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# Ukrainian Food Journal

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### Ukrainian Food Journal is an

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# Consumer properties of biodegradable edible cups for hot drinks

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### Abstract

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Iryna Koretska E-mail: tac16@ukr.net **Introduction.** Determining the biodegradable edible cup consumer properties will allow finding an ecological alternative to disposable cups for hot drinks.

**Materials and methods.** Butter cookies were made in the shape of a cup, followed by the application of a waterproof layer, the basis of which was pectin or polyvinyl alcohol. A 10-point scale for evaluating cups by individual indicators was developed, and the Harrington's function was used.

Results and discussion. Biodegradable edible cups in terms of organoleptic, ergonomic and geometric parameters fully correspond to disposable cups that are currently used. Due to the thicker walls and the material (biscuits) properties, the edible cup allows you to hold a cup with a hot drink in your hands without an additional layer. The absence of an additional layer is important from an economic and environmental point of view. The edible cup could keep the drink inside from 20 minutes to 1 hour. The cup appearance did not change under the influence of drinks at different temperatures: 10-15 °C (Glaze, Frappe, Cold Americano, Ice Cream), warm, 60-65 °C (Latte, Cappuccino, Mochaccino, Macchiato, Flat White, Raff) and hot, 85-90 °C (Americano, Espresso and their varieties). The biodegradable edible cup is resistant to acetic acid and ethyl alcohol that is important because some types of coffee-based drinks require the addition of alcoholic beverages, in particular, Irish coffee, Farisei, Karsk, Coffee punch. The cups can be used for a wide drinks range with a temperature from 10 to 90 °C. It has excellent ergonomic performance and is environmentally friendly, as it can be decomposed like any other confectionery product.

**Conclusions.** The biodegradable edible cup has excellent ergonomic indicators, is environmentally friendly because it can be easily decomposed like any other confectionery.

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### Introduction

The problem of the accumulation of plastic materials in the environment requires society to reconsider its habits, including the refusal of using polymer disposable cups when consuming hot drinks. A possible solution for utilization of disposable non-biodegradable coffee cups could be replacement them with (a) reusable cups; (b) biodegradable non-edible cups and (c) biodegradable edible cups. Reusable cups are the most optimal way, but the least convenient in practice, since in this case you need to carry the cup with you, have enough space for it in your bag, protect the cup from breakage, and follow sanitary rules (Allison et al., 2021). To follow this way, Starbucks, the largest coffee house chain in the world, offers a discount to consumers, who bring a reusable coffee cup (Ziada, 2009). Some coffee shops propose discount for use the ceramic mugs available in store instead of using paper cups (Jung et al., 2011). Additional motivating factors include environmental commitments, financial support, corporate initiatives, customer demand, and public pressure (Ma, 2014).

There is a worldwide increase in coffee consumption in disposable cups-to-go. For example, about 3 billion disposable coffee to-go-cups are used each year in Germany (Loschelder et al., 2019). However, encouraging café customers to become part of the 'choose a reusable mug' movement in a 14-week intervention trial in which 23,946 hot drinks were sold resulted in a reduction in the use of disposable cups and an increase in the use of reusable cups by 17.3% (Loschelder et al., 2019)

Different approaches to the classification of disposable cups such as by purpose, by the number of uses, by the presence of a barrier coating, and by the type of main raw material for cup making were proposed (Bidiuk et al., 2020). Paper cups are coated with a thin layer of plastic, usually petro-plastic, to prevent liquid to intrude into the cup. Particularly petro-plastic cups are frequently associated with an unnecessary use of limited resources and superfluous production of waste (Van der Harst et al., 2013). However, polyethylene can be replaced with a biodegradable material, for example, natural polymers (Mahinpei, 2020). Waste paper cups can be pyrolyzed at temperatures ranging from 325 to 425°C to produce commercial fuel (Biswal et al., 2013) or used for production of the bio-eco-based cellulose nanomaterials by citric acid hydrolysis (Nagarajan et al., 2020).

Another type of disposable cups are foam cups, which are cheaper compared to paper ones. However, Styrofoam cups are also not biodegradable and should be landfill or incinerated (Jung et al., 2011).

Sazeli et al. (2021) proposed to make disposable tableware and cutlery from organic material such as corn and barley to develop fully biodegradable tableware.

Kaya et al. (2016) proposed the production of chitin and chitosan cups from the abdominal exoskeleton of the insect *Pimelia sp.* as an alternative to synthetic materials in the food industry. Both chitin and chitosan cups exhibited antimicrobial activity against two common food pathogens *Candida albicans* and *Listeria monocytogenes*. Liu et al. (2020) developed an all-natural biodegradable, hygienic, water- and oil-resistant, mechanically strong, and low-cost tableware using environmentally friendly sugarcane fiber and bamboo fiber using a scalable molding method cellulose. Choeybundit et al. (2022) propose the production of cutlery from soy protein isolate with the addition of 5-20 % crude fiber from morning glory stems. The production of these biodegradable cutlery involves hydraulic hot pressing at a temperature of 160 °C for 5 min. Production of environmentally friendly, biodegradable plates made of areca leaves were proposed (Nayak et al., 2021). The technology of a new disposable cups type made from bioplastics based on spent coffee grounds and using natural binding food components has been developed (Bidiuk et al., 2020).

Currently, there are already developments of alternatives to disposable cups that have been implemented into production. The Inferra Pack Company produces biodegradable tableware from various natural raw materials: sugar cane cake, corn starch, and coffee waste. Using the innovative secondary processing technology of cellulose, kraft tableware contains 30 % more recycled raw materials than ordinary paper tableware. The uniqueness of this ecological product is that the term of its complete disposal in the soil is about 180 days. The advantage of using environmentally friendly single-use bioplastic packaging is its computability. Manufacturers who are already engaged in the production of such disposable tableware most often position them as a confectionery product and offer coffee in such cookies in the shape of a cup/glass.

Although a straw is not a mandatory attribute for drinking coffee, many consumers prefer to drink it by this way. For example, only in Taiwan three billion non-degradable plastic straws are consumed annually (Cheng et al., 2022). Currently many countries are already planning to increase strict legislation of plastic straws.

The literature analysis showed that scientists all over the world are actively engaged in the replacing non-biodegradable tableware with biodegradable ones. Because currently quite a limited number of developments have been put into production, the development and research of edible cups is still relevant.

Therefore, the development of technology for ecological biodegradable disposable tableware for widespread use is currently an extremely important task to ensure sustainable development and environmental protection.

### Materials and methods

### Materials

Wheat flour (40–60%); native potato starch or native corn starch (10–25%); white sugar (5–20%); margarine (5–20%); leavening agents (0.5–2%); drinking water (10–30%) were used as raw materials for the biodegradable edible cup production.

Pectin (E440) or polyvinyl alcohol (E1203), and drinking water served as raw materials for making a waterproof layer.

### **Production of experimental cups**

From the specified raw materials, dough for butter cookies having shape of cups was prepared. The shape of the cup could be changed according to the customer's requirements. The formed cup was baked, after which a waterproof layer was applied to the inner surface of the baked product by praying, glazing or brushing on the surface, and the cup was kept for  $10\pm 2$  h until the waterproof coating is dry. The variants of the developed biodegradable cups is shown (Figure 1).



Figure 1. The biodegradable edible cups

### **Research methods**

The properties of the developed biodegradable tableware were studied according to Bidiuk et al. (2020): organoleptic (color, appearance, physical condition, presence of smell) according to the technical specifications for the corresponding disposable cups; the sound when struck with a wooden stick was determined by hitting the edge of the cup, indirectly this method indicates the presence of voids inside the material); defects (heterogeneity of the surface, uneven and visible loose seam for a paper cup, color heterogeneity for an edible cup, indistinct pattern for paper and polymer tableware); ease of use was determined by the subjective perception of holding and consuming the drink; actual capacity, ml, distilled water was poured into the cup under study, after which it was poured into a measuring cylinder and the volume was determined; height, mm, was measured with a caliper; the diameter in the upper part, mm, was measured with a caliper; the diameter in the lower part, mm, was measured with a caliper; wall thickness, mm, was measured with a caliper; the thickness of the bottom, mm, was measured with a caliper; mass fraction of moisture, %, method of drying to constant mass; short-term exposure to hot water: for this, the tested product is immersed in distilled water with 85-90 °C, the water should not change color or a sediment should appear, the tableware were removed from the water, wiped dry and compared with a similar sample that was not immersed in water; short-term exposure to an alcohol solution: for this, the tested product is immersed in a 30 % solution of ethyl alcohol at 60–65 °C, the alcohol solution should not change color or a sediment should appear, the tableware were removed from the water, wiped dry and compared with a similar sample that did not was immersed in an alcohol solution; short-term exposure to an acetic acid solution: for this, the product under investigation is immersed in a 9% acetic acid solution at 85-90 °C, the acetic acid solution should not change color or a sediment should appear, the dishes were removed from the water, wiped dry and compared with a similar sample, which was not immersed in an acid solution; the method of *temperature influence* on disposable cups.

The waterproofness of the developed biodegradable cups was checked according to the method, which involves dense placement of filter paper on the outer surface. After that, a liquid (distilled water or a drink of the appropriate temperature) is poured into the cup. The duration of aging is determined by the usual drink consumption duration. The minimum acceptable duration is  $40\pm5$  min, which is due to the coffee consumption traditions. The spots appearance on the filter paper indicates cup water penetration. The waterproofing performance was tested empirically by placing a beverage in a designed cup and placing the cup in filter paper and visually observing the appearance of spots on the filter paper surface. The biodegradable edible cup does not pass liquid, because not a single plume appeared on the filter paper during the entire research duration. The research duration was determined by the keeping expediency the liquid inside the drink – the time until the drink is consumed. A hot drink is consumed on average within 20-30 min according to personal observations. If the drink is consumed in the consumer company, the duration may increase to 1 hour.

### Processing of research results

The degree of expert opinions consistency regarding product quality indicators was assessed using the average determining sum ranks method and the squared sum ranks deviations from the average sum (Koretska et al., 2018).

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The grades variance was calculated using the formula:

$$\sigma_{j} = 1/(n_{j} - 1) \cdot \sum_{i=1}^{n} (C_{ij} - N_{j})^{2} , \qquad (1)$$

where  $N_j = \sum_{i=1}^{n} C_{ij} / n_j$  - average value in points;  $C_{i,j}$  - evaluation of the *i* expert in the *j* 

direction;  $n_i = 5$ 

The grades variation coefficient in the *j* direction was calculated using the formula:

$$\gamma_j = \sqrt{\sigma_j / N_j} \cdot 100\% \tag{2}$$

Melnik et al. (2020) and Chen et al. (2012), which allows universalizing the general approach regarding the expediency of using and/or replacing paper and polymer disposable cups with biodegradable edible ones. One of E. K. Harrington's logistic functions was taken as a basis – the «desirability curve», which is the geometric mean of individual desirability functions:

$$D = \sqrt[q]{d_1 \cdot d_2 \cdot \dots d_q} \tag{3}$$

where  $d_1$ ,  $d_2...d_q$  – desired level (separate desirability function) of the 1-st, 2-nd, etc. optimization parameter (changes from 0 to 1); q – the parameters number.

### Harington's scale

To construct a significance scale, was used a well-known table of ratings correspondence in empirical or numerical systems. Standard correspondences of the significance scale (Harington's scale) are given in Table 1.

Table 1

Grade	Value intervals
Very good	0.80 - 1.00
Good	0.63-0.80
Satisfactory	0.37-0.63
Poor	0.20-0.37
Very bad	0.00-0.20

### Harrington's scale desirability for individual assessments

The values choice 0.63 and 0.37 is partially explained by the calculation convenience:

$$\approx 2.7; \quad 0.37 \approx 1/e; \quad 0.63 \approx 1-1/e$$

For further research, the scale was presented in the form of a graph.

To determine the reliability of such a replacement, was proposed the restrictions introduction on the main quality indicators of experimental samples and the restrictions formation on the values of individual indicators (Kim et al., 2002; Marinković, 2021; Sharma et al., 2022). To do this, Harington's scale was transformed into a ten-point scale and superimposed it on the ordinate scale (Figure 2).

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Figure 2. The quality indicators generalized analysis of the researched tableware for drinks samples

On this scale, five intervals were used in the general scale interval from 10 to 0: very good (VG), 10.0-8.0; good (G), 8.0–6.3; satisfactory (S), 6.3–3.7; bad (B), 3.7–2.0; very bad (VB), 2.0–0.0. The Figure 2 shows the desired indicators limits, according to which the samples quality will be within the range of «good – very good». The estimate arrival at the point  $d_1$  will indicate the submission to the minimum value point of the given critical limit.

A 10-point scale to evaluate cups was developed. Groups of main indicators  $P_i$  were used for evaluation: 1, temperature of the cup surface with a drink; 2, environmental friendliness; 3, organoleptic indicators; 4, structural and mechanical properties and their descriptors ( $P_{ij}$ ) (Table 2).

The obtained objective indicators were transformed into objective values by converting them into points.

The profilograms area (S) (quality criterion) is equal to the areas sum of the triangles formed by the corresponding rays of individual (partial) quality indicators:

$$S = \sum_{j=1}^{n} \left(\frac{1}{2} f_1 f_{j+1} \cdot \sin \frac{2\pi}{n}\right) = \frac{1}{2} \sin \frac{2\pi}{n} \sum_{j=1}^{n} (f_1 f_{j+1}), f_{n+1} = f_1$$
(4)

Instead of the function S, the function F was used, which differs from S only by a constant factor that does not affect the choice of the largest value. To choose the most successful option with the largest value of the complex criterion, it is enough to use the criterion definition formula:

$$F = f_1 f_2 + f_2 \cdot f_3 + \dots + f_{n-1} f_n + f_n f_1$$
(5)

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#### Table 2

Indicators (notation)	Test method	Weighting factor	Evaluation, points		Assessment, points	
			min	max	min	max
The temperature of the cup surface with a drink (P <sub>1</sub> )	objective	1.5	6	10	9	15
Environmental friendliness (P <sub>2</sub> )	objective or organoleptic	2.5	6	10	15	25
Organoleptic properties (P <sub>3</sub> )	organoleptic or objective	3.5	6	10	21	35
Sensation when touching the cup surface	organoleptic	4.0	6	10	24	40
Color (P <sub>32</sub> )	organoleptic or objective	2.0	6	10	12	20
Odor (P <sub>33</sub> )	organoleptic	1.0	6	10	6	10
Taste (P <sub>34</sub> )	organoleptic	1.0	6	10	18	30
Structural and mechanical properties (P <sub>4</sub> )	organoleptic or objective	2.5	6	10	15	25
<i>The structure</i> (P <sub>41</sub> )	organoleptic	2.0	6	10	12	20
Deformation under the temperature influence 85-90 °C (P <sub>42</sub> )	organoleptic	5.0	6	10	30	50
<i>Waterproof</i> (P <sub>43</sub> )	objective	3.0	6	10	18	30
Product quality based on the sum of all indicators	calculated	10.0	24	40	60	100

General scoring scale for evaluation the quality of polymer, paper and biodegradable cups taking into account the weighting factors of the main indicators

Notes: The deformation index was determined for two temperatures, which are typical for cold and hot drinks. The temperature of 60-65  $^{\circ}$ C (Table 2) was not used, because if the cup does not deform at 85-90  $^{\circ}$ C, then at a lower temperature (60-65  $^{\circ}$ C) there will be no deformation.

In the case of a one-sided restriction on the optimization parameters, a separate desirability function  $d_i$  takes the known form (Fig. 2):

$$d_i = exp[-exp(-y_i')] \tag{6}$$

 $y_i$ ' – some dimensionless quantity related to the optimization parameter  $y_i$  by a linear law:

$$y_i = b_0 + b_1 y_i$$
 (7)

 $b_0$ ,  $b_1$  – coefficients that can be determined if two values of the optimization  $F = f_1 f_2 + f_2 f_3 + f_{n-1} f_n + f_n f_1$  parameter  $y_i$  are given corresponding values of a separate desirability function.

The indicator average value was calculated for the series of each experiment:

$$Y = \frac{\sum_{n=1}^{n} Y_1}{n} \tag{8}$$

Y – the indicator average value;  $Y_I$  – the indicator value in each experiment; N – the parallel number experiments.

Next, was evaluated the variance of the arithmetic mean  $S^2$  for each series of experiments:

$$S^{2} = \frac{\sum_{n=1}^{n} (y_{1} - \overline{y}_{2})^{2}}{n-1}$$
(9)

The repeating experiments frequency was 3-5 times.

### **Results and discussion**

### **Consumer properties of experimental samples**

The disposable cup is represented by edible and non-edible samples. Biodegradable edible tableware should also have similar mechanical, ergonomic and operational indicators as non-edible tableware. The most daring idea is the complete replacement of paper, polymer (polystyrene and polypropylene) cups by edible tableware. Authors of this research have opinion that replacing non-edible tableware by edible ones is not an equivalent replacement, therefore, non-edible tableware should be replaced with biodegradable ones, in particular, the polyethylene layer in paper cups should be replaced with ecological ones, and polypropylene and polystyrene should be replaced with natural polymers. An edible cup also serves as a snack and a sweet for a drink.

Quality indicators are determined by the technical specifications of the market operator. Characteristic of disposable tableware was provided according to the following indicators: (1) organoleptic (color, appearance, physical condition, smell); (2) sound when struck with a wooden stick; (3) the presence of defects; (4) ease of use; (5) the actual capacity, ml; (6) the height, mm; (7) the diameter of the upper part, mm; (8) the diameter of the bottom part, mm; (9) the wall thickness, mm; (10) the bottom thickness, mm;(11) the moisture mass fraction, %; (12) short-term hot water influence (60–65 °C and 85–90 °C); (13) short-term cold water influence (10–15 °C); (14) short-term alcohol solution influence; (15) short-term influence of acetic acid solution.

### Organoleptic, ergonomic and geometric properties of biodegradable edible cups

Organoleptic, ergonomic and geometric properties of biodegradable edible cups were compared with properties of disposable paper and polymer cups (Table 3).

The developed cup fully corresponds in terms of organoleptic, ergonomic and geometric parameters to the disposable cups that are currently in use. In addition, the developed edible cup also has an advantage over paper and polymer analogues, because due to the greater thickness of the walls and the material properties (cookies), it is possible to hold a cup with a hot drink in your hands without an additional layer. From the outside, the designed cup can be coated with edible polymer, which can be consumed or thrown away without negative impact on the environment.

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Indicators	Paper cup	Polymer cup	Biodegradable edible cup
Organoleptic	White, opaque, no	Transparent,	The color inherent in
(color,	smell, soft, visible	polypropylene glass is	baked butter cookies, the
appearance,	side seam of	soft in the hands,	pleasant smell of cookies,
physical	gluing, there is a	deforms with a slight	there is a border on top, it
condition, smell,	border on top	effort; polystyrene cup	is firm in the hands and
taste*)		is hard in the hands,	does not deform, the
		does not deform.	pleasant taste,
		There is a border on	characteristic of butter
		top	cookies
Sound when	Unvoiced		
struck with a			
wooden stick			
Presence of	Absent in the studie	ed samples	
defects			
Ergonomic	It is comfortable	It is convenient to hold	The shape is similar to a
indicators	to hold in the	in the hand,	paper or polymer cup
	hand, but needs an	polystyrene cups do	
	additional	not need an additional	
	intermediate layer	thermal insulation	
	to hold a hot drink	layer, unlike	
	T 1 1	polypropylene	
Actual capacity,	In accordance with	the standard size and con	nsumer needs
IIII Height mm	00+0.1	00+0 1	90+0 1
Diameter of the	90⊥0.1	90±0.1	90±0.1
upper part mm	70±0.1	70±0.1	70±0.1
Diameter of the			
bottom part mm	40±0.1	42±0.1	$40{\pm}0.1$
Wall thickness			
mm	$1\pm0.1$	$0.5{\pm}0.1$	5±0.1
Bottom			
thickness, mm	$1\pm0.1$	$0.5{\pm}0.1$	8±0.1
Moisture mass		50.05	14:0.5
fraction, %	5.5±0.5	5.0±0.5	14±0.5
Short-term hot	The shape and appe	earance of the cups remain	ined unchanged
water influence			-
Short-term cold			
water influence			
Short-term			
alcohol and			
acetic acid			
solution			
influence			

### Properties of various disposable cups for hot drinks

\* the taste was determined only for edible cups

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Coffee contains organic acids, and some types of coffee-based drinks, such as Irish coffee, Dumas, Karsk, Espresso Martini, and Coffee punch, are prepared with the addition of alcohol. Therefore, the effect of alcohol and acetic acid on the developed biodegradable cup was studied.

The key characteristic of the biodegradable edible cups is their waterproofness, which allows to completely replace disposable cups. As mentioned above, the cup waterproofness was created due to the inner natural polymer layer. The manufactured biodegradable cup with a drink was kept for 2 hours and no place of liquid leakage was found.

### Effect of temperature on the biodegradable edible cup properties

Bidiuk et al. (2020) recommend to test tableware at temperatures for typical drinks offered by restaurants: 10-15 °C (Glaze, Frappe, Cold Americano, Ice Cream); 60-65°C (Latte, Cappuccino, Mochacino, Macchiato, Flat White, Raff), and 85-90°C (Americano, Espresso and their varieties). The effect of different temperatures on the change in appearance on disposable cups are present in Table 4.

Table 4

Experimental conditions	Paper cup	Polymer cup	Biodegradable edible cup
Drink with a temperature of 10-15 °C		No changes in appearance	8
Drink with a temperature of 60-65°C	No changes in the appearance for holding in hand	No changes in appearance, the polypropylene cup needs an additional heat- insulating layer to hold in the hand	No changes in appearance, it is possible to hold without an additional insulating layer to hold in the hand
Drink with a temperature of 85-90°C	No changes in the appearance of the cup, but it is impossible to hold it in your hands without an additional layer	A beverage with a temperature above 70 °C cannot be placed in a polypropylene cup (manufacturer's recommendation on the label); polystyrene cup unchanged	No changes in appearance, it is possible to hold without an additional insulating layer for holding in the hand

### Temperature influence on disposable cups

The results are quite acceptable since the polymers used are insoluble in water and the water drink will not dissolve the inner waterproof layer of the biodegradable edible cup. Naik et al. (2019) substantiated the importance of possible burn depending on the drinkware type. That is why in the Table 4 presents the sensations characteristics when holding a cup with a drink in your hands. The results agree with Bidiuk et al. (2020): the developed cups do not show any changes at 85-90 °C, therefore a lower temperature does not change the cups appearance.

### Quality assessment of test samples

The quality criterion determination of test samples was carried out in several stages: (1) determination of characterize indicators, conversion of measurement units into dimensionless units (if necessary); (2) mathematical model development and calculation of product quality criteria.

A critical limit was set for a specific indicator for 0.6 points during the determinations. If the absolute value of the property indicator corresponds to the limit provided for in the regulatory documentation for the product, then a score of  $K_{ij} = 0.37$  is an unacceptable indicator quality for the sample.

An important issue for the scientific substantiation of expert evaluation is the degree consistency assessment of expert opinions using the indicator system (Koretska et al., 2003). To assess the generalized agreement opinions measure from all directions, it was calculated the expert opinions agreement degree. The calculations results are given in the Table 5.

Table 5

The samples	The indicator	The average value (rank)
Diadagradable adible aun	The variance	0.0236
biodegradable edible cup	Rate of variation, %	2.657
Danar aun	The variance	0.0187
raper cup	Rate of variation, %	2.784
	The variance	0.0192
Polymer cup	Rate of variation, %	2.852

### Assessment results of the consistency experts' opinions degree

It can be seen that the variance value the estimates is close to zero from the obtained data (Table 5), and the variation estimates coefficient is no more than 3%. Such a result indicates a high assessment by experts of the finished product quality and shows consistency in the samples assessment between experts. This is explained by the fact that the group of experts was selected correctly. For the first time was calculated the conformity assessment results of experts' opinions for disposable cups.

### Quality assessment of disposable cups using Harington's scale

Table 6 presents the results of s disposable polymer, paper and biodegradable edible cups evaluation.

The edible cup is completely biodegradable compared to other ecological cups, because it is a food. Tableware made of polylactide can completely decompose into water and carbon in 3 months (Ziada, 2009). There is an indicator environmental friendliness (P<sub>2</sub>) in Table 6 that is why decomposition requires temperatures of at least from 55 to 70 °C and humidity, which can only be provided by commercial composting plants (Changwichan et al., 2020). In addition, much less carbon dioxide and other greenhouse gases are released during biopolymer production. Switching from conventional plastic to bioplastic would reduce greenhouse gas emissions by up to 25% (Ziada, 2009). The use of polylactide will reduce dependence on petroleum-based polymer materials, which, in turn, will reduce demand for it.

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Table 6

Indicators (notation)	Biodegradable edible	Disposable cups	
Indicators (notation)	cup	polymer	paper
The temperature of the cup surface with a			
drink (P <sub>1</sub> )	13.5	9	10.5
Environmental friendliness (P <sub>2</sub> )	25	15	15
The organoleptic properties (P <sub>3</sub> )	31.9	24.5	25.0
Sensation when touching the cup surface			
(P <sub>31</sub> )	36	28	30.4
Color (P <sub>32</sub> )	18	18	18
Odor (P <sub>33</sub> )	8.9	6*	6*
Taste (P <sub>34</sub> )	28.8	18	18
Structural and mechanical properties (P <sub>4</sub> )	22.1	25.0	24.8
The structure (P <sub>41</sub> )	17	20	20
Deformation under the temperature			
influence 85-90 °C (P <sub>42</sub> )	45	50	50
Waterproof (P <sub>43</sub> )	27	30	29.4
Criterion of product quality, point	92.52	73.50	75.36

### Score evaluation quality of disposable cups taking into account the importance of the main indicators

\* to avoid the exclusion of the indicator, its value was taken as the critical limit

### Polygon of the quality assessment of disposable cups

The quality criterion were used to construct diagrams, according to the planar principle, that is, the value of the complex quality criterion corresponds to the area of a polygon in which the distances from its center to the vertices are equal to the normalized values of individual quality indicators (Koretska et al., 2003) (Figure 3).

This is because the biodegradable edible cup has the advantage of being environmentally friendly. Other researchers have obtained the similar results (Liu et al., 2020).

The generalized quality criterion determined by the desirability function is calculated and presented in Table 7.

Table 7

### Ranking of disposable cups according to the generalized desirability function

Type of cup	Biodegradable cup	Polymer cup	Paper cup
Quality criterion	92.52	73.50	75.36

When rating the tested samples, the quality criterion of a paper cup, 75.36 points, was accepted as 100%. The quality criterion of the developed biodegradable edible cup was higher, 122.8% = 92.50 points, compared to the polymer cup, 97.53% = 73.5 points.

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biodegradable edible cup (a); polymer cup (b); paper cup (c).

 $P_1 \mbox{ is the temperature of the cup surface with a drink;} \label{eq:posterior}$ 

P2 is environmental friendliness;

- P<sub>3</sub> is the organoleptic indicators:
- P<sub>31</sub> is sensation when touching the cup surface,

P<sub>32</sub> is color,

P<sub>33</sub> is odor,

P<sub>34</sub> is taste;

- P4 is structural and mechanical properties;
- P<sub>41</sub> is the structure,
- P<sub>42</sub> is deformation under the temperature 85-90 °C, P<sub>43</sub> is waterproof.

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### **Environmental benefits**

The expediency of replacing the existing disposable cups with a biodegradable edible cup is evident because nowadays environmental friendliness is a priority compared to other characteristics of the cups. In addition, do not forget about the capital investment required for the collection and processing of used paper and polymer cups. The biodegradable edible cup does not need to be recycled, because it is edible and is consumed completely with a drink or could be completely decomposed like any other confectionery product.

Environmental initiatives will have a positive effect only under the condition of a comprehensive approach and the population's awareness of the need for such initiatives. It is more difficult to convince the adult population to change their preferences and habits, and that is why ecological products are becoming the subject of sociological research in the field of marketing (Ivanov et al., 2021; Kim et al., 2019; Lee et al., 2023; Marinova et al., 2022). Today, consumers are becoming more aware of the importance of caring for the environment, which will be reflected in their attitude towards quality disposable edible new cups (Bertossi et al., 2023). Environmentalism must be brought up in children, so that in adulthood it is already perceived as a norm, a habit and a necessity of everyday life.

A separate segment that also needs to be provided with eco-friendly tableware is hotels (Subbiah et al., 2011). Such dishes keep their shape well when hot dishes are packed into them and can be used to store liquid dishes and sauces for 24 h without getting wet or softening. Biodegradable tableware is an ideal solution for catering and is suitable for heating in microwave ovens of different power. Bioware can be easily disposed of together with other food scraps, which will greatly facilitate the work of the restaurant by reducing the amount of waste and will allow to completely abandon the sorting of plastic.

### Conclusions

- 1. The biodegradable edible cup is a full-fledged replacement of currently used polymer and paper cups. It has been confirmed using the Harrington's function.
- 2. The biodegradable edible cup is a cookie formed in the form of a cup. It is possible to change the shape of the cup according to the consumer requirements. The cup's waterproofness is provided due to the waterproof layer inside it.
- 3. The developed cup can be used for a wide range of drinks with a temperature from 10 to 90  $^{\circ}\mathrm{C}.$
- 4. The biodegradable edible cup is resistant to the action of acetic acid and ethyl alcohol.
- 5. The biodegradable edible cup has excellent ergonomic indicators, is environmentally friendly and can be easily decomposed like any other confectionery.

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# Effect of prolonged proteolisis on biochemical composition of the malt wort

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Abstract

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**DOI:** 10.24263/2304-974X-2023-12-3-4 **Introduction.** Changes in mashing modes can increase extraction of biological compounds from malt. Biochemical composition of the malt wort with prolonged action of proteolytic enzymes during the mashing process was determined.

**Materials and methods.** The raw material composition made from three types of malt: base malt, melanoidin malt and roasted malt. Analysis of the amino acid, carbohydrate and lipid profile in modified proteolytic wort was done by chromatographic methods.

**Results and discussion.** Wort analysis identified 19 amino acids; ten of them were essential amino acids. Total content of amino acids in control wort was 1349 mg/l. Total amino acid content in wort modified by mashing mode 1 increased by 55% to 2096 mg/l. Total content of amino acid in wort modified by mashing mode 2 increased by 90% to 2572 mg/l. Proline content in modified wort was the highest: 437 mg/l in wort modified by mashing mode 1; 568 mg/l in wort modified by mashing mode 2. The high content of leucine, arginine, and phenylalanine was observed in modified wort. The percentage increase in the content of individual amino acids in the modified wort varied significantly. Thus, the content of valine and phenylalanine increased by 101-102%, meanwhile the amount of glycine increased only by 18%.

Chromatographic analysis showed that prolonged proteolysis has no effect on the carbohydrate and lipid composition of the wort. Total fatty acid content was in the range from 5.6 to 11.4 mg/l. Difference in total content of fatty acids between samples of modified proteolytic wort was more than 30%. Analysis of wort showed the presence of 11 fatty acids. Palmitic and linoleic acids were predominant in fatty acid profile in modified proteolytic wort: the content of palmitic acid was 38-48%, and the content of linoleic acid (omega-6) was 30-37% from total fatty acid amounts.

Carbohydrate profile of the modified proteolytic wort presented by maltose, 53.1%; dextrins, 23.4%; maltotriose, 15.2%, and glucose, 8.3%. Carbohydrate profile of modified proteolytic wort had no difference with traditional malt wort.

**Conclusions.** Modified proteolytic wort after prolonged proteolysis during mashing is a good source of amino acids. Long proteolytic rest significantly increases the content of amino acids in malt wort, which can be further used in the technology of dietary supplement or functional drinks.

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### Introduction

Malt wort is an intermediate in the production of beer, obtained by mashing malt with water. Next technological stages for the malt wort is boiling with hops and fermentation. Mashing is the most important process in wort production, providing the compounds such as sugars and amino acids necessary for yeast activity during wort fermentation. Sugars and amino acids play a special role in the final taste, aroma and color of the beer or other malt beverages (Castro et al., 2021; Jukic et al. 2022; Stewart et al., 2013). Main goal of the mashing process is to transfer, partially or completely, hydrolyzed compounds from malt and/or unmalted raw materials to water. Hydrolyzed compounds in wort are necessary for the yeast reproduction and activity. Optimal concentration of fatty acids (FA) and free amino nitrogen (FAN) in prepared wort are being considered as crucial for the final taste and aroma of beer (Ferreira et al., 2018). However, it is proven that high concentration of fatty acids has a negative effect on the quality of beer. Content of long-chain fatty acids is usually very low in the final product. Increase of long-chain fatty acid concentration may lead to increase of compounds formed through their oxidation, which affects beer's qualities (Vanderhaegen et al., 2006). Fatty acids are needed for the yeast cells activity and play a significant role in fermentation. Another important group of compounds determines yeast growth is the free amino nitrogen substances, presented by amino acids, ammonium ions, peptides, and tripeptides (Lei et al., 2012). However, overdosed amino acids could be converted by yeasts to higher alcohols, which are highly toxic (Ferreira et al., 2018). The substitution of malt with unmalted barley, wheat, rye, corn or other is a common practice in brewing, but addition of unmalted materials decreases enzyme activity (Bogdan et al, 2020; Kok et al., 2019).

Mashing process to extract malt raw materials is primarily focused on beer production technology. Under conditions of traditional beer production, the action of proteolytic enzymes is strongly limited. Longer action of proteolytic enzymes negatively affects on the stability of the foam in the beer and can cause turbidity, which significantly reduces the quality of the final product (Bamforth, 2023; Cvengroschová et al., 2003). The malt extract for the beer brewing must acquire the minimum amount of free amino nitrogen required for yeast reproduction. Enough quantity of free amino nitrogen, depending on the yeast culture, is in the range of 150-250 mg/l (Bogdan et al, 2020; Hill et al., 2019). Protein rest phase is usually limited by a lower limit level of free amino nitrogen content. With a high quality of malt protease, the rest phase can be skipped, because content of soluble proteins is high enough after malting. With a mash heating rate of 1 °C/min, the duration of heating in the range of action of proteolytic enzymes is enough to obtain a minimal level of free amino nitrogen (Ledley et al., 2023; Montanari et al., 2005).

Longer mashing allows a deeper extraction of biologically active substances to the final wort. Proteolysis can be prolonged, if the wort should not be used for beer production and can be not completely transparent because in the classical method the amount of amino acids in the wort used for beer production, should be minimal. High amino acid content in the wort increases the nutritional value of malt beverages prepared on its base or malt extract used for production of dietary supplements. Therefore, a study of modified malt wort with an extended rest at optimal temperature for proteolytic enzyme activity followed with quantitative and qualitative determination of the amino acids, carbohydrates and fats profiles of the final product was conducted.

The aim of the present research was to determine the amino acid, carbohydrate and lipid composition of modified proteolytic wort. Extraction was done by long-term mashing with long protein rest phase for the deeper malt proteolysis.

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### Materials and methods

### Materials

To prepare raw material composition a mixture of three types of malt was used: (a) barley brew malt Weyermann Pilsner Malt (Germany); extract (dry basis)  $\leq 80$  %; the color of the laboratory wort was 3 EBC; malt was produced according to the classical method of malting and next drying; (b) barley specialty melanoidin malt Bel-Her melanoidin (Ukraine); extract  $\leq 77$  %; the color of the laboratory wort was 57 EBC; malt was produced according to the classic method of malting followed by fermentation at a temperature of 50 °C and drying; (c) barley roasted malt Castle malting chocolate (Belgium); extract  $\leq 70\%$ ; the color of the laboratory wort was 590 EBC; the malt was produced using the classic method of malting followed by roasting at a temperature of 220 °C and rapid cooling when the malt reaches the needed intense of color. To prepare raw material composition next ratio of malts was used: Weyermann Pilsner Malt, 50 %; Bel-Her melanoidin malt, 42 %; Castle malting chocolate malt, 8 %.

### Methods

*Moistening* of malt was provided before grinding to give elasticity to the shells: malt was kept in the water for 15 minutes at a temperature of 30 °C.

*Grinding* of moistened malt was carried out on a LZM-1 laboratory mill, followed by passing through a sieve with a hole size of 1.0 mm.

*Mashing ratio* between malt and water was 1:3; for the all experiments malt portion was 250 g, water portion was 750 ml. First portion of the water was added at the stage of hydration before grinding. Rest of the water was added just before mash preparation.

*Mashing* goal was to extract maximal amount of amino acids and other biological compounds to the malt wort and compare with a control wort. Mashing process was carried out using the following modes (for first mash temperature was 20 °C):

- 1. Control mode the temperature of the first mash rest was 63 °C, the duration of first rest phase for  $\beta$ -amylase catalysis during mashing was 25 min. At the end, the mash was heated to 72 °C for the saccharification rest phase followed by enzyme inactivation. Finally, wort had mass fraction of dry substances of 15 °P;
- 2. Mode 1 the temperature of the first mash rest was 50 °C (heating of the mash with a rate of 1 °C/min). Duration of the first rest phase for proteolytic catalysis during mashing was 30 min. Next heating was to 63 °C. It took 13 minutes, and the mash was holding at this temperature for the rest phase for 25 min. At the end, the mash was heated to 72 °C for the saccharification rest phase followed by enzyme inactivation. Finally, wort had mass fraction of dry substances of 15 °P;
- 3. Mode 2 the temperature of the first mash rest was 50 °C (heating of the mash with a rate of 1 °C/min). Duration of the first rest phase for proteolytic catalysis during mashing was 60 min. Next heating was to 63 °C. It took 13 minutes, and the mash was holding at this temperature for the rest phase for 25 min. At the end, the mash was heated to 72 °C for the saccharification rest phase followed by enzyme inactivation. Finally, wort had mass fraction of dry substances of 15 °P.

Mashing was carried out with constant stirring on a laboratory reactor LR-2.ST the Compact Power IKA (Germany). The reactor is equipped with a stirrer EUROSTAR 100 control for intensification of mashing. According to each mashing condition were prepared three samples of wort, the average value for each condition is indicated in the results.

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The schematic difference in the duration and temperature indicators at different mashing conditions is shown in Figure 1.



Figure 1. Schematic difference of mashing conditions

### Determination of amino acid content

The content of amino acids in malt extracts was determined as an indicator of the effectiveness of prolonged protein rest phase (proteolysis) during the extraction of malt raw materials. According to each mashing condition, three samples of wort were prepared and analysed.

Amino acids in wort were analyzed using ion exchange liquid chromatographic method by system LC-40D Shimadzu (Japan). The column chromatography method was used to determine the qualitative and quantitative amino acid composition. The chromatography conditions included the use of mobile phase, ninhydrin with added sodium citrate buffer (pH 2.2), eluent flow rate of 15 mL h–1 and a chromatography cycle of 120 min. Standard amino acids were used in parallel, while qualitative amino acid composition was determined from retention times. A mixture of 21 amino acids was used as an internal standard. The colorimetric measurement of the complex resulting from the ninhydrin reaction was carried out at 570 nm (440 nm for proline). The qualitative composition of the mixture of amino acids was determined by comparing the aminograms of the standard mixture of amino acids LAA21-1KT (Sigma-Aldrich) and the studied samples of malt wort. Quantitative analysis was done by automated determination of peak areas for identified acids by chromatograph software (Yilmaz et al., 2018).

To calculate the number of amino acids in wort from malt raw materials, the peak area of each amino acid (or peak height) is calculated on the aminogram. The number of micromoles of each amino acid  $(X_1)$  in the test solution is determined by the next formula:

$$\mathbf{X}_1 = \mathbf{S}_1 / \mathbf{S}_0$$

where  $S_1$  is the peak area (or peak height) of the amino acid in the test sample;  $S_0$  is the peak area (or peak height) of the same amino acid in a solution of a standard mixture of amino acids, which corresponds to 1 micromole of each amino acid (Savchuk et al., 2017).

### Determination of carbohydrate profile

The carbohydrate profiles of the worts were determined using high-performance liquid chromatography (HPLC) methods. Samples were centrifuged using laboratory centrifuge type MPW-351R (10 min, 5000 rpm) and subjected to 2-times dilution. Separation of the mixture was made using Rezex ROA Organic Acid H+ ( $300 \times 7.8$  mm) column made by Phenomenex. Respectively, a method of high-duty chromatography HPLC, using Shimadzu Prominence apparatus, was used to analyze concentration of glucose, maltose, maltotriose, dextrins and amount of produced glycerol and ethanol. A volume of 0.02 cm<sup>3</sup> injection, celerity of the flow of eluent 0.6 cm<sup>3</sup>/min, temperature of separation 60 °C, solution H<sub>2</sub>SO<sub>4</sub> 0.005 mol/l as eluent and refractometric method of detection were used (Gasior et al., 2020).

### Determination of fatty acid profile

Fatty acid profile was determined by gas chromatography. The analysis was performed by extracting the total fatty acid content from the sample (0.25 g) using 5 ml of a 9:1 chloroform: methanol solution. Heptadecanoic acid, 400  $\mu$ g, as an internal standard was added to the extracted lipids and methylated by heating at 70 °C for 3 hours in a solution of 1 % H<sub>2</sub>SO<sub>4</sub> in methanol. The composition of methyl esters of fatty acids was determined by gas chromatography on a Shimadzu GC 2010 Plus chromatograph under the following conditions: capillary column 50 m, flame ionization detector, carrier gas - nitrogen. The results of the research were processed using the computer software of the chromatograph (Cozzolino et al., 2014).

### Statistics

Means with standard deviations ( $\pm$ SD) were calculated from the data obtained from three independent experiments. The univariate analysis of variance (ANOVA) of means was performed using SPSS (version 16.0). Multiple-means comparisons were determined with the Duncan's multiple range test at the p <0.05 confidence level.

### **Results and discussion**

### Amino acid profile

The effect of prolonged protein pause (proteolysis) on the content of amino acids and other biological components in wort from malt composition was studied. Three samples of wort were prepared according to each mashing condition. Result is indicated as average value of each amino acid made under the same mashing modes. All wort samples had a final concentration of dry substances of  $15\pm0.5$  °P.

Analysis of wort identified the presence of 19 amino acids. Amount of each amino acid was calculated by specialized software. The average content of amino acids in different samples of wort is given in Table 1.

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### Table 1

Amino opid	The content of amino acids in wort, mg/l			
Amino acid	Control	Mode 1	Mode 2	
Proline	288 ±9.8 ª	$437 \pm 14.0^{b}$	568 ±15.9 °	
Leucine	112 ±4.2 <sup>a</sup>	166 ±6.3 <sup>b</sup>	208 ±6.7 °	
Arginine	99 ±3.5 <sup>a</sup>	156 ±4.4 <sup>b</sup>	187 ±7.3 °	
Phenylalanine	91 ±2.5 <sup>a</sup>	146 ±3.9 <sup>b</sup>	184 ±6.6 °	
Valine	94 ±3.7 <sup>a</sup>	150 ±5.5 <sup>b</sup>	190 ±4.7 °	
Glutamine	68 ±2.3 <sup>a</sup>	124 ±2.6 <sup>b</sup>	133 ±4.9 <sup>b</sup>	
Alanine	82 ±2.5 <sup>a</sup>	126 ±4.4 <sup>b</sup>	157 ±6.0 °	
Tyrosine	80 ±2.2 ª	123 ±4.4 <sup>b</sup>	151 ±4.5 °	
Lysine	58 ±2.2 ª	92 ±3.0 <sup>b</sup>	114 ±3.3 °	
Isoleucine	62 ±1.9 <sup>a</sup>	$98 \pm 3.8^{b}$	121 ±3.8 °	
Asparagine	48 ±1.8 <sup>a</sup>	75 ±2.7 <sup>b</sup>	90 ±2.5 °	
Aspartic acid	37 ±1.2 ª	47 ±1.6 <sup>b</sup>	49 ±2.3 <sup>b</sup>	
Serine	37 ±1.4 ª	58 ±1.6 <sup>b</sup>	62 ±2.6 <sup>b</sup>	
Glutamic acid	41 ±1.3 <sup>a</sup>	71 ±1.7 <sup>b</sup>	77 ±2.5 <sup>b</sup>	
Threonine	40 ±1.6 <sup>a</sup>	63 ±2.4 <sup>b</sup>	80 ±2.1 °	
Tryptophan	32 ±1.2 <sup>a</sup>	50 ±1.2 <sup>b</sup>	64 ±1.8 °	
Histidine	38 ±1.3 <sup>a</sup>	59 ±1.8 <sup>b</sup>	70 ±2.3 °	
Glycine	22 ±0.9 <sup>a</sup>	25 ±0.9 <sup>a</sup>	26 ±1.5 ª	
Methionine	20 ±0.7 <sup>a</sup>	31 ±1.1 <sup>b</sup>	40 ±1.5 °	
Total amino acid content, mg/l	1349	2096	2572	

Content of amino acids in	wort with different	mashing modes
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Results expressed as mean values  $\pm$  SE (n = 3); values with superscript different letters in the same line are significantly different (p < 0.1)

The highest content of amino acids was in the samples of wort prepared according to the second modification mashing mode 2. Chromatogram of the amino acid content in malt wort prepared with a mash mode 2 is shown in Figure 2.

### Carbohydrate profile

The main aim of the research was to select and determine regime for wort mashing providing the highest content of biologically active components. Wort prepared according to the second modification mashing mode had the highest content of amino acids, so it was used for further determination of carbohydrate and fatty acid profiles. All samples before the chromatographic study had  $15\pm0.5$  °P extract. The results of the carbohydrate profile analysis in modified proteolytic wort samples are shown in Table 2.

### Fatty acid profile

Analysis made by high-efficiency capillary column identified 11 fatty acids and their isomers from C10:0 to C22:0 in modified proteolytic wort samples. Fatty acid profiles in modified wort samples are shown in Table. 3.

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**Figure 2.** Chromatogram of amino acids of wort (15±0.5 °P): aspartic acid (ASP), glutamic acid (GLU), asparagine (ASN), serine (SER), glutamine (GLN), histidine (HIS), glycine (GLY), threonine (THR), arginine (ARG), proline (PRO), alanine (ALA), tyrosine (TYR), methionine (MET), valine (VAL), tryptophan (TRP), phenylalanine (PHE), isoleucine (ILE), leucine (LEU) and lysine (LYS). Unsigned peaks are not identified.

Table 2

#### Carbohydrate profile in the malt wort samples

Samula	Carbohydrates content, g/l			
Sample	Maltose	Dextrins	Maltotriose	Glucose
1	43.91 <sup>a</sup>	21.07 ª	13.21 <sup>a</sup>	8.11 <sup>a</sup>
2	50.06 <sup>b</sup>	21.05 ª	13.17 <sup>a</sup>	7.9 <sup>b</sup>
3	47.42 °	20.2 <sup>b</sup>	14.05 <sup>b</sup>	6.14 °

Values with superscript different letters in the column are significantly different (p < 0.05)

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Fatty	Concentration, %			
acid	Sample 1	Sample 2	Sample 3	
C10:0	0.99±0.05 <sup>a</sup>	n/d	n/d	
C12:0	0.92±0.05 <sup>a</sup>	n/d	n/d	
C14:0	3.09±0.1 <sup>a</sup>	2.50±0.1 <sup>b</sup>	2.44±0.1 <sup>b</sup>	
C16:0	48.45±0.25 <sup>a</sup>	38.21±0.25 <sup>b</sup>	38.21±0.25 <sup>b</sup>	
C16:1	2.47±0.1 <sup>a</sup>	2.32±0.1 <sup>a</sup>	2.7±0.1 <sup>b</sup>	
C18:0	5.31±0.1 <sup>a</sup>	11.6±0.15 <sup>b</sup>	8.56±0.1 °	
C18:1	2.59±0.1 <sup>a</sup>	5.53±0.1 <sup>b</sup>	2.88±0.1 ª	
C18:2	30.78±0.25 <sup>a</sup>	33.92±0.25 <sup>b</sup>	37.64±0.25 °	
C18:3	0.55±0.05 <sup>a</sup>	$0.80{\pm}0.05^{\text{ b}}$	0.45±0.05 °a	
C20:0	3.46±0.1 <sup>a</sup>	5.18±0.1 <sup>b</sup>	2.70±0.1 <sup>a</sup>	
C22:0	1.23±0.1 <sup>a</sup>	n/d	1.31±0.1 <sup>a</sup>	
Total fatty acid content, mg/l	8.09±0.1 ª	5.6±0.1 <sup>b</sup>	11.45±0.15°	

Fatty acid profile of malt wort samples

Not determined (n/d) refers means amount was < 0.5%; values with superscript different letters in the same line are significantly different (p < 0.05)

### Discussion

The raw material for the production of the studied wort was barley malt. According to the traditional technology of malt production and its subsequent processing, one of the most important quality indicators is the high content of carbohydrates needed for the fermentation during next processing (Duke et al., 2009; Yousif et al., 2020). However, the aim of the present research was to determine the effect of the long protein rest phase on the content of amino acids in the wort. It was shown that pause in the mash at 50 °C for 60 minutes significantly increased the content of all amino acids. The highest increase in the amounts of amino acids in modified wort, by 101-102%, was observed for valine and phenylalanine. The content of proline, leucine, and arginine increased by 97.5, 85.7, and 89.4%, respectively. The smallest changes, an increase of the content by 31% and 18%, were recorded for aspartic acid and glycine, respectively. The content of total amino acids of modified proteolytic wort increased by 90.5% due to long-term proteolysis during mashing.

Holding the mash at 50 °C for 30 minutes also increased the content of amino acids in the wort samples. The highest content increases, 81% and 74%, were observed for glutamine and glutamic acid, respectively, but the increase in the amounts of the most amino acids in the wort was within the range from 50 to 60%. However, the increase of the amount of aspartic acid was only 25%. The smallest quantitative change, 13%, was recorded for glycine. Under these mashing conditions, the total content of amino acids increased by 55.3%.

The temperature of 50 °C, the main mode of mash rest phase in the present study, is below the limit of activation of cytolytic enzymes. Therefore, the carbohydrates profile of the malt wort did not have significant changes. The proportion of carbohydrate content in the wort remained close to the types of wort prepared by the classic mashing mode (Ferreira, 2009). The malt extract samples had a content of reducing carbohydrates in the range from 86 to 92 g/l. Chromatography analysis showed that the main sugar in mashed wort was maltose, 53.1%, followed by dextrins, 23.4%, and maltotriose, 15.2%, and glucose 8.3%. Graphically, the carbohydrate profile of the obtained malt extract is shown in Figure 3.

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Figure 3. Diagram of the carbohydrates profile in the malt wort

Analysis of modified wort showed the presence of 11 fatty acids. Total content of fatty acids was in the range of from 5.6 to 11.4 mg/l. The largest amount of fatty acids was presented by palmitic, 38-48%, and linoleic acids, 30-37%. Low content of fatty acids in the wort can be related to the laboratory filtration technique due to the filter layer formed by crushed malt. The content of fatty acids in wort filtrated on industrial vacuum filter presses was significantly higher (Kühbeck et al., 2006).

Total protein content in the grain crops ranges from approximately 10 to 15 % of the dry weight of the grain. Barley grains contain four different classes of proteins: albumin, globulin, prolamin (hordein), and glutelin. The storage proteins, prolamin and glutelin, account for approximately 50% of the total protein in mature cereal grains. With the exception of oats and rice, the main reserve proteins of the endosperm of all cereal grains are prolamins. Prolamins have a relatively high content of proline and amide nitrogen, and other specific amino acids, such as histidine, glycine, methionine, and phenylalanine. Prolamins are mostly deficient in lysine, threonine, and tryptophan (Allai et al., 2022; Sterna et al., 2022).

Amino acid profile of the wort showed the biggest changes under different mashing modes. Amount of amino acids in the wort was influenced by many factors: biological characteristics of cereals, optimal conditions for enzymes during mashing, method and equipment for filtering of mash. Starchy endosperm contains approximately two-thirds of the grain's total protein, and its internal pH during malting is 5.0-5.2. Carboxypeptidases, which are present in the endosperm at a high content, are very active pH and likely play a central role in the mobilization of reserve proteins during malting and fermentation in the technology of special melanoidins malt. The high activity of peptidase in the modified cotyledon (shield) of the grain indicates absorption in the form of peptides of some hydrolysis products, which are further hydrolyzed to amino acids in this tissue (Geißinger et al., 2022).

Generally known that up to 70% of wort free amino nitrogen is formed during malting. Accordingly, barley with a higher nitrogen content is used to produce FAN-rich extracts; meanwhile barley with a low nitrogen content is used for the production of extracts rich with carbohydrates. Although nitrogen levels vary by grain variety, the general types of amino acids present are similar (Thompson-Witrick et al., 2020; Wefing et al., 2021). There are some researchers reporting about possible relationship between the amount of free amino nitrogen and the content of amino acids (Nie et al., 2010).

During malting, germination of barley grain is started with water absorption (soaking). Hydration initiated production of enzymes needed to convert starch reserves into fermentable sugar. Proteolytic enzymes are also activated and protein degradation occurs after hydration of grain (Arif et al., 2011). Proteolysis is important during malting because soluble nitrogen is needed for enzyme synthesis, and it results in the release of the associated  $\alpha$ -amylase enzymes required for starch breakdown. Amount of proteolytic enzymes including at least 40 different endoproteinases with high activity. Proteolytic enzymes were founded in the aleurone layer and endosperm during the germination process of barley grains (Benešová et al., 2017; Jones, 2005).

Use of plant hormones, such as gibberellic acid, during germination caused a change of the ratio of amino acid synthesized to their utilization in the germinating grains that can have effect on the final amino acid composition of the wort (Liu et al., 2013). Bromate, a substance used to limit the growth of roots, also affects the relative amount of free amino acids (especially proline and methionine) in malt and wort (Dufková, 2020). Malt drying conditions also influence on the content of amino acids in the wort. An increase of the temperature in the dryer from 82 to 104 °C causes a noticeable decrease in amino acid content (Chursinov et al., 2015). All these should be taken into account providing a mashing process with prolonged action of proteolytic enzymes. Based on the obtained research results, it is possible to increase total content of amino acid over 3 g/l in modified proteolytic wort.

### Conclusions

Technology of long-term proteolysis during wort preparation can be used in technology of dietary supplements or functional drinks. Modified wort has higher content of amino acids. High amino acid content has a strong impact on the functional properties of the wellness products. Research results:

- 1. Modified proteolytic wort prepared according to the two mashing modes: with 30 min of proteolysis and 60 min of proteolysis. Control wort was prepared without protease rest phase. Analysis of wort identified 19 amino acids, and ten of them were essential amino acids. Total amino nitrogen content in control wort was 1349 mg/l. Total amino acid content of wort modified by mashing mode 1 increased by 55% to 2096 mg/l. Total amino acid content of wort modified by mashing mode 2 increased by 90% to 2572 mg/l.
- 2. Individual amino acid content in the modified wort increased in a different range. Valine and phenylalanine content in modified wort increased by 101-102%, at the same time the amount of glycine increased only by 18%.
- 3. Total amino acid content in wort could exceed 3000 mg/l by using gibberellic acid and bromate in the malting process, by making prolonged proteolysis rest phase and using industrial separation machines.
- 4. Carbohydrate profile of modified proteolytic wort had no difference compared to traditional malt wort because prolonged proteolysis rest temperature mode is below the activation limit of cytolytic enzymes. Carbohydrate profile of the modified wort presented by maltose, 53.1%; dextrins, 23.4%; maltotriose, 15.2%, and glucose, 8.3%.
- 5. Wort analysis identified 11 fatty acids. Total content of fatty acids was not higher than 11.4 mg/l.

Therefore, prolonged proteolysis rest phase allows a significant increase of total amino acid content. Malt wort with high amino acids content can be used in production of dietary supplements or functional drinks.

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### Influence of foliar fertilizers application on the volatile composition of red wines

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	Abstract
Keywords:	
Foliar Ferilizers Red wine Volatile compounds	Introduction. The aim of the present study was to determine the influence of different foliar fertilizers (in four harvests – 2019, 2020, 2021, and 2022) on the composition of volatile and aromatic compounds of red wines from the Bulgarian hybrid variety Storgozia. Materials and methods. Gas chromatography-flame ionization detection was used to determine the volatile compounds in red wines.
	Results and discussion. The results obtained regarding the
Article history: Received 20.04.2023 Received in revised form 16.07.2023	total content of volatile compounds in the analyzed wines showed that the variant of vine treatment with fertiliser MaxGrow NS, consist of N, 20%, S, 7%, Mg, 14%, P, 5%, Mn, 1%, Zn, 0.01%, Cu, 0,05%, Fe, 0.05), Cu, 0.01%, Zn, 0.01%, B, 0.025%, Mo, 0.002%, demonstrated final high levels of volatile and aroma compounds in wines of three from the four investigated harvests (2019, 2020 and 2021). As individual representatives from the
Accepted	group of higher alcohols, 2-methyl-1-butanol and 3-methyl-1-
29.09.2023	butanol dominated in all four harvests. The two alcohols are the
Corresponding author: DimitarDimitrov E-mail: dimitar_robertov@ abv.bg	main metabolites of the yeast microflora. The application of different foliar fertilizers did not negatively affect the acetaldehyde content in the resulting wines. Its presence corresponded to the appearance of its positive influence. From the obtained results, it could be concluded that in the 2020 and 2022 harvests, the foliar application of nitrogen and mineral sources affected the content of esters. In the other two harvests, individual variants showed higher ester accumulation than the untreated control, but others demonstrated lower levels. As a result, no specific conclusion could be made about the influence of foliar
<b>DOI:</b> 10.24263/2304-	fertilizers on the final wines ester concentrations. A major ester from this fraction was ethyl acetate. Foliar fertilization affects the synthesis of β-citronellol, leading to increased levels of this terpene. Conversely, for geraniol, a decrease in its concentrations was observed in the wines obtained after the application of foliar fertilization. <b>Conclusions</b> . The application of foliar fertilizers led to changes in composition of volatile compounds of the researched red wines and influenced both on the different groups of volatiles and individual compounds.

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### Introduction

The need for organic and ecological agriculture at the current stage is fundamental due to the significant climate changes. They are a stress factor not only for vine plants but also for different crops. This stress is formed because of extreme, unpredictable and unexpected changes in weather, which can lead to a significant reduction in the yield of the vine and the composition of the grapes (Azabard, 2022; van Leeuwen and Dariet, 2016). The vine is a plant with high sensitivity to extreme high temperatures, and the heat stress caused by them significantly affects the content of organic acids, the titratable acidity and pH of the grapes (Hewitt et al., 2023; Venios et al., 2020). Increasing temperatures and decreasing rainfalls, intensifying grape ripening, lead to a deterioration of the aromatic wine profile (Gutiérrez-Gamboa, 2018). This necessitates the application of various viticultural practices to ensure controlled metabolic development of the vine, improving the yield and quality of its grapes. One of the essential treatments in this aspect is mineral fertilization. Nitrogen has a significant impact on the metabolomics and life cycle of the grapevine, and directly affects the yield, composition and quality of its fruit (Delas et al., 1991; Soubeyrand et al., 2014). Foliar fertilization is an effective strategy for improving crop nutrition when the soil is poor in nutrients and under dry climatic conditions (Lv et al., 2017). It essentially represents spraying of nutrients on the canopy of the crops, which leads to the direct absorption of these substances by the crop leaves and stems (Cooper, 2003). It is actively entering in viticulture, due to the improved and efficient assimilation of nitrogen and mineral components, improving the vitality of the vine and the qualitative composition of grapes and wine (Rubio-Bretón et al., 2018).

Wine, being a product of grape juice fermentation by the yeast belonging to the genus *Saccharomyces*, contains many volatile compounds produced mostly due to the yeast metabolic activity. These compounds includes higher alcohols, aldehydes and ketones, esters, terpenes, lactones, phenols, the amounts of which varied from a few ng/l to hundreds of mg/l (Delgado et al., 2020; Manolache et al., 2018; Tang et al., 2019). However, it was reported that some of volatile compounds present in wine come into wine directly from the grapes (Cordente et al., 2012; González-Barreiro et al., 2015).

Higher alcohols are synthesized by yeasts that metabolize sugars and amino acids (direct precursors for higher alcohols formation) (Bell and Henschke, 2005). The biological synthesis of esters is also associated with yeasts metabolism during alcoholic fermentation. This is a clear indicator that the presence of nitrogen in the must (vitally necessary for the yeasts) is extremely important for the proper course of fermentation and generating aromatic compounds that determine the wine quality (Lacroux et al., 2008; Miller et al., 2007).

In the scientific literature, there are not many studies determining the influence of foliar fertilization directly on the wine's aromatic profile. Trdenić et al. (2020) determined the effect of foliar application of K, B and other microelements on the content of aromatic compounds in grapes of the white variety Škrlet bijeli from Croatia. The team identified the presence of 24 volatile compounds in the three years study. In two of the harvests (2013 and 2014) Trdenić et al. (2020) found high contents of 1-hexanol, linalool, β-damascenone, trans-2-hexenal and geranyl-acetone. Lacroux et al. (2008) found that N and S foliar fertilization of Sauvignon Blanc vines improved the aromatic profile of wines by increasing the volatile thiols and glutathione. On the other hand, Rubio-Bretón et al. (2012) when studying the foliar application of two nitrogen sources – phenylalanine and urea, applied as fertilizers to vines of the red Tempranillo variety, concluded that the studied nitrogen sources did not change the wine aromatic profile. Gutiérrez-Gamboa et al. (2018) investigated the effect of applying different foliar nitrogen fertilizers on Cabernet Sauvignon wines. The team found that the
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urea + sulfur treatments and the application of a commercial BA preparation had a positive effect by increasing the amounts of three very important wine esters – ethyl hexanoate, ethyl octanoate and ethyl decanoate. When treated with urea and arginine, the team found an accumulation of high total levels of terpenes, which are a product of the vine.

The aim of the present study was to determine the influence of different foliar fertilizers on the production of volatile and aromatic compounds in red wines from the Bulgarian hybrid variety Storgozia.

## Materials and methods

#### Variety and plantation

The red hybrid grapevine variety Storgozia grafted on the rootstock Berlandieri x Riparia selection Oppenheim 4 was used in the study. The plantation was located on the territory of the Experimental Base of Institute of Viticulture and Enology (IVE)-Pleven in the area "Tomovskoto". The variety was planted according to a scheme of 1 m intra-row and 2.5 m inter-row distance, in which there were 4000 vines per hectare. They were trained monostemmed and with a single cordon at a height of 1 m. The rows were oriented in the southeast-northwest direction. The exposure had a slight slope to the north. The support structure consisted of one supporting wire 100 cm from the ground, and two pairs for shoots tucking in, respectively 30 and 60 cm above it. The load on the vines was leveled at 20 buds provided through spurs and fruit canes.

#### Experimental variants of foliar fertilization

The Ka-Bor liquid fertilizers (Agro-Bio Trading Ltd., Bulgaria) and the Max Grow series (Maxgrow Chemical Ltd. Bulgaria), recommended for vineyards, were tested. Applied fertilizers differed by their elemental composition. Content of macronutrients – nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) and micronutrients – iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), chlorine (Cl), molybdenum (Mo). The influence of every single nutrient on vine is related with: nitrogen stimulates growth, grape berries size, bunch weight, it is component of enzymes, vitamins and chlorophyll; phosphorus participates in nucleic acids, phosphoric esters, lipids and nucleotides, participates in energy transfer and has a direct effect on the grapes quality and yield; potassium increases the growth of the vine root system, increases the disease resistance and the content of sugars in grapes; sulfur is a component of enzymes and proteins, participates in photosynthesis, increases resistance to diseases; calcium is extremely important for grape berries strength, leaf growth and disease resistance; magnesium participates in photosynthesis, protein synthesis, grapes quality and yield; boron improves the growth of the root system and aerial parts, increases the content of sugars and increases the yield; zinc stimulates general root growth, increases sugar content, berries strength and yield; Mn, Cu, and Fe increase photosynthetic activity and the development of leaf mass.

The scheme of the experiment contained six variants; each with four repetitions of five vines is shown (Table 1).

The soil type was chernozem. The vineries were grown without irrigation. For the purpose of the research, soil fertilization was excluded from the plantation agrotechnics. Foliar feeding was carried out four times and with doses recommended by the manufacturer.

Vines	Fertilizer	Content of elements, %	Amount of fertilizer, ml/ha
V1	Control –	-	-
	untreated		
V2	Ka-Bor	K, 12; Ca, 6; B, 1.5	600
V3	Max Grow	N, 9; P, 9; K, 9; Fe, 0.05; Mn, 0.025;	5000
	999	Cu, 0.01; Zn, 0.01; B, 0.025; Mo, 0.02	
V4	Max Grow	N, 3; P, 20; K, 22; Fe, 0.05; Mn,	4000
	РК	0.025; Cu,0.01; Zn, 0.01; B, 0.025;	
		Mo, 0.002	
V5	Max Grow	N, 20; S, 7; Mg, 14; P, 5; Mn, 1; Zn,	5000
	NS	0.01; Cu, 0.5	
V6	Max Grow	Max Grow 999*, Max Grow PK, Max	Dose for fertilizer
	Co.	Grow NS as 2V3 + V4 + V5	was indicated above

#### Experimental vines variants and doses of fertilizers application

\* Max Grow 999 was taken in double dose

**Vinification.** The study was conducted at the IVE – Pleven. The object of the present study were red wines obtained from four harvests (2019, 2020, 2021 and 2022) of the Storgozia variety. The grapes for each studied variant (30 kg each) were picked and vinified in the IVE Experimental Wine Cellar in the conditions of microvinification, according to the classic scheme for the production of red wines (González-Neves et al., 2013): crushing – destemming – sulphitation (50 mg/kg SO<sub>2</sub>) – alcoholic fermentation with SIHA Rubio Cru yeast *Saccharomyces cerevisiae* from Eaton's Begerow: 20 g of dry yeast per 100 1 at temperature  $28^{\circ}$ C – separation from solids – additional sulphitation – storage.

Gas chromatography-flame ionization detection (GC-FID) of the wine's volatile composition. The content of the main volatile and aromatic compounds was determined based on a stock standard solution prepared in accordance with IS 3752:2005 method. The method describes the preparation of a standard solution of one congener, but the preparation step was followed to prepare a solution of more compounds. The standard solution in the present study included the following compounds (purity > 99.0%): acetaldehyde, ethyl acetate, methanol, 2-propanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 1-butanol, isobutyl acetate, ethyl butyrate, 2-butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, 4-methyl-2-pentanol, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, dimethyl succinate, phenyl acetate, linalool , ethyl caprylate, 2-phenylethanol,  $\alpha$ -terpineol, nerol,  $\beta$ -citronellol, geraniol, ethyl decanoate.

The prepared standard solution containing all compounds was injected in an amount of 2  $\mu$ l into a gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF= 0.25  $\mu$ m), equipped with flame ionization detector (FID). The carrier gas was helium. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manual, using a microsyringe.

The gas chromatographic determination parameters were: injector temperature -220 °C, detector temperature -250 °C, initial oven temperature -35 °C/1 min retention, rise to 55 °C with a step of 2 °C/min for 11 min, rise to 230 °C with a step of 15 °C/min for 3 min. Total chromatography time -25.67 min. After the retention times of the compounds in the

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standard solution were determined, the identification and quantification of the volatile compounds in the wines were conducted. The volatile composition was determined based on the injection of wine distillates. Samples were injected in an amount of 2  $\mu$ l into a gas chromatograph and identification and quantification of volatile compounds was performed.

**Statistical data processing.** Statistical processing of the data was performed, including determination of average content and standard deviation ( $\pm$ SD), with four replications (four harvests) for the main compounds identified in all of the four analized harvests. The statistical data processing was carried out using the Excel 2007 program (Microsoft Corporation, USA).

#### **Results and discussion**

The obtained results of the performed gas-chromatographic analysis of the wines from the respective four harvests (2019, 2020, 2021 and 2022) are presented in Tables 2 - 5.

#### Changes of the wines total volatile content by harvests

The highest total content of volatile compounds in the wines of the 2019 harvest was found in the variant V6. The determined concentration of volatile compounds in this combined variant of foliar fertilization was more than 4 times higher, compared to the untreated control. High levels of total volatile compounds were also reported in variants V5 and V3. Variant V2 showed a lower content of volatile components than the control, and variant V4 demonstrated a slightly lower level than the control. For the total amount of volatile compounds in the wines from the next harvest (2020) a different trend was observed. In this harvest, all experimental wines accumulated a significantly higher amount of volatile compounds, compared to the control. Very high concentration of total volatile compounds was found here in variants V2 and V5. They were respectively more than 24 times and more than 14 times higher compared to the untreated control. In the third harvest (2021), the wine of variant V5 dominated in terms of total volatile compounds concentration. In this harvest, it was noticeable that the levels of volatile compounds in the experimental variants were not significantly higher than those of the control variant. A correlation could be drawn here with the first harvest (2019), where variants V2 and V4 also showed lower total volatile content than the control variant. In contrast, for all three harvests (2019, 2020 and 2021) a high final amount of volatile compounds was observed for variant V5. In the last harvest (2022), only variant V2 showed levels of volatile compounds lower than the control. The highest total content of volatile compounds in this harvest was found in variant V3. The V4 variant also showed high levels.

The results obtained regarding the total content of volatile compounds in the analyzed wines indicated that in three of the harvests – 2019, 2021 and 2022, the V2 variant showed levels of total volatile compounds lower than the control. The most constant in terms of high final content of volatile compounds turned out to be variant V5, which demonstrated final high levels in wines of three from the four investigated harvests (2019, 2020 and 2021). In this variant of used foliar fertilizer, the amounts of nitrogen are the highest. This led to more nitrogen in the grapes and during fermentation has led to the stimulation of the yeasts metabolic action and the secretion of higher amounts of volatiles.

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Table 2

	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Ethyl alcohol, vol.%	13.63	14.25	13.02	13.06	12.56	12.66		
Acetaldehyde	45.81	22.85	52.08	10.39	308.85	511.77		
Methanol	7.33	7.01	35.27	65.74	198.72	115.14		
2-methyl-1-butanol	45.69	3.85	28.16	46.28	110.94	302.46		
3-methyl-1-butanol	102.25	8.39	17.07	96.20	230.08	71.57		
2-phenyl ethanol	nd	24.75	320.65	nd	nd	nd		
1-propanol	nd	nd	22.59	9.00	ND	153.48		
2-propanol	nd	nd	19.92	7.23	304.51	nd		
Total higher alcohols	147.94	36.99	408.39	158.71	645.53	527.51		
Ethyl acetate	22.17	38.55	247.35	6.31	155.36	286.95		
Pentyl acetate	nd	nd	54.13	nd	nd	nd		
Isopentyl acetate	23.48	ND	66.74	31.54	nd	116.86		
Propyl acetate	27.69	12.46	119.69	77.03	nd	nd		
Isopropyl acetate	nd	nd	5.63	1.51	nd	nd		
Ethyl hexanoate	50.58	nd	nd	nd	nd	nd		
Phenyl acetate	nd	nd	85.20	nd	nd	168.59		
Ethyl caprylate	16.62	nd	nd	nd	nd	nd		
Hexyl acetate	28.13	nd	nd	2.67	nd	nd		
Total esters	168.67	51.01	578.74	119.06	155.36	572.40		
Nerol	nd	nd	0.10	0.20	nd	nd		
$\beta$ – citronellol	nd	0.56	nd	0.63	nd	nd		
Geraniol	0.75	nd	0.14	0.12	nd	nd		
Total terpenes	0.75	0.56	0.24	0.95	nd	nd		
Total content	370.50	118.42	1074.72	354.85	1308.46	1726.82		

#### Gas-chromatographic analysis, harvest 2019

\*nd – not detected

\*\*Statistical data (Average ± SD) for four main compounds (acetaldehyde, methanol, 3-methyl-1-butanol and ethyl actate) identified in all harvests analysed is shown in Table 6.

# Table 3

Gas-chromatographic analysis of wines, harvest 2020

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Idout:God commony da	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Ethyl alcohol, vol.%	12.64	12.18	12.30	12.02	12.35	13.01		
Acetaldehyde	8.58	102.44	200.88	119.41	162.63	81.25		
Methanol	9.47	193.44	86.03	166.11	270.62	199.69		
2-methyl-1-butanol	8.69	586.46	134.12	nd	169.13	nd		
3-methyl-1-butanol	18.89	1500.35	137.58	126.60	439.75	66.36		
1-propanol	0.05	0.05	nd	0.05	68.36	nd		
2-propanol	nd	nd	nd	nd	nd	56.26		
1-pentanol	nd	0.05	0.05	nd	nd	nd		
1-heptanol	0.05	nd	nd	nd	nd	nd		
2-phenylethanol	0.05	nd	nd	305.88	nd	0.05		

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Identified compounds	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Total higher and aromatic alcohols	27.73	2086.91	271.75	432.53	677.24	122.67		
Ethyl acetate	47.78	72.30	174.94	263.15	212.37	205.71		
Pentyl acetate	nd	nd	0.05	0.05	nd	nd		
Isopentyl acetate	6.03	86.35	119.10	109.13	167.90	72.68		
Propyl acetate	9.37	199.35	nd	nd	138.97	nd		
Isopropyl acetate	nd	0.05	0.05	0.05	0.05	0.05		
Phenyl acetate	nd	nd	nd	nd	0.05	nd		
Ethyl caprylate	nd	nd	nd	nd	0.05	0.05		
Total esters	63.18	358.05	294.14	372.38	519.39	278.49		
Linalool oxide	0.32	nd	nd	0.05	nd	nd		
$\alpha$ – terpineol	0.05	nd	nd	nd	nd	0.05		
Nerol	0.05	nd	nd	0.65	nd	0.74		
$\beta$ – citronellol	0.05	0.05	0.14	nd	0.05	0.05		
Geraniol	0.53	0.69	nd	0.41	0.10	0.23		
Total terpenes	1.00	0.74	0.14	1.11	0.15	1.07		
Total content	109.96	2741.58	852.94	1091.54	1630.03	683.17		

\*nd - not detected

\*\*Statistical data (Average  $\pm$  SD) for four main compounds (acetaldehyde, methanol, 3-methyl-1-butanol and ethyl actate) identified in all harvests analysed is shown in Table 6.

Table 4

Gas-chromatographic	analysis	of wines.	harvest 2021
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Identified compounds	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Ethyl alcohol, vol.%	11.69	12.11	12.36	12.10	10.83	15.44		
Acetaldehyde	13.92	13.45	8.77	67.26	27.56	19.33		
Methanol	64.95	79.20	50.08	33.00	173.43	173.35		
2-methyl-1-butanol	36.45	19.66	19.28	13.63	30.45	21.35		
3-methyl-1-butanol	62.91	35.31	33.88	38.85	41.83	36.54		
2-propanol	nd	nd	13.35	9.74	nd	nd		
1-pentanol	nd	nd	18.79	nd	21.43	nd		
2-butanol	ND	24.32	nd	nd	18.12	31.77		
1-heptanol	43.20	nd	76.02	nd	nd	nd		
2-phenylethanol	193.93	70.17	257.14	nd	nd	nd		
Total higher and aromatic	336.49	149.46	418.46	62.22	111.83	89.66		
alcohols								
Ethyl acetate	58.82	27.27	20.04	18.18	71.06	58.71		
Pentyl acetate	nd	nd	nd	nd	39.78	nd		
Isopentyl acetate	5.22	nd	nd	6.30	30.98	6.91		
Propyl acetate	nd	nd	nd	14.68	nd	nd		
Isopropyl acetate	nd	6.52	7.98	11.41	20.03	7.67		
Phenyl acetate	nd	29.41	nd	nd	38.68	9.48		

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Table 4 (Continue)

Table 5

Identified compounds	Content in wines, mg/l, from vines						
Identified compounds	V1	V2	V3	V4	V5	V6	
Ethyl hexanoate	nd	nd	nd	nd	112.74	nd	
Total esters	64.04	63.20	28.02	50.57	313.27	82.77	
Linalool oxide	0.42	nd	0.43	0.96	nd	nd	
$\alpha$ – terpineol	nd	0.15	0.20	0.55	nd	nd	
Nerol	nd	0.10	nd	nd	nd	nd	
$\beta$ – citronellol	0.12	0.24	0.33	0.15	nd	0.12	
Geraniol	0.29	nd	0.15	nd	0.94	0.17	
Total terpenes	0.83	0.49	1.11	1.66	0.94	0.29	
Total content	480.23	305.80	506.44	214.71	627.03	365.40	

\*nd - not detected

\*\*Statistical data (Average ± SD) for four main compounds (acetaldehyde, methanol,

3-methyl-1-butanol and ethyl actate) identified in all harvests analysed is presented in Table 6.

T.J	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Ethyl alcohol, vol.%	13.15	12.77	11.59	11.90	12.53	12.76		
Acetaldehyde	68.44	46.98	69.02	67.22	103.75	7.83		
Methanol	7.64	2.07	18.26	8.00	8.02	4.14		
2-methyl-1-butanol	22.26	13.41	16.21	12.07	6.61	12.99		
3-methyl-1-butanol	26.09	45.53	90.81	52.78	43.61	94.34		
4-methyl-2-pentanol	nd	nd	nd	nd	2.57	nd		
1-propanol	4.84	2.49	nd	12.31	3.05	3.49		
2-propanol	nd	nd	2.18	nd	nd	nd		
1-pentanol	nd	nd	nd	nd	4.36	nd		
1-butanol	21.44	nd	nd	112.75	nd	44.19		
2-butanol	nd	nd	139.39	5.89	nd	nd		
Isobutanol	nd	nd	nd	1.55	nd	nd		
1-heptanol	15.81	8.13	nd	nd	nd	nd		
1-hexanol	nd	12.97	nd	nd	nd	nd		
2-phenylethanol	55.11	nd	nd	60.86	165.28	40.65		
Total higher and	145.55	82.53	248.59	258.21	225.48	195.66		
aromatic alcohols								
Ethyl acetate	21.68	4.96	106.55	31.91	15.18	29.77		
Pentyl acetate	13.79	14.18	7.81	nd	nd	nd		
Diethyl succinate	nd	nd	49.85	ND	18.97	nd		
Propyl acetate	nd	nd	1.69	nd	nd	nd		
Isopropyl acetate	nd	nd	nd	10.44	nd	nd		
Butyl acetate	nd	nd	12.60	nd	1.79	nd		
Isobutyl acetate	36.73	nd	nd	nd	102.56	nd		

# Gas-chromatographic analysis of wines, harvest 2022

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#### Table 5 (Continue)

Idont: Cod commons do	Content in wines, mg/l, from vines							
Identified compounds	V1	V2	V3	V4	V5	V6		
Phenyl acetate	nd	nd	72.39	220.26	nd	nd		
Ethyl hexanoate	nd	nd	182.85	78.21	nd	46.87		
Ethyl butyrate	nd	nd	70.76	nd	nd	nd		
Ethyl caprylate	23.59	173.25	nd	nd	nd	nd		
Hexyl acetate	nd	nd	153.84	nd	nd	90.27		
Ethyl decanoate	23.43	nd	nd	nd	nd	nd		
Total esters	119.22	192.39	656.65	340.82	138.50	166.91		
Linalool oxide	nd	nd	nd	nd	nd	0.36		
Linalool	0.11	0.64	nd	nd	0.16	0.86		
$\beta$ – citronellol	0.19	nd	nd	nd	nd	nd		
Geraniol	nd	0.16	0.82	0.14	nd	nd		
Total terpenes	0.30	0.80	0.82	0.14	0.16	1.22		
Total content	341.15	324.77	993.34	674.39	475.91	375.76		

\*nd - not detected

\*\*Statistical data (Average  $\pm$  SD) for four main compounds (acetaldehyde, methanol, 3-methyl-1-butanol and ethyl actate) identified in all harvests analysed is shown in Table 6.

# Changes of total and individual content of higher and aromatic alcohols in the analyzed wines by harvests.

Higher alcohols are a product of yeast amino acid metabolism. In the present study, the analysis of their total and individual concentrations was directly related to the presence of free nitrogen and amino acids in the grapes of the vine, which were directly affected by the applied foliar fertilizers. The concentration of higher alcohols in red wines can reach 600.00 mg/l (Chobanova, 2012).

In Storgozia wines from the 2019 harvest, only variant V2 showed total levels of higher alcohols significantly lower than the control. The highest content of higher alcohols was found in variants V5 and V6, which was respectively 4.36 and 3.56 times higher than that generated in the control wine.V3 variant also showed high final levels of higher alcohols. In the next harvest (2020), in all experimental wine variants, significantly higher quantitative levels of higher alcohols were obtained, comparing them with the control variant. Very high final level in variant V2 was obtained – 75 times higher than that found in the control variant. High final levels of higher alcohols in this harvest were also found in the variants V5 and V4. They were respectively 24 and 15 times higher than the control. In the wines of the 2021 harvest, however, the trends were significantly different. Almost all experimental variants in this harvest showed total higher alcohol levels lower than the control. Only variant V3 accumulated more in comparison with the control. In the last harvest, only variant V2 accumulated a lower amount of total higher alcohols than the control. The highest levels of higher alcohols in this year had the wines of variants V4, V3 and V5.

The different foliar fertilizers had a positive effect on the accumulation of higher alcohols in three of the four investigated harvests. Significant amounts of these wine aroma components were established. Variant V5 showed consistent accumulation of high amounts

higher alcohols in three of the four harvests examined -2019, 2020 and 2022, and variant V4 showed high levels in two of the harvests examined -2020 and 2021. Moreover, since the formation of higher alcohols by yeasts during alcoholic fermentation is directly influenced by the accumulation of assimilable nitrogen in the must, the positive influence on the higher alcohols yeasts production based on the application of foliar fertilization of vines from the hybrid variety Storgozia could be outlined.

As individual representatives from the group of higher alcohols, 2-methyl-1-butanol and 3-methyl-1-butanol dominated in all four harvests. The two alcohols are the main metabolites of the yeast microflora. Only two of the experimental variants of the investigated wines from the 2019 harvest showed a significantly higher amount of 2-methyl-1-butanol -V5 and V6, compared to the control variant. Variant V4 showed a very close (almost comparable) concentration of this compound to the control, while the other two variants -V2 and V3 showed lower levels of this higher alcohol and in the V2 variant they were very low. The trend of the presence of 2-methyl-1-butanol in the next harvest (2020) was not identical. In this particular harvest, high levels of this higher alcohol were found in variant V2, exceeding those found in the control. In the wines of the 2021 harvest, a trend towards lower levels of 2-methyl-1-butanol was found in the experimental variants, compared to the control. Thus, in the experimental wines of this harvest, the content of 2-methyl-1-butanol was lower than the control V1. In the last harvest (2022), the trend in the content of 2-methyl-1-butanol was analogous to the previous one. The level found in the control sample V1 dominated those of the experimental wine variants. The influence of this higher alcohol on the wine aroma has been associated with fruity nuances (Carpena et al., 2021). Analyzing the results for this individual component (given the results obtained in the more significant part of the experimental variants from the four harvests) of the higher alcohols fraction, it could be concluded that the application of the different foliar fertilizers did not affect the content of 2-methyl-1-butanol, because the results indicated that in the most cases the untreated control showed higher levels of this component. The obtained results correlated with the data of Rubio-Bretón et al. (2018), who applied foliar different nitrogen fertilizers to vines of the red Tempranillo variety and found that wines obtained from grapes of untreated vines showed a higher content of 2-methyl-1-butanol, compared to treated ones. Gutiérrez-Gamboa et al. (2018) in a study of foliar fertilization of Cabernet Sauvignon found that wines from vines treated with Arg, Ur and commercial preparations - NT and BA accumulated a smaller amount of 2-methyl-1-butanol than the untreated control variant. These data are also consistent with what was found in our study.

Regarding the content of 3-methyl-1-butanol in the wines of the 2019 harvest, a higher content than the control was found only in the variant V5. All other experimental variants showed levels of this alcohol lower than the control, and in variant V2 and V3 they were very low. In the next harvest (2020), however, the trend was reversed. The lowest levels of this higher alcohol were found in control V1. All experimental variants showed higher level of 3-methyl-1-butanol, and in variant V2 it was very high. Experimental variant V5 also showed a much higher concentration than the control. The trend in the 2021 harvest was the same as in 2020. The detected amounts of 3-methyl-1-butanol in the experimental wines were lower than that found in the lowest concentration in wine of the control variant V1. In the experimental wines, its concentration was higher. The data in two of the studied harvests (2019 and 2021) correlated with the results of Rubio- Bretón et al. (2018), who found that the concentration of 3-methyl-1-butanol in Tempranillo red wines, under foliar treatment with nitrogen fertilizers, was lower in the experimental variants than in the control. 3-methyl-

1-butanol is a higher alcohol that has a strong aromatic fruity influence on wine (Cameleyre et al., 2015; Cometto-Muñiz and Abraham, 2008).

This reduction in the two major higher alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) was also been demonstrated with soil nitrogen fertilization (Webster et al., 1993). The data on the content of 3-methyl-1-butanol in the 2020 and 2022 harvests were correlated with data obtained by Gutiérez-Gamboa et al. (2018) who applied foliar fertilization with different nitrogen sources to Cabernet Sauvignon vines. In the wines of the Urea + Sulfur treatment variants, they proved a higher concentration of 3-methyl-1-butanol and 2-methyl-1-butanol.

2-phenylethanol was the only aromatic alcohol that was identified in the studied wines. In the wine of the 2019 harvest, it was found only in two of the experimental variants – V2 and V3. In the wines from the next harvest (2020), it was identified in traces in the control variant V1 and variant V6. A high amount of 2-phenyl ethanol was found in the wine of variant V4 from this harvest. In the wines of the 2021 harvest, 2-phenylethanol was identified in three of the variants – V1, V2 and V3. In the last harvest (2022), this aromatic alcohol was found in four of the variants – V1, V4, V5 and V6. This aromatic alcohol has a strong influence on the wines aroma, giving a rose aroma, and its concentrations vary widely – on average 10.00 - 150.00 mg/l (Chobanova, 2012).

1-propanol was identified in three experimental wine variants from the 2019 harvest – V3, V4 and V6. Its concentrations between these variants varied from 9.00 mg/l to 153.48 mg/l. In the wines of the next harvest (2020), it was identified in four variants – V1, V2, V4 and V5, and in the first three, it was in very low amounts (traces). In the wines of the 2021 harvest, 1-propanol was not identified. In the last harvest (2022), this compound was identified in almost all variants examined, except for V3. In the wines of this harvest, its concentrations ranged from 2.49 mg/l to 12.31 mg/l. 1-propanol is a compound that imparts alcoholic-bitter notes in wine aroma (Zhang et al., 2015).

Other higher alcohols identified in individual samples between harvests were 2-propanol, 1-pentanol, 2-butanol, 1-heptanol, 1-hexanol, isobutanol.

The changes of the aldehyde content in the analyzed wines by harvests. Acetaldehyde was identified in all analyzed wines by variants and harvests. This compound is the main dominant representative of the wine aldehyde fraction.

In the wines of the 2019 harvest, only the variants V2 and V4 showed levels of this aldehyde lower than that found in the control variant V1. The rest of the experimental wines demonstrated higher aldehyde concentrations, and in the variants V5 and V6 they are very high, 308.85 mg/l and 511.77 mg/l, respectively. Analyzing the results of the next harvest (2020), it was found that all experimental wines accumulated acetaldehyde levels higher than that found in the control. The variation in the presence of aldehyde between the experimental wines ranged from 81.25 mg/l (V6) to 200.88 mg/l (V3), compared to 8.58 mg/l in the control V1. The trend in the next harvest (2021) was almost similar. Of all the analyzed experimental wines, only variants V2 and V3 showed aldehyde levels lower than the control V1, and even the concentration in variant V2 was marginally lower than the control. In the remaining variants of this harvest, the aldehyde was present in higher concentrations than the control, varying between samples from 19.33 mg/l to 67.26 mg/l. In the last harvest (2022), a lower concentration of acetaldehyde than the control was found in variants V2, V6 and V4. Variant V3 showed a concentration slightly higher, but almost comparable to the control. Of all the examined wines of this harvest, only variant V5 showed a distinctly higher aldehyde content than the control.

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Acetaldehyde is a compound with dual nature with respect to the dry wines aroma. At concentrations up to 130.00 mg/l it gives a fruity character to the wine, while at higher concentrations it causes an oxidized tone in the aroma (Ribéreau-Gayon et al., 2000; Noble et al., 1987). In almost all the dry red wines examined in the present study, the aldehyde was identified in concentrations positively influencing the aromatic quality. The only exceptions were variants V5 and V6 from the 2019 harvest (where high aldehyde levels were found) and variants V3 and V5 from the 2020 harvest.

The conducted research indicated that the application of different foliar fertilizers did not negatively affect the acetaldehyde content in the resulting wines. Its presence corresponded to the appearance of its positive influence on the wine's aromatic profile.

The changes of total and individual content of esters in the analyzed wines by harvests. The ester fraction has an important and strong impact on the wine aromatic profile. The total amount of esters in young wines varies from 200.00 to 400.00 mg/l (Chobanova, 2012).

In the wines of the 2019 harvest, only two variants showed significantly higher than the control level of total esters – variant V3 and V6. All other variants accumulated less esters than the control wine. In the next harvest (2020), the trend was different. In it, all experimental variants showed levels of total esters significantly exceeding that found in the control. In the 2021 harvest, three of the variants (V2, V5 and V6) accumulated higher amounts of esters than the control, with the highest concentration of esters found in variant V5. The last researched harvest (2022) showed a similar trend to that found in the 2020 harvest. The amount of esters accumulated in the control was the lowest, compared to the experimental variants, where it varied from 138.50 to 656.65 mg/l.

From the obtained results, it could be concluded that in the 2020 and 2022 harvests, the foliar application of nitrogen and mineral sources affected the content of esters. In the other two harvests, individual variants showed higher ester accumulation than the untreated control, but others demonstrated lower levels. As a result, no specific conclusion could be made about the influence of foliar fertilizers on the final wines ester concentrations. Further studies are needed to determine how fertilization affects the metabolic accumulation of esters in grapes and their biosynthesis by yeasts during fermentation.

A major ester from this fraction was ethyl acetate. In the 2019 harvest, it was found in a lower concentration than the control only in the wine of variant V4. All other variants showed higher levels, ranged from 38.55 to 286.95 mg/l. In the next harvest (2020), higher content of this ester was noticed in the experimental wines, compared to the control. In the 2021 harvest, only variant V5 showed ethyl acetate levels higher than the control. In the other experimental variants, the ester varied from 18.18 to 58.71 mg/l. In the last harvest (2022), two of the variants – V2 and V5 accumulated less ethyl acetate, compared to the control. In the other variants, its quantities were higher.

Ethyl acetate has a dual effect on the wine's aromatic quality and it is synthesized normally in young wines biologically by the yeast microflora. In concentrations up to 80.00 - 100.00 mg/l, it positively affects the wine aroma; above them, the wine acquires a characteristic unpleasant acetic-acidic and chemical nuance in the aroma (Plata et al., 2003). Based on this, it was clear from the obtained results that in the 2019 harvest, variants V3, V5 and V6 accumulated ester in high levels (155.36 mg/l - 286.95 mg/l), over the upper limit of its positive influence. In the 2020 harvest, variants V3, V4, V5 and V6 also demonstrated high ester levels ranging from 174.94 mg/l to 263.15 mg/l. It was noteworthy that in both harvests the wines obtained from variants with applied foliar fertilization with the presence of Mg in the fertilizer accumulated high levels of ethyl acetate. The last two harvests (2021)

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and 2022) showed normal ester levels, which in the first harvest ranged from 18.18 mg/l to 71.06 mg/l, and in the second from 4.96 mg/l to 106.55 mg/l. Within these concentrations, it had a positive effect on the wine's aromatic quality.

Two other esters with a more substantial presence in the analyzed wines were propyl acetate and isopentyl acetate. In the wines of the 2019 harvest, propyl acetate was identified in four of the studied variants (V1 – V4). Its levels varied from 12.46 mg/l to 119.69 mg/l. In the next harvest (2020), the ester was found in three variants – V1, V2 and V5, with variations from 9.37 mg/l to 199.35 mg/l. In the wines of the 2021 harvest, propyl acetate was identified only in the V4 variant. In the last harvest (2022), this ester was identified again in only one variant – V3.

Isopentyl acetate in the 2019 harvest was identified in three of the experimental variants – V1, V3 and V4. Its amounts ranged from 23.48 mg/l to 66.74 mg/l. The lowest concentration was found in the control. In the next harvest (2020) it was identified in all studied variants, making the impression that in the control V1 it was present in a lower concentration (6.03 mg/l) compared to the experimental wines, where it varied from 72.68 mg/l to 167.90 mg/l. In the wines of the 2021 harvest, isopentyl acetate was found in four experimental variants – V1, V4, V5 and V6, and again, as in the other two harvests, its concentration in the control sample V1 was lower compared to the experimental variants. In the last harvest (2022), isopentyl acetate was not identified. Isopentyl acetate, like ethyl acetate, is a key compound for the wine aroma profile (Plata et al., 2003). From the results in the present study, it was evident that the application of foliar fertilizers affects the synthesis of this ester, leading to an increase in its concentrations in the experimental wines. Such a tendency was also observed for other ethyl esters. The data correlated with studies of Martinez-Gil et al. (2012), who reported a relationship between the ethyl esters formed in wines and the must nitrogen content.

Pentyl acetate, ethyl hexanoate, phenyl acetate, ethyl caprylate, hexyl acetate, ethyl butyrate, butyl acetate, diethyl succinate, isobutyl acetate and ethyl decanoate were also identified in the wines by harvests in separate variants.

#### Changes of total and individual terpene content in the analyzed wines by harvests

The terpene fraction has an important, fundamental relation to the aroma of wines from muscat varieties and formes so-called muscat aroma. Storgozia variety investigated in the present study is non-muscat. However, terpenes, as a product of the vine, exert an aromatic influence on the wines and the study of their presence is an important indicator of the complex wine aroma.

Regarding the total amount of terpenes identified in the wines of the 2019 harvest, only variant V4 accumulated an amount higher than the control. Terpenes were not identified in variants V5 and V6, and the other two variants showed a total terpene concentration lower than control. In the next harvest (2020), variant V4 showed again total terpene levels higher than control (1.00 mg/l). Variant V6 also accumulated more terpenes. All other experimental variants were characterized by terpene levels lower than control. In the wines of the 2021 harvest, variants V3, V4 and V5 accumulated amounts of total terpenes ranging from 0.94 mg/l to 1.66 mg/l, higher than control. In this harvest as well, it was noticeable that variant V4 accumulated a high amount of terpenes, as in previous two harvests. In the last studied harvest (2022), higher total terpenes were found in variants V2, V3 and V6, ranging from 0.80 mg/l to 1.22 mg/l, comparing them with the control level for this harvest (0.30 mg/l). According to Chobanova (2012), the average content of total terpenes in wine is about 2.00 mg/l. The data in the present study were in absolute correlation with this concentration range.

The data also correlated with the results of Vilanova et al. (2007) who investigated Albariño wines from northern and southern Spain. They found a concentration of total free terpenes in northern wines of 1757.15  $\mu$ g/L<sup>-1</sup> (1.75 mg/l) and in the south – lower, respectively 458.99  $\mu$ g/L<sup>-1</sup> (0.45 mg/l).

From the obtained results, it could not be concluded that the foliar fertilizers applied to the vines have a constant effect on increasing the concentration of total terpenes in the wines obtained from them.

Geraniol was found as the representative species of terpenes in the 2019 harvest in most of the tested variants (three of them). It was noteworthy that in the control its concentrations were higher compared to the other two experimental variants - V3 and V4. In the next harvest (2020) a dominant presence was reported, in addition to geraniol, and also  $\beta$ -citronellol. The latter was found in almost all samples (except variant V4), but only in the wine of variant V3 it was in a higher amount. In the remaining variants (including control) it was present in low concentrations. Geraniol, on the other hand, in this harvest was also identified in almost all the wines examined (with the exception of variant V3). It was noteworthy that of all the experimental variants, only variant V2 showed levels of this terpene higher than the control. All other variants contained it in lower amounts. For the 2021 harvest, the situation was identical. Again, the dominant terpenes were ß-citronellol and geraniol. ß-citronellol was identified in almost all the wines analyzed (except variant V5). Its concentration in the experimental wines was comparable in one variant (V6) and higher (0.12 mg/l - 0.33 mg/l)than the control V1 (0.12 mg/l). Geraniol in this harvest was identified in variants V1, V3, V5 and V6 and only variant V5 showed levels higher than the control. The last harvest (2022) was distinguished by a dominant presence of linalool and geraniol. Linalool was identified in four of the investigated wines - V1, V2, V5 and V6. Its concentration in the control wine was lower than that found in the experimental variants. Geraniol was identified in three of the investigated variants - V2, V3 and V4, where it varied from 0.14 mg/l to 0.82 mg/l.

It looks that foliar fertilization affects the synthesis of ß-citronellol, leading to increase levels of this terpene in the experimental wines. Conversely, for geraniol, a decrease in its concentrations was observed in the wines obtained after the application of foliar fertilization, compared to the untreated ones.

#### Changes in the amounts of methanol in the analyzed wines

Methyl alcohol is a normally present component of wines that is produced from fruit pectin by pectinesterase enzymatic degradation, and in red wines, it is accumulated to a maximum of 350.00 mg/l (Chobanova, 2012). It was identified in all examined wines from the four harvests. In the wines of the 2019 harvest, only variant V2 showed methanol levels slightly lower than the control. All other variants showed higher levels of this component, ranging from 35.27 mg/l to 198.72 mg/l. In the next harvest (2020), the trend was maintained. Control V1 accumulated an amount of methyl alcohol, which was lower than that found in all experimental variants. In the wines of the 2021 harvest, lower methanol levels than the control were found in two of the experimental variants – V3 and V4. The rest showed a methanol content higher than the control. In the last harvest (2022), only variants V2 and V6 contained this component in amounts lower than the control. The tendency for the remaining variants was towards a higher accumulation, which varied from 8.00 mg/l to 18.26 mg/l.

In all wines, in all four harvests, normal levels of methanol, inherent in red wines, were found. It was noteworthy, however, that most experimental variants accumulated higher levels of this component compared to the untreated controls. It is likely that foliar fertilization

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accelerates the metabolism of the vine and has the effect of increasing the pectin content of the fruit, which is a factor for the final wines methanol levels.

## Statistical data analysis of the main compounds identified in all four harvests

The statistical data is presented on table 6. Statistical processing of the data was performed, including the determination of the average values and standard deviation of four compounds (acetaldehyde, methanol, 3-methyl-1-butanol and ethyl acetate) that were identified practically in all studied variants and in the four harvests. The four replicates were taken from each single concentration of the compound found in a particular harvest. The results processed in this way can give an idea of whether these compounds show high variability considering the many factors that influence their presence in the wine – climatic conditions of the year, soils, agrotechnical measures and others. Acetaldehyde showed a very high standard deviation for variants V3, V5 and V6. In the V6 variant, it even surpassed the average value. This is an indicator that led to the conclusion that the content of this aldehyde in these variants was influenced by various factors and can vary significantly between different harvests. For methanol, a distinctly high standard deviation was observed for variants V2 and V4. And here it could be concluded that these variants showed a high variability in the concentration of methanol, which could vary significantly between harvests. The trend was similar for variants V2 and V5 for 3-methyl-1-butanol and V4 for ethyl acetate (basic wine ester).

Table 6

Compounds,	Content in wines, mg/l, from vines							
mg/l	V1	V2	V3	V4	V5	V6		
Acetaldehyde	34.18	46.43	82.68	66.07	150.69	155.04		
	$\pm 28.13$	$\pm 39.92$	$\pm 82.77$	$\pm 44.52$	$\pm 119.05$	$\pm 239.99$		
Mathanal	22.25	70.43	47.41	68.21	162.69	123.08		
Wiethanoi	$\pm 28.23$	$\pm 89.26$	$\pm 28.84$	$\pm 69.41$	$111.03\pm$	$\pm 86.80$		
2 mathul 1 hutanal	52.53	397.35	69.83	78.60	188.81	67.20		
3-methyl-1-butanol	$\pm 38.34$	$\pm 735.47$	$\pm 55.09$	$\pm 40.25$	$\pm 189.17$	$\pm 23.77$		
Ethyl acetate	37.36	35.77	137.22	79.88	113.49	145.28		
	$\pm 18.94$	$\pm 28.06$	$\pm 96.99$	$\pm 122.62$	$\pm 87.55$	$\pm 121.87$		

Statistical data (average  $\pm$  SD) of main compounds for four harvests analyzed

# Conclusions

- It was proved that the variant of vine treatment with fertilizer MaxGrow NS, demonstrated final high levels of volatile and aroma compounds in wines of three from the four investigated harvests (2019, 2020 and 2021).
- A positive influence of foliar fertilization on the content of higher alcohols was outlined. This was due to the likely higher amounts of assimilable nitrogen in the must, which directly increased the final higher alcohol content of the analyzed wines.
- Dominant representatives of the higher alcohols fraction were 2-methyl-1-butanol and 3-methyl-1-butanol. The application of foliar fertilizers did not affect an increase in the content of 2-methyl-1-butanol in wines. In most cases, the untreated control showed

higher levels of this higher alcohol than the experimental variants. No increasing trend could be outlined in the content of 3-methyl-1-butanol either. In two of the harvests (2019 and 2022) this higher alcohol was present in lower amounts than the control. In the other two harvests (2020 and 2021) the trend was reversed and it dominated quantitatively in the experimental wines compared to their controls.

- A single aromatic alcohol was identified 2-phenyl ethanol. 1-propanol was also found to be a major component of the higher alcohols fraction.
- From the group of aldehydes, its main representative acetaldehyde was present in the volatile fraction. The different foliar fertilizers did not have negative effect on the acetaldehyde secretion.
- No specific conclusion can be drawn about the influence of foliar fertilizers on the final total ester concentrations of the wines. Further studies related to the influence of fertilization on the metabolic accumulation of esters in grapes and their biosynthesis by the yeasts during fermentation are needed.
- Ethyl acetate was the main one, followed with propyl acetate and isopentyl acetate. The application of foliar fertilizers affected the accumulation of isopentyl acetate, leading to an increase in its concentrations in the experimental wines.
- The obtained results indicated that foliar fertilizers did not consistently affect increased total terpene content in the wines, but foliar fertilization affected the concentrations of β-citronellol, leading to an increase in the levels of this terpene in the experimental wines. A decrease of concentration of another terpene geraniol in the experimental wines (treated with foliar fertilizers) compared to the untreated control was observed.
- Normal levels of methanol inherent in red wines were found. Most experimental variants accumulated higher levels of this component compared to the untreated controls. It is likely that foliar fertilization accelerates the metabolism of the vine and had the effect of increasing the pectin content of the fruit, which is a main factor for the final wines methanol levels.

The application of foliar fertilizers led to changes in contents of volatile compounds in the researched wines.

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# Ground beef waste in Mexican households

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**DOI:** 10.24263/2304-974X-2023-12-3-6 Abstract Introduction. Household food waste is a primary worldwide concern; however, in developing countries, quantitative information on this matter is scarce. This study aimed to provide baseline information on consumer behaviour, practices, beliefs, and attitudes associated with ground beef waste in Mexican households.

**Materials and methods.** Field data came from a survey of 740 consumers from two major Mexican cities. A survey was administered to consumers willing to answer, and they had five fixed options to select from in the questions related to reasons for awareness and for disposing of minced beef at home. Statistical analysis was performed using Chi-square.

Results and discussion. Consumers were asked to choose from the survey the option closest to their opinion. The results indicate that more than 75% of the respondents were households with 3-5 members, comprising mostly children  $\leq 10$  years old and a smaller proportion of people aged 70 and over. Only 33% buy ground meat packaged and displayed in open refrigerated cabinets in supermarkets, while the rest, 67%, purchase it from local butchers. The best-before date was considered an important parameter as it reports the level of freshness of the meat and its positive impact on health. In contrast, for >50% of respondents, meat waste is associated with cleaning activities. Discarding or throwing away ground meat in Mexican households can occur within a few days of purchase, and can also be related to inadequate preservation and refrigeration practices. Major reasons for consumer awareness of household food waste were associated with guilt feelings (73.0%) and damage to the environment (71.6%), whilst rotten meat and leftovers not eaten were linked to discarding ground meat. Household refrigerator malfunctions and buying in excess occasionally influenced perishable food waste. There are significant relationships between food wastage and various activities, such as purchasing practices, preparation methods, attitudes, and lifestyles associated with eating habits. Reasons that could be related to wastage are prolonged storage of raw meat, finished of the expiry date and disgust. In addition, other related aspects focus on compulsive buying, coupled with a need for more planning and prioritizing the purchase of large packages.

**Conclusion**. Any program related to reducing minced meat waste in households must include the design of governmental and non-governmental policies that impact all links in the supply chain, especially on the final consumer.

# Introduction

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At least one-third of the world's food production is lost as food waste (Vilariño et al., 2017); so then to feed the growing human population, an increase in food production is not enough, but a significant reduction in food waste has to be addressed as an essential topic in academia. Food discard has been pointed out as a threat to food security, economic losses, increase in carbon footprint, soil erosion, salinization and nutrient depletion and air and water pollution (Buzby et al., 2014; Grizzetti et al., 2013;United Nations, Department of Economic and Social Affairs, 2017). Food waste and loss impact the entire food chain (Mokrane et al., 2023); furthermore, it is essential that links of specific food chains be determined and protocols to avoid or reduce them validated (Elimelech et al., 2018; Parfitt et al., 2010). Households in high-income countries, the last common link in the food chain, have been identified as the link with the highest proportion of food waste (Kummu et al., 2012). In contrast, (FAO, 2011) reports that at the consumer level, food waste is much lower in low-income countries.

Richter and Bokelmann (2018) stated that many consumers do not see food waste as an economic loss or as a negative environmental impact issue; however, a proportion of consumers are aware of food-borne diseases and the negative impacts of food waste on their economy and the increasing damages on the environment (Principato et al., 2021). Consumer experiences and broader distribution of information on food waste impacts might be sources of consumers' decisions on frequency and amount of food purchasing to avoid food waste at home (Farr-Wharton et al., 2014). In this case, the theory of planned consumer behaviour is proposed as a basis for explaining food waste are mostly from primary production and immediate post-harvest links to consumers' homes. Consequently, it leads to the relevance of studying food losses and wastes along all food chain links in different countries (Kummu et al., 2012).

Focusing on household food waste Block et al. (2016) suggested that factors associated with food waste should be grouped into time-purchase, storage, preparation and consumption. In addition to time-grouping, factors related to household food waste should be classified into social, economic, and environmental concerns (Hebrok and Boks, 2017; Dobernig and Schanes, 2019). Household food waste is produced and processed for human consumption; however, once at home, the food is not consumed by humans but thrown away, given to pets, or sent to composting processes (Stancu et al., 2016). It is suggested that bestbefore dates, over-preparation, food presentation and food preferences directly impact food waste (Mokrane et al., 2023). Another relevant factor is that single people are the ones who waste the most significant amounts of food (Silvennoinen et al., 2014). Additionally, the decision on food destination at home, either human consumption or waste depends on the consumer (Kranert et al., 2012); then, if household food waste is to be reduced or avoided, depth studies should be done on consumers' factors or drivers to discard food (Jörissen et al., 2015). Ananda et al. (2023) argue that households' repetitive and routine patterns of food management, which include the activities related to checking stocks before shopping and meal planning schedules, create a series of habits that directly affect the amount of food waste. Another problem associated with food waste is related consumers' religious values. If religious norms, for example those rules involved in food consumption and fasting are restrictive, they lead to significant food waste (Minton et al., 2020).

The study presented by Schanes et al. (2018) concluded that household food waste reduction starts with prevention, then studies should be addressed to identify household factors drivers of food waste. Among such components there are: household size, with the

largest ones wasting more food (Jörissen et al., 2015); older householders trended to show a lower amount of food waste (Quested et al., 2011); high-income families with children showed more significant amounts of food waste (McCarthy and Liu, 2017; Parfitt et al., 2010); religious beliefs (Abdelradi, 2018); traditional servings at home celebrations (Fajans, 2006; Porpino et al., 2016); and, buying food above the needs (Quested et al., 2011; Stancu and Lähteenmäki, 2022). Some food waste comes from leftovers that are kept under refrigeration for up to four days and then thrown away (Halloran et al., 2014; Stancu et al., 2016). Sustainable food systems depend not only on the production and processing links along the food chain; the reduction and elimination of food waste by final consumers is also an essential link toward this goal (Matzembacher et al., 2020). Therefore, it is crucial to identify in-home food waste drivers and barriers to reducing or eliminating them (Wakefield and Axon, 2020).

The highest proportion of food waste is reported for perishable products such as vegetables and fruits, meat, and fish (FAO, 2019). Ground beef goes through intensive and elaborate food processing and handling without a peel, skin, or any other external protection; then, high hygienic standards, cold chain maintenance and proper packaging should be provided at processing time. The responsibility of the consumer at home when handling ground meat is to apply good preservation practices, avoid overcooking and home storage beyond the "best before" date (Halloran et al., 2014; Milne, 2012; Stefan et al., 2013; Wakefield and Axon, 2020). Consequently, the aim of the study is to provide baseline information on conditions and drivers to ground beef waste in some Mexican households from field research based on the application of a questionnaire to consumers that buy this meat product.

# **Materials and methods**

A survey of ground beef consumers was conducted in two cities in Mexico. At the core of the questionnaire was a set of 14 reasons for awareness and for discarding ground beef at home. The consumer was asked to select one of five options for each cause. The questionnaire was applied through face-to-face questions, and the respondents were approached when leaving the supermarkets. Sampling was non-probabilistic of convenience (Kalton, 2021) since the respondents were those willing to provide all the answers to the questions. Data analysed were the proportion expressed as a percent of respondents in each option within each question/reason. Statistical analysis of ratios within each question was by Chi-square (Stokes et al., 2012) under the null hypothesis that respondents were evenly distributed across all options within each question/reason to declare significant effect p-value was equal to or less than 0.05.

# **Results and discussion**

Questionnaires thoroughly answered came from 740 consumers interviewed, demographic information of them is shown in Table 1. Consumers interviewed were mainly ( $p\leq0.05$ ) female, between 20 and 40 years old, with at least high school completed and a middle monthly income of \$5,500 or above. In a study conducted in the Czech Republic (Filipová et al., 2017), the food waste rate is related to age, income, and level of education; the latter is associated with the person in charge of the household's food. A relevant finding is that people with a higher level of education, such as students, employees, and — Economics and Management —

entrepreneurs, tend to waste significant amounts of food. Psychological factors are also pointed out as the leading cause of household food wastage. However, in a Danish consumer survey, Stancu et al. (2016) reported a more significant correlation between consumers' awareness of the economic consequences of food waste than the damage caused to the environment.

Table 1

Item	Variable	Percentage of responds (% ± SE)*			
Gender	Female	$69.0\pm2.0^{\mathrm{a}}$			
	Male	$31.0 \pm 3.0^{b}$			
Age (years)	Less than 20	7.8± 3.53°			
	20	22.8± 3.2 <sup>b</sup>			
	30	$33.8\pm2.9^{\rm a}$			
	40	$22.4\pm3.2^{\mathrm{b}}$			
	50	8.5± 3.5°			
	60	$3.5{\pm}3.6^{d}$			
	70	$1.2 \pm 3.6^{d}$			
Educational level	Junior high or lower	$19.3 \pm 3.3^{b}$			
	High school	$20.1 \pm 3.2^{\circ}$			
	Technical degree	$37.2 \pm 2.9^{a}$			
	College degree	$23.4 \pm 3.2^{\circ}$			
Monthly income (MXN)	Less than 2,700	$21.0\pm3.2^{\rm b}$			
	2,800 - 5,000	$27.3 \pm 3.1^{a}$			
	5,500 - 8,000	$25.7 \pm 3.17^{a}$			
	8,500 - 10,000	$14.3 \pm 3.4^{\circ}$			
	10,500 - 15,000	7.8±3.5°			
	15,500 - 20,000	$3.6 \pm 3.6^{d}$			
	More than 20,000	$0.3\pm3.6^{\text{d}}$			
*a, b within item means with one letter in common are not different ( $p \le 0.05$ ). SE= Standard error					

Demographic indicators of consumers interviewed and fully answered the questionnaire (n = 740)

In addition, in this study, almost 75% of the consumer sample was made up of people of 3 to 5 family members ( $p \le 0.05$ ); of them 46.8 and 22.4%, first and second places ( $p \le 0.05$ ) had at least one family member 10 years old or younger or 70 years old or older, respectively. For 67% of the consumers ( $p \le 0.05$ ), the source of ground beef is local butcher's shops, and grinding is done when buying it, while 33% get the ground beef from supermarkets displayed in open refrigerated displays and already packed (Figure 1; Figure 2; Figure 3). Ground beef was cooked and consumed by 98% of the consumers within the first three days after the purchase of it. When analysing the relationship between food waste and consumption, it can be determined that price has a significant relationship, where age, education and economic activity are directly related (Hazuchova et al., 2020). In contrast, 37% of Egyptian consumers reported being concerned about food waste regularly (Abdelradi, 2018).

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Figure 1. Meat on refrigerated shelves in Mexican supermarkets



Figure 2. Different types of meat in a traditional Mexican market



Figure 3. Traditional Mexican food market

Respecting reasons for awareness of meat product waste expressed by the respondents (Table 2) is relevant to indicate that 71.6% agreed or strongly agreed in associating meat waste with the environment, while 73.0% related it to resource waste and feelings of guilt. Concerning the expiry date, respondents strongly agreed and agreed that it is an important parameter; 70.4% specified that respecting the expiry date impacts health, while 60.3% indicated that it represents the level of meat freshness. In contrast, more than 50.0% of the group respondents in this study strongly agreed that wastage of ground meat is not a problem

but rather an act of cleanliness. For example, Alongi et al. (2019) expressed that undisputed the use-by date label represents a factor in consumer decisions to dispose of food. Bosnian households reported a low rate of food waste and paid favourable attention to prevention. Most respondents reported understanding the content of labels and usually scheduled their purchases and consumption due to the pandemic (Vaško et al., 2023). Consumer awareness plays a relevant role in food waste and is aggravated by behavioural patterns (Preka et al., 2020). It is documented that Albanian consumers manage household waste in various ways: more than 86.0% stated that they throw it in the rubbish, 60% use it as food for their pets, and smaller proportions 10.3% channel the waste to needy families and 9.7% use it as compost in the garden. On the other side, American consumers stated that their primary motivations for reducing food were saving money and setting an example to children, and lastly, they placed importance on the environment (Neff et al., 2015a).

Table 2

Consumers	Frequency of the reason to be awareness				
awareness	Completely disagree	Partially disagree	Agree	Completely agree	I do not know
Fresher packed	28 7+3 1ª	13 2+3 4 <sup>b</sup>	20 3+3 3 <sup>b</sup>	17 3+3 3 <sup>b</sup>	20 5+3 3 <sup>b</sup>
one expiring earlier	20.7±3.1	13.2±3.4	20.3±3.3	17.3±3.3	20.5±3.5
Expiration date of packed minced meat is a freshness indicator	13.4±3.4ª	14.9±3.4ª	35.0±3.0 <sup>b</sup>	25.3±3.2ª	11.5±3.5ª
Expiration date of packed minced meat is useful for health	6.0±3.6ª	7.6±3.5ª	38.1±2.9 <sup>b</sup>	32.3±3.0ª	16.1 ±3.4ª
When I throw food away, I feel it like wastage, and I get a bad conscience	9.3±3.5ª	7.7±3.5ª	35.4±3.0 <sup>b</sup>	37.6±2.9 <sup>b</sup>	10.0±3.5ª
When I throw food away, I feel it as cleaning out the refrigerator	26.8±3.2ª	13.8±3.4ª	18.0±3.3ª	25.4±3.2ª	16.1±3.4ª
If I throw food away, it is not a problem	26.9±3.1 <sup>b</sup>	13.8±3.4ª	18.0±3.3ª	25.5±3.2 <sup>b</sup>	15.8±3.4ª
Food waste make pollution increasing	$31.4\pm3.1^{a}$	19.1±3.3ª	21.8±3.3ª	21.1±3.3ª	6.8±3.6 <sup>a</sup>
When I throw food away, I think about environment	6.7±3.6 <sup>b</sup>	6.8±3.6 <sup>b</sup>	31.5±3.0 <sup>b</sup>	40.1±2.8ª	14.9±4.0 <sup>b</sup>
a, b, c, means within row with at least one letter in common are not different ( $p>0.05$ )					

Sorting (%) of consumers by frequency of reason of awareness wasting ground beef at home

There was a relatively short time between buying and cooking/eating ground beef in Mexican households. Then, the decision to discard ground beef and throw it away could be taken a few days after purchasing. In addition, this short time could lead consumers to overlook basic conservation practices, such as proper refrigeration and clean containers. Table 3 shows the sorting of consumers by frequency of each reason to discard ground beef. In 12 out of the 14 reasons offered, discarding ground beef, it was found that the option never grouped the highest ( $p\leq 0.05$ ) proportion of consumers, and the consumer range was from 24.2 up to 42.7%. Respondents indicated that the two reasons for always discarding ground beef with the highest proportion ( $p\leq 0.05$ ) are related to keeping leftover food in the refrigerator and not eating it, and the other is that it becomes rotted. Similarly, another study reports that the leading causes of food waste are storing food in the refrigerator that is not consumed, removing leftovers from the plate or overcooking food (Abdelradi, 2018). Another essential aspect to consider is food portions, e.g. large quantities have an unfavorable effect on consumption, but at the same time, lead to a large amount of leftovers on the plate (Lorenz and Langen, 2018).

	Frequency of the reason to discard ground beef					
Reason	Never	Seldom	Often	Always	I do not know	
Bought in excess	36.4±2.9ª	18.8±3.3 <sup>b</sup>	19.6±3.3 <sup>b</sup>	15.6±3.3 <sup>b</sup>	9.6±3.5°	
Poor package size diversity	37.3±2.9 <sup>a</sup>	$20.8 \pm 3.2^{b}$	18.8±3.3°	15.9±3.3°	7.2±3.5 <sup>d</sup>	
Package difficult to empty completely	37.9±2.9ª	18.8±3.3 <sup>b</sup>	18.8±3.3 <sup>b</sup>	12.2±3.4°	12.3±3.4°	
Not the right ground beef	42.7±2.8ª	19.7±3.2 <sup>b</sup>	13.6±3.4 <sup>b</sup>	14.6±3.4 <sup>b</sup>	9.4±3.4°	
Kitchen appliance breakdown	37.0±2.9ª	23.4±3.2 <sup>b</sup>	15.5±3.3 <sup>b</sup>	11.4±3.4°	12.7±3.4°	
Passed suggested date for consumption	28.4±3.1ª	15.9±3.3ª	20.2±3.2ª	24.6±3.1ª	10.9±3.4 <sup>b</sup>	
Broken package	35.1±2.9 <sup>a</sup>	20.9±3.2 <sup>b</sup>	16.1±3.3°	14.5±3.4°	13.4±3.4°	
Meat rotten	27.8±3.1ª	12.6±3.4°	14.2±3.1°	31.9±3.0 <sup>a</sup>	13.5±3.4°	
Saving leftovers was not possible	36.8±2.9ª	16.3±3.3 <sup>b</sup>	18.9±3.3 <sup>b</sup>	15.7±3.3 <sup>b</sup>	12.3±3.4°	
No intention to save leftovers	36.8±2.9ª	16.3±3.3°	18.9±3.3 <sup>b</sup>	15.7±3.3°	12.3±3.4°	
Dislike	36.5±2.9 <sup>a</sup>	$16.1 \pm 3.3^{b}$	16.6±3.3 <sup>b</sup>	21.2±3.2 <sup>b</sup>	9.6±3.5°	
Mistake at cooking	36.1±2.9ª	$16.9 \pm 3.3^{b}$	$18.2 \pm 3.3^{b}$	$15.7 \pm 3.3^{b}$	13.1±3.4°	
Saved leftovers but not eaten	23.9±3.2 <sup>b</sup>	15.1±3.3°	15.2±3.3°	33.5±3.0ª	12.3±3.4 <sup>d</sup>	
Meat stored refrigerator but never cooked	24.2±3.2 <sup>b</sup>	14.9±3.3°	20.0±3.2 <sup>b</sup>	28.8±3.1ª	12.1±3.4 <sup>d</sup>	
a, b, c, means within row with at least one letter in common are not different (p>0.05)						

Sorting (%) of consumers by frequency of reason to discard ground beef at home

Table 3

Another study suggests that three main reasons for food wastage are identified, leaving food in the refrigerator for extended periods (Preka et al., 2020), unpleasant appearance and taste, and mould presented (Vaško et al., 2023). Food spoilage is also another reason that Finnish consumers point to as being of significant importance for food waste. However, at the same time, other important reasons are also the best-before date, dish leftovers and overcooking (Silvennoinen et al., 2014). In addition, Koivupuro et al. (2012) also highlighted the socio-demographic, attitudinal and behavioural factors influencing food waste as relevant parameters. It is noted that household size, gender of the person in charge of food shopping, and frequency of purchase of products with a reduced-price offer were the only factors that recorded a clear correlation with food waste in the household. In contrast, the same study reported no clear correlation with the eldest person in the family, type of residence, education level, shopping habits, food, and food preparation. The purchase frequency was the variable that influenced the amount of food wasted. However, the existence or lack of a ready-to-eat shopping list determined the food wasted (Giordano et al., 2019).

The reason meat rotten showed in this study a high ( $p\leq0.05$ ) proportion in both frequencies' extremes, never and always, the mean proportion for both frequencies was 29.8%. It is essential to point out that the lowest ( $p\leq0.05$ ) proportions of consumers in all 14 reasons were found in the lack of knowledge category (Table 3). Concurrently, there are significant relationships between food waste production and consumer purchasing practices, such as the way food preparation, the type of practices used to manage household waste, as well as beliefs, attitudes and lifestyles associated with their eating habits. Mainly related to food waste is the awareness of various aspects of the consumer, such as the correct way of eating and the production of food waste (Parizeau et al., 2015). However, one risk factor of food loss was influenced during COVID-19 because of supply chain disruption and the decrease in demand in many countries. Such phenomena were most problematic in small and medium-sized enterprises in developing countries (Laborde et al., 2020). On the other hand, food consumption practices changed during the Covid-19 pandemic. In Mexico was reflected in cooking habits and household food management changes. For example, Mexican households increased food purchases and decreased food waste (Vargas-Lopez et al., 2022).

However, three additional reasons in this study are also recorded in the always category, with a smaller proportion, such as the fact that raw meat is stored and not cooked on time, the suggested date for consumption has passed and for displeased. Porpino et al. (2015) identified five categories of food waste in Brazilian households: buying and preparing too much food, showing care for pets, lack of knowledge to preserve food, and avoiding having leftovers in the refrigerator. It also goes deeper and reports two interesting subcategories that, at the same time, influence food waste, such as compulsive buying and lack of planning, together with a preference for large packages. Two prominent factors, per capita income and single-person households, report a strong relationship with the amount of food wasted, resulting from an interaction of behaviour that influence the planning of food purchase, preparation, storage, and consumption (Quested et al., 2011). The relevant reasons American consumers put forward for discarding food focused on concerns about foodborne illness and being fresh food consumers (Neff et al., 2015 b). Concerning Dutch household consumers gave several reasons for discarding food from the refrigerator, firstly the expiry date, followed by product spoilage, excessive food preparation and forgetting the food in the refrigerator (Janssen et al., 2017). A study conducted in eight countries, Germany, Italy, Canada, the UK, China, Russia, Spain, and the USA, concerning the type of diets, showed a difference between smart diets and vegetarian diets, indicating that the former contributes to more food waste (Iori et al., 2022).

Another study also reported that packaging seldom becomes a key factor for discarding ground meat for consumption in the household; however, prevention of food waste also involves proper handling by retail sellers or supermarkets from processing to the selling point (Ali et al., 2022). For example, Neff et al. (2019) detailed that consumers aged 18-34 most frequently eliminate meat foods, and reasons pointing to buying food in more oversized packaging and misunderstanding the meaning of food labels. A study reports that the main drivers of food waste are associated with consumers' shopping routines, the reuse of leftovers, and the food purchase program (Stancu et al., 2016). By consensus, it has been argued that oversized food portions have an unfavorable impact on consumer food consumption because, in most cases, it leads to many leftovers on the plate (Giordano et al., 2019). There are typical characteristics such as the personal dimension associated with socio-demographic factors, the level of food waste knowledge and personal beliefs in each household that impact on food consumption (Schanes et al., 2018), and the influence linked to social norms and the overall cultural context of the consumer (Roodhuyzen et al., 2017).

Then, of the 14 reasons reported in this study to discard ground beef, 12 are avoidable in households if consumers are made aware of the negative impact of food waste. It is also essential to consider the importance of behavioural and attitudinal patterns and general lifestyle in reducing household food waste (Barr, 2007; Parizeau et al., 2021). Once the leading causes of household food waste are found, a sound program addressing the problem should be done (von Massow et al., 2019). This program should consider habits and emotions individually (Russell et al., 2017) and could also work on the guilt feelings of food buyers as a driver to reduce household food waste (Neff et al., 2015a). However, in today's life, consumers' concern for their economy, time and money exerts a distaste for wasting food (Watson and Meah, 2012). Consequently, food waste reduction is most feasible in prevention at the consumption stage because the most significant potential exists (Kummu et al., 2012).

Relating food waste in the meat sector is highly significant, and it is necessary to implement measures to reduce losses of this type of food. Although the consumption of meat and meat products is high, the level of wastage is also significant, both in production and at the consumer level (Karwowska et al., 2021). While the reasons to discard ground beef at home are avoidable, then programs should promote positive consumer behaviour, adopting strategies for saving, storing, and consuming leftover food at home (Jribi et al., 2020). Furthermore, Aydin and Yildirim (2021) stressed the direct and indirect effects of consumers' moral attitudes, as well as their behavioural habits and patterns on food waste and their practical knowledge of how to conserve food at home. Proper management and use of a food shopping list could help make consumers aware of the amount and expense of food wasted in their household, and then be a driver to reduce food waste (Principato et al., 2015). Regarding consumers' attitudes, it is reported that people with higher incomes are the ones who waste the highest proportion of food. In contrast, the retired sector registers lower amounts of wasted food. The same study points out that young people may have better food management because they are frequently in primary contact with social networks and technical devices (Filipová et al., 2017).

Another factor that affects food reduction is associated with religious values of positive influence when messages supporting food consumption are highlighted. In contrast, the opposite occurs when messages restrict food consumption (Minton et al., 2020). This program should consider habits and emotions individually (Russell et al., 2017) and could also work on the guilt feelings of food buyers as a driver to reduce household food waste (Neff et al., 2015a). However, in today's life, consumers' concern for their economy, time and money exerts a distaste for wasting food (Watson and Meah, 2012). Consequently, food waste reduction is most feasible in prevention at the consumption stage because the most

significant potential exists (Kummu et al., 2012). Beef and dairy products are characterized by higher reported resource consumption and adverse environmental effects (Moult et al., 2018). Therefore, there is a need for more information regarding losses and wastage in the meat products sector. Therefore, it is advisable to apply a systematic approach to quantify meat product wastage (Caldeira et al., 2019).

Several studies report waste in a variety of foods, for example high amounts of waste, in most cases avoidable, were recorded in British households and three categories of motivation to reduce waste were identified. These relate to environmental damage, implementation of appropriate action, and the last links to people training (Graham-Rowe et al., 2014). Additionally, Ilakovac et al. (2020) deduced that any food waste reduction program needs to go through household food handling and management because this end link could be responsible for up to 50% of food wasted at home. Other factors as consumer age and income, and the number of children under 18 in the household, could influence the amount of food waste. A study conducted in Turkey by Aydin and Aydin (2022) indicated that meat was the second most wasted. For example, in this case, the effect of food donation behaviour on the intention of not wasting was negligible. It is attributed to the fact that in that country, the concept of food donation is not ingrained in society as an effect on food waste reduction. On the other hand, consumers are also unaware that the temperature for meat products needs to be significantly colder or that freezing is required to avoid decomposition. Other reasons identified focus on confusion or misunderstanding about the meaning of food labels or dishes that did not meet consumer expectations (Lipinski, 2020).

Plazzotta and Manzocco (2019) postulate that there are potential barriers to valorising food waste control strategies. Five main reasons are put forward, the first of which is associated with the characteristics of food waste; another focuses on the lack of information on the components, where the involvement of science and technology plays a predominant role; the next one refers to the response of consumers, especially the level of commercial and marketing knowledge on food waste; the possibility to scale up and standardize the process is also envisaged, the reason being the high investment cost in the valorisation of industrial processes; finally there is the compliance with the regulatory framework, in terms of governmental environmental guidelines and those proposed by the public. It is conclusive that food waste in households is a matter of concern. The most important reasons why consumers resort to food disposal are due to expired food products and purchase lack of planning, inadequate quality, or excessive quantities, not knowing conservation techniques, and inadequate preparation of portions for consumption (Vieira et al., 2021; Żukiewicz et al., 2022).

# Conclusions

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- 1. Food waste is currently a significant concern concerning hunger alleviation and negative environmental impact. Our findings show that ground meat is preferably consumed on the same day of purchase or the next day among the Mexican middle-income population.
- 2. Awareness factors about wasting meat products are related to environmental impact, waste of resources and guilt. The use-by date is related to the wholesomeness and freshness of meat. The main reasons for discarding minced meat are putrefaction and uneaten leftovers.
- 3. Future initiatives should then focus on helping consumers to cope with current purchasing and handling trends. Thus, implementing programs to reduce product wastage should involve retailers and consumers.

- 4. Therefore, it is necessary to determine the feasibility of implementing and operability of food waste control strategies, and it is essential to consider economic, social, and environmental factors.
- 5. Finally, it is necessary to continue this type of research in Latin America since there is a lack of information on the subject. Therefore, it is relevant to point out that this study provides the first data on food waste in Mexico.
- 6. However, more research is needed on the behaviour of Mexican consumers to address the adverse effects and to pay more attention to the factors that drive food waste, and thus address the problem in a preventive manner in the Mexican population.

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# Beliefs about plant-based diet based in a sample of Hungarian females

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#### **Keywords:**

#### Abstract

**Introduction**. The aim of research is to examine the knowledge about plant-based diets, what beliefs and misconceptions exist about plant-based diets (PBDs), and how these differ between lifestyle groups among Hungarian females.

Materials and methods. Data were collected through an online survey on social media. These data were processed using univariate statistics (general description of the sample), exploratory factor analysis (identification of healthy lifestyles), cluster analysis (segmentation purposes), chi-square statistics (cluster profiling), F-statistics (comparing attitudes toward PBDs), and cross tabulation (knowledge and perceptions of PBDs).

Results and discussion. Four health-related lifestyle dimensions (health-conscious eating, mindfulness, carbohydrate avoidance, red meat avoidance) were identified, and four segments emerged (healthy food choosers, red meat avoiders, stress-free women, rejecters). Healthy food choosers (40.9%) prioritize healthy eating, avoid sugary snacks, and monitor carbohydrate intake. Red meat avoiders (27.9%) are neutral about healthy eating, but avoid red meat and processed foods; don't focus on carbohydrates. Stress-free women (20.8%) value mindfulness, relaxation, and outdoor physical activity for a stress-free life. Rejecters (10.4%) have a negative attitude toward healthy eating, mindfulness, carbohydrates, and red meat. Red meat avoiders live in the capital city, eat fruits and vegetables more often or at least once a day. Rejecters live in villages and eat fruits and vegetables every 4-5 days in a week or do not eat fruits and vegetables in a week. Healthy eaters eat fruits and vegetables more times a day. Stressfree people eat fruits and vegetables every 2-3 days in a week. They differed in their knowledge, attitudes and perceptions of PBDs.

72.1% of healthy food choosers, 84.8% of red meat avoiders, 75.8% of stress-free people and 71.9% of rejecters thought that plant-based diet was similar to vegan and vegetarian diet. The attitudes range from "may have health benefits for certain diseases" as the attitude with the highest mean level of agreement (4.26), especially among red meat avoiders, to "encourages diary consumption" as the attitude with the lowest mean level of agreement (1.69), especially among red meat avoiders. Red meat avoiders, healthy food choosers, and stress-free women had more positive attitudes toward PBDs than did rejecters. The majority of females were thinking about trying out PBDs. Red meat avoiders, healthy eaters, and stress-free women had more positive attitudes toward PBD than did rejecters. Healthy eaters perceived PBD as healthy. Red meat avoiders perceived the plant-based diet as healthy, safe, varied, exciting, environmentally friendly, and a complete diet. Stress-free women thought the plant-based diet was unhealthy and environmentally unfriendly. Rejecters attached more negative attributes to the PBD. They perceived the diet as unhealthy, dangerous, meatless monotonous, boring. environmentally unfriendly, difficult to digest, and malnutrition.

**Conclusions** The results contribute to the literature by adding empirical evidence to the emerging trends (PBD, vegan, vegetarian diets), as well as generating suggestions for nutrition and dietetics professionals and the government, as targeted marketing programs can be planned to change dietary behavior.

Health Lifestyle Plant-based diet Vegan Vegetarian diet

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# Introduction

Research by the United Nations (UN) World Health Organization (WHO) worldwide has shown that inadequate fruit and vegetable consumption may play a role in the deaths of 2.7 million people each year (OECD, 2016). However, with a balanced diet reduces the risk of heart disease, cancer and stroke (Jenkins et al., 2021).

While it is clear that a myriad of factors (in addition to fruit and vegetable consumption) influence an individual's health and years of healthy life, the data show that the "situation" of men in the country is alarming in terms of life expectancy at age 65. Almost 2/3 of Hungarian men do not even reach this age.

Estimates of current fruit and vegetable consumption vary widely around the world, ranging from less than 100 g/day in less developed countries to approximately 450 g/day in Western Europe (Liu et al., 2021). In terms of global fruit and vegetable consumption, the majority of countries do not meet the 2003 WHO/FAO recommendation of at least 400 grams of fruit and vegetables per day. Recognizing this problem, the UN has designated 2021 as the International Year of Fruit and Vegetables, hoping to raise awareness of this global issue (UN, 2019; WHO, 2020). 55% of the Hungarian population does not eat fruit daily, another 35% at least once a week and 9.4% less than once a week or never, while 45% eat vegetables daily, 46% weekly and 8.8% less than once a week or never. While the EU average is 32.9% of the population not consuming fruit and vegetables every day, in Hungary it is 36.3% (Kocsis, 2022).

Daily consumption of fruits and vegetables varies by age group, education, and income level (European Health Interview Survey - EHIS, 2019). Older people, women, those with higher incomes and those with higher levels of education are more likely to consume fruit and vegetables (KSH, 2018). Hungary's poor performance in this area can be partly explained by the relatively high price sensitivity of the Hungarian population, as price increases, especially for food, including fruit and vegetables, have increased significantly in 2021 and 2022 (Szabó et al., 2022). 60% of shoppers say they eat at least one portion of fresh fruit and vegetables a day, according to a GfK survey of 3,500 people in 2022. The amount bought per shopping trip has not increased in the summer of 2021 compared to previous years, with the average family taking home 2.36 kg of fruit and 1.75 kg of vegetables per trip. The drastic increase in prices in recent years and other effects (job losses, changes in family cost structures) are clearly visible: although the average household in Hungary will spend about 41,000 HUF on fruit in 2021, the amount purchased will decrease from 89 kg in 2014 to 83 kg in 2021. The same applies to vegetables, on which the average Hungarian household spent HUF 71,716 per year last year (GfK, 2022).

In Hungary, the total amount of food available to the population in 2020 (these data are not included in Figure 1) is 683.7 kg, a decrease of almost 0.6 percent compared to the average of the previous 5 years. The structure of the food supply has not changed significantly compared to previous years, with vegetables and fruits accounting for almost 30 percent of consumption.

Per capita consumption of fruits and vegetables in 2020 is 189.9 kg, slightly lower than in previous years. On the positive side, however, consumption of fruits and vegetables has increased by about 20 percent since 1970, and the trend is upward (Figure 1).

The aim of this study is to explore the knowledge about plant-based diets, the beliefs and misconceptions about plant-based diets (PBD), and how these differ between lifestyle groups among Hungarian females. Females are more concerned about their body weight and eat less and more fruits and vegetables (Wah, 2016). Graca et al. (2015) and Beacom et al. (2021) highlighted that meat is more important for males, while females are willing to adopt a more plant-based diet to be healthier (Lea et al., 2006).



Figure 1. Per capita consumption of fruit and vegetables (kg/year) Source: Own construction based on KSH data

The article is organized as follows: first, in relation to the research question theory and literature on healthy eating and plant-based diet. Then, the methodology is explained, followed by the results. Finally, the theoretical and practical implications of the findings are discussed, and the paper concludes with an exploration of research limitations and future research opportunities.

# **Theoretical background**

#### Healthy eating

An increasing number of international studies have focused on segmenting people based on their healthy or unhealthy eating behavior (André et al., 2017; Heerman et al., 2017; Lv et al., 2011; Psouni et al., 2016). Some papers focused on children (Sabbe et al., 2008; Smith et al., 2011) or adolescents (Cuenca-García et al., 2013; Honaken et al., 2004; Matias et al., 2018) and clustered adolescents based on eating behavior and physical activity or sedentary behavior. These studies analyzed the demographics of each group and found gender differences. Wah (2016) and Lv et al. (2011) also highlighted the healthy eating behaviors of females and the unhealthy eating behaviors of males. Men consume more high-calorie foods, junk foods, and oily foods with carbohydrates.

Among the international researches, more results that studied a specific consumer group (André et al., 2017; Benedet et al., 2017; Tanton et al., 2015). Tanton et al. (2015) analyzed the eating habits of British university students, whose lifestyle is considered risky in terms of eating. The researchers used the consumption of snacks, convenience and fast foods, and fruits and vegetables. They identified four segments: risky, mixed, moderate, and favorable eating patterns. The majority of university students had unfavorable eating habits. André et al. (2017) categorized older Norwegian citizens (65+) based on similarities in food consumption. They distinguished between people with an unhealthy eating pattern (21.5%) and people with a healthy eating pattern (78.5%). The first group of people consumed a greater amount of foods and beverages such as chocolate/candy, pasta, sausage, sugar-free and sugary soft drinks, whole milk, juice, white bread and half-wheat bread. The second group consumed more fruits, vegetables, boiled potatoes, oily fish, whole wheat bread and

water. Benedet et al. (2017) used clustering of four unhealthy food habits (low fruit intake, low vegetable intake, high candy intake, and high fried snack intake) among Brazilian workers. They found that unhealthy food habits were more common among female workers, those with lower levels of education, and those living without a partner.

Heernan with coauthors (2017) identified eating styles from six eating behaviors (frequency of eating healthy foods, frequency of eating unhealthy foods, frequency of breakfast, frequency of snacking, overall diet quality, and problem eating behaviors) and tested their association with BMI in adults. Four eating styles were identified and defined by healthy vs. unhealthy eating patterns and engagement in problem eating behaviors. Unhealthy and unhealthy problem eating groups had significantly higher BMI than healthy eaters. Psouni et al. (2016) examined patterns of eating and physical activity attitudes and behaviors in relation to BMI. They also identified healthy and unhealthy groups of Greek people. Healthy people were associated with healthier physical activity and eating behaviors. They had normal BMI. Unhealthy people were associated with lower levels of exercise and healthy eating. They belonged to the overweight category considering their BMI.

Jenkins and coauthors (2021) highlighted the importance of behavioral segmentation in the case of healthy lifestyles. De Vries et al. (2008) identified clusters based on five important preventive health behaviors, namely non-smoking, alcohol consumption, fruit consumption, vegetable consumption and physical activity in the Netherlands. They distinguished healthy, unhealthy, and poor diet clusters. They found a strong relationship between cluster membership and education level. The higher the education, the healthier the behavior. Later, Bennasar-Veny et al (2020) did a similar behavior-based clustering. They found that men consumed more alcohol, had a less healthy diet, were more likely to be overweight, and were more physically active. Women had a higher prevalence of underweight and were less physically active. They distinguished between moderate-risk unhealthy lifestyles, high-risk unhealthy lifestyles, and low-risk healthy lifestyles. Ly with coauthors (2011) identified three distinct health-related lifestyle clusters: an unhealthy (25.7%), a moderately healthy (31.1%), and a healthy (43.1%) group among Chinese adults (ages 18 and 64). Lifestyle variables analyzed included tobacco use, physical activity, fruit and vegetable consumption, and eating out. Men were more likely than women to lead an unhealthy lifestyle. Adults aged 50 and 64 were more likely to have a healthy lifestyle. Adults aged 40 and 49 were more likely to have an unhealthy lifestyle. They also highlighted the relationship with cluster membership and education level and wealth index.

On the other hand, many researchers focused on the lifestyle of health and sustainability (LOHAS), which can be characterized by health, well-being, and environmental sustainability (Choi and Feinberg, 2021). Sung and Woo (2019) highlighted that individuals who value health and sustainability in their lifestyle by purchasing local products and thus helping the environment have been a popular consumer trend in recent years. LOHAS consumers tend to value green living, which includes organic foods, local products, and healthier products, which further influences their family and friends to adopt sustainable living and healthier lifestyles as well. Lendvai et al. (2022) found that this group of people strives for a sense of naturalness, aims to behave ethically, and seeks to hold authentic and individualistic values. They surveyed 357 Hungarian members of Generation Z. They distinguished three personas within this generation: (1) personas that fit LOHAS, (2) personas that may fit LOHAS, and (3) personas that do not fit LOHAS. Tam et al. (2021) studied the adoption of LOHAS by Chinese consumers. They found that women, older people, and people with higher incomes are most likely to be in this group. Environmentally conscious consumers tend to be more quality and price conscious in their shopping behavior. Health and fitness conscious consumers tend to look for quality and novelty products.

#### — Economics and Management ——

#### Plant-based diet

According to European Health Interview Survey (EHIS, 2019) data, one fifth of the Hungarian population consciously follows some kind of diet, 14% follow one and 4.3% several at the same time. The most common special diets are diabetic (6.4%) and lactose-free (4.1%). As people age, the number of special diets increases, which, in addition to health-conscious lifestyles, has been linked to the development of food allergies, food sensitivities and other conditions requiring special diets. One in ten 15- to 17-year-olds and 21% of people aged 65 and older follow a special diet. Regardless of age, women are more likely to follow a special diet than men do. The proportion of people following a special diet increases with education and income. Only 1.1% of the population follows a vegetarian diet. According to a survey conducted by the TÉT (Diet, Lifestyle and Physical Activity) Platform a few years ago, only 2-2% of people consider a vegan or vegetarian diet to be healthy. Hungarians are typically meat lovers and find it difficult to give up animal products, but the vegan lifestyle has a growing number of followers (NAK, 2019). Schmidt (2019) noted that in recent years, vegetarian and vegan diets have become an increasingly popular dietary trend, which is actually a separate lifestyle as well.

An important consumption trend is "vegetable stars", which is about the positioning of vegetables, i.e. the spread of the plant-based food trend. Vegetables have taken over the role of meat, in part because of growing questions about meat consumption, especially in terms of environmental impact and sustainability. As a result, "vegetables are moving from side dish status to main course." Strict veganism, which rejects all animal products, is gaining more and more adherents (Törőcsik, 2019).

The reasons for low fruit and vegetable consumption may be personal, economic, or cultural, and changing them is not an easy task. Szabó and Lehota (2020) conducted a focus group study on the main reasons for consumption and identified love of fruits and vegetables, healthy lifestyle and healthy diet as the main reasons.

Plant-based diets are one of the trendy diets of our time (Törőcsik, 2016), and their impact on health is attracting the attention of an increasing number of researchers. In fact, plant-based diets have been used for centuries. Vegetarianism first appeared in India and the ancient Greek world, and was part of the religious aspirations of both cultures. A plant-based diet consists of "all minimally processed fruits, vegetables, whole grains, legumes, nuts and seeds, herbs, and spices and excludes all animal products, including red meat, poultry, fish, eggs, and dairy products" (Ostfeld, 2017; Szabó et al., 2016). Plant-based diets range from strict plant-based (vegan) to semi-vegetarian. Today, vegetarianism is "no longer simply a health-conscious dietary trend, but a holistic worldview whose proponents have a closed philosophical, ethical-moral, environmental, and agro-technological view; others appeal to socio-economic reasons" (Balogh, 2017). The previous thought is reinforced by Törőcsik and Szűcs (2021) cited in Forbes, who found that members of Generation Z are going to climate protests instead of school and adopting vegan lifestyles. The authors also highlight the more positive environmental impact of a plant-based diet based on the traditional food chain.

People who follow a vegan lifestyle completely exclude the consumption of animal derivatives, so they do not consume eggs and dairy products apart from meat. In Hungary, an advocacy group (Hungarian Vegan Association) was founded in 2017. Veganism is a "non-violent way of life" that opposes the exploitation of animals of both sexes. This is reflected in the way they eat (avoiding meat, dairy products, eggs, gelatin, honey, etc.), dress and have fun. Vegans do not wear leather shoes or use cosmetics that have been tested on animals. Balogh classifies veganism as a form of vegetarianism.
In addition, there are several other trends (Balogh, 2017):

- Semi-vegetarian: The diet includes poultry and fish.
- Ovo-vegetarianism: Eggs are eaten along with plant foods.
- Ovo-lacto vegetarianism: Eggs and dairy products are consumed along with plant foods.
- *Lactovegetarianism*: Dairy products of animal origin are consumed along with plant foods.
- *Pesco-vegetarianism*: Where fish is eaten along with plant foods.
- Raw vegetarianism: Followers eat 75-100% of vegetarian foods raw.

Looking at the motivations of people who follow a plant-based diet, it can be seen that the most common reasons for choosing this lifestyle are ethical, ecological, environmental, economic, political, animal welfare, spiritual or health-related. Several religious traditions -Brahmanism, Judaism, and Hinduism - also favor a meatless or meat-free diet (Szabó et al., 2016).

Increased competition in the market has also prompted retailers to review their business policies, and as Törőcsik and Szűcs (2021) have pointed out, the battle for customers' money is no longer just between identical types of stores, but also between different types of stores. The authors pointed out that the success of discount stores, for example, is due to their expansion and the targeting of specialized segments (organic, vegan, vegetarian, free, etc.).

Several recent national studies (Fehér et al., 2020; Szabó et al., 2016; Véha et al., 2019) have been conducted on plant-based diets. They mainly present the literature background and the characteristics of the diet. Krause and Williams (2017) conducted an empirical study to investigate the knowledge of university students on plant-based diets. In their systematic literature review, Fehér et al. (2020) developed a conceptual model based on the planned behavior model, taking into account objective supporting and inhibiting factors for switching to a plant-based diet, which they intend to test empirically in the future. Several researchers have also investigated the factors that hinder (Lea et al., 2006; Véha et al., 2019a) and support (Reipurth et al., 2019; Véha et al., 2019b) plant-based diets. According to Véha et al. (2019b), the main benefits of plant-based diets can be explained by factors that positively influence health and positive effects on well-being, satisfaction, and quality of life. In addition, ethical environmental benefits have been identified, including aspects such as more efficient use of economic resources, reduced global pollution, and animal welfare. From an economic perspective, the benefits of a plant-based diet are linked to sustainability, as it has a much smaller ecological footprint than meat consumption, i.e. an omnivorous diet. Today, one of the main reasons people are switching to a plant-based diet is to mitigate climate change (Reipurth et al., 2019). A plant-based diet produces 2.5 times less carbon emissions than a meat-based diet. Second, there are ethical arguments in favor of meat-free diets (Véha et al., 2019b). One is the rights-based or deontological approach, which argues that animals have intrinsic value because of their ability to "experience life" and should therefore be seen as ends in themselves rather than means to an end. The other approach is utilitarian or consequentialist, which argues that every sentient being should have at least one right - the right not to be treated as property. One of the characteristics of ethical vegans is their total rejection of the commodification of animals. They also do not use animal products for clothing, fragrances, or any other purpose, and try to avoid animal-tested ingredients (Balogh, 2017). The way in which animals are kept is the main subject of ethical objections. Animals are forced to live in overcrowded stalls and confined spaces that do not allow them to express their natural behaviors. Animals are deprived of exercise, which burns energy and wastes material resources. They also unnaturally force animals to gain maximum weight, causing internal organ disease, physical deformities, and suffering. Vegetarian principles do not consider any form of animal slaughter to be humane. A plant-based diet can have many beneficial, positive effects on health and therefore quality of life (Lea et al., 2006), but people need to be prepared. Plant-based diets are generally lower in energy, fat, and therefore saturated fat, reducing the risk of overweight, obesity, and cardiovascular disease (Szabó et al., 2016). Beacom, Bouge and Repar (2021) highlighted that Irish and British females were almost twice as likely as males to consume plant-based products (PBPs). The main motivations for consumption were 'sustainability,' 'animal welfare' and 'health.'

A predominantly or exclusively plant-based diet has its own dangers, as it can lead to deficiency diseases due to the lack of protein intake (Reipurth et al., 2019). To compensate for deficiencies, dietary supplements or functional foods should be consumed (Szakály, 2008). It is always advisable to consult a dietician before switching to a plant-based diet. Lea et al (2006) found that the main perceived barrier to adopting a plant-based diet was lack of information. In a literature review of barriers to plant-based diets, Véha et al. (2019a) found that in addition to negative physiological effects, enjoyment of eating meat and difficulty in giving it up, convenience and time constraints, information acquisition, social barriers and associated negative discrimination, and financial constraints all hindered the adoption of a plant-based diet. Barriers to consumption for non-PBP consumers were 'not seeing the need to change their diet' and 'taste' (Beacom et al., 2021). Havermans et al. (2021) found a similar result among Dutch adolescents. Pohjolainen et al. (2015) found that enjoyment of meat, eating routines, health beliefs, and difficulties in preparing vegetarian foods hindered PBD adaptation.

Based on the literature review the authors defined the following research questions:

- 1. What kind of factors can be distinguished on the basis of healthy eating?
- 2. Is it possible to segment consumers based on healthy eating?
- 3. Is it possible to profile each segment based on demographics (age, location, occupation, family life cycle and diet, online lifestyle group)?
- 4. What knowledge does each group have about vegetarian, vegan and plant-based diets? Are they planning to try a plant-based diet?
- 5. What are the respondents' attitudes toward plant-based diets?
- 6. What associations does each group have with plant-based diets?

#### Materials and methods

#### **Data collection**

Data was collected through an online survey in Hungary. The questionnaire was pretested in order to ensure consistency, and understanding. Data was collected through nonprobability snowball sampling by distributing to Hungarian consumers through social media during one week in April 2023. The survey was created in the Google Sheet platform, and SPSS 26.0 was used for statistical analysis.

The questionnaire was divided into three sections: (1) health behaviors; (2) knowledge and beliefs about plant-based diets, (3) sociodemographic characteristics. Participants reported their attitudes toward *health behaviors* by expressing their level of agreement on a 5-point Likert scale with the endpoints 1: strongly disagree, 5: strongly agree. The 15 statements were as follows: (1) 'I take care of my health', (2) 'I eat fresh fruit every day', (3) 'I eat fresh vegetables every day, (4) 'I watch my fluid intake', (5) 'I avoid sugary foods', (6) 'I watch my carbohydrate intake', (7) 'I do not snack', (8) 'I eat high-fiber foods', (9) 'I avoid red meat', (10) 'I avoid processed foods', (11) 'I make sure I get fresh air every day', (12) 'I try to avoid stressful situations', (13) 'I exercise regularly, (14) 'I consciously seek out things that relax me and make me feel good', (15) 'I make a conscious effort to relax''. These statements are based on a previous study by Keller (2019).

*Knowledge about plant-based diet* was measured on nominal scale that was used by Krause and Williams (2017).

Attitudes toward a plant-based diet were measured by 15 attitude statements using a 5 point Likert scale (1) does not provide the body with enough protein, (2) avoids processed foods, (3) encourages consumption of diary products, (4) eliminates or minimizes the consumption of animal products, (5) promotes oil consumption, (6) limits or excludes eggs, (7) discourages complex carbohydrates, (8) does not get enough omega-3 fatty acids, (9) helps prevent chronic diseases, (10) facilitates digestion, (11) good for extra energy, (12) perfect for muscle building, (13) is beneficial for some illnesses, (14) more environmentally friendly, (15) reduces saturated fat consumption. These statements are based on a previous study by Krause and Williams (2017).

Pairwise comparisons were used to measure the *associations toward a plant-based diet*. 10 contrasting attributes were questioned: (1) healthy – unhealthy, (2) cheap – expensive, (3) dangerous – safe, (4) monotonous – varied, (5) boring – exciting, (6) old-fashioned, fashionable, (7) simple – complicated, (8) environmentally unfriendly – environmentally friendly, (9) difficult to digest – easy to digest, and (10) malnutrition – full diet.

*Sociodemographic characteristics* included age (in years), place of residence (village, town, city, capital), occupation (student, white-collar worker, blue-collar worker, unemployed, retired), marital status (single, cohabiting, married without children, family with children).

*Categories of fruit and vegetable* consumption were measured on a weekly basis: more than once a day, once a day, every 2-3 days, every 4-5 days, not eating fresh fruit and vegetables on a weekly basis.

#### Data analysis

The sample was not representative - the authors would like to emphasize that this is an exploratory study and that the main conclusions apply only to this sample. Data analysis was conducted using SPSS 26.0 software. Multivariate statistical analysis was used to answer the research questions. To answer the first research question, the authors used factor analysis. The method of factor extraction was principal components analysis. The number of factors was determined by a priori determination (3) and approaches based on eigenvalues (5), scree plot (5), and percentage of variance accounted for (5). The number of factors was determined by the eigenvalues. The benchmark of factor loading above 0.4 was used as a criterion for inclusion of items in each factor. Factor rotation was assessed using the Varimax method. To answer the second research question, the method of cluster analysis, specifically the method of Ward's hierarchical cluster analysis, namely agglomerative clustering, was used (Malhotra, 2018). Since the aim was to highlight the main differences, the square Euclidean distance was used to measure the distances. After investigating the prerequisites, the researchers considered different clustering solutions, but finally they decided to apply the four clustering solution. In the next step, they considered these clusters as nominal variables. To answer the third research question to analyze the relationship between cluster membership and basic demographics, chi-square analysis was performed. In this case, the authors considered the expected value and the state of the variables measured on nominal scales. Cross-tabulations were used to analyze knowledge, attitudes, and associations toward a plant-based diet across cluster membership.

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#### **Results and discussion**

In relation to healthy eating 15 statements were examined. In this case the value of Cronbach  $\alpha$  was 0.921, which means that this scale is consistent. The values of the indicators that prove the adequacy of the factor analysis were adequate (KMO: 0.893, Bartlett's Test: 3820.136, Sig. 0.000). The number of factors was determined by Scree test that is four factors could be distinguished explaining 65.101% of the total variance (Table 1).

#### Table 1

	Statements	Loading	Factor
1	I eat fresh vegetables every day (2)	0.830	
2	I eat fresh fruit every day (3)	0.824	health-
3	I eat high-fiber foods (8)	0.644	conscious eating
4	I take care of my health (1)	0.626	(20.14%; 0.85)
5	I watch my fluid intake (4)	0.560	
6	I make a conscious effort to relax (15)	0.810	
7	I consciously seek out things that relax me and	0.768	
	make me feel good (14)		mindfulness
8	I try to avoid stressful situations (12)	0.724	(17.35%; 0.76)
9	I make sure I get fresh air every day (11)	0.531	
10	I exercise regularly (13)	0.449	
11	I watch my carbohydrate intake (6)	0.791	avoidance of
12	I avoid sugary foods (5)	0.767	carbohydrate
13	I do not snack (7)	0.763	(16.42%; 0.77)
14	I avoid red meat (9)	0.834	avoidance of red
15	I avoid processed foods (10)	0.740	<i>meat</i> (11.17%;
	_		0.75)

#### **Results of factor analysis**

Source: Own research, n = 614 respondents. Method: Main component analysis, Rotation: Varimax Note: First number behind the factors is the explained variance; second number is the value of Cronbach alpha.

The *first factor* included five variables, which represented a *health-conscious eating* (HCE) behavior and the choice of vegetables, fruits, nutritious foods, and fluid intake that are good for the body. The *second factor* contained five variables representing *mindfulness* (MF), the importance of relaxation, and physical activity in fresh air, and the avoidance of stressful situations. The third factor included items related to the *avoidance of carbohydrate* (ACH) intake and the refusal of sugar and snacks. The last factor included two items that are the *refusal of red meat and processed food* (ARM). It can be stated that four factors can be distinguished based on health behavior, namely health-conscious eating, mindfulness, avoidance of carbohydrates, and refusal of red meat.

In the next step using the results of factor analysis were used for clustering. Based on the results of Elbow criterion and Agglomeration schedule the four-cluster solution was selected. Count and frequency in case of each cluster was the following: first cluster 251 people (40.9%), 2nd cluster 171 people (27.9%), the 3rd cluster 128 people (20.8%) and the 4th cluster 64 people (10.4%). In order to make a typology of the different clusters, it was necessary to analyze the means. The method of one-way ANOVA was used to check the

category means of the health behavior factors (HCE, MF, ACH, ARM) in case of each cluster and significant differences (FCHF: 509.468, p: 0.000,  $\eta^2$ :0.472; FRUF: 748.369, p: 0.000,  $\eta^2$ : 0.568). There were significant differences between groups for all variables. To test the homogeneity of variables post-hoc tests (Dunnett T3 and LSD) were performed. according to the results there were statistically significant differences between variables.

- 1. *Healthy food choosers* (40.9%): they try to eat in a healthy way and they typically refuse unhealthy foods like sugary odd and snacks and the pay attention to their carbohydrate intake.
- 2. *Red meat avoiders* (27.9%): they are mainly neutral when it comes about healthy eating, they do not pay attention to their carbohydrate intake, but they avoid red meat and processed foods.
- 3. *Stress-free women* (20.8%): could be characterized by the importance of mindfulness. They pay attention to relaxation and physical activity in the fresh air, so a stress-free life is important to them.
- 4. *Rejecters* (10.4%): they could not be characterized by health-conscious eating, mindfulness, avoidance of carbohydrates and red meat. They have a negative attitude towards all the four aspects of health behavior (Table 2).

Ward Met	nod	HCE	MF	ACH	ARM
	Mean	0.346	-0.054	0.8368129	0.001
1, heathy food	Ν	251	251	251	251
choosers (40.9%)	Std. Deviation	0.783	0.942	0.683	0.842
	Variance	0.614	0.888	0.467	0.711
	Mean	0.195	0.232	-0.629	0.840
2, red meat avoiders	Ν	171	171	171	171
(27.9%)	Std. Deviation	0.773	0.955	0.743	0.565
	Variance	0.598	0.913	0.553	0.319
	Mean	-0.064	0.228	-0.562	-1.076
3, stress-free women	Ν	128	128	128	128
(20.8%)	Std. Deviation	0.882	0.891	0.707	0.753
	Variance	,779	,795	0.500	0.568
	Mean	-1.751	-0.865	-0.474	-0.098
4 main stars (10, 49/)	Ν	64	64	64	64
4, rejecters (10.4 <i>7</i> 6)	Std. Deviation	0.634	1.055	0.800	0.788
	Variance	0.403	1.114	0.641	0.621
	Mean	0.000	0.000	0.000	0.000
Total	Ν	614	614	614	614
10181	Std. Deviation	1.000	1.000	1.000	1.000
	Variance	1.000	1.000	1.000	1.000

#### Results of cluster analysis

Table 2

Source: Own research, n=614 respondents

It can be seen that consumers can be divided into homogeneous groups based on health behavior factors (HCE, MF, ACH, and ARM). Four groups of consumers were identified: healthy food choosers (40.9%), red meat avoiders (27.9%), stress-free women (20.8%), neutrals (26.2%) and rejecters (10.4%).

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#### **Profiling consumer groups**

Table 3 shows the sociodemographic characteristics of the sample. To analyze the relationship between cluster membership and basic demographics, cross tabulation (chi-square analysis) was performed. The relationship between cluster membership and occupation, family life cycle, income level, and weekly fruit and vegetable consumption were analyzed. Significant associations were found for residence ( $\chi^2=20.377$ ; p=0.01), fruit ( $\chi^2=150.496$ ; p=0.00) and vegetable consumption ( $\chi^2=190.084$ ; p=0.00). However, these associations were weak (Cramer's V<sub>residence</sub>=0.105; Cramer's V<sub>fruit</sub>=0.286; Cramer's V<sub>vegetable</sub>=0.321) (Table 4).

Based on the results of the adjusted standardized residuals, we can say that red meat avoiders live in the capital city, eat fruits and vegetables more often or at least once a day. Rejecters live in villages and eat fruits and vegetables every 4-5 days in a week or do not eat fruits and vegetables in a week. Healthy eaters eat fruits and vegetables more times a day. Stress-free people eat fruits and vegetables every 2-3 days in a week (Table 4).

Table 3

	Healthy food choosers	Red meat avoiders	Stress-free women	Rejecters	Total
Age (median)	28.0	30.0	28.0	30.5	29.0
Place of residence					
Capital city	23.9%	30.4%	16.4%	12.5%	23.0%
City	21.5%	17.5%	21.9%	23.4%	20.7%
Town	31.5%	31.0%	33.6%	23.4%	30.9%
Village	23.1%	21.1%	28.1%	40.6%	25.4%
Occupation					
Student	29.9%	24.6%	38.3%	25.0%	29.6%
White-collar workers	49.8%	55.6%	43.0%	37.5%	48.7%
Blue-collar workers	16.7%	16.4%	15.6%	31.3%	17.9%
Unemployed	2.8%	2.9%	1.6%	3.1%	2.6%
Retired	0.8%	0.6%	1.6%	3.1%	1.1%
Marital					
status					
Single	33.5%	26.9%	30.5%	29.7%	30.6%
Cohabiting	31.5%	34.5%	33.6%	26.6%	32.2%
Married without children	16.7%	14.6%	18.0%	28.1%	17.6%
Family with children	18.3%	24.0%	18.0%	15.6%	19.5%

Sociodemographic characteristics of the four segments and the total sample

#### Table 4

		Adjusted st	tandardized		Sign.	
Demog	ranhics	Healthy	Red	Stress-	Rejecters	relations
Demographies		food	meat	free		with
		choosers	avoiders	women		clusters
	capital city	0.5	27	-2.0	-2.1	read meat
Residence	capital enty	0.5	2.1	-2.0	-2.1	avoiders
	village	-1.1	-1.5	0.8	3.0	rejecters
						healthy
	more than					food
	once a day	3.6	2.1	-3.5	-4.2	choosers
	once a day					red meat
						avoiders
	once a dav	0.8	29	-15	-3.6	red meat
Fruit	once a day	0.0	2.9	-1.5	-5.0	avoiders
consumption	every 2-3	-29	-2.6	54	12	stress-free
in a week	days	2.9	2:0	5.4	1.2	people
	every 4-5	-2.4	-15	0.8	5.0	rejecters
	days	2.1	1.5	0.0	5.0	rejecters
	not eating					
	iresn iruit	-0.8	-3.8	-0.6	7.6	rejecters
	basis					
	04515					healthy
						food
	more than	47	32	-43	-6.6	choosers
	once a day		5.2	-4.5	-0.0	red meat
						avoiders
						stress-free
Vegetable	every 2-3	-3 5	-37	43	53	neonle
consumption in a week	days	-5.5	-5.7	4.5	5.0	rejecters
	every 4-5					10,000015
	days	-0.8	-2.1	-0.4	4.9	rejecters
	not eating					
	fresh fruit	2.6	2.4	0.6	<u> </u>	
	on a weekly	-2.6	-2.4	-0.6	8.4	rejecters
	basis					

#### Clusters and basic demographics

Source: Own research

It is possible to profile each segment based on demographics, especially based on place of residence and fruit and vegetable consumption.

#### Vegetarian, Vegan and Plant-Based Diet

In the next session, knowledge of the different types of plant-based diets was analyzed. The majority of respondents (80.1% of red meat avoiders, 77.7% of healthy food choosers, 67.2% of rejecters, and 66.4% of stress-free people) believed that a vegetarian diet meant rejecting white and red meat and fish, while eating other foods of animal origin such as eggs

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and dairy products. 20.3% of the rejectionists, 18.0% of the stress-free women, 13.1% of the healthy food choosers, and 11.7% of the red meat avoiders believed that vegetarians should not have eaten meat, but could eat fish. The rejection of all animal foods was believed to be true by 8.6% of the women. The refusers were not aware of the principles of the vegetarian diet, 7.8% of them thought that animal products could be consumed (Table 5).

#### Table 5

	Healthy food choosers	Red meat avoiders	Stress- free women	Rejecters	Total
Do not eat (red, white) meat, fish, dairy products and eggs.	9.2	7.0	11.7	4.7	8.6
Do not eat meat (including red meat, white meat, and fish), but do eat other animal products, including dairy and eggs.	77.7	80.1	66.4	67.2	74.9
Eat all animal products.	0.0	1.2	3.9	7.8	2.0
Do not eat red or white meat, but do eat fish.	13.1	11.7	18.0	20.3	14.5

#### Attributes of Vegetarian Diet, %

Source: Own research

Note:  $\chi^2 = 28.76$ ; p = 0.00, Cramer's V = 0.12; p=0.00

Regarding the vegan diet, the majority knew that not all foods of animal origin could be consumed, especially those who avoided red meat. 9.4% of the rejecters thought that the refusal of meat but the consumption of fish was typical of a vegan diet (Table 6).

Attributes of Vegan Diet, %

Table 6

	Healthy food choosers	Red meat avoiders	Stress- free women	Rejecters	Total
Do not eat (red, white) meat, fish, dairy products and eggs.	84.1	90.1	76.6	78.1	83.6
Do not eat meat (including red meat, white meat, and fish), but do eat other animal products, including dairy and eggs.	7.2	3.5	8.6	6.3	6.4
Eat all animal products.	6.0	5.3	7.8	6.3	6.2
Do not eat red or white meat, but do eat fish.	2.8	1.2	7.0	9.4	3.9

Source: Own research

Note:  $\chi^2 = 18.27$ ; p = 0.03, Cramer's V = 0.10; p=0.03

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72.1% of healthy food choosers thought that plant-based diet was similar to vegan and vegetarian diet, and 27.9% of them admitted that it was a diet free of products derived from animals. 84.8% of red meat avoiders thought that plant-based diet was similar to vegan and vegetarian diet, and 14.6% of them admitted that it was a diet free from foods derived from animals; only 0.6% thought that PBD allowed diary consumption. 75.8% of stress-free people admitted that PBD was similar to vegan and vegetarian diets, 22.7% of them thought it was a meat-free diet. 71.9% of rejecters thought it was similar to vegan and vegetarian diets, 25% thought it was a meat-free diet, and 3.1% mentioned that diary consumption was allowed in this diet.

#### Attitude towards plant-based diet

37.6% of respondents rejected the plant-based diet and the rest thought about trying it. 50.6% of the healthy food choosers thought about following a PBD, 4.4% thought about it only for a short period, and 31.9% rejected this diet. The proportion of undecided women was 13.1%. 73.7% of the red meat avoiders planned to follow a PBD, 3.5% only for a short time. 16.4% refused the diet and 6.4% did not know. Taking into account the stress-free women, they abstained from a plant-based diet, as 61.7% of them refused this type of diet. The refusers were attached to meat, as 68.8% of them did not think of trying a meat-free diet.

Table 7 shows the sample's mean level of agreement with 15 attitudinal statements about plant-based diets. The attitudes range from "may have health benefits for certain diseases" as the attitude with the highest mean level of agreement (4.26), especially among red meat avoiders, to "encourages diary consumption" as the attitude with the lowest mean level of agreement (1.69), especially among red meat avoiders. No significant results based on cluster membership were found in two cases, "excludes the consumption of processed foods" and "encourages the consumption of complex carbohydrates". Red meat avoiders, healthy food choosers, and stress-free women had more positive attitudes toward PBD than did rejecters.

#### **Perception about Plant-Based Diet**

Women viewed a plant-based diet as healthy, safe, varied, exciting, fashionable, simple, environmentally friendly, easy to digest, and satisfying. The majority associated it with positive attributes. However, 47.4% perceived it as cheap and 52.6% perceived it as expensive. Healthy eaters perceived PBD as healthy. Red meat avoiders perceived the plant-based diet as healthy, safe, varied, exciting, environmentally friendly, and a complete diet. Stress-free women thought the plant-based diet was unhealthy and environmentally unfriendly. Rejecters attached more negative attributes to the PBD. They perceived the meatless diet as unhealthy, dangerous, monotonous, boring, environmentally unfriendly, difficult to digest, and malnutrition (Table 8).

#### Table 7

Statement – A plant based diet	Healthy food choosers	Red meat avoiders	Stress- free women	Rejecters	Total
is beneficial in case of some	4.27	1 15	4 17	2.02	1 26
illnesses* (13)	(1.02)	4.43	4.17	3.93	4.20
F=4.26, p=0.00	(1.02)	(0.93)	(1.07)	(1.19)	(1.04)
eliminates or minimizes the					
consumption of products of	4.27	4.36	4.02	3.93	4.21
animal origin* (4) F=2.89,	(1.19)	(1.24)	(1.37)	(1.47)	(1.28)
p=0.03					
facilitates digestion* (10)	4.17	4.36	4.03	3.75	4.15
F=5.51, p=0.00	(1.10)	(0.99)	(1.18)	(1.15)	(1.10)
is more environmentally	1 10	1 31	4.01	3 70	1 14
friendly* (14)	(1.00)	(1.09)	(1.15)	(1.20)	(1.14)
F=5.97, p=0.00	(1.09)	(1.09)	(1.15)	(1.29)	(1.14)
reduces saturated fat	4.11	4.15	3.82	3 73	4.02
consumption* (15)	(1.07)	(1.00)	(1.13)	(1.27)	(1.12)
F=4.08, p=0.00	(1.07)	(1.07)	(1.15)	(1.27)	(1.12)
helps prevent chronic diseases*	3.88	4 02	3 82	3 28	3 84
(9)	(1.27)	(1.26)	(1.21)	(1.20)	(1.26)
F=5.52, p=0.00	(1.27)	(1.20)	(1.21)	(1.20)	(1.20)
good for extra energy* (11)	3.76	4.00	3.60	3.25	3.74
F=6.03, p=0.00	(1.30)	(1.24)	(1.31)	(1.20)	(1.29)
limits or excludes eggs* (6)	3.59	3.92	3.47	3.15	3.63
F=6.46, p=0.00	(1.44)	(1.40)	(1.38)	(1.40)	(1.43)
is perfect for muscle building*	3 48	3 76	3.07	2 70	3 39
(12)	(1.37)	(1.36)	(1.37)	(1.28)	(1.39)
F=12.38, p=0.00	(1.57)	(1.50)	(1.57)	(1.20)	(1.57)
avoids processed foods (2)	3.38	3.12	3.28	3.45	3.29
F=1.35, p=0.25	(1.43)	(1.58)	(1.33)	(1.42)	(1.45)
discourages complex	3.17	3 19	3.08	3 25	3 17
carbohydrates (7)	(1.35)	(1.38)	(1.21)	(1.32)	(1.32)
F=0.27, p=0.84	(1.55)	(1.50)	(1.21)	(1.52)	(1.52)
does not get enough omega-3	2.19	1 90	2 20	2.71	2.16
fatty acids* (8)	(1.24)	(1.21)	(1.21)	(1.26)	(1.24)
F=6.90 p=0.00	(1121)	(1121)	(1121)	(1120)	(112.1)
promotes oil consumption* (5)	2.04	1.99	2.39	2.45	2.14
F=5.26, p=0.00	(1.09)	(1.14)	(1.22)	(1.19)	(1.15)
does not provide the body with	1.98	1.83	2.25	2.65	2.06
enough protein* (1)	(1.23)	(1.20)	(1.21)	(1.28)	(1.24)
F=8.25, p=0.00	(1)	(1.20)	()	(1.20)	()
encourages dairy consumption*	1.67	1.47	1.87	2.01	1.69
(3)	(1.03)	(1.01)	(1.17)	(1.21)	(1.09)
r=5.48, p=0.00		` '	· /	`` '	· /

Attitude towards Plant-Based Diet

Source: Own research

Note: Values are means measured on a 5-point Likert scale from strongly disagree (1) to strongly agree (5), and values in parentheses are standard deviations.

#### Table 8

Plant based diet is	Healthy food choosers, %	Red meat avoiders, %	Stress-free women, %	Rejecters, %	Total, %	χ2 p
healthy	78.5	89.5	54.7	45.3	73.1	74.26
unhealthy	21.5	10.5	45.3	54.7	26.9	0.00
cheap	47.4	48.5	43.8	51.6	47.4	
expensive	52.6	51.5	56.3	48.4	52.6	
dangerous	35.9	25.7	39.1	46.9	34.9	11.45
safe	64.1	74.3	60.9	53.1	65.1	0.01
monotonous	35.1	21.1	35.9	48.4	32.7	18.97
varied	64.9	78.9	64.1	51.6	67.3	0.00
boring	38.2	22.8	43.0	56.3	36.8	27.12
exciting	61.8	77.2	57.0	43.8	63.2	0.00
old-fashioned	20.3	16.4	18.0	25.0	19.2	
fashionable	79.7	83.6	82.0	75.0	80.8	
simple	68.9	66.1	61.7	54.7	65.1	
complicated	31.1	33.9	38.3	45.3	34.9	
environmentally unfriendly	13.9	8.8	27.3	34.4	17.4	32.54
environmentally friendly	86.1	91.2	72.7	65.6	82.6	0.00
difficult to digest	14.3	10.5	15.6	28.1	15.0	11.46
easy to digest	85.7	89.5	84.4	71.9	85.0	0.00
malnutrition	33.5	23.4	40.6	56.3	34.5	24.96
full diet	66.5	76.6	59.4	43.8	65.5	0.00

#### Perception of PBD

Note: Based on the results of the adjusted standardized residuals, the attributes of the PBD are related to the groups of consumers shown in bold.

#### Discussion

Healthy lifestyle is an important multidisciplinary topic, as it is not only addressed by health studies, but also by social sciences in general. Most previous studies have classified consumers or specific consumer groups, such as children (Smith et al., 2011), adolescents (Matias et al., 2018), Generation Z (Tanton et al., 2015), or seniors, based on their eating habits (André et al., 2017) or their healthy lifestyle. As more and more researchers (Beacomet al., 2021; Lea et al., 2006) have highlighted the openness of women to healthy lifestyles, the present study focused only on women. The authors first distinguished various health factors, such as health-conscious eating, mindfulness, carbohydrate avoidance, and red meat avoidance. As previous studies such as Jenkins et al. (2021) have highlighted the importance of behavioral segmentation in the case of healthy lifestyles, we also adopted this approach. The present quantitative study identified four consumer segments among women based on their health behaviors. Healthy food choosers (40.9%), red meat avoiders (27.9%), stress free women (20.8%), and rejecters (10.4%). The majority of the sample considers a healthy lifestyle, either eating behavior or mindfulness, to be somewhat important. This could be explained by the fact that the half of the sample follows some kind of online social media group. Red meat avoiders live in the capital city, eat fruits and vegetables more often or at least once a day. Rejecters live in villages and eat fruits and vegetables every 4-5 days in a week or do not eat fruits and vegetables in a week. Healthy eaters eat fruits and vegetables several times a day. Stress-free people eat fruits and vegetables every 2–3 days in a week.

Many researchers (Schmidt, 2019; Törőcsik, 2016) have noted that plant-based diets have become an increasingly popular dietary trend in recent years due to health and sustainability concerns. Plant-based diets aim to marginalize animal and highly processed foods and include mostly raw, unprocessed, or minimally processed plant foods in the diet (Ostfeld, 2017; Szabó et al., 2016). Plant-based diets range from strictly plant-based (vegan) to semi-vegetarian. The majority of healthy food choosers, red meat avoiders, stress-free women, and rejecters thought that plant-based diets were similar to vegan and vegetarian diets. The majority of respondents believed that a vegetarian diet meant avoiding white and red meat and fish, while eating other foods of animal origin such as eggs and dairy products. Regarding vegan diets, the majority knew that not all foods of animal origin could be consumed, especially those who avoided red meat. The majority of women thought about trying PBDs. Red meat avoiders, healthy food choosers, and stress-free women had more positive attitudes toward PBDs than did avoiders. Women acknowledged the positive health effects of this diet, such as good for digestion and prevention of chronic disease, as previous studies have confirmed (Beacom et al., 2021; Véha et al. 2019b). Sustainability is a major advantage of the plant-based diet, which is why many researchers currently started to deal with this topic (Fehér et al., 2020, Havemnans et al., 2021; Véha et al., 2019b). In this empirical research, women admitted that PBD is environmentally friendly. More authors emphasized the negative effect of plant-based diet (Pohjolainen et al., 2015; Reipurth et al., 2019; Véha et al., 2019a). The respondents were not aware of the fact what kind of nutrition can be missed with this diet.

Women viewed a plant-based diet as healthy, safe, varied, exciting, fashionable, easy, environmentally friendly, digestible, and satisfying. Rejecters attached more negative attributes to the PBD than did healthy eaters, red meat avoiders, and stress-free women. As Havermans et al. (2021) and Pohjolainen et al. (2015) emphasized that the taste of plant-based foods could be a barrier in the adaptation process, we also found that rejecters perceived the meatless diet as unhealthy, dangerous, monotonous, boring, environmentally unfriendly, difficult to digest, and malnutrition.

#### Conclusions

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The aim of this research was to explore the perceptions of healthy eating in Hungary There are some educational initiatives and programs targeted at the young generation by the national government, and public education related to eating behavior (smart plate, school lunch reform and school fruit program) and physical activity (mandatory daily physical education). At the same time, the attitudes and behaviors of the Hungarian population are slowly changing, and there is a large gap between recommendations and actual eating habits. The present study is useful for the health sector (physicians, dieticians and nutritionists) and the government, as targeted marketing programs can be planned to change dietary behavior.

- 1. In the case of the government, social marketing programs would be necessary. It is also important to raise health awareness among the rejecters.
- 2. To improve people's quality of life and well-being, it is necessary to improve their health. Health is not just a state of being free from disease or injury, but a more complex category.
- 3. A healthy mind in a healthy body should be emphasized in social marketing campaigns. Reducing overweight and obesity is the goal of society as a whole (especially in

developed countries, where this phenomenon is considered an epidemic), so it is necessary to educate people and use social marketing campaigns.

- 4. People should be informed about the progress of more PBDs and the principles of a healthy lifestyle (food pyramid, smart plate, WHO principles). These applications could be developed in collaboration with doctors, dieticians and nutritionists.
- 5. It is in the interest of society to educate people and change their unhealthy habits. Not only the individual level, but also the national economic interest such as sustainability issues should be emphasized. The social marketing campaign aims to change people's attitudes and behaviors.

This paper has limitations. First, the sample was not representative. There is little age bias in the sample. Mainly young women, students and white-collar workers could be reached by the online survey. However, previous studies have highlighted the importance of age and educational level in relation to PBDs. We focused mainly on a regional sample, people living in the western part of Hungary, which is considered to be more developed economically and in terms of quality of life. On the other hand, the survey was shared in different online social media groups, so the respondents were engaged in health issues at a higher level.

In the future, we would like to analyze men as well. Comparing the knowledge, beliefs, attitudes and perceptions of men and women would also be an interesting research question. An intercultural study comparing the beliefs, knowledge, attitudes and perceptions of people from different cultures would be interesting.

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# Determination of ejection coefficient of liquid-gas ejector with combined mixing chamber

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Abstract

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**Introduction.** The aim of the research was to develop a method for determining the actual ejection coefficient of a liquid-gas ejector with a combined mixing chamber.

**Materials and methods.** Theoretical calculation methods were used (balance equations of mass and energy in the form of the Bernoulli equation and the laws of hydrodynamics), experimental methods (ejection coefficients of an ejector with a combined mixing chamber were experimentally determined on a hydraulic bench in order to determine the experimental constant). The Sokolov-Zinger graph-analytical method was used to compare the ejection coefficients.

**Results and discussions.** A characteristic feature of the ejector with a combined mixing chamber is the presence of the initial conical and subsequent cylindrical parts of the mixing chamber. The angle of opening of the conical part is  $3-8^{\circ}$  less than the angle of the flame of spraying liquid from the nozzle. Such a design reduces the hydraulic resistance to the entry of liquid and prevents the formation of back-circulation flows. The ejection coefficient of a jet device with a combined mixing chamber is by 15–55% higher than that of an ejector with a cylindrical mixing chamber.

The joint solution of the balance equations for the conical and cylindrical parts of the mixing chamber, taking into account the energy losses in each, makes it possible to determine theoretical flow rates of the phases in the ejector for different operating modes.

The coefficient k takes into account the influence of energy redistribution between phases during ejection and the design of the mixing chamber. When the pressure increases from 0.05 to 0.25 MPa coefficient k increases from 3.6 to 4.8 in a rational exponent function.

The effective ejection coefficient is defined as the product of the theoretical ejection coefficient and the experimental constant, while the error does not exceed 5%.

**Conclusions.** The proposed calculation method allows you to determine the effective ejection coefficient of a liquid-gas ejector with a combined mixing chamber.

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#### Introduction

Liquid-gas ejectors (jet devices) are characterized by high intensity of processes in jet streams (Balamurugan et al., 2007; Liu, 2014). Considerable interest in these devices is caused by their indisputable advantages: simplicity of design, absence of tribological problems, compactness, the possibility of installation in any place of the production premises, minimal needs for maintenance and repair (Ivanov et al., 2021; Kong et al., 2015; Sliusenko et al., 2021).

Such devices are widely used in various industries for heat and mass exchange processes (Tashtoush et al., 2019), in particular during sulfitation of liquids in the sugar industry (Ponomarenko et al., 2015). The sulfitation process (treatment of water or sugar solutions with sulfite gas with an SO<sub>2</sub> content of 10-15%) is one of the main technological processes in the sugar production. Traditionally, it was carried out in bubbling apparatus by blowing gas through the juice layer. Due to the significant disadvantages of such devices, jet ejection devices with high intensity of mass transfer processes were introduced into the technological process and miscalculations in the design, they had a number of disadvantages: insufficient processing efficiency due to the use of ejectors with a compact liquid jet, low SO<sub>2</sub> utilization rate, significant air pollution with harmful emissions.

To carry out such processes, a design of a liquid-gas ejector with a conical-cylindrical (combined) mixing chamber was developed and patented (Ponomarenko V.V., Sliusenko A. M. (2020), *Liquid-gas ejector, UA Patent 122296*; Riffat et al., 2005). Such a jet device at moderate pressures of liquid supply to the working nozzle (0.1-0.3 MPa) allows increasing the ejection coefficient *k* (ratio consumption of the passive flow to the flow of the active flow) in comparison with an ejector with a cylindrical mixing chamber (Sliusenko et al., 2021).

It should be noted that the ejection coefficient is the main operating characteristic of jet devices (Riffat et al., 2005), needed in analyzing the functioning of ejectors, their revision and design. To determine the ejection coefficient of jet devices, various methods are used, which are divided into several main groups (Elbel and Lawrence, 2016; Zegenhagen et al., 2015):

- Methods based on balance equations (Sun et al., 1995)
- Empirical dependencies (Zhu et al., 2008)
- Theoretical dependencies obtained because of solving the equations of non-separability, amount of motion, energy and state using experimental constants (Ismagilov et al., 2016).

Combined methods of calculations with the use of balance equations of mass and energy and the introduction of empirical coefficients (test constants), in particular, coefficients of energy loss, resistance, and friction (Aidoun et al., 2019; Chen et al., 2020; Zhu et al., 2008).

Since the liquid-gas ejector with a combined mixing chamber is innovative (Ponomarenko V.V., Sliusenko A. M. (2020), *Liquid-gas ejector*, UA Patent 122296), there is no method that allows to determine the k, taking into account its design features (the initial conical part of the mixing chamber).

The purpose of the research was to develop a method for determining the actual ejection coefficient of a liquid-gas ejector with a combined mixing chamber based on mathematical modeling of the ejection process using mass and energy balance equations and an empirical coefficient (research constant).

#### Materials and methods

The subject of research was an ejector with a conical-cylindrical mixing chamber, the object of research was the ejection processes that take place in it.

Theoretical and experimental research methods were used to achieve the defined goal.

#### **Theoretical methods**

Mathematical modeling of the ejection process in a liquid-gas ejector with a combined mixing chamber was carried out on the basis of the equations of conservation of mass and energy (the law of conservation of energy is written in the form of the Bernoulli equation) and using the laws of hydrodynamics.

For mathematical modeling, a calculation scheme of the ejector (Figure 1) was developed and characteristic of cross-sections were determined (cross-section I-I – at the nozzle section; cross-section II-II – the point of connection of the conical and cylindrical parts of the mixing chamber; cross-section III-III – exit from the chamber mixing of the mixture, in which the parameters of the two-phase flow acquire a constant value).





Flows in the mixing chamber of the ejector are characterized by the following parameters (the upper index shows which section the indicator refers to):

$P_l^I$ – fluid pressure at the outlet of the	$V_g^I$ – gas velocity at the entrance to the
working nozzle;	mixing chamber;
$P_g^I$ – pressure (reduction) in the receiving	$V_{\scriptscriptstyle mix}^{\scriptstyle II}$ – the velocity of the liquid-gas
chamber; $P_{mix}^{II}$ – the pressure of the liquid-gas mixture at the boundary of the conical and cylindrical parts of the mixing chamber; $P_{mix}^{III}$ – the pressure of the liquid-gas mixture at the exit from the mixing chamber; $V_l^I$ – liquid speed at the exit from the working nozzle;	<ul> <li>mixture at the boundary of the conical and cylindrical parts of the mixing chamber;</li> <li>V<sup>III</sup><sub>mix</sub> – is the velocity of the liquid-gas mixture at the exit from the mixing chamber.</li> <li>Subscripts: <i>I</i> – liquid, <i>g</i> – gas, <i>mix</i> – mixture.</li> <li>Superscripts: <i>I</i> – section <i>I-I</i>, <i>II</i> – section <i>II-II</i>, <i>III</i> – section <i>III</i>-<i>III</i>.</li> </ul>

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#### **Experimental methods**

The ejector with a conical-cylindrical mixing chamber (Figure 2) is a receiving chamber 2 with a cover 4 and gas phase inlet nozzles 3, to which a conical adapter 5 with an expansion angle of  $25^{\circ}$  is attached. The cylindrical part of the glass-mixing chamber 6 was installed coaxially to it. The joint between them was sealed.



#### Figure 2. Ejector with a combined mixing chamber (schematic image): 1 – injector nozzle; 2, 4 – cover and housing of the reception chamber; 3 – inlet pipe; 5 – conical part of the mixing chamber; 6 – cylindrical part of the mixing chamber

The working nozzle of the ejector is a centrifugal jet nozzle with inclined inlet channels (Figure 3). Such a nozzle has a liquid spray torch angle of 29°. Since the angle of expansion of the conical part of the mixing chamber is 25°, the condition is ensured when it is by 3-8° smaller than the angle of the liquid spray torch (Ponomarenko et al. 2015; Sliusenko et al., 2021).

The main dimensions of the nozzle: nozzle diameter  $-d_n = 4$  mm, length of the nozzle channel  $l_n = 5$  mm, diameter of the nozzle twisting chamber -10 mm. The nozzle is made according to the seventh quality of accuracy. The roughness of the surfaces of the twisting chamber and supply channels is Ra = 1.25, and the nozzle channel is Ra = 0.16 (grinding).

With the adopted dimensions, the main geometric parameter of the ejector m (the ratio of the area of the mixing chamber with a diameter of  $D_{m.c} = 19$  mm to the area of the nozzle nozzle) is m = 22.56.

A detailed description of the experimental set up and research methodology can be found in (Sliusenko et al., 2021). The experimental installation consisted of a liquid tank, a pump, a system of pipelines, shut-off and regulating valves and was equipped with control and measuring devices: liquid flow meter KV-1.5, gas flow meter PREMA G 1.6, manometer OBM1-160, differential manometer.

Figure 3. 3-D model of a centrifugal jet nozzle with two inclined inlet channels: 1 – liquid inlet pipe; 2 – housing; 3 – vortex chamber; 4 – nozzle; 5 – supply channel; 6 – plunger

Changing the working characteristics of the ejectors was carried out by adjusting the liquid pressure in the working nozzle (in the range of 0.05-0.25 MPa), which was controlled by a manometer.

Processing of experimental data was carried out by well-known methods (exclusion of gross errors according to the Student's criterion at a significance level of 0.05, the result was reduced to the arithmetic mean value).

To compare the obtained results of determining the ejection coefficient for the ejector with a combined mixing chamber with the grapho-analytical method of Sokolov-Zinger (Shestopalov et al., 2016; Wang et al., 2023), the value  $\Delta p_c / \Delta p_p$  was found for the pressures in the control sections and the main geometric characteristic of the ejector  $m = D_{m,c}^2/d_n^2$  was determined. According to the graphs, at the corresponding values of  $\Delta p_c / \Delta p_p$  and m, the ejection coefficients were determined.

#### **Results and discussion**

#### Justification of the design of the ejector with a combined mixing chamber

Analysis of the liquid flow in the initial part of the ejector (Han et al., 2018; Kandakure et al., 2005) showed that the conical expansion of the mixing chamber (its initial part) leads to a decrease in hydraulic resistance and, accordingly, an increase in the useful energy of the liquid jet for gas ejection. At the same time, the opening angle of the diffuser is taken in the range of  $3-8^\circ$ , at which minimum hydraulic losses and uninterrupted movement of liquid (Bi et al., 2017; Lu et al., 2010; Smyk et al., 2010). It should be noted that the proposed opening angle of the diffuser depends on the type of nozzle through which the liquid flows out (the specified angle is relevant for the flow of liquid through the hole in the form of a compact jet of liquid).

Based on our own preliminary calculations and experimental studies, the mixing chamber is made of a combined one – with an initial conical and subsequent cylindrical

sections. The opening angle of the conical part is  $3-8^{\circ}$  smaller than the angle of the liquid spray torch (Ponomarenko V.V., Sliusenko A. M. (2020), Liquid-gas ejector, *UA Patent 122296*). With this design, the annular gap between the torch of the atomized liquid and the conical wall at the beginning of the receiving chamber ensures guaranteed ejection of the gas phase and contributes to the creation of a zone of high rarefaction (Singh et al., 2019).

The transition to the cylindrical part of the mixing chamber of the ejector takes place at the point of impact of liquid drops against its walls, which ensures the operation of the ejector without the formation of reverse circulation flows (Tang et al., 2019). The diameter of the cylindrical mixing chamber is selected from the condition of achieving the maximum ejection coefficient (optimal value of the main geometric parameter of the ejector) (Al-Manea and Al-Jadir, 2021; Yan et al., 2022). Thus, this leads to an increase in the efficiency and k cone of the ejector.

When the opening angle of the conical part of the mixing chamber is less than  $3^{\circ}$  for the angle of the liquid spray torch, liquid drops will fall on the conical wall and move along it, which leads to an increase in hydraulic resistance (Wang et al., 2019). If the angle of expansion of the conical part of the mixing chamber is greater than  $8^{\circ}$  of the angle of the liquid spray torch, the liquid drops will not touch the conical walls, the gap between the inner surface of the conical part of the mixing chamber and the outer surface of the spray torch will increase, reverse circulation flows will occur, and the ejection coefficient will decrease (Lu et al., 2010).

#### Mathematical modeling of the ejection process in an ejector with a conicalcylindrical mixing chamber

For mathematical modeling of the ejection process in the ejector with a combined mixing chamber, two characteristic zones were chosen (Figure 1).

Zone I – passive flow ejection zone (conical section of the mixing chamber between sections I - I and II - II).

$$0 < y < l_{c.p}$$
,

where  $l_{c,p}$  – length of the conical part of the mixing chamber.

#### Boundary conditions for the section I - I.

The conical part of the mixing chamber also serves as the receiving chamber of the ejector. In this zone, due to the friction between the phases, part of the energy is converted into heat (Lu et al., 2010). However, in the first approximation, we will not consider these transient processes.

The speed of the liquid from the nozzle of the nozzle is found from the expression:

$$V_l^I = \mu \cdot \sqrt{\frac{2 \cdot \Delta P}{\rho \cdot_l}}, \qquad (1)$$

and fluid consumption ( $Q_l = V_l^I \cdot f_n$ ):

$$Q_l = \mu f_n \sqrt{\frac{2 \cdot \Delta P}{\rho_l}}, \qquad (2)$$

where  $\mu$ -injector flow rate;  $f_n$ -area of the nozzle;  $\Delta P$ - the pressure difference under which the liquid is sprayed from the nozzle of the nozzle;  $\rho_l$ -density of the liquid.

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The gas phase is ejected from the receiving chamber of the ejector, which moves along with the active flow. Let's write down the mass balance equation for the input *I-I* and output *II-II* sections:

$$m_l^I + m_g^I = m_{mix}^{II} = m_{mix}^{III}$$
 (3)

Note that the mass of the mixture at the end of the first zone  $m_{mix}^{II}$  is equal to the mass of the mixture at the exit from the ejector  $m_{mix}^{III}$ .

The mass flow of each of the flows can be found if its speed in the given section is known:

$$m_i = \rho_i \cdot V_i \cdot F_i \,, \tag{4}$$

where  $\rho_i$  – density of the *i*-th phase;

 $V_i$  – velocity of the corresponding phase in the *i*-th section;

 $F_i$  – area of the *i*-th section.

Express the mass flow of liquid in the inlet section I - I:

$$m_l^I = \rho_l \cdot V_l^I \cdot f_n = \rho_{l} \cdot \mu \cdot \sqrt{\frac{2 \cdot \Delta P}{\rho_l}} \cdot \frac{\pi \cdot d_n^2}{4}.$$
 (5)

Mass flow of the gas phase in the inlet section I - I:

$$m_g^I = \rho_g \cdot V_g^I \cdot f_{c.p} = \rho_g \cdot V_g^I \cdot \frac{\pi}{4} \cdot \left( D_{c.min}^2 - d_n^2 \right), \tag{6}$$

where  $\rho_g$  – density of the gas;

 $f_{c.p}$  – the area of the annular channel (gap) between the nozzle and the beginning of the conical part of the mixing chamber.

Zone 2 is a zone of simultaneous movement of phases (cylindrical part of the mixing chamber between sections *II* - *II* and *III* - *III*).

In this zone, there is a compatible movement of phases to the exit from the mixing chamber with the alignment of all kinematic and energy characteristics (Yan et al., 2022). If the liquid and gas at the beginning of the zone in section II - II have different velocities, then at the exit in section III - III, the flow rate of the phases does not change, but only their redistribution along the cross section and equalization of velocities occurs.

The mass flow rate of the mixture in the initial section III – III:

$$m_{mix}^{III} = \rho_{mix} \cdot V_{mix}^{III} \cdot F_{m.c} = \rho_{mix} \cdot V_{mix}^{III} \cdot \frac{\pi \cdot D_{m.c}^{2}}{4}.$$
 (7)

where  $D_{m.c}$  – diameter cylindrical part of the mixing chamber.

The energy balance equation in the form of Bernoulli's equation (Sliusenko et al., 2021)

(the energy balance is not related to weight, but to a unit volume of liquid  $V = \frac{m}{\rho}$ ) for

sections I-I and III-III:

$$\frac{\left(V_{l}^{I}\right)^{2}}{2 \cdot g} + \frac{\left(V_{g}^{I}\right)^{2}}{2 \cdot g} = \frac{\left(V_{mix}^{III}\right)^{2}}{2 \cdot g} + \Delta p , \qquad (8)$$

where g – acceleration of gravity;

 $\Delta p$  – specific losses of flow energy between sections *I* - *I* and *III* - *III* 

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$$\Delta p = \Delta p^{I-II} + \Delta p^{II-III} , \qquad (9)$$

where  $\Delta p^{I-II}$  – specific input energy losses of the phases between sections *I*–*I* and *II*–*II*;

 $\Delta p^{II-III}$  – specific energy losses of the mixture between sections *II-II* and *III-III* (along the length of the cylindrical part of the mixing chamber).

Energy losses in the conical ejection zone are determined by Bord's formula:

$$\Delta p^{I-II} = \varsigma \cdot \frac{\left(V_l^I\right)^2}{2 \cdot g},\tag{10}$$

where  $\varsigma$  – total resistance coefficient of the conical section:

$$\varsigma = \varsigma_e + \varsigma_{c.p}, \tag{11}$$

where  $\varsigma_e$  – coefficient of resistance to expansion;  $\varsigma_{c.p}$  – the resistance coefficient along the length of the conical part can be determined by the formula:

$$\varsigma_{c.p} = \lambda \cdot \frac{l_{c.p}}{D_{c.p}}, \qquad (12)$$

where  $\lambda$  – resistance coefficient along the length of the pipe (depends on the viscosity and roughness of the pipe);

 $D_{c.p}$  – the average diameter of the conical part of the ejector:

$$D_{c.p} = \frac{3D_{c.\min} + D_{c.\max}}{4}$$

Energy losses in the cylindrical part of the mixing chamber  $\Delta p^{II-III}$ :

$$\Delta p^{II-III} = \varsigma_c \frac{\left(V_{mix}^{III}\right)^2}{2 \cdot g}, \qquad (13)$$

where  $\varsigma_c$  – resistance coefficient along the length of the cylindrical part of the mixing chamber:

$$\varsigma_c = \lambda \cdot \frac{l_c}{D_{m,c}},\tag{14}$$

where  $l_c$  – length of the cylindrical part of the mixing chamber.

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The length of the mixing chamber is chosen so that the velocity of the liquid and gas phases at its exit are the same:

$$V_g^{III} = V_l^{III} = V_{mix}^{III}$$

We also note that the mass flow rate of both gas and liquid phases in the cross section *III–III* is equal to the mass flow rate of these phases in the cross section *II–II*. The cylindrical part of the mixing chamber is intended only for transporting phases and equalizing flow characteristics (velocities, cross-sectional concentrations) due to exchange processes between them (Yan et al., 2022).

After writing down all the balance equations, we will find one of the main characteristics of the ejector (Ponomarenko et al., 2015) – the consumption of the gas phase, and therefore the ejection coefficient.

The mass flow rate of the gas phase is determined from the mass balance equation (3):

$$m_g^I = m_{mix}^{III} - m_l^I . (15)$$

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In order to determine the mass of the mixture at the exit from the mixing chamber (section *III–III*), it is necessary to determine the speed  $V_{mix}^{III}$ . The latter can be found from equation (8):

$$\frac{\left(V_{mix}^{III}\right)^2}{2\cdot g} = \frac{\left(V_l^{I}\right)^2}{2\cdot g} + \frac{\left(V_g^{I}\right)^2}{2\cdot g} - \Delta p$$

After the transformation, we have:

$$V_{mix}^{III} = \sqrt{\left(V_l^I\right)^2 + \left(V_g^I\right)^2 - 2 \cdot g \cdot \Delta p} . \tag{16}$$

From the mass balance equation (3), substituting the corresponding expressions:

$$V_g^I \cdot \rho_g \cdot \frac{\pi}{4} \cdot \left( D_{c.min}^2 - d_n^2 \right) = V_{mix}^{III} \cdot \rho_{mix} \cdot \frac{\pi \cdot D_{m.c}^2}{4} - \mu \cdot \rho_l \cdot \frac{\pi \cdot d_n^2}{4} \cdot \sqrt{\frac{2 \cdot \Delta P}{\rho_l}}$$
(17)

we will find the velocity of the gas phase.

The density of the mixture depends on:

$$\rho_{mix} = \beta \cdot \rho_l + (1 - \beta) \cdot \rho_g \tag{18}$$

where  $\beta$  – liquid content per unit volume of the mixture.

Let's perform a number of simple transformations and enter the notation of expressions:

$$a = \rho_g^2 \cdot f_{c,p}^2 - \rho_{mix}^2 \cdot F_{m,c}^2, \qquad (19)$$

$$b = 2 \cdot \rho_g \cdot f_{c.p} \cdot (V_l^I) \cdot \rho_l \cdot f_n , \qquad (20)$$

$$\boldsymbol{c} = \left(V_l^I\right)^2 \cdot \rho_l^2 \cdot f_n^2 - \left(V_l^I\right)^2 \cdot \rho_{mix}^2 \cdot F_{m.c}^2 + 2 \cdot \Delta p \cdot \boldsymbol{g} \cdot \rho_{mix}^2 \cdot F_{m.c}^2, \qquad (21)$$

Equation (17) will take the form:

$$a \left( V_g^I \right)^2 + b \left( V_g^I \right) + c = 0.$$
<sup>(22)</sup>

Its roots:

$$(V_g^I)_{1,2} = \frac{-b \pm \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}.$$
 (23)

One of the roots, as the calculations show, has a negative value and it is not accepted in the further calculation.

After finding the speed of the gas phase at the entrance to the mixing chamber, its flow rate is determined:

$$Q_g = \left(V_g^I\right) \cdot f_{c.p} \,. \tag{24}$$

With a known flow rate of the gas phase, the ejection coefficient is unambiguously found.

Thus, the proposed calculation method allows you to determine the flow rate of the gas phase, and therefore the ejection coefficient of the liquid-gas ejector with a conicalcylindrical mixing chamber.

#### Model validation

The adequacy of the mathematical model is checked by comparing the calculated ejection coefficient with its experimental value.

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The algorithm for finding the ejection coefficient according to the proposed by us mathematical model is as follows.

1. Given the known nozzle flow coefficient  $\mu$  and the pressure under which spraying  $\Delta P$  occurs, determine the fluid velocity at the exit from the injector nozzle according to equation (1)

$$V_l^I = \mu \cdot \sqrt{\frac{2 \cdot \Delta P}{\rho_l}} \,.$$

2. With a given diameter of the injector nozzle, determine the volume flow of liquid according to equation (2)

$$Q_l = \mu \cdot f_n \cdot \sqrt{\frac{2 \cdot \Delta P}{\rho_l}}$$

3.Determine the energy losses in the conical part of the mixing chamber according to formula (10)

$$\Delta p^{I-II} = \varsigma \cdot \frac{\left(V_l^I\right)^2}{2 \cdot g}.$$

4. Find the total resistance coefficient  $\varsigma$  of the conical part, if all the structural dimensions of the ejector are known.

5. Determine the energy losses in the cylindrical part of the mixing chamber according to formula (13)

$$\Delta p^{II-III} = \varsigma_c \frac{\left(V_{mix}^{III}\right)^2}{2 \cdot g}$$

6.Calculate the total energy losses in the ejector mixing chamber according to formula (9):

$$\Delta p = \Delta p^{I-II} + \Delta p^{II-III} \ .$$

7.Determine the unknowns *a*, *b*, *c* using formulas (19-21):

$$a = \rho_g^2 \cdot f_{c.p}^2 - \rho_{mix}^2 \cdot F_{m.c}^2,$$
  

$$b = 2 \cdot \rho_g \cdot f_{c.p} \cdot (V_l^1) \cdot \rho_l \cdot f_n,$$
  

$$c = (V_l^1)^2 \cdot \rho_l^2 \cdot f_n^2 - (V_l^1)^2 \cdot \rho_{mix}^2 \cdot F_{m.c}^2 + 2 \cdot \Delta p \cdot g \cdot \rho_{mix}^2 \cdot F_{m.c}^2.$$

8. If *a*, *b*, *c* are known, determine the gas velocity at the entrance to the mixing chamber according to formula (23)

$$\left(V_g^I\right)_{1,2} = \frac{-b \pm \sqrt{b^2 - 4 \cdot a \cdot c}}{2 \cdot a}$$

9.Calculate the volume flow of gas at the entrance to the conical part of the mixing chamber according to formula (24)

$$Q_g = \left(V_g^I\right) \cdot f_{c.p}.$$

10. With known volume flow rates of liquid and gas, determine the volume coefficient of ejection of the liquid-gas ejector according to the formula:

$$k = Q_g / Q_l$$
.

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Comparative data of the results of calculating ejection coefficients based on the developed mathematical model and experimental data (own research) for an ejector with a conical-cylindrical (combined) mixing chamber are presented in Table 1. As for the accuracy of the obtained results, the calculations were performed with accuracy to the fourth significant digit. Experimental studies were carried out at least three times, the exclusion of gross errors was carried out according to the Student's criterion at a significance level of 0.05, and the average arithmetic value of the ejection coefficient was taken.

The work of the ejector is characterized by accompanying processes (cavitation, shock waves, liquid evaporation) (Besagni et al., 2017; Jafarian et al., 2016), which are accompanied by redistribution of energy and its losses, which are extremely difficult to take into account analytically. Therefore, to correlate the calculated ejection coefficient, an experimental constant was found and its average value was determined.

Table 1

No of	Liquid supply	Ejection	Correction	
experiment	pressure P, MPa	calculated	experimental	factor
1	0.05	1.152	3.652	3.17
2	0.1	1.208	3.908	3.235
3	0.15	1.254	4.295	3.425
4	0.2	1.305	4.728	3.622
5	0.25	1.361	4.825	3.545
Average value	-	-	-	3.4

Comparative data of calculated ejection coefficients with experimental ones

The graph of the dependence of the ejection coefficient (experimental and calculated) on the liquid pressure with the average value of the experimental constant equal to 3.4 is presented in Figure 4.





At the nominal operating mode of the ejector of 0.1-0.2 MPa, the error in determining the ejection coefficient does not exceed 5%.

A comparison of own results of determination of ejection coefficients with results of other researchers was carried out. When determining the ejection coefficient according to the data of Sokolov-Zinger (Shestopalov et al., 2016; Wang et al., 2023) when using the theoretical pressure characteristic (graph of the dependence of the ejection coefficient on the pressure) for an ejector with the main geometric characteristic according to our researches  $m = D_{m.c}^2/d^2_n = 19^2/4^2 = 22.56$  the maximum value of the ejection coefficient reaches a value of 4.8 when:

$$\Delta p_c / \Delta p_p = (p_c - p_l) / (p_p - p_l) = 0.038,$$

where  $p_c$  – pressure of the mixture at the exit from the ejector, MPa ( $p_c = 0.1$  MPa);

 $p_p$  – pressure of the active medium, MPa ( $p_p = 0.25$  MPa);

 $p_l$  – pressure of a low-pressure medium, MPa ( $p_l = 0.006$  MPa).

This value of the ejection coefficient was recorded by us during experimental studies of ejection processes in an ejector with a combined mixing chamber at the pressure of the active medium  $p_p = 0.25$  MPa.

When the pressure of the active medium increases, the ejection coefficient of the innovative ejector reaches higher values than the k ejector with a cylindrical mixing chamber (Ponomarenko et al., 2020), which is explained by the presence of the initial conical part of the mixing chamber, as a result of which the formation of reverse-circulation flows of phases is prevented.

#### Conclusions

- 1. The selection of the main dimensions of the innovative ejector with a conical-cylindrical mixing chamber is justified. In particular, the angle of the conical part of the ejector is taken to be 3-8° smaller than the angle of the liquid spray plume, which ensures the operation of the ejector works without the formation of reverse circulation flows.
- 2. A mathematical model of the ejection process based on the mass and energy balance equations is proposed (the energy balance is written in the form of the Bernoulli equation) and an algorithm for finding the main operating characteristic of the ejector the theoretical ejection coefficient is given.
- 3. The average value of the experimental constant is set, which is equal to 3.4, which allows determining the valid ejection coefficient.
- 4. The ejector with a combined mixing chamber is recommended for use in mass transfer processes of food production, in particular during sulfitation of sugar production liquids, which is explained by its higher ejection coefficient by 15-55% compared to the ejector with a cylindrical mixing chamber.

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### Intensification of water jet cutting process in deepfrozen food products

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#### Abstract

**Introduction.** The study aims to enhance the hydro-cutting process by modifying the working fluid using water, water-ice and water-nitrogen jets when cutting deep-frozen food products.

**Materials and methods.** *Materials:* Hake fish fillets, beef meat, and ice samples at -30 °C as a model sample of meat at -25 °C. *Experimental Methods:* Hydro-cutting unit is utilized to cut food products with a water jet. The experiments were conducted at food temperatures ranging from -3 to -25 °C, with pressure variations from 50 MPa to 500 MPa, nozzle diameters of  $0.2 \cdot 10^{-3}$  to  $0.8 \cdot 10^{-3}$  m and the speed of movement of the jet of the working liquid relative to the sample of the food product of 0.01 to 0.07 m/s.

**Results and discussions.** It was found that the increasing the pressure from 200 to 500 MPa results in a nearly 21-fold and 6fold increase in the depth of cut for nozzles with diameters of  $0.2 \times 10^{-3}$  and  $0.4 \times 10^{-3}$  m, respectively. Additionally, increasing the nozzle opening diameter leads to an increase in the depth of cut in the frozen food product. At the same time, the maximum cutting depth in the food product at the temperature of -11 °C did not exceed  $82,5 \times 10^{-3}$  m with the pressure of 500 MPa. Reducing the temperature of the food product to -11 °C and below excludes the possibility of using water jet cutting at the pressure of 250-300 MPa, and the necessity of creating pressures above 300 MPa causes a sharp increase in the cost of water jet cutting equipment. Experimental verification was conducted to evaluate the feasibility of using a water jet with small ice particles to enhance the hydro-cutting. The process of hydro-cutting deep-frozen food products can be intensified by adding small ice particles to the water jet, which is highly effective but also expensive. Alternatively, a water-nitrogen jet that employs ice microparticles as an abrasive, formed by cooling the water jet with liquid nitrogen vapour, is a costeffective way to significantly improve the water jet cutting process for deep-frozen food products.

**Conclusions.** To significantly intensify the hydro-cutting process of deep-frozen food products, it is most expedient to use a water-nitrogen jet as the working fluid, in which ice microparticles are formed in the process of cooling a water jet with vapours of liquid nitrogen.

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#### Introduction

The development of innovative cutting equipment poses a significant challenge to the food industry. The current methods and equipment utilized presents significant drawbacks. These include equipment maintenance hazards, high noise and vibration levels, unsatisfactory sanitary conditions, potential introduction of metal microparticles, rapid dullness and the need for frequent sharpening and replacement of the working tool, wide cutting widths resulting in additional product loss, difficulties in cutting of deep-frozen food products, and others (Cui et al., 2022; Xu et al., 2022).

The XX century brought numerous ground-breaking discoveries to humanity, including the technology of water jet cutting, which was hailed as the future three decades ago. Currently, this technique is widely utilized across several industries. If a thin water jet increases its velocity and energy to cause erosion of a material, it can have a cutting effect on any material (Duspara et al., 2018; Gyliene et al., 2014). Hence, water jet cutting process can be an alternative method of cutting food products, particularly at low temperatures, without the mentioned drawbacks. The hydro-cutting method's high manufacturability compared to traditional ones is the mail reason for its widespread use. However, the lack of comprehensive research on the process of food water jet cutting and equipment development hinders its implementation in the food industry (Ranjan et at., 2022; Xu et al., 2022).

One of the main benefits of water jet technology is the computerized control of the water jet cutting process, enabling 3D food processing. Utilizing servos, the water jet cutting head can process the food product from various sides, while making cuts of any complexity, at any location, and with programmed changes in the parameters of hydro-cutting food products. This will enable a traditional robotic conveyor line for primary processing to be streamlined into one (or more) processing points, where the water jet cutting head can execute multiple operations simultaneously (Xu et al., 2022).

Hydro-cutting is a thin, high-speed liquid jet that serves as the cutting tool for food. The properties of the working fluid determine the water jet's ability to acquire the necessary hydrodynamic features that guarantee optimal productivity and the highest cutting surface quality while consuming minimum energy for jet formation. Minimizing energy costs should involve reducing the working pressure of the fluid in front of the nozzle to its lowest possible value, while also meeting the technological requirements for cutting the product. Determining the type and composition of the working fluid is a key point in the development of a technological process for water jet cutting of deep-frozen food products. The relevance of the study is also due to the fact that lowering the temperature of meat, fish and other food products to -11 °C excludes the possibility of using water cutting at pressures less than 250–300 MPa, meanwhile the use of higher pressures is economically unprofitable (Pogrebnyak, 2020; Ranjan et at., 2022).

The aim of the research was to intensify the water jet cutting process by using a water jet with ice particles and a water-nitrogen jet when cutting deep-frozen food products.

#### Materials and methods

#### Materials

Hake fish fillets and beef meat were used in the study. The meat samples were obtained by cutting pieces of muscle from a medium-fat adult cow and then combined into desired sizes. For the fish samples, multiple layers were combined to achieve the desired size. As a model for meat samples at - 25 °C, ice samples at - 30 °C were used. It was shown that the most accurate criterion for measuring the resistance of frozen food products to water jet cutting is their uniaxial compression strength. Thus, the use of ice as a model for meat samples in water jet cutting experiments is justified, as the uniaxial compressive strength of ice at -  $30 \,^{\circ}$ C and meat frozen to -  $25 \,^{\circ}$ C is identical.

#### **Experimental research methods**

Water jet cutting unit with a maximum working pressure of 500 MPa (Pogrebnyak et al., 2020) that was able to alter and regulate integral and differential parameters during the food-cutting process using a water jet. In order to achieve the required low temperatures (of up to - 40 °C), utilized customized thermostatization and cooling system for the receiver filled with a working fluid (Pogrebnyak et al., 2017, 1992). The temperature was maintained at the specified level with precision up to  $\pm 0.1$  °C.



## Figure 1. Schematic diagram of the technological process for obtaining a water jet containing ice particles as an abrasive

The error in determining (in %) was: for the depth of cutting  $-\pm 0.5 \div 1$ ; for the velocity of movement of the jet of the working liquid relative to the sample of the food product  $-\pm 1$ ; for the temperature of the food product  $-\pm 0.5$ ; for the pressure  $-\pm 1$ .

#### **Results and discussion**

#### Pure water jet cutting

The application of pure water jets as cutting tools for food products has proven to be advantageous. Table 1 displays results from hydro-cutting of frozen fish.

d₀, m	Depth of cut, h, m×10 <sup>-3</sup> , at ΔP, MPa								
	100	150	200	250	300	400	500		
0.2 x 10 <sup>-3</sup>	0.6	1.1	3.0	5.1	10.2	38.1	61.8		
0.3 x 10 <sup>-3</sup>	2.9	4.5	7.2	13.6	21.4	48.8	69.3		
0.4 x 10 <sup>-3</sup>	5.9	10.6	14.6	19.2	34.9	62.5	82.5		
0.6 x10 <sup>-3</sup>	16.7	21.1	25.7	35.3	-	-	-		
0.8 x10 <sup>-3</sup>	23.6	31.4	36.7	-	-	-	-		

Water pressure effect on depth of cut in block samples from hake fillets at temperature -11°C

Table 1

Table 2

Note: d<sub>o</sub> is a nozzle diameter

The velocity of the water jet (Vn) remained constant at  $25 \times 10^{-3}$  m/s compared to a block sample of hake fillet, while the distance (l) between the nozzle cutter and the sample surface was  $5 \times 10^{-3}$  m. The analysis of experimental data indicates that the depth of cut (h) in frozen fish rises with increasing water pressure across the entire range of values. For instance, elevating the pressure ( $\Delta P$ ) from 200 to 500 MPa leads to an increase in the depth of cut for nozzles with diameters (d<sub>o</sub>) equal to  $0.2 \times 10^{-3}$  m and  $0.4 \times 10^{-3}$  m by almost 21 and 6 times, respectively. Enlarging of the nozzle orifice diameter, results in a greater depth of cut in frozen fish (Table 1). In this case, the maximum depth of cut in a block sample from hake fillet with a temperature (t) of -11 °C was  $82.5 \times 10^{-3}$  m, which was observed at a pressure of 500 MPa and a nozzle diameter of  $0.4 \times 10^{-3}$  m. The depth of cut in frozen fish samples at - 25 °C will be even smaller.

The impact of high-speed water jet displacement velocity on the depth of cut in a block sample of hake fillet are shown in Table 2.

	Depth of cut h, m x $10^{-3}$ , at V <sub>n</sub> × $10^{-3}$ m/s							
ΔP, MPa	1	5	10	25	40	50		
150	76.4	47.1	36.1	9.2	6.25	2.3		
200	98.6	72.3	47.2	27.4	17.25	11.8		
250	127.2	105.7	84.1	64.1	40	21.7		
300	145.1	128.6	102.4	84.2	55	28.4		

#### Influence of jet velocity on depth of cut in block samples of hake fish fillet at temperature -3°C

The depth of cut decreases rapidly with increasing water jet travel speed throughout the investigated pressure range from 150 to 300 MPa. The working depth of cut are achieved on frozen fish samples up to -3 °C only at relatively low velocities of water jet movement ranging from  $10^{-3}$  to  $10^{-2}$  m/s. The data indicate that water jet cutting at pressures below 250–300 MPa is not possible when food product temperature decreases to -11 °C and below. The working conditions, including high pressure and low water jet velocity, do not yield maximum productivity for water cutting and result in high energy consumption for jet formation. These factors led to the need for an intensified cutting process for frozen foods with temperatures ranging from -11 to -25 °C and below, which is of practical importance.

It is important to note one significant negative effect that arises at high operating pressures -a substantial rise in water jet temperature passing through the nozzle of the jet-forming head. Increasing the water jet pressure results in a rise in temperature. However, this

poses limitations on the use of high pressures (max 700 MPa), as exceeding 100 °C would impair water jet functionality. Moreover, elevated water jet temperature can compromise the quality of the cut food product.

One way to enhance the efficiency of the water jet cutting process, which significantly expands its technological capabilities, is to introduce abrasive additives into the cutting fluid jet (Liu et al., 2019; Natarajan et al., 2020). Water jet cutting involves abrasive particles being propelled by a liquid jet to affect the material being cut.

#### Ice abrasive water jet

Pure water jet cutting and especially abrasive water jet cutting are a complex physical process that depends on various factors. The influence of these factors on the cutting process is complex and interdependent, with a result that is difficult to predict, depending on the material to be cut. Currently, specific hydro-cutting units are developed for each industry, which can work with pure water or by accelerating abrasive particles. Each hydro-cutting unit design has a distinctive characteristic parameter that signifies the efficiency of the hydro-cutting process. The parameter depends on the design, geometric features, and manufacturing quality of individual parts of the hydro-cutting equipment, the material properties of the substance being cut, and the choice of abrasive used in hydro abrasive cutting (Liu, 2019; Ranjan et al., 2022; Wang and Shanmugam, 2009).

Currently, the following abrasive materials are used: garnet, aluminium oxide, silicon carbide, steel shot, copper slag, silica sand (silicon dioxide), and glass chips (Perec and Tavodova, 2016; Ranjan et at., 2022). Silica sand is the most commonly used, primarily because of its low cost. Obviously, none of the abrasive materials used in practice can be applied to the abrasive water jet cutting of frozen foods. In this situation, a promising way to increase the efficiency of the process of water jet cutting frozen food products can be the use of ice abrasive water jet, i.e. when the abrasive material is small ice particles.

It is also important to note that ice abrasive water jets in the food industry may also have prospects for use in cleaning the internal surfaces of food equipment and in the development of technology for hydrodemolition of meat from bones.

Experimental verification was conducted to explore the use of a water jet, infused with small ice particles, to enhance hydro-cutting of deep-frozen food products. The process model, as depicted in Figure 1, was implemented on model meat samples using ice with a temperature of -30 °C. It has been found that significant depths of cut are achievable in frozen meat at -25 °C and model meat samples (made from ice) at -30 °C at working pressures of 100 MPa and water jet velocities of  $(15-25)\times10^{-3}$  m/s. At a pressure of 150 MPa and with a nozzle diameter of  $0.4 \times 10^{-3}$  m, the hydro-cutting process using a hydro-abrasive jet on frozen meat samples and model meat samples at -25 °C and -30 °C, respectively, resulted in a 0.15–0.20 m cut depth. The use of small ice particles as abrasives significantly increased the efficiency of the process under these conditions. Thus, for instance, if in the model meat sample the cutting depth with a jet of pure water was  $2.3 \times 10^{-3}$  m at  $\Delta P=100$  MPa; d<sub>o</sub>=0.6·10<sup>-3</sup> m; V<sub>n</sub>=25×10<sup>-3</sup> m/s, then using small ice particles as abrasive material under the same conditions we obtained a through cut of the sample (the sample thickness 0.4 m) with a high quality of the cut surface.

Consequently, it can be concluded that the process of intensifying the water jet cutting of deep-frozen food products, by adding small ice particles to the water jet, is highly efficient. However, due to the complexity and high cost of obtaining an ice abrasive water jet (Figure 1), their utilization in the food industry is still economically limited (Kuzkin et al., 2019; Zhuravka et al., 2023).

#### Thermal effects in the flow of a water through a nozzle

The proper selection of the water jet formation system design and thermophysical conditions for water flow through the hydraulic cutting head should result in enhanced efficiency of the hydraulic cutting process. Misunderstandings and misconceptions in the interpretation of experimental results surrounding the phenomena that occur in the jet-forming hydro-cutting head during water flow are largely due to the presence of thermophysical effects. At the formation of a water jet during water flow through the jet-forming head, various thermal effects can influence both the technological process of hydro-cutting of food products and the parameters of hydro-cutting equipment. To intensify the process of water jet cutting of frozen food products, it is necessary to investigate the thermal effects arising from the flow of water through the water jet cutting jet-forming head. The temperature change of the water jet during formation is a crucial consideration.

Water flows out of the jet-forming nozzle due to a decrease in pressure to atmospheric levels, resulting in the expansion of the water flow and a process of water throttling. Consequently, a change in temperature of the water jet should occur. This expansion of a liquid flowing through a small hole from high to low pressure is known as Joule-Thomson effect in thermodynamics (Schroeder, 2000). During a flow of water, the enthalpy remains constant while the temperature of the water adjusts. Every fluid has a Joule-Thomson inversion temperature, where expansion at constant enthalpy results in a temperature rise when above the threshold and cooling when below it. The inversion temperature varies according to pressure and is below room temperature for water. Therefore, isoenthalpic expansion can raise the temperature of water stream generated by passing through a water jet's nozzle at isoenthalpic throttling between two pressures:  $P_1$  (pressure in the inlet domain of the water jet nozzle) and  $P_2$  (atmospheric pressure).

The function below expresses the total enthalpy differential, I.

$$dI = C_{p}dT + V\left(1 - T / V\left(\frac{\partial V}{\partial T}\right)_{p}\right)dP, \qquad (1)$$

where C<sub>p</sub> is water heat capacity at constant pressure;

T is temperature, °K;

V is volume of water,  $m^3$ .

For isentropic processes when l = constant, the relationship between pressure and temperature is as follows:

$$dT = V / C_{p} \left( 1 - T / V \left( \frac{\partial V}{\partial T} \right)_{p} \right) dP$$
(2)

Coefficient

$$\mathbf{K} = \mathbf{V} / \mathbf{C}_{p} \left( 1 - \mathbf{T} / \mathbf{V} \left( \frac{\partial \mathbf{V}}{\partial \mathbf{T}} \right)_{p} \right) \mathbf{dP}$$
(3)

is referred to as the Joule-Thomson differential coefficient. It can be approximated that

$$\Delta \mathbf{T} = -\mathbf{K} \cdot \Delta \mathbf{P} \,, \tag{4}$$

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where  $\Delta T = t_2 - t_1$  represents the temperature difference between the water jet at the nozzle outlet and in the receiver, while  $\Delta P = P_1 - P_2$  refers to the pressure disparity between the inlet region of the water jet nozzle and the atmