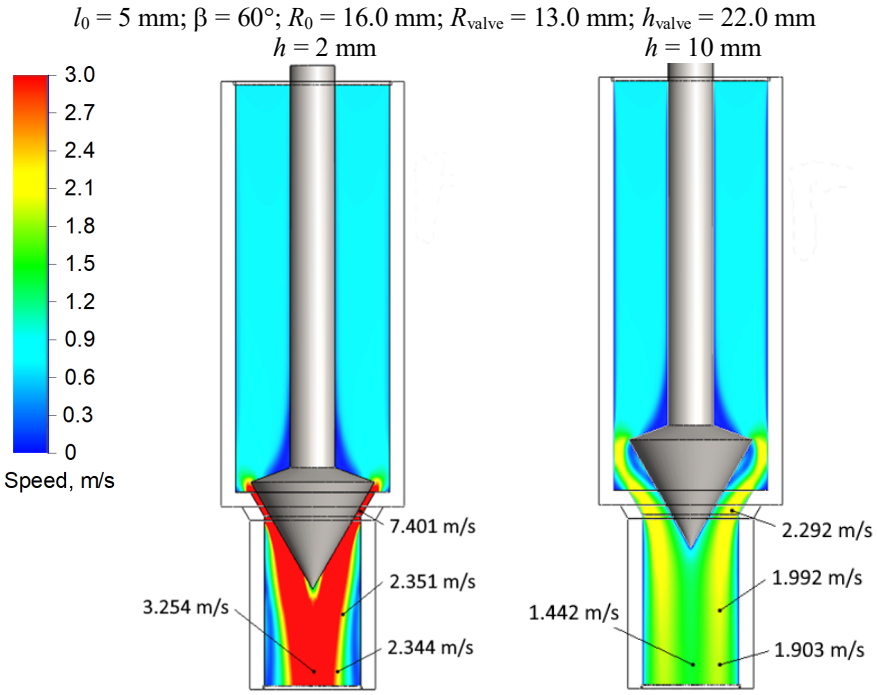


Figure 9. Change in the field of vectors of the speed of movement of liquid products in the intervalve channel of the feeder for different values of the base of the saddle: a – l_0=25 mm; b – l_0=15 mm; c – l_0=5 mm



c

Figure 9 (*continue*). Change in the field of vectors of the speed of movement of liquid products in the intervalve channel of the feeder for different values of the base of the saddle: a – l_0=25 mm; b – l_0=15 mm; c – l_0=5 mm

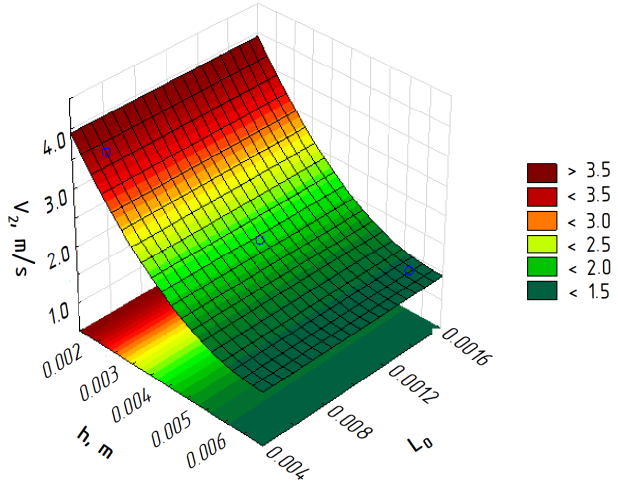


Figure 10. Generalized results of simulated modeling of fluid movement in the intervalve channel when changing the nozzle seat base

The analysis of the results of the simulation makes it possible to state that the execution of the conical valve with an angle at the base of 50–60º is the most rational, because it provides the maximum allowable stroke of the valve (7–10 mm), which makes it possible to effectively implement the given laws of changing the throughput of the feeder with servo drives. Along with this, it is important to eliminate possible fluid turbulence in the intervalve channel and at the exit from the seat. Such conditions are realized also when the angle at the base of the valve cone is within 50–60º.

The rational design of the valve feeder also depends on the length of the nozzle seat base. Figure 9 shows a graphical interpretation of the results of simulated modeling of fluid movement in the intervalve channel of the feeder for different values of the saddle base, and Figure 10 shows their generalizations.

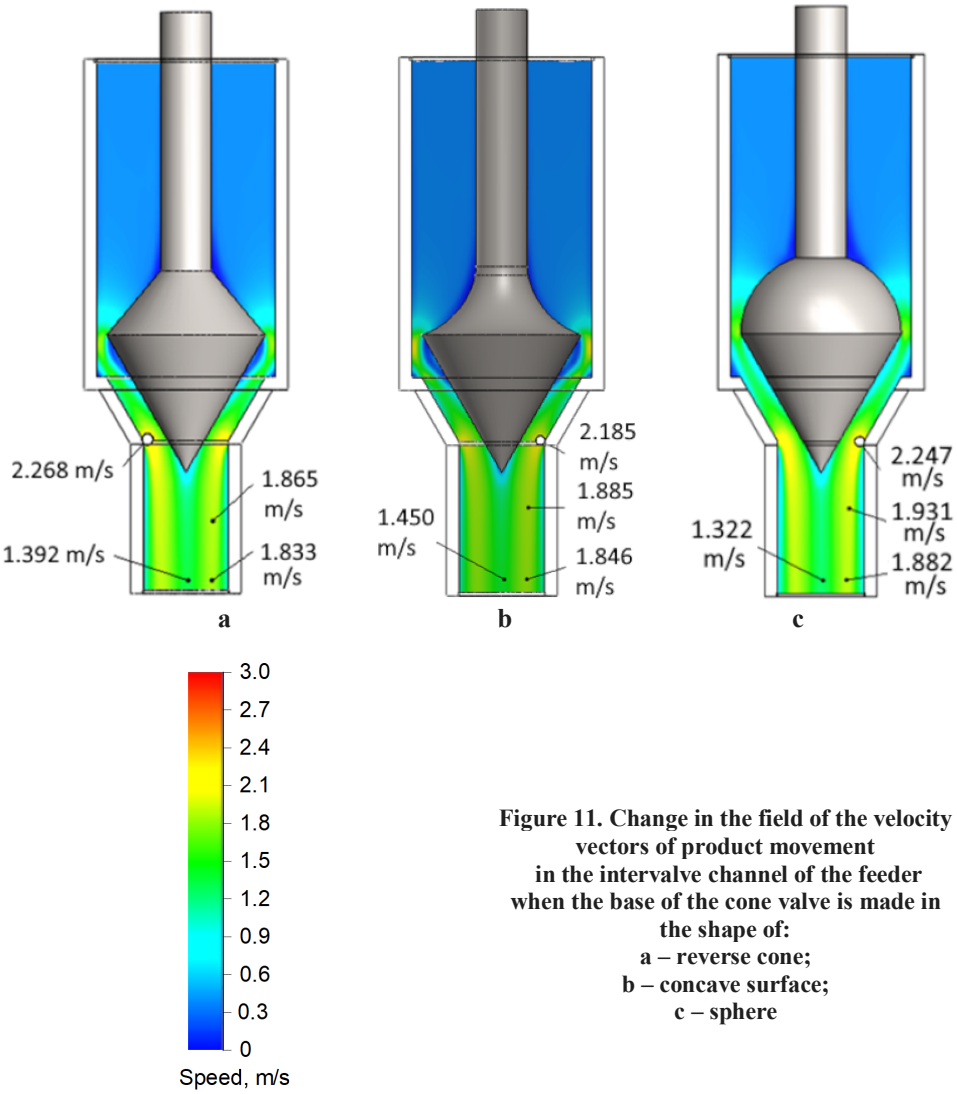


Figure 11. Change in the field of the velocity vectors of product movement in the intervalve channel of the feeder when the base of the cone valve is made in the shape of:
a – reverse cone;
b – concave surface;
c – sphere

The results of the study show that the length of the base of the saddle makes it possible to stabilize parallel fluid flows and minimize the factors of the emergence of turbulence centers. Therefore, it is appropriate to choose the length of the saddle base within 20–25 mm at β=50–60º.

As can be seen from Figure 7 and Figure 9, the contact of the liquid with the surface of the base of the valve cone, which is located perpendicular to the body of the feeder, creates a slight decrease in the pressure of the liquid, which leads to the effect of its reverse movement.

All this affects the parallel laminar mode of liquid movement, which must be implemented in dosing systems to ensure the specified accuracy of dosing and duration of product storage. To eliminate this phenomenon, it is appropriate to replace the flat surface of the base of the cone with a convex or concave one.

Figure 11 shows the results of the base of the cone in the form of reverse cone; concave and convex spheres.

From the point of view of fluid flow stability, the obtained results demonstrate that the best result is achieved when the surface of the base of the cone is made in the form of a sphere with a radius equal to the radius of the base of the cone $R_{sp}=R_1$. Along with this, on the graphic images of the change in the speed of movement of the liquid in the feeder, it can be seen that at the places where the liquid enters the intervalve channel, there is additional resistance, that is, it is a zone of possible formation of turbulence.

The next negative factor is the formation of an annular cross section of the liquid flow after its exit from the valve channel into the cylindrical nozzle. To stabilize the flow of liquid across the nozzle, it is possible to increase the length of the nozzle or make the valve in the form of a truncated cone (Figure 12).

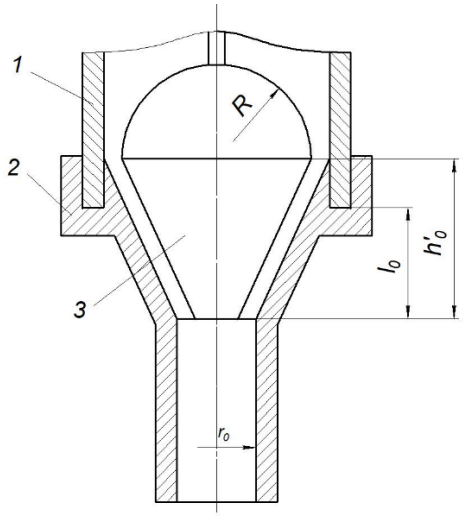


Figure 12. Improved design of the feeder valve of the adaptronic liquid product dosing module: 1 – measuring capacity; 2 – nozzle; 3 – cone-spherical valve

The reliability of the results obtained by analytical and simulation modeling is confirmed by the results of experimental studies (Figure 13). The error in the throughput capacity of the valve feeder for different positions of the valve, relative to the nozzle seat, did not exceed 4.3%.

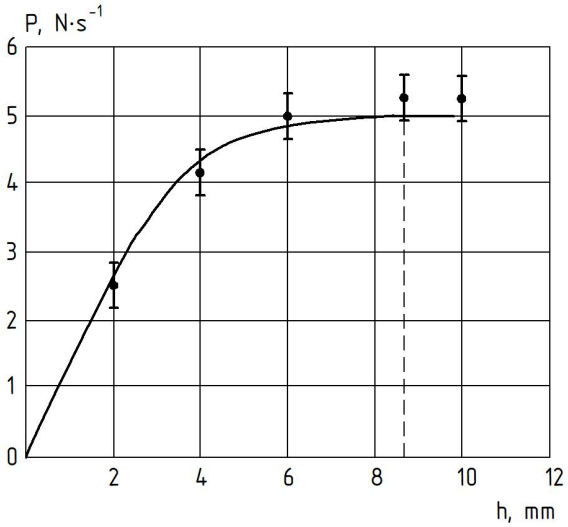


Figure 13. Change in the throughput capacity of the valve feeder at different positions of the valve relative to the nozzle seat

Conclusions

1. The hydrodynamic phenomena of fluid movement in valve feeders are described by complex nonlinear differential equations, with the help of which it is practically impossible to choose rational values of the geometric parameters of the valve, and therefore it is appropriate to use simulation modeling based on computational fluid dynamics (CFD) for the corresponding group of liquid products.
2. On the basis of the analytical dependencies between the parameters of the valve and the feeder seat, and the simulation of fluid movement in the intervalve channel in the program Solid Works – Flow – Simulation 2023, it was established that according to the criteria of the minimum dimensions of the feeder, the possibility of implementing the given law of changing the throughput and eliminating possible cells turbulence, it is recommended to take the angle at the base of the valve cone in the range of 50°–60°, and the base of the nozzle seat in the range of 20–25 mm.
3. During the movement of the liquid in the valve feeder, three negative factors affecting the parallel laminar movement of the liquid were also found: reverse movement of the liquid when it comes into contact with the surface of the base of the valve cone; turbulence cells at the entrance of the liquid into the valve channel and the tubular form of the liquid flow in the nozzle. These negative factors can be eliminated by using a ball-conical valve with a truncated top.
4. The obtained research results need clarification for a wide range of liquid products with different values of physical and mechanical properties.

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Foreign direct investment and sugar production in Africa: a review

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Abstract

Introduction. This review provides a comprehensive evaluation of the role of Foreign Direct Investment (FDI) on sugar production, efficiency, and competitiveness in African countries, based on empirical evidence from multiple studies.

Materials and methods. Drawing from peer-reviewed journal studies, databases, and unconventional sources, the review focuses on the nexus between FDI and its impact on the sugar industries of African countries.

Results and discussion. The study highlights the historical significance of FDI in bolstering sugar production and competitiveness across Southern and Northern African regions. Empirical evidence suggests that FDI has the potential to drive sustainable investment, foster inclusive growth, and enhance competitiveness within Africa's sugar production value chain. The key mechanisms through which FDI boosts sugar production and export competitiveness in certain African nations, as revealed in surveyed literature, include expanding cultivated sugarcane areas and modernizing milling infrastructure through significant investments in property, plant, and equipment. This expansion is propelled by intra-regional investors for Southern African countries and investors from the Middle East in the case of North African countries. However, East and West African countries continue to face challenges, including inadequate investment flows and heavy state intervention, which have inhibited efficiency in both sugarcane production and processing. Despite these insights, there is limited conclusive evidence to determine whether FDI directly improves productivity, yields, and agricultural intensification in African countries. Current literature lacks methodological rigor to quantitatively measure and assess the extent to which FDI impacts sugar production, exports, efficiency, and competitiveness of African sugar industries.

Conclusion. African governments need to balance fostering investor-friendly environments with ensuring effective governance, by strengthening institutional frameworks and promoting synergies for sustainable industry growth and competitiveness.

Introduction

African countries have grappled with prolonged challenges related to savings and foreign exchange gaps. These issues have been instrumental in impeding the region's progress and hampering the accumulation of capital. Several scholars have proposed augmenting financial inflows from external sources as a strategy to bolster the domestic capital reservoir (Koomson-Abekah and Nwaba, 2018; Wamboye and Sergi, 2019). In this context, Foreign Direct Investment (FDI) emerges as a conduit through which Africa could potentially redress its persistent issues of sluggish growth rates and elevated poverty levels. In Africa, the transfer of production technologies through FDI has the potential to transform the agricultural sector, increase domestic production capacity, and boost the overall economy.

The utilization of contemporary agricultural technologies, sophisticated irrigation systems, and mechanization associated with FDI has the potential to increase productivity while concurrently lowering production costs, thereby fostering improvements in comparative advantage (Daum, 2023). This is particularly important given that agriculture, the mainstay for most African countries, is marked by inefficiencies and low productivity. Despite the sector employing over half of the sub-Saharan population (International Labour Organization, 2017) and boasting the largest expanse of untapped arable land globally, productivity is low owing to 95 per cent of agriculture being rain-fed (International Monetary Fund, 2016), limited use of irrigation, low public investment and limited access to finance (Shimeles et al., 2018). The impacts of climate change, growing population and rapid urbanization are likely to compound the status quo (Dogan, 2022). Africa has historically experienced numerous severe and prolonged droughts posing a significant threat to the steady supply of agricultural produce (Masih et al., 2014). Consequently, there is an escalating urgency not only for essential but also critical investments in agriculture and food systems to effectively address these multifaceted demands. FDI could, therefore, play a pivotal role in African agriculture in two distinct ways: firstly, by enhancing productivity across presently cultivated land, and secondly, through its potential contribution to the cultivation of underutilized land in regions where efficiency remains suboptimal (Gunasekera et al., 2015).

The sectorial distribution of greenfield FDI inflows in Africa's agro-food sector shows the dominance of crop production, with sugar and confectionary products, alongside breweries and distilleries, following in importance (Morgan et al., 2022). The cultivation of sugarcane, in particular, has recently attracted considerable attention from investors who perceive this industry as a promising avenue to enhance productivity and yields in previously exploited agricultural regions, as well as to establish new arable land in pivotal African nations (Gunasekera et al., 2015). Investors are also seeking to exploit lucrative markets under existing preferential regional trade agreements such as the European Union (EU) for a crop whose global production exceeds that of any other crop (McKay et al., 2016). The importance of sugar cannot be overemphasized, as sugar remains as one of the preeminent globally consumed, traded, highly sensitive, and protected commodities on the international market (Bouët et al., 2022). Sugar production is a capital-intensive venture requiring investments to sustain a designated level of competitiveness (Galović and Bezić, 2019). Consequently, owing to the substantial capital demands inherent in this sector, investments become imperative for the modernization of equipment, enhancement of infrastructure, and implementation of advanced technologies aimed at augmenting productivity and efficiency within the industry.

Given the escalating influence of FDI in the African sugar industry, it is imperative to evaluate the extent to which it contributes not only to sugar production but also to efficiency and competitiveness. Therefore, this paper reviews existing literature to discern the nexus

between FDI and sugar production, efficiency, and competitiveness. The objective is to investigate whether FDI leads to sugar production, and if so, through what channels—whether through efficiency and productivity gains, encompassing cultivation techniques, introduction of new varieties of sugarcane, irrigation systems, mechanization, technology transfer, and processing methods, or through the expansion of cultivated areas. This review will provide valuable insights for policymakers and industry stakeholders to boost the sugar industries of African countries.

Materials and methods

Search strategy

The study utilized information sourced from peer-reviewed journal studies and other databases and registers conducted on Africa to understand how FDI impacts sugar production, efficiency and competitiveness. To this effect, a meticulous search was conducted on scientific databases Google Scholar, Scopus and Web of Science using key terms such as "Impact of FDI on production, productivity and efficiency of African countries," "effects of FDI on sugar competitiveness of African countries," and "FDI and technological transfer in African sugar industries." Additionally, the search also encompassed unconventional sources and grey literature, such as websites, dissertations, and annual reports of sugar-producing companies in Africa. The inclusion of unconventional sources helped uncover valuable insights, including current data, and emerging trends, and practical implications from a policy perspective that may have been overlooked in traditional research literature. The keywords were delimited within the Scopus and Web of Science databases using Boolean operators OR and AND. For instance, FDI AND sugar production OR FDI AND sugar productivity OR FDI AND sugar competitiveness OR FDI AND sugar production efficiency in Africa. Additional literature was sourced by employing the snowballing technique, wherein the bibliographies or reference lists of analogous studies published in the English language were scrutinized.

Data extraction

This search yielded 96 records published from 1995 up to 2023. Duplicate records were removed, followed by a screening process using the titles and abstracts. The ultimate selection of studies occurred after the retrieval of full texts for comprehensive evaluation, which was based on the explicit reporting of the nexus between FDI and the key terms in question.

Eligibility criteria

Both qualitative and quantitative and quantitative studies were included. Systematic and meta-analyses studies were excluded. Only studies that reported on FDI and the key outcome variables of interest; sugar production, efficiency, exports and competitiveness were included in the synthesis. The two authors independently screened studies for potential inclusion by accessing information in the title and abstract. Following this initial screening, relevant studies aligned with the research questions underwent further evaluation to determine eligibility for full-text review. The ultimate selection of studies occurred after the retrieval of full texts for comprehensive evaluation, which was based on the explicit reporting of the

nexus between FDI and the key terms in question. Studies that provided substantiated evidence or explicitly reported on the effects of FDI on sugar production, efficiency, exports and competitiveness were considered for inclusion. The papers adjudged pertinent by both reviewers were incorporated into the analysis. In instances where disparities arose concerning the selection of papers, these were mitigated through consensus facilitated by an independent arbitrator. Only eleven (11) studies were included in the final analysis, indicating the paucity of information on the impact of FDI on the African sugar industry.

Definition of thematic areas

The study adopted the World Bank definition of FDI. In this regard, FDI refers to all net inflows of capital aimed at establishing a lasting interest or acquiring managerial control over a business entity resident in another country outside the investor's home country. It encompasses the total amount of equity investments, retained profits, other forms of long-term capital, and short-term financial resources. Sugar production and exports are measured in terms of cane or beet sugar and chemically pure sucrose, in solid form, based on data from the United Nations Comtrade (UN COMTRADE), the United Nations Conference on Trade and Development (UNCTAD), and the Food and Agriculture Organization (FAO).

Scope and limitations

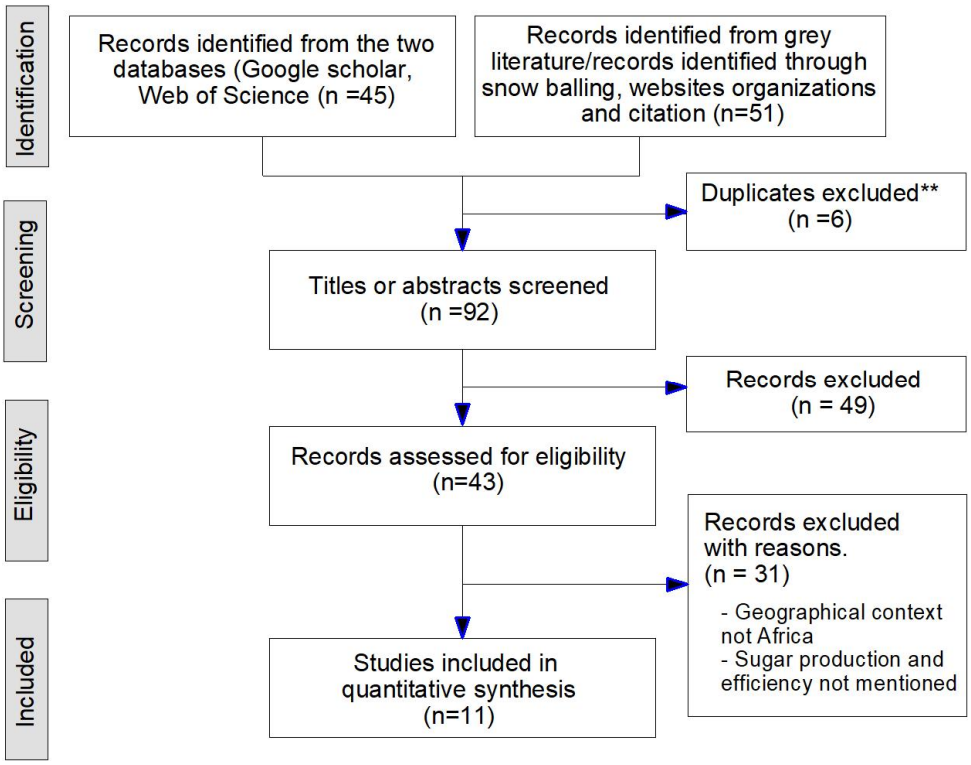

Figure 1. Records retrieval process using the PRISMA flow diagram

Two noteworthy limitations of this study merit discussion. Firstly, the employed methods, although aligned with certain aspects, deviate from the conventional systematic review analysis approaches by incorporating grey literature. The inclusion of grey literature in the analysis may introduce potential bias, as it is often not subject to the same rigorous peer-review process as traditional academic publications. Secondly, while the study relies on evidence from Africa, the reviewed studies do not encapsulate the entirety of the region; rather, there is a bias towards Northern, Eastern and Southern Africa. The limited focus on certain regions of Africa may limit the generalizability of the findings to other parts of the continent. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1) flow diagram visually illustrates the study's record identification and selection process.

Results and discussion

General overview of FDI inflows

Africa has experienced an upsurge in FDI over the past few decades. The continent's vast natural resources, accounting for about a third of the world's resources (Gupta and Singh, 2016; Koomson-Abekah and Nwaba, 2018), including oil, gas, and minerals, have also attracted significant FDI from foreign investors seeking to tap into these resources. Figure 2 shows the trends in FDI inflows to Africa from 1970 to 2021. FDI inflows have increased by over 6000% between 1970 and 2021. During the 1970s, FDI inflows into Africa averaged $1.1 billion and increased twofold in the 1980s to about $2.2 billion. The 1990s recorded significant increases in FDI inflows. FDI inflows to Africa tripled from $2.2 billion to an average of $6.6 billion.

Prior to the 1990s, the majority of African governments implemented trade and capital controls to protect domestic businesses and the country's limited foreign reserves as a result of the events that occurred prior to independence (Ibrahim and Acquah, 2021). However, the shift from protectionism, import substitution and command driven economy to a more liberalized and market driven economy following the economic structural adjustment programs (SAPs) is one crucial aspect that galvanized FDI inflows in the region during this period (Chih et al., 2022). At the beginning of the 21st century, FDI inflows to Africa increased exponentially averaging $31 billion and $49 billion between 2000 and 2010, and 2011 and 2020, respectively. However, these increases have been interspersed with periods of fluctuation, particularly after the global 2008 financial crisis and the COVID-19 pandemic.

Since the 2000s, African governments instituted policies to foster FDI, including liberalized regulatory frameworks, introduction of fiscal and non-fiscal incentives (Malikane and Chitambara, 2017), and the establishment of special economic zones (SEZs) (UNCTAD, 2022). These measures are designed to ramp up industrial production and exports, attract FDI, and create employment opportunities. In addition, Africa offers many opportunities, including its vast natural resources (Mourao, 2018), large and growing population, and an expanding middle class (Odusola, 2018), which together provide a larger market than both Europe and Japan combined. Such deliberate policies account for the upward trajectory in FDI in African countries. The anticipated implementation of the African Continental Free Trade Area Agreement (AfCFTA) is poised to provide an additional impetus to the influx of FDI as it provides investors with an opportunity to tap into a larger consumer base across the African continent (Illovo, 2017; UNCTAD, 2019).

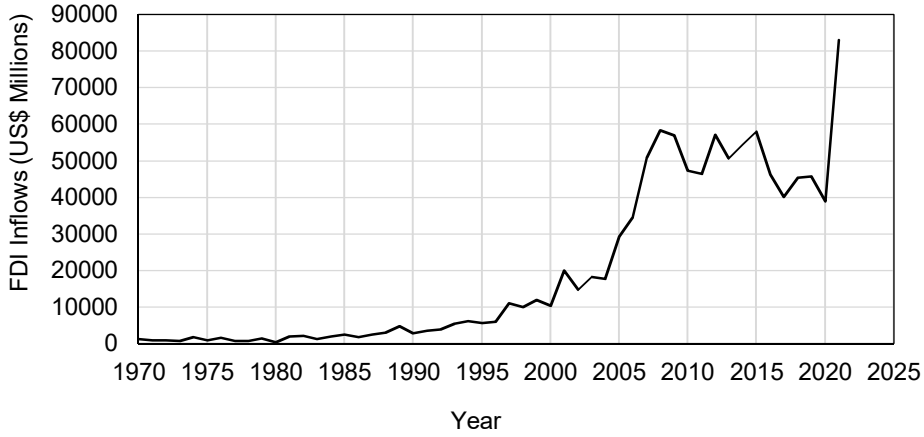

Figure 2. FDI Inflows to Africa: 1970–2021.
Source: Authors' computation based on data from the World Bank

Such policies have coincided with the increase in FDI inflows. The continent's agro-food sector attracted FDI of 773 million US dollars in 2003, and by 2014, this figure had escalated to 1,106 million US dollars (Idsardi et al., 2016). In 2022, FDI inflows to the agro-food sector amounted to 1186 million US dollars (UNCTAD, 2023). Despite the upward trajectory in FDI inflows, UNCTAD (2014) assessment reveals that the necessary investment in agriculture and food security between 2015 and 2030 is $480 billion USD, with a projected deficit of $260 billion USD. The assessment underscores the critical role of FDI in addressing this funding gap to increase food production and enhance agricultural productivity (Dogan, 2022).

Despite the increase in FDI inflows, Africa has continued to attract the least FDI compared to other regions(Anyanwu and Yaméogo, 2015; Morgan, 2021). For instance, between 1980 and 2010, the total value of FDI stock in just the Netherlands alone exceeded that of FDI stock on the entire continent of Africa (Morgan, 2021). This is particularly ironic given that most African countries possess untapped natural resources that may be appealing to potential investors (Mourao, 2018). Africa is still at the bottom end of the list of FDI recipient regions with Europe accounting for nearly half of total global FDI inflows (Morgan, 2021). Africa's share of global FDI inflows was a paltry has 5.2% in 2021 (UNCTAD, 2022). Political instability, policy uncertainty and weak institutions, corruption, poor infrastructure, human capital deficiencies, and underdeveloped financial markets have been cited as the major contributors to the low FDI inflows to the African region compared to other regions (Okafor et al., 2017).

Within the African region, there are considerable variations in FDI inflows. Table 1 shows the FDI inflows by region. The high standard deviation of 22553.37 and coefficient of variation of 1.15 confirms the great deal of variability and heterogeneity among the African countries. FDI inflows to Africa vary widely by region and intra-regionally, and are irregular at any given period. Historically, the Northern Africa region has attracted larger FDI inflows compared to other regions. The Western Africa region ranked second while Middle Africa received the least over the period 1970 to 2021. Over the past decade, the Southern Africa region has attracted the highest share of FDI inflows, albeit with high volatility compared to the West Africa, East Africa and Central African regions. For instance, in 2021 alone,

Southern Africa alone accounted for over 50% of total FDI inflows in Africa. This could be attributed to the region's stable economic and political environment, as well as its abundance of natural resources. There are also intra-regional variations. Large and resource rich countries tend to attract higher FDI inflows compared to smaller and resource deficient countries. It is envisaged that the African Continental Free Trade Area (AfCFTA) may make it possible for other sub-regions to gain from FDI inflows through regulatory harmonization across nations, decreased costs associated with cross-border transactions, and expanded intra-African market access.

Table 1

Descriptive statistics of FDI inflows to Africa by region: 1970-2021 (million US$)

	Mean	Sigma	CV	Minimum	Maximum
Africa	19687.87	22553.37	1.15	400.35	82990.54
Northern Africa	5827.70	6795.84	1.17	-397.98	23096.86
Sub-Saharan Africa	13860.16	16903.78	1.22	247.98	73722.84
Eastern Africa	3931.65	5430.12	1.38	38.99	16348.02
Middle Africa	2145.14	3256.80	1.52	-1957.79	17934.71
Southern Africa	3048.27	6180.32	2.03	-361.58	41508.04
Western Africa	4735.10	5286.08	1.12	-434.39	19038.31

Source: Authors' computation based on data from the UNCTAD

Table 2 shows the trends in FDI inflows to Africa and at World level between 2017 and 2021. The trends corroborate the earlier observation that the Southern African region has been an attractive investment hub. During the period from 2017 to 2021, FDI inflows increased by over 4500 percent. These inflows have been primarily in the agricultural and extractive sectors.

Table 2

Foreign Direct Investment Inflows (millions of dollars and per cent)

	2017	2018	2019	2020	2021	2017-2021 Growth Rate
Africa	40176	45384	45678	38952	82991	107%
North Africa	13275	15407	13550	9800	9335	-30%
Central Africa	8946	9353	8858	9506	9409	5%
East Africa	8784	8054	7893	6062	8179	-7%
Southern Africa	-941	4469	4514	4244	42219	4586%
West Africa	10112	8102	10863	9340	13849	37%
World	1632639	1448276	1480626	963139	1582310	-3%

*Source: Authors' computation based on data from the UNCTAD

The major recipients of FDI in Africa in 2023 are presented in Figure 3. The top ten recipients are Egypt (North Africa), Democratic Republic of Congo (DRC) (Central Africa), Ethiopia and Uganda (East Africa), Namibia, Mozambique and South Africa (Southern Africa), Senegal, Nigeria and Ivory Coast (West Africa). This reinforces the notion that large, resource-rich African nations are attractive markets for investment. These countries on average account for about 38% of total FDI inflows in Africa. It is also worth noting that

Mauritius has experienced a surge in FDI inflows due to favorable investment-related taxation laws and incentives (Morgan, 2021). However, it is important to note that investing in smaller and less resource-rich countries can also provide unique opportunities for growth and diversification. It ultimately depends on the specific goals and strategies of the investor. Smaller economies with growing markets and favorable business environments can also offer promising investment prospects, potential for growth and profitability, albeit with different risks and challenges.

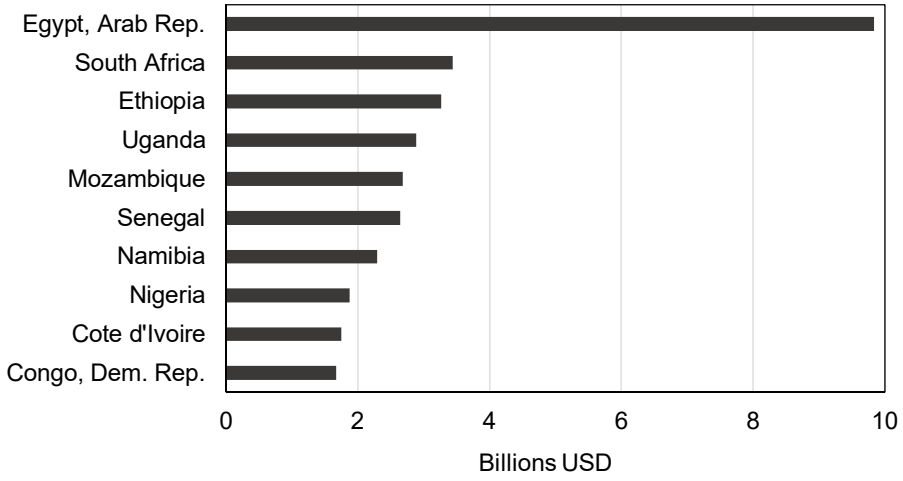

Figure 3. Top FDI recipients in 2023
Source: Authors' computation based on data from the World Bank

Territorial and sectorial distribution of FDI

Most of the FDI inflows have targeted extractive industries, particularly mining and fossil fuels expansion, renewable energy, manufacturing and services sectors (Meniago and Lartey, 2021). Traditional investors such as the European Union have continued to dominate stocks of FDI in Africa. In particular, the United Kingdom ($65 billion) and France ($60 billion) are the two European countries with by far the largest holdings of foreign assets in Africa (UNCTAD, 2022). While Europe remains a dominant source of FDI inflows in Africa, its share declined to below 50% in 2018 (Qiang et al., 2021). At the same time, Chinese investments in Africa increased significantly after the 2000s, following the adoption of the Go-Global Policy by the Chinese government, which strengthened diplomatic ties with African governments (Drogendijk and Blomkvist, 2013; Xu et al., 2019). Other non-traditional investors such as India and the United Arab Emirates have emerged to be the major contributors of FDI in Africa in recent years, especially in greenfield FDI projects (UNCTAD, 2022).

Sugar production and exports

About thirty-seven African countries are involved in sugar production (FAO, 2023). Most of the sugar produced comes from sugarcane while a small proportion is derived sugar beet (Hinke et al., 2018). Sugar from sugarcane is mainly from sub-Saharan countries while the north-African countries mostly produce sugar from sugar beet. However, countries like

Egypt, Morocco, and Tunisia generally combine the cultivation of sugar beet and sugarcane to satisfy domestic sugar demand. Sugar beet cultivation is more prevalent in North Africa, given the Mediterranean climate, which offers more favorable growing conditions compared to the tropical climates in southern Africa that favor sugarcane cultivation. The major sugar-producing countries on the African continent are predominantly located in the northern and southern regions. These two regions account for over 50% of the total sugar production in Africa (Hinke et al., 2018). Several reasons have been cited for this dominance, including favorable climatic conditions, supportive government policies, abundant water for irrigation, readily available arable land and a surge in large-scale investments in the sugar industry (Chudasama, 2021; Mabeta and Smutka, 2023b).

The prominence of the northern and southern African regions in sugar production is further demonstrated by their competitive advantage in sugar exports. Nearly 70% of the countries with a competitive advantage in sugar exports are located in the southern and northern African regions (Mabeta and Smutka, 2023b). While some countries, such as Algeria, Sudan, and Ethiopia, have emerged as significant contributors to sugar production and exports on the African continent, overall sugar exports from African countries have stagnated over the past two decades. The volatility in sugar production and, consequently, exports arises from various factors, including droughts due to climate change, fluctuating market prices, and a lack of investment in modern sugar production and processing methods, particularly by large economies such as Nigeria and Kenya, as well as many West African states. Figure 4 illustrates the trends in sugar production and exports among African countries over the past two decades.

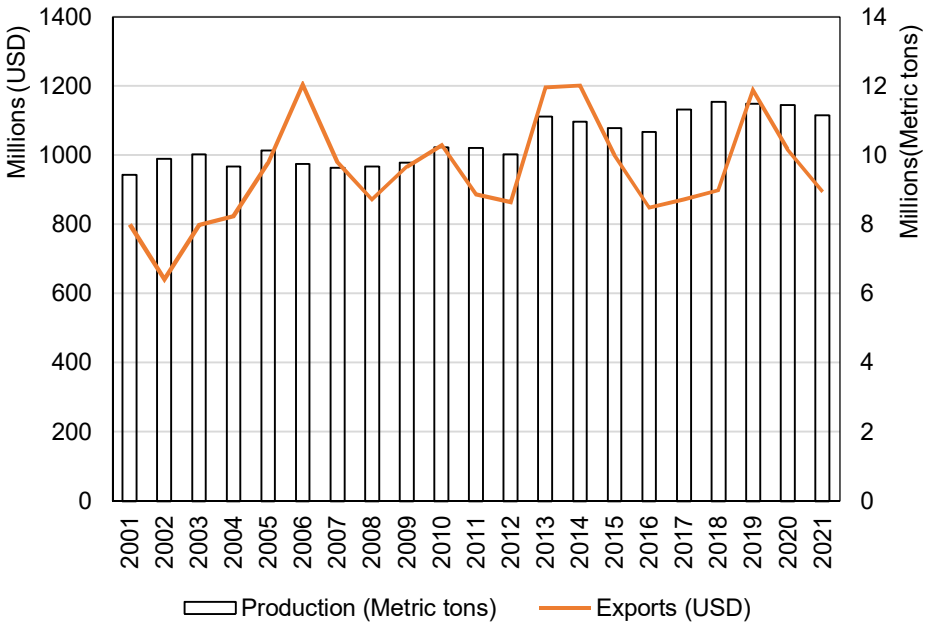

Figure 4. Sugar production and exports of African countries-2001 to 2021
Source: Authors' computation based on data from FAO

Major foreign investors in African sugar industries

In the past decade, approximately 3 billion USD has been earmarked for investment in the sugarcane industries, predominantly in Southern African countries (Richardson, 2010b). These countries' comparative advantage lies in their favorable climatic conditions, access to irrigated fresh water, and availability of arable land making them low-cost and globally competitive sugarcane producers (Dubb et al., 2017; Richardson, 2010b). The major source of recent investments in has emanated from a combination of South African and European capital. These investments have been initiated by foreign entities seeking to engage in the production of sugar and ethanol biofuel, primarily for exportation purposes. There is also growing sugar demand for human consumption (Hess et al., 2016). African sugar consumption has experienced an annual growth rate of almost 3% during the period from 2012 to 2018, surpassing the growth rate of the rest of the world by more than twofold. The present deficit in African sugar amounts to over 70% of the current sugar production on the continent, estimated at $15 billion (Illovo, 2017). Africa stands as the global region with the greatest potential for the growth of sugar consumption, characterized by a below-average per capita consumption and above-average population growth. Therefore, the sugar industry in Africa offers a distinct prospect for sustainable investment and inclusive growth across an extensive value chain.

The increasing level of intra-regional investment is of particular interest, as three long-standing sugar-producing companies – Illovo, Tongaat Hulett, and Transvaal Suiker Beperk (TSB) – have accounted for over 90 percent of sugar production in the entire Southern Africa region (Dubb et al., 2017). During the late 1990s and well into the 2000s, these multinational corporations have significantly escalated their operational expansion into neighboring countries within the region, such as Malawi, Tanzania, Mozambique, Eswatini, and Zambia (Chisanga et al., 2014). Further expansion is anticipated through international aid financing, which is aimed at supporting investments that create opportunities for outgrowers (Dubb et al., 2017). Table 3 provides a summary of the major foreign investors across the African continent.

Characteristics of the reviewed studies

The following information was extracted from the 11 articles as shown in Table 4: author and year of publication, study objective(s), research design, study location, and key outcomes. The review reveals a paucity of information regarding the nexus between FDI and agricultural production or exports. The search indicates that the studies were published between 2007 and 2022, with none explicitly focused on the sugar industry. Furthermore, the review uncovers significant shortcomings concerning the methodological rigor of the studies and the limited robust quantitative evidence on the impact of FDI on various indicators. With the exception of Gunasekera et al. (2015), which utilized simulation through the dynamic Global Trade Analysis Project model (GDyn), all other studies were qualitative in nature and failed quantify the impact the impact of FDI on the selected indicators. Additionally, all studies, except for those by Chisanga et al. (2014), Das Nair et al. (2017), and Msuya (2007), were published in peer-reviewed journals. Most studies were conducted in Southern African countries, underscoring the region's competitive advantage and dominance in sugar production on the African continent.

Table 3

Major foreign sugar investors in Africa

Investor/Company	Countries of investment	Sugar production per annum[1]
Illovo (1.7 million)	South Africa	550,000
	Malawi	250,000
	Mozambique	80,000
	Eswatini	300,000
	Tanzania	130,000
	Zambia	450,000
Tongaat Hulett (1.1 million)	South Africa	535,000
	Zimbabwe	408,000
	Mozambique	195,000
	Botswana	60,000
	Eswatini, Namibia[2]	
Al Khaleej Sugar	Egypt	900,000
Kenana Sugar Company	Sudan	400,000
Somdiaa Group	Côte d'Ivoire	135,000
	Cameroon	130,000
	Congo	70,000
	Chad	35,000
Terra	Côte d'Ivoire	90,000
Wilmar International	Morocco	340,000

[1] Adapted from Dubb et al. (2017). Some data represent estimates derived from the most recent peak production levels from company annual reports: Illovo Sugar Africa - About Us, 2021IAR.pdf (tongaat.com), Profile - Tongaat Hulett, Home 8 – Canal Sugar, Our operational regions: West and Central Africa - Somdia (groupe-somdia.com), PRESENTATION (cosumar.co.ma), Investors | Terra Mauricia Ltd, Cane, Power Brands, Property, Construction, Investment, Leisure, Mauritius.
[2]Operations in these two countries were sold in 2020.

The documented literature tacitly acknowledges the role of FDI in advancing numerous facets of these elements; production, exports, efficiency and competitiveness. FDI has the potential to enhance a country's production by facilitating the transfer of technological knowledge, managerial expertise, efficient production methods and instigating heightened market competition. Furthermore, FDI has the capacity to engender enhancements in infrastructure and fostering an environment conducive to increased export activities (Chih et al., 2022; Doku et al., 2017; Kusairi et al., 2023) and stimulate exports (Ayenew, 2022; Bieleń et al., 2024). The subsequent sections provide a comprehensive analysis of the findings of the reviewed studies in relation to various thematic areas.

Table 4

Summary of studies included in the review

Author /year	Study objectives	Research design	Study location	Key outcomes
Msuya, 2007	The study assessed the impact of FDI on agricultural productivity and poverty reduction.	Descriptive assessment based on a literature review	Tanzania	Considerable positive impact of FDI on productivity and efficiency but with limited benefits to smallholder farmers.
Chisanga et al., 2014	The study compared the dynamics of competition in the sugar industries of selected African countries.	Descriptive assessment based on a literature review	Kenya, South Africa, Tanzania and Zambia	Multinational firms have intensified and expanded operations into neighboring countries, resulting in stable sugar production and inhibiting competition in the host countries.
Sulle, 2017	The study examined the impacts of large-scale commercial agricultural initiatives on the livelihoods of small-scale sugarcane farmers.	Cross-sectional, descriptive assessment that employed qualitative methods, including key informant interviews (KIIs).	Tanzania	Although the outgrower model has led to an increase in overall sugarcane production, it has led to significant social differentiation among farmers.
Gunasekera et al., 2015	The study examined the potential impacts of FDI in African agriculture.	Quantitative cross-sectional study using the dynamic Global Trade Analysis Project model (GDyn) to conduct simulations.	Twenty-one (21) countries from all the five African regions.	FDI enhances agricultural productivity, boosts crop output, and significantly increases exports.
Richardson, 2010a	The study explored the impact of large-scale sugarcane production investments on rural development in southern Africa.	Cross-sectional study assessment that employed secondary data, structured interviews and qualitative methods, including key informant interviews (KIIs).	Zambia	FDI has substantially expanded production capacity and focused on large-scale, export-oriented production to target EU markets.

Author /year	Study objectives	Research design	Study location	Key outcomes
Smalley et al., 2014	The study explores broader questions about the role of foreign capital and outgrower arrangements in promoting economic growth and reducing poverty and inequality.	Cross-sectional study assessment that employed secondary data, structured interviews and qualitative methods, including key informant interviews (KIIs).	Tanzania	Foreign capital caused surplus sugarcane production, overwhelming the mills' capacity and creating harvesting delays and financial instability for outgrowers.
Buur et al., 2011	The study seeks to demonstrate the Mozambican state's role in the rehabilitation of the sugar industry.	Cross-sectional study assessment that employed secondary data, structured interviews and qualitative methods, including key informant interviews (KIIs).	Mozambique	The inflow of foreign capital, including management and technical expertise from foreign corporations, facilitated the industry's modernization of infrastructure and expansion of production capacity.
Ngcobo and Jewitt, 2017	The study examines the drivers and impacts of sugarcane expansion on water resources in Southern Africa.	Literature review and secondary data.	South Africa, Swaziland and Tanzania	Substantial investments in new processing plants, distribution infrastructure, and improved sugarcane varieties have resulted in the expansion of sugarcane cropland, intensification and higher yields.
Ibrahim and Workneh, 2022	The study examines the main technical factors that have contributed to the decline in sugar productivity in Sudan.	Qualitative study using KIIs and quantitative analysis using secondary data.	Sudan	Absence of sufficient investments, particularly in agricultural inputs, equipment, and infrastructure, has hampered sugarcane productivity

Author /year	Study objectives	Research design	Study location	Key outcomes
Dubb et al., 2017	The study analyzed the factors shaping the contemporary dynamics of corporate capital and agricultural production in Africa through the lens of the sugar industry.	Qualitative study using secondary data sources.	Malawi, Mozambique, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe	The study concluded that while FDI can lead to increased sugar production and efficiency, its impact is contingent on local political, economic, and social dynamics hence the need for a context-specific analysis.
Das Nair et al., 2017	The study examined the factors restricting the growth of downstream industrial activities and competitiveness in the sugar-to-confectionery value chain.	Cross-sectional study assessment that employed secondary data, structured interviews and qualitative methods, including key informant interviews (KIIs).	South Africa and Zambia	Investments by large sugar millers in both South Africa and Zambia in expanding their refining and storage capacity have generally led to increased sugar production, However, profits and hence competitiveness have in some years declined due to drought conditions, increased sugar imports, and lower global sugar prices.

FDI and sugar production

FDI has the potential to impact sugar production through three main channels: the rehabilitation of existing facilities, acquisition of new land, and improvements in productivity or efficiency. These channels of impact are crucial as they address different aspects of sugar production. The rehabilitation of existing sugar mills can help improve their infrastructure and technology, leading to increased efficiency and productivity. Acquiring new land for sugar production can expand the industry's capacity and meet growing demand. Additionally, focusing on increasing productivity and efficiency can optimize resource utilization and reduce costs in the production process. These aspects are discussed in detail in the ensuing sections.

A preponderance of studies has been conducted in Southern, Eastern, and Northern Africa. There is paucity of literature on Western African countries. The Southern African countries in particular dominate sugar production in Africa. Mabeta and Smutka (2023) posit that FDI has played a critical role in fostering the development and competitiveness of the sugar industry across many Southern African countries. One strand of literature attributes this to substantial commercial investments, both domestic and international, leading to significant upswings in sugar production (Chudasama, 2021; Dunne and Masiyandima, 2017). According to Dubb et al. (2017) and Das Nair et al. (2017), FDI countries that attract significant FDI inflows in the sugar industry are characterized by low production costs and high yields.

Several studies posit that the conduit through which FDI has enhanced sugar production is mainly through expansion in area cultivated or sugarcane cropland. The expansion involves the acquisition of new land in close proximity to the nucleus estates, much of which falls under 'traditional' or 'communal' tenure under outgrower schemes as is the case in Zambia, Swaziland, Mozambique, Zimbabwe, Malawi, South Africa and Tanzania (Chinsinga, 2017; Scoones et al., 2017). This expansion is propelled by commercial investments facilitated by South Africa-based companies through acquisitions (Dubb et al., 2017) in the case of Southern Africa, and from the United Arab Emirates (UAE) for the majority of North African countries of Morocco, Algeria, and Egypt (Morgan et al., 2022) culminating into the establishment of some of the world's largest sugar factories (Arezki et al., 2019; Sandrey et al., 2018).

Empirical literature consistently underscores the influence exerted by South African capital, particularly the predominant presence of three major companies—Illovo, Tongaat Hulett, and TSB—in the sugar industry of Southern Africa. For instance, Illovo Sugar Africa, the largest sugar producer in Africa and a wholly-owned subsidiary of Associated British Foods plc (ABF), has made substantial investments in six African nations, all located in Southern Africa, primarily through the acquisition of sugar mills (Sandrey and Moobi, 2015). These countries include Malawi, South Africa, Tanzania, Mozambique, Eswatini, and Zambia. Tongaat Hulett, on the other hand, has expanded northwards to Zimbabwe and Mozambique, with a combined installed milling capacity of over one million tons per annum (Dubb et al., 2017). With the increased FDI inflows, studies highlight how this has affected the hectarage under sugar cultivation. Ngcobo and Jewitt (2017) and Viljoen (2014) have documented the increase in sugar production resulting from the expansion and intensification of large-scale investments in Southern Africa. Dubb et al. (2017), for instance, indicates that this expansion propelled Swaziland ahead of Zimbabwe as the region's largest producer outside South Africa, with sugar constituting 43 percent of Swaziland's exports. Unlike other African countries, investments in the Ugandan sugar industry form a hybrid blend of foreign and local investors, with local investors dominating and contributing to over 60% of domestic

sugar production (Martiniello et al., 2022). Ugandan investors play a significant role in intra-regional investments, particularly in other East African countries such as Rwanda, South Sudan and Tanzania. Despite its concentrated emphasis on the local market, the expansion has been so substantial that sugarcane currently holds the second position in terms of tonnage production, trailing only banana plantain, the traditional food staple with production recording an impressive annual growth rate of almost 20% over the span of a decade (Martiniello et al., 2022).

In Zambia, Chisanga et al. (2016) and German et al. (2020) highlight how injection of capital investment of USD 3 billion in 2009 resulted into massive expansion in area cultivated by 54% through the integration of outgrower farmers. The expansion project resulted in a more than twofold increase in sugar production capacity from 200,000 tons to about 450,000 tons per annum. Das Nair et al. (2017) also attribute the significant increase in sugar production in Zambia to substantial investments, doubling in the period 2010 to 2014 compared to the period 2005 to 2008. While Tanzania remains a net importer of sugar and is not yet self-sufficient, Sulle (2017) and Smalley et al. (2014) document the growing role of FDI in expanding the area under sugarcane cultivation and, consequently, sugar production, especially through outgrower farmers. Illovo's investment in factory rehabilitation, infrastructure enhancement, and farmer incentivization led to a significant expansion in both the number of outgrowers and the area dedicated to sugarcane cultivation (Smalley et al., 2014). Investments in the Kilombero Valley expanded the area under outgrower production by over 12,000 hectares between 1990 and 2014, resulting in a substantial increase in the quantity of sugarcane sourced from small-scale, medium-scale, and large-scale outgrowers. A recently announced expansion project by Illovo Sugar Africa in the Kilombero region in Tanzania is projected to increase sugar produced by the nucleus estate from 127,000 tons to 271,000 tons per annum and from 600,000 tons to 1,700,000 tons of sugarcane supplied by outgrowers (Illovo, 2020). Table 5 provides a comprehensive summary of key production indicators associated with sugar production in selected African countries, where literature has documented the trends and status of sugar production.

Table 5

Sugarcane harvested area and production for selected African countries, 1991-2021

	Area harvested (' 000 ha)				Sugarcane production (' 000 tons)			
	1991	2001	2011	2021	1991	2001	2011	2021
Egypt	112	131	137	128	11624	15572	15765	12361
Eswatini	40	41	54	59	3941	4000	5288	5715
Kenya	48	48	79	92	4600	3551	5307	7783
Madagascar	64	68	88	97	1950	2208	2805	3123
Malawi	18	21	25	29	1900	2200	2700	3157
Mauritius	76	73	57	42	5621	5792	4230	2670
Morocco	15	18	13	9	1026	1114	764	613
Mozambique	20	36	43	50	253	676	3396	2987
Nigeria	22	23	49	85	888	705	989	1498
South Africa	276	326	253	279	20078	21157	16800	17991
Tanzania	16	16	57	49	1420	1500	3021	3515
Uganda	22	20	53	85	845	1543	3650	5369
Zambia	12	19	40	49	1150	2000	4200	5102
Zimbabwe	32	41	43	47	3236	4630	3058	3450

Source: Authors' computation based on data from FAO

The data suggest varying trends in sugarcane cultivation and production across the selected African countries, with the majority experiencing consistent growth, while others show fluctuations or declines. Notable fluctuations are observed for Mauritius, Morocco, South Africa, and Zimbabwe. The fluctuations in both the cultivated area and production can be explained by high production costs coupled with low sugar prices on the international market, leading to the exit of smallholder farmers from the sugar industry (Chudasama, 2021). There is also a trade-off between the cultivated areas for sugar beet and sugarcane, particularly in North African countries, as well as the impact of land redistribution reforms in Zimbabwe and South Africa.(Dubb et al., 2017; James and Woodhouse, 2017).

FDI, sugar productivity, efficiency gains and competitiveness

Another strand of literature has highlighted the critical role of technological spillovers from FDI, resulting into enhanced efficiency (Ngcobo and Jewitt, 2017). According to these studies, the surge in FDI in the sugar industry has enhanced sugar production through technological spillovers, leading to the adoption of efficient production methods. Increase in sugar production, particularly among Southern African countries can be ascribed to significant FDI, particularly investments in sugar mills or new processing plants, advancements in sugarcane seed varieties, and the enhancement of supply chain and distribution infrastructure. Technological advancements associated with FDI not only boost productivity but also improve access to high-return markets (Osano and Koine, 2016), playing a crucial role in shaping the competitiveness of the sugar industry . Several studies in recent years have attributed the increase in sugar production to predominant strategies implemented by sugar companies to extend their operations. These strategies involve assuming control of and revitalizing existing sugarcane plantations and processing facilities, often formerly state-owned, as observed in Mozambique, Tanzania, and Zambia (Dubb et al., 2017; Gunasekera et al., 2015). By acquiring these plantations and facilities, companies can leverage their expertise and resources to modernize their operations, improve efficiency, and experience swift expansion in production, as evident in most Southern African countries, except South Africa and Swaziland. In recent years, Illovo and Tongaat Hulett have allocated substantial capital expenditure primarily for expansion in plant, property, and equipment and storage capacity in Malawi, South Africa and Zambia (Das Nair et al., 2017). This investment, for instance, increased Zambia's sugar production capacity by 90,000 tons (Das Nair et al., 2017).

Mozambique is another success story, with substantial FDI in the rehabilitation of the sugar industry lifting the country from its nadir and dependency on contraband imports from its Southern African neighbors to a surplus country, with exports exceeding domestic consumption (Buur et al., 2011). Another pivotal role of FDI involves the creation of infrastructure, including the construction of roads, port facilities, and irrigation dams (Gunasekera et al., 2015). This is evident in countries such as Malawi and Zambia. Dubb et al. (2017) further argues that expansion takes place through the acquisition of assets offered for sale, as evidenced in instances like Lonrho in Malawi and Anglo American in Zimbabwe. This approach helps manage costs and can avoid perceptions of forcefully taking land from local communities, thereby allowing sugar companies to navigate potential conflicts and maintain a more positive reputation in the countries where they operate.

Empirical evidence on the impact of FDI on productivity is scanty. However, the limited research available suggests a positive correlation between FDI and productivity growth. Richardson (2010) suggests that the Southern African countries of Malawi, Tanzania, and Zambia have achieved superior cane yields compared to Australia and Brazil due to their

recognized comparative advantage in sugarcane production. These countries are characterized by abundant sunlight and access to irrigated freshwater, availability of arable land and have extended seasons in subtropical climates conducive to emerging as globally competitive producers (Hess et al., 2016; Macháček et al., 2017). The existence of extensive estates also lowers haulage costs combined with concentrated management practices (Dubb et al., 2017). Mabeta and Smutka (2023) opine that while most sub-Saharan African countries have low sugarcane yields, nine other African countries exhibit ideal yields ranging between 70 and 100 tons per hectare. These include Senegal, Burkina Faso, Eswatini, Chad, Zimbabwe, Kenya, Mauritius, Mali, and Burundi. The prospect of recording high yields further catalyzes investments in their sugar industries (Chudasama, 2021).

Owing to investments made in the past few decades, some African countries have witnessed a rapid surge in sugar production, with a significant portion of the output targeted for export markets. The increase in sugar production of these countries has enhanced their competitiveness on the international market. The gain in sugar export competitiveness is highlighted in several studies (Mabeta and Smutka, 2023b; Seleka and Dlamini, 2020). These studies reveal that the best performers in terms of export competitiveness are predominantly from Southern Africa, with some of them outcompeting the largest sugar exporters from the EU. These countries, including Eswatini, Malawi, Mauritius, Mozambique, South Africa, Zambia, and Zimbabwe, have effectively maintained their competitiveness in the sugar industry over the past two decades. On the other hand, North African countries such as Morocco, Algeria, and Egypt have transitioned from a state of comparative disadvantage to that of comparative advantage. One commonality across these countries is the influx of large-scale commercial investments in their sugar industries. Morgan et al. (2022) posit that the Southern and Northern African regions have historically accounted for the largest proportion of FDI inflows. Studies by Dunne and Masiyandima (2017) and Ngcobo and Jewitt (2017) attribute the increase in sugar production and the subsequent competitiveness of sugar exports in these countries to the impact of FDI. Five of the top 10 lowest cost producers of sugar in the world are countries in which Illovo and Tonga Hulett have a significant footprint (Tongaat Hulett, 2021). Given that global sugar prices continue to be low and volatile, especially after the European sugar reforms, maintaining cost efficiency is essential for sustaining a viable business model.

The underperforming countries are mostly from the East and West African regions (Mabeta and Smutka, 2023b; Seleka and Dlamini, 2020). The sugar industries in these countries are marred by a lack of investments, high barriers to entry, heavy government involvement, and mismanagement, thereby contributing to inefficiencies, low sugar production, and uncompetitiveness (Bomett et al., 2020; Chisanga et al., 2014).

3.2.3 Correlation analysis of FDI and sugar production and exports

FDI data in agriculture, forestry, and fisheries is used as a proxy for investments in the sugar industry. This decision is justified given that a considerable portion of FDI in the agriculture, forestry, and fisheries sector is allocated to sugar production. (Morgan et al., 2022). Table 6 presents Pearson's correlation coefficient matrix of FDI and several indicators, namely sugar production, area harvested, yield, exports, and sugar competitiveness.

Table 6

Correlation analysis

Country	Sugar production	Area harvested	Yield	Exports	Competitiveness
Egypt	-0.2126	-0.3382	0.0153	-0.0670	-0.1559
Morocco	0.4859	-0.1261	-0.7097*	0.4188	0.4426
Kenya	-0.0605	-0.0740	0.0442	-0.1627	-0.3640
Madagascar	-0.0499	-0.1620	0.3954	0.4099	0.6288*
Mauritius	0.2571	0.1037	0.1002	0.1741	0.0350
Mozambique	-0.1700	-0.0617	-0.1073	-0.3157	-0.3787
Nigeria	-0.2660	0.3004	0.0880	-0.0157	-0.4447
Rwanda	-0.3854	0.0545	-0.1536	0.5927*	0.4933
Uganda	-0.2226	-0.1917	-0.0774	-0.1173	0.0364
Tanzania	0.5028	0.3224	-0.3277	-0.3203	-0.5047
Zambia	0.5440*	0.5883*	-0.2447	0.4299	-0.0916

Note * denotes statistical significance at 5% level.
Source: Authors' computation based on data from the FAO

The examined correlations shed light on the associations of FDI with pivotal sugar-related variables, including production, exports, and competitiveness within the sugar industries across the diverse landscape of African countries. Notably, Morocco stands out with robust positive correlations between FDI and sugar production, exports, and competitiveness, highlighting a promising synergy between foreign investments and the growth and competitive edge of its sugar sector. Madagascar, too, portrays a positive scenario, displaying strong correlations with FDI across various dimensions, particularly emphasizing the positive impact on yield and competitiveness. Mauritius and Zambia echo this pattern, with positive correlations in sugar production and area harvested, suggesting a constructive relationship with FDI. Tanzania displays strong positive correlations with production, area harvested, and competitiveness, indicating potential positive influences from FDI. These insights underscore the potential for FDI to catalyze positive developments in sugar production and competitiveness, signaling opportunities for strategic investments and growth in these nations. However, amidst these positive patterns, challenges emerge in countries like Egypt, Mozambique, Kenya, Nigeria, and Uganda, where negative correlations with FDI in key aspects raise questions about potential hurdles and the need for nuanced strategies to leverage foreign investments effectively. These countries face issues such as political instability, inadequate infrastructure, mismanagement and corruption, which can deter foreign investors (Bomett et al., 2020; Chisanga et al., 2016). Therefore, it is crucial for these countries to address these challenges and implement targeted policies to create a conducive environment for foreign investments.

Impact of institutional and production arrangements on sugar production

While empirical literature acknowledges the impact of FDI sugar industries of some African countries, the role of institutional arrangements cannot be overlooked. The two are complementary to each other. The presence of better institutional and production arrangements is more likely to be catalytic to FDI inflows and ultimately enhance the growth and competitiveness of the sugar industries. Outgrowers have become integral to investors, playing a crucial role not only in the expansion of capital within the sugar production sector but also making substantial contributions to the national fiscus of various countries (Dubb et

al., 2017). The nature of outgrower schemes exhibit considerable variability, ranging from individually owned small-scale plots or communal land under irrigation, as evidenced in Eswatini, Kenya, Malawi, Mozambique, and Zambia, to relatively larger-scale growers in South Africa and Zimbabwe (Dubb et al., 2017; von Maltitz et al., 2019). Given that sugarcane production in most sub-Saharan countries is heavily dependent on outgrower farmers during production start-up or in expansion efforts of investors, issues related to land acquisition becomes of paramount importance (Chisanga et al., 2014; Mabeta and Smutka, 2023a). Without secure land rights for outgrower farmers, investors may face challenges in expanding their operations. Additionally, clear, and transparent land acquisition policies can help build trust between investors and local communities, fostering a conducive environment for investment in the sugar industry.

The state, therefore, plays a critical role in this regard by establishing and enforcing land laws that protect the rights of both outgrower farmers and investors. This includes ensuring fair compensation for land acquisition and providing a streamlined process for resolving any disputes that may arise. Moreover, the state can also facilitate partnerships between investors and local communities, promoting sustainable development and mutually beneficial relationships in the sugar industry. For instance, Msuya (2007) findings indicate that smallholder sugar outgrowers located in close proximity to foreign-owned sugar milling companies in Tanzania achieve higher levels of efficiency compared to those that are far away through adoption of advanced skills and techniques. However, African countries such as Kenya, Nigeria and Sudan, where government intervention has extended beyond providing a conducive environment, have experienced poor performance and production inefficiencies in their sugar industries (T. S. Ibrahim and Workneh, 2022; Onyango et al., 2018; Sandrey and Moobi, 2015; USDA, 2023). Privately run sugar companies perform better and are more efficient compared to state-owned mills in these countries (Chisanga et al., 2014; Onyango et al., 2018). These countries have experienced challenges such as corruption, mismanagement, and lack of accountability in their sugar industries (Bomett et al., 2020). This has hindered the growth and profitability of the sector, leading to negative impacts on both outgrower farmers and investors, and likely to impede potential FDI inflows in the sugar industry. Therefore, it is crucial for African governments to strike a balance between creating a favorable business environment and ensuring effective governance to foster a thriving sugar industry.

FDI and potential conflicts

In recent years, there has been a surge in interest regarding the nexus between corporate investment and agricultural production in Africa, sparking contentious debates, particularly evident in the discourse surrounding 'land grabs.' These discussions hinge on the impact of capital investment in large-scale agriculture, the involvement of the state, and the balance between associated costs, such as displacement of existing land users. In contrast to more recent corporate-driven agricultural ventures, sugarcane cultivation in the region boasts a deep-rooted history, intricately tied to sustained governmental support in the form of financing, infrastructure development, and political endorsement over the long term. The expansion of agricultural production, often facilitated by public-private partnerships, raises questions about access to land and water resources and the distributional impacts of such investments. Furthermore, the reliance on customary land ownership in many African countries complicates negotiations for large-scale projects like sugar estates, often leading to unequal bargaining power and disputes over land rights. Despite resistance from local communities and safeguards enshrined in land laws, the pursuit of corporate interests in the

sugar industry continues to shape land use and tenure across the continent, highlighting the complex interplay between state policies, corporate investments, and community resistance in the agricultural sector.

Sugar production in southern Africa serves as a focal point for examining these debates. The region has seen significant expansion in sugarcane production, driven by large-scale corporate investments, which have both positive and negative consequences. Scholars such as Dubb et al. (2017) argue that while such investments have led to employment opportunities and economic growth, they have also resulted in deforestation, loss of biodiversity, and displacement of indigenous communities. Several other scholars have highlighted how this phenomenon is particularly pronounced in Southern African countries. For instance Chinsinga (2017) and Smalley et al. (2014) in Tanzania highlight how the 'Green Belt Initiative' and the Southern Agriculture Growth Corridor (SAGCOT) Initiative, respectively, have intensified political tensions over land acquisition and reallocation for irrigated outgrower plots. There have been reported cases of eviction and displacement of local people as companies have exhausted their designated estate land (Smalley et al., 2014). Hess et al. (2016) and Richardson (2010) have documented how 1100 households were displaced in Mozambique due to land and water appropriation by sugarcane companies. Similar patterns are observed in Zambia by German et al. (2020) in which the displacement of smallholder agriculture, accounting for 9.3% of the expansion, primarily entailed the substitution of rainfed farmland with sugarcane cultivation on smallholdings during intensification thereby threatening food security. In addition, German et al. (2020) raise some concerns over water availability and stress in Zambia's lower Kafue River basin as expansion of irrigated cane cultivation has led to increased consumptive use of irrigation water. These concerns regarding the impact of sugarcane expansion on water resources have been echoed by Hess et al. (2016) not only in terms of water availability but also water quality, particularly in relation to the pollution of surface and groundwater bodies in several sub-Saharan countries.

The political economy of sugar production in Africa also prompts critical inquiries into the distribution of benefits—whether they accrue more significantly to the host economy or are disproportionately skewed in favor of investors. There are reported cases of rent-seeking behavior by investors arm twisting government in Zambia and Mozambique. This raises concerns about the predatory nature of international capital and its long-term viability and sustainability, particularly regarding its potential impact on the national fiscus of African countries. For instance, Illovo Sugar has obtained substantial tax concessions from the Zambian government, significantly reducing its tax obligations. Notably, the company succeeded in being reclassified as an agricultural entity, securing a nearly 50% reduction in its corporate tax rate, while also capitalizing on additional benefits such as duty exemptions on imported machinery and preferential financing rates through an agreement under the Investor Promotion and Protection Act (Richardson, 2010a) . There are also concerns that major milling companies have managed to assert a level of market dominance in the markets in which they operate, such as Zambia and South Africa, thereby creating significant barriers to entry. The competitive dynamics among milling companies, both within individual countries and across the region, are therefore compromised by both implicit and explicit barriers to entry and growth, primarily driven by regulatory frameworks and protectionist policies (Chisanga et al., 2014) . The strong connections between these multinationals and government entities have led to stable, non-competitive markets, enabling these firms to capitalize on the favorable regulatory landscape for the benefit of their international shareholders (Dubb et al., 2017). The dearth of competition stifles the prospects for growth and innovation within the sugar industry.

Conclusions

Africa's sugar production is low, with consumption outstripping current supply, despite the continent's comparative advantage in terms of favorable climatic conditions, access to irrigate freshwater, and the availability of arable land. FDI has the potential to transform the sugar industries of African countries through technological transfers and adoption of efficient production and processing methods. The continent has experienced a surge in FDI inflows in the sugar industry. This review was aimed at assessing the impact of such FDI inflows on the performance of African sugar industries, particularly on sugar production, productivity, and competitiveness. There is strong evidence that FDI has contributed to increased sugar production and enhanced competitiveness in the global market, especially for countries in the Southern and Northern African regions. The reviewed literature contends that FDI, particularly in Southern African countries, has increased sugar production mainly through the expansion of cultivated cropland. This expansion is propelled by intra-regional investors for Southern African countries and investors from the UAE in the case of North African countries. The expansion of cultivated cropland has yielded both favorable and adverse effects on the environment and local communities. While it has generated employment opportunities and bolstered economic growth, it has concurrently triggered deforestation, biodiversity depletion, and the displacement of indigenous communities.

The reviewed literature also attributes the increase in sugar production to efficiency gains resulting from sugar companies' strategies to modernize and expand their operations. These strategies involve acquiring state-owned sugarcane plantations and facilities, leveraging expertise, and making investments in plant, property, and equipment, which have increased sugar production capacity and enhanced export competitiveness in Southern African countries such as Mozambique, Tanzania, Zambia, Eswatini, Malawi and Zimbabwe. Empirical evidence also suggests that North African countries such as Morocco, Algeria, and Egypt have gained a comparative advantage in sugar production due to a surge in FDI inflows.

There is weak evidence to establish whether FDI has enhanced the productivity and yields of African countries. However, studies do document how FDI has improved the efficiency of sugar milling companies. The studies highlight that sugar milling companies run by multinational corporations perform better and are more efficient compared to state-owned mills in these countries, underscoring the importance of institutional arrangements in sugarcane and sugar production. The role of governments in the sugar industry should be limited to creating a favorable business environment and ensuring effective governance under the existing production arrangements to foster a thriving sugar industry.

While this review provides valuable insights into the relationship between FDI and sugar production, efficiency, and competitiveness, it is important to acknowledge the limitations of the study, including the focus on specific regions of Africa due to lack of empirical evidence and the inclusion of grey literature, which may introduce potential bias. This review, however, underscores the potential of FDI to drive sustainable investment, inclusive growth, and enhanced competitiveness across the sugar production value chain in Africa, offering valuable insights for policymakers and industry stakeholders. These insights highlight the potential for FDI to positively impact key facets of the sugar industry in selected African countries, emphasizing the need for further in-depth analyses to unravel the causation and complexities of these associations for each nation's sugar sector.

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From production to regulation: the comprehensive role of hyaluronic acid in the food and cosmetic industry

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Abstract

Introduction. Hyaluronic acid (HA) is now approved as a food additive in many countries of Europe, Asia and America and is used as an important element in the food industry. Also, HA is one of the most popular cosmetic ingredients. The aim of this research was to review the comprehensive role of hyaluronic acid in the food industry, from production methods to regulatory aspects.

Materials and methods. The study utilized international and domestic scientific publications from leading periodicals and specialized global journals, focusing on hyaluronic acid applications in the food and cosmetic industry and suitable producers. Scientific articles were sourced using global scientometric databases such as Google Scholar and PubMed.

Results and discussion. The review revealed that hyaluronic acid has broad applications in the food industry, including as a modifier for dairy and starch-based products, a natural flavor enhancer, and a salt reducer. In the cosmetic industry, HA is used as an anti-aging component that promotes skin hydration. Various microbial strains for HA production were compared, with *Corynebacterium glutamicum* showing the highest yield (32 g/l over 60 hours). Moreover, international regulations are essential in ensuring the quality and safety of HA-containing products. In the European Union, Regulation (EC) No 1223/2009 sets the standards for the use of hyaluronic acid in cosmetic products, outlining guidelines for product safety, labeling, and permissible concentrations. Globally, HA used in cosmetics and food products must comply with various international standards such as the Codex Alimentarius, which governs food additives, and the ISO 22716:2007 for Good Manufacturing Practices (GMP) in cosmetics production, ensuring consistency in product quality and consumer safety

Conclusions. Hyaluronic acid presents significant potential for use in the food and cosmetic industry, with promising production methods using GRAS-status (Generally Recognized As Safe) microorganisms. Adherence to regulatory requirements is crucial for manufacturers and importers of HA-containing products. Further development of the regulatory framework is expected as technologies and research in HA application continue to advance.

Introduction

Hyaluronic acid is a natural substance that plays a crucial role in maintaining skin health and youthfulness. While it is best known for its use in cosmetics, hyaluronic acid has recently been gaining wider application in the food industry.

The commercial use of hyaluronic acid in baked goods as a substitute for egg whites was discovered in 1942, long before its initial use in medical and cosmetic products in 1960 and 1979, respectively. In 2011, the Japan Association for Healthy Nutrition and Food, and in 2014, the Korean Ministry of Food and Drug Safety officially approved HA for use as a food additive. Subsequently, China and the European Union also made decisions allowing its use as a food ingredient. Currently, HA is actively promoted as a food additive in countries like the USA, Canada, Italy, and Belgium (Joshi et al., 2024).

Since 2021, China has permitted the use of HA as a food additive in common foods such as yogurt, fruit juice, green tea, carbonated beverages, as well as in snacks, including soft candies and jelly. These products are marketed as having health benefits, including weight control, relaxation, liver protection, stomach support, skin whitening, and antioxidant effects. Studies have confirmed that oral intake of HA can improve skin hydration, elasticity, reduce roughness, and wrinkle depth, and help in treating osteoarthritis. Pharmacokinetic studies have shown that after oral administration, HA is degraded in the gastrointestinal tract, and its bioavailability is significantly influenced by the gut microbiota (Hu et al., 2023).

This unique molecule has the ability to retain a large amount of water, making it a valuable ingredient for improving the texture and consistency of various food products. Moreover, beyond its primary function, hyaluronic acid is used as an emulsifier, thickener, and stabilizer. It helps extend the shelf life of products, enhances their taste, and gives them a more appealing appearance. Additionally, hyaluronic acid can be used to create functional foods aimed at supporting joint and skin health. It is added to beverages, yogurts, confectionery, and other products to increase their nutritional value (Cheng et al., 2023).

Furthermore, research is ongoing into the use of hyaluronic acid in food products, including dietary supplements, food safety testing, food packaging, food delivery systems, and food quality enhancers. Overall, hyaluronic acid has great potential in the food industry for improving the functional properties of food products.

In addition, hyaluronic acid is a unique glycosaminoglycan (GAG) and stands apart from other GAGs because it is non-sulfated, has a remarkably large molecular size, and is synthesized in the cytoplasm of cells rather than within the Golgi apparatus (Figure 1). Its structure and properties make it essential in a variety of biological processes, especially in maintaining the skin's hydration. Hyaluronic acid's ability to retain large amounts of water is one of its most notable features, which contributes significantly to the moisture content and elasticity of the skin. This exceptional water-binding ability makes HA a key molecule not only in cosmetic applications but also in medical treatments aimed at improving skin quality and healing (Mendoza-Muñoz et al., 2023).

In terms of its role in the body, hyaluronic acid plays several vital functions. It is involved in cell adhesion, where it assists cells in attaching to the extracellular matrix (ECM), and in fixing cells to specific locations within the skin. These processes are crucial for maintaining skin structure and integrity, as well as for ensuring that cells function correctly within their given environment. HA also plays a significant role in cellular communication, growth, and migration. It acts as a signaling molecule in a variety of cellular activities, including cell motility, inflammation, and tissue repair. Furthermore, it participates in more complex processes like cancer metastasis, where cell movement is critical, and in the regulation of cell metabolism, where it influences cellular energy use and repair mechanisms (Mendoza-Muñoz et al., 2023).

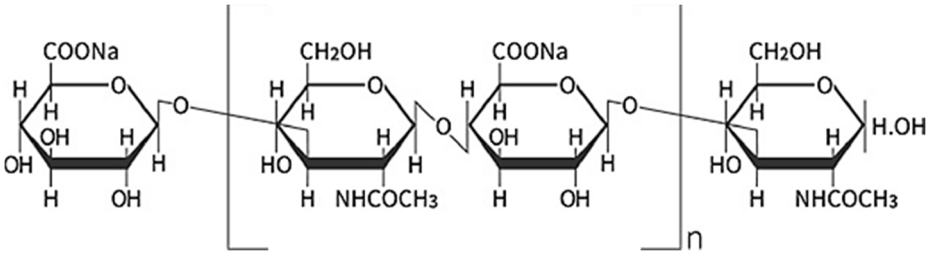

Figure 1. Structural formula of hyaluronic acid

Within the skin, hyaluronic acid is found in different concentrations depending on the layer. It is present in much higher amounts in the dermis, where its concentration can reach 0.5 mg per gram of tissue, compared to 0.1 mg per gram in the epidermis. This uneven distribution is significant because the dermis is the thicker layer of the skin, providing structural support, while the epidermis serves as the protective barrier. Because of this, HA's moisturizing properties are especially important in the dermis. It serves as a popular ingredient in dermal fillers used in aesthetic medicine to smooth wrinkles and restore volume to the face. These fillers, such as Hylaform®, Restylane®, and Dermalive®, typically contain HA in concentrations ranging from 0.025% to 0.050%, ensuring that they provide long-lasting moisture retention and volume to the treated areas. Topically applied HA can also improve the function of the outer layer of the skin by restoring its barrier function, which is crucial for protecting the skin from external stressors and preventing moisture loss (Kanlayavattanakul & Lourith, 2015).

Another important factor that determines the biological effects of hyaluronic acid is its molecular weight. HA molecules can vary greatly in size, with molecular weights ranging from 2 × 10^5 to 10^7 Daltons. This broad range of sizes allows HA to perform a diverse array of functions within the body, depending on whether it is in its high-molecular-weight form or low-molecular-weight form.

High-molecular-weight hyaluronic acid (HMWHA) primarily works through passive mechanisms. One of its most well known properties is its ability to retain water, making it highly effective in promoting tissue hydration. This property is essential not only in maintaining skin moisture but also in protecting joint cartilage. In joints, HMWHA acts as a lubricant, ensuring smooth movement and protecting cartilage from wear and tear. It also helps maintain osmotic balance in tissues and contributes to the stabilization of the extracellular matrix, which provides structural support to cells. While HMWHA cannot easily penetrate cells due to its large size, it can still influence cellular activity by interacting with receptors on the cell surface. Through these interactions, HMWHA can activate signaling pathways that regulate processes like cell proliferation (the growth and division of cells) and angiogenesis (the formation of new blood vessels). Angiogenesis is a critical process for both wound healing and the growth of tumors, as new blood vessels are needed to supply tissues with nutrients and oxygen. HMWHA, however, acts as an inhibitor of angiogenesis, which helps prevent the uncontrolled growth of blood vessels associated with cancer progression. This inhibitory effect can be beneficial in preventing tumor growth but may also have a downside, as excessive inhibition of angiogenesis can slow down wound healing and tissue regeneration. Thus, while HMWHA offers protective benefits, its overuse

could potentially interfere with the body's ability to repair itself after injury (Juncan et al., 2021).

Low-molecular-weight hyaluronic acid (LMWHA), on the other hand, behaves quite differently. Unlike HMWHA, which primarily inhibits angiogenesis, LMWHA actually promotes it. This angiogenic activity is useful in situations where tissue repair is needed, such as in wound healing or after an injury. However, it can also contribute to tumor progression, as new blood vessels can supply a growing tumor with the resources it needs to expand. Despite this potential risk, LMWHA also has anti-inflammatory properties, which can be beneficial in reducing inflammation and promoting tissue healing. This dual role makes LMWHA a molecule of interest in both therapeutic and cosmetic applications. By carefully controlling its concentration and molecular weight, LMWHA can be used to manage both tissue repair and inflammation without encouraging undesirable side effects such as tumor growth (Juncan et al., 2021).

Overall, hyaluronic acid is a highly versatile molecule with a wide range of applications in both medical and cosmetic fields. Its ability to influence hydration, tissue repair, inflammation, and even cancer progression underscores its importance in modern skincare and therapeutic treatments. As research into HA continues, new applications and formulations are likely to emerge, making it an even more indispensable component in health and beauty products.

Since hyaluronic acid has such a wide range of applications in the cosmetic, medical, pharmaceutical, and food industries, there is a pressing need to examine the regulatory documentation that governs this glycosaminoglycan. Considerable attention is paid to documentation that is relevant for Ukraine, while there are no hyaluronic acid production facilities, but national companies do use HA as a raw material in their manufacturing processes (Kumar, 2024).

Materials and methods

This review utilized scientific publications from leading periodicals and specialized global journals, focusing on the application of hyaluronic acid in the food and cosmetics industry with a consideration of suitable producers.

Results and discussion

Hyaluronic acid in nanotechnology and encapsulation

In a study by Guo et al. (2018), nanocapsules were developed to improve the stability and effectiveness of poorly soluble antioxidants in products such as juices, yogurts, and dietary supplements. These nanocapsules are created based on a special polymer that combines molecules of hyaluronic acid and curcumin. The inner part of the capsules is filled with curcumin and resveratrol, antioxidants beneficial for cardiovascular diseases.

Nanocapsules have the ability to form spherical particles of very small size - approximately 134.5 nanometers. Due to this, they can penetrate cells more effectively. Another important characteristic of these capsules is their stable electric charge (zeta potential), which is -29.4 mV at pH 7.4. This ensures the stability of nanocapsules in the gastric environment, where they are able to maintain their properties and gradually release active substances.

During testing in conditions simulating the stomach environment, these nanocapsules demonstrated high stability and the ability to gradually release curcumin and resveratrol. In addition, they showed higher activity in neutralizing free radicals compared to other known formulations and liposomes. Therefore, it would be good to use this development in health food products.

In another study (Wang et al., 2024), the authors also used HA in combination with gelatin to create a multilayer coating for probiotics to improve their delivery and effectiveness. Layer-by-layer (LBL) technology allowed obtaining encapsulation with an efficiency of 78%–92%, which was confirmed by FT-IR (Fourier transform infrared spectroscopy) and XRD (X-ray diffraction) analysis methods. Multilayer microcapsules demonstrated high biocompatibility, lack of immunogenicity and toxicity, significant improvement in probiotic survival in adverse conditions such as high acidity and temperature. In addition, multilayer microcapsules with HA showed improvement in antioxidant properties and viability of probiotic cells compared to non-encapsulated forms. These results confirm the effectiveness of LBL technology and HA not only in protecting probiotics, but also in potentially enhancing their beneficial properties.

Hyaluronic Acid Coatings for Food Preservation

As it turned out, HA-based coatings are effective not only for probiotics but also for many food products, as they allow increasing their shelf life. Thus, the authors (Al-Hilifi et al., 2024) managed to create an edible coating with HA, chitosan, and gelatin for strawberries. It was noted that coated fruits lost less weight and had a stable pH, indicating preservation of their juiciness, unlike uncoated hyaluronic acid analogues. In addition, the inclusion of hyaluronic acid significantly increased the antioxidant properties of the coating. This was confirmed by measurements of total phenol content, changes in ascorbic acid content and DPPH analysis (this test evaluates the antioxidant activity of substances by measuring their ability to neutralize free radicals through color change of the stable radical DPPH - 2,2-diphenyl-1-picrylhydrazyl).

A similar study was conducted by Zhou et al. (2024). A simple and effective method was developed for creating a multifunctional coating for fruit preservation by incorporating a complex of CIN and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) into hyaluronic acid, a natural polysaccharide with excellent film-forming properties. The finished HA/CIN@HP-β-CD coating demonstrated universal adhesion ability, excellent antimicrobial properties and good antioxidant activity without toxicity. Studies have shown that CIN from the coating is released gradually and constantly. Freshness tests on bananas and apples showed that this coating effectively preserves their color, reduces weight loss, prevents the development of microorganisms and significantly extends shelf life, making it promising for fruit preservation.

In addition, similar composite films of hyaluronic acid were created by adding curcumin and cellulose nanofibers (CNF) to the HA base, and their effectiveness was tested on egg preservation at 25 °C and 70% humidity. The addition of composite materials increased the thickness of the films. Curcumin at a concentration of 0.025% provided the best antimicrobial properties, while CNF significantly affected the viscosity, permeability and mechanical properties of the films. In a 56-day egg preservation test, a composite film with 0.5% HA, 0.025% curcumin and CNF, with a coating time of 2 minutes, showed the least weight loss - 13.88%. The final Haugh unit was 52.08, which exceeded control values by approximately the 35th day and extended the shelf life of eggs by at least 14 days (Fan et al., 2023).

Hyaluronic acid's role in modifying dairy and starch-based products

Due to its physicochemical properties, it is an excellent modifier and plasticizer for food products. Thus, Joshi et al., 2024 analyzed the effect of HA of different molecular weights on the properties of skimmed milk. Scientists studied the effects of HA in four sizes: 8, 320, 980 and 2550 kilodaltons (kDa), adding it to milk at a concentration of 0.25%. It was found that with increasing molecular weight of HA, the viscosity of milk and its pseudoplasticity increased, i.e. the ability to reduce viscosity when shaken. In milk samples, the formation of a weak gel-like structure was observed, caused by the interaction between polymeric particles of HA and the milk environment (Figure 2).

Hyaluronic acid with higher molecular weight (except for the smallest than 8 kDa) also improved milk's ability to retain water, its emulsifying properties and foaming ability. Interestingly, HA with the lowest molecular weight (8 kDa) did not significantly affect milk functionality, making it suitable for addition to dairy drinks to improve health without changing their properties.

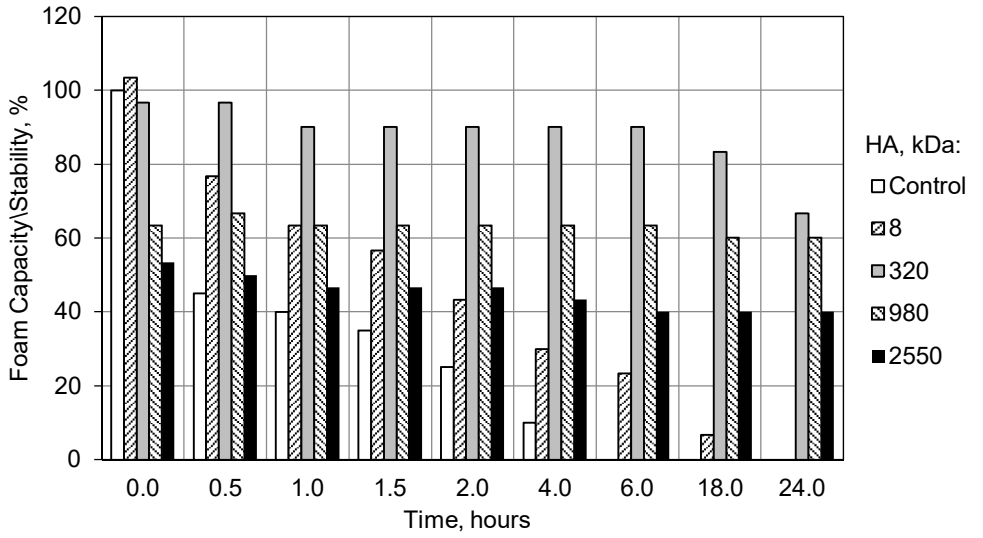

Figure 2. Foaming capacity and stability of milk samples treated with different molecular weights of hyaluronic acid at 0.25% (w/w) concentration

The study also showed that HA with a molecular weight of 980–2550 kDa did not cause significant differences in the effect on viscosity, protein separation and heat resistance of milk, which opens up possibilities for using HA in this range without additional fractionation, reducing production costs. The mechanism of HA action on milk is mostly related to the ability to bind water, while no changes were detected in the structure of milk protein fractions, and the hydrodynamic diameter of particles remained unchanged.

In another source (Wu et al., 2024), the property of HA to change the structure of cornstarch was considered. The results showed that the addition of HA significantly increased peak viscosity, solubility and water retention capacity in starch mixtures. At the same time, a decrease in pasting temperature, swelling power and amylose leaching was observed. Thus, hyaluronic acid can be used even as a conditional preservative for starch-containing products.

Hyaluronic acid as a natural flavor enhancer and salt reducer

In addition, it was noticed that HA contributes to changes in the taste properties of food (Huang et al., 2023). It discussed the effect of hyaluronic acid of different molecular weights on the perception of sweet taste in neutral and acidic solutions. HA with molecular weights of 100 kDa, 400 kDa and 1090 kDa increased the intensity and duration of sweet taste sensation at pH 4.0. This phenomenon is explained by the rupture of glycosidic bonds in HA in acidic environment, which changes its viscosity and interaction with mucin. In acidic solutions, the perception of sweet taste was slightly weaker, probably due to suppression by sour taste. Different molecular weights and concentrations of HA significantly improved the quality and thickness of the mucin layer, while the penetration of sucralose into the mucin layer decreased in acidic solutions. Hyaluronic acid with a molecular weight of 100 kDa showed the best results in enhancing sweet taste, probably due to its rigid rod-like structure, which allows more sucralose to penetrate the mucin layer. This study demonstrates the potential of using HA as a natural flavor enhancer to reduce sugar consumption without losing the desired level of sweetness.

Table 1

Applications of hyaluronic acid in food industry

Application	Description	Results/Effects	Source
Nanocapsules for antioxidants	Hyaluronic acid + curcumin for creating nanocapsules with antioxidants (curcumin, resveratrol)	Stability in gastric environment, gradual release of active substances, enhanced antioxidant activity	Guo et al., 2018
Multilayer coating for probiotics	Hyaluronic acid + gelatin for probiotic coating	Improved probiotic survival in high acidity and temperature, antioxidant properties	Wang et al., 2024
Edible coating for fruits	Hyaluronic acid + chitosan and gelatin for strawberry coating	Reduced weight loss, pH maintenance, and antioxidant properties	Al-Hilifi et al., 2024
Modification of dairy products	Impact of HA molecular weight on the properties of skimmed milk	Increased viscosity, foaming ability, and water retention with higher molecular weight	Joshi et al., 2024
Modification of starch	Hyaluronic acid as a modifier for corn starch	Increased peak viscosity, solubility, and water retention capacity	Wu et al., 2024
Enhancing flavor properties	Effect of different HA molecular weights on the intensity and duration of sweet taste	Enhanced sweet taste, reduced need for sugar	Huang et al., 2023
Salt reduction in products	Use of HA to reduce salt content in dishes (steaks, sauces)	10% salt reduction without loss of saltiness perception	Hu et al., 2023

Hu et al. (2023) noticed that adding hyaluronic acid to dishes such as steak, black pepper sauce, sour fish and packaged sour fish sauces allows reducing salt content by 10% while maintaining the sensation of saltiness. This discovery has significant potential for the food industry and health care, as excessive salt consumption is associated with an increased risk of cardiovascular diseases and hypertension. Using HA as a natural flavor enhancer can be an effective way to create low-sodium products that remain attractive to consumers.

Characteristics of modern cosmetic products containing hyaluronic acid

The beauty industry, particularly in the realm of cosmetic skincare, is undergoing a revolutionary transformation. The modern beauty paradigm has permeated deeply into human health and aesthetic consciousness, with contemporary pop culture acting as a powerful catalyst for this phenomenon. This symbiosis of demand and supply has catapulted the beauty sector into one of the most dynamic and lucrative industries globally (Lee et al., 2022). The digital age, characterized by the omnipresence of social media and the meteoric rise of e-commerce, has fundamentally altered consumer behavior, leading to increased expenditure on beauty and skincare products.

The landscape of skincare has undergone a seismic shift, transcending mere superficial enhancements to embrace a holistic approach to skin health. This paradigm shift is driven by an unprecedented level of consumer awareness and a collective aspiration to defy the aging process. Consequently, there is a surging demand for multifunctional products that not only enhance aesthetic appeal but also contribute significantly to skin health and longevity. In this context, hyaluronic acid has emerged as a revolutionary ingredient, heralded for its remarkable ability to retain moisture, boost skin elasticity, and exhibit potent antioxidant properties (Rathod et al., 2020).

One of the most popular cosmetic forms for GAGs is hydrogels. Hydrogels are gaining popularity as an innovative medium for the cosmetic application of GAGs, such as hyaluronic acid and alginate. These biocompatible polymers can form three-dimensional cross-linked networks that absorb and retain a large amount of water, making them ideal for moisturizing and regenerating skin care products. The hydrogel form provides prolonged action and optimal release of active ingredients like GAGs onto the skin over a long period. Additionally, it creates a pleasant cooling and refreshing sensation upon application, which appeals to consumers looking for effective and comfortable cosmetic products (Mitura et al., 2020).

Despite these promising outcomes, penetrating GAGs into the deeper layers of the skin remains a challenge for topical exogenous preparations. Consequently, new forms and delivery technologies are being actively developed to enhance the bioavailability of topical GAGs, including nano/microemulsions, liposomes, microneedles, and conjugation strategies. While these technologies are still in the experimental stage of development, there are already many scientific publications on this topic.

For instance, the authors point out that for the penetration of hyaluronic acid (the most popular GAG in cosmetics) into the deeper skin layers, auxiliary delivery methods are necessary (Wang et al., 2021). Most commonly used methods include microneedles with dermarollers, sliding or stamping microneedles, radiofrequency-based thermal needles, and fractional lasers.

A new method for producing microneedles from hydrolyzed hyaluronic acid could open new opportunities in the cosmetic and pharmaceutical industries. Research has shown that using a gel substance based on hyaluronic acid in combination with a vacuum imprinting method allows for the production of high-resolution microneedles with impressive precision,

a diameter of 13 micrometers, and a height of 24 micrometers, making them ideal for use in cosmetic and pharmaceutical products (Miura et al., 2022).

Microneedles have several advantages over conventional subcutaneous injections. Firstly, they are less painful; secondly, they do not require medical training, and many patients have a phobia of regular needles, making this method of HA delivery an excellent alternative.

Another alternative is nanoemulsions. These are transparent or semi-transparent substances with the following properties: low viscosity, effective penetration of active ingredients, increased interfacial surface area, high solubilization capacity, high kinetic stability, and the ability to carry both hydrophilic and hydrophobic substances. These characteristics make this type of nanocarrier an excellent candidate for delivering cosmetic ingredients to the skin. A study demonstrated that lipophilic hyaluronic acid could be encapsulated in nanoemulsions and effectively used as a transdermal delivery system for cosmetic applications. Nanoemulsions can also provide controlled release of hyaluronic acid over a long period, increasing the effectiveness and duration of its action (Oliveira et al. 2022).

Jegasothy et al. (2014) used nanotechnology to improve HA penetration. It was hypothesized that better HA penetration could be achieved by reducing its molecular weight and forming a polymer in the form of nanoparticles (nano-HA, 5 nm). Treatment based on several formulations (lotion, serum, and cream) containing nano-HA was tested on thirty-three women, and after 57 days, improvements in skin hydration and elasticity, along with reduced roughness, were noted. Although the effectiveness of products based on nano-HA was demonstrated, this study did not compare its results with treatment using regular-sized HA.

However, this was addressed in the next article (Tokudome et al., 2018). This study aimed to evaluate the passive delivery of HA nanoparticles, formed by polyionic complex formation, into the skin. HA nanoparticles were prepared by mixing and stirring anionic HA with the cationic polymer protamine at a charge ratio of 55:45. The penetration of fluorescent-labeled HA nanoparticles (HANPs) or free HA through the skin of hairless mice was characterized in vitro. HANPs or free HA were applied to the skin of UV-irradiated mice in vivo, and trans-epidermal water loss was measured after 4 days. The HA that penetrated the skin was separated and characterized using gel-permeation chromatography. The results showed that HANPs could deliver HA to the dermis both in vitro and in vivo, whereas free HA did not penetrate beyond the stratum corneum. After applying HANPs, HA in the skin was present as free HA, not nanoparticles. In vivo application of HANPs significantly reduced UV-induced trans-epidermal water loss.

Today, tissue filling procedures using collagen and HA fillers are becoming increasingly popular as they are affordable and produce noticeable long-lasting effects. They account for 9 out of 10 cosmetic procedures and 75% of the market value of cosmetic interventions, with the market size estimated at £2.27 billion.

There are many injectable HA-based products on the market for wrinkle correction, especially for nasolabial folds. Since the effect of these fillers is still temporary (usually less than 12 months for tissue fillers), many formulations have been proposed to increase the retention time, both for topical cosmetic creams and tissue fillers. For example, the preparation of partially depolymerized GAGs, gold or silver salts (Salgado et al., 2017).

This invention represents a revolutionary step in developing pharmaceutical and cosmetic products based on glycosaminoglycans. Partially depolymerized gold and silver salts of glycosaminoglycans obtained through a patented process have unique advantages over native high-molecular-weight forms of these compounds.

Firstly, the partial depolymerization process allows reducing the molecular weight of glycosaminoglycans, significantly increasing their ability to penetrate the skin barrier. This makes these compounds much more effective for topical use in pharmaceutical and cosmetic compositions for skin treatment and care.

Secondly, the presence of noble metal ions, such as gold and silver, adds additional therapeutic properties. These metals are known for their antimicrobial, anti-inflammatory, and regenerative effects on the skin, making these compounds ideal for treating wounds, burns, scars, and skin infections. Finally, these innovative compounds are promising for use in skin care cosmetics. Due to their moisturizing, regenerative, and anti-inflammatory properties, they can improve skin condition and appearance by reducing wrinkles and signs of aging.

Nevertheless, the risks associated with the use of dermal fillers include infections, lump formation, filler migration, the need for surgical intervention, scarring, blood vessel blockage, and even blindness (Pervez, 2021).

Thus, based on the above information, it can be concluded that one of the most optimal cosmetic forms for home use remains creams with hyaluronic acid. Unlike injectable fillers, cream is easy and safe to apply at home, without the risks of infection, lumps, surgical intervention, and so on. Moreover, high-molecular-weight hyaluronic acid is the most similar to the HA produced in the human body, and although it does not penetrate beyond the stratum corneum, it works quite effectively on the outer layers of the skin, acting as a barrier. The disadvantages of nanoemulsions, liposomes, and microneedles are that these technologies are still insufficiently studied, and using them on a large scale is risky, as we do not yet fully understand the possible negative effects. Compared to hydrogels, after application to the skin, creams feel more pleasant, particularly because they lack stickiness and viscosity.

Additional active components can be added to the HA cream formula, which in combination will provide better results. In the article (Juncan et al., 2021), it is noted that HA cream helps moisturize the skin and increase elasticity, thereby reducing wrinkle depth. It is assumed that when applied to the skin surface, HA solutions form an occlusive layer, absorbing moisture and thereby moisturizing the skin, filling in wrinkles. Additionally, the occlusive properties provided by HA may allow biologically active substances contained in the cosmetics to be retained in the skin layers, possibly facilitating their penetration into the epidermis.

Manufacturers of hyaluronic acid

As already mentioned, HA has quite a wide potential for use in the food and cosmetic industry, but its production must also meet standards so that this glycosaminoglycan can be safe for consumption. Since extraction from animal tissues is a somewhat inhumane method and more vulnerable to external contamination than microbial synthesis, it is obviously less common on an industrial scale now.

Since microbial synthesis has many more advantages, today many scientific sources are considering ways to improve the efficiency and reduce the cost of this process. Thus, in the article (Ferreira et al., 2021) they talk about improving the production process of this polysaccharide by *Streptococcus zooepidemicus*, because in the basic scenario HA is produced by batch fermentation, reaching 2.5 g/l after 24 hours. It is then centrifuged, diafiltered treated with activated carbon and precipitated with isopropanol. The product is suitable for local application, and its production cost is estimated at $1115/kg. Therefore, a similar production scenario was developed, based on fed-batch cultivation, which led to a higher product titer - 5.0 g/l and a lower cost of $946/kg.

In another study (Kumar et al., 2021), they optimized the production of hyaluronic acid by the *Streptococcus equisimilus* MK156140 strain. Using statistical optimization methods (Plackett-Burman design and central composite design), they determined the optimal cultivation conditions: pH 7.38, 12.15% meat extract, 7.64% yeast extract, 3% sucrose, temperature 35-38 °C, stirring speed 180 rpm, incubation time 28 hours. Under these conditions, a maximum HA yield of 7.16 g/l was achieved, which is close to the predicted value of 7.21 g/l. This is approximately 4 times higher than previous studies. The authors believe that the optimized process can be promising for industrial production of HA.

In another article (Jafari et al., 2022), the production of hyaluronic acid by a group G *Streptococcus* strain was optimized. Chemical mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was used in two rounds with concentrations of 120 and 200 μg/ml. Optimal cultivation conditions included pH 5.5 and a cultivation time of 4 hours. The original strain produced 1241±2.1 μg/ml of HA, while the best mutants Gm2-120-21-3 and Gm2-120-21-4 achieved 2470±8.1 μg/ml and 2856±4.2 μg/ml respectively, which is 16.1-45.5% higher. The molecular weight of the obtained HA was below 160 kDa. The mutants maintained stable production for 10 generations. This approach allowed obtaining strains with high yield of low molecular weight HA in a short cultivation time.

In the above-mentioned articles, representatives of the *Streptococcus* family are used as the target microorganism, as they are one of the most well known natural producers with a good yield of the target product. Despite this, this family of bacteria is quite pathogenic and produces exotoxins, and the HA obtained in this way must undergo additional processing stages, which increases the cost of production. Therefore, new producers are actively being sought, or they are being created using genetic engineering.

One such microorganism is *Lactococcus lactis*. The article (Sheng et al., 2015) describes the creation of an HA producer that has GRAS (Generally Recognized As Safe) status. The process of creating the HA producer involved cloning HA synthesis genes (hasA, hasB, hasC) from *Streptococcus zooepidemicus* into *L. lactis.* Several variants of recombinant strains were created with different combinations of genes, of which the best was strain NFHA03, which contained the hasA gene and enhanced expression of the endogenous ugd and glmU genes of *L. lactis*. Cultivation conditions included the use of LM17 medium with 1% lactose, cultivation at 30 °C for 12 hours, and induction of expression by adding nisin (10 ng/ml). The highest HA yield was 0.594 g/l for strain NFHA01, while strain NFHA03 produced 0.492 g/l of HA. The main advantages of the created system lie in the use of the food-grade *L. lactis* strain, the food-grade NICE expression system, and the food-grade selective marker lacF, making the process fully suitable for the production of food-grade hyaluronic acid.

In another study (Muthukrishnan et al., 2020), *Lactococcus lactis* was also used. The researchers used the non-pathogenic strain *L. lactis NZ9000*, widely used in the food industry, as a basis for genetic manipulations. A plasmid vector pNZ8148 containing the hasA and hasB genes from *Streptococcus zooepidemicus,* which encode key enzymes for HA synthesis, was introduced into this strain. Cultivation of the modified *L. lactis* was carried out in a specially adapted GM17 medium, enriched with glucose and chloramphenicol to maintain the plasmid. Optimal growth conditions included a temperature of 30˚C and static conditions. Induction of HA synthesis genes was carried out using nisin at a concentration of 2 ng/ml, which was determined to be optimal for maximum expression. Bioreactors were used to scale up the process, where cells were grown at 30˚C, with stirring at 200 rpm and pH maintained at seven. To ensure cell growth and HA synthesis, sterile glucose and chloramphenicol were periodically added to the medium.

In addition, this species was mentioned in the source (Jeeva et al., 2022). It studied the effect of respiratory metabolism on the production of hyaluronic acid by the modified strain *Lactococcus lactis* GJP5Δldh. Experiments were conducted at different levels of dissolved oxygen and with the addition of hemin. It was found that increasing oxygen levels and the presence of hemin positively affect HA production, reaching a maximum titer of 4.6 g/l. The researchers established a correlation between the concentrations of intracellular HA precursors and its final yield.

Recently, a study (Shaheen et al., 2023) for the first time revealed the ability of *Enterococcus durans* K11 and *Lactiplantibacillus plantarum* St3 strains to produce hyaluronic acid. These strains, isolated from natural sources - kefir and breast milk respectively, demonstrated high productivity: *E. durans* K11 synthesized HA at a concentration of 4.8 mg/ml, and *L. plantarum* St3 - 4.4 mg/ml. Strain identification was performed using 16S rRNA gene sequencing. HA obtained from both strains showed high antioxidant activity (about 66%) and significant protective effect against UV irradiation on human keratinocyte culture. It is important to note that these strains are natural isolates and have not undergone genetic modification to obtain the ability to synthesize HA. This study expands the range of known microorganisms capable of producing HA and opens new perspectives for the use of probiotic bacteria in the production of HA for the cosmetic and pharmaceutical industries.

In a study of Ahmed et al. (2023), *Klebsiella pneumoniae* H15 was identified as the best hyaluronic acid producer among 108 bacterial isolates analyzed. The strain was characterized morphologically, culturally, and biochemically, with its identity confirmed by 16S rDNA sequencing. Optimization of fermentation conditions showed the highest HA production at pH 8.0, 30 °C, 180 rpm, for 30 hours. The optimal medium contained 7% sucrose, 1.25% yeast extract, and 1.25% peptone, allowing a maximum HA concentration of 1.5 g/L. The bacterial HA demonstrated an inhibitory effect on MCF-7, HepG-2, and HCT cancer cells at concentrations of 0.98–3.91 μg/ml. On BHI agar, colonies were large, opaque, mucoid, and grayish-white, while on blood agar they were non-hemolytic, 3-4 mm in diameter. Alkaline medium favored higher HA production, with an optimal initial pH of 8.0, resulting in a final medium pH of 3.5-4.3. A temperature of 30 °C proved optimal, with satisfactory results at 25 °C and decreased production at 35-45 °C. The optimal fermentation time was 30 hours, after which a decrease in HA production was observed.

In addition, Du et al. (2021) optimized hyaluronic acid production in *Corynebacterium glutamicum* through metabolic engineering. Key strategies included improving glucose uptake by knocking out the iolR gene, intensifying cardiolipin synthesis by overexpressing pgsA1/pgsA2/cls genes, expressing Vitreoscilla hemoglobin (vgb) to enhance oxygenation, and supplementing the medium with glutamine. Cultivation conditions: medium with 40 g/L glucose, pH 7.2, temperature 28 °C, aeration 200 rpm, with periodic glucose addition and pH correction. These methods allowed achieving an HA yield of 32 g/L over 60 hours of fermentation, significantly exceeding previous indicators for *C. glutamicum.*

Radchenkova et al. (2020) optimized hyaluronic acid production by the halophilic bacterium *Chromohalobacter canadensis* 28, isolated from salt lakes of Pomorie. The strain was cultivated in a medium with 1% lactose, 15% NaCl, 1% tryptone, 0.5% yeast extract at pH 7.5. Optimal cultivation conditions included a temperature of 55 °C, pH 7.4, aeration 1.0 vvm, stirring 900 rpm. A continuous cultivation method was applied with an optimal dilution rate of 0.035 h^{-1}, ensuring stable production of high-purity exopolymer. This approach allowed avoiding the drawbacks of batch fermentation and optimizing HA production by a halophilic microorganism.

Kluyveromyces lactis as a safer alternative. The researchers inserted the xlhasB gene and created four strains with different hasA genes (three human, one bacterial). Transcript analysis confirmed hasA gene presence in three strains. The strain with the bacterial pmHAS gene produced HA, verified by electron microscopy. In bioreactor cultivation, this strain achieved maximum HA levels of 1.89 g/L with a molecular weight of 2.097 MDa. This represents the first report of HA production in *K. lactis* and the highest HA titers reported in yeast (Gomes et al., 2019).

Generalized data on hyaluronic acid producers are shown in Table 2.

Table 2

Hyaluronic acid production methods and yields across different bacterial strains

Producer	Modification method	HA yield	Cultivation conditions	Glucose source	Reference
Streptococcus zooepidemicus ATCC 39920	Fed-batch cultivation	5 g/l	pH 7, 37 °C, 12 h	Glucose	Ferreira et al., 2021
Streptococcus equisimilus MK156140	Statistical optimization	7.16 g/l	pH 7.38, 35-38 °C, 180 rpm, 28 h	Sucrose	Kumar et al., 2021
Streptococcus group G	Chemical mutagenesis (NTG)	2.856 g/l	pH 5.5, 4 h	Not specified	Jafari et al., 2022
Lactococcus lactis NFHA01	Cloning of hasA, hasB, hasC genes	0.594 g/l	30 °C, 12 h	Lactose	Sheng et al., 2015
Lactococcus lactis NZ9000	Introduction of pNZ8148 plasmid	2 g/l	30 °C, 200 rpm, pH 7	Glucose	Muthukrishnan et al., 2020
Lactococcus lactis GJP5Δldh	Introduction of pGJP5 plasmid	4.6 g/l	Various O_2 levels, hemin addition	Glucose	Jeeva et al., 2022
Enterococcus durans K11	Natural isolate	4.8 g/l	37 °C, 24 h	Skimmed milk	Shaheen et al., 2023
Lactiplantibacillus plantarum St3	Natural isolate	4.4 g/l	37 °C, 24 h	Skimmed milk	Shaheen et al., 2023
Klebsiella pneumoniae H15	Not specified	1.5 g/l	pH 8.0, 30 °C, 180 rpm, 30 h	Sucrose	Ahmed et al. 2023
Corynebacterium glutamicum	iolR gene knockout, overexpression of pgsA1/pgsA2/cls genes	32 g/l	pH 7.2, 28 °C, 200 rpm, 60 h	Glucose	Du et al., 2021
Kluyveromyces lactis.	pm *hasA*	1.89 g/l	24 hours, 30 °C and 200 rpm. 2 vvm, pH 6.0 maintained with 2 M NaOH.	Glucose	Gomes et al., 2019
Chromohalobacter canadensis 28	Not specified	2.1 g/l	55 °C, pH 7.4, 1.0 vvm, 900 rpm	Lactose	Du et al., 2021

Regulatory framework and standards

The use of HA in Ukraine is regulated by several key documents. In the cosmetics industry, the main one is the Technical Regulation on Cosmetic Products, approved by the Resolution of the Cabinet of Ministers of Ukraine on January 20, 2021, No. 65. This document establishes requirements for the composition, safety, and labeling of cosmetic products, including those containing HA.

In the pharmaceutical sector, DSTU 4765:2007 plays an important role, defining research methods, composition requirements, and quality standards for HA polymers used in pharmaceutical products. Additionally, the National List of Essential Medicines, approved by the Order of the Ministry of Health of Ukraine, includes information about medicinal products containing HA and their uses.

For medical devices containing HA, registration requirements and the Technical Regulation on Medical Devices (Resolution of the Cabinet of Ministers of Ukraine No. 753 of October 2, 2013) apply. These documents outline requirements for the safety, effectiveness, and labeling of medical devices, including those containing HA (Hryshchenko et al., 2024).

Chemical properties and quality standards

According to international standards, HA is described as a water-soluble substance available in the form of highly purified, lyophilized powder or aqueous solution. The molecular weight of HA can range from 5 to 1800 kDa, depending on the production procedures. Sodium hyaluronate, commonly used in cosmetic and medical preparations, has a molecular weight ranging from 80.2 to 4010 kDa, according to the Food Chemicals Codex (FCC) standards (Belsito et al., 2023)

Production and purification requirements

The production of HA can be carried out using various methods, including bacterial fermentation and extraction from natural sources such as rooster combs. Purification processes include solvent extraction (ethanol, acetone, etc.), ultrafiltration, ion-exchange chromatography, and other methods to remove impurities such as proteins, peptides, and nucleic acids.

It is important to note that for dermal fillers used in aesthetic medicine, the FDA requires that HA be cross-linked. This must be clearly stated in product descriptions and summaries to avoid misleading information.

Application in the food industry

In the food industry of Ukraine, the use of HA is regulated by several key documents. The main one is the Law of Ukraine "On the Basic Principles and Requirements for Food Safety and Quality," which sets out general safety requirements for food products, including additives and ingredients.

DSTU 4161-2003 "Food Additives. General Requirements" defines general requirements for food additives, their quality, labeling, and use in food products. This standard is important for manufacturers planning to use HA as a food additive.

The Order "On Approval of Requirements for Food Flavors, Food Additives, and Food Enzymes" (Resolution of the Cabinet of Ministers of Ukraine No. 133 of January 26, 2024)

defines the list of permitted food additives, including their acceptable levels and conditions of use in various food products. This document is key to determining the legality and conditions for the use of HA in the food industry in Ukraine.

Ukraine also adheres to international standards, including the Codex Alimentarius, which regulates the safety of food products and food additives at the international level. This means that the use of HA in the food industry in Ukraine must comply not only with national but also with international safety standards.

The List of Permitted Food Additives and Processing Aids, approved by the Order of the Ministry of Health of Ukraine, is another important document. It describes specific food additives, including HA, that are permitted for use in food products in Ukraine, as well as their allowable concentrations.

Safety considerations

The safety of HA use is a key aspect of its regulation. According to available data, various safety tests are conducted, including in vitro skin and eye irritation tests, human repeat insult patch tests (HRIPT), and comprehensive toxicity data.

It is important to note that clinical and safety studies on HA must clearly indicate and accurately describe the type of HA used (cross-linked or non-cross-linked) and the context of its use (e.g., cosmetic or medical applications). This is particularly important for ensuring transparency and accuracy of information for consumers and regulatory authorities.

The regulation of hyaluronic acid use in Ukraine is a complex process that covers various areas of application, from cosmetics to the food industry. The regulatory framework ensures quality control, safety, and effectiveness of HA in different products.

One of the key aspects of introducing hyaluronic acid into industrial production for cosmetic products is the compliance with regulatory requirements, including both international and national standards. In Ukraine, the production and circulation of hyaluronic acid for the cosmetic industry will be governed by the Technical Regulation on Cosmetic Products, which is harmonized with European legislation. This document will come into force on August 3, 2024. It is important to note that most national standards, which previously regulated the quality of cosmetic products, have already been repealed.

The primary regulator in the European Union that controls the use of hyaluronic acid in cosmetics is the European Parliament and Council Regulation (EC) No 1223/2009 of November 30, 2009, "On Cosmetic Products" (European Parliament & Council of the European Union, 2009). This regulation outlines a list of permitted ingredients, labeling requirements, packaging, storage, and procedures for evaluating the safety of cosmetic ingredients. Ukraine, as part of its legislative harmonization with European norms, is also implementing a Technical Regulation on cosmetic products, which will take effect on August 3, 2024. The document is based on EU Regulation No 1223/2009 and is aimed at ensuring the functioning of the domestic market while maintaining a high level of consumer health protection (Кабінет Міністрів України, 2021).

According to EU Regulation No 1223/2009, the inclusion of hyaluronic acid and its salts (sodium hyaluronate, potassium hyaluronate) is permitted in cosmetic formulations in concentrations of up to 100%. This gives manufacturers the flexibility to use HA in various cosmetic applications, such as moisturizers, serums, and anti-aging products, in concentrations optimized for specific skin types and purposes.

ISO Standards for GMP in Cosmetics

In addition to the EU regulatory framework, there are several standards within the ISO (International Organization for Standardization) series that regulate Good Manufacturing Practices (GMP) in cosmetic production. These include:

1. ISO 22716:2007 "Cosmetic Products. Good Manufacturing Practices (GMP). Guidelines" – This is the primary standard that defines the requirements for GMP in the production of cosmetics (International Organization for Standardization, 2007).
2. ISO 22717:2015 "Cosmetic Products. Microbiological Testing. Detection of *Pseudomonas aeruginosa*" – This standard outlines methods for detecting microbial contamination by *Pseudomonas aeruginosa*, a common bacterium that can contaminate cosmetic products (International Organization for Standardization, 2015a).
3. ISO 22718:2015 "Cosmetic Products. Microbiological Testing. Detection of *Staphylococcus aureus*" – This standard describes procedures for identifying *Staphylococcus aureus*, a bacterium responsible for skin infections, which can compromise product safety (International Organization for Standardization, 2015b).
4. ISO 29621:2017 "Cosmetic Products. Microbiological Testing. Guidelines for Risk Assessment and Identification of Products with Low Microbiological Risk" – This standard addresses methods for identifying pathogenic microorganisms in cosmetic products and provides guidance on risk assessment (International Organization for Standardization, 2017).

In Ukraine, two ISO standards have been harmonized for GMP and microbiological control in cosmetic production. These include DSTU EN ISO 22716:2015 "Cosmetics. Good Manufacturing Practice (GMP). Guidelines on Good Manufacturing Practice (EN ISO 22716:2007, IDT)", and DSTU EN ISO 29621:2016 "Cosmetics. Microbiology. Guidelines for risk assessment and identification of low microbiological risk products," which is harmonized with ISO 29621:2010 (although this version of the standard is no longer in effect in the EU).

Quality Standards for hyaluronic acid in cosmetics

There are also specific standards and pharmacopeial articles that establish quality requirements for hyaluronic acid. The European Pharmacopoeia includes a monograph on "Hyaluronic Acid," which outlines quality criteria for HA in both pharmaceutical and cosmetic applications. These criteria include appearance, solubility, identification, water content, heavy metals, and microbial limits (European Directorate for the Quality of Medicines & HealthCare, n.d.). In the United States, the USP-NF standard <1276> "Hyaluronan" provides similar requirements for hyaluronic acid quality, applicable to both pharmaceutical and cosmetic use (United States Pharmacopeial Convention, n.d.).

In Ukraine, the State Pharmacopoeia does not contain a separate monograph on hyaluronic acid. However, there is a general article on "Cosmetic Products" that includes requirements for microbiological and toxicological parameters (State enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products", 2015). Specific requirements in Ukraine for hyaluronic acid as an ingredient in cosmetics are defined in the upcoming Technical Regulation on cosmetic products, which will govern product safety, labeling, and consumer protection.

Regulation of cosmetic products in the United States

In the United States, the primary regulatory body overseeing the safety and marketing of cosmetic products, including those containing hyaluronic acid, is the U.S. Food and Drug Administration (FDA). Under the Federal Food, Drug, and Cosmetic Act, manufacturers are responsible for ensuring that their products are safe and that their labeling is truthful and not misleading. The FDA does not require premarket approval for cosmetic products, except for color additives, but it monitors adverse events and issues guidance on best practices for manufacturing and labeling.

The FDA has established specific quality standards for individual ingredients, including hyaluronic acid, when used in pharmaceutical and cosmetic formulations. In the case of hyaluronic acid, its production is expected to comply with GMP, which in the U.S. is regulated by standards such as ISO 22716, ensuring that products meet safety and quality requirements before reaching consumers.

Ukrainian regulatory authorities and harmonization with EU standards

In Ukraine, the regulation of cosmetic products is overseen by the State Service of Ukraine on Food Safety and Consumer Protection. Unlike in the United States, where the regulatory environment is somewhat less formalized, the regulation of hyaluronic acid in Ukraine is more structured, as it is based on the requirements of the Technical Regulation on cosmetic products. This regulation, harmonized with European standards, ensures that Ukrainian cosmetic products meet the safety and quality benchmarks of the EU.

Dermal fillers based on hyaluronic acid are becoming more and more popular in the world for cosmetic procedures. However, regulatory requirements for these medical devices still vary between jurisdictions, raising questions about their safety and efficacy.

For many years, there has been a problem of insufficient regulation of the safety of filers in the UK and the European Union (EU). Non-medical fillers were not subject to the same strict requirements as medical products and had less stringent safety standards than even toys or cosmetics (Bowes, 2014). This posed risks to consumers due to potentially unsafe products, unskilled filler insertion, lack of proper training and certification of providers, and unclear requirements for facilities and management of complications (Rowland-Warmann, 2020).

Approval for the sale of fillers in the US requires extensive clinical studies, while in the EU limited data was sufficient. The presence of CE marking for fillers did not guarantee a proper assessment of product safety. This has raised concerns about the lack of clear safety standards compared to the US.

However, the situation is changing with the release of the new EU Medical Device Regulation (MDR) 2017/745, which will replace the outdated Directive (MDD). The MDR imposes stricter requirements on Class III medical devices, particularly fillers (Kelso, 2020). This involves stricter requirements for pre-market clinical studies, post-market surveillance, product identification and transparency.

While the FDA approval process for fillers as prescription products may still be more rigorous at the pre-market stage, the new MDR brings EU regulation significantly closer to US standards. Both systems have their advantages and disadvantages, so the authors call for global harmonization of requirements (Kelso, 2020).

In summary, although previous EU filler regulation has been insufficient, the new MDR promises to raise safety and efficacy requirements to levels close to those of the US.

However, there is room for further improvement and harmonization of standards at the global level.

In Ukraine, regulation of dermal fillers is carried out in accordance with the legislation on medical products.

The main normative acts are Law of Ukraine "On technical regulations and conformity assessment"; Technical regulation on medical devices, approved by the resolution of the CMU No. 753 of October 2, 2013.

According to this Technical Regulation, dermal fillers are classified as class III medical products - products with a high degree of risk.

Basic requirements:

- Mandatory certification of the medical product in the authorized conformity assessment body before putting it into circulation on the territory of Ukraine.
- Clinical approval based on evaluation of clinical data obtained from relevant clinical studies.
- The manufacturer's quality management system must meet the requirements of technical regulations.
- Proper labeling of the product in accordance with the requirements of the regulation (name, manufacturer, instructions, etc.).

The sale and use of dermal fillers in Ukraine is possible only by those manufacturers/distributors who have passed the above-mentioned conformity assessment procedure. However, unlike the EU and the USA, Ukraine does not yet have specialized requirements specifically for dermal fillers. They are governed by the general rules for medical devices.

Post-fermentation stages in glycosaminoglycan production: an essential but complex process

In the biotechnological production of glycosaminoglycans, biosynthesis represents just the initial phase of a much longer and more complex process. Once the cultivation of microorganisms or cell cultures has been completed, glycosaminoglycans such as hyaluronic acid must be isolated from the supernatant and purified to remove impurities, including bacterial cell debris and proteins. This post-fermentation stage is crucial for producing a final product that is both high in quality and non-immunogenic, meaning it will not provoke an immune response when used in medical or cosmetic applications. However, this step is not only labor-intensive but also costly, especially at a large industrial scale. The purification and isolation process can be technically challenging due to the delicate nature of glycosaminoglycan molecules and the need to maintain their structure and biological activity (Sharma et al., 2022).

One of the most widely used methods for the extraction of hyaluronic acid from the supernatant involves precipitation techniques that closely resemble those used for protein precipitation. This approach typically requires the use of organic solvents. The principle behind this technique lies in the reduction of the dielectric constant of the aqueous phase by introducing an organic solvent. This modification in the system encourages macromolecular interactions, which, in turn, lead to an increase in the molecular weight of the hyaluronic acid molecules. As the molecular weight increases, the hyaluronic acid becomes less soluble in the solution, ultimately resulting in its precipitation.

According to recent research (Ucm et al., 2022) isopropanol is often the preferred organic solvent for hyaluronic acid precipitation. Isopropanol is particularly effective due to its properties that promote efficient precipitation while maintaining the structural integrity of

the hyaluronic acid. In laboratory settings, however, cold ethanol is sometimes used as a more accessible and cost-effective alternative. This precipitation method, while effective, requires a careful balance between the solvent concentration, temperature, and process duration to ensure that the hyaluronic acid does not degrade during the process.

Another study conducted by Abbas Mohammed and Niamah (2022) investigated similar precipitation methods using isopropanol. In this process, the authors employed 1% trichloroacetic acid (TCA) to remove protein contaminants, which is a common step in the refinement of biological molecules. Trichloroacetic acid denatures proteins, making them easier to separate from the target molecule—in this case, hyaluronic acid. Following protein removal, the hyaluronic acid was dialyzed against ultrapure water to further purification of the product. Dialysis is a method that utilizes a semi-permeable membrane to remove small impurities while retaining larger molecules like HA. The ultrapure water used in this stage is specifically treated to have extremely low mineral content, ensuring that no ions or particles interfere with the purification process. Finally, the dialyzed product was subjected to lyophilization, a freeze-drying technique that removes water from the sample by sublimation. This method allows the hyaluronic acid to be converted into a stable, dry form, which can then be processed into a commercial product.

An alternative approach to HA purification was described by Güngör et al. (2019). Their method began with the removal of cellular debris after fermentation using a 0.15% sodium dodecyl sulfate (SDS) solution, followed by centrifugation to separate the solid and liquid phases. SDS is a surfactant that helps to break down cell membranes and solubilize proteins, making it easier to remove unwanted cellular material from the mixture. After centrifugation, the supernatant containing hyaluronic acid was processed through a dialysis column equipped with a cellulose membrane. The column's dimensions were 25 mm × 16 mm, and the membrane had a molecular weight cut-off of 14,000, allowing only smaller molecules to pass through while retaining larger hyaluronic acid molecules. The column was immersed in a NaCl solution for five days to facilitate the gradual removal of impurities. Following the dialysis step, the dialysate was filtered through a series of increasingly fine filters, including cellulose acetate filters with pore sizes of 0.45 and 0.2 micrometers, and mixed cellulose ester filters with a pore size of 8 micrometers. This multi-step filtration process ensured the thorough removal of any remaining contaminants. The hyaluronic acid was then precipitated using 96% ethanol, resulting in an impressive yield of 12 g/L, which is considered a very high concentration for HA production.

A common theme across these studies is the reliance on organic solvents like isopropanol or ethanol to precipitate hyaluronic acid from the supernatant. While these solvents are highly effective, they are also expensive and may not be the most economical option for large-scale industrial production. Moreover, the use of organic solvents can pose environmental concerns and increase the complexity of waste management.

Recognizing these challenges, Gözke et al. (2017) explored the possibility of using electrodialysis as an alternative to organic solvent-based precipitation. Electrodialysis is a process in which ions are moved across a membrane using an electric field, allowing for the selective removal of charged impurities while retaining neutral molecules like hyaluronic acid. The study found that electrodialysis was able to preserve the molecular weight and structural integrity of the hyaluronic acid, which are critical for its biological function and commercial value. Additionally, the researchers reported an improvement in the overall yield of the product compared to traditional filtration methods. This technique offers a promising alternative for large-scale HA purification, potentially lowering costs and minimizing environmental impact.

Justification of methods for the extraction and purification of hyaluronic acid

Taking into account the processed literature, the most common methods of hyaluronic acid extraction at each purification stage were summarized and considered (Figure 3).

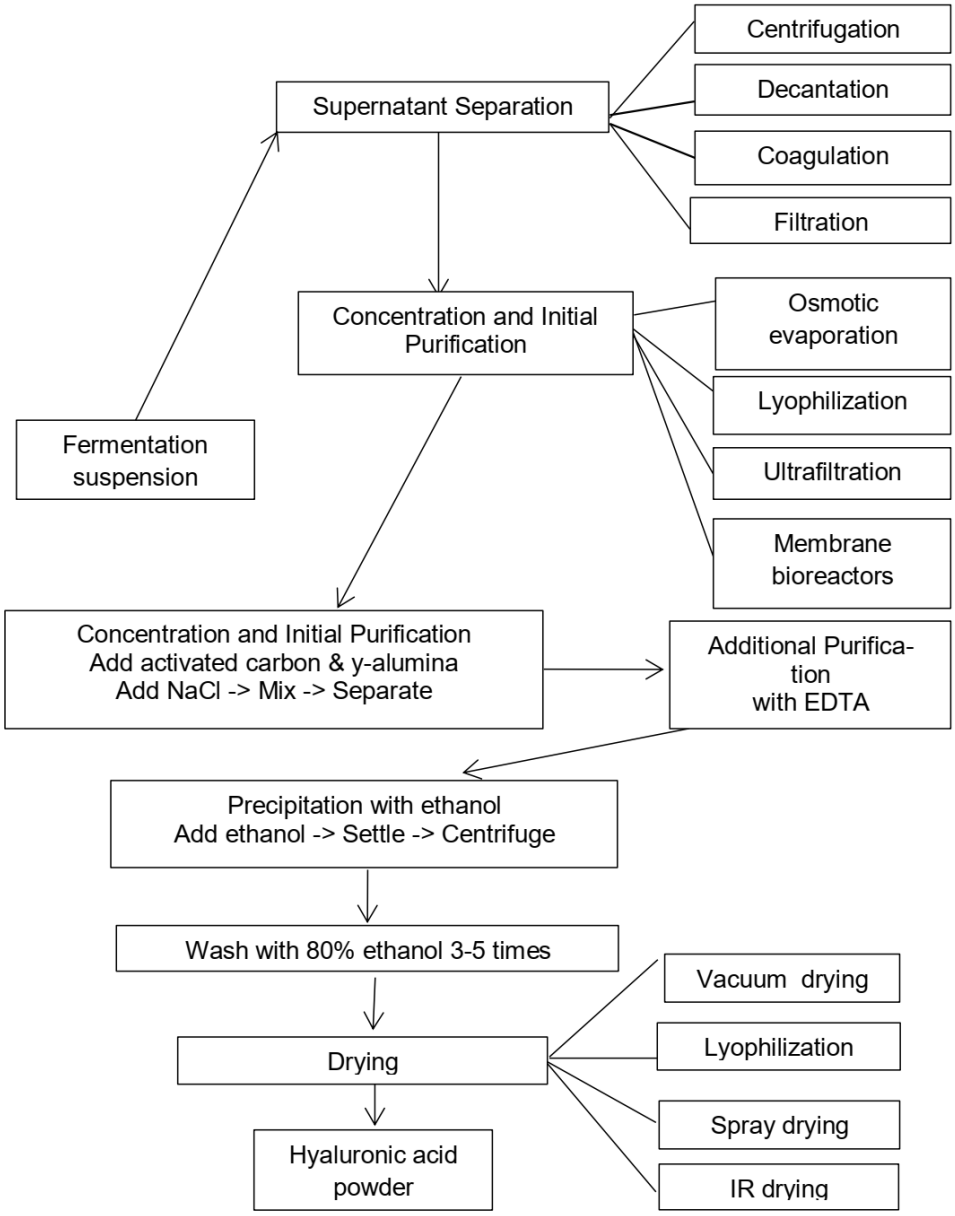

Figure 3. Common methods of hyaluronic acid extraction

Supernatant separation

The initial step in the hyaluronic acid extraction process is the separation of the supernatant from the bacterial culture. This step is critically important as it determines the quality and quantity of HA that can be obtained in subsequent stages. Several methods can be employed for this purpose, each with its own advantages and disadvantages (Choi et al., 2015):

Centrifugation:

- This is the most common and efficient method.
- Advantages include rapid separation, reproducibility of results, and scalability for industrial production.
- Typical conditions involve centrifuging at 5000-7000 g for 10-15 minutes.
- These parameters may vary depending on the specific bacterial strain and desired purity of the final product.
- It is crucial to optimize centrifugation speed and time to achieve maximum cell separation without causing cell lysis.
- Modern continuous-flow centrifuges can be used for large-scale operations, allowing for uninterrupted processing of large volumes of bacterial culture (Rodriguez-Marquez et al., 2022).

Filtration:

- While less common for bacterial cultures due to potential clogging issues, filtration can be useful for smaller-scale operations or specific bacterial strains.
- Diatomaceous earth can be added to improve filtration efficiency by creating a porous filter cake that enhances particle retention.
- Cross-flow filtration systems can be employed to minimize fouling and extend filter life in larger operations.
- The choice of filter pore size is critical: it must be small enough to retain bacterial cells but large enough to allow HA molecules to pass through (Cavalcanti et al., 2019).

Decantation:

- This method is simple but time-consuming and less efficient, making it unsuitable for large-scale production.
- It relies on gravity to separate the cells from the supernatant, which can take several hours to achieve adequate separation.
- While not practical for industrial processes, it might be used in laboratory settings for small-scale extractions or when specialized equipment is unavailable (Rossatto et al., 2023).

Coagulation:

- The addition of coagulants like aluminum or iron salts can aid in cell separation but may introduce unwanted impurities.
- This method can be particularly useful when dealing with difficult-to-separate bacterial strains.
- The choice and concentration of coagulant must be carefully optimized to ensure efficient separation without compromising HA quality.
- A subsequent purification step may be necessary to remove residual coagulants from the HA solution (Choi et al., 2014).

Centrifugation is generally preferred due to its efficiency, speed, and minimal risk of contamination. However, care must be taken to optimize centrifugation conditions to prevent cell lysis, which could release intracellular contaminants. The choice of separation method

may also depend on the specific characteristics of the bacterial strain used for HA production, as some strains may be more prone to cell lysis or may produce HA with different molecular weights that affect separation efficiency (Rodriguez-Marquez et al., 2022).

In industrial settings, a combination of methods may be employed. For instance, a preliminary filtration step might be used to remove large debris before centrifugation, enhancing the overall efficiency of the separation process.

Concentration and initial purification

Following supernatant separation, the HA solution undergoes concentration and initial purification. This step is crucial for removing water and low-molecular-weight impurities while retaining and concentrating the HA molecules. Several methods can be employed, each with its own advantages and considerations:

Ultrafiltration:

- This is the preferred method due to its efficiency in concentrating large molecules like HA while removing smaller impurities.
- A two-stage ultrafiltration process is often employed:
- First stage: Concentrates the solution to half its original volume using a 30,000 Da membrane.
- Second stage: Diafiltration with distilled water to reduce conductivity to around 2 mS/cm.
- The choice of membrane molecular weight cut-off is critical: it must be small enough to retain HA but large enough to allow impurities to pass through.
- Tangential flow filtration can be used to minimize membrane fouling and maintain high flux rates.
- Temperature control during ultrafiltration is important to prevent HA degradation.
- The process can be optimized by adjusting parameters such as transmembrane pressure, cross-flow velocity, and concentration factor (Cavalcanti et al., 2019).

Lyophilization:

- While effective in preserving HA structure, this method is time-consuming and energy-intensive, making it less suitable for large-scale production.
- It involves freezing the HA solution and then removing the ice by sublimation under vacuum.
- The freezing rate and temperature must be carefully controlled to minimize damage to the HA structure.
- Secondary drying may be necessary to remove residual bound water.
- While not ideal for initial concentration, lyophilization might be used as a final step to produce a dry, stable HA product (Aguilera-Bulla et al., 2022).
- Osmotic evaporation:
- This method uses osmotic pressure differences to remove water but can be slow and challenging to scale up.
- It involves using a concentrated salt solution separated from the HA solution by a semipermeable membrane.
- The osmotic gradient drives water from the HA solution into the salt solution, concentrating the HA.
- While gentle on the HA molecules, this method requires careful management of the salt solution and may introduce salt contamination if the membrane integrity is compromised (Lambe et al., 2021).

Membrane bioreactors:

- These combine fermentation and filtration but require significant initial investment.
- In this system, HA is produced continuously while being separated from the bacterial cells and concentrated in real-time.
- This approach can lead to higher productivity and reduced processing time but requires careful design and operation to maintain optimal conditions for both HA production and separation.

Ultrafiltration is typically chosen for its balance of efficiency, scalability, and preservation of HA quality. The process not only concentrates HA but also removes low-molecular-weight impurities, enhancing the purity of the product. The multi-stage approach allows for fine-tuning of the concentration and purification process, with the diafiltration stage being particularly effective at removing salts and other small molecules.

In industrial settings, the ultrafiltration process may be further optimized by using a cascade of membranes with different molecular weight cut-offs, allowing for more precise fractionation of the HA based on molecular weight. This can be particularly useful when producing HA for specific applications that require a narrow molecular weight distribution.

The choice of concentration and initial purification method will depend on factors such as the desired final HA concentration, the nature and quantity of impurities present, the molecular weight distribution of the HA, and the intended application of the final product. Careful optimization of this stage can significantly affect the efficiency of subsequent purification steps and the quality of the final HA product (Karami et al., 2021).

Adsorption of impurities

The concentrated HA solution is then subjected to an adsorption process to remove high-molecular-weight impurities. This step is crucial for achieving high-purity HA suitable for cosmetic and pharmaceutical applications. Common adsorbents include activated carbon granules (e.g., Norit C Gran), gamma-alumina, diatomaceous earth.

The process typically involves:

- Adding 2% activated carbon granules and 1% gamma-alumina to the HA solution.
- Adding sodium chloride to a concentration of 1 M to enhance impurity adsorption.
- Mixing the solution for about 5 hours to allow adsorption of proteins, nucleic acids, and endotoxins.
- Separating the adsorbents by centrifugation or filtration.
- The use of both activated carbon and gamma-alumina provides a synergistic effect: activated carbon effectively removes high-molecular-weight proteins and nucleic acids, while gamma-alumina is particularly effective at removing endotoxins.
- The presence of sodium chloride aids in detaching impurities from HA and increases the adsorption capacity of the adsorbents. After adsorption, the adsorbents can be regenerated for reuse, typically by heating to 900 °C in the absence of oxygen and in the presence of steam (Choi et al., 2015).

Additional purification

An additional purification step using EDTA (ethylenediaminetetraacetic acid) is often employed to further enhance the purity of the HA solution. This step serves multiple purposes:

- Removal of divalent cations: EDTA chelates divalent metal ions that may be present in the solution.
- Inhibition of protease activity: EDTA can inhibit metalloproteinases that might degrade HA.
- Removal of residual aluminum: EDTA helps in removing any remaining aluminum from the previous adsorption step.
- The process typically involves:
- Adding EDTA to the HA solution to a final concentration of 1 mM.
- Mixing the solution for about 10 minutes to allow chelation and impurity removal.

This step is particularly important for cosmetic-grade HA, where high purity is essential for product safety and efficacy (Mónico et al., 2017).

Precipitation

The purified HA is then precipitated using an organic solvent, most commonly ethanol. This step serves to isolate the HA from the aqueous solution and remove any remaining water-soluble impurities.

The precipitation process typically involves:

- Adding ethanol to the HA solution in a ratio of 2:1 (ethanol to HA solution).
- Allowing the mixture to stand for a period to ensure complete precipitation.
- Separating the precipitate by centrifugation (typically at 6000 g for 5 minutes).

Ethanol is preferred over other organic solvents due to its lower cost and reduced toxicity. The 2:1 ratio is chosen to balance efficient HA precipitation with minimizing HA losses.After precipitation, the ethanol can be recovered through distillation for reuse, improving the process's economic efficiency (Cavalcanti et al., 2020)

Washing

The HA precipitate is washed with 80% ethanol to remove any remaining impurities. This step is crucial for achieving a high-purity final product suitable for cosmetic applications.

- The washing process typically involves:
- Resuspending the HA precipitate in 80% ethanol.
- Mixing thoroughly to ensure all surfaces of the precipitate are exposed to the ethanol.
- Separating the washed precipitate by centrifugation.
- Repeating the process 3-5 times to ensure thorough purification.

The 80% ethanol concentration is chosen to effectively remove impurities while minimizing HA loss. The ethanol used in this step can also be recovered and reused after appropriate purification (Cavalcanti et al., 2020).

Drying

The final step in the process is drying the HA precipitate to obtain the final powder product. Several drying methods can be employed (Aguilera-Bulla et al., 2022):

Vacuum drying:

- This method is often preferred due to its balance of efficiency, cost-effectiveness, and preservation of HA quality. The process involves:
- Placing the HA precipitate in a vacuum chamber.

- Applying vacuum to lower the boiling point of residual water.
- Gently heating to facilitate water removal without degrading the HA (Collins et al., 2013).

Lyophilization:

- While effective in preserving HA structure, this method is time-consuming and energy-intensive. It involves:
- Freezing the HA precipitate.
- Applying vacuum to sublime the ice directly to vapor.
- Secondary drying to remove bound water.

Spray drying:

- This method is fast and suitable for large-scale production but may lead to some HA degradation due to high temperatures. The process involves:
- Atomizing the HA suspension into a hot air stream.
- Collecting the dried powder at the outlet of the drying chamber.

Infrared drying:

- This method provides rapid heating and drying but may result in uneven drying and potential HA degradation (Fallacara et al., 2019).
- Vacuum drying is often chosen for its ability to dry the HA at relatively low temperatures, preserving its quality while still being efficient and scalable for industrial production.
- The dried HA powder is then collected and packaged under sterile conditions to maintain its purity and quality.

Each of these steps in the extraction and purification process plays a crucial role in obtaining high quality HA suitable for cosmetic and pharmaceutical applications. The choice of methods at each stage depends on factors such as required purity, scale of production, available equipment, and economic considerations. Continuous optimization of these processes contributes to more efficient and cost-effective production of this valuable biopolymer.

The production of glycosaminoglycans, particularly hyaluronic acid, involves several post-fermentation steps that are essential for obtaining a high-purity, biologically active product. While traditional methods such as organic solvent precipitation, dialysis, and filtration are effective, they present challenges in terms of cost, scalability, and environmental sustainability. As the demand for hyaluronic acid continues to grow in fields such as medicine, cosmetics, and biotechnology, the need for more efficient and sustainable purification methods is becoming increasingly important. New technologies, like electrodialysis, show promise in addressing these challenges, offering a more cost-effective and environmentally friendly approach to glycosaminoglycan production.

This expanded version provides a more in-depth analysis of the various post-fermentation techniques and the innovations aimed at improving the efficiency and sustainability of glycosaminoglycan purification.

Conclusions

Based on the conducted review, it can be concluded that hyaluronic acid has broad commercial applications in the food industry, medical and cosmetic fields. In particular, the most promising strain for industrial production of HA is *Corynebacterium glutamicum* (32

g/l during 60 hours of cultivation), which has received GRAS status, confirming its safety for use in food products.

For the food industry, it is especially important to comply with the requirements established by the Law of Ukraine "On Basic Principles and Requirements for Food Safety and Quality", DSTU 4161-2003, and the Technical Regulation on Food Additives. These documents define the conditions for using HA as a food additive, its permissible concentrations, and labeling requirements.

Manufacturers and importers of products containing HA must carefully adhere to all regulatory requirements, ensure proper quality and safety of products, and provide consumers with accurate and complete information about the composition and properties of the products.

Given the constant development of technologies and research in the field of HA application, further improvement of the regulatory framework governing its use can be expected. This may include updating quality standards, expanding areas of application, and implementing new safety control methods.

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Comparative analysis of functional and technological properties of β-glucans from oats and yeast in whey ice cream

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Abstract

Introduction. The effect of β-glucans from oats and yeast incorporation on the quality parameters of whey ice cream during one month of storage was studied.

Materials and methods. The viscosity of the ice cream mixes was determined using an IKA ROTAVISC lo-vi Complete viscometer, texture parameters were studied using a Brookfield CT-3 texture analyzer, and ice cream structural elements were analyzed using microstructure analysis. Overrun, melting rate, and sensory evaluation were also performed to characterize the overall ice cream quality.

Results and discussion. The incorporation of β-glucans, a natural stabilizing ingredient, into whey ice cream significantly effects on its distinctive characteristics. Addition of oat β-glucan increased the viscosity of the ice cream mix to 623.07 mPa·s. Incorporation of β-glucan derived from yeast exhibited a viscosity of 542.14 mPa·s. The addition the mixture of oat and yeast β-glucans softened the texture of the ice cream. However, the effects of the glucans on the elasticity and extensibility of the ice cream matrix were different. This is attributed to the difference in molecular weight and structural properties between the β-glucans. This effect ensures a higher overrun and melting resistance of ice cream with oat β-glucan compared to yeast β-glucan. Addition of β-glucan from yeast significantly inhibited the growth of ice crystals, reaching a size of 9.52 μm, and provided long-term stabilization of the ice cream air phase. Conversely, β-glucan derived from oats exerted a comparatively mild influence on the recrystallization of free water in ice cream, resulting in the formation of ice crystals no larger than 16.31 μm.

Incorporation of oat and yeast β-glucans typically result in a softer ice cream, but oat β-glucan has been shown to significantly enhance the elastic properties of ice cream, which may contribute to an improved structure. The incorporation of β-glucans into ice cream also improved flavor characteristics, such as creaminess and stickiness, and provided the desired level of sweetness and cold taste when consumed.

Conclusions. The addition of oat and yeast β-glucan has been demonstrated to exert a considerable influence on the rheological and physicochemical attributes of ice cream, including its texture parameters, structural elements, and taste perception.

Introduction

Ice cream production that meets modern consumer preferences for nutritious foods and transparent labeling has become popular around the world over the past decade (Kumari et al., 2020). Manufacturers are increasingly producing ice cream that is low in calories and fat, functional, enriched with proteins and dietary fiber, and free of synthetic structure stabilizers (Blassy & Abdeldaiem, 2024; Guler-Akin et al., 2021; Lai et al., 2024). However, such products frequently fail to meet consumer expectations for quality. The replacement of ingredients or the addition of new technological additives without adequate research on their impact on quality merely formalizes demand but fails to account for the potential occurrence of defects (Samsalee et al., 2024).

In a preliminary study, it was demonstrated that β-glucan derived from oats (facilitates a regulated process of free water recrystallization in low-fat milk ice cream (Buniowska-Olejnik et al., 2023). Nevertheless, further research is required to ascertain the influence of these polysaccharides on the quality of ice cream produced using liquid hydrolyzed whey concentrate.

β-Glucans are polysaccharides that occur naturally in the cell walls of various organisms, including cereals, bacteria, and fungi (Mykhalevych et al., 2022). In the food industry, β-glucans are of interest due to their functional properties, particularly their capacity to enhance texture and stabilize emulsions. For example, oat β-glucan has been successfully employed in the production of yogurt to enhance its viscosity and creaminess without adversely affecting the product's flavor (Qu et al., 2021). Similarly, β-glucan derived from yeast is incorporated into breads to enhance dietary fiber content and facilitate the formation of a softer crumb structure (Martins et al., 2015; Mohebbi et al., 2019).

β-Glucans influence product texture through a range of mechanisms.They function as hydrocolloids, which are substances that form gels when mixed with water, thereby imparting the desired flavor and stability to foods (Cao et al., 2023). β-glucans, by additionally converting free water into a bound state, also assist in preventing the formation of large ice crystals in ice cream (Hamed et al., 2014), which is essential for maintaining a smooth and creamy texture of the product. Furthermore, β-glucans influence the viscosity of ice cream mixes (Akal, 2023), which can enhance its texture and prevent the formation of a coarse crystalline structure during storage.

Furthermore, it is crucial to comprehend the influence of the employed processing additives on the flavor profile of ice cream. A number of food companies employ sensory profiling to provide qualitative and quantitative representation of the attributes perceived by humans, thereby enabling them to quantify the sensory response to stimuli derived from the consumption of a product (Varela & Ares, 2015). The ability of these polysaccharides to significantly improve the rheological properties and imitate the milk taste to the level of full-fat ice cream analogues has been reported (Mousakhani-Ganjeh and Goli, 2021; Piotrowska et al., 2009).The aforementioned evidence underscores the considerable potential of β-glucans in enhancing the texture of low-fat ice cream.

Since the reduction of fat and sugar content in the developed whey ice cream (Mykhalevych et al., 2024; Shevchenko et al., 2022) will affect its texture and taste properties, it is important to characterize these properties by sensory and instrumental evaluation in order to determine the magnitude of the expected effects. For this reason, the impact of β-glucans from diverse origins on the texture, physicochemical, and sensory attributes of ice cream was investigated. To this end, the initial step was to ascertain the rheological properties of ice cream mixes incorporating β-glucans. The subsequent step was to conduct a texture profile analysis and sensory evaluation of whey ice cream with these polysaccharides.

Materials and methods

Raw materials

For the production of liquid hydrolyzed concentrate of demineralized whey with a solids content of 40%, whey powder with a degree of demineralization of 90% (Milk Alliance JSC, Ukraine), an enzyme preparation lactase (β-D-galactosidase) with an activity of 5000 NLU/g (GODO-YNL2, Danisko, Denmark), activated starter based on *L. acidophilus* LYO 50 DCU-S (Danisko, Denmark), and water were used. For ice cream preparation the following ingredients were used: water, white sugar, vanillin, Cremodan SI 320 stabilization system (Danisco A/S, Denmark), whey protein isolate 90% (SPOMLEK, Radzyń Podlaski, Poland), highly soluble β-glucan (1-3, 1-4) extracted from oats (OBG) with a purity of 72% (Grupa Feniks 2050, Ćmielów, Poland), and β-glucan from yeast (YBG) *Saccharomyces cerevisiae* with a purity of 70% (GOLDCELL, Biorigin, Sao Paulo, Brazil).

Production technology

The starter was activated in ultra-pasteurized skim milk at a temperature of 38–42 °C until a pH of 5.4–5.2 was reached. A liquid whey concentrate with a solids content of 40% was obtained by reconstituting demineralized whey powder in water at 40–42 °C and subsequently adding β-D-galactosidase and starter L. acidophilus. The enzymatic hydrolysis was conducted at 38–42 °C until a degree of lactose hydrolysis of 85% was achieved. The technology of liquid hydrolyzed concentrates is described in work of Osmak et al. (2021).

To prepare the ice cream, the dry components, as specified in Table 1, were combined with water (40–45 °C) and stirred until a homogeneous mix was achieved.

Table 1

Formulations of the ice cream samples

Ingredients, %	Ice cream samples		
	Control	0.5% OBG	0.5% YBG
Hydrolyzed concentrate of demineralized whey	75.0	75.0	75.0
White sugar	9.0	9.0	9.0
Whey protein isolate (90%)	3.0	3.0	3.0
Stabilization system	0.6	–	–
β-glucan from oats	–	0.5	–
β-glucan from yeast	–	–	0.5
Activated starter	3.0	3.0	3.0
Vanillin	0.1	0.1	0.1
Water	9.3	9.4	9.4
Total	100.0	100.0	100.0

OBG, extracted from oats; YBG, β-glucan from yeast *Saccharomyces cerevisiae*.

Subsequently, the mix was incorporated with the liquid whey concentrate. The resulting mixes were filtered through a 1 mm mesh filter prior to pasteurization at 83-87 °C for 5 min, followed by homogenization at 12.0±2.5 MPa using a laboratory homogenizer-disperser 15M-8TA "Lab Homogenizer & Sub-Micron Disperser" (GAULIN CORPORATION, Massachusetts, USA). Homogenized mixes were cooled to 38–42 °C, and a 3% of activated starter was added. The fermentation process was conducted until a pH of 5.25–5.10, after

which the mix was cooled to 2–6 °C, vanillin was added, and maturation was carried out for 12 h. The maturated mixes were freezed using a laboratory freezer FPM-3.5/380-50 "Elbrus-400," (JSC ROSS, Kharkiv, Ukraine). In the initial phase of freezing, the mix was cooled in a cooling cylinder (volume – 7 L) to a temperature of –1 °C at a rotation speed of the scraper stirrer of 4.5 s^{-1} for 120 s. Subsequently, in the second stage, the mix was frozen and whipped at a rotational speed of 9 s^{-1} for 180 s to a temperature of –5.0±0.5 °C. The ice cream samples (3 kg each) were hardened and stored in a Caravell A/S freezer (Løgstrup, Denmark) at –22±1 °C for one month. To ensure the reliability of the results, ice cream samples of the same chemical composition were prepared 3 times.

According to the above formulations (Table 1) of the ice cream samples, the solids content is 42.33–42.61%, of which protein is 5.98–6.01% and fat is 0.35–0.73%.

The content of β-glucans at 0.5% was chosen in accordance with the available information in scientific works on their use in ice cream (Aljewicz et al., 2020b; Tomczyńska-Mleko et al., 2024).

Research methods

The viscosity of ice cream mixes was determined using an IKA ROTAVISC lo-vi Complete viscometer (IKA, Staufen, Germany) (Nazarewicz et al., 2022). For the measurement, a T-SP-2 spindle was used, which was immersed in the prepared sample at 18±1 °C and a shear rate of 200 rpm. Viscosity values were read after 2 min. A power law model was used to determine the flow behavior index (n) and the consistency coefficient (K). The flow behavior index shows how close the mix is to Newtonian. The consistency coefficient gives an idea of the flow properties of the mix (Muse and Hartel, 2004).

Analysis of the texture profile. Ice cream texture parameters were determined using a Brookfield CT-3 texture analyzer (Middleboro, Massachusetts, USA). Measurements were performed using Pro CT V1.6 software (Brookfield Engineering Laboratories Onc., ABD, Middleboro, MA, USA). For the analysis, a TA27 conical probe (on the first day of storage) and a TA 15/1000 (on the 1th month of storage) were used. The speed was 2 mm/s, the distance was 15 mm, the trigger load was 1.08 H, the length was 40 mm and the diameter was 60 mm.

Determination of overrun. Ice cream overrun was determined by the weight method by the difference in weight of samples of the same volume of the mix and ice cream, expressed as a percentage (Sofjan and Hartel, 2004).

Determination of the melting rate. The ice cream samples were stored at –22±1 °C, selected and placed on a special melting grid at room temperature of 19±1 °C. The weight of the melted ice cream was recorded after one hour every 10 min for 2 h. The melting rate (R, %) was calculated using the formula (Yeon et al., 2017):

$$R = (\text{weight of melted ice cream}) / (\text{weight of ice cream before melting}) \times 100,$$

Analysis of structural elements. The process of free water recrystallization and air bubbles in ice cream were studied using an Olympus BX53 microscope with a Linkam LTS420 cooling system (measuring temperature range from –196 °C to –420 °C) and an Olympus SC50 digital camera. For each sample, 300 to 500 crystals were labeled and the area, equivalent diameter, and standard deviation were calculated using NIS Elements D Imaging software (version 5.30.00, Nikon). The method has been reported in works related to the study of ice cream (Kamińska-Dwórznicka et al., 2022; Kamińska-Dwórznicka et al., 2020).

Sensory evaluation. The sensory evaluation of the ice cream was carried out on a five-point scale for such indicators as creaminess, milk taste, sweet taste, cold taste, stickiness, and hardness. Based on the results of the evaluation, profilograms were made.

Statistical processing. The significance of the test was set at $\alpha = 0.05$. The data are expressed as mean values with standard deviations (±SD).

Results and discussion

Determination of rheological parameters of ice cream mixes

Viscosity is a critical parameter in ice cream formulations that affects texture and flavor. The obtained viscosity values indicate significant differences between the ice cream mixes (Table 2).

Table 2

Rheological parameters of ice cream mixes ($p \leq 0.05$, n = 3)

Indicator	Control	0.5% OBG	0.5% YBG
Viscosity, mPa·s	372.14^{a}±10.54	623.07^{b}±29.87	542.14^{ab}±15.40
Consistency coefficient (K), dynes/cm^2	102.03^{a}±2.56	155.98^{b}±5.21	137.19^{ab}±3.68
Flow behavior index (n)	0.214^{a}±0.01	0.244^{b}± 0.02	0.236^{ab} ±0.02

Due to their ability to interact with water and form viscous solutions, β-glucans can significantly increase the viscosity of foods with a high water content, with oat β-glucan being the most effective. On the contrary, β-glucan from yeast increases the viscosity of mixes less pronounced, which is associated with differences in molecular weight and structure (Frank et al., 2004; Sammalisto et al., 2024). Studies indicate that addition of yeast β-glucan can increase the viscosity of beverages and some dairy products, although to a lesser extent than oat β-glucan (Chiozzi et al., 2021; Rose et al., 2023).

The values of the consistency coefficient (K) show statistically significant differences between the groups. The 0.5%OBG sample has a significantly higher K value (155.98±5.21) than the control group (102.03±2.56), indicating an increase in viscosity with the addition of 0.5% oat β-glucan. The 0.5%YBG sample also exhibits a higher K value (137.19±3.68) compared to the control. The flow behavior index (n) for the samples with β-glucans indicates a significant change in flowability compared to the control.

The observed differences in viscosity between the samples demonstrate how the choice of β-glucan additive and its concentration can affect the rheological properties of ice cream.

Study of texture parameters of whey ice cream

To understand the changes in ice cream texture in the presence of β-glucans, a comparative analysis of the texture parameters of the control and experimental samples was conducted (Table 3).

Table 3

Texture parameters of whey ice cream ($p \leq 0.05$, n = 3)

Indicator	Control	0.5% OBG	0.5% YBG
1 day			
Hardness, N	9.79±0.24	7.13±0.15	3.43±0.10
Adhesiveness, mJ	1.70±0.05	1.02±0.08	1.67±0.04
Adhesive Force, N	0.75±0.02	0.48±0.02	0.45±0.01
Stringiness, mm	1.22±0.02	0.80±0.04	1.61±0.07
Stringiness Work Done, mJ	0.70±0.01	0.30±0.01	0.50±0.02
Recoverable Deformation, mm	0.60±0.01	0.41±0.02	0.82±0.02
1 month			
Hardness, N	14.46±0.24	9.98±0.27	5.63±0.19
Adhesiveness, mJ	1.02±0.01	2.30±0.08	0.91±0.02
Adhesive Force, N	0.47±0.02	0.79±0.01	0.32±0.01
Stringiness, mm	1.41±0.01	1.81±0.02	1.86±0.04
Stringiness Work Done, mJ	0.57±0.02	1.05±0.01	0.30±0.01
Recoverable Deformation, mm	1.23±0.03	1.01±0.04	1.43±0.01

The presence of β-glucans in ice cream mixes leads to noticeable changes in several key texture parameters. Both β-glucans generally soften ice cream, consistent with the demonstrated ability of polysaccharides as structural modifiers to reduce the force required to penetrate the ice cream matrix (Tolve et al., 2024). In particular, ice cream with 0.5% yeast β-glucan showed a significant reduction in hardness on day one compared to the control, indicating the ability of this polysaccharide to effectively reduce the textural stiffness of the product. Oat β-glucan also reduces the hardness of ice cream, but this effect is less pronounced.

With regard to adhesion and adhesive force, which refer to the stickiness and the force required to separate the ice cream from the surface, both parameters showed trends consistent with the softening effect observed for hardness. The decrease in stickiness and adhesion in ice cream with β-glucans indicates a possible change in the way the ice cream interacts with surfaces, which will certainly affect the organoleptic perception of the product by consumers.

The elasticity, which measures the tendency of ice cream to exhibit elastic behavior when stretched, shows quite different results. On the first day, the ice cream with oat β-glucan showed less elasticity than the control and the sample with yeast β-glucan. This suggests that oat β-glucan may affect the elasticity and extensibility of the ice cream matrix in a different way than yeast β-glucan. This phenomenon may be because oat β-glucan typically exhibits pseudoplastic behavior in dairy food systems (Aljewicz et al., 2021; Li et al., 2023), while yeast β-glucan provides a less robust gel network (Tomczyńska-Mleko et al., 2024).

The recoverable deformation, which measures the ability of ice cream to return to its original shape after deformation, indicates that β-glucans influence the recovery process, with oat β-glucan showing a more pronounced effect on day 1 compared to yeast β-glucan. The observed pattern confirms that oat β-glucan can improve the elastic properties of ice cream, potentially contributing to improved structure and shape retention during storage, which requires further research.

Analysis of the overrun of the ice cream indicates that β-glucans affect the distribution of the air phase in the product. The control sample had an overrun of 75.25%, while the oat and yeast β-glucan samples had overrun of 83.12% and 77.39%, respectively (Figure 1).

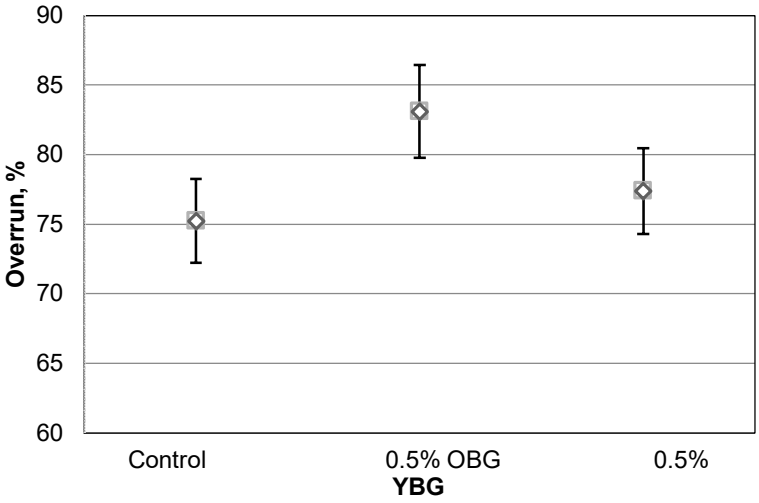

Figure 1. Overrun of whey ice cream

Oat β-glucan has been demonstrated to be more effective than yeast β-glucan in stabilizing air bubbles. Akal (2023) reported that the incorporation of soluble fibers, such as inulin and oat β-glucan, into ice cream formulations results in an increased rate of overrun. This phenomenon may be attributed to the stabilizing effect of soluble fibers, which enhance foam stability by trapping air within the ice cream matrix (Burkus and Temelli, 2000).

While yeast β-glucan also demonstrated an increase in overrun compared to the control sample, its ability to stabilize the air phase was less pronounced than that of oat β-glucan. These findings align with those of other researchers (Aljewicz et al., 2020b; Sadiq and Mousa, 2024) who have observed that different types of β-glucans exert varying effects on the physicochemical properties of dairy products, including the capacity to stabilize emulsions and air bubbles.

The incorporation of β-glucans markedly influenced the melting behavior of ice cream (Figure 2). The sample containing 0.5% oat β-glucan exhibited the lowest melting rate, with only 55% of the thawed mass melting after 120 minutes, indicating enhanced stability and resistance to melting. The sample with 0.25% yeast β-glucan also slowed the melting of ice cream by up to 64%.

The findings demonstrate that oat β-glucan is more efficacious in impeding the melting process of ice cream in comparison to yeast β-glucan. The higher water-binding and gel-forming ability of oat β-glucan contributes to the formation of a more stable ice cream matrix. Similarly, Aljewicz et al. (2020b) reached a comparable conclusion, indicating that the incorporation of hydrocolloids, particularly oat β-glucan, enhances the melting resistance of ice cream due to its stabilizing properties.

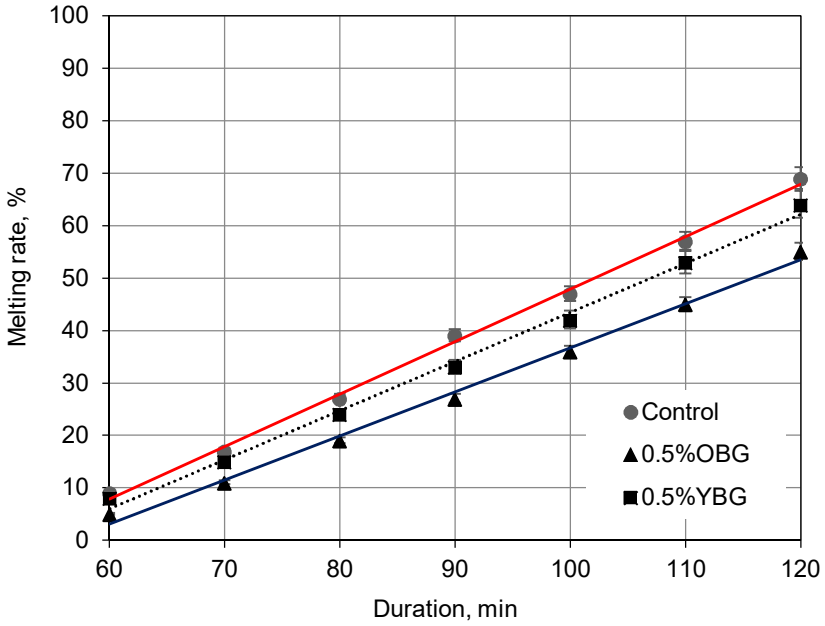

Figure 2. Melting rate of whey ice cream

In order to identify patterns of change in the dispersed crystalline and air phases of ice cream during storage, the dynamics of ice crystalls and air bubbles growth in the tested samples were studied (Table 4).

Table 4

Structural elements of the dispersion phase of whey ice cream ($p \leq 0.05$, n = 3)

Sample	Average diameter of air bubbles, μm	Average diameter of ice crystals, μm
1 day		
Control	6.60±0.03	15.80±0.67
0.5% OBG	11.51±0.21	11.38±0.17
0.5% YBG	8.56±0.18	8.49±0.37
1 month		
Control	14.92±0.10	32.15±1.18
0.5% OBG	13.54±0.55	16.31±0.15
0.5% YBG	10.47±0.12	9.52±0.16

The average diameter of air bubbles on the first day of storage was the smallest in the control sample (6.60 μm). In the samples with yeast and oat β-glucan, it was 8.56 μm and 11.51 μm, respectively. After 1 month, an increase in the size of air bubbles was observed in all samples, in particular, the largest bubble diameter was in the control sample (14.92 μm). This indicates that β-glucans stabilize the air phase more effectively over a long period in

contrast to the sample with a commercial stabilization system (Santipanichwong and Suphantharika, 2009). This is in line with the findings of Izydorczyk and McMillan (2019), who reported that dietary fiber, such as β-glucans, can stabilize air bubbles, improving the overall texture of the product.

Analyzing the process of free water recrystallization in ice cream it can be noted that significant differences were observed between the samples. On the first day, the control sample had the largest ice crystals (15.80 μm), while the smallest were found when using β-glucan from yeast. After 1 month, the control sample showed a significant increase in ice crystal size – up to 32.15 microns. In contrast, the sample with 0.5% oat β-glucan showed significantly smaller ice crystals (16.31 μm), and the sample with 0.5% yeast β-glucan had the smallest ice crystals at 9.52 μm). Yeast β-glucan is the most effective in inhibiting the growth of ice crystals during long-term storage. In a study of Soukoulis et al. (2014), it was found that the inclusion of hydrocolloids and dietary fiber in ice cream reduces the growth of ice crystals, which improves the texture and sensory characteristics of the product. Similar conclusions were reported by Ng et al. (2022), where hydrocolloids helped to soften the texture of ice cream and reduce the rate of recrystallization.

At the last stage of the study, a sensory evaluation of ice cream samples was performed (Figure 3).

Figure 3. Sensory evaluation of whey ice cream

Samples with β-glucans received higher scores than the control sample for their more pronounced milk flavor. The presence of β-glucans effectively mimics milk fat in ice cream, which is likely due to their ability to improve texture by forming a more stable and homogeneous ice cream matrix. Other scientists who have established the ability of polysaccharides to improve the taste of low-fat products (Bealer et al., 2020; Kaur and Riar, 2020) have drawn similar conclusions.

The control sample received the highest score for its sweet taste, which is typical for ice cream. The decrease in sweetness perception in samples with β-glucans may be due to

their specific interaction with monosaccharides (Aljewicz et al., 2020a) present in the product.

The ice cream firmness score correlates with the measured firmness value in the texture analysis. Thus, β-glucans reduce the hardness of ice cream, which improves the perception of the product. β-Glucans also increase the stickiness of ice cream, possibly due to their gelling properties, which improve the cohesion of the product. Aljewicz et al. (2020b), studying ice cream with 1% oat β-glucan, found that it led to a sticky mouthfeel due to excessive viscosity. However, we did not observe such an effect, which can be explained by the lower mass fraction of the additive (0.5%) used in this study.

In terms of cold taste, all samples received an equally high score of 4.5, indicating that the addition of β-glucans did not affect this characteristic. The inclusion of oat and yeast β-glucans in ice cream improves several sensory characteristics, including creaminess, stickiness, and milky flavor, while maintaining the desired level of sweetness and cold taste. Such properties could potentially lead to the development of ice cream that consumers would prefer in further commercial testing.

Overall, the differences found between oat and yeast β-glucans emphasize the importance of understanding the specific structural and functional properties of each additive in the development of new ice cream formulations. The results of the experiment emphasize the significant influence of β-glucans, primarily on the parameters of ice cream texture. This makes it possible to rationally use β-glucans to control the characteristics of new types of ice cream.

Conclusions

The substantial impact of β-glucans on the rheological and physicochemical attributes of whey ice cream has been demonstrated. The addition of oat β-glucan (0.5%) to mixes has the effect of increasing their viscosity, as well as improving verrun and melting resistance, thus enhancing the quality of the resulting ice cream. β-Glucan from yeast (0.5%) has a less pronounced effect on these indicators, but it provides long-term stabilization of the crystalline and air phases of ice cream. Specifically, in the presence of β-glucan from yeast, after one month of ice cream storage, the diameter of air bubbles in the ice cream did not exceed 10.47 μm, and ice crystals did not exceed 9.52 μm. In contrast, the ice cream sample containing 0.5% oat β-glucan exhibited a significantly higher ice crystal size of 16.31 microns after one month of storage.

β-Glucans of disparate origins typically result in a softer ice cream, but oat β-glucan markedly enhances the elastic properties of ice cream, which has a beneficial impact on the structure of the product. The incorporation of oat and yeast β-glucan into ice cream formulations has been demonstrated to enhance the creaminess, stickiness, and milky flavor of the product, while maintaining the desired level of sweetness and cold taste.

Further research is needed to elucidate the long-term effects of β-glucans on ice cream quality.

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Determination of the bioactive properties, mineral and phenolic composition of different solvent-based propolis extracts and their evaluation according to existing regulations

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Abstract

Introduction. The propolis is a natural remedy and a popular food supplement worldwide. Botanical origin of raw propolis, bee types, extraction methods, parameters and solvents effect the propolis extract bioactivity and attributes. In this study, 14 propolis extracts (water and ethanol based) were analyzed and compared with the results evaluated according to current regulations.

Materials and methods. The physicochemical properties (pH, titration acidity, color, °Brix), antioxidant activity (inhibition %), total phenolic content, and phenolic and mineral compositions were determined, and the results were statistically compared ($p<0.05$). Additionally, the sensory profiles of the propolis extracts were evaluated using principal component analysis.

Results and discussion. A significant ($p<0.05$) correlation was identified between the ºBrix values of the samples and their total phenolic content, suggesting that the ºBrix value could serve as an initial indicator for estimating the polyphenol content in propolis extracts. Among the phenolic compounds, chrysin, apigenin, and caffeic acid phenyl ester were found to be the most abundant. The study showed that phenolic content was generally higher in alcohol-based samples compared to water-soluble ones. None of the propolis extracts complied with the "phenolic content" requirement set out in the Turkish Food Codex (TFC) Bee Products Communiqué. Potassium and calcium were identified as the most prevalent minerals across all samples, while lead and cadmium were present within the limits specified by TFC regulations on contaminants. Although arsenic is not regulated in the national legislation, it was detected, particularly in alcohol-based samples.

Conclusions. It was recommended to establish a limit for As content in bee product regulations. Additionally, similar to the regulations in countries such as Brazil and Ukraine, it was suggested to include standards for total phenolic content or total flavonoid content as quality classification criteria in bee product regulations.

Introduction

Propolis is a natural material collected by honey bees (*Apis mellifera* L.) from various plants and is composed of resin, 50%, and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and other organic compounds such as polyphenols and terpenoids (Anjum et al., 2019; Rocha et al., 2023). Overall, more than 300 different bioactive components, including polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes, and ketones have been identified in propolis (El-Guendouz et al., 2019). It has been proven to possess numerous beneficial pharmacological effects and has been used in complementary medicine due to its anti-inflammatory, antimicrobial, immunomodulatory, antioxidant, and antitumor activities (Mele et al., 2023). Currently, propolis is a natural remedy and a popular food supplement worldwide. It is available either in extract form or combined with other natural products, such as vitamins, minerals, or herbal syrup preparations, and as a constituent of health foods.

Raw propolis cannot be consumed directly due to its wax, resin, herbal balsam content, and bioactive compounds; therefore, it must be extracted appropriately. There are numerous studies in the literature on propolis extraction methods, parameters and solvents. The most well known form of propolis consumption is the liquid drop form. For this purpose, different solvents, such as glycol, ethyl alcohol, glycerin, essential oils and olive oil, and several extraction techniques, such as maceration, reflux, ultrasound or microwave-assisted extraction, are used. These factors, including the botanical origin of propolis, have been reported to influence the bioactivity and pharmacological properties of the final propolis extract (Özdal et al., 2023). Because propolis is a natural remedy and popular food supplement worldwide, it is possible to find many different brands of propolis from different companies in the national and international markets. These products are presented to customers by pharmacies and e-commerce platforms in a variable price range and in different forms, such as extract, tincture, capsule or powder. It is a requirement that propolis extracts, which are preferred by consumers as natural food supplements, can be offered to consumers within certain quality limits. For this purpose, several national and international regulations have been established by governmental associations and many scientific studies. In this study, the quality properties of propolis extracts consumed as food supplements were compared. They were evaluated according to the national regulations established in our country, and their suitability was discussed.

Materials and methods

Materials

The propolis extract samples were sourced from the domestic market in Turkey. Of these, seven were water-soluble (with no alcohol as the final solvent), while the remaining seven were alcohol-based propolis extracts. These samples were purchased from markets and e-commerce sites in their original commercial packaging, boxed, tightly sealed, and stored in the dark until analysis. The chemicals used in this study were obtained from Merck (Darmstadt, Germany), and the DPPH radical was sourced from Sigma (Darmstadt, Germany).

Physicochemical properties

pH meter (Ohaus) was used to measure the pH of the samples. Each sample and distilled water were homogenized (1:9, w/v) with an Ultra-Turrax homogenizer (IKA, T25, Germany). The mixture was kept for 10 min, and then titrated with 0.1 N NaOH to a pH of 8.3. The total titration acidity (TTA) was subsequently calculated. The color profile was determined by the colorimetric method (3nh Colorimeter, NR145, China) as *L*, a*,* and *b** in five replicates. The results are presented as the means and standard deviations. A refractometer (Atago, Germany) was used to determine the percentage dissolved solids of the commercial propolis samples supplied, and the measured values were given in Brix° (Keskin and Kolaylı, 2019).

Total phenolic content

The total phenolic content of the propolis extracts was determined according to the Folin–Ciocalteu assay (Shahidi and Naczk, 1995). Briefly, 0.1 mL of extract (diluted 100 times with ethanol) was transferred to a glass tube, and 0.5 mL of Folin–Ciocalteu's reagent (0.2 N) was added. After mixing the tube using a vortex, 0.4 mL of sodium carbonate solution and 4 mL of distilled water were added. After incubating the mixture in darkness for 1 h, the absorbance was recorded at 760 nm using a UV–VIS spectrophotometer (Shimadzu Corporation, Japan) against the blank prepared using ethanol instead of extracts. The total phenolic content (calculated as mg gallic acid equivalent (GAE)/100 ml) of the extract was calculated using the calibration curve [concentration = (Abs + 0.009x)/0.020] obtained using gallic acid standard solutions.

Antioxidant activity

For determining the antioxidant activity (inhibition %), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used. A volume of 4900 µl of DPPH solution (0.025 g/l in ethanol) was combined with 0.3 mL of the extracts, which had been diluted 100-fold with ethanol, and the mixture was thoroughly vortexed. The mixture was then incubated in the dark for 30 minutes. Following incubation, the absorbance was measured at 517 nm using a UV–VIS spectrophotometer.The results were calculated as Trolox equivalent (µg/ml) and inhibition (%) with the following equation:

$$\text{Inhibition } \% = [1 - (\frac{Abs\ Sample}{Abs\ Control})] \cdot 100 \quad (1)$$

Phenolic composition

Phenolic content analysis was performed on an Inertsil ODS4 (3 µM, 2.1 × 50 mm) column. LC–MS/MS was carried out according to a validated method using 18 polyphenols (catechin, cinnamic acid, gallic acid, 2-5 dihyroxy benzoic acid, tannic acid, caffeic acid, trans ferulic acid, routine trihydrate, myricetin, naringenin, ellagic acid, quercetin, luteolin, chrysin, naringin, triacetin, apigenin, and caffeic acid phenyl ester). The propolis samples were diluted 100 times with ethanol and filtered. For LC–MS/MS analysis (8030 Plus, Shimadzu Corporation), a gradient phase was applied using mobile phase A (ultrapure water containing 1% formic acid) and mobile phase B (methanol containing 1‰ formic acid).The mobile flow rate was 0.4 ml/min , the injection volume was 10 µl, and the column oven

temperature was 40°C. The results are expressed as μg standard phenolic substance per ml extract sample.

Mineral composition

The mineral content of the samples was assessed using a microwave-assisted nitric acid digestion procedure (CEM MARS6, USA) followed by analysis with inductively coupled plasma–optical emission spectrometry (ICP–OES) (SpectroBlue, Germany). Approximately 1 mL of each sample was mixed with 1 ml of H_2O_2 (30% v/v) and 10 ml of HNO_3 (67% v/v) in PTFE flasks. The digestion program included heating to 200°C for 15 min, followed by a 15-min hold at 200°C. Once cooled to room temperature, the solutions were transferred to 50 ml polyethylene flasks and brought to volume with ultrapure water. The prepared samples were then filtered through microfilters and analyzed using ICP–OES (Al Khalifa and Ahmad, 2010). Calibration standards were prepared from a multi-element standard stock solution (Merck, Germany). All measurements were conducted in triplicate.

Sensory profile analysis

Propolis extracts were evaluated for turbidity (It should have a homogeneous appearance and should not contain any sediment), taste (After swallowing, a resinous and clean propolis taste should remain in the mouth), flavor (It should not contain any chemically or unusual odor notes), color/appearance (It should have a distinctive, yellowish and uniform color), and aftertaste (There should be no foul or bitter taste in the mouth after swallowing). For this purpose, the samples were coded with three-digit codes and served to panelists. 10 drops of propolis were added to plain glasses containing 100 ml of drinking water. Individuals who had previously consumed propolis, were not allergic to bee products, and did not smoke were selected as panelists. The panel group was informed about the evaluation criteria and scoring system. The samples were assessed using a hedonic scale ranging from 1 (I did not like it at all) to 5 (I liked it very much) based on sensory properties.

Statistical analysis

The data were reported as the mean±standard deviation. The statistical evaluation of the results was performed using analysis of variance (ANOVA) (IBM SPSS 1.0.0.781). After optimization, the obtained results were validated experimentally. Independent samples t-tests (SPSS 17.0.1, Chicago, IL, USA) were used for comparisons ($p<0.05$) of the two groups. Principal component analysis (PAST 4.03 Statistical Analysis App for Windows) was applied to determine product similarity and clustering tendencies according to the liking scores of the sensory evaluation results.

Results and discussion

Label Declarations and Contents of Propolis Samples

The label information and contents declared by their producers on the packages of 14 liquid propolis extracts were given in Table 1.

Table 1

Label declarations and contents of propolis samples

Sample	Volume (ml)	Form	Food supplement approval number	Final main solvent	Content	Shelf life (year)
P1	30 ml	Glass dropper bottle	+	Water	Water, propolis extract (18%)	2
P2	30 ml	Glass dropper bottle	+	Water	Pure water, raw propolis extract powder (14.4%)	3
P3	20 ml	Glass bottle	-	Water	Propolis, water	3
P4	20 ml	Glass dropper bottle	+	Water	Pure water, glycerin, propolis (20%)	3
P5	20 ml	Glass bottle	-	Water	Pure water, propolis extract (40%)	4
P6	20 ml	Glass dropper bottle	+	Water	Water, propolis extract (7.5%)	2
P7	20 ml	Glass dropper bottle	+	Water	Pure water, organic propolis	3
P8	30 ml	Glass dropper bottle	+	Ethanol	Propolis, Zn, D vitamine	3
P9	30 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), ethanol	3
P10	20 ml	Glass dropper bottle	-	Ethanol	Propolis (38%), water, ethanol	2
P11	20 ml	Glass dropper bottle	-	Ethanol	Propolis (20%), water, ethanol	2
P12	20 ml	Glass dropper bottle	+	Ethanol	Propolis (30%), water, ethanol	3
P13	20 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), water, ethanol	3
P14	20 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), water, ethanol	3

The liquid propolis samples were classified according to the final solvent type of the end product; the samples between P1-P7 were called "water-based propolis", and the samples between P8-P14 were called "ethanol-based". Twelve of the 14 propolis samples were collected in glass bottles with droppers, and the P3 and P5 samples were collected in glass bottles without droppers. The net volumes of the samples varied between 20 and 30 ml.

According to the Turkish Food Codex (Official Gazette dated 02.05.2013 and No. 28635), "Regulation on the Import, Production, Processing and Placing on the Market of Food Supplements" (TFC, 2013), food supplements cannot be produced, processed, imported or placed on the market without approval. Despite the regulation, 10 of the 14 liquid propolis samples had a food supplement number on the label, while the approval number of 4 samples was not declared on the label.

According to the Turkish Food Codex Regulation on Labeling and Provision of Food Information to Consumers (TFC, 2017), declarations such as the main ingredient contents, net amount, origin, business registration or approval number, expiration date or shelf life must be included in the packaging. The raw propolis content (%) of extracts is important for standardization. In this study, the main components of 14 propolis samples were listed on the label, and the propolis ratio was stated on the label in 11 samples, except for the P3, P7, and P8 samples. The propolis contents of the samples varied between 7.7-40%. In the P8 example, there is mineral (Zn) and vitamin D supplementation. Shelf life was stated on the label for all samples, and this period varied between 2 and 4 years.

Physicochemical properties of propolis samples

The physical properties of the 14 propolis extract samples were given in Table 2.

The total titratable acidity values of 14 liquid propolis samples were significantly different ($p<0.05$). The difference between the total titration acidity and pH values of the water-based and alcohol-based groups was found to be significant ($p<0.05$) according to the independent variables t-test. The pH values of water-based propolis extracts varied between 3.93 and 8.26, and those of alcohol-based propolis extracts varied between 4.52 and 5.93. In general, the alcohol-based group is more acidic than the water-based group. According to the multiple variance analysis performed within the water-based and alcohol-based groups, the total titration acidity values of the propolis samples showed a significant difference within the groups ($p<0.05$).

Kara et al. (2022) found the pH of ethanolic propolis samples to be between 4.29 and 5.82 and reported that the pH may vary depending on the ethanol content in the solvent used for extraction. Additionally, propolis has an acidic pH due to the phenolic acids it contains. This suggests that the phenolic acid compositions of alcohol-based and water-based propolis extracts will differ, and alcohol-based extracts will have a higher level and greater diversity of phenolic acid profiles. The acidic pH of propolis due to phenolic acids is significant because the effects of these substances in propolis on microorganisms decrease as the pH increases (Ivančajić et al., 2010).

Table 2

Some physicochemical properties of the propolis samples

				Color			
		TTA, %	**pH**	***L****	***a****	***b****	**Brix°**
Water based	P1	0.35 ±0.07^{iiD}	8.08 ±0.09^{bA}	1.51 ±0.51cB	0.89 ±0.05^{efD}	1.68 ±0.28^{cdCD}	8.29 ±0.02^{jD}
	P2	0.00^{iE}	8.26 ±0.08^{aA}	1.73 ±0.08^{cB}	1.75 ±0.18^{defD}	2.38 ±0.20^{cdCD}	8.62 ±0.02^{iC}
	P3	2.5 ±0.14^{hC}	3.93 ±0.03^{hD}	15.70 ±0.51^{aA}	7.17 ±0.11^{aA}	20.08 ±0.37^{aA}	10.62 ±0.03^{tB}
	P4	0.00^{iE}	8.26 ±0.05^{aA}	0.42 ±0.04^{cB}	1.90 ±0.17^{cdefD}	0.56 ±0.05^{cdD}	62.69 ±0.07^{dA}
	P5	21.0 ±0.30^{aA}	4.19 ±0.03^{gC}	2.90 ±0.62^{cB}	3.62 ±0.52^{bcdeC}	4.17 ±0.92^{cC}	10.56 ±0.05^{tB}
	P6	19.3 ±0,30^{abB}	4.39 ±0.01^{fBC}	12.92 ±0.28^{aA}	5.08 ±0.20^{abcB}	14.14 ±0.17^{bB}	1.93 ±0.02^{kE}
Ethanol based	P7	17.95 ±0.20^{bB}	4.45 ±0.03^{fB}	14.97 ±4.15^{aA}	6.99 ±0.09^{aA}	20.42 ±0.58^{aA}	8.52 ±0.04^{ijC}
	P8	6.00 ±0.07^{fF}	5.93 ±0.04^{cA}	0.28 ±0.04^{cC}	0.38 ±0.21^{efB}	0.31 ±0.07^{cdC}	73.81 ±0.12^{cC}
	P9	17.10 ±0.20^{bB}	4.52 ±0.03^{fD}	2.12 ±0.61^{cC}	7.10 ±4.70^{aA}	3.49 ±1.05^{cdC}	39.64 ±0.08^{fE}
	P10	13.90 ±0.30^{dD}	4.52 ±0.01^{fD}	14.38 ±1.05^{aA}	7.51 ±0.37^{aA}	21.63 ±1.37^{aA}	86.98 ±0.03^{bB}
	P11	16.60 ±0.14^{bB}	4.79 ±0.04^{deC}	2.11 ±0.23^{cC}	7.91 ±0.62^{aA}	3.18 ±0.12^{cdC}	36.52 ±0.04^{gF}
	P12	9.7 ±0.14^{eE}	4.96 ±0.02^{dB}	6.47 ±1.20^{bB}	6.68 ±0.76^{abA}	10.21 ±1.60^{bB}	91.64 ±0.08^{aA}
	P13	15.2 ±0.30^{cC}	4.90 ±0.03^{deB}	0.15 ±0.01^{cC}	0.23 ±0.02^{fB}	0.11 ±0.1^{dC}	57.18 ±0.02^{eD}
	P14	17.7 ±0.30^{bA}	4.76 ±0.01^{eC}	1.33 ±0.13^{cC}	4.99 ±0.56^{abcdA}	2.14 ±0.23^{cdC}	35.11 ±0.02^{hG}
Between two groups	*P** value	<0.001	<0.001	0.009	0.279	0.142	0.127

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

Although ºBrix measurements are mostly applied to aqueous solutions, it is also used for ethanolic propolis extracts (Popova et al., 2017). In this study, it was determined that the ºBrix values of the samples were significantly different ($p<0.05$). The difference between the two water-based and alcohol-based groups was not significant ($p>0.05$) according to the independent samples t-test. In this study, the ºBrix values ranged between 1.93 and 62.69 for the water-based samples and between 35.11 and 91.64 for the alcohol-based samples. In a study, it was determined that the ºBrix values of commercial propolis samples obtained from the market varied between 0 and 100, and it was reported that this change was caused by the impurities contained in the propolis or different solvents affecting its solubility. In one study, it was reported that there was a high correlation (r^2: 0.9) between the ºBrix and total polyphenolic content of ethanolic propolis extracts, and the ºBrix value could be used to estimate the total amount of polyphenols contained in the extract (Keskin and Kolaylı, 2019).

While the differences between the samples were found to be significant in terms of the L*, a* and b* values, the difference between the two groups based on water and alcohol was significant for the L* value ($p>0.05$) but was not found to be significant in terms of a* and b* values ($p>0.05$). In addition to the effect of solvent and process parameters on the final product color of liquid propolis extracts, the botanical origin and region of the raw propolis used as a raw material are also important. In fact, color can be used as a distinguishing feature in identifying origin. Contieri et al. (2022) examined the color qualities of propolis in their study. They reported that although commercial propolis extracts should have amber tones, reddish or greenish according to Brazilian legislation, all samples exhibited a light tone, and samples were detected with "reddish pink and gray tones" that did not match the characteristic color.

Antioxidant activity and total phenolic content

The total phenolic content and antioxidant activity of the 14 propolis extract samples were given in Table 3.

The difference between the total phenolic content and antioxidant activity of all the samples was determined as statistically significant ($p<0.05$). Moreover, they varied within a wide range of data. The lowest Trolox Equivalent Antioxidant Capacity (TEAC) was 88.9 µg/ml (P5), the highest was 24205.6 µg/ml (P10), the lowest total phenolic content was 372.22 µg GAE/ml (P5), and the highest was 30333.33 µg GAE/ml (P12). The differences between the two sample groups, water-based and alcohol-based, were found to be significant ($p<0.05$) according to the independent variables t-test. The antioxidant activity and total phenolic content of alcohol-based propolis were greater than those of water-based propolis. Keskin (2018) determined the total phenolic content to be 16.13-178.38 mg GAE/g in the raw propolis samples collected from Turkey when ethanol was used as a solvent, similar to the findings of this research. On the other hand, they reported that the total phenolic content was much lower (0.07-0.22 mg GAE/g propolis) when water was used as a solvent. Kubiliene et al. (2015) reported that the total phenolic content of propolis obtained from Lithuania was 12.7 mg GAE/ml because of alcohol extraction, 1.6 mg GAE/ ml because of water extraction, and 0.5 mg GAE/ml because of olive oil extraction.

The flora plays a crucial role in the antioxidant activity of propolis. Shehata et al. (2018) observed significant variations in the chemical compositions of brown and green propolis collected from different regions, such as Egypt, China, Bulgaria, and Brazil. Studies indicate that the variety, geographical location, climate, and vegetation of the region where propolis is collected are essential in determining its antioxidant capacity.

Table 3

Antioxidant activity and total phenolic content values

	Samples	Antioxidant activity		Total phenolic content
		Inhibition (%)	TEAC*** (μg/ml)	GAE (μg/ml)
Water based	P1	26.15±0.31^{fB}	6155.6±88.89^{fB}	3250.00±50.00^{gB}
	P2	43.15±0.86^{eB}	11011.1±244.44^{eB}	3427.78±16.67^{gB}
	P3	8.74±0.21^{hB}	1183.3±61.11^{hB}	916.67±5.56^{ijB}
	P4	49.38±0.27^{dB}	12788.9±77.78 ^{dB}	7511.11±133.33^{eB}
	P5	4.90±0.31^{iB}	88.9±88.89^{iB}	372.22±5.56^{jB}
	P6	6.05±0.25^{iB}	416.7±72.22^{iB}	1066.67±66.67^{iB}
	P7	22.18±0.39^{gB}	5022.2±111.11^{gB}	2094.44±127.78^{hB}
Ethanol based	P8	78.05±0.74^{cA}	20977.8±211.11^{cA}	10138.89±72.22^{dA}
	P9	84.71±0.51^{bA}	22877.8±144.44^{bA}	18472.22±150.00^{bA}
	P10	89.36±0.10^{aA}	24205.6±27.78^{aA}	29961.11±116.67^{aA}
	P11	87.24±0.43^{aA}	23600.0±122.22^{aA}	13444.44±33.33^{cA}
	P12	83.74±0.47^{bA}	22600.0±133.33^{bA}	30333.33±222.22^{aA}
	P13	82.57±0.04^{bA}	22266.7±11.11^{bA}	5983.33±127.78^{fA}
	P14	89.28±0.02^{aA}	24183.3±5.56^{aA}	13372.22±227.78^{cA}

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

TEAC - Trolox Equivalent Antioxidant Capacity; * GAE - gallic acid equivalent.

The extraction method and conditions, such as solvent type and extraction time, also affect the extraction efficiency and final product properties. In a study conducted by Mokhtar (2019), the use of ethanol as a solvent in maceration extraction resulted in higher phenolic and flavonoid contents than did the use of water. It has also been reported that the highest bioactivity was achieved with a solid/liquid ratio of 1/5. The chemical composition and bioactivity of propolis vary according to seasonality, the flora of the region where hives are located, dominant botanical origin, bee species, and extraction type and parameters (Calegari et al., 2017).

In this study, the botanical origin or geographical location of the commercially available propolis extracts was unknown. However, according to the results, it is possible that the substance used as the extraction solvent and the extraction process used accordingly have an effect on the antioxidant capacity of the final product. On the other hand, the ratio of crude propolis in the propolis extract may also have an effect on antioxidant activity. However,

although the P5 sample was the product with the highest propolis content (40%) according to the label information, it was found to have the lowest antioxidant activity. The antioxidant activities of propolis extracts, which are water-based and commercially available for consumption, show a very heterogeneous distribution. Although many different solvents and processes have been tested for the extraction of bioactive substances from propolis, studies have shown that alcohol extraction is the best method and that alternative solvents and methods used in the production of nonalcoholic extracts are insufficient.

It was reported that there is a positive correlation between the total phenolic content and the antioxidant activity of propolis (Aliyazıcioglu et al., 2013). Many studies have shown that, in addition to antioxidant capacity, all other biological activities, such as antimicrobial, antitumoral, anti-inflammatory, antiviral and antifungal activities, increase in proportion to the amount of phenolic substances (Kolaylı et al., 2010; Tezcan et al., 2011). In this study, a positive correlation was detected between the antioxidant activity (inhibition %) and total phenolic contents (μg/ml) of the examined samples according to the Pearson correlation test and was found to be statistically significant ($p<0.05$). It is known that the positions of the hydroxyl groups of flavonoids, which are phenolic components, change their antioxidant capacity (Cai et al., 2006). As a result, the presence of a specific flavonoid can increase the total amount of phenolic substances, which can increase the total antioxidant capacity.

Identification of phenolic compounds

In this study, the phenolic compounds in propolis extracts were characterized via LC–MS/MS. The results were given in Table 4a and b. Phenolic compounds, which are secondary metabolites of plant origin and are found in many natural products, are agents responsible for antioxidant activity (do Nascimento et al., 2016; Zhang et al., 2018). According to previous studies, at least 300 different propolis compounds have been identified; their biological activities are attributed mainly to phenolic components, such as flavonoids (flavonols, flavones, flavonones, dihydroflavonols, and chalcones); aromatic aldehydes; terpenes; alcohols; and beta-steroids. Phenolic compounds have biological activities, such as antioxidant potential, because they have hydroxyl groups and aromatic compounds in their chemical structures (Milene Angelo and Jorge, 2007; Gülcin, 2012). According to different studies, the main phenolic compounds identified in propolis are hydroxycinnamic and hydroxybenzoic acids (Calegari et al., 2017; Xavier et al., 2017), chrysin, galangin, pinocembrin, apigenin, quercetin, luteolin, ferulic acid, benzoic acid, cinnamic acid, flavones, flavonols, and flavanones (Kar et al., 2019; Peter et al., 2017; Woźniak et al., 2020). In this study, catechin, myricetin, naringenin, quercetin, rutin trihydrate, chrysin, apigenin and CAPE were detected in all the samples.

Luteolin was not detected in water-based samples (except P7) but was detected in all alcohol-based samples. Triacetin was detected in all the samples except P8. In general, the total flavonoid content of alcohol-based propolis extracts was greater than that of the water-based samples in this study. Several studies have shown that the ethanol ratio in the solvent affects the phenolic compound composition of propolis extracts. While the phenolic acid content is high in solvents with high water content, no significant change is observed in solvents containing 70% or more ethyl alcohol (Kara et al., 2022).

In terms of phenolic contents of the chestnut propolis extracts, CAPE, apigenin and chrysin were determined to be the most abundant compounds, followed by quercetin and naringenin. The beneficial biological activities of propolis, such as antimicrobial, anti-inflammatory, antiulcer, and anticancer properties, are closely related to its bioactive compounds. Flavonoids are effective against various bacteria and protect against ulcers

(Ruiz-Hurtado et al., 2021). While chrysin is known to have anti-inflammatory and antineoplastic effects and functions as an important antioxidant and hepatoprotective agent (liver protector) (Shahbaz et al., 2023), another major component of propolis that causes its anticancer effect is caffeic acid phenyl ester (CAPE) (Keskin, 2018). The sample with the highest CAPE, chrysin, quercetin and naringenin contents among the alcohol-based samples was P10, which had the highest raw propolis content (38%). A study comparing the phenolic compositions and antioxidant activities of propolis samples extracted with different ethanol concentrations and extraction conditions revealed caffeic acid to be the main component in extracts prepared with solvents containing 0-40% ethanol and revealed chrysin and pinocembrin to be the main components in extracts containing 60% or more ethanol (Kara et al., 2022). Therefore, the percentage of raw propolis and alcohol used in the preparation of the extract has an effect on the phenolic composition of the final product.

Table 4b shows the phenolic acid contents of the liquid propolis samples. Although tannic acid could not be detected in any water-based chestnut propolis extracts, it was detected only in P9 and P13 in the alcohol-based samples. The major phenolic acids in the water-based samples were trans ferulic, caffeic, cinnamic and gallic acids, while in the alcohol-based samples, they were trans ferulic, caffeic, cinnamic and ellagic acids. P4 from water-based samples and P10 from alcohol-based samples were the propolis extracts with the highest phenolic acid content. In this study, the P4 and P10 samples, which used chestnut propolis on their labels, had the highest CAPE, ferulic acid, and ellagic acid contents and the highest antioxidant activity (inhibition %). In another study, propolis samples containing caffeic acid phenyl ester (CAPE) presented stronger radical scavenging activity (Sulaiman et al., 2014). This indicates that the botanical origin of propolis used as a raw material has an impact on the bioactivity of the propolis extract.

According to the Turkish Food Codex Bee Products Communiqué, liquid propolis consumed as a food supplement (coumaric acid (total of o, m and p-), pinobanksin, galangin, cinnamic acid and 3-4 dimetoksi cinnamic acid (total), chrysin, pinosembrin, caffeic acid, CAPE, caempferol, ferulic acid (total of izo and trans), quercetin, naringenin, rutin, apigenin, protocatechuic acid and artepilin C) must contain at least 5 phenolic substances at a minimum level of 500 mg/l. In Tables 5a and 4b, these phenolic compounds foreseen in the Communiqué were marked (*). As seen from the tables, none of the propolis extracts obtained from the national market meet the specified phenolic substance limits in the codex. Moreover, it is considered that the phenolic compound composition alone is insufficient in the bee products regulation. In addition, similar to the regulations in various countries concerning propolis, it is necessary to establish the total phenolic content or total flavonoid content as a quality criterion.

Mineral composition and heavy metal contents

Propolis is an important food supplement for human nutrition because of its antibacterial, antifungal, antiviral, and antioxidant activities. Therefore, it is crucial to determine propolis's content in terms of essential minerals and heavy metals. In this study, the concentrations of several elements (Ca, Mg, K, Mn, Fe and Zn) and heavy metals (Hg, As, Cd and Pb) in propolis extracts were determined using ICP–OES (Table 5). Heavy metals and other elements demonstrated a heterogeneous distribution in propolis extracts. Therefore, the difference between the means of the two alcohol and water-based groups was not found to be statistically significant ($p<0.05$) in terms of Ca, Mg, K or Mn. Fe and Zn were found to be significant ($p<0.05$) because these two elements were below the detection limits in most of the propolis samples. The amount of Zn in the P8 sample was found to be very high

because it was already declared on the label as Zn supplemented. While the K content was found to be highest for all the samples, Fe and Zn were rarely found. The mineral contents of the two groups, regarding the solvent type, could not be compared clearly because the data were quite heterogeneous. Although P7 is water-based and P13 is alcohol-based, they have similar contents. Soós et al. (2019) determined that the elemental content of propolis extracts is significantly affected by the composition of the extraction solvent, and in most cases, the extraction time has a large impact. It has been reported that most of the analyzed elements dissolve better in aqueous solutions than in solvents containing ethanol; however, with a few exceptions, elements essential to the human body are also well extracted in solvents containing 50% or 80% (v/v) ethanol. Görkem (2022) reported that in commercial propolis drop samples, in addition to Fe and Na, other elements (Ca, Mg, K, Al, B, Cu, Mn, P and Zn) were found to be more abundant in a sample extracted with water than in samples extracted with alcohol and water + glycerol. Ristivojević et al. (2023) determined that microelements were variable, macroelements (Na, Ca, Mg, K and P) were more common, and the Ca content was the highest in twentytwo propolis samples that differed in terms of geographical and botanical origins in Serbia. Mineral diversity is passed on to the composition of propolis by transferring the mineral composition of the soil to the plants from which the propolis is obtained. Therefore, plant sources strongly influence the elemental composition of propolis [38]. As a result, it is thought that the botanical origin, parameters such as time, temperature, and several applications in propolis extraction impact the elemental distribution and concentrations.

The heavy metal contents of the samples were given in Table 5b. The heavy metal contamination limits for food supplements in the Turkish Food Codex Regulation of Contaminants (TFC, 2023) are 3 mg/kg for Pb, 1.0 mg/kg for Cd, and 0.1 mg/kg for Hg. There is no limit value for arsenic (As). Although arsenic was not detected in water-based propolis extracts, it was detected in all alcohol-based propolis extracts except for the P8 and P13 samples. The lowest amount of arsenic was 1031.25 µg/kg (P10), and the highest was 2445.80 µg/kg (P11).

Since the Hg contents of all propolis samples in this study were below the detection limit (0.4 mg/kg), they could not be evaluated statistically, and comparisons could not be made according to the TFC Regulation of Contaminants. The Cd values of the samples were determined to be the lowest at 185.6 µg/kg (P8) and the highest at 232.40 µg/kg (P12), and since all samples were below the limit value (<1 mg/kg), they were appropriate according to the contaminant regulations. The differences between the samples were not significant in the water-based group ($p>0.05$) but were significant in the alcohol-based group ($p<0.05$). The Pb values of the samples were determined to be the lowest at 502.7 µg/kg (P1) and the highest at 855.2 µg/kg (P8), and since all samples were below the limit value (<3 mg/kg), they were appropriate according to the contaminant regulations. The Pb content of the water-based group was lower than that of the alcohol-based group ($p<0.05$).

Because plant sources and soil strongly influence the elemental composition of propolis, the basic propolis content can be used to develop reliable traceability methods and distinctive features of the geographical areas where it is produced to indicate environmental pollution (Golubkina et al., 2016). However, the detection of As, especially in alcohol-based samples, in this study suggested that extract production factors such as solvent qualities, in addition to plant and soil qualities, are also effective for detecting heavy metal contamination.

Table 4

Flavonoid (a) and phenolic acid (b) contents of liquid propolis samples

	(ppb)	Catechin	Mirisetin	Naringenin*	Quercetin*	Rutin*	Luteolin	Chrysin *	Apigenin*	CAPE*	Triacetin	Total flavonoid (ppm)
Water based	P1	1698.28	1109.37	241.54	723.62	480.69	-----	163171.53	52191.19	1582.39	1938.53	223.137
	P2	425.66	1216.36	822.65	820.53	495.27	-----	83540.17	61255.76	2475.28	3338.48	154.390
	P3	2278.98	1231.35	29137.31	12119.98	1211.38	-----	42383.26	15946.98	30759.63	2662.11	137.731
	P4	1594.73	1223.18	50326.08	1226.59	1302.52	-----	183255.37	62563.74	302348.06	2677.54	606.518
	P5	668.87	108.82	1275.95	163.28	50.16	-----	12132.26	3441.37	65.90	5351.08	232.576
	P6	171.21	1109.21	15384.22	1505.74	503.31	-----	56712.49	11724.32	44490.57	2773.36	134.374
	P7	1554.47	2538.24	31700.26	28273.76	8653.00	1409.32	31654.46	38448.91	62663.48	2259.10	209.155
Ethanol based	P8	501.66	1894.13	41203.99	43532.22	30997.50	5330.91	88643.11	122669.46	136380.31	-----	471.153
	P9	1517.46	7192.16	92555.68	110944.03	25826.11	11508.03	83669.99	103957.15	431734.70	1733.27	870.639
	P10	1006.02	27877.75	187138.01	335933.89	4582.85	68736.66	173957.97	97849.53	804898.84	3481.76	1705.463
	P11	1030.00	1516.66	91773.18	154905.19	6606.80	40295.97	125398.19	152157.10	476212.41	2430.03	1052.326
	P12	1464.30	8707.65	146142.00	122668.44	2088.98	112053.92	172442.71	313710.50	697909.15	11001.99	1588.190
	P13	823.28	2051.87	67871.83	42315.71	12538.39	11459.01	84767.58	73791.34	222212.11	3534.82	521.366
	P14	1195.56	2283.71	63196.52	62623.03	3083.76	856.75	107240.42	43526.17	474079.80	9511.22	767.597

a

	(ppb)	Cinnamik acid*	Gallic acid	Tannic acid	Cafeic acid*	2-5, Dihydroxy benzoic acid	Trans ferulic acid*	Ellagic acid	Total phenolic acid (ppm)
Water based	P1	58450.54	13753.26	-----	4782.78	6.79	4473.17	2347.21	83.814
	P2	53905.98	18036.81	-----	8577.11	205.52	5541.38	9614.61	95.881
	P3	17967.96	2426.63	-----	120729.46	-----	9274.57	10387.19	160.786
	P4	81810.10	4044.50	-----	114837.86	50.59	728639.38	2074.71	931.457
	P5	584.38	4898.46	-----	4198.82	28.49	1557.38	223.80	11.491
	P6	8631.01	6729.90	-----	147326.29	-----	75817.91	1848.80	240.354
	P7	10538.64	4279.65	-----	352788.11	208.05	257101.52	24222.41	649.138
Ethanol based	P8	99732.59	20968.94	-----	25871.21	763.58	57368.14	46999.98	251.705
	P9	213153.99	9250.30	176506.07	407085.56	91.28	71362.26	96421.70	973.871
	P10	96462.05	5914.59	-----	515156.69	308.98	1348264.31	251768.28	2217.875
	P11	250661.62	10022.72	-----	317015.62	39.28	140639.74	142636.50	861.016
	P12	141871.22	4392.37	-----	356322.53	-----	260053.17	120861.73	883.501
	P13	87747.12	11753.58	53549.92	524699.46	267.67	141010.21	33164.36	852.192
	P14	184581.14	24595.99	-----	451022.94	146.21	874694.26	56777.39	1591.818

* Phenolic compounds that must be present in a propolis extract according to the Turkish Food Codex Bee Products Communiqué

b

Table 5

Mineral (a) and heavy metal (b) contents of the liquid propolis samples

		Ca (mg/kg)	Mg (μg/kg)	K (mg/kg)	Mn (μg/kg)	Fe (μg/kg)	Zn (μg/kg)
Water based	P1	59.40 ±1.00^{deB}	4628.35 ±167.40^{aD}	179.45 ±10.0^{ghC}	< 0.076	884.90 ±43.40^{bA}	< 0.554
	P2	27.20 ±0.30^{deB}	1615.2 ±9.75^{aEF}	151.00 ±3.20^{hD}	< 0.076	118.05 ±1.50^{dC}	< 0.554
	P3	24.30 ±0.15^{deB}	1225.45 ±5.75^{aF}	99.80 ±0.95^{iE}	< 0.076	< 1.243	< 0.554
	P4	28.00 ±0.30^{deB}	2350.45 ±18.70^{aE}	160.4 ±3.^{20hCD}	36.45 ±0.080^{efC}	< 1.243	< 0.554
	P5	57.55 ±0.20^{deB}	11003.6 ±15.10^{aB}	58.05 ±1.10^{iF}	< 0.076	< 1.243	< 0.554
	P6	212.15 ±3.50^{abA}	8038.75 ±63.45^{aC}	235.00 ±3.65^{fB}	231.05 ±4.6^{cB}	263.45 ±2.95^{cB}	127.6 ±7.50
	P7	257.75 ±30^{aA}	35350.3 ±406.12^{aA}	1097.65 ±6.35^{bA}	449.5 ±29.64^{aA}	< -5.616	89.15 ±34.84
Ethanol based	P8	111.6 ±1.55^{cC}	12863.75 ±178.35^{aA}	642.7 ±4.20^{dC}	132.35 ±2.40^{dD}	5472.4 ±38.25^{a}	15582.8 ±91.00
	P9	30.3 ±0.05^{deE}	2596.5 ±32.50^{aA}	153.6 ±1.70^{hF}	< 0.076	< 1.243	< 0.554
	P10	155.4 ±0.85^{cB}	15635.75 ±166.8^{aA}	720.2 ±2.30^{cB}	363.75 ±2.80^{fF}	< 1.243	< 0.554
	P11	16.30 ±0.15^{eF}	2373.00 ±21.30^{aA}	198.70 ±2.05^{gE}	< 0.076	< 1.243	< 0.554
	P12	63.05 ±0.40^{dD}	18029.00 ±2.42^{aA}	571.8 ±4.95^{eD}	61.55 ±0.03^{eE}	< 1.243	< 0.554
	P13	209.30 ±2.75^{bA}	51504.50 ±133.50^{aA}	1831.00 ±6.65^{aA}	403.40 ±2.95^{bA}	< 1.243	142.3 ±8.45
	P14	57.5 ±0.55^{deD}	5767.10 ±46.05^{aA}	194.4 ±0.35^{gE}	225.15 ±2.50^{cC}	< 1.243	< 0.554
	*p**	0.140	0.817	0.238	0.940	0.007	0.001

a

		Hg (mg/kg)	As (µg/kg)	Cd (µg/kg)	Pb (µg/kg)
Water based	P1	<0.4	< 3.034	199.80 ±4.10^{aA}	502.7 ±51.60^{eB}
	P2	<0.4	< 3.034	211.40 ±1.75^{aA}	662.35 ±17.35^{deAB}
	P3	<0.4	< 3.034	214.15 ±1.05^{aA}	684.00 ±13.05^{cdAB}
	P4	<0.4	< 3.034	197.85 ±0.05^{aA}	688.30 ±21.70^{bcdAB}
	P5	<0.4	< 3.034	227.90 ±1.65^{aA}	832.1 ±41.00^{abcA}
	P6	<0.4	< 3.034	223.30 ±1.80^{aA}	812.60 ±0.10^{abcdA}
	P7	<0.4	< 3.034	227.40 ±47.84^{aA}	824.45 ±70.20^{abcdA}
Ethanol based	P8	<0.4	< 3.034	185.60 ±0.10^{aE}	855.15 ±22.75^{aA}
	P9	<0.4	1898.35 ±182.80^{bAB}	211.00 ±2.80^{aD}	761.9 ±19.75^{abcdB}
	P10	<0.4	1031.25 ±56.3^{cC}	227.25 ±1.90^{aAB}	806.3 ±14.7^{abcdAB}
	P11	<0.4	2445.80 ±76.95^{aA}	209.40 ±1.10^{aD}	764.20 ±17.10^{abcdB}
	P12	<0.4	1129.25 ±3.51^{cC}	232.40 ±0.08^{aA}	805.40 ±0.20^{abcdAB}
	P13	<0.4	< 3.034	216.45 ±2.15^{aCD}	849.45 ±9.40^{abA}
	P14	<0.4	1336.75 ±182.1^{cBC}	221.15 ±1.45^{aBC}	781.15 ±7.60^{abcdAB}
	*P**	-	<0.001	0.416	0.006

b

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

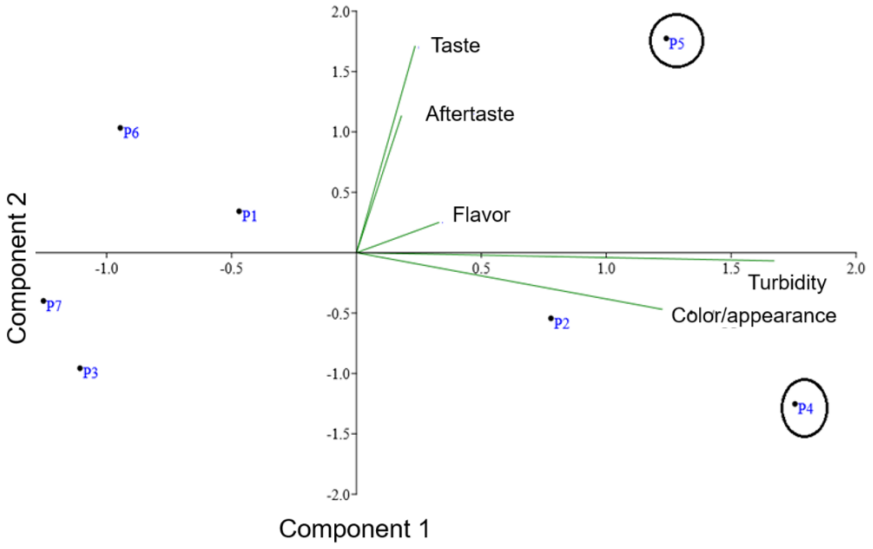
It is crucial that propolis extracts offered for consumption as food supplements adhere to common criteria and are standardized. For the standardization of these products, not only the phenolic substance composition but also the characteristics of the raw propolis ratio, total polyphenol content, phenolic composition, contaminant limits (such as heavy metals and pesticides), solvent types, packaging, and labeling must be determined. This study found that alcohol- and water-based commercial propolis offered by different companies varied significantly in terms of label declarations, physical and bioactive properties, mineral and heavy metal contents, and sensory properties. While alcohol-based extracts generally show higher values, especially in terms of bioactive properties, intragroup variability exists in both alcohol- and water-based groups. Standardization of the content of these products will only be achievable by complying with legislation, ensuring consistency in consumption recommendations, pricing, and labeling. Given the variability in propolis extracts offered for commercial consumption, the publication of common legislation containing quality limits has become important and a priority. This study recommends that unusual situations, such as the detection of arsenic in alcohol-based extracts, be taken into consideration, relevant regulations be revised, and the results of this and similar scientific studies be used to establish quality limits in legislation. Similar to regulations in various countries, such as Brazil, it is recommended to establish a limit for arsenic (As) content in our national legislation for propolis.

Sensory Properties

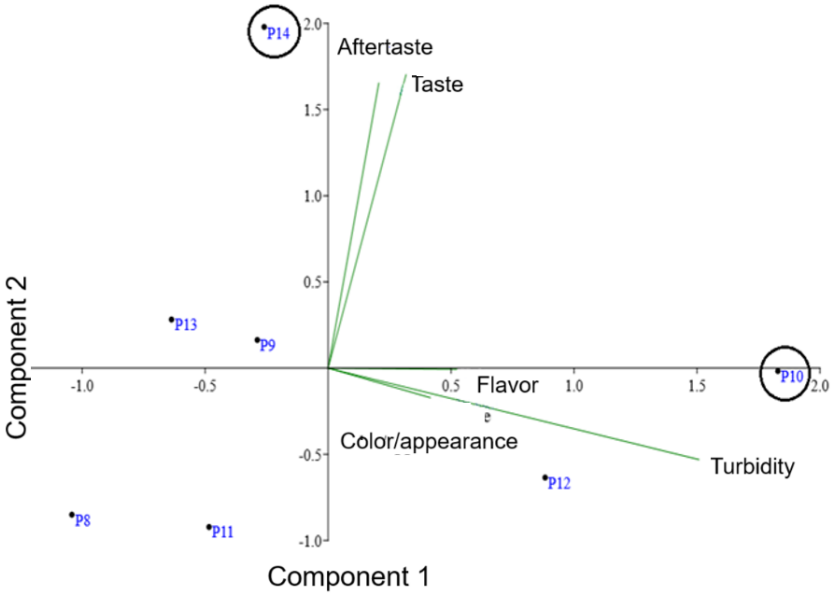
Water and alcohol-based propolis extracts were prepared in separate groups at a concentration of 10 drops/100 ml of water and were evaluated for sensory activity by panelists at different times. The sensory evaluation was based on five basic criteria: taste, flavor, turbidity, color/appearance, and aftertaste. The panelists rated each criterion on a scale from 1 (I did not like it at all) to 5 (I liked it very much). The average scores for each criterion were calculated, and principal component analysis (PAST 4.03 Statistical Analysis App for Windows) was applied to determine product similarity and clustering tendencies according to the sensory evaluation results.

To determine the clustering tendency and similarities of the ethanol-based and water-based propolis samples according to their sensory analysis scores, the resulting water-based products (Figure 1a) and alcohol-based products (Figure 1b) were evaluated by principal component analysis. For water-based products, the most popular product was P4 in terms of color/appearance and turbidity qualities and P5 in terms of taste, flavor, and aftertaste properties. Although these samples did not show a clustering tendency with the others, the P1 and P6 samples and the P3 and P7 samples were found to be similar and showed clustering.

When the ethanol-based products were evaluated, P10 was the most liked product in terms of color/appearance, turbidity, and flavor properties, while P14 was the most liked product in terms of taste, aroma, and aftertaste properties. Although these samples did not show a clustering tendency with the others, the P9 and P13 samples and the P8 and P11 samples were found to be similar and showed clustering among themselves.



a



b

Figure 2. Principal component analysis graphs of water-based (a) and alcohol-based (b) propolis samples

Conclusions

It is crucial that propolis extracts offered as food supplements meet common criteria and are standardized. For effective standardization, it is necessary to determine key characteristics such as the raw propolis content, total polyphenol content, phenolic composition, contaminant limits (e.g., heavy metals and pesticides), solvent types, packaging, and labeling requirements. Although the Bee Products Communiqué has only recently been published, it is considered necessary to review the characteristics and limit values of raw propolis and propolis extracts. This study found that commercial alcohol- and water-based propolis extracts offered by various companies vary significantly in terms of label declarations, physical and bioactive properties, mineral and heavy metal contents, and sensory characteristics. While alcohol-based extracts generally exhibited higher values, particularly in bioactive properties, variability was observed within both alcohol- and water-based groups. To achieve standardization in consumption recommendations, pricing, and labeling, compliance with finalized legislation is essential. Given the variability in commercially available propolis extracts, the publication of legislation with quality limits has become a priority. This study highlights the need for unusual findings, such as the detection of arsenic in alcohol-based extracts, to be addressed in the relevant regulations. Revising these regulations based on the results of this and similar scientific studies will be critical for establishing quality standards in future legislation.

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Chemical composition of seeds of industrial Ukrainian hemp varieties

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Abstract

Introduction. The aim of the work was to study the chemical composition and characteristics of seeds of industrial Ukrainian hemp varieties.

Materials and methods. The research materials are seeds of the industrial hemp varieties Hliana, Hlesiia, Hlukhivs`ki 51, Artemida, and Harmoniia. The content of moisture, protein, oil, fiber, and ash were determined by standard methods. The mineral composition was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Results and discussion. The chemical composition of industrial hemp seeds of the analyzed varieties averaged, %: oil – 37.38, protein – 24.22, fiber – 34.71, ash – 5.15, moisture – 8.31. The highest content of oil (38.90 %) and fiber (37.63 %) was found in the seeds of Artemida, and the highest content of protein (25.68 %) and ash (5.63 %) was detected in Hlukhivs`ki 51. The average oil content in seeds of Ukrainian hemp varieties was by 5.5 % higher than the average oil content in the seeds of the best known world hemp varieties. The average content of macroelements in seeds of Ukrainian hemp varieties was, g/kg of dry weight: calcium, 1.67, phosphorus, 8.08, sodium, 0.06, potassium, 6.82, magnesium, 4.04. The average content of trace elements in the studied seeds was, mg/kg of dry weight: copper, 12.62, zinc, 54.27, iron, 106.28, cobalt, 0.06, manganese, 95.85, meanwhile content of lead was low, 0.08 mg/kg of dry weight and cadmium was not detected. The average content of calcium and copper in the seeds of Ukrainian industrial hemp varieties exceeded the average content of these elements in the seeds of world industrial hemp varieties by 21% and 6%, respectively. The phosphorus content in seeds of studied Ukrainian hemp varieties was almost the same as in the seeds of known world varieties. The average content of sodium, potassium, magnesium, zinc, iron, and manganese in seeds of tested Ukrainain hemp varietiers was lower than the average content in seeds of the known world varieties.

Conclusions. The presented characteristics of seeds of Ukrainian hemp varieties and the comparison with well-known world analogues confirm the high nutritional value of the varieties and its great potential for application.

Introduction

Hemp has been cultivated for thousands of years as a fiber, grain, and medicinal plant. However, strict controls on the cultivation of cannabis for illicit use, the proliferation of other threads and oilseeds, and the emergence of cheap synthetic fiber have led to a decline and even eradication of hemp production. Hemp has been banned in most countries for more than seven decades, and it missed out on the Green Revolution and the adoption of new technologies and varieties (Viskovic et al., 2023).

In the late 60s of the last century, Ukrainian selectionists began working on the creation of cannabis varieties with a minimized content of tetrahydrocannabinol, which is a psychotropic substance. The first varieties (YuSO-14, YuSO-16) that did not cause narcotic excitement were developed at the Institute of Bast Crops of the National Academy of Agrarian Sciences of Ukraine in 1980. In these hemp varieties, the content of tetrahydrocannabinol did not exceed 0.2 %. The year 2011 was a landmark in hemp selection, when scientists at the Institute of Bast Crops of the National Academy of Agrarian Sciences of Ukraine developed the world's first hemp variety, Victoriia, which did not contain tetrahydrocannabinol (Holovii et al., 2024). Such hemp was called non-narcotic hemp, and a little later technical or industrial hemp, depending on the direction of processing. Since then, the hemp industry, previously banned in most European countries due to the rise of drug addiction, has been developing rapidly.

In recent years, the area allocated for hemp cultivation in the EU has increased by 60 % (Figure 1). During the same period, hemp production increased by 84.3 %. France is the largest producer of hemp in the EU with more than 60 % of production, followed by Germany with 17 % and the Netherlands with 5 % (An official website of the European Union: Farming. Crop productions and plant-based products, 2024).

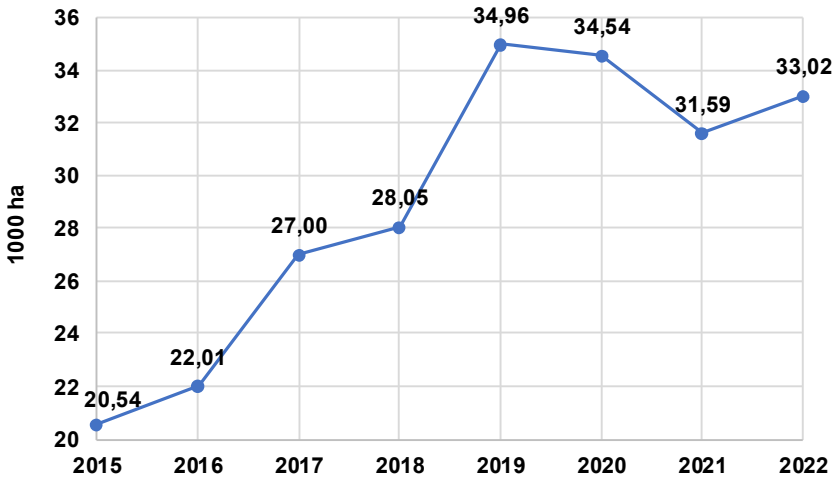

Figure 1. The area of land in the EU devoted to hemp cultivation, according to EUROSTAT

The rapid growth of industrial hemp and its use in the production of various goods is of interest to many scientists and manufacturers. Hemp is used to produce a variety of goods, including textiles (fabrics, yarns, carpets, canvas, and ropes), paper, medicines, food, animal feed and bedding, paints, biofuels, biodegradable plastics, biocomposites, and building

materials. The rapid production of biomass and the ability to grow in a variety of conditions make hemp a good candidate for remediation of contaminated land (Rehman et al., 2021; Viskovic et al., 2023).

Hemp fiber differs from others in its aseptic properties, high absorption capacity, protection from ultraviolet radiation, and lack of allergic effects. According to a study by Schumacher, hemp is a promising alternative to cotton, and growing hemp will reduce agricultural costs by 77.63 % compared to cotton (Schumacher et al., 2020).

Hemp seeds were originally considered a by-product of the industrial fiber industry (Farinon et al., 2020). Hemp seeds began to be used about 3000 years ago, when they were the main source of food for the population of the region that is now China and Nepal (Viskovic et al., 2023). Hemp seeds have a valuable composition, which can be influenced by various factors, including varietal characteristics and the region of cultivation (Arango et al., 2024; Mamadaliev et al., 2023). Hemp seeds contain 25–35 % oil with a balanced composition of fatty acids, 20–25 % proteins that are easily digestible by the human body and contain all essential amino acids, 20–30 % carbohydrates, as well as a significant amount of minerals, deficient coarse dietary fiber, vitamins, antioxidants and other biologically active substances (Capcanari et al., 2024; Oseyko et al., 2021). Given the biochemical composition of hemp seeds, it should be positioned as the most nutrient-rich food product. Hemp seeds can be consumed both as a whole and as processed products – kernels, oil, flour, protein concentrates, and fiber (Oseyko et al., 2021; Sheichenko et al., 2024).

In modern technologies, in order to increase the nutritional and biological value of food products, industrial hemp seeds and its processing products are used, in particular in the production technologies of bakery, dairy, meat, confectionery products, as well as products for children and special nutrition (Oseyko et al., 2021; Xu J. et al., 2022).

It is important to continue the active study of the biochemical composition of seeds of new varieties of industrial hemp to ensure its competitiveness.

The aim of the work was to study the chemical composition of industrial hemp seeds grown in Ukraine, in particular, the varieties Hliana, Hlesiia, Hlukhivs`ki 51, Artemida, and Harmoniia.

Materials and methods

Materials

Ukrainian industrial hemp varieties Glyana, Glesia, Glukhovskaya 51, Artemida, and Garmoniya, from which the seeds for the study were obtained (Figure 2), were grown in the research areas of the Institute of Bast Crops (Glukhov, Sumy region, Ukraine) in compliance with the conditions of spatial isolation.

Methods

Determination of moisture in hemp seeds

The moisture in hemp seeds was determined by drying at 105 °C (± 2 °C) to a constant weight (Kumar et al., 2013).

Figure 2. Seeds of Ukrainian industrial hemp varieties

Determination of total nitrogen and crude protein content

The total nitrogen and crude protein content in the hemp seeds was determined by the Kjeldahl method (Cruz, 2013). A conversion factor equal 6.25 was applied to calculate crude protein content that was based on measured total nitrogen (Mariotti et al., 2008).

Determination of crude oil content

The crude oil content in hemp seeds was determined by extraction in a Soxhlet apparatus (Kumar et al., 2013).

Determination of crude fiber content

Defatted material, 1 g, was treated with 200 ml of 1.25 % sulfuric acid solution in commercial fiber determination bags (Marichal et al., 2011). The residue after filtration and washing with hot distilled water was treated with 1.25 % sodium hydroxide solution and filtered. The residue was washed in the following sequence: hot distilled water, 1 % nitric acid solution 3 times with hot distilled water. The remaining fiber was dried, weighed, and burned in a muffle furnace. The weight of the ash residue was subtracted and the weight of crude fiber in the sample was obtained (Kumar et al., 2013).

Determination of crude ash content

The ash content was determined after heating and burning in a muffle furnace for 5–6 hours at a temperature of 525±25 °C to a constant weight (Kumar et al., 2013).

Determination of mineral content

Approximately 0.5 g of the pre-ground material was placed in a mineralization vessel and 10 ml of 65 % nitric acid and 1 ml of concentrated hydrochloric acid (Sigma-Aldrich reagents) were added. The sample was microwave digested for 45 minutes (including cooling time) at 185 °C in a commercial pressurized microwave digestion system (Multiwave GO Plus, manufactured by Anton Paar, Austria).

The solution was diluted with ultrapure water to a specified volume. Mineral concentrations were determined by inductively coupled plasma atomic emission spectrometry ICP-AES (Agilent 5110) as presented by Khan et al. (2022). The analysis was performed using an external standard (Agilent, multi-element standard solution). All calibration curves were obtained for 6 different concentrations.

Statistics

All experiments were performed in triplicate and mean±standard deviation was shown.

Results and discussion

Chemical composition of seeds of Ukrainian industrial hemp varieties

The content of moisture, protein, oil, fiber and ash in seeds of industrial Ukrainian hemp varieties are shown in Table 2.

It was shown that: (a) the moisture content in the seeds of industrial hemp varieties was on average 8.31 %, oil – 37.38 %, protein – 24.22 %, fiber – 34.71 %, and ash – 5.15 %; (b) the highest content of oil (38.90 %) and fiber (37.63 %) was found in the seeds of Artemida; protein (25.68 %) and ash (5.63 %) in Hlukhivs`ki 51 varieties; (c) the lowest content of oil (35.11 %) was in the seeds of Hliana, protein (22.92 %) in Harmoniia, fiber (31.49 %) in Hlukhivs`ki 51, and ash (4.55 %) in Artemida varieties. Thus, the seeds of industrial Ukrainian hemp varieties can be positioned as modern raw materials for efficient complex processing.

Table 2

Chemical composition of seeds of Ukrainian industrial hemp varieties

Variety	Moisture, %	Content, % dry weight			
		oil	protein	fiber	ash
Hliana	8.93±0.13	35.11±1.89	25.58±0.32	36.11±1.12	5.55±0.21
Hlesiia	8.55±0.18	37.16±1.25	23.57±0.41	33.73±1.25	4.71±0.42
Hlukhivs`ki 51	7.86±0.11	37.59±1.37	25.68±0.26	31.49±1.08	5.63±0.38
Artemida	7.98±0.21	38.90±1.54	23.34±0.52	37.63±1.41	4.55±0.29
Harmoniia	8.24±0.22	38.15±1.48	22.92±0.17	34.58±1.33	5.32±0.40
Average value	8.31	37.38	24.22	34.71	5.15
Minimum value	7.86	35.11	22.92	31.49	4.55
Maximum value	8.93	38.90	25.68	37.63	5.63

Chemical composition of the seeds of Ukrainian industrial hemp varieties was compared with the published data on world varieties. The seeds of industrial hemp varieties Alyssa, CanMa, Anka, Jutta, Yvonne, Delores, CFX-1, CFX-2, CRS-1, and Finola, grown in Canada, contained 26.9–30.6 % oil; 23.8–28.0 % protein, 32.7–38.8 % fiber, and 5.1–5.8 % ash. The richest in oil, protein, and ash content was Finola, and the richest in fiber content was Anka (Vonapartis et al., 2015).

Seeds of industrial hemp varieties CFX-2, X-59, CRS-1, Grandi, Picolo, CFX-1, Katani, Canda, Delores, and Joey grown in the USA (North Dakota) contained 32.75–35.88 % oil; 24.30–28.13 % protein; 32.47–37.53 % carbohydrates, and 4.86–6.08 % ash. Variey CFX-1 had the highest oil and ash content, CFX-2 in protein, and Delores in carbohydrates (Lan et al., 2019).

Seeds of industrial hemps cultivars grown in Greece - Santhica 27 (France), Fedora 32 (France), Felina 32 (France), Futura 75 (France), Tygra (Poland), Bialobrzeskie (Poland), and Finola (Finland) - contained 8.5–29.2 % oil, 12.2–25.4 % protein, 4.4–5.3 % ash, and 40.8–74.5 % carbohydrates. The highest content of oil and protein was in Finola, and the highest carbohydrate content was in Santhica 27 (Irakli et al., 2019).

Seeds of industrial hemp grown in the USA (Kansas) of the varieties Fedora 17, Helena, Joey, Hlukhivs`ki 51, Katani, Felina 32, Futura 75, Tygra, Hlesiia, CRS-1, Canda, YuSO 31, and CFX-1 contained 28.03–33.23 % oil, 26.48–32.03 % protein, 28.78–36.55 % fiber, and 5.43–6.32 % ash. Among the analyzed varieties the highest content was found: oil in Katani, protein in CRS-1, fiber in Canda, and ash in Fedora 17 (Xu Y. et al., 2021).

Seeds of hemp grown in Spain of the varieties Bialobrzeskie, Carmagnola, Fedora 17, Felina 32, KC Dora, Kompolti, Santhica 27, Tiborszallasi contained 29.1–32.66 % oil, 18.3–23.0 % protein, 32.5–40.4 % fiber, and 8.7–10.4 % ash. Bialobrzeskie has the highest oil and protein content among the analyzed varieties, Kompolti has the highest fiber content, and Santhica 27 has the highest ash content (Alonso-Esteban et al., 2022).

Seeds of Henola and Bialobrzeskie hemp varieties grown in Poland contained an average of 32.52 % oil, 23.47 % protein, 28.15 % fiber, and 4.66 % total ash. Henola seeds contained more oil, 32.75±1.7 %, Bialobrzeskie seeds contained more protein, 23.54±0.59 %; fiber, 28.88±0.18 %, and ash, 4.66±0.47 % (Teleszko et al., 2022).

Seeds of hemp varieties Felina 32, Santhica 27, and Rodnik, grown in Uzbekistan, contained 29.10–31.70 % oil, 23.44–26.95 % protein, and 4.95–6.10 % ash. Among the analyzed varieties, seeds of Santhica 27 had the highest oil and ash content, while Rodnik had the highest protein content (Mamadaliev et al, 2023).

Industrial hemp seeds of Antal, Bacalmas, Fibrol, KC Dora, KC Virtus, KC Zuzana, Kompolti, Monoica, Tiborszallasi, Tisza (Hungary), Carmagnola (Italy), Chameleon (Netherlands), Dioica 88, Epsilon 88, Fedora 17, Felina 32, Ferimon FR 8194, Futura 75, Santhica 23 (France), Helena, Marina, Novosadska, Novosadska+, Simba (Serbia), Kina (China), Lovrin 110, Srcuienijubilee (Romania), Silesia, and Wojko (Poland) varieties, grown in different countries, contained 21.12–35.67 % oil, 21.63–28.92 % protein, 25.49–43 % carbohydrates, and 4.4–7.49 % ash. Industrial hemp seeds of the Fibrol variety had the highest content of oil, Tisza –of protein, Epsilon 88 – of carbohydrates, and KC Virtus – of ash (Arango et al., 2024).

The chemical composition of the best samples of industrial hemp seeds of world selection are given in Table 3.

Table 3

Chemical composition of seeds of world industrial hemp varieties

Variety	Cultivation region	Content , % DW				Reference
		Oil	Protein	Fiber	Ash	
Finola	Greece	29.20	25.40	n.d*	4.80	Irakli et al., 2019
Finola	Canada	30.60	28.00	33.20	5.80	Vonapartis et al., 2015
Anka		28.80	23.80	38.80	5.70	
CFX-1	USA (North Dakota)	35.88	25.97	n.d	6.08	Lan et al., 2019
CFX-2		35.52	28.13	n.d	5.34	
Katani	USA (Kansas)	33.23	30.07	28.85	6.00	Xu Y. et al., 2021
CRS-1		31.53	32.03	28.78	5.95	
Canda		28.92	26.81	36.55	6.03	
Fedora 17		30.24	26.48	35.02	6.32	
Rodnik	Uzbekistan	29.10	26.95	n.d	5.82	Mamadaliev et al, 2023
Santhica 27		31.70	23.44	n.d	6.10	
Santhica 27	Spain	29.90	18.30	37.44	10.40	Alonso-Esteban et al., 2022
Kompolti		31.70	18.80	40.40	9.40	
Bialobrzeskie		32.66	23.00	32.50	10.28	
Bialobrzeskie	Poland	32.28	23.54	28.88	4.66	Teleszko et al, 2022
Henola		32.75	23.39	27.42	4.40	
Tisza	Hungary	33.91	28.92	n.d	4.55	Arango et al., 2024
Fibrol		35.67	25.46	n.d	5.54	
KCVirtus		33.04	23.33	n.d	7.49	
Average value		31.93	25.36	33.44	6.35	
Minimum value		28.80	18.30	27.42	4.40	
Maximum value		35.88	32.03	40.40	10.40	

*n.d, not determined.

Thus, the highest content of oil (35.88 %) was found in seeds of industrial hemp CFX-1 (Lan et al., 2019), protein (32.03 %) in CRS-1 (Xu Y. et al., 2021), fiber (40.40 %) in Kompolti, and ash (10.40 %) in Santhica 27 (Alonso-Esteban et al., 2022).

Comparison of the chemical composition of the seeds of industrial Ukrainian hemp varieties with the best ones of industrial world hemp varieties revealed that (a) the oil content in seeds of industrial Ukrainian hemp varieties Hlesiia (37.16 %), Hlukhivs`ki 51 (37.59 %), Harmoniia (38.15 %), and Artemida (38.90 %) exceeds oil content in the world varieties Finola, Anka, CFX-1, CFX-2, Katani, CRS-1, Canda, Fedora 17, Rodnik, Santhica 27, Kompolti, Bialobrzeskie, Henola, Tisza, Fibrol, and KCVirtus, listed in Table 3; (b) oil content in seeds of industrial hemp variety Artemida (the highest among the analyzed Ukrainian varieties) is 3 % higher than the oil content in seeds of industrial hemp variety CFX-1 (the highest among the analyzed world varieties); (c) the average oil content in the seeds of industrial Ukrainian hemp varieties is by 5.5 % higher than the average oil content in the seeds of industrial world hemp varieties; (d) the content of protein, fiber, and ash in Ukrainian and world hemp varieties was the same.

Mineral composition of seeds of Ukrainian industrial hemp varieties

The content of macroelements – calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and magnesium (Mg) - in seeds of Ukrainian industrial hemp varieties are given in Table 4.

Table 4

Content of macroelements in seeds of Ukrainian industrial hemp varieties

Variety	Content, g/kg dry weight				
	Ca	P	Na	K	Mg
Hliana	1.72±0.09	8.81±0.21	0.06±0.002	7.77±0.31	4.09±0.15
Hlesiia	1.61±0.06	7.53±0.30	0.06±0.002	6.44±0.24	3.90±0.22
Hlukhivs`ki 51	1.94±0.10	9.07±0.25	0.05±0.003	6.23±0.22	4.33±0.20
Artemida	1,49±0,08	6.72±0.12	0.05±0.003	6.32±0.27	3.77±0.12
Harmoniia	1.58±0.05	8.25±0.29	0.07±0.002	7.36±0.31	4.12±0.17
Average value	1.67	8.08	0.06	6.82	4.04
Minimum value	1.49	6.72	0.05	6.23	3.77
Maximum value	1.94	9.07	0.07	7.77	4.33

The highest content of calcium (1.94 g/kg DW), phosphorus (9.07 g/kg DW), and magnesium (4.33 g/kg DW) was determined in seeds of Hlukhivs`ki 51 variety; sodium (0.07 g/kg DW) in Harmoniia variety, and potassium (7.77 g/kg DW) in Hliana variety. The lowest content of calcium (1.49 g/kg DW), phosphorus (6.72 g/kg DW, and magnesium (3.77 g/kg DW) was found in seeds of Artemida variety; sodium (0.05 g/kg DW) in Hlukhivs`ki 51 and Artemida varieties, and potassium (6.23 g/kg DW) in Hlukhivs`ki 51 variety. According to Awuchi et al. (2020), 100 g of industrial hemp seeds fully meet the recommended daily requirement for adults for phosphorus and 96 % for magnesium.

The content of trace elements – copper (Cu), zinc (Zn), iron (Fe), cobalt (Co), manganese (Mn), cadmium (Cd), and lead (Pb) - in seeds of Ukrainian industrial hemp varieties is shown in Table 5.

Table 5

Content of trace elements in seeds of Ukrainian industrial hemp varieties

Variety	Content, mg/kg dry weight						
	Cu	Zn	Fe	Co	Mn	Cd	Pb
Hliana	8.42 ±0.27	43.58 ±1.33	111.92 ±1.54	0.02 ±0.002	101.35 ±1.85	n.d*	0.12 ±0.002
Hlesiia	10.76 ±0.38	52.87 ±0.61	120.51 ±1.12	0.10 ±0.003	97.29 ±1.24	n.d	n.d
Hlukhivs`ki 51	13.75 ±0.41	61.21 ±0.84	104.03 ±0.98	0.06 ±0.004	89.74 ±1.15	n.d	0.07 ±0.002
Artemida	13.01 ±0.55	55.81 ±0.72	93.12 ±1.05	0.06 ±0.002	89.68 ±0.92	n.d	0.09 ±0.003
Harmoniia	17.15 ±0.60	57.88 ±0.35	101.83 ±1.21	0.08 ±0.007	101.19 ±1.19	n.d	0.11 ±0.004
Average	12.62	54.27	106.28	0.06	95.85	0	0.08
Minimum	8.42	43.58	93.12	0.02	89.68	0	0
Maximum	17.15	61.21	120.51	0.10	101.35	0	0.12

*not detected.

The highest content of trace elements in hemp seeds was found: copper (17.15 mg/kg) in Harmoniia; zinc (61.21 mg/kg) in Hlukhivs`ki 51; iron (120.51 mg/kg), cobalt (0.10 mg/kg) in Hlesiia; manganese, (101.35 mg/kg) and lead (0.12 mg/kg) in Hliana varieties. The lowest content of copper (8.42 mg/kg), zinc (43.58 mg/kg), and cobalt (0.02 mg/kg) was in the seeds of the Hliana variety, iron (93.12 mg/kg) and manganese (89.68 mg/kg) in Artemida, lead (absent) in Hlesiia. 100 g of industrial hemp seeds fully meet the recommended daily requirement (Awuchi et al., 2020) for copper; 59 % for iron, and 49 % for zinc.

Minerals are essential for the human body to maintain biochemical processes. They play a functional and structural role and are electrolytes. Phosphorus is a component of bones, cells and is important for bioenergy transformations, maintains the pH of the body. Magnesium is necessary for bones and ATP conversion, participates in muscle contraction and control of acid-base and water-salt balance (Awuchi et al., 2020; Quintaes et al., 2015). Seeds of Ukrainian industrial hemp varieties should be considered as a valuable source of minerals, especially in terms of phosphorus, magnesium, manganese, copper, zinc, and iron.

The population of many developed countries faces the problem of food contamination with toxic metals. Consumption of products with a high content of toxic metals has serious consequences for human health, as it can lead to disruption of some cellular processes due to the displacement of essential metals from the relevant biological structures. Toxic metals such as lead, cadmium, mercury, and arsenic are very common in the environment (Nedzvetsky et al., 2022). Lead damages and causes dysfunction of the kidneys, liver, reproductive, nervous, urinary, and immune systems. Cadmium toxicity is mainly manifested in organs such as the liver, kidneys, brain, lungs, and bones. Cobalt affects lung function, vision and hearing impairment. Copper, nickel, zinc and iron, on the other hand, are vital trace elements in the human body (Okereafor et al., 2020). Copper is a necessary component of various redox reactions, participates in the synthesis of collagen and elastin, the formation of hemoglobin and red blood cells. Zinc is essential for the functioning of many enzymes,

for normal sense of taste and participates in protein synthesis. Iron is necessary for the synthesis and functioning of many enzymes and proteins, in particular hemoglobin, to prevent anemia, and is involved in cellular metabolism and oxygen transport. Manganese is a cofactor of enzymes, participates in bone formation and reproductive function (Awuchi et al., 2020; Quintaes et al., 2015). However, excessive consumption of these microelements is harmful to the human body. Prolonged exposure to copper often leads to severe irritation of the mucous membranes and central nervous system, damage to capillaries, liver, and kidneys. Systemic dysfunctions that lead to impaired growth and gastrointestinal irritation are associated with an excess of zinc. The development of Parkinson's disease, changes in cardiovascular function may be associated with an excess of manganese in the body (Okereafor et al., 2020).

The mineral compositions of seeds of Ukrainian industrial hemp varieties and world varieties were compared. Seeds of industrial hemp varieties CFX-2, X-59, CRS-1, Grandi, Picolo, CFX-1, Katani, Canda, Delores and Joey grown in the USA (North Dakota) contained, mg/100 g DW: calcium, 94.12–120.60; phosphorus, 910.38–1014.30; magnesium, 430–482.14; sodium, 22.09–26.49; potassium, 727.03–866.17; iron, 10.88–13.40; manganese, 12–14.59; copper, 0.78–0.92; zinc, 9.87–11.01, and selenium, 0.28–0.30. The highest content of calcium, sodium, potassium, manganese and selenium was determined in Delores, of phosphorus and magnesium in Grandi, of iron and zinc in Joey, and of copper in X-59 varieties (Lan et al., 2019).

Seeds of Felina 32 variety grown in the Northern Europe and Baltic region contained 0.69–0.9 % potassium; 0.41–0.51 % phosphorus; 0.42–0.46 % magnesium; 0.10–0, 19 % calcium; iron, 181–334 mg/kg DW; manganese, 58–81 mg/kg DW; zinc, 55–68 mg/kg DW; copper, 13–20 mg/kg DW; boron, 7–16 mg/kg DW; aluminum, 6–12.6 mg/kg DW, and sodium, 0.9–16.1 mg/kg DW (Barcauskaitė et al, 2022).

Seeds of industrial hemp varieties Antal, Bacalmas, Fibrol, KC Dora, KC Virtus, KC Zuzana, Kompolti, Monoica, Tiborszallasi, Tisza (Hungary), Carmagnola (Italy), Chameleon (Netherlands), Dioica 88, Epsilon 88, Fedora 17, Felina 32, Ferimon FR 8194, Futura 75, Santhica 23 (France), Helena, Marina, Novosadska, Novosadska+, Simba (Serbia), Kina (China), Lovrin 110, Srcuienijubilee (Romania), Silesia, Wojko (Poland), grown in different countries contained, mg/100 g DW: calcium 70.99–177.21; magnesium, 274.66–499.90; potassium, 509.96–1182.65; iron, 5.06–32.37; manganese, 2.84–12.48; copper, 0.57–1.47, and zinc, 2.21–7.93 (Arango et al., 2024).

The mineral compositions of the seeds of world industrial hemp varieties are given in Tables 6 and 7.

Thus, the highest content of calcium (1.9 g/kg DW) was found in the seeds of industrial hemp variety Felina 32 (Barcauskaitė et al., 2022), phosphorus (10.14 g/kg DW) in Grandi, sodium (0.26 g/kg DW) in Delores (Lan et al., 2019), potassium (11.83 g/kg DW) in Fibrol, magnesium (5.00 g/kg DW) in KC Dora (Arango et al., 2024).

The average content in the seeds of world industrial hemp varieties were, mg/kg DW: copper, 11.86; zinc, 77.75; iron, 159.36; and manganese, 110.6. The highest contents of copper (20 mg/kg DW) and iron (334 mg/kg DW) were found in the seeds of the Felina 32 (Barcauskaitė et al., 2022); zinc (110.1 mg/kg DW) in Joey; manganese (145.9 mg/kg DW) in Delores (Lan et al., 2019) varieties.

Table 6

Content of macroelements in the seeds of world industrial hemp varieties

Variety	Cultivation region	Content, g/kg DW					Reference
		Ca	P	Na	K	Mg	
Delores	USA (North Dakota)	1.21	9.64	0.26	8.66	4.54	Lan et al., 2019
Grandi		1.08	10.14	0.25	8.37	4.82	
Felina 32	Northern Europe, the Baltic states	1.90	5.10	0.17	9.00	4.60	Barcauskaitė et al., 2022
KCDora	Hungary	1.19	n.d	n.d	7.44	5.00	Arango et al., 2024
Fibrol		1.14	n.d	n.d	11.83	3.75	
Helena	Serbia	1.77	n.d	n.d	7.40	4.26	
Average value		1.38	8.29	0.23	8.78	4.49	
Minimum value		1.08	5.10	0.17	7.40	3.75	
Maximum value		1.90	10.14	0.26	11.83	5.00	

n.d., not determined.

Table 7

Content of trace elements in the seeds of world industrial hemp varieties

Variety	Cultivation region	Content, mg/kg dry weight				Reference
		Cu	Zn	Fe	Mn	
Delores	USA (North Dakota)	8.5	103.2	133.9	145.9	Lan et al., 2019
Joey		8.9	110.1	134.0	135.0	
X-59		9.2	98.7	108.8	120.0	
Felina 32	Northern Europe and the Baltic states	20.0	68.0	334.0	81.0	Barcauskaitė et al., 2022
Antal	Hungary, mg/100 g	11.3	45.6	323.7	105.1	Arango et al., 2024
Tiborszallasi		10.8	53.8	116.3	124.8	
Helena	Serbia	14.7	63.3	84.6	95.5	
Novosadska		11.5	79.3	119.6	77.5	
Average value		11.86	77.75	159.36	110.6	
Minimum value		8.5	45.6	84.6	77.5	
Maximum value		20.0	110.1	334.0	145.9	

As a result of comparing the mineral composition of the studied seeds of Ukrainian industrial hemp varieties with the best samples of industrial hemp seeds of world varieties, it was found that: (a) the average content of calcium and copper in the seeds of Ukrainian industrial hemp varieties exceeded the average content of these elements in the seeds of world industrial hemp varieties by 21% and 6%, respectively. The phosphorus content in the tested Ukrainian varieties was close in value to known world varieties. The average content of such elements as sodium, potassium, magnesium, zinc, iron and manganese in the studied seeds of Ukrainian industrial hemp varieties was lower than in world varieties by 22, 10, 23.5, 33, and 13%, respectively.

Conclusions

1. The chemical composition of seeds of industrial Ukrainain hemp varietiers Hliana, Hlesiia, Hlukhivs`ki 51, Artemida, and Harmoniia was determined. The average content of moisture content was 8.31 %. The content of oil, protein, fiber, and ash were, g/100 g of dry weight: 37.38; 24.22; 34.71, and 5.15, respectively. The highest content of oil (38.90 g/100 g DW) and fiber (37.63 g/100 g DW) was determined in the seeds of Artemida variety, and the highest content of protein (25.68 g/100 g DW) and ash (5.63 g/100 g DW) was found in Hlukhivs`ki 51. The average oil content in the seeds of industrial Ukrainian hemp varieties was by 5.5% higher than the average oil content in the seeds of the known world hemp varieties.
2. The average content of macroelements in the seeds of the analyzed Ukrainian hemp varieties was, g/kg of dry weight: calcium, 1.67; phosphorus, 8.08; sodium, 0.06; potassium, 6.82, and magnesium, 4.04. The average content of trace elements in the studied seeds was, mg/kg of dry weight: copper, 12.62; zinc, 54.27; iron, 106.28; cobalt, 0.06; manganese, 95.85; lead, 0.08; cadmium was not detected. The highest content of calcium (1.94 g/kg), phosphorus (9.07 g/kg), magnesium (4.33 g/kg) and zinc (61.21 mg/kg) was in the seeds of Hlukhivs`ki 51; sodium (0.07 g/kg) and copper (17.15 mg/kg) in Harmoniia; potassium (7.77 g/kg), manganese (101.35 mg/kg) and lead (0.12 mg/kg) in Hliana; iron (120.51 mg/kg) and cobalt (0.10 mg/kg) in Hlesiia.
3. 100 g of industrial hemp seeds fully meet the recommended daily requirement of the adult human body for phosphorus, copper, and manganese, 96 % for magnesium, 59 % for iron, and 49 % for zinc. The average calcium content in the seeds of Ukrainian hemp varieties was by 21 % higher than the average calcium content in the seeds of the studied known varieties. The phosphorus content in the seeds of the analyzed Ukrainian hemp varieties was almost the same as in the seeds of known world hemp varieties. The average content of sodium, potassium, magnesium, zinc, iron, and manganese in the seeds of Ukrainian hemp varieties was lower than their average content in the known world hemp varieties.
4. Comparison of the chemical compositions of seeds of industrial Ukrainian hemp varieties with known world analogues confirmed the high value of the varieties and its great potential for application.

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Extruded snacks from maize flour with red grape pomace

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Abstract

Introduction. Grape pomace is a promising ingredient that can be valorized to obtain functional foods. The development of new ready-to-eat products with increased nutritional value is a current research topic.

Materials and methods. Seedless (SGPR) and whole (GPR) red grape pomace was used to obtain maize-based snacks by extrusion. The protein, ash, fiber, lipids, total polyphenols, starch fractions, color, cutting force, and sensory acceptability parameters were investigated to evaluate the impact of grape pomace type (seedless and whole) and addition level (10-40%) on maize snacks quality.

Results and discussion. The amounts of added grape pomace showed significant ($p < 0.05$) effects on almost all the characteristics considered, while grape pomace type had a significant effect only on polyphenol content, antioxidant activity, starch fractions, cutting force and color parameters. The raise of grape pomace level led to the increase of the content of protein, lipids, ash, fiber, total polyphenols, and slowly and resistant digestible starch (up to 30% addition level) in the extruded snacks. The luminosity of the extruded snacks decreased along with the a* color parameter, while b* increased as the amount of grape pomace was higher. A significant reduction of the cutting force was observed as the addition level of grape pomace increased. As for sensory acceptability, it increased with increasing content of seedless or whole grape pomace up to 30%, and decreased with further SGPR or GPR content increase. The best amount of additive was 30% for seedless grape pomace and about 20% for whole grape pomace. The improved samples exhibited higher nutrients content, antioxidant properties and polyphenols content compared with the control. The improved maize-based snacks presented higher FT-IR absorbances and an enhanced amino acid profile compared with the control. Among the essential amino acids in the improved and control extruded snacks, threonine and methionine predominated, and among the nonessential ones, the highest content was noted for aspartic acid, alanine, and tyrosine.

Conclusion. Addition of seedless grape pomace, 30%, or whole grape pomace, 20%, allowed to produce extruded maize snacks with increased nutritional value.

Introduction

The demand for healthier and more nutritious snacks is increasing, and for this reason, various approaches to enhance the quality of snacks production from maize flour are presented in the scientific papers. The maize snacks products are usually have high glycemic index and low nutrients. The partial substitution of maize flour with grape by-products in the extrusion process represents a significant opportunity for wine by-products upcycling. At the same time, the extrusion processing is a technology recognized for its functionality and versatility that looks as a promising option for the incorporation of various by-products types (Garcia-Amezquita et al., 2018; Mironeasa et al., 2019). It produces dietary fiber enriched foods or products improved nutritionally which cannot be made easily by any other process (Grasso, 2020).

Red grape pomace, a by-product of red wine production, available in high amount at low prices, is characterized by a high dietary fiber content and phenolic compounds because during winemaking only a minor part of grape phytochemicals are extracted into the wine (Grevtseva et al., 2023; Rainero et al., 2022; Stabnikova et al., 2023). The pomace is still rich in phenolic compounds, dietary fibres, proteins of high value, lipids, and minerals (Beres et al., 2017; Yu and Ahmedna, 2013), being suitable to enhance the nutritional value of various food products (Iuga and Mironeasa, 2020). Moreover, the bioactive molecules from grape pomace have been proven to be of vast health benefits, including their ability to influence carbohydrates metabolism (Rudra et al., 2015). The dietary fibers include pectin, cellulose and hemicelluloses (Deng et al., 2011) and were associated with lower rate of starch hydrolysis (Hardacre et al., 2016), reduction in glucose absorption (Villemejane et al., 2016), helping in obesity prevention, blood cholesterol levels reduction and fecal transit time decrease (Dhingra et al., 2012; Rainero et al., 2022). Phenolic compounds containing mainly condensed tannins, anthocyanins, and resveratrol with antioxidant and radical scavenging properties (Yu and Ahmedna, 2013) play a crucial role in preventing of some diseases (Rothwell et al., 2017). Some studies have reported that phenolic compounds can affect carbohydrate hydrolysis (Zhu, 2015), are premised to bind to active or secondary sites of digestive enzymes (Barrett et al., 2015) and/or bind to substrate thus reducing starch hydrolysis (Podsedek et al., 2014).

During extrusion cooking, snacks ingredients and their components undergo different changes resulting extrudates with enhanced functionalities. The transformations in the functional features are related to the nutritional characteristics of the extruded snacks (Alam et al., 2016). There have been carried out researches on improving the nutritional properties of extruded snacks based on corn grits by adding tomato skin, seed, and paste (Dehghan-Shoar et al., 2010). The authors reported that starch digestibility decreased as the proportion of tomato derivates was higher. This was attributed to the complex formation between starch and pectin that limited the contact of starch with the digestive enzyme like alpha-amylase. In another study (Karkle et al., 2012), when cornmeal was substituted with apple pomace, it was found that this pomace limits starch digestibility by reducing starch transformation and creating a more compact structure. The diversity in the composition of the vegetable pomace determined different hardness and expansion between the different pomace-incorporated samples obtained in the same processing conditions (Potter et al., 2013).

Grape pomace at levels of 10 and 20% was extruded with cassava-soy composite by Oladiran and Emmambux (2018). The authors reported that the addition of grape pomace lowers the rate of starch digestion, increasing slowly digestible starch and decreasing rapidly digestible starch. An increase in grape pomace level determines an increase in protein and lipid content of extrudates, which can cause decreased digestibility of extrudates (Oladiran

and Emmambux, 2018). The total phenolic content and antioxidant activity also increased in extruded snacks as the level of grape pomace increased. As the amount of grape pomace increased, FTIR spectra showed a significant decrease in α-helical conformation, promoting an increase in β-sheet formation (Oladiran and Emmambux, 2018). Grape peel flour has been used in extrusion cooking (Fontoura et al., 2022) by mixing with brown sorghum flour in proportions of 10%-20%. Using response surface methodology, responses including proximate composition, pasting and expansion properties were influenced by the addition of grape peel, but acceptable extrudates were obtained. The resulting products presented significant values of antioxidants and phenolic compounds and can contribute to the health of consumer.

Most of the studies from recent years found that the addition of pomace at levels up to 100 g/kg by weight of the final product shows no considerable negative effects on the eating quality, whereas detrimental impact was often observed beyond this incorporation level (Altan et al., 2008a; Altan et al., 2008b; Bajerska et al., 2016). However, the valorization of grape by-products has become a growing trend in the food industry due to these nutritionally valuable raw materials, and thus reducing the total waste and determining increase the nutritional value of the new products. By incorporating high amounts of pomace in corn extrudates, changes in the nutritional and functional properties, and also color, texture parameters and acceptability of these products occur.

Conducting research on the incorporation of different grape by-products in high percentages into extrudates can enhance our understanding of how various ingredients interact in the extrusion process and affect extruded snacks quality. Therefore, this study aims to investigate the impact of red seedless and whole grape pomace in various amounts (10-40%) in a mixture with maize flour on some features of extruded snacks. Furthermore, the properties of the best snacks from nutritional and functional properties point of view were investigated.

Materials and methods

Materials

The red grape pomace was sourced from the Iași Research and Development Center for Viticulture and Vinification, while the maize flour, a commercial variety, was obtained from a producer in northern Romania. The grape pomace was dried in a ZRD-A5055 convection oven (Zhicheng Analysis Instrument, Shanghai, China) at 50°C for 20 h, reducing its moisture content to below 10%. After drying, the pomace was divided into two categories: seedless grape pomace and whole grape pomace. Both were ground using a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden) and passed through 0.20 mm sieves to achieve a uniform particle size. Maize flour, initially sieved to a uniform size of 1.00 mm, was then thoroughly mixed with the seedless grape pomace (SGPR) and whole grape pomace (GPR). Maize flour alone served as the control (M). During the mixing process, water was gradually added at medium speed using a Bosch MFQ3520 mixer (Gerlinger, Germany) to achieve a final moisture content of 15% (wet basis) in the mixtures.

Extrusion process

The flour mixtures were processed using a laboratory single-screw extruder (Kompakt extruder KE 19/25, Brabender, Duisburg, Germany). This extruder featured a barrel with a

19 mm diameter, a length-to-diameter ratio of 25:1, and a 2 mm nozzle diameter. The extrusion was carried out at a constant feeding speed of 24 rpm, a screw speed of 150 rpm, which operate, with a compression ratio of 3:1. The barrel temperature, divided into four sections from the feeder to the nozzle, was set at 50°C, 95°C, 175°C, and 180°C. After extrusion, the snacks were left to cool at room temperature for 16 h, then cut into small pieces before being packaged in polyethylene bags for future analysis. When needed, the dried samples were ground, using a mortar and pestle, and passed through a 250 mm sieve to ensure uniform particle size distribution.

Methods

Chemical composition

The proximate composition was analyzed using both International and Romanian standard methods: ash content was measured according to SR ISO 2171:2023, protein content following SR EN ISO 20483:2014, and lipid content using SR 91:2007. Total dietary fiber was determined with a Megazyme kit (K-TDFR-200a 04/17) based on the AACC 32-05.01 method.

Total polyphenols content (TPC) and DPPH antiradical activity

The total polyphenols were extracted from the samples using a solution of 70% acetone, 28% water, and 2% acetic acid (v/v/v) with a solid-to-liquid ratio of 1:10 (w/v). The extraction process was carried out in an ultrasonic bath for 60 min at 45 Hz. After extraction, the liquid phase was separated from the solid phase by centrifugation (3234 g for 15 min). The extraction of the solid residue was repeated twice, and the resulting supernatants were combined and filtered.

The total polyphenol content (TPC) was determined using the Folin-Ciocalteu method (FAO/IAEA, 2000). A 0.2 mL aliquot of the extract was diluted with 0.8 mL distilled water, mixed with 0.5 mL of 1N Folin–Ciocalteu reagent, and 2.5 mL of 20% sodium carbonate. The samples were then incubated at room temperature for 40 min. Absorbance was measured at 725 nm using a Shimadzu 3600 UV-Vis-NIR spectrophotometer (Tokyo, Japan), with gallic acid (GAE) as the standard.

The antiradical activity (AA) of the grape-maize snack extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, based on previous studies (Bajerska et al., 2016). A 0.5 mL aliquot of the sample extract, diluted with 0.5 mL of 80% methanol, was added to 5 mL of DPPH solution and incubated in the dark at 25°C for 30 min. Absorbance was measured at 517 nm using the same Shimadzu 3600 spectrophotometer, with gallic acid as the standard. A blank sample was prepared, and the results were calculated using Equation (1), where A sample represents the absorbance of the analyzed sample at 517 nm, and A blank represents the absorbance of the blank at 517 nm.

$$AA(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \quad (1)$$

Rapid (RDS), slowly (SDS) digestible and resistant (RS) starch

The analysis of starch fractions was conducted according to the international AOAC 2017.16 protocol, using a Megazyme kit (K-DSTRS; Megazyme, Bray, Ireland), following the manufacturer's instructions. Spectrophotometric methods were used to measure the

contents of rapidly digestible starch (RDS, after 20 min), slowly digestible starch (SDS, after 120 min), and resistant starch (RS, not digested after 240 min) in the extruded snacks.

Color parameters

The color parameters of the flour mixes were evaluated using a CR-400 chromameter (Konica Minolta, Tokyo, Japan).

Cutting force

The cutting force of the extruded snacks was evaluated using a TVT 6700 texturometer (Perten Instruments, Hägersten, Sweden), equipped with a 10.00 kg load cell. A Warner-Bratzler shear blade probe was used for cutting, with the cut made perpendicularly to the main axis of the snack. The test was conducted at a speed of 1 mm/s until the snack was completely broken.

Determination of acceptability

A sensory analysis to determine the acceptability of the snacks was carried out by 65 semi-trained panelists. Prior to evaluating the products, panelists were provided with a brief explanation of the 9-point hedonic scale and instructed on how to score the extruded snacks. To neutralize their taste buds, panelists were asked to rinse their mouths with water before each evaluation.

Amino acids determination

The amino acid content of the control and improved samples was quantified using a high-performance liquid chromatography (HPLC) system (Shimadzu LC40-PDA-40). The samples were homogenized with 0.1 N HCl in a 1:5 (w/w) ratio and then centrifuged at 10,000 x g for 20 min at 4°C. The supernatant was filtered through a 0.45 μm membrane filter (Igual et al., 2021). A 300 μL portion of the supernatant was deproteinized by adding 900 μL of acetonitrile and then filtered again using a 0.45 μm membrane filter. Pre-column derivatization was carried out following the Agilent Amino Acid Analysis protocol, using OPA (o-phthalaldehyde) and FMOC (9-fluorenylmethyl chloroformate) reagents.

Separation was achieved on a 4.6 x 100 mm LC column, with quantification performed on the Shimadzu LC40-PDA-40 system (Shimadzu, Japan) at a wavelength of 338 nm (bandwidth 10 nm). The mobile phase A was composed of 10 mM Na2HPO4 and 10 mM Na2B4O7 at pH 8.2, while mobile phase B was a mixture of acetonitrile, methanol, and water (45:45:10).

FT-IR analysis

Fourier transform infrared (FTIR) spectroscopy was employed to investigate the molecular properties of the improved and control samples using a Thermo Scientific Nicolet iS20 spectrophotometer (Waltham, MA, USA) in attenuated total reflection (ATR) mode. The spectral range analyzed was from 4000 to 650 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans per spectrum. Molecular characteristics were assessed by analyzing the peak areas reported in the literature using OMNIC software (version 9.9.549, Thermo Fisher Scientific, Waltham, MA, USA).

Experimental design and statistical analysis

The effects of the addition level (A) at 4 levels (10, 20, 30, 40%) and type of red grape pomace (B) at 2 levels (seedless - SGPR and whole - GPR) on maize extruded snacks characteristics (protein, ash, lipids, fibers content, TPC, DPPH, SDS, RDS, RS, L*, a*, b*, acceptability and cutting force) were investigated.

The experimental data for each characteristic was fitted to polynomial cubic regression equation. Model adequacy was evaluated by using sequential *F*-test, coefficients of determination (R^2), adjusted coefficients of determination ($Adj.\text{-}R^2$), and significant probabilities. The significance of the coefficients of the models were evaluated by using ANOVA at a confidence level of 95%.

The best formulations were chosen based on the highest level of fiber, TPC, DPPH, SDS, RS, and L*, and the lowest values of RDS and cutting force. XL STAT and Ease Design-Expert software (trial version) were used for data processing.

Results and discussion

The impact of type of grape pomace and addition level on maize extruded snack properties was evaluated by means of ANOVA and the results are presented in Table 1. Protein content was influenced significantly ($p < 0.05$) by the addition level and its interaction with grape pomace type. On the other hand, the fibers content was affected significantly ($p < 0.05$) by both addition level and its interaction with type of grape pomace. The increase of grape pomace amount led to the increase of protein, ash, lipids, and fiber content. Alshawi (2024) also reported a significant enrichment of wheat snacks fiber, lipids and ash content when grape pomace was added in 5-15% proportion. The snacks with more than 20% SGPR or GPR can be considered as „high-fiber products" because they contain > 6g/100g fibers (Alshawi, 2024). The dietary fiber from grape pomace is mainly formed by pectin, cellulose and hemicelluloses (Deng et al., 2011). The intake of nutrients from grape pomace is responsible for the increasing trends obtained in this study. The chemical composition of the enriched extruded snacks depend on the nutrients profile of the ingredients added. For instance, the addition of brewer's spent grain in corn snacks resulted in higher protein, and fat content, while when apple and sugar beet pulp were incorporated the protein content decreased (Jozinović et al., 2021).

The TPC and DPPH antioxidant activity was significantly influenced ($p < 0.05$) by both type of grape pomace and addition level (Table 1). The incorporation of grape pomace determined the increase of TPC and antioxidant activity due to the intake of polyphenols from the ingredient added. Yagci et al. (2022) also obtained a significant proportional increase of the snacks TPC as the amount of tomato powder incorporated was higher, with the DPPH antioxidant activity being 1.3–1.8 fold higher compared with the control. It was proved that the incorporation of rosehip and apple pomace in extrudates led to positive changes of total polyphenols content and antioxidant activity of corn extruded snacks, depending on the addition level and fruit pomace variety (Drozdz et al., 2014). The main polyphenols from grape pomace are represented by condensed tannins, anthocyanins, and resveratrol which exert high antioxidant and radical scavenging activity (Yu and Ahmedna, 2013).

Starch digestibility registered changes depending on the type of grape pomace and the amount used. Grape pomace type and its interaction with grape pomace addition level exhibited a significant effect ($p < 0.05$) on starch fractions content (Table 1). The RDS

content increased as the addition level raised, while SDS and RS increased first, then decreased. In another study, Altan et al. (2008b) used extrusion cooking of barley flour-grape pomace blend, grape pomace being added at levels of 2 to 10% (w/w). The authors reported a reduction in starch digestibility of extrudates with the increasing level of grape pomace. It was suggested that this might be due to fiber content, which tends to reduce starch digestibility by trapping starch granules within a viscous protein-fiber-starch network (Altan et al., 2008b). Dehghan-Shoar et al. (2010) demonstrated that starch digestibility decreased with the increase of tomato by-product amount due to the formation of a complex between starch and pectin, which led to a less contact surface with the digestive enzyme like alpha-amylase. In another study (Karkle et al., 2012), the addition of apple pomace in maize snacks resulted in starch digestibility reduction due to the modifications of starch grains and the formation of a more compact structure.

Sensory acceptability was significantly affected ($p < 0.05$) by the addition level and type of grape pomace and their interaction and increased proportionally with the addition level increase up to 30%, and then it decreased. The extrudates with 2% grape pomace in barley flour has found to present higher preferences for overall product quality, while rising grape pomace above 6% diminished the expansion (Altan et al., 2008b). The acceptability of the maize-soy-apple pomace extruded snacks obtained by Shahzad et al. (2018) ranged between 6.4 and 7.4, which is in agreement with our results. The decrease of acceptability at high grape pomace levels could be due to the presence of polyphenols (Shahzad et al., 2018).

The color parameters (L*, a* and b*) of the extruded snacks were significantly influenced ($p < 0.05$) by both type and addition level of grape pomace, as well as by their interaction (Table 2). The L* and b* values decreased as the amount of grape pomace raised, while a* parameter exhibited the opposite trend. These changes could be related with the pigments of the grape pomace, as well as the Maillard reactions that occur during extrusion. Similar results were reported by Yu et al. (2018) which demonstrated that by adding grape pomace in maize snacks resulted in smaller L* (lightness) and b* values (yellowness), and raised a* value (redness).

The cutting force was significantly affected ($p < 0.05$) by both grape pomace types and level and their interaction, and it was reduced proportionally with the increase of grape pomace amount (Table 1). Grape pomace contains soluble pectin which may determine the reduction of snacks hardness by playing a lubrification role, and thus, leading to a crispier rather than a harder product. Similar finding was reported by Singha et al. (2019) for snack made from maize, soy, and apple pomace.

Figure 1 illustrates the variations of protein, ash, lipids and fiber with the addition level of grape pomace.

The relationship between the addition level of grape pomace and the values obtained for TPC and DPPH are displayed in Figure 2. The decreasing trend of TPC and DPPH antiradical activity at addition levels > 30% could be possibly related to a more intense binding of polyphenols on maize starch and proteins. Antiradical activity of polyphenols can be modulated through intra or intermolecular hydrogen linkage of active or neighboring –OH groups (Stojadinovic et al. 2013). The reduction in antiradical activity was observed when polyphenols are non-covalently linked to proteins for example (Stojadinovic et al. 2013).

Figure 3 represents the variations of starch fractions with the addition level of grape pomace.

Table 1

ANOVA results for the cubic model fitted for the extruded snacks properties

Factor	Protein (%)	Ash (%)	Lipids (%)	Fibers (%)	TPC (mg GAE/g)	DPPH (%)	RDS (%)	SDS (%)	RS (%)	Acceptability	L*	a*	b*	Cutting force (g)
Intercept	9.13	1.28	1.74	7.36	28.97	92.70	34.74	4.82	3.54	6.74	49.84	6.90	6.48	1007.32
A-Type	0.03	-0.12	0.04	-0.49	1.76**	-0.78*	-1.91**	-0.21*	5.19*	-0.01	0.84**	0.64**	0.75**	80.33**
B-Level	2.22*	0.03	0.26	9.65**	8.37**	3.62**	0.34	-1.08*	-0.26*	0.60**	-6.64**	1.53**	-1.37**	108.49**
AB	-1.76**	-0.61**	-0.65**	-3.80**	0.80**	3.34**	-0.94**	-0.70**	-0.74**	0.02*	0.26	0.68**	0.42**	177.83*
B^2	2.44**	0.72**	0.31*	6.22**	-1.60**	-3.50**	3.56**	-1.34**	-0.84**	-0.14**	1.87**	-0.43	0.97**	-69.88
AB^2	1.11**	0.65**	0.56**	7.53**	-0.65	3.50**	5.32**	1.10**	-1.35**	0.07**	0.82**	-0.84**	-0.33**	-56.21
B^3	-1.47	0.29	0.11	-7.78**	-4.87**	-4.98**	0.37**	1.10	1.23	-0.40**	-0.21	-0.72	-0.61**	-30.06
***p*-value**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R^2	0.89	0.91	0.92	0.98	0.97	0.96	0.99	0.96	0.96	0.98	0.99	0.94	0.99	0.88
Adj.-R^2	0.85	0.88	0.89	0.97	0.96	0.94	0.99	0.94	0.95	0.97	0.99	0.92	0.98	0.84
* - significant at $p < 0.05$, ** - significant at $p < 0.01$														

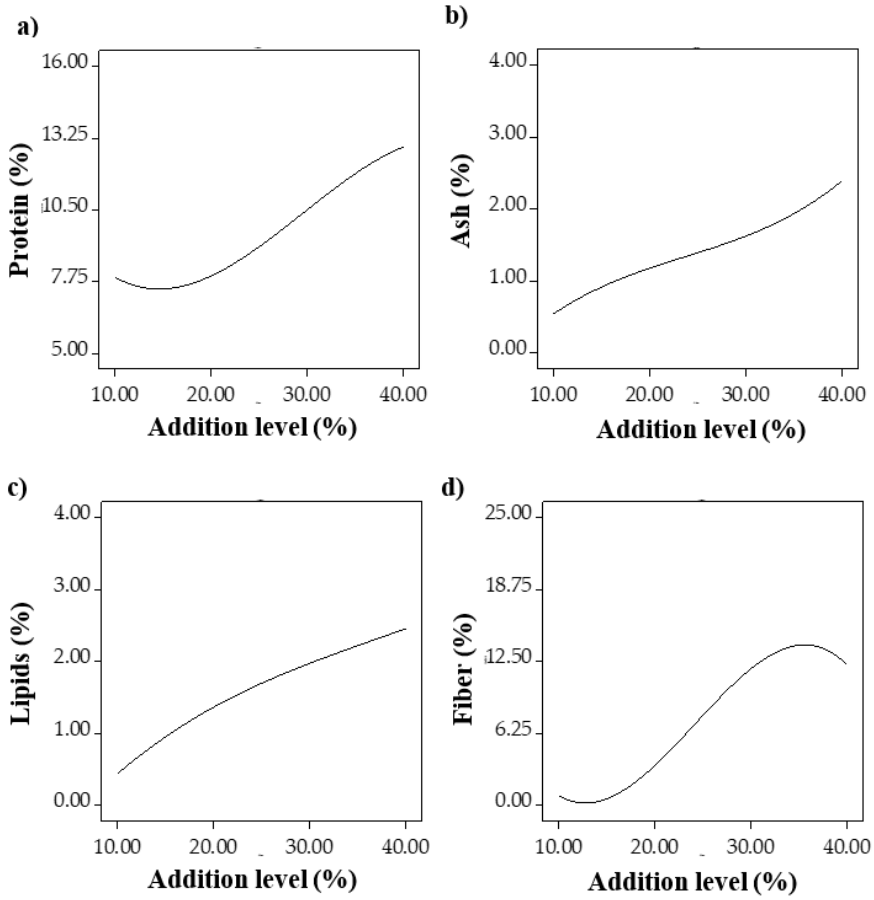

Figure 1. **Variations of protein (a), ash (b), lipids (c), and fiber content (d) with the addition level of grape pomace**

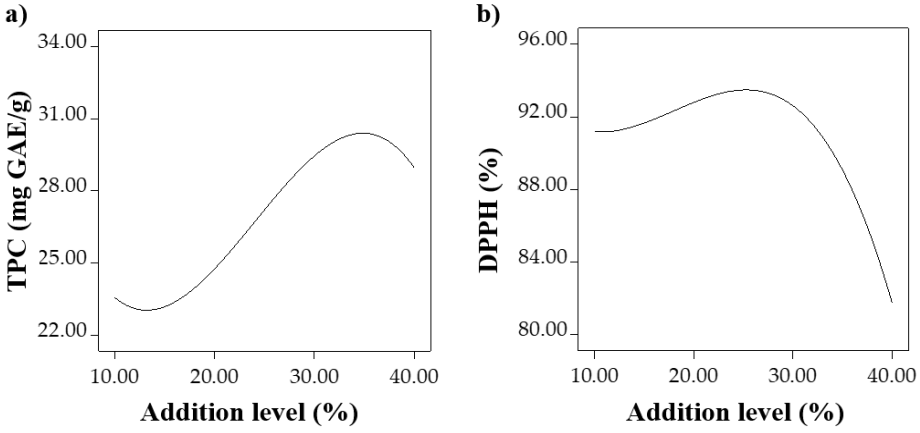

Figure 2. **Variations of TPC (a) and DPPH (b) in function of the addition level of grape pomace**

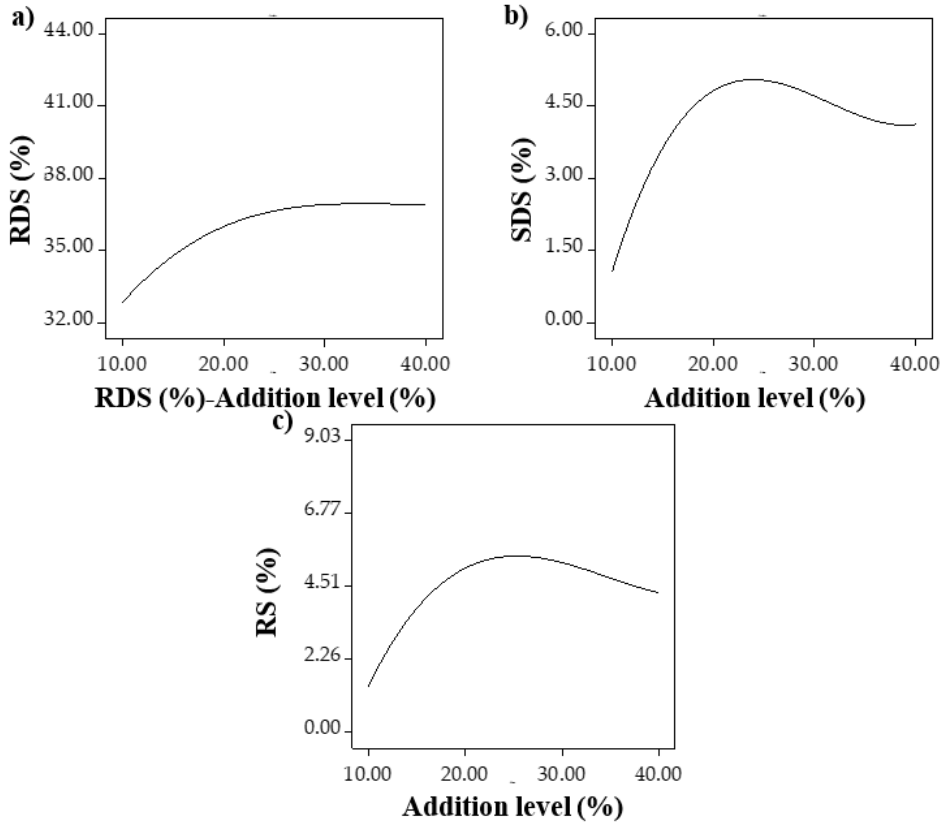

Figure 3. **Variations of RDS (a), SDS (b), and RS (c) with the addition level of grape pomace**

The variations of color parameters (L*, a*, and b*) with grape pomace addition level are shown in Figure 4.

Figure 5 shows the relationships between the addition level of grape pomace and the values obtained for acceptability and cutting force.

Significant correlations ($p < 0.05$) were found between some of the chemical components and the color parameters, acceptability and cutting force. Similar to our results, Altan et al. (2008a) also obtained a negative correlation between L* and a* values of barley-tomato pomace snack.

The best formulations were chosen based on the highest level of fiber, TPC, DPPH, SDS, RS, and L*, and the lowest values of RDS and cutting force. Thus, the recommended addition level for the seedless grape pomace (O_SGPR) was found to be around 30%, while for whole grape pomace (O_GPR) was about 20% (Figure 6).

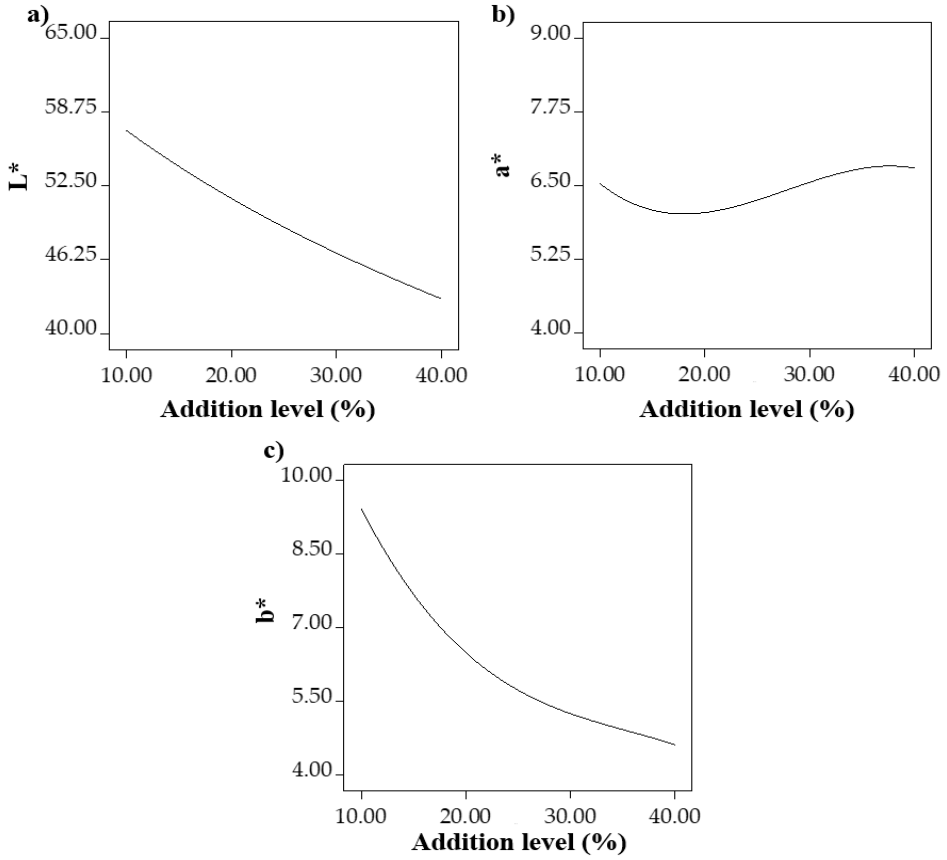

Figure 4. Variations of L* (a), a* (b), and b* (c) with the addition level of grape pomace

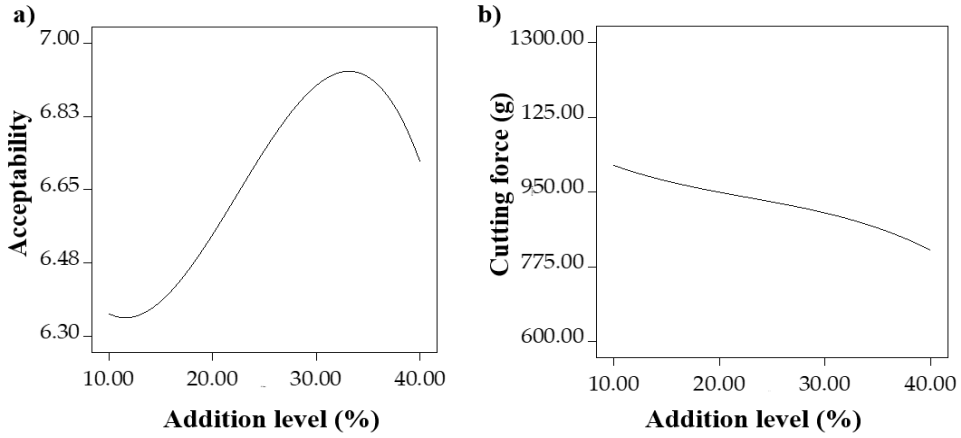

Figure 5. Variations of acceptability (a) and cutting force (b) with the addition level of grape pomace

M O_SGPR O_GPR

Figure 6. Control and improved snacks appearance

Compared to the control (M), both improved samples were richer in protein, ash, lipids, fiber, TPC, RDS, RS and had lower L*, b* and cutting force value. Compared with the O_GPR, the sample with seedless grape pomace (O_SGPR) had higher protein, ash, fiber, antioxidant activity (DPPH), SDS, RS, and acceptability, while the TPC, cutting force, and color parameters registered lower values (Table 2).

Table 2

Characteristics of the best seedless (O_SGPR with 30%) and whole (O_GPR with 20%) grape pomace containing snacks *vs.* control (M) properties

M	O_SGPR	O_GPR	M
Protein (%)	10.16±0.48^{a}	9.15±0.48^{b}	7.61±0.03^{c}
Ash (%)	1.57±0.13^{a}	1.21±0.13^{b}	0.45±0.01^{c}
Lipids (%)	1.92±0.77^{a}	1.82±0.77^{a}	0.35±0.05^{b}
Fiber (%)	11.04±1.02^{a}	6.52±1.02^{b}	0.46±0.03^{c}
TPC (mg GAE/g)	28.47±0.68^{a}	29.57±0.68^{a}	28.85±0.04^{a}
DPPH (%)	93.29±0.95^{a}	91.08±0.95^{b}	38.04±0.10^{c}
RDS (%)	36.87±0.49^{a}	32.92±0.49^{b}	30.03±0.03^{c}
SDS (%)	4.81±2.70^{a}	4.73±2.70^{a}	0.88±0.03^{b}
RS (%)	5.33±0.09^{a}	5.04±0.09^{b}	1.21±0.01^{c}
Acceptability	6.84±0.03^{a}	6.65±0.03^{b}	-
L*	47.84±0.45^{c}	51.51±0.45^{b}	78.48±0.12^{a}
a*	6.41±0.29^{b}	7.24±0.29^{a}	0.71±0.03^{c}
b*	5.45±0.17^{c}	7.36±0.17^{b}	21.75±0.20^{a}
Cutting force (g)	914.45±69.42^{b}	1050.46±69.42^{b}	1625.43±147.86^{a}

Mean values followed by different letters then as are significantly different ($p < 0.05$)

The differences between the two types of grape pomace-containing snacks could be due to the different types of fiber and the lipid content of the two ingredients. It was obvious the enhancement of snacks nutritional value by increasing the nutrients and phenolic compounds content of the maize snacks, which was in agreement with previous studies regarding the fortification of gluten free snacks with different vegetal by-products (Dehghan-Shoar et al., 2010; Oladiran and Emmambux, 2018).

The changes of color properties after grape pomace incorporation could be due to the Maillard reactions which involves a complex series of reactions, the first one being the conjugation between an active carbonyl group (coming from a reducing sugar for example) with an amino group (usually from proteins or amino acids) (Rolandelli et al., 2021). Thus, the presence of sugars in grape pomace could have been promoted the Maillard reactions during extrusion, which generated color differences compared with the control. Another possible explanation can be the caramelization reactions which imply the direct conversion of reducing sugars by 1,2-enolization, dehydration, and cycling reactions (Rolandelli et al., 2021).

Characterization of the improved samples

The molecular characteristics of the improved and control samples are represented in Figure 7. The lowest absorbances intensities were observed for the control sample (M), followed by O_GPR, and O_SGPR. Compared to the control, the enriched samples presented an additional peak at 2855 cm^{-1}, which was more pronounced in O_SGPR snack. This band is due to CH_2 stretching and it suggests the presence of grape pomace cutin, waxes and cutan (Nogales-Bueno et al., 2017). The most prominent peaks were observed in the lipid (2800-3050 cm^{-1}) and carbohydrates region (900-1200 cm^{-1}), similar with the results obtained by Amador-Rodríguez et al. (2019). The peak at 2925 cm^{-1} corresponding to lipids confirm the higher abundance in the grape pomace samples compared to the control.

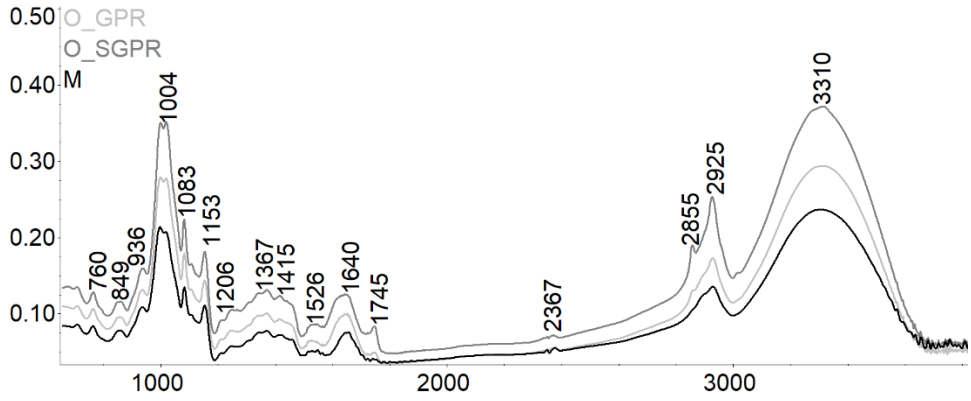

Figure 7. FT-IR spectra for the improved and control samples

The protein regions (1700–1600 and 1570–1534 cm^{-1}) showed changes of the intensities in the grape pomace-containing samples compared with the control. The increase of peak intensity at 1745 cm^{-1} corresponds to pectin (Amador-Rodríguez et al., 2019) and the increase in its intensity after grape pomace addition indicated the intake from the ingredients added.
The amino acid content of the control and the improved samples are shown in Figure 8. Among the essential amino acids, methionine and lysine was found in all the samples, while phenylalanine and isoleucine were present only in O_GPR. Leucine and threonine were identified in both enhanced samples, and O_SGPR had the highest threonine level. Valine was found in M and O_SGPR sample, with the improved sample presenting the highest value (Figure 8a). A study reported some of the main essential amino acids found in grape pomace

are leucine and lysine (Hanušovský et al., 2023), which may explain the increase of these compounds in the improved samples compared with the control. Since lysine is a limiting amino acid in cereals, the incorporation of grape pomace led to an enhanced amino acid profile of the final product. Phenylalanine was also reported in grape pomace and thus the amino acid profile of bakery goods can be improved (Antoniolli et al., 2024). Chikwanha et al. (2018) reported high levels of threonine in grape pomace, which may explain the presence of this amino acid in the improved samples.

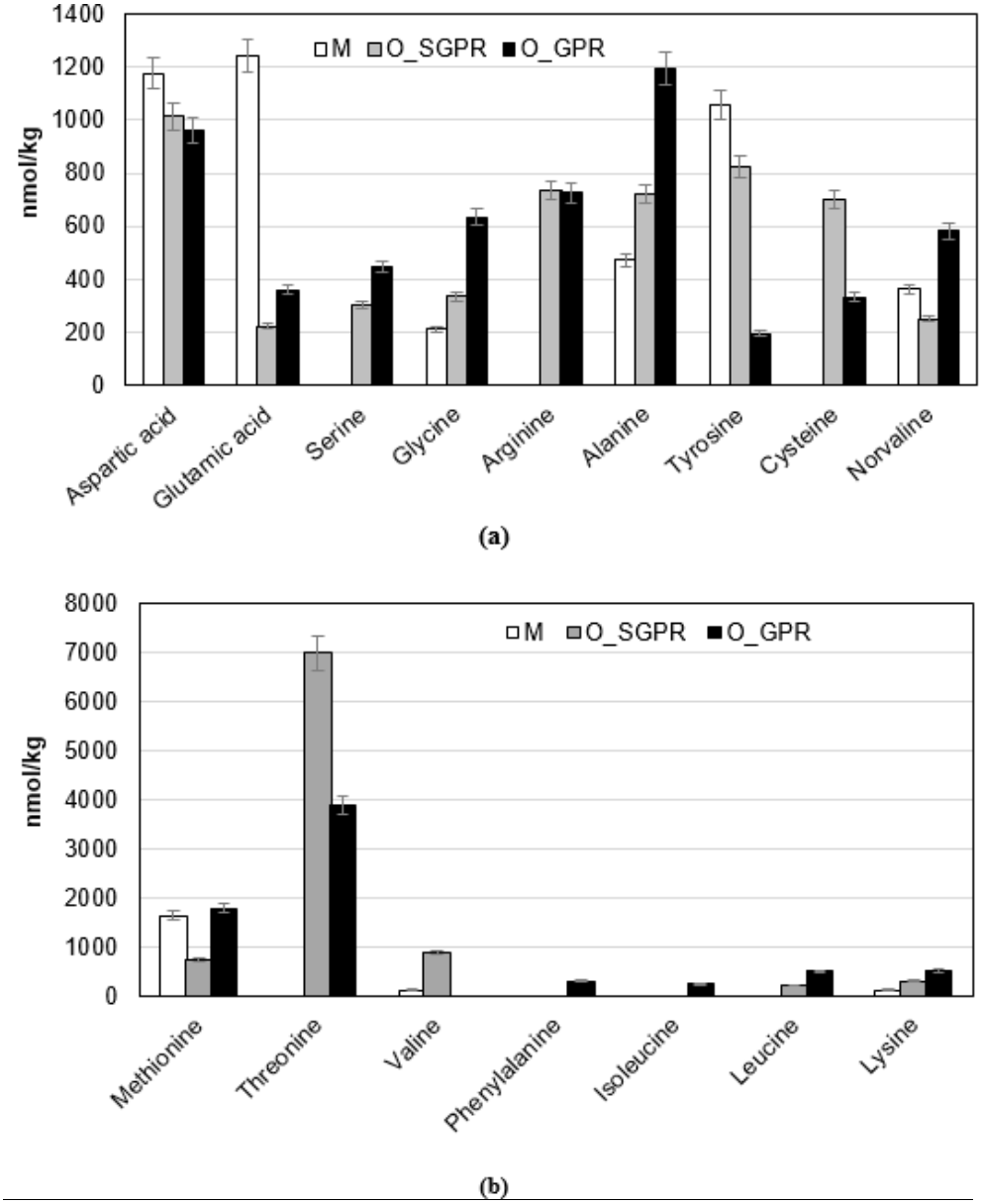

Figure 8. Essential (a) and non-essential (b) amino acid content of the improved and control samples

Regarding the non-essential amino acids content, aspartic acid, alanine and tyrosine were the most abundant amino acids in all the samples (Figure 8b). Control M exhibited the highest concentration of aspartic acid, glutamic acid, and tyrosine. O_GPR was the richest in glycine, alanine and norvaline, while O_SGPR had the biggest concentration of cysteine. Grape pomace was proved to be rich in glycine (Hanušovský et al., 2023), which may explain the higher values found in the improved samples compared with the control. Alanine was identified as being one of the amino acids found in grape pomace (Chikwanha et al., 2018), a fact proved by the increase of this compound content in the improved samples compared with the control. The amino acid composition of grape pomace depend on the grape variety and type (seedless or with seeds) and thus influences the composition of the final product in different ways.

Conclusions

This study highlighted the possibility to valorized two types of grape pomace (seedless and whole) in high amounts to obtain functional extruded snacks. The results obtained revealed the enhancement of the nutritional profile after grape pomace incorporation, with the increase of protein, ash, lipids, fiber, total phenolic content, and antioxidant activity being proportional with the addition level increase for both types of grape pomace. The color, starch digestibility, texture and sensory acceptability of the extruded snacks were also affected by the addition level, depending on the type of grape pomace. The best amount of seedless grape pomace was found to be about 30%, while for whole grape pomace it was about 20%. The improved samples exhibited enhanced nutritional profile compared with the maize snacks used as a control sample. FT-IR molecular characteristics and the amino acid profile were changed after grape pomace addition, depending on the type used.

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Patent
The results were first used in a patent application entitled “Process for obtaining an extruded non-gluten product, direct-expanded and product so obtained” (“Procedeu de obţinere a unui produs aglutenic extrudat, direct-expandat şi produs astfel obţinut”).

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Combined food additive based on iron oxide nanoparticles and kombu in a rye-wheat bread technology

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Abstract

Introduction. The aim of the study was to develop a technology for rye-wheat bread with a combined food additive (CFA) consisting of iron oxide nanoparticles (IONPs) and powder of dried brown algae *Laminaria japonica* L. (kombu) to improve baking performance and the nutritional value of the end-product.

Materials and methods. The synthesized IONPs and kombu powder were used to prepare a combined food additive that was added in the amounts of 1.0, 1.5, and 2.0% of the flour weight during the manufacturing of rye-wheat bread. Physical and textural characteristics of dough and bread were evaluated using standard methods. Sensory analysis of bread was performed to compare it with a traditional one.

Results and discussion. Preliminary treatment of kombu powder by hydration for 10 min and boiling in water for 5 minutes allowed reducing the iodine content by 78.9% to avoid the risks to exceed the recommended iodine daily intake. The inclusion of the CFA in the amounts of 1.0, 1.5, and 2.0% of the flour weight reduces the dough spreadability index from 4.6 to 11.4%, increases the accumulation of carbon dioxide in the dough from 6 to 11% and the specific volume of the dough from 6 to 13%, while reducing the dough formation time by 8.0±1.5 min. Rye-wheat bread with CFA had an increased specific volume by 17 to 33%, crumb porosity by 16 to 37%, shape stability by 19 to 59% compared to the control bread. A comparative analysis of physicochemical characteristics and sensory indicators determined the optimal amount of CFA 1.5% as for inclusion as an ingredient in rye-wheat bread formulation. The calculation of the biological value indicates an improvement in the nutritional value of the developed rye-wheat bread. Fortification of bread at a level of 55±10 μg/100 g of product with iodine allows recommending it to consumers with iodine deficiency diseases.

Conclusion. Combined food additive based on iron oxide nanoparticles and kombu powder can be used as an ingredient in the formulation of rye-wheat bread to stabilize its structure and improve the textural characteristics. The presence of valuable nutrients in its composition allows it to enhance the nutritional profile of rye-wheat bread.

Introduction

One of the modern trends in food production is application of novel natural additives is the development of functional foods with health-promoting properties (Ivanov et al., 2021). Among them, edible seaweeds (macroalgae) attract attention as supplements to the different traditional food products to increase their nutritional value and shelf life (Healy et al., 2023; Kryzhova et al., 2021; Polat et al., 2023; Stabnikova et al., 2021). Seaweeds are considered to be one of the most promising food resources due to their remarkable adaptability and short development period (Adeyemi and Fawole, 2023; Buschmann et al., 2017). This is evidenced by the steady increase in seaweed production, which has more than tripled worldwide over the past 20 years, according to the Food and Agriculture Organization (FAO, 2022).

Brown algae (*Phaeophyceae*) are among the most consumed seaweeds in human nutrition (Leandro et al., 2020). There are a number of reasons for this:

- high content of valuable nutrients such as sulfated polysaccharides, minerals, vitamins, dietary fibers, fatty acids, pigments, polyphenols, and polyunsaturated fatty acids (Biancarosa et al., 2018; Costa et al., 2021);
- specific technological functions allowing their use for stabilization and thickening in the food production;
- their use as nutraceuticals and pharmaceutical preparations due to the presence of bioactive compounds that exhibit various medical activities (Tagliapietra and Clerici, 2023).

Kombu (*Saccharina japonica*, formerly *Laminaria japonica*), a very popular brown seaweeds, can be classified as a valuable dietary product due to its low energy value, low content of fat and sugar (Mahadevan, 2015; Mohammed et al., 2021; Salido et al., 2024). These algae contain iodine in the form of iodides and iodates, as well as iodinated amino acids (Blikra et al., 2022; Romarís-Hortas et al., 2011), being one of the important natural sources of iodine and its organic compounds in human nutrition (Healy et al., 2023; Matos et al., 2024; Salido et al., 2024; Smyth, 2021). As is known, iodine is important for human health due to its fundamental role in the functioning of the thyroid gland and the associated production of thyroid hormones (Wells et al., 2017). Therefore, the use of algae-enriched products can be considered as a way to prevent iodine deficiency disorders (Matos et al., 2024). The content of this microelement in kombu significantly depends on growing conditions and time of harvesting and can change widely from 2500 to 10,000 μg/g dry weight (dw) (Smyth, 2021). At the same time, much lower number of 241 μg/g dw was presented in the review (Blikra et al., 2022). The iodine content in commercially available kombu powders varied from 3940 to 7430 μg/g dw depending on the batch of product purchased at different times (Gubskyi et al., 2015). This fact must be taken into account when fortifying food products and control of the iodine content in the finished product must be strictly observed. Consuming iodine in food in amounts exceeding the recommended daily allowance (RDA) of 150 μg (NIH, 2023) and especially the tolerable upper intake level (UL) of 600 μg/day for adults established in Europe (WHO, 2007) may have negative effects on human health (Correia et al., 2021; Roleda et al., 2018).

Another recent trend in food production is the implementation of nanotechnologies (Ameta et al., 2020; Sanguansri and Augustin, 2006). Unique and new functional properties of nanomaterials can find applications in food preparation, including improvement of the sensory characteristics of finished products and extension of their shelf life. All this fully applies to metal and metal oxide nanoparticles, which have a huge potential for use in the food industry, both in the development of innovative food products and in food packaging (Adeyemi and Fawole, 2023; Joshi et al., 2024).

Bread is one of the most widely consumed staple food throughout the world. Thus, it has significant potential for fortification with many functional ingredients and rapid dissemination of the developed functional foods with improved nutritional properties to the general population (Aabel et al., 2023). However, the addition of certain fortifying agents, including seaweeds, can have a significant impact on the textural and sensory properties of baked goods.

Despite the rich chemical composition of seaweeds, their traditional use in wheat or rye bread technologies has been limited (Polat et al., 2023). Meanwhile, there has been an increase in the research on this topic recently, especially for wheat flour products (Aabel et al., 2023). It was noted that the inclusion of seaweed in bread creates a number of new problems and complexities with significant changes in textural properties, sensory acceptability, and overall quality of the bread. Other studies have reported significant effects on the textural characteristics of bread, such as increased firmness and chewiness with decreased final porosity, and a consistent decrease in overall consumer ratings in sensory analysis (Arufe et al., 2018; Mamat et al., 2023; Sasue et al., 2023). The authors note that these effects increase with increasing amounts of seaweed powder added to the product formulation. However, some studies have shown consumer acceptability of bakery products containing up to 6% seaweed (Jönsson et al., 2024; Lamont and McSweeney, 2021; Mamat et al., 2023). All of these arguments support the conclusion of the review authors (Aabel et al., 2023) that the inclusion of seaweed as an ingredient in bread formulation requires careful balance and precise formulation to ensure that the desired characteristics are maintained.

The possibility of using iron oxide nanoparticles (IONPs) as a food additive with certain functional and technological properties was shown (Tsykhanovska et al., 2020; 2021; 2022a, b). The inclusion of IONPs into bread formulation improved its functional and technological properties during storage.

An analysis of the literature confirmed the lack of research on the simultaneous use of algae and nanoparticles in bread technologies. Therefore, the purpose of the present research was to study the possibility of using a combined food additive based on iron oxide nanoparticles and kombu powder in the technology of rye-wheat bread. This improvement should occur by taking advantage of both ingredients, namely kombu as a natural source of bioactive substances, and the unique properties of iron oxide nanoparticles.

Materials and methods

Chemicals and raw materials

All reagents used in the synthesis of iron oxide nanoparticles and analysis were provided by Merck (Darmstadt, Germany). Kombu (kelp powder, food grade, moisture less than 12%, crude protein 11.4%) was purchased from Fuzhou Beautiful Agricultural Development Co., Ltd (Fujian, China). Commercial grade wheat and rye flour, table salt, and pressed baker's yeast were bought in local markets in Kharkov, Ukraine. Natural concentrated rye sourdough "Sapore Othello" in powder form was purchased from Puratos, Belgium.

Preparation of combined food additive

The general flow chart of the CFA preparation and the wheat-rye bread, as well as their analysis using standard methods, is shown in Figure 1.

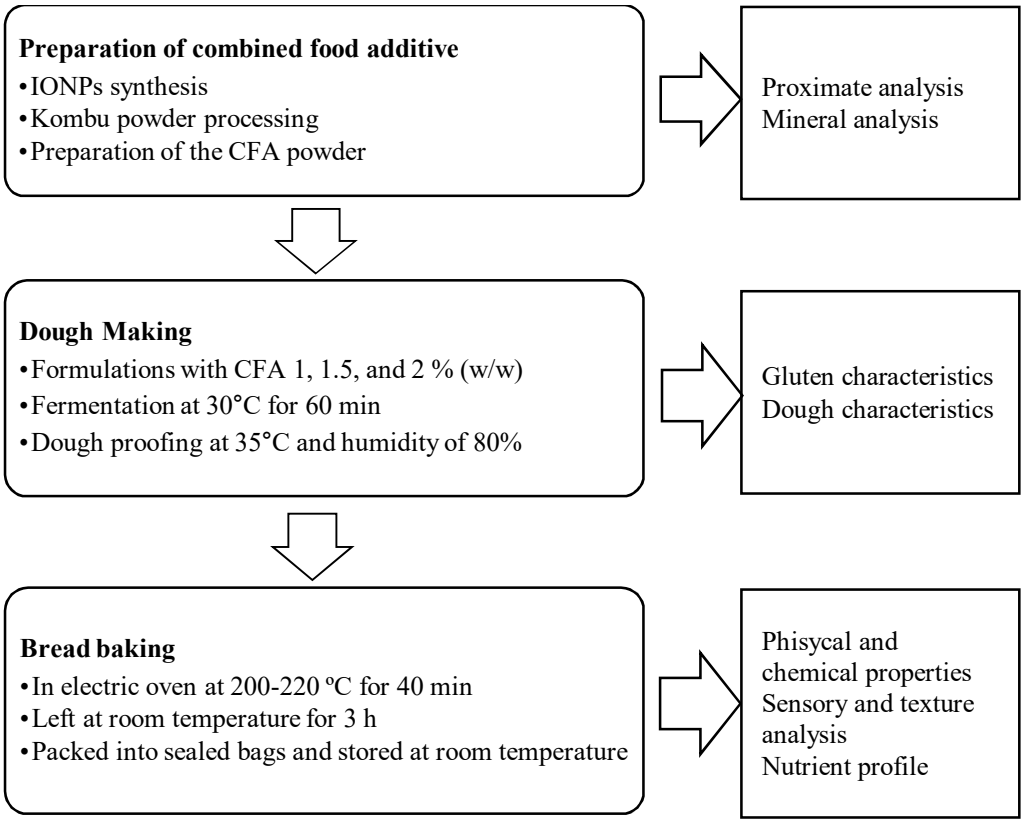

Figure 1. A chart flow of the research

Nanoparticles IONPs were obtained by precipitation of iron (II) and (III) salts with ammonia solution. The precipitate was washed with distilled water and dried at 100 ºC for 60 minutes. The obtained dark brown powder is a mixture of iron oxides FeO and Fe_2O_3 with an iron content of at least 90% and about 10% moisture. An average particle size was 75±10 nm with a zeta potential in aqueous suspensions 40±5 mV. The synthesized nanoparticles are stable to the action of external factors and oxidation.

Considering the significant iodine content in seaweed, kombu powder was pre-treated by rehydration and boiling according to the method (Correia et al., 2021) with slight modifications. Dry kombu powder was rehydrated for 10 min using tap water (ratio by 1:10 w/w) at room temperature. Then water was drained, and the rehydrated kombu was placed into boiling water and boiled for 5 min. Water was drained, and the seaweed biomass was dried in an oven at 40 °C for 12 hours. Finally, a dehydrated kombu was ground using a household coffee grinder Bosch TSM6A013B (BSH Hausgeräte GmbH, Germany) for 2–3 min to obtain a homogeneous fine powder. The average volume diameter of powder particles measured by laser diffraction on a PSA 1190 particle size analyzer (Anton Paar, Austria) was about 200±15 μm.

Next, the crushed kombu powder was ground in a mortar with IONPs for 3–5 minutes and the mixture was dried at 105 °C to a moisture content of no more than 5%. The complex

food supplement prepared in this way contained 15% (w/w) iron oxide nanoparticles and 85% (w/w) kombu powder and was used as the ingredient in the formulation of rye-wheat bread.

Bread production

To prepare rye-wheat bread, a formulation with peeled rye flour, 60 g; wheat flour first grade, 40 g; dry rye sourdough, 3.0 g; table salt, 1.4 g, and water, 45 g, was used as a control called BC. In the developed bread, the formulation included a combined food additive in the amount of 1.0, 1.5, and 2.0% w/w of the total amount of flour, and these samples were named B1, B2, and B3, respectively. The dough was kneaded in a kitchen machine Tefal Bake Essential QB160138 (Tefal, France) for 20 min. The homogeneous dough was subjected to fermentation at a temperature of 30±2°C for 60 min. After giving the final shape to dough manually, it was allowed to be proofed at 35±2°C and humidity of 80±5%. Baking was performed in an oven KIY-V ShZh-3 (KYI-V, Ukraine) at temperature 200–220°C for 40 min. The breads were left at room temperature for 3 h. The samples were then packed into sealed bags and stored at room temperature for sensory analysis.

Proximate analysis

Protein content was determined by the Kjeldahl method (ISO 1871:2009, 2009) and a conversion factor of 6.25 was used for protein calculation. Fat content was determined by the Soxhlet extraction method (ISO 11085:2015, 2015), the fat was extracted with petroleum ether boiling range of 40–60°C and determined gravimetrically. The moisture and ash content of the samples was determined according to the official procedures 44-16.01 humidity-air-oven (aluminum plate) and 08-01.01 ash-basic method, respectively (AACC, 2000). The results were presented on a dry weight basis (dw).

Mineral analysis

The mineral contents of bread samples were determined with an inductively coupled plasma atomic-emission spectrometer Thermo iCAP 6300 Duo ICP-AES Spectrometer (Thermo Scientific, USA) with the operating parameters as in (Yurchenko et al., 2020). Sample preparation was performed according to (Bilgiçli and İbanoğlu, 2015) without modification. Determination of iodine content in the samples was carried out by galvanostatic coulometric titration using equipment (Gubsky, 2023) according to the procedure (Gubskyi et al., 2015). Preliminarily studied samples were subjected to mineralization using the method of dry alkaline ashing to convert all chemical forms of iodine into iodide.

Dough characterization

The effect of the combined food additive on gluten was assessed by the amount of raw gluten, its compressebility and extensibility, which were determined using standard methods according to Drobot (2015).

The titratable acidity of the dough was determined by the volumetric titrimetric method with a 0.1 M sodium hydroxide solution as titrant in the presence of phenolphthalein as an indicator. The result was expressed in degrees (Neumann). For the dough, two acidity values were determined as the initial acidity at the beginning immediately after the preparation of the bread dough and as the final acidity at the end of fermentation

Dough development and gas production were carried out using rheofermentometer CHOPIN Technologies Rheo F4 (KPM Analytics, USA). From the dough development and gas release curves, which were recorded in real time, the total volume of carbon dioxide released Vt in mL, value of maximum dough height Hm in mm, dough height at the end of the measurement h in mm, decline in dough development (Hm-h)/Hm in % and time to maximum dough development T1 in min were quantified.

Specific volume of dough was determined from the data on the initial volume of the dough and its change during fermentation according to the methodology (Drobot, 2015). In the experiment, a sample weighing 50 g was placed in a 250 ml cylinder and kept in a thermostat at a temperature of 30°C.

Dough spreading was determined by observing the change in the diameter of a dough ball weighing 100 g during its fermentation at a temperature of 30°C for 180 min (Drobot, 2015). When calculating the yield stress in Pa, data on the degree of penetration determined by the Labor penetrometer (LABOR machine s.r.o., Czech Republic) were used.

Bread quality assessment

The total titratable acidity, moisture content, specific volume and porosity were estimated by standard methods according to Drobot (2015). The total titratable acidity of the bread was determined by the titrimetric method with a 0.1 M sodium hydroxide solution as titrant in the presence of phenolphthalein as an indicator. Briefly, freshly crushed bread weighing 25±0.01 g was placed in a 500 ml flask. Then add 50 ml of distilled water and grind the bread into pulp. 200 ml of water was added to the resulting mixture and the flask with the sample was shaken for 5 minutes. It was allowed to settle for 30 minutes and filtered. The supernatant was titrated with alkali solution and the result was expressed in degrees.

Bread moisture was determined by drying 5 g of ground bread crumbs in an OlisLab 4300 drying oven (Chizhova's device) (Olis, Ukraine). The weight and volume of bread were measured after baking. Specific volume was calculated as weight/volume. The mass of the bread by weighing with the help of Certus Balance CBA-300-0,05 scales (Certus, Ukraine) and the volume of the bread (ml) were measured according to the principle of measuring the volume of loose filler (fine grain) squeezed out of the bread, using the device RZ-BIO (Laboratorna Technika, Ukraine). The porosity of rye-wheat bread in (%) was determined using the UOP-01 (Zhuravleva) device (ThermoLab, Ukraine) and calculated according to the expression

$$\text{Porosity} = \frac{V_x - (\frac{m_B}{\rho_M})}{V_x}$$

where V_x is total volume of the bread recess in ml;
m_B is the mass of the recesses in g;
ρ_M is the density of the non-porous crumb mass in g/ml.

The shape stability of rye-wheat bread (F in the standard edition) was determined by expression

$$F = \frac{H}{D}$$

where H is the bread height in cm;
D is the bread diameter in cm.

The effect of the additive on the shelf life of bread freshness was studied by crumbliness of the crumb determined after 72 hours of storage. For this purpose, two parallelepiped-shaped pieces weighing 5 g each were cut out of the crumb and placed in a 250 ml conical flask that was moved on a vibrating mixer for 5 min. The crumbs formed as a result of friction of the two pieces were collected and weighed on a scale with an accuracy of 0.01 g (Drobot, 2015). The crumbliness index X in % of the crumb weight was determined by the expression

$$X = \frac{G_1}{G_2} 100$$

where G_1 is the crumb weight in g;

G_2 is the weight of the bread sample in g.

Analysis of the crumb texture profile of rye-wheat bread samples was evaluated using a TA.HDplusC Texture Analyzer (Stable Microsystems, UK) with a cylindrical probe (P/25) at a compression ratio of 25% at a speed of 1 mm/s according to the method (Bourne et al., 1978). Ten different parts for three different batches were pre-selected. The samples were cut from the center of the product into squares of 2.0×2.0 cm and kept for 2.0 hours at 20±2ºC and relative air humidity of 45–50% before conducting the test for standardization of water absorption. The analysis was carried out on such textural properties as hardness in g, cohesiveness in g, springiness in g, chewennes in %, elasticity in g according to Szczesniak (2002).

Indicators of the biological value of proteins were evaluated within the framework of approach (Mitchelll and Block, 1978) in the form of the unbalance coefficient of the amino acid composition UCAAC in %, the coefficient of biological value BV in % and the coefficient of utilitarianism CU in the magnitude ranges from 0 to 1 according to the methodology presented in the study (Sokolovskaya et al., 2020)

$$UCAAC = \sum_i \frac{(SCORE_i - SCORE_L)}{8}$$

$$BV = 100 - UCAAC$$

$$CU = \frac{SCORE_{min} \times \sum_i^8 AAC_{st}}{\sum_i^8 AAC_s}$$

where $SCORE_i$ is the *i*th essential amino acid score in %;

$SCORE_L$ is the limiting essential amino acid score in %;

$SCORE_{min}$ is the minimum of the score's of essential amino acids in %;

AAC_{st} is the total content of essential amino acids in the reference protein in mg/g of protein;

AAC_s is the total content of essential amino acids in the sample protein in mg/g of protein.

The sensory properties of bread were determined by scoring the quality of baked goods (Drobot, 2015). A preliminary acceptance test was used to evaluate the following attributes in the bread samples: crust surface, crust and crumb color, bakedness, porosity, chewiness and elasticity of crumb, taste, and odor by the ISO's methods (ISO, 2012, 2016). The sensory analysis was performed at State Biotechnological University (Kharkiv, Ukraine) as in (Aksonova et al., 2022). The panelists stayed in the room with temperature 25±2 ˚C and the

relative humidity 55±3%. The samples were prepared 1 h before the evaluation. Samples were kept in coded plates covered with aluminium foil. Ten trained panellists were selected to guarantee the evaluation accuracy. The intensity of each sensory characteristic was recorded on a 5-point hedonic scale after 1 h orientation sessions. In this session, the panelists had specified the terminology and anchor points on the scale. The coded samples were shown simultaneously and evaluated in random order.

Statistical analysis

A one-factor analysis (ANOVA) for a series of parallel measurements (at least 3) was used. The data in tables represents the mean±standard deviation. Value of $p < 0.05$ was considered statistically significant. The Tukey's HSD test was used to determine significant differences between means. Basic statistics and ANOVA were performed using the statistical software package Minitab ver. 18.1 (Minitab Inc., USA).

Results and discussion

Combined food additive characteristics

Combined food additive was a finely dispersed uniform dry powder with a mean volume particle size of 197±15 μm (Figure 2). The powder has a greenish-brown color and a characteristic taste and odor for algae.

Figure 2. Combined food additive

An experimental study of the proximate composition of the developed additive was conducted. The water content in the additive powder was 4.12±0.04%. A characteristic feature of the additive was a significant protein content of 11.6±0.4%, while the lipid level was 1.75%.

Significant fluctuations in the iodine content in raw materials and the requirement regarding the limiting indicators of RDA and UL necessitate a quantitative assessment of the iodine content in the additive. This indicator may be a limiting factor in determining the maximum possible amount of CFA additive to be included in the recipe for rye-wheat bread. The initial iodine content in the manufacturer's kombu powder was 1012±25 μg/g. This value is within the range of iodine content in kombu discussed above. After appropriate treatment of the powder by hydration and boiling, the iodine content was reduced to 213±15 μg/g by dry weight of the additive. This decrease accounts for 78.9% of iodine loss. This value agrees well with the results for *Laminria digitite* in the study (Correia et al., 2021). According to the

authors, the applied processes significantly reduce the iodine content because of its water-soluble form, which is extracted from the raw material. In addition to the decrease in iodine content, it should be noted that the sodium and potassium contents decreased to 47 and 55 mg/g (dw), which amounted to a decrease of 40 and 50%, respectively.

Particular attention was paid to the quantitative determination of algae polysaccharides in the additive, taking into account their positive effect on the quality of bakery products (Fu et al., 2021; Jönsson et al., 2024; Koh et al., 2020). The content of the main polysaccharides of the CFA, namely laminarin, fucoidan and alginic acid, was 3.35, 3.45 and 28.55%, respectively. Thus, CFA is a valuable source of dietary fiber to provide the recommended daily intake of 25-35 g for adults (Stephen et al., 2017). On the other hand, the said polysaccharides have important functional and technological properties. For example, alginic acid and its derivatives have gelling, structure-forming and emulsifying properties.

With regard to rye-wheat bread, the presence of the specified polysaccharides in the additive is a factor in improving the functional and physical properties of both the dough and the bread as a final product. This conclusion is based on some experience in studies related to the use of algae in the bakery products formulation. Thus, the inclusion of algae polysaccharides for partial replacement of 0-1.5% of the wheat flour in the bread recipe slowed down the process of starch retrogradation from amorphous to a more structured form (Fu et al., 2021). As a result, the bread prepared from frozen dough with 1% of *Laminaria japonica* L. polysaccharides had increased specific volume, water content and texture quality, while stalling processes during the storage were reduced.

Another study examined the effect of sulfated polysaccharide fucoidan as a fortifying agent at a concentration of 0.4 g and 0.80 g per 100 g of flour. Fortification of flour with fucoidan improved the gluten structure and gas-forming capacity of yeast, and the baked bread had a significantly higher specific volume and softer breadcrumbs. In addition, fucoidan-fortified bread was found to retain both antioxidant and anticancer activities *in vitro* (Koh et al., 2020).

Dough characteristics

An important technological factor in the formation of the quality of rye-wheat bread is the properties of wheat flour gluten. During hydration of gluten proteins, glutenin and gliadin interact with the formation of a viscoelastic three-dimensional matrix (Wrigley et al., 2006). The quantity and quality of gluten largely determines the texture of the dough and its ability to hold carbon dioxide. Effect of combined food addition on gluten characteristics is presented in Table 1.

Table 1

Gluten characteristics

Indicator	Dough sample			
	BC	B1	B2	B3
Wet gluten, %	26.8 ± 0.3^{a}	27.9 ± 0.5^{b}	28.6 ± 0.4^{bc}	29.4 ± 0.3^{c}
Compressibility, CU	78 ± 2^{a}	73 ± 1^{b}	70 ± 2^{c}	66 ± 2^{cd}
Extensibility, cm	15.0 ± 0.6^{a}	14.0 ± 0.5^{b}	12.5 ± 0.3^{c}	11.0 ± 0.4^{d}

[a-d] Means within the same row with different superscripts are significantly different at $p < 0.05$.

The analysis of the data in Table 1 allows confirming the improvement of the quantity and quality of gluten with the inclusion of CFA in the bread formulation compared to the control, regardless of the added amount. Thus, there is a statistically significant increase from 4% to 10% of the wet gluten quantity ($p < 0.05$). The inclusion of the additive increased the compressibility by 7-18% and decreased extensibility by 7-36% of gluten, corresponding to samples B1-B3. The probable cause of these facts is the ability of iron oxide nanoparticles of additives to form protein-ligand complexes (Tsykhanovska et al., 2022c, 2023) with protein molecules and, in particular, with gluten proteins (Tsykhanovska et al., 2018). An additional factor may be the interaction between wheat flour proteins and algal polysaccharides (Fu et al., 2021). In general, it can be assumed that the combined food additive forms bonds between molecules of proteins and carbohydrates with the formation of a spatially stable matrix.

The positive effect of CFA on physical-chemical and rheological characteristics of rye-wheat dough was established because of rheofermentometer measures. The inclusion of the additive in the recipe of bread had a positive effect on the improvement of the gas-forming ability in rye-wheat dough (Figure 3).

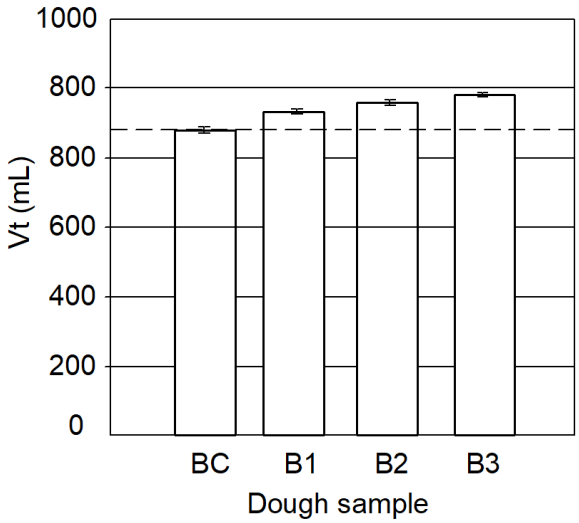

Figure 3. Total amount of CO_2 produced in the dough samples

Thus, the amount of carbon dioxide released from the gaseous release curve during the fermentation period of the experimental dough increased by 6-11% ($p<0.05$) compared to the control, depending on the sample. Intensification of alcoholic fermentation in the dough can occur due to the activation of yeast cells in the presence of additive's bioactive compounds. According to the dough development time curve, with increasing addition content in the bread formulation, the time to maximum dough development T1 decreases (Figure 4). The maximum dough height Hm and dough height h at the end of fermentation period are by 8.5-19.0% and by 10.5-26.0% higher compared to the control sample (Figure 5).

A decrease in decline in dough development (Hm-h)/Hm is also observed in samples B1-B3 as the amount of additive increases. These parameters are caused both by an increase in the gas-retaining capacity of test samples as a result of the discussed gluten strengthening, and by the acceleration of gas formation in it, as was said above.

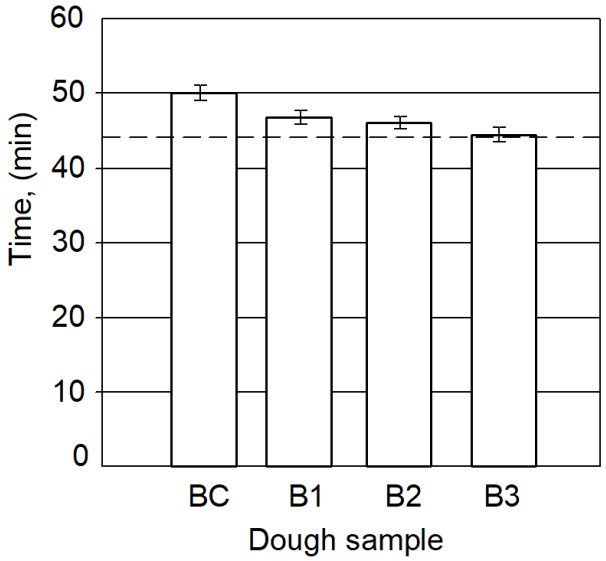

Figure 4. Time to maximum dough development T_1

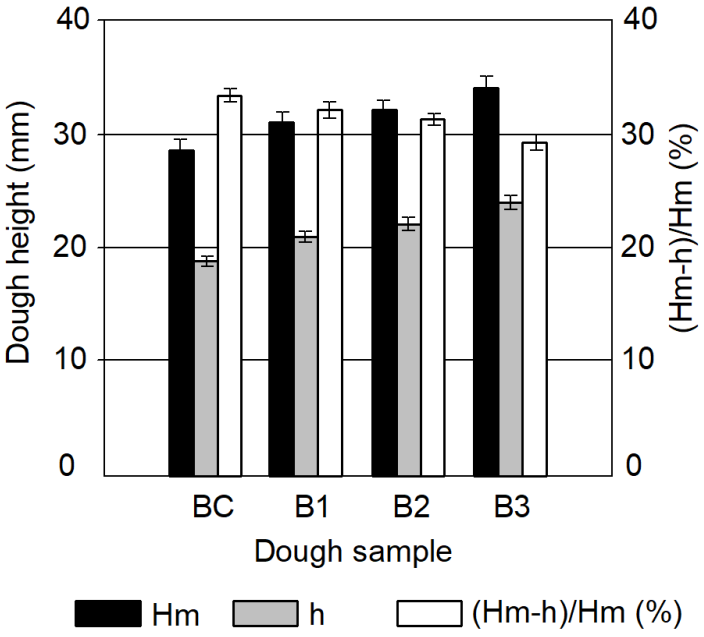

Figure 5. Magnitudes of maximum dough height Hm, dough height h at the end of the measurement after 3 hour and decline in dough development (Hm-h)/Hm of the dough samples

The inclusion of CFA in the formulation of rye-wheat dough leads to a slight decrease in both the initial and final acidity by 0.1–0.3 grades, which may be a consequence of the amphoteric properties of iron nanoparticles (Table 2).

Table 2

Physicochemical and rheological characteristics of rye-wheat dough

Indicator	Dough sample			
	BC	B1	B2	B3
Initial acidity, grades	6.9±0.1[a]	6.8±0.3[a]	6.7±0.3[a]	6.6±0.2[a]
Final acidity, grades	8.0±0.4[a]	7.9±0.3[a]	7.9±0.2[a]	7.8±0.3[a]
Spreading, mm	88.0±1.2[a]	84.0±1.1[b]	81.0±1.0[c]	78.0±1.0[d]
Specific volume, ml/g	2.48±0.1[a]	2.64±0.11[b]	2.72±0.11[c]	2.80±0.12[d]
Yield stress, Pa	452±8[a]	478±9[b]	492±9[c]	508±7[d]
Adhesive strength*, kPa	2.3±0.1[a]	2.0±0.2[b]	1.8±0.2[c]	1.7±0.2[cd]

[a-d] Means within the same row with different superscripts are significantly different at $p < 0.05$.
*The value is determined using steel plates

However, the observed decrease is statistically insignificant ($p>0.05$) and thus requires additional research. An increase of the additive content when moving from sample B1 to B3 changes some of the physical properties of the dough. The data in Table 2 indicate a decrease in the spreading index of rye-wheat dough from 11 to 13% ($p<0.05$). This fact is caused to the strengthening of wheat gluten and the increase in the water-retaining capacity of the dough. There is also an increase in the specific volume of the dough from 6 to 13% ($p<0.05$), which confirms the data shown in Figures 4 and 5. This parameter is caused both by an increase in the gas-retaining capacity of test samples as a result of the discussed gluten strengthening, and by the acceleration of gas formation in it, as was said above.
A study of the rheological properties of rye-wheat dough with the addition of an additive indicates an increase in yield stress from 6 to 12% ($p<0.05$). This fact shows the water-binding ability of the additive components and is the reason for the reduction in adhesion strength (to steel as the platinum material for research) by 15-35% ($p<0.05$). There results are consistent with the data of other research (Różyło et al., 2017; Tsykhanovska et al., 2022a).

Rye-wheat bread samples characteristics

The produced samples of rye-wheat bread were subjected to physicochemical analysis for moisture content and acidity (Table 3).

These results indicate that the inclusion of the additive in the bread formulation contributes to an increase in bread moisture due to the greater water-holding and water-absorbing capacity of the CFA. The amphoteric properties of the iron-containing component of the complex food additive slightly reduce the acidity of bread by 0.1–0.3 degrees. However, the observed decrease is not statistically significant ($p>0.05$).

Table 3

Characteristics of rye-wheat bread

Characteristics	Bread sample			
	BC	B1	B2	B3
Moisture content, %	47.0±0.2[a]	47.8±0.1[b]	48.2±0.3[bc]	48.6±0.2[c]
Acidity, degrees	7.4±0.3[a]	7.3±0.2[a]	7.2±0.q[a]	7.1±0.2[a]
Specific volume, ml/g	1.7±0.1[a]	1.8±0.1[ab]	2.0±0.1[bc]	2.1±0.1[cd]
Porosity, %	58±1[a]	61±1[b]	63±1[c]	65±1[d]
Shape stability, H/D	0.32±0.02[a]	0.35±0.01[b]	0.37±0.01[bc]	0.40±0.02[c]
Crumbliness after baking, %	4.7±0.2[a]	4.3±0.3[ab]	4.0±0.2[b]	3.8±0.2[bc]
Crumbliness after storage*, %	6.1±0.4[a]	5,5±0.3[ab]	4.9±0.2[c]	4.6±0.3[cd]

[a-d] Means within the same row with different superscripts are significantly different at $p < 0.05$.

* The value is determined after 72 h storage

The inclusion of CFA had an effect on changing some of the physical properties of rye-wheat bread compared to the control sample. Due to the improvement of the dough structure, its gas-forming and gas-retaining capacity (Figure 3), there was an increase in the specific volume from 6 to 24% and the crumb porosity from 5 to 12%, and the shape stability from 9 to 25% with an increase in the additive content in samples B1-B3. At the same time, a decrease in crumbliness was observed from 9 to 24% immediately after baking and from 11 to 33% after 72 hours of storage.

As mentioned above, this analysis was carried out on a 5-point scale, taking into account the contribution factors of each quality indicator. Recipients ranging from 4.5 to 5.0 rated the proposed quality indicators. Therefore, for better visualization, Figure 6 presents the results of the ratings on the minimum rating-maximum rating scale. The use of a combined food additive had a positive effect on the taste, aroma and surface of the crust of products. Sensory analysis of rye-wheat bread samples with different amounts of addition compared to the control sample is shown in Figure 6.

The elasticity, volume, porosity of products and chewiness increased, which is consistent with the data on the improvement of the physical and chemical properties of bread with the additive (Table 3). However, the addition of more than 2% of the additive leads to the appearance of a specific taste and smell of algae in the bread, which led to a decrease in these indicators.

Inclusion of the complex food additive, 1.0, 1.5, and 2.0%, to the formulation of rye-wheat bread resulted in improving of the sensory characteristics in comparison with control bread without CFA, namely, the average score increased by 1.06; 1.08, and 1.07 times, respectively. Based on a comparative analysis of physicochemical, rheological and sensory indicators, it was concluded that the optimal amount of inclusion of a combined food additive in the formulation of rye-wheat bread as an ingredient is 1.5%. This bread sample was subjected to further quality assessment called BD (bread development).

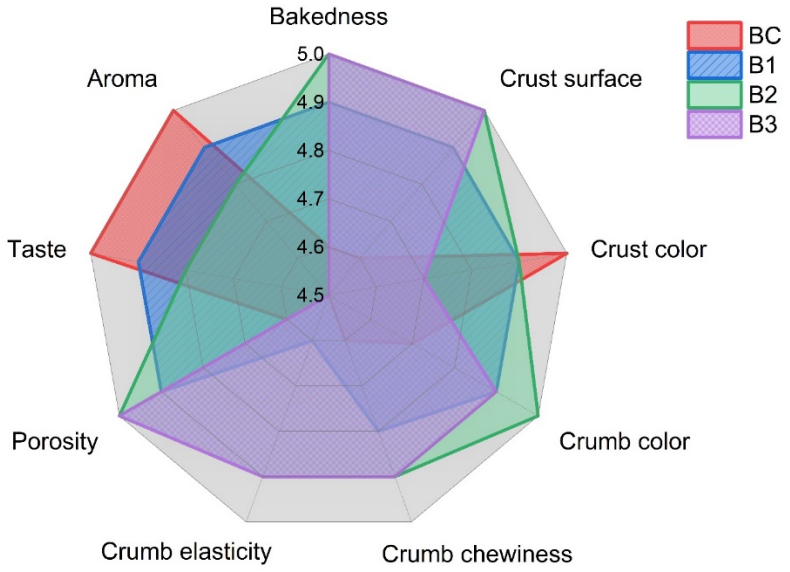

Figure 6. Spider diagram of sensory characteristics of bread samples

Bread quality assessment

One of the important factors influencing consumer preference for a food product, in addition to its appearance, aroma and taste, is its texture. Formed because of the interaction of the main components such as proteins, polysaccharides, and lipids, it should promote their release from the food matrix to reach the corresponding taste buds. The development of taste is closely related to both the destruction of the initial texture of the food product in the mouth and its changes during chewing. An experimental study of the texture of the bread under development with the inclusion of CFA in an amount of 1.5% by weight of flour compared to the control is shown in Table 4.

Table 4

Texture properties of bread samples

Characteristic	Bread sample	
	BC	BD
Hardness, g	714.2±1.8[a]	667.1±1.7[b]
Cohesiveness, g	0.343±0.001[a]	0.369±0.001[b]
Elasticity, g	0.48±0.01[a]	0.51±0.01[b]
Chewiness, %	75.1±0.9[a]	77.8±0.8[b]
Springiness, g	0.064±0.00[a]	0.069±0.00[b]

[a-d] Means within the same row with different superscripts are significantly different at $p < 0.05$.

The data in Table 4 indicate a significant (p<0.05) decrease in bread hardness by 6.6% and an increase in elasticity by 6.7%). A higher loaf specific volume (Table 3) results in lower hardness due to a less dense crumb and less compact cells (Table 4) (de la Hera et al., 2014). It was found that the inclusion of CFA in BD sample formulation contributes to a significant (p<0.05) decrease in bread hardness by 6.9% and an increase in elasticity by 6.68% compared to the control (Table 4). This is associated with a higher mass fraction of moisture (Table 3) and the plasticizing ability of water molecules. At the same time, springiness and cohesiveness of bread with CFA increased compared to the control by 7.3% and 7.1%, respectively, due to strengthening and stabilizing the structure through intermolecular interactions of biopolymer molecules (proteins, fats, and carbohydrates). The consequence of the higher cohesiveness of the bread with CFA (1.5%) compared to the control is an increase in the chewing index by 2.7% and an improvement in the chewing ability of the product.

Analysis of the nutrient profile and value of samples of rye-wheat bread shows an improvement in the nutritional value of the BD compared to the control (Table 5).

Table 5

Nutrient profile and value of rye-wheat bread

Nutrient or indicator	Bread	
	BC	BD
Macronutrients, g/100 g dw		
Protein	7.5±0.2[a]	7.9±0.3[b]
Fat	0.90±0.01[a]	0.91±0.02[a]
Starch	39.2±0.3[a]	40.6±0.4[b]
Fiber	0.40±0.01[a]	0.46±0.01[b]
Minerals, mg/100 g dw		
Sodium	343.5±0.9[a]	349.3±1.1[b]
Potassium	98.6±0.7[a]	101.0±0.8[b]
Iodine	nd[**]	0.055±0.010
Nutrition value		
UCAAC, %	28.75±0.04[a]	19.50±0.04[b]
BV, %	71.25±0.87[a]	80.50±0.92[b]
UC	0.54±0.02[a]	0.62±0.02[b]

[a-d] Means within the same row with different superscripts are significantly different at $p < 0.05$.

[*]nd is not detected.

Analysis of the nutrient profile and value of rye-wheat bread samples shows an improvement in the nutritional value of the BD compared to BC (Table 5). The data in Table 5 indicate a significant increase in the content of protein, starch, dietary fiber, and some minerals ($p < 0.05$). Kombu was characterized above as a natural source of iodine. The control sample did not contain iodine. The inclusion of the additive in the bread formulation made it possible to obtain a final product fortified with iodine with a content of 55±10 μm/100 g of bread. This magnitude allows calculating the daily consumption of the developed bread without exceeding the RDA value.

To assess the biological value of rye-wheat bread according to the Mitchell-Block method (Block and Mitchell, 1946), the protein content was preliminarily determined, and the amino acid score was estimated. The limited amino acid in both samples is lysine, but in the BD sample, this indicator is improved by 37.5% compared to the control. The score of other essential amino acids also increases in the range from 4.75 to 20.05% compared to the control. Data on the amino acid composition were used to calculate the coefficient of imbalance of the amino acid composition according to formula (1). The obtained values of this indicator for both BC and BD samples are 32.8 and 28.2%, respectively, which indicate a satisfactory biological value of the samples. This conclusion is confirmed by calculating the value of the balance of essential amino acids in relation to the physiologically necessary norm as a coefficient of utility. The calculated values of this coefficient are 0.54 and 0.62 for the BC and BD samples, respectively, which testify to the improvement of the balance of essential amino acids in the developed sample of rye-wheat bread. Thus, fortification of rye-wheat bread with a combined additive made it possible to improve its food value.

Conclusions

1. Iron oxide nanoparticles with an iron content of at least 90% and an average particle size of 75±10 nm were synthesized.
2. Commercial kombu powder was treated by hydration for 10 min and boiling in water for 5 minutes, which reduced the iodine content in the sample from 1012±25 μg/g to 213±15 μg/g dw.
3. Dry powders of nanoparticles of iron oxide and kombu in a ratio of 15:85 (w/w) were used to develop a combined food additive (CFA) as an improver of technological properties and a fortifying agent when included in the formulation of rye-wheat bread as an ingredient.
4. The inclusion of the additive in the amount of 1.0, 1.5, and 2.0% of the flour weight (bread samples B1, B2, and B3) improves the quality of gluten. This is confirmed by a statistically significant increase in raw gluten by 4% to 10%, increased compressibility by 7–18% and decreased extensibility by 7–36% of gluten for bread samples B1-B3.
5. The gas formation curves showed an increased release of carbon dioxide during the fermentation period of the experimental dough by 6–11% ($p<0.05$) compared to the control, depending on the sample, due to the activation of yeast cells in the presence of biologically active compounds of the additive.
6. According to the curve of dough development time, with an increase in the content of the additive in the bread recipe, the time of maximum dough development decreases, and the maximum height of the dough and the height of the dough at the end of the fermentation period are 8.5–19.0% and 10.5–26.0% higher compared to the control sample.
7. Analysis of the physicochemical properties and sensory characteristics of rye-wheat bread samples allowed to determine the optimal level of fortification of rye-wheat bread with CFA at a level of 1.5% dw.
8. The inclusion of a combined food additive in the rye-wheat bread formulation increased its nutritional value, as evidenced by an increase in the unitarity index from 0.54 to 0.62.
9. Fortification of rye-wheat bread with iodine at a level of 55±10 μg/100 g allows it to be considered as a functional product for the prevention of iodine deficiency diseases.

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Ultrasound degumming of sunflower oil

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Abstract

Introduction. The degumming of vegetable oils is a crucial stage of oil refining aimed at phospholipid removal. The objectives of this study were to investigate the possibility of ultrasound acceleration of the sunflower oil degumming.

Materials and methods. The ultrasound degumming of vegetable oil was carried out on the mixer MEDITON (Ukraine) and ultrasound thermostat Bandelin Sonorex (Austria) under addition of 5 % water from 5 to 15 minutes. The volume of degummed oil was measured after gum separation by centrifugation. Phosphorus content in oil ash was detected photometrically. Acid and peroxide values were determined by standard methods. Antioxidant properties of the oil were estimated as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity.

Results and discussion. The ultrasound degumming leads to an increase in the yield of degummed oil by 2-2.5 % compared to water degumming with maximal yield at ultrasonic treatment duration from 5 to 11 min. The ultrasonic degumming as well as water degumming decreased the phosphorus content from 98 ppm in crude oil to 28-32 ppm in the degummed sunflower oil. The oil acidity was reduced substantially due to ultrasound degumming, the peroxide value demonstrated some increase, and the antioxidant capacity of the sunflower oil varied from 18.6 to 26.9 % of DPPH radical scavenging as a result of degumming.

Ultrasound degumming at fixed temperatures of 30 and 60 °C resulted in close phospholipids content in degummed oil. The minimal phospholipid content in the range from 24 to 35 ppm of phosphorus was observed after ultrasound degumming during 6-9 min at both temperatures. Ultrasound degumming at 60 °C resulted in an acid value increase from 0.64 mg KOH/g at the beginning to 1.1 mg KOH/g after treatment for 15 min. The acid value of oil after ultrasound degumming at 30 °C was not higher than in the control at any duration of treatment and was in the range from 0.5 to 0.7 mg KOH/g. The peroxide values of oil after ultrasound degumming at 60 °C were higher compared to the control oil and samples after ultrasound degumming at 30 °C.

Conclusion. The ultrasound degumming accelerated the removal of phospholipids from sunflower oil and increased the yield of degummed oil. Ultrasound degumming can be recommended for the oil industry and it is important to carry out ultrasound treatment of the oil at low temperatures.

Introduction

The degumming of vegetable oils removes various hydrophilic substances from oils, such as phospholipids and other gums. The phospholipids of vegetable oils have different levels of hydrophilicity, one part is more hydrophilic (more polar) and another, which is less hydrophilic. More hydrophilic phospholipids are called hydratable, and they could be removed by water degumming. The other part of the phospholipids are nonhydratable, and acid degumming is required to remove them from oil. Water degumming is a sustainable method of oil processing (Hamm et al., 2013) and it is possible to obtain food-grade lecithin. However, water degumming does not provide a high level of phospholipid removal and oil yield as well as requires a long exposition time. Thus, improving oil degumming is important for the oil industry.

One of the methods for phospholipids removal from oil is enzymatic degumming with various types of phospholipases (Dijkstra, 2010; Gofferjé et al., 2014; Guerrand, 2017). The effectiveness of new types of phospholipase preparations with phospholipase A and C activity for improving sunflower oil degumming was demonstrated (Nosenko and Zhupanova, 2023). However, phospholipase preparations are sensitive to degumming conditions and are still expensive for the industry.

Various methods of physical treatment are used for improving oil degumming as well as other processes of oil processing. In particular, ultrasonic treatment is widely used in food technology to accelerate chemical reactions and technological processes, such as extraction (Sun et al., 2023; Tiwari, 2015) and emulsion obtaining (Bernardi, et al, 2021; Soria and Villamiel, 2010). Ultrasound treatment is relatively cheap, simple, and energy saving and has become a promising technology to be used for food processing (Chew and Ali, 2021).

Ultrasound is a sound wave with a frequency greater than the upper limit of the human hearing range. An ultrasonic wave is a mechanical wave with a frequency range of $10^4 \sim 10^{12}$ Hz. When the ultrasonic wave spreads in the medium, it produces a pressure wave, which forms a high-pressure region (compression region) and a low-pressure region in the medium. In the region of high pressure and low pressure, the medium molecules contract and expand respectively. In the process of expansion, the bounds between molecules are pulled apart to produce tiny cavities or microbubbles. When the critical radius is reached, a very high local energy density is released due to the explosion, which is called the cavitation effect. At the moment of collapse of ultrasonic cavities, local high temperature, high pressure, and accompanying strong shock waves are generated around it (Ashokkumar and Mason, 2007; Legay, et al., 2011; Chemat et al., 2016). The shock wave, shear, and vibration enhance the movement of macromolecules, and particles and significantly increase the mass transfer rate, which is very important for chemical reactions in heterogeneous systems.

Several studies were devoted to the ultrasound-assisted enzymatic degumming of crude oil. Jiang et al (2014) detected that ultrasonic treatment had increased the rate of enzymatic degumming reactions of rape oil resulting in phosphorus content of less than 10 ppm during 2 h. At the same time, 4 h is needed to achieve the same phosphorus content under mechanical mixing of the reaction mixture. However, it was shown that the use of ultrasonic treatment could reduce the oxidative stability of rapeseed oil.

The authors (More and Gogate, 2018) studied the ultrasound-assisted enzymatic degumming of crude soybean oil, quantifying the extent of degumming. The influence of different operating parameters such as enzyme content, pH, presence of water, temperature, and ultrasonic power on the enzymatic degumming of crude oil has been investigated. Ultrasound combined with enzyme at a dose of 2.0 ml/L resulted in an extent of degumming of 92.2% under ambient conditions. The addition of water (5%) in combination with

ultrasound and enzyme at 2.0 ml/l and pH of 5 resulted in maximum extent of degumming (98.4%) during 120 min of treatment. Scale-up studies were performed at 500 ml and 1 l operating volume under optimized conditions of 2.0 ml/l as the enzyme loading, pH of 5, 5% water addition, and ultrasonic power of 100 W where 93.63% and 91.15% phospholipid separation respectively was obtained. The effects of ultrasonic treatment on the acid value reduction and oxidative stability for the processed oil were estimated too. It was demonstrated the reduction in acid value (final value less than 1) and oxidative stability (TOTOX less than 4) were obtained using enzymatic degumming. Overall, the approach of enzymatic degumming was established to show much higher efficacy for soybean oil processing as compared to only ultrasound or only enzymatic treatment.

Ultrasound-assisted enzymatic degumming was applied also to the arachidonic acid oil produced by *Mortierella alpina* (Guo et al., 2021). The conditions of the degumming process were optimized by response surface methodology. The efficiency of degumming 98.82% was achieved under optimum conditions of 500 U/kg of phospholipase A_1 (PLA_1) dosage, 2.8 mL/100 g of water volume, 120 min of ultrasonic time, and 135 W of ultrasonic power. The phosphorus content of ultrasonic-assisted enzymatic degumming oil was 4.79 mg/kg, which was significantly lower than that of enzymatic degumming oil (17.98 mg/kg). The oxidation stability of the same oil after enzymatic degumming of crude oil was equivalent to that after enzymatic degumming and weaker than crude oil.

As Ukraine is a main sunflower oil producer, improvement of its processing is important for oil industry. In our previous work, the influence of the different types of phospholipases on the efficacy of sunflower oil degumming was shown (Nosenko and Zhupanova, 2023). However, industrial use of phospholipases is still complicated by their high sensitivity to degumming conditions and oil composition. Thus, it is important to improve the oil degumming efficacy by simple and accessible methods. The objectives of this study were to investigate the influence of the ultrasound itself on the sunflower oil degumming.

Materials and methods

Crude sunflower oil was purchased from the local market.

Ultrasound degumming

Crude sunflower oil, 100 g, with 5 % of distilled water was placed into the ultrasound mixer MEDITON (Ukraine) and treated for 5 to 15 minutes at ultrasonic treatment frequency 44 kHz. Every 3 min the samples were taken for analysis. Mixture was kept for 30 min at room temperature, 10 ml of the reaction mixture was put into a graduated centrifuge tube and the gums were separated from the oil by centrifugation at 2000 rpm for 10 min.

For thermostated degumming ultrasound thermostat Bandelin Sonorex (Austria) was used and a mixture of 100 g crude sunflower oil with 5 % of distilled water was treated at 30 or 60 °C at ultrasonic treatment frequency 28 kHz. Every 3 min the samples were taken for analysis.

The water degumming (control sample) was carried out according to the next procedure. Crude sunflower oil, 100 g, was placed into a 250-ml conical flask. The oil was heated to about 60 °C followed by the addition of 5 % of distilled water. The mixture was thoroughly mixed for 1 min and stirred at 60 °C for 1 h. After that, reaction mixture was kept for 30 min

at room temperature. The 10 ml of the reaction mixture was put into a graduated centrifuge tube and the gums were separated from the oil by centrifugation at 2000 rpm for 10 min.

Oil yield determination

The volume of degummed oil was measured after gums separation and oil yield (X, % of crude oil) was calculated as

$$X = (b/9.52)\cdot 100,$$

where 9.52 was the volume of the crude oil in 10 ml degumming mixture before centrifugation in ml, and b was the volume of separated oil in ml.

Phosphorus content analysis

The oil samples were ashed for phosphorus content determination according to (Yang et al., 2006). 0.7 g of MgO and 0.6 to 0.7 g of oil were weighed and heated in the oven at 110 °C for 10 min. After that, the samples were carbonized by heating on the hot plate and then ashed in an electric muffle furnace at 600 °C until a constant mass was achieved.

The phosphorus content of the ash was determined according to the AOCS method Ca 12–55 (1997). Cold ash was transferred to a 100 ml flask, 10 to 12 ml water, and 20 ml 2N H_2SO_4 were added. The solution was heated to dissolve the ash. After that 20 ml molybdenum reagent was added and the solution was heated in boiling water bass for 30 min. The volume of the cold solution was adjusted to 100 ml. Photometric analysis was carried out on UV/VIS spectrometer PerkinElmer Lambda 35 at 750 nm.

A standard solution of KH_2PO_4 (concentration 10 μg/ml) was used for the calibration curve obtaining. The calibration solution set was prepared in the same condition as the ash samples. The concentration of KH_2PO_4 in the calibration solution set was adjusted from 0.05 to 2.0 μg/ml.

Phosphorus content (P, % of oil mass) was calculated as follows:

$$P = 0.01\ d/m,$$

where d was the phosphorus content in ash solution according to a calibration curve, μg/ml, m was oil mass, g.

The content of phospholipids as stearoiloleil phosphatidylcholine (PC, % of oil mass) was calculated as follows:

$$PC = 25.4\ P,$$

where P was the phosphorus content, % of oil mass, and 25.4 was a coefficient for calculation of the stearoiloleil phosphatidylcholine mass on the base of phosphorus content.

Chemical parameters of oils

The acid value of oil samples was determined by the titrimetric method according to ISO 660:2020.

The oil's peroxide value was determined using the iodometric method according to ISO3960:2017.

Antioxidant activity

The radical scavenging capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. For the DPPH test, DPPH was dissolved in a small volume of ethyl acetate and diluted with ethyl acetate by adjusting the absorbance to 0.700 ± 0.020 at 520 nm. 100 mg of oil was weighed in a test tube and 15 ml DPPH$^{\bullet}$ free radical solution was added (Vovk et al., 2023). The sample was agitated and absorbance was measured at 520 nm against ethyl acetate. After 30 min of incubation in darkness, absorbance was measured against ethyl acetate on UV/VIS spectrometer PerkinElmer Lambda 35 at 520 nm.

The results of the DPPH test were expressed as % of DPPH$^{\bullet}$ free radicals that were scavenged by antioxidants in 100 mg oil (A, %):

$$A = (1 - D_1/D_0) \cdot 100,$$

where D_0- was the absorbance of the reaction solution at 520 nm before incubation, and D_1- was the absorbance of the reaction solution at 520 nm after incubation.

Statistical analysis

Each sample was analyzed in triplicate, and the results were reported as mean ± standard deviation. Differences were considered to be significant at validity α=0.95.

Results and discussion

Effect of the ultrasound degumming on the sunflower oil yield and properties

To evaluate the effect of ultrasonic treatment on the effectiveness of sunflower oil degumming, we determined the yield of oil after ultrasound degumming. The duration of ultrasonic treatment was from 5 to 15 minutes in the presence of 5 % water. The control oil sample was treated with the same amount of water with constant stirring for 1 h at 60 ºC.

The obtained results demonstrated that using ultrasound degumming leads to an increase in the yield of degummed oil by 2–2.5% (Figure 1). The maximal yield was observed under ultrasonic treatment duration from 5 to 11 min followed by a slight decrease in oil yield. The last phenomenon may be due to the enhancement of the oil emulsifying under the continued ultrasonic treatment.

The most important parameter of the oil degumming is the phospholipid content in the oil. It is known that water degumming itself can remove only part of phospholipids from vegetable oils, which are called hydratable phospholipids. The ratio of hydratable phospholipids to nonhydratable phospholipids in vegetable oils depends on different factors - oil nature, method of obtaining, condition, and duration of storage. For entrance, in our previous work about half of sunflower oil phospholipids were hydratable phospholipids (Nosenko and Zhupanova, 2023). In the present study, content of phospholipids had dropped sharply after water degumming of sunflower oil (Figure 2). About 68 % of phospholipids were removed by water degumming.

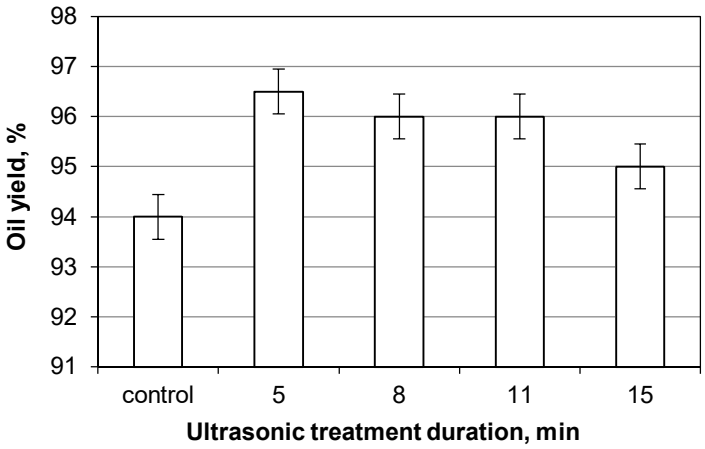

Figure 1. Influence of the ultrasound degumming on the sunflower oil yield.

Other researchers had obtained that about 80 % of the initial phospholipids content in sunflower oil were hydratable phospholipids (Zufarov et al., 2008; Zufarov and Serkayev, 2023) and the residual amount of phosphorus after water degumming at 80 °C during 15 min was in the range from 50.1 to 56.9 ppm. At the same time, phosphorus content in water-degummed soybean oil was 127 ppm (Dijkstra and Opstal, 1989).

The ultrasonic treatment did not sufficiently affect the oil phospholipid content compared with water degumming. The phospholipid content in the degummed sunflower oil was in the range of 0.07-0.08 % of stearoiloleil phosphatidylcholine, which corresponds to 28-32 ppm phosphorus (Figure 2).

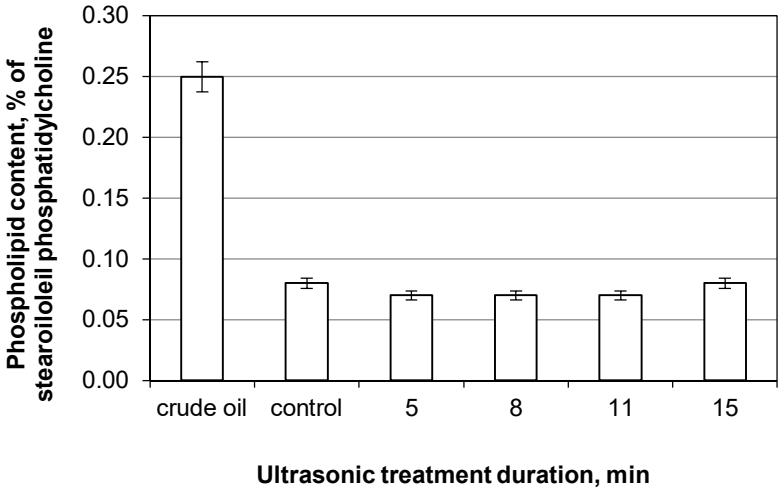

Figure 2. Influence of degumming on the phospholipid content of the sunflower oil (ultrasonic treatment frequency 44 kHz)

Simultaneously with the phospholipid content decrease, we observed the reduction of oil acidity (Figure 3). The acid value of crude oil was high 5.6 mg KOH/g and declined substantially due to ultrasound degumming. Thus, it was 3.1 mg KOH/g in oil after 11 min ultrasound treatment. Such as there was a correlation between phospholipid content and the acid value of the oil, we can suppose, that the oil acidity decrease was mainly due to acid phospholipids removing, first, phosphatidic acid.

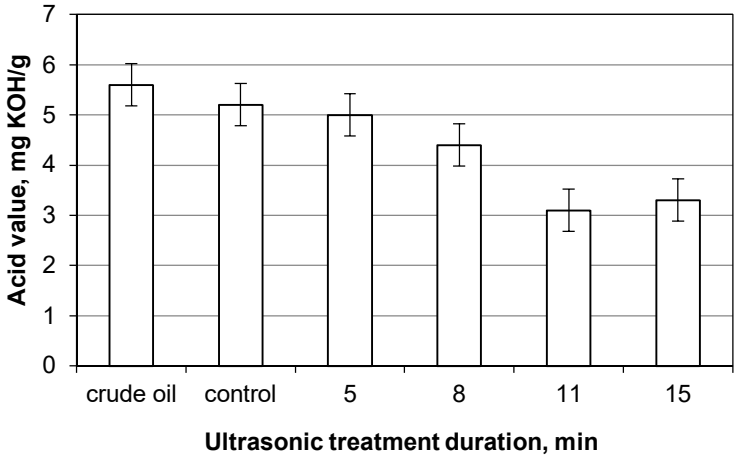

Figure 3. Influence of ultrasound degumming on the acid value of the sunflower oil (ultrasonic treatment frequency 44 kHz)

The peroxide value of crude sunflower oil in this experiment was 4.2 meq O/kg. There was a slight rise in peroxide value because of degumming (Figure 4). The influence of ultrasound treatment was unimportant; the peroxide value was in the range from 5.1 to 5.9 meq O/kg.

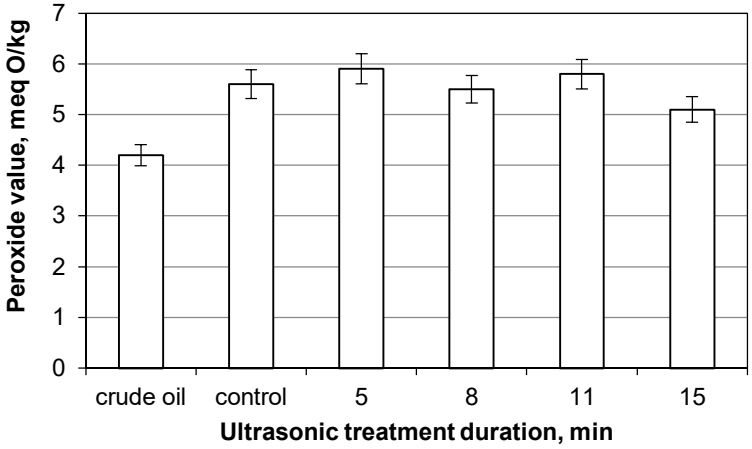

Figure 4. Influence of ultrasound degumming on the peroxide value of the sunflower oil (ultrasonic treatment frequency 44 kHz).

The water and ultrasound degumming had no substantial effect on the antioxidant capacity of the sunflower oil (Figure 5). The antioxidant capacity of the degummed sunflower oil varied from 18.6 to 26.9 % of DPPH scavenging, the crude oil antioxidant capacity was 20.4 % of DPPH. A slight rise in the antioxidant property after degumming could be caused by metal removal, which is known as prooxidants (Zufarov and Serkayev, 2023). On the other hand, long ultrasonic treatment could result in antioxidant inactivation.

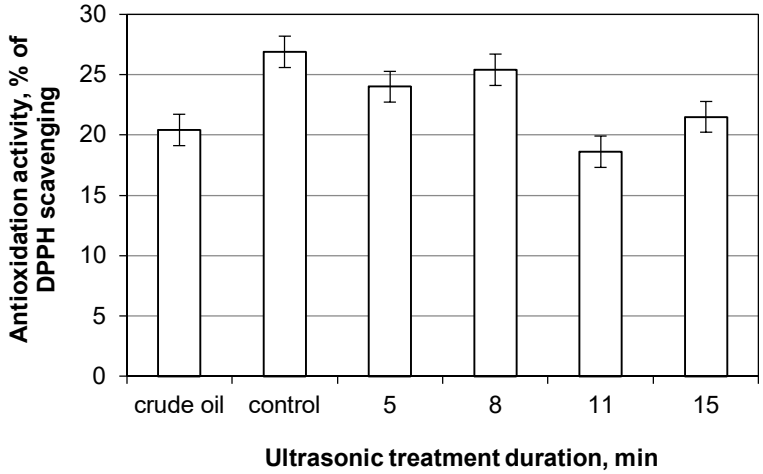

Figure 5. Influence of ultrasound degumming on the antioxidant capacity of the sunflower oil (ultrasonic treatment frequency 44 kHz).

Effect of the ultrasound degumming at different temperatures on the sunflower oil quality

Such as ultrasonic treatment results in an increase in the oil temperature to 55- 60 °C it is important to separate the influence the ultrasound and heating on the effectivity of the oil degumming. For this, we carried out ultrasound degumming at the constant temperature and ultrasonic treatment frequency of 28 kHz. Ultrasound degumming at fixed temperatures of 30 and 60 °C results in close phospholipids content in degummed oil (Figure 6). The minimal phospholipids content in the range of 0.06 to 0.09 % of stearoiloleil phosphatidylcholine was observed after ultrasound degumming during 6-9 min. There was a drop of phospholipids content in degummed oil only after ultrasonic treatment for 9 min at 60 °C. It was the lowest phospholipids content, which corresponds to 24 ppm phosphorus in sunflower oil. Prolongation of ultrasound treatment was accompanied by a phospholipid content increase, which could be due to phospholipids stabilization in oil because of emulsifying.

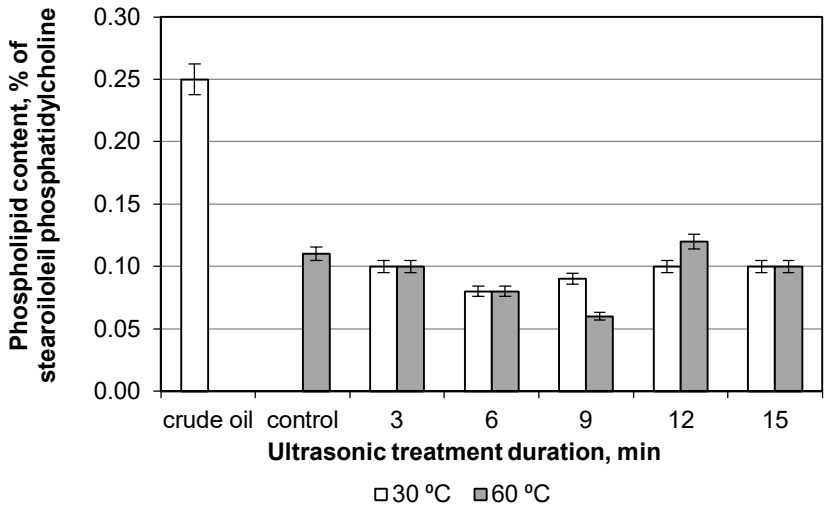

Figure 6. Influence of the ultrasound degumming at different temperatures on the phospholipid content in the sunflower oil (ultrasonic treatment frequency 28 kHz)

It is important also to study the effect of ultrasound degumming on the other parameters of oil quality, first, the acidity of the oil. Increasing the free fatty acid content in the oil could result in the loose of oxidation stability followed by a shelf life decrease. Ultrasound degumming at 60 °C was accompanied by acid value increase from 0.64 mg KOH/g at the beginning to 1.1 mg KOH/g after 15 min treatment (Figure 7).

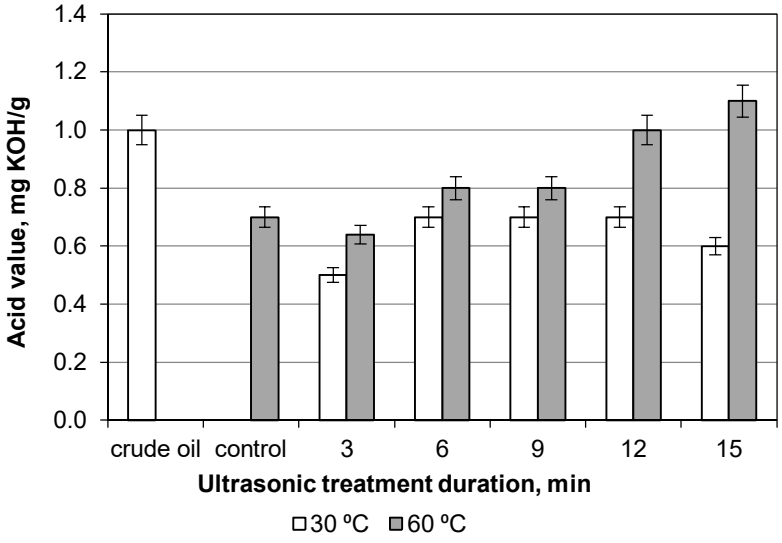

Figure 7. Influence of the ultrasound degumming at different temperatures on the acid value of the sunflower oil (ultrasonic treatment frequency 28 kHz)

It is supposed that higher temperature together with ultrasound could accelerate the hydrolysis of acylglycerols followed by free fatty acids release. It is obvious, that under ultrasound degumming at low temperature acid value of oil was not higher than in the control at any duration of treatment and was in the range from 0.5 to 0.7 mg KOH/g.

It was shown, that water degumming, acid degumming, and TOP (total degumming process) degumming result in removing of metals from rapeseed and sunflower oils enhancing the oxidative stability of these oils (Zufarov and Serkayev, 2023). In our previous study, we detected that water and enzymatic degumming were accompanied by a decrease in peroxide content in the sunflower oil (Nosenko and Zhupanova, 2023).

The data obtained in the present study have demonstrated that ultrasound degumming results in a decrease in the peroxide value of sunflower oil in every sample compared with crude oil (Figure 8). However, oil samples after ultrasound degumming at 60 °C had higher peroxide values than control oil and samples after ultrasound degumming at 30 °C except the samples of long ultrasound degumming. The long ultrasound treatment usually was accompanied by a reduction of quality parameters of oil. Thus, we observed higher phospholipids content, and acid value of oil after ultrasound degumming at 60 °C during 12-15 min compared with 6-9 min treatment.

The content of oxidation products and oxidation stability in vegetable oil depends on numerous internal and external factors, such as fatty acid composition, antioxidant capacity, quality of oil seeds, processing technology, and conditions of storage. Thus, the content of oxidation products in the same oil can be very different.

Commonly, the ultrasound degumming did not have a prominent negative effect on the peroxides content in the oil. We are suggesting that the removal of phospholipids because of degumming can be accompanied by the removal of peroxides and prooxidants, mainly metals from the oil. The increase of peroxide content in some samples could be the result of stable emulsion creation after long ultrasound treatment.

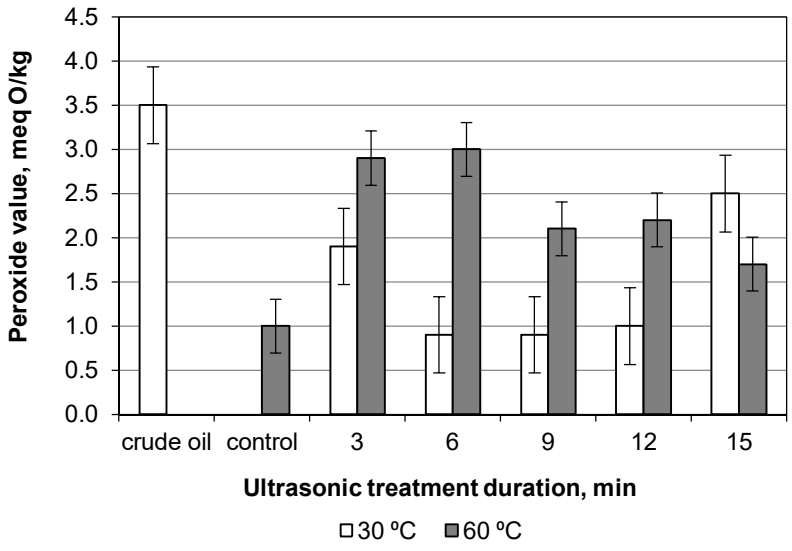

Figure 8. Influence of the ultrasound degumming at different temperatures on the peroxide value of the sunflower oil (ultrasonic treatment frequency 28 kHz)

Conclusions

1. The ultrasound degumming is a prominent tool to reduce the duration of this process and increase the oil yield. The maximal degummed oil yield was 96–96.5 % at ultrasonic treatment duration from 5 to 11 min which was higher by 2–2.5% compared to water degumming during 60 min.
2. The water degumming for 60 min and ultrasound degumming for 5 min had the same effect on the phospholipid content in the oil. Because of degumming, the phosphorus content decreased from 98 ppm in crude oil to 28–32 ppm in degummed sunflower oil.
3. Ultrasound degumming at fixed temperatures of 30 and 60 °C resulted in close phospholipids content in degummed oil. The minimal phospholipids content in the range from 24 to 35 ppm of phosphorus was observed after ultrasound degumming during 6-9 min.
4. It is worth carrying out ultrasound treatment of the oil at low temperatures such as the simultaneous effect of ultrasound and high temperature can stimulate the hydrolytic and oxidative reactions in the oil.

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Dealcoholized wine from the grape of the Citronny Magaracha variety: Physico-chemical parameters and the formation of a sensory profile

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Abstract

Introduction. The aim of the study was to determine the technology of dealcoholized wines produced from the Citronny Magaracha grape variety, ensuring maximum preservation of the natural sensory properties of the original wine.

Materials and methods. White dry wine from the grape of the Citronny Magaracha variety (Ukraine) was used. Dealcoholization was carried out using vacuum or osmotic distillation. The physico-chemical parameters of the dealcoholized wine were determined using nuclear magnetic resonance and chromatography. The sensory properties were evaluated using specialized methods.

Results and discussion. Complex physico-chemical processes occur during dealcoholization of wine. The removal of alcohol results in the concentration of both volatile (aldehydes, acids, higher alcohols, and ethers) and non-volatile (glycerol, organic acids, and amino acids) substances, which alters the overall flavor profile of the wine, causing flavor imbalances and sensory disharmony.

Concentration of organic acids increases during dealcoholization, which changes the acid balance and affects the microbiological stability of wine. Content of malic acid increases by 30-38%, which changes the acidity of the wine. Content of glycerin increases by 24-26%, which has a positive effect on the sensory characteristics of wine. Content of amino acid proline increases significantly after dealcoholization, which can affect the taste properties of wine. The presence of volatile components, such as acetaldehyde and 3-methyl-butanol, may contribute to the aromatic and taste characteristics of the wine. An important finding is also the reduction of methanol concentration during dealcoholization, which improves product safety.

Sensory evaluation showed that dealcoholization of wine obtained by two different methods (vacuum or osmotic distillation) did not have a significant effect on the peach, citrus or nutmeg aroma characteristic of the wine produced from the Citronny Magarach grape variety, and the main aromatic characteristics remained almost unchanged. However, dealcoholization significantly affected other sensory characteristics such as sweetness, acidity, bitterness, and wine body. Overall, the treated wines had less sweetness and bitterness, increased acidity, and reduced fullness.

Conclusion. The process of dealcoholization of wine changes its physical and chemical composition and sensory properties, which requires the improvement of technologies to preserve the taste balance.

Introduction

In recent years, there has been a significant increase in demand for non-alcoholic beverages, including non-alcoholic wines, which is associated with modern ideas about healthy lifestyle. In addition, non-alcoholic wines can be considered as drinks that can be an excellent alternative to alcoholic holiday drinks for drivers, pregnant women, athletes, and those consumers who, for various reasons or personal beliefs, do not drink alcohol (Uspalenko and Kucherenko, 2023).

According to the latest statistics and forecasts, the global non-alcoholic wine market is valued at 20 billion US dollars with a compound annual growth rate (CAGR) of more than 45% in 2018. It is projected to increase at a significant CAGR of more than 7% during the forecast period (2019-2027), reaching a value of more than 30 billion US dollars (Deore, 2024). According to other forecasts, the global market will continue to grow steadily, with a CAGR of 10.4% from 2021 to 2031, compared to a CAGR of 8.8% from 2016 to 2020. Therefore, in order for wine producers to meet consumer demands and adapt to the growing non-alcoholic wine market, they need to produce high-quality non-alcoholic or low-alcohol wines (Fact9199MR, 2023).

In view of this, there are many discussions at the legislative and scientific level regarding this type of drink. This therefore constitutes two important key aspects: legal constraints and technical challenges. Regarding the first, laws in different countries may determine the maximum permissible amount of alcohol in soft drinks, which limits the options of winemakers. Regarding the second, the technical difficulties are the difficulty of preserving the sensory characteristics of the wine after dealcoholization.

Despite the difficult path of legalization and production of non-alcoholic wine, the market still has a large range of products, creating competition among producers and pushing enterprises to constantly search for new and improved products. Currently, among the producing countries, Germany is the leader in the production of non-alcoholic wines (Schulz et al., 2023). Ukraine also has the potential for the development of this industry, especially in the aspect of creating these wines using its own aromatic grape varieties, which will favorably distinguish domestic non-alcoholic wines from foreign analogues. This direction in expanding the range of wine products is an important task for the further development of the Ukrainian grape and wine industry, especially during climate change and consumer preferences.

Alcohol-free wine is not really a new type of wine, as it was first mentioned in 1869, when the American Thomas Bramwell Welch, a strong supporter of the alcohol moderation movement, produced alcohol-free wine by pasteurization. Later, in 1908, the German scientist Carl Jung managed to produce non-alcoholic wine without a "boiled" taste using vacuum distillation at a temperature of 35 °C (Schulz et al, 2023).

In Ukraine in the 1980s, during the anti-alcohol campaign, Ukrainian scientists conducted scientific research on the development of non-alcoholic beverage technology based on grape raw materials and grape wines but did not have significant success (Tyshchenko and Bozhko, 2023).

In addition, at the beginning of the war in 2022, the situation for Ukrainian winemaking was complicated by a complete ban by local military administrations on the sale of winemaking products in Ukraine for more than 40 days, which led to significant losses for winemaking enterprises that remained in operation.

Currently, various ways of reducing the alcohol content in wines are proposed. These are membrane distillation; reverse osmosis; vacuum distillation; dilution of wort with water;

application of the enzyme glucose oxidase in the wort before fermentation, which oxidizes the fermented sugars in the wort; the use of yeast, which contributes to the production of wines with a lower alcohol yield; ultrafiltration and nanofiltration by reducing the sugar content in the wort before fermentation (Heux et al., 2006; Röcker et al., 2016).

It is extremely important to form harmonious sensory characteristics in alcohol-free wines during dealcoholization or the use of other methods that contribute to the reduction of alcohol. The goal of the present study was determination of the technology of non-alcoholic wines from Citronny Magaracha grapes with maximum preservation of the natural sensory properties of the original wine.

Materials and methods

Materials

50 liters of processed dry white table wine made from white grapes of the Citronny Magaracha variety of Ukrainian selection and grown in the Zaporizhzhia region (Ukraine) were used.

Experimental setup and dealcoholization conditions

The trials were carried out at the Getränke Technologie Zentrum (GTZ) of Hochschule Geisenheim University, in Germany.

A Vacuum Distillation plant, Hei-VAP Industrial Rotary Evaporators (Heidolph Instruments GmbH & Co. KG; Walpersdorfer STR.12, D-91126, Schwabach, Germany), equipped with a Vacuum Pump, Hei-Vac Valve Industrial, a touch screen control panel displaying all process parameters, programmable ramps, an ascending condenser, a chilling system, Hei-Chill 3000, one 20 L evaporating flask, and two 10 L receiving flasks, was used for the dealcoholization of 18 L of wine. The wine was divided into three batches for the distillation. The rotation of the flask was set at 60 rpm, the water bath temperature at 40°C, the vacuum pressure at 50 mbar, and the condensation temperature was kept constant at 1.8°C throughout all dealcoholization experiments. Each dealcoholization process was completed in 2 hours after reaching the set temperature and pressure indicated above. This process resulted in 12 L of dealcoholized wine at 0.18% vol and 5.5 L of spirits at 43.49% vol. The spirits were then redistilled under the same conditions as the previous distillations, but with a water bath temperature of 50°C. This resulted in 1 L of water at 0.20% vol and 4 L of spirits at 59.75% vol. The latter were blended with the dealcoholized wine, resulting in 13 L of dealcoholized wine.

The osmotic distillation was conducted with the WineBrane Lab Gas/Alc plant (Inoxpa Deutschland, C/Telers 60, 17820, Banyoles, Spain), equipped with a membrane contactor, Liqui-Cel® 2.5x8 (3M Deutschland GmbH, Ohder STR.28, 42289, Wuppertal, Germany). The membrane was always stored and cleaned according to the manufacturer's instructions. The feed solution, wine, was pumped by a membrane pump Flojet (Flojet Corporation, Icon 20, 92610, Foothill Ranch, U.S.A.) with a capacity of 300 L/hour, and the stripping solution, distilled water, was performed by a peristaltic pump (Verder Deutschland GmbH & Co. KG, Retsch-Allee 1-5, 42781, Haan Germany) with a capacity of 83 L/hour. The temperature of the two liquids during the trials was 20°C. The experiment needed 23 L of wine and two batch of 100 L each of distilled water. Due to the processing time (2 days), there was a problem of turbidity caused by microbiological contamination. To avoid possible blockage

of the machine, it was decided to finish the last 2.21% vol by Vacuum Distillation (as described above). The 19 L of wine were divided into four batches, resulting in 4.3 L of dealcoholized wine (0.12% vol) per batch. Another distillation was performed for further concentration of spirits, resulting in 0.6 L of water at 0.15% vol and 1.2 L of spirits at 33.20 % vol from 1.8 liters of spirits. These were blended with the dealcoholized wine, yielding 17.8 L of dealcoholized wine.

An addition of SO_2 in liquid form was made to bring the free SO_2 to 30 mg/L in both types of dealcoholized products. In order to stabilize the dealcoholized wines, they were bottled using an instantaneous liquid heater, Getrankedurchlauferhitzer 03-0318 (Schankanlagen Koch GmbH, Dagstuhler STR.62, 66687, Wadern-Morscholz, Germany), bringing the temperature to 62°C. The wine was bottled into 750 mL brown glass bottles, corked with a screw cap, and stored in a warehouse at temperatures between 12 and 15°C for further analyses.

Setting up the experiment

During the experiment, the physico-chemical and sensory indicators of the quality of non-alcoholic wines obtained by various methods of dealcoholization were studied in comparison with the control wine:

- **control** – wine with an alcohol content of 13.41% vol;
- **wine 1** – dealcoholized wine by vacuum distillation with an alcohol content of 0.18% vol.;
- **wine 2** – dealcoholized wine by osmotic distillation with an alcohol content of 0.12% vol.

The first stage of the experiment was the production of dealcoholized wine by two different methods: vacuum or osmotic distillation.

At the second stage, a physico-chemical analysis using NMR and a sensory analysis by a specialized tasting commission were carried out.

Determination of sensory properties

Tastings took place in a special room equipped with individual booths and air conditioning at 20 °C. The testing was conducted by two independent tasting commissions.

For wine tasting, a standard tasting glass made of thin, clean, transparent glass with a capacity of 210–220 mL was used, which makes it possible to operate 60–70 mL of wine for a comprehensive sensory assessment of all elements of quality.

Wine with alcohol content and de-alcoholized wine were evaluated according to the Standards of the International Organization of Vine and Wine (Resolution OIV/Competition ECO 332A/200). The maximum tasting rating of the experimental wines was 100 points and was determined as the sum of points for each indicator: appearance (transparency, color) – 14; aroma (authenticity, intensity, quality of aroma) – 30; taste (authenticity, intensity, harmonic stability, taste quality) – 44; harmony (general impression) – 11.

To create the aromatic profiles of the experimental wines, a descriptive method was used according to a 10-point rating scale based on the following descriptors: citrus, fruity, nutmeg, floral, sweetness, acidity, bitterness, body/fullness, intensity, astringency. Graphical representations of experimental data were performed using Microsoft Excel 2010.

Comparing the profiles of the experimental wines made it possible to determine their differences and draw conclusions about the change in wine quality during dealcoholization.

Determination of physical and chemical parameters

Determination of physical and chemical parameters was carried out in accordance with the methods and regulations of the International Organization of Grapes and Wine (OIV). Standard protocols and analytical methods recommended by the OIV were used to conduct the research, ensuring the accuracy and reproducibility of the results obtained.

Processing research results

Determination of physico-chemical indicators of non-alcoholic wine wines was carried out in two repetitions. Results are shown as mean, including standard deviation.

Statistical data processing

Statistical data processing was performed, including determination of mean content and standard deviation (±SD), with four replicates (four collections) for the main compounds identified in all four collections analyzed. Statistical data processing was carried out using Excel 2007 (Microsoft Corporation, USA)

Results and discussion

Changes in the physical and chemical parameters of wine during dealcoholization

Changes of ethanol content, % vol., in wine during osmotic distillation is shown in Figure 1.

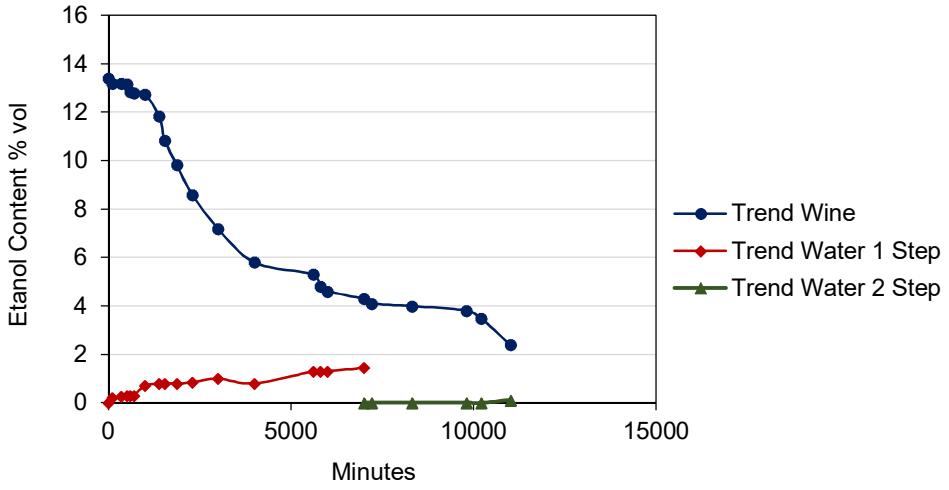

Figure 1. Changes of ethanol content, % vol., in wine during osmotic distillation

During dealcoholization of wine, complex physico-chemical processes take place in which, in addition to the removal of ethyl alcohol, other components of wine undergo changes

– both volatile – aldehydes, acids, higher alcohols, ethers, acetates, and non-volatile – glycerol, organic acids, and amino acids (Diban et al., 2008).

The alcohol content during the experiment, as expected, was reduced from 13.29% vol. (control) up to 0.18% vol. (wine 1 – dealcoholized wine by vacuum distillation) and 0.12 % vol. (wine 2 – dealcoholized wine by osmotic distillation).

It should be noted that because of removing alcohol from wine, wine components were concentrated, because of which the concentration of non-volatile compounds increased (Table 1).

Table 1

Comparison of physical and chemical parameters of the studied wines

Indicator	Control	Dealcoholized wine	
		1	2
Total alcohol, mg/L	105872.75±0.10	1402.15±0.10	966.35±0.10
Total alcohol, % vol.	13.39±0.07	0.18±0.07	0.11±0.07
Organic acids, mg/L:	4820.85±0.02	5866.53±0.02	5797.21±0.02
Glycerin, mg/l	4761.70±0.02	5853.95±0.02	5923.60±0.02
Alcohols, mg/L:			
– Methanol	50.22±0.20	<30	<30
– Proline	592.76±0.10	724.52±0.10	711.38±0.10
– 2-phenylethanol	<25	31.80±0.30	26.86±0.30
– 2,3-butanediol	384.25±0.20	483.95±0.20	467.36±0.20
– 3-methyl-butanol	141.00±0.70	<100	<100
Acetaldehyde, mg/L	232.09±0.40	238.19±0.40	166.93±0.40
Acetoin, mg/L	21.20±0.40	20.52±0.40	15.48±0.40
Trigonelline, mg/L	13.58±0.70	17.26±0.70	17.93±0.70
Energy value, kJ/L	3241.88±0.03	147.28±0.03	135.92±0.03

The total content of organic acids undergoes significant changes during dealcoholization, this is due to the change in concentration caused by the removal of ethanol and water. Glycerin content increases by 24-26%, which has a positive effect on the feeling of the wine extract. Amino acids are the main component of nitrogen compounds in grapes and wine, and make up approximately 50% of the total nitrogen content. They play an important role in shaping the sensory properties of wine, its stability and biological value. The study of amino acid content in wine is an important aspect for understanding the chemical processes that occur during wine production and storage.

In the study, the concentrations of amino acids such as alanine, arginine, and proline were determined in wine wines before and after the dealcoholization process. The results showed that the content of alanine and arginine remained stable and did not undergo significant changes after dealcoholization. This indicates that these amino acids are not subject to significant losses or transformations during this technological process.

However, the concentration of proline in the wines after dealcoholization increased. In the wine where vacuum distillation was applied, the proline content increased by 22%, and in the second wine after membrane distillation – by 20%. This may be due to various factors, such as changes in the distribution of amino acids between the liquid and solid phases or other chemical reactions occurring during the removal of alcohol from the wine. A higher

proline content can influence the taste properties of wine, as proline is an important metabolite in winemaking and can affect the formation of aromatic compounds.

Concentrations of volatile substances also changed after dealcoholization of wines. For example, the content of acetaldehyde increased slightly when vacuum distillation was used, but after membrane distillation it decreased by 28%. Aldehydes play an important role in the formation of sensory indicators of wine (aroma, taste). One of the main aldehydes formed during fermentation is acetaldehyde. An increase in the content of this aldehyde gives the wine a sharp smell that has shades of toasted bread, but a decrease led to less expression of fruity aromas and flavors, making it less saturated and complex in sensory perception (Sam et al., 2021).

Trigonelline, an alkaloid, an aromatic compound that is not an important component, but also plays a role in the formation of aroma and taste, giving the wine light tones of roasted coffee, and acts as a natural biological stabilizer of wine during dealcoholization in both wines, respectively, its content increased by 1.27 and 1.32 times.

The dealcoholization process also affects other volatile compounds that affect the aroma and taste of wine. Wine contains more than 1,000 volatile compounds of various chemical classifications (alcohols, esters, fatty acids, aldehydes, terpenes, ketones, sulfur compounds), where about 400 volatile compounds are formed during wine fermentation (Esteras-Saz et al., 2021). Higher alcohols in wine are formed during alcoholic fermentation, which is carried out by yeast. They are by-products of yeast metabolic processes. Higher alcohols can be formed from amino acids via the so-called Ehrlich pathway. During this process, amino acids are deaminated to the corresponding aldehydes, which are then reduced to higher alcohols.

A representative of monoatomic alcohols is methyl alcohol, which is synthesized before and during alcoholic fermentation due to the hydrolysis of pectin's by methylpectinesterase, which is contained in grapes. Pectinase catalyzes the cleavage of ester bonds in pectin, resulting in the formation of methyl alcohol and pectinic acid (Kucherenko and Bilko, 2020). It is known that methanol is a toxic substance that can accumulate in the body and have a harmful effect on various organs and systems. In white wines, the methanol content is up to 250 mg/L, the results of the study showed the methanol content in the control wine – 50.22 mg/L, which is 4.97 times less than the maximum permissible level, and both non-alcoholic wines have an even lower methanol content – < 30 mg/L. This indicates that methanol is removed along with ethyl alcohol during the dealcoholization process of the wine.

During dealcoholization, the 3-methyl-butanol content of both wines decreased by a factor of 1.41, thereby reducing the fruity and floral notes compared to the original control wine. The volatile compound 2,3-butanediol at low concentrations can give aromatic notes of caramel or honey, but higher values can lead to a feeling of immaturity of the wine and to too high acidity, as can be seen from Table 1, the content of 2,3-butanediol increased in wines 1 and 2 on 1.26 and 1.22 times, respectively, it could be influenced by the fact that this substance was formed as a result of yeast metabolism under the influence of stress (for example, under the influence of high temperatures).

Dealcoholization also has a significant effect on the change in energy value. According to the research results, the energy value in the control wine was 3241.88 kJ/L, while in experimental wines 1 and 2 it was 147.28 kJ/L and 135.92 kJ/L, respectively, that is, the decrease occurred in 22.02 and 23.85 times, respectively. The most obvious decrease in energy value is a significant decrease in alcohol concentrations. This emphasizes the dominant effect of alcohol on energy content.

Impact of dealcoholization on content of organic acids in wine

Organic acids are an essential group of compounds in wines, as they influence the physicochemical and microbiological stability of wines, as well as their sensory properties (Coelho et al., 2018).

Table 2

Change in the content of organic acids in dealcoholized wines compared to control

Organic acids, mg/L	Control	Dealcoholized wine	
		1	2
Tartaric acid	2184.15±0.04	2408.60±0.04	2425.80±0.04
Citric acid	284.93±0.30	362.97±0.30	388.82±0.30
Caftaric acid	119.10±0.80	150.44±0.60	152.32±0.60
Succinic acid	361.71±0.20	459.19±0.20	445.37±0.20
Acetic acid	237.03±0.40	267.39±0.40	297.13±0.40
Malic acid	1430.45±0.06	1977.75±0.06	1855.15±0.06
Galacturonic acid	203.48±0.40	240.19±0.40	232.62±0.40

Thus, the intent of tartaric acid increases by 10–11%, acetic acid by 12–25%, malic acid by 30–38%, citric acid by 27–34%, caftaric acid by 26–27%, succinic acid by 24–27%. Such transformations lead to a change in the general taste profile of non-alcoholic wine, which is expressed in the imbalance of taste and disharmony of sensory perception of wine. Thus, some researchers observed that dealcoholized wines have higher acidity and less bitterness compared to the original (control) wine (Corona et al., 2019).

However, organic acids have the ability to interact with alcohols, forming complex esters that add a rich aroma and taste to wines. They create taste harmony, which is critically important for consumers' perception of wine quality.

In addition, these acids affect not only the acidity and taste, but also the stability and microbiological safety of wine. For example, acetic acid can be formed in wine during the process of aerobic bacterial activity, which can lead to undesirable sensory characteristics, such as a strong vinegar smell. At the same time, low concentrations of organic acids can contribute to the formation of a pleasant aroma and taste.

Thus, the content of tartaric acid increases by 10–11%, acetic acid by 12–25%, malic acid by 30–38%, citric acid by 27–34%, caffeic acid by 26–27%, and succinic acid by 24–27%. These transformations lead to changes in the overall flavor profile of non-alcoholic wine, resulting in taste imbalance and sensory disharmony. Some researchers note that dealcoholized wines exhibit higher acidity and less bitterness compared to the original (control) wine (Corona et al., 2019).

It is worth noting that organic acids can interact with alcohols to form complex esters, which contribute rich aromas and flavors to the wine. These acids create a harmonious taste, which is critical to consumers' perception of wine quality.

These acids also influence not only the acidity and taste but also the stability and microbiological safety of the wine. For instance, acetic acid can develop in wine through aerobic bacterial activity, potentially causing undesirable sensory traits, such as a strong vinegar aroma. At the same time, low concentrations of organic acids may enhance the aroma and flavor profile.

During dealcoholization, the galacturonic acid content remained relatively unchanged. This is because galacturonic acid forms through the degradation of pectin substances, which occurs at elevated temperatures. However, the relatively low temperature during dealcoholization does not promote intensive pectin breakdown or a significant increase in galacturonic acid (Robles et al., 2019).

A slight increase, compared to other acids, was observed in tartaric acid, which helps maintain acidity, reduce pH, inhibit bacterial growth, and preserve long-lasting freshness in the wine. Additionally, tartaric acid is essential for enhancing the structure and flavor profile of the wine (Yang, 2021).

Acetic acid also significantly affects the sensory profile and stability of wine. In low concentrations, up to 300 mg/L, it is a natural component of wine, adding subtle, pleasant acidic notes that lend freshness. However, concentrations above 600–700 mg/L can cause off-flavors and aromas, such as vinegar or what is commonly known as "volatile acidity" (Vilela-Moura et al., 2010). Studies have shown that with both methods of dealcoholization, the acetic acid content increased but remained within low levels, providing pleasant acidic notes to the non-alcoholic wine.

As shown in Table 2, malic acid increased by 30–38% during dealcoholization. This may lead to the formation of lactic acid, which negatively affects wine stability and can introduce an unpleasant bitter taste. Therefore, it is essential to control malic acid levels during dealcoholization.

Other acids, such as citric, caffeic, and succinic acids, were affected by a process of preservation, resulting from the simultaneous removal of ethanol with water and certain volatile compounds. This led to an imbalance in acidity levels, making these acids appear more acidic to consumers (Gawel et al., 2013).

Changes in sensory parameters of wine during dealcoholization

During the study, no changes in the color of the wine were observed compared to the initial (control) wine. Wine 1, obtained by vacuum distillation, retained its light straw color and transparency, remaining unchanged. Meanwhile, wine 2, obtained by osmotic distillation, also showed no color changes but exhibited slight turbidity (Figure 2). The turbidity arose due to a shift in the colloidal equilibrium of the system following the removal of alcohol, which was associated with the treatment duration of two days. Further technological stabilization processes will help achieve the desired appearance, clarity, and stability of wine.

However, the greatest impact of dealcoholization was found on the wine's aromatic and flavor characteristics, highlighting the importance of selecting processing methods that preserve the sensory properties of the product.

The results of the tasting analysis of the experimental wines showed that dealcoholization using two different methods did not lead to a significant change in the perception of peach aroma in wines (Figure 3).

A careful analysis of the changes in the main descriptors of the experimental wines made it possible to establish the following. Dealcoholization of wine is generally associated with the loss of volatile aromatic components (Kumar et al., 2024). On the other hand, decreasing ethanol levels enhances the perception of aromatic components, which can compensate for aroma losses (Escudero et al., 2007; Goldner et al., 2009). In this case, both effects probably resulted in the perception of the peach aroma not being significantly altered.

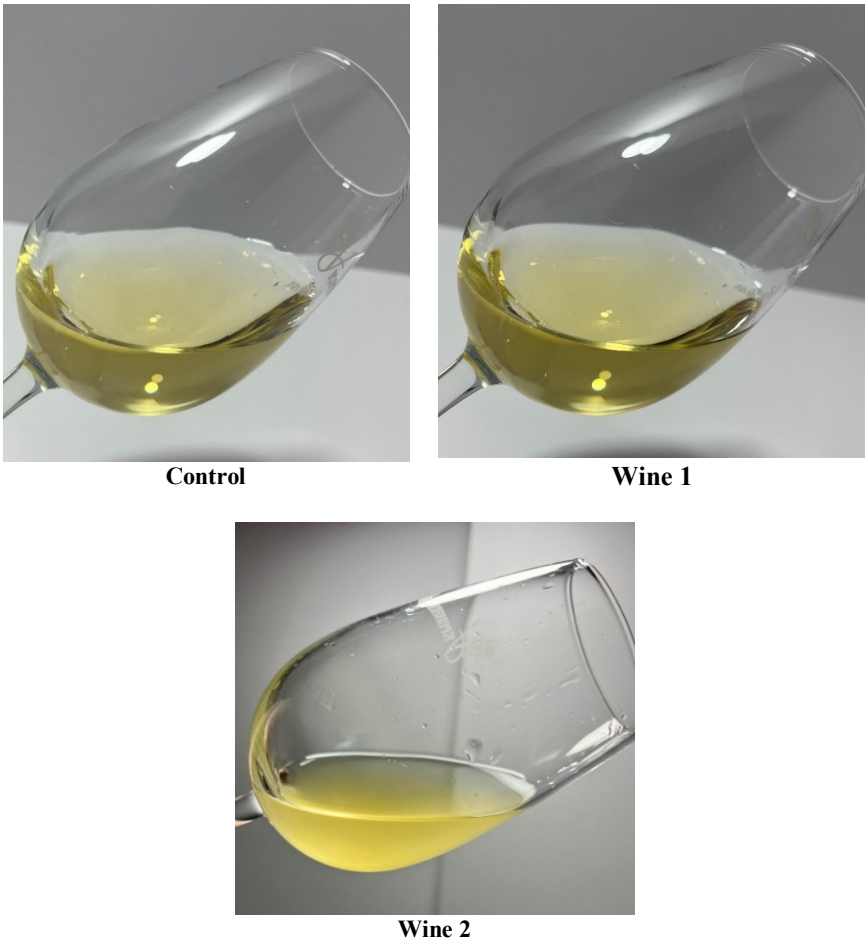

Figure 2. Comparison of the color of the control with the dealcoholized wines

Both treated wines showed similar expressions of citrus tones. Dealcoholized wines were generally described as having a slightly reduced, but not significantly altered, intensity of this descriptor.

Nutmeg tone was rated almost equally for both dealcoholized wines. Unprocessed wine showed a more pronounced sense of this descriptor, although not at a significant level.

The overall floral sensation was scored higher for the untreated control wine compared to the dealcoholized variants, which again showed similar expressions.

The dominant aromatic attributes investigated were rated almost identically for the dealcoholized wines and did not differ significantly from the original untreated wine (Schmitt et al., 2023).

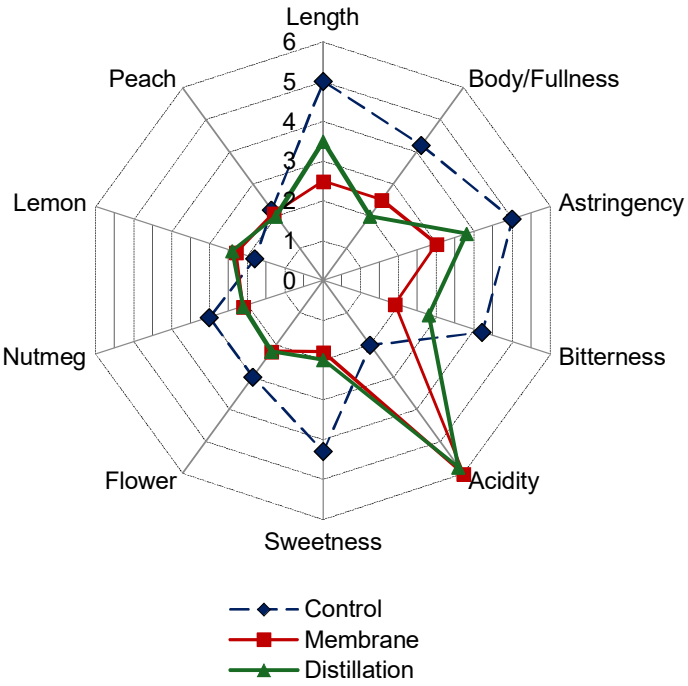

Figure 3. Profilogram of aroma and taste of dealcoholized wines

Sweetness perception was clearly altered by dealcoholization. Dealcoholized wines had significantly less sweetness compared to the original wine. This is due to the direct sensory effects of ethanol, which adds sweetness to wine (Martin and Pangborn, 1970; Nurgel and Pickering, 2006). Based on this, it becomes clear why de-alcoholized wines are usually produced with an increased level of sugar, that is, semi-dry or semi-sweet. To compensate for this change due to dealcoholization, average residual sugar in dealcoholized wines is around 40 g/L (Schmitt, 2023). Perception of acidity was rated significantly higher for dealcoholized wines. Both wines had almost the same indicators. This increase in perceived acidity is consistent with a direct effect of ethanol in wine, as alcohol reduces perceived acidity in wine (Fischer and Noble, 1994). In addition, the loss of volume during dealcoholization increases the acidity value even more. Therefore, when choosing wines for dealcoholization, this factor should be considered and wines with lower than usual acidity values should be chosen to obtain a balanced product.

The bitterness of the initial wine was significantly higher compared to the two dealcoholized variants. A direct effect of ethanol in wine is to increase bitterness (Noble, 1994).

Regarding astringency, tasters rated the initial wine as having the highest level of this sensation. The distilled version scored the second highest, while the membrane-processed version scored the lowest for astringency. Some scientists report a decrease in astringency with increasing alcohol levels in model wine solutions. Wines were used in this study, and the treatment was accompanied by a concentration effect. Further research should focus on

how the complex perception of astringency is modified by wine dealcoholization to optimize the sensory characteristics of still wines (Fontion et al., 2008).

The perception of body and fullness in wine is clearly correlated with alcohol content (Amerine and Roessler, 1983; Gawel et al., 2007; Grainger, 2009; Yu, 1998). The higher the alcohol content, the higher this perception. The results of this study show a significant difference between the treated variants and the original wine. As body and fullness are clearly considered positive sensory characteristics for wine (Hoffmann and Szolnoki, 2008), oenological strategies such as adding mannoproteins or tannins can partially compensate for the reduction of these sensory parameters due to dealcoholization. Similarly, tasters noted that the initial wine had significantly more aftertaste compared to the treated wines. This corresponds to the findings obtained using the method of assessing the temporal dominance of sensations in partially dealcoholized wines (Meillon et al., 2009).

The study shows that dealcoholization of wine significantly affects its sensory characteristics. The differences between the two processing methods were not so pronounced. The sensory characteristics of the aromatic parameters were changed less than the attributes affecting the perception of the wine on the palate. The main effects of dealcoholization are consistent with the complex sensory characteristics of ethanol in wine.

Conclusions

1. The process of dealcoholization is accompanied by complex physical and chemical changes in the composition of wine, which affects its sensory properties. Reducing the alcohol content to the level of 0.18% or 0.12% in the wine led to the concentration of other wine components, which, in turn, affected the balance of taste and the harmony of sensory perception.
2. The study confirmed that various methods of dealcoholization, such as vacuum or osmotic distillation, had its own influence on wine quality. However, with the decrease in the level of alcohol, an increase in the concentration of non-volatile compounds was recorded, which required further improvement of technologies to preserve the harmony of taste.
3. The results show the need for further research to optimize dealcoholization technologies to preserve the natural sensory properties of wine.
4. To ensure the stability of non-alcoholic wines, all stages of the technological process must be considered, including the addition of stabilizers such as SO_2 and the storage conditions. This will help preserve the quality of dealcoholized wine during its storage.

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Nutritional properties of wild edible mushrooms sold in Aegean region

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Abstract

Introduction. This study investigated the nutritional value and antioxidant capacity of Çıntar mushrooms cultivated in Muğla, providing data intended to enhance the current literature on mushrooms with significant dietary benefits for human health.

Materials and methods. Çıntar mushrooms, which hold significant nutritional value for human diets, were sourced from Türkiye and physicochemical and antioxidant properties of the ten Çıntar mushrooms samples were analyzed.

Result and discussion. The findings obtained were evaluated in terms of nutritional composition, functional properties, and antioxidant capacity of these mushrooms. The dry matter, moisture, ash, titratable acid, pH, total carbohydrate and protein contents of the Çıntar mushroom samples were determined in the range of 6.381–17.920%, 82.079–93.618%, 0.264–0.821%, 0.334–1.015%, 6.58–7.41, 0.062–1.066%, 3.125–5.694%, respectively. Among the antioxidant capacity properties, the total phenolic content was determined to range between 0.0017–0.0029 mg GAE/g, FRAP values were between 3.854–8.267 μmol Trolox/g, and TEAC values ranged from 0.0014–0.0146 μmol Trolox/g. Upon examining the obtained results, it is expected that the samples with the highest total phenolic compound content would also exhibit the highest antioxidant capacities in the FRAP and TEAC analyses. However, the results do not support this theory. It is hypothesized that the differences in the antioxidant capacities of the samples may be attributed to the reduction of different compounds. The compositions of the fungi depend on the type of fungus, growing conditions, chemical and physical characteristics of the growing medium, developmental stage, and location. Additionally, to accurately determine their antioxidant capacities and the specific compounds they can reduce, various capacity analyses should also be conducted.

Conclusions. More research on these mushrooms is needed in Türkiye, a country abundant in wild mushrooms, to better understand these variations.

Introduction

Macrofungi have been used as medicine and food since the times of Chinese, Roman, and Greek civilizations and can be an important food source for the rapidly growing world population (Dülger and Gücin, 1999). Mushrooms, which consist of three basic groups: *Zygomycetes*, *Ascomycetes* and *Basidiomycetes*, show different shapes, sizes and living conditions depending on whether they grow underground or above ground. Unlike other plants, they do not require sunlight and thrive in high humidity environments (Dimopoulou et al., 2022). Natural mushrooms are valued for their high content of protein, fiber, vitamins, minerals, and unsaturated fatty acids, making them useful both as medicine and as food (Acay, 2018; Stabnikova et al., 2024). On average, mushrooms contain about 90% water. The dry weight of mushrooms consists of 16-85% carbohydrates, 0.2-8.7% lipids, 14-44% proteins, and 1-29% ash (Karasüleymanoğlu, 2014). The chemical compositions of macrofungi can vary based on the type of fungus, growing conditions, characteristics of the growing medium, developmental stage, and location (Turfan et al., 2016). Macrofungi are an excellent food source for low-calorie diets due to their richness in essential amino acids and low fat content. Their nutritional properties also include antioxidant, antibacterial, antifungal, antitumor, immunomodulatory, anti-inflammatory, and antiviral effects (Acay, 2018; Dulay et al., 2015; Ghahremani-Majd and Dashti, 2015).

Wild mushrooms are collected and consumed during specific season in nearly all countries (Obodai et al., 2014). Türkiye boasts around 40 wild mushroom species, but only 25 are sold commercially. In each region, local people typically recognize and consume 3 to 5 mushroom species as food (Adanacıoğlu et al., 2016). The *Lactarius* species is one such wild mushroom that naturally grows in autumn. It is known by various names such as "Çıntar," "melki," "kanlica," and "termit" in many regions of Türkiye (Altuntaş et al., 2016).

Free radicals are produced in the human body due to various physicochemical and pathological conditions. These radicals can damage important biomolecules, like lipids, proteins, and DNA that play crucial roles in human metabolism. Consuming antioxidant-rich substances daily can help prevent damage from highly reactive oxygen species, reducing the risk of diseases such as cancer, atherosclerosis, diabetes, arthritis, malaria, AIDS, and heart diseases. Antioxidants have a protective effect against naturally occurring toxic processes in the body. Many synthetic antioxidants, however, can cause side effects like hepatotoxicity, pneumotoxicity, and cancer. Therefore, it is important to find natural non-toxic antioxidants to help prevent chronic diseases and protect the body from free radicals (Gupta and Sharma, 2006).

The natural antioxidants found primarily in vegetables include phenolic compounds (such as phenolic acids and flavonoids), carotenoids, tocopherols, and ascorbic acid, all of which are vital for human health (Barros et al., 2008; Cheung et al., 2003). Ascorbic acid and phenolic compounds are common antioxidants in fungi and considered secondary metabolites of macrofungi (Barros et al., 2008).

This study investigated the nutritional value and antioxidant capacity of Çıntar mushrooms cultivated in Muğla, providing data intended to enhance the current literature on mushrooms with significant dietary benefits for human health.

Materials and method

Collection of samples

Ten Çıntar mushroom samples were purchased from the marketplace in Muğla province in Türkiye, in the autumn of 2020. All samples were transported to the laboratory in a thermobox while still in their original packaging. The Çıntar mushroom samples were stotred at -18°C until analysis.

Reagents

The chemicals used in this study were as follows: phenolphthalein, hydrochloric acid (HCl), glucose ($C_6H_{12}O_6$), cupper (II) sulphate (Cu SO_4*$5H_2O$), potassium sodium tartarate tetrahydrate ($C_4H_4KNaO_6$*$4H_2O$), gallic acid ($C_7H_6O_5$), iron chloride ($FeCl_3$), and potassium peoxydisulphate ($K_2O_8S_2$) (Merck, Germany), sodium hydroxide (NaOH), phenol (C_6H_6O), methanol (CH_3OH), ethanol (C_2H_5OH), sodium carbonate (Na_2CO_3) acetate (CH_3COOH) (Tekkim, Turkey), folin ($C_{19}H_{19}N_7O_6$), Trolox ($C_{14}H_{18}O_4$), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (Sigma-Aldrich, Germany), 2,4,6-Tri(2-pyridyl)-1,3,5-triazine (TPZT) (Alfa Aesar, Germany), oxalic acid ($H_2C_2O_4$) (Aldrich, USA), 2.6-dichlorophenoldophenol (Acros Organic, USA), sulfiric acid (H_2SO_4) (ISOLAB, Germany), bovine serum albumin (BSA) (Sigma, USA).

Physicochemical properties

The content of dry matter, total ash content, titratable acidity (citric acid, %), and pH of the research samples were analyzed using the methods established by the Association of Official Analytical Chemists (AOAC, 1995; 1997). The total carbohydrate content was determined using the phenol-sulfuric acid method (Taylor, 1995) and The total protein content of the products were analyzed using the Biuret method (Gornall et al., 1949).

Antioxidant properties

The extraction process was conducted prior to determining the antioxidant capacity. For extraction, 10 g of samples were mixed with 25 mL of the extraction solution (HCI %1: CH_3OH 80%). The mixtures were then placed in a shaking incubator at room temperature for 30 minutes, followed by storage at 4°C for 24 hours. The pellets were discarded after centrifugation (10000 × g, 5 min). The resulting supernatants were collected and stored at 4°C.

The extracts from Çıntar mushrooms were used to determine the total phenolic content (expressed as gallic acid equivalent) and antioxidant activity.

The total phenolic content was determined using the method established by Franke et al. (2004), the ferric reducing ability of plasma (FRAP) assay was measured spectrophotometrically following Benzie and Strain (1996), while the trolox equivalent antioxidant capacity (TEAC) assay was conducted spectrophotometrically according to Re et al. (1999).

Statistical analyses

All experiments were conducted with two replicates and two parallels, resulting in a total sample size of n=4. All statistical analyses were carried out with the SPSS statistical software (IBM SPSS Statistics Version 22; USA) through ANOVA variance analysis. The significance levels of $P<0.05$ were used for statistical differences. Duncan's test was used to determine significant differences between means. The relationship between variables was assessed by using Pearson's correlation and presented as a correlation matrix.

Result and discussion

Physicochemical properties

The result and statistical differences of total dry matter, moisture, total ash, pH, titratable acidity (citric acid %), protein, and total carbohydrate, are presented in Table 1. Statistically significant differences ($P\leq0.05$) were observed between the samples for total dry matter, moisture, total ash, pH, titratable acidity, and protein content. However, there was no significant difference in the total carbohydrate content ($P>0.05$).

Table 1

Physicochemical properties of Çıntar mushrooms

Sample	Total moisture %	Total dry matter %	Total ash %	pH	Titratable acid (citric acid) %	Total carbohydrate mg/g	Protein mg/g
1	82.079± 0.865^{a}	17.920± 0.865^{a}	0.546± 0.072^{abc}	6.65± 0.03^{ab}	0.457± 0.06^{abc}	0.787± 0.194^{a}	4.745± 1.049^{ab}
2	89.526± 0.595^{bc}	10.473± 0.595^{bc}	0.636± 0.182^{bc}	6.59± 0.04^{a}	0.569± 0.048^{bc}	0.411± 0.424^{a}	4.398± 0.996^{ab}
3	89.456± 0.343^{bc}	10.543± 0.343^{bc}	0.550± 0.141^{abc}	6.73± 0.06^{b}	0.607± 0.143^{c}	0.211± 0.323^{a}	4.421± 0.358^{ab}
4	89.692± 0.504^{bc}	10.307± 0.504^{bc}	0.365± 0.092^{ab}	6.58± 0.005^{a}	0.789± 0.226^{d}	0.749± 1.125^{a}	3.125± 0.243^{a}
5	90.420± 0.238^{cd}	9.579± 0.238^{cd}	0.821± 0.370^{cd}	6.72± 0.05^{b}	0.419± 0.117^{ab}	0.062± 0.074^{a}	3.611± 0.239^{ab}
6	93.618± 0.550^{f}	6.381± 0.550^{f}	0.391± 0.098^{ab}	7.41± 0.13^{c}	0.334± 0.114^{a}	0.254± 0.146^{a}	4.027± 1.172^{ab}
7	91.020± 0.387^{d}	8.979± 0.387^{d}	0.264± 0.131^{a}	6.69± 0.07^{b}	0.914± 0.069^{de}	0.427± 0.370^{a}	5.231± 3.081^{ab}
8	92.143± 0.252^{e}	7.856± 0.252^{e}	0.948± 0.309^{d}	6.60± 0.04^{a}	0.43± 0.036^{ab}	0.371± 0.323^{a}	5.694± 1.866^{b}
9	88.872± 1.221^{b}	11.127± 1.221^{b}	0.591± 0.151^{bc}	6.72± 0.02^{b}	1.015± 0.062^{e}	1.066± 1.327^{a}	4.282± 1.078^{ab}
10	90.349± 0.474^{cd}	9.650± 0.474^{cd}	0.474± 0.066^{ab}	6.64± 0.04^{ab}	0.609± 0.052^{c}	0.365± 0.209^{a}	4.259± 0.872^{ab}
MEAN	89.718	10.282	0.559	6.73	0.6143	0.471	4.379
Min	82.079	6.381	0.264	6.58	0.334	0.062	3.125
Max	93.618	17.920	0.821	7.41	1.015	1.066	5.694

* n=4 (± standard deviation). Different lowercase letters indicate differences between rows.

Mushrooms with high moisture content typically have a limited shelf life of two days (Lin et al., 2019). The average dry matter content of the samples was found 10.282%. The moisture content of the samples ranged from 82.079% to 93.618%. For comparison, *Lactarius deliciousus* samples from China were reported to have a moisture content of 92.00% (Xu et al., 2019). Similarly Altuntaş et al. (2016) found the moisture content of *Lactarius* genus to range between 86 and 92% in their study. The results are compatible with the data in the literature that have obtained.

Ash content is a measure of the total mineral substance present in food, including minerals like calcium, copper, iron, zinc, potassium, phosphorus and magnesium. The average ash content of the samples ranged from 0.264 to 0.821%, with an average value of 0.559%. The ash content of mushroom species *Pleuroyus, Termitomyces, Lentinus, and Auricularia* (3.05-6.38%) were higher than this study results (Obodai et al., 2014).

The pH value of mushrooms plays a crucial role in their storage. In this study, the pH values of the mushroom samples ranged from 6.58 in sample 4 to 7.41 in sample 6, indicating low acidity. (Table 1, Figure 1). Kumar and Ray (2016) reported a pH value of 6.67 for white button mushrooms used in Tomato-Mushroom ketchup production, which aligns well with our findings. These results are also in line with pH values reported for sample pickled in Kastamonu (pH 6.51) and for sample grown in a research center in Istanbul (pH 7.26) (Çavuşoğlu, 2018; Gül et al., 2021). In addition, obtained results are compatible with pH values (6.82-7.54) of fresh mushrooms collected from Muğla and microbiologically analyzed (Öncül and Çiftçi, 2023).

The organic acid composition of Çıntar mushrooms was examined, with citric acid identified as the main acid (Eissa, 2008). The average citric acid content in our samples was 0.6143%. In comparison, Kumar and Ray (2016) reported a titratable acidity value of 0.31% for white in terms of the citric acid. Sample no. 9 had the highest titratable acidity at 1.015%, while sample no. 6 had the lowest at 0.334%. In a study on Shiitake mushrooms from China, the titratable acidity ranged from 0.70% to 0.93% (Khaskheli et al., 2017). Additionally, another study found titratable acidity values to be 0.32 g lactic acid/100 g (Çavuşoğlu, 2018).

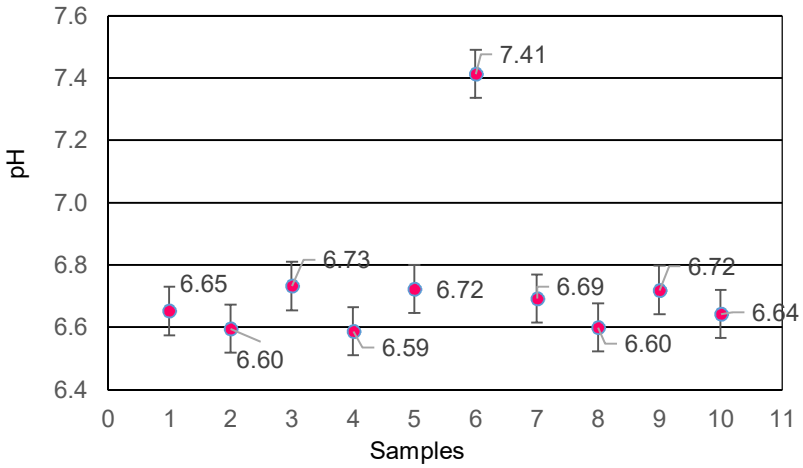

Figure 1. Average pH values of Çıntar mushrooms

Carbohydrates are often recommended for people with diabetes and anemia due to their low content in certain fungi (Adejumo and Awosanya, 2005; Turfan et al., 2016). The highest total sugar content was found in mushroom sample 9 at 1.066 mg/g, while the lowest was in sample 5 at 0.062 mg/g (Table 1, Figure 2). According to the Pearson correlation analysis, there was a positive correlation between carbonhydrate content and dry matter (R=0.521) as well as between carbohydrate content and titratable acidity (R=0.626) (Table 2). Vieira et al. (2014) reported an average sugar content of 77.22 g/100g in freeze-dried mushrooms, which was significantly higher than the result of this study (1.066 mg/g). Similarly, Xu et al. (2019) and Altuntaş et al. (2016) reported carbohydrate contents of 66.61 g/100 g and between 71.8 and 83.9%, respectively.

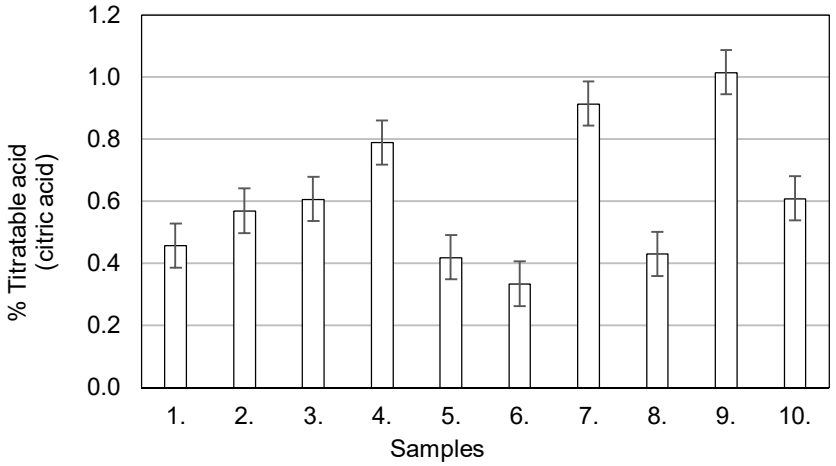

Figure 2. Average titratable acidity values of Çıntar mushrooms (% citric acid)

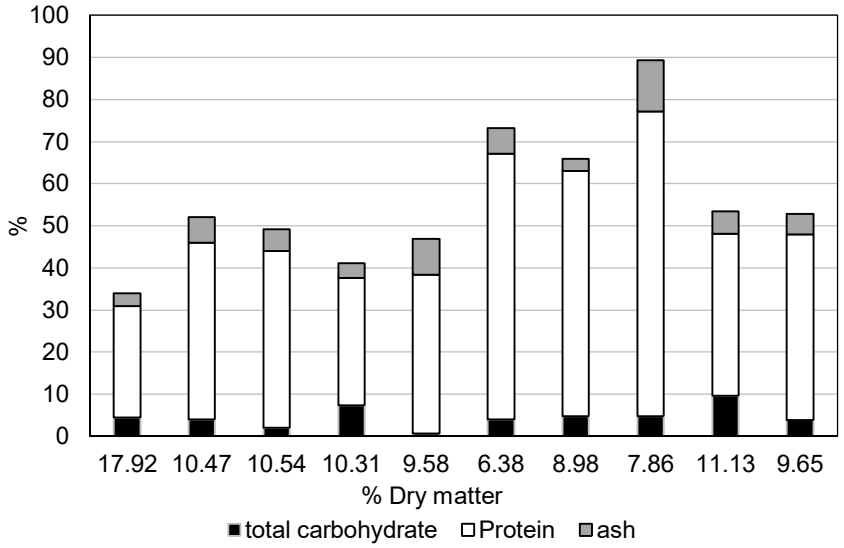

Figure 3. Total carbohydrate, protein and ash ratios of Çıntar mushrooms in dry matter

Considering the various components found in mushrooms, species that are high in protein tend to be low in carbohydrates, while those rich in carbohydrates are generally low in protein (Altuntaş et al., 2016).

The protein content of the mushroom can vary based on factors such as substrate composition, heap size, harvest time, and mushroom species. Mushrooms contain protein levels twice that of vegetables and some researchers suggest that their amino acid composition can rival that of animal proteins (Dulay et al., 2015).

Figure 3 illustrates the percentages of total carbohydrate, protein, and ash in the dry matter of the analyzed mushrooms.As shown in the figure, protein makes up the majority of the dry matter. For instance, *L. deliciousus* samples from China were found to contain 17.19 g/100 g of protein (Xu et al., 2019). Dulay et al. (2015) reported in their study that the protein content of *P. antillarium* genus mean was 15.01%. The protein content of samples 4 (3.125 mg/g) and 8 (5.694 mg/g) was found below from the value mentioned above (Table 1). Given that our samples were fresh, the lower protein content is likely attributable to storage conditions and/or spices. The mean protein values of the mushroom are 4.379 mg/g (Table 1).

Table 2

Correlation matrix for the physicochemical properties of Çıntar mushroom samples

R-Pearson Values*		**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. Total moisture		1.00						
2. Total dry matter		-1.00**	1.00					
3. Total ash		0.018	-0.018	1.00				
4. pH		0.430	-0.430	-0.283	1.00			
5. Titratable acid		-0.068	0.068	-0.442	-0.378	1.00		
6. Total carbohydrate		-0.521	0.521	-0.226	-0.273	0.626	1.00	
7. Protein		-0.019	0.019	0.265	-0.166	0.036	-0.34	1.00

** Correlation is significant at the 0.01 level (2-tailed).

Antioxidant capacity

Free radicals can cause cell-damage, leading to DNA mutations, protein damage, lipid peroxidation, modification of low-density lipoproteins, and various diseases such as diabetes, cancer, neurodegenerative and cardiovascular diseases. Consuming antioxidant-rich foods can help prevent damage from free radicals. The antioxidant capacity of foods and their effectiveness are determined by various mechanisms. Therefore, it is essential to use multiple evaluation methods to assess the antioxidant capacity of food (Xu et al., 2019). Due to this effect, we evaluated the antioxidant activity of Çıntar mushroom samples collected from Mugla using FRAP assays, TEAC values, and total phenolic content. The results are presented in Table 3.

The total phenolic method using the Folin-Ciocalteu Reagent (FCR) relies on electron transfer from phenolic compounds and other reducing agents to molybdenum. This method

is not suitable for lipophilic compounds and only allows phenolic compounds to react in a basic medium (Okan et al., 2013). The FRAP method is based on the reduction of a ferric tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. In this process, antioxidant compounds act as electron donors and function as both primary and secondary antioxidants by reducing the oxidized intermediates of lipid peroxidation (Ghahremani-Majd and Dashti, 2015). The TEAC method is based on the inhibition of the absorbance of the 2-2-azinobis (3-ethyl-bezothiazoline 6 sulfonate) (ABTS) radical cation by antioxidants. This method is relies on the discoloration of ABTS by antioxidant compounds. One advantage of this method is its applicability to both lipophilic and hydrophilic components (Büyüktuncel, 2013; Okan et al., 2013).

The lowest values for total phenolic content, FRAP, and TEAC were obtained in sample 7. Sample 5 had the highest total phenolic content, sample 1 had the highest FRAP value, and sample 8 had the highest TEAC value. While the lowest values were consistent across all three analyses in sample 7, the highest values varied. The highest total phenolic content recorded was 0.0029 mg GAE/g, and the lowest was 0.0017 mg GAE/g. FRAP values ranged from 3.854 to 8.267 µmol Trolox/g, and TEAC values ranged from 0.0014 to 0.0146 µmol Trolox/g (Table 3). A strong positive correlation was found between TEAC values and both total phenolic content (R=0.785) and FRAP values (R=0.868) (Table 4).

Table 3

Antioxidant capacity of Çıntar mushrooms samples

Samples	Total phenolic content mg GAE/g	FRAP µmol Trolox/g	TEAC µmol Trolox/g
1	0.0023 ± 0.0002^{cde}	8.267 ± 0.958^{e}	0.0141 ± 0.0008^{cde}
2	0.0019 ± 0.0008^{abc}	7.210 ± 1.122^{cde}	0.0125 ± 0.0007^{cde}
3	0.0024 ± 0.0001^{de}	6.397 ± 2.978^{bcde}	0.0143 ± 0.002^{de}
4	0.0023 ± 0.00005^{cde}	5.604 ± 0.284^{abcd}	0.0115 ± 0.002^{bcd}
5	0.0029 ± 0.0002^{f}	6.027 ± 1.274^{abcde}	0.00135 ± 0.001^{cde}
6	0.0018 ± 0.00003^{ab}	4.157 ± 0.780^{ab}	0.0092 ± 0.0011^{ab}
7	0.0017 ± 0.00008^{a}	3.854 ± 0.547^{a}	0.0075 ± 0.0007^{a}
8	0.0027 ± 0.0001^{ef}	7.512 ± 1.054^{de}	0.0146 ± 0.0003^{e}
9	0.0022 ± 0.0001^{bcd}	5.686 ± 1.304^{abcd}	0.0128 ± 0.0009^{cde}
10	0.0021 ± 0.0002^{abcd}	5.109 ± 2.136^{abc}	0.0113 ± 0.0005^{bc}
MEAN	0.0022	5.982	0.0121
Min	0.0017	3.854	0.0014
Max	0.0029	8.267	0.0146

* n=4 (± standard deviation). Different lowercase letters indicate differences between rows (P<0.05).

Table 4

Correlation matrix for the antioxidant capacity of Çıntar mushroom samples

R-Pearson Values*	1	2	3
1 Phenolic compound	1.00		
2 FRAP	0.532	1.00	
3 TEAC	0.785**	0.868**	1.00

** Correlation is significant at the 0.01 level (2-tailed).

The average phenolic content was 1.84 mg/g on a dry mass basis for eight popular edible mushrooms in Hungary, which is higher than the findings of our study (Krüzselyi et al., 2020). Bozdogan et al. (2018) reported the phenolic content 15.66 and 34.55 mg GAE/kg for two different species in Türkiye. In the same study, antioxidant capacity was assessed using the FRAP method and recorded as 0.57 and 0.28 mmol Fe^{2+}/L. In Romania, the phenolic content of eight different mushroom species ranged from 16.55 to 28.74 mg GAE/100 g (Avramiuc, 2018). TEAC and FRAP values for eight edible macrofungal species varied from 1.07 to 12.57 μmol Trolox/g dry matter and 0.10 to 16.68 μmol Trolox/g dry matter, respectively (Kıvrık et al., 2022). For six different species in Türkiye, ABTS values ranged from 44.58 to 101.36 mg Trolox/g extract (Akata et al., 2019). In the same study, antioxidant capacity assessed by the FRAP method was between 9.61 and 31.87 mg Trolox/g extract. Comparing our results with those of other studies is challenging due to various factors, such as differences in extraction methods, expression of results (based on dried or fresh mushrooms), mushroom type, harvest time, and climatic conditions (Salihović et al., 2019; Gül et al., 2021).

Conclusion

The physicochemical properties and antioxidant activities of 10 Çıntar mushrooms collected from Muğla province and its surrounding region were examined. Our results showed variations between the samples, which we believe are influenced by factors such as the carbon and nitrogen content of the environment where the mushrooms grew, temperature, and other conditions. Therefore, more research on these mushrooms is needed in Türkiye, a country abundant in wild mushrooms, to better understand these variations.

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Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:
Автори А.А. (рік видання), Назва статті, *Назва журналу (курсивом)*, Том (номер), сторінки, DOI.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
Приклад:
Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108, https://doi.org/5533.935-3.

2. Посилання на книгу:
Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
Приклад:
Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

3. Посилання на розділ у редагованій книзі:
Автори (рік), Назва глави, In: Редактори, *Назва книги (курсивом)*, Видавництво, Місто, сторінки.
Приклад:

Fordyce F.M. (2013), Selenium deficiency and toxicity in the environment. In: O. Selinus (Ed.), *Essentials of Medical Geology*, Springer, pp. 375–416, https://doi.org/10.14453/10.1007/978-94-007-4375-5_16

4. Тези доповідей конференції:

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52–57, https://doi.org/5533.935-3.

5. Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.

Приклад:

Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з української мови використовується паспортний стандарт.

Зручний сайт для транслітерації з української мови: http://translit.kh.ua/#lat/passport

Стаття надсилається за електронною адресою:
ufj_nuft@meta.ua

УДК 663/664

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

Ukrainian Food Journal індексується наукометричними базами:

- Index Copernicus (2012)
- EBSCO (2013)
- Google Scholar (2013)
- UlrichsWeb (2013)
- CABI full text (2014)
- Online Library of University of Southern Denmark (2014)
- Directory of Open Access scholarly Resources (ROAD) (2014)
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- InfoBase Index (2015)
- Chemical Abstracts Service Source Index (CASSI) (2016)
- FSTA (Food Science and Technology Abstracts) (2018)
- Web of Science (Emerging Sourses Citaton Index) (2018)
- Scopus (2022)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

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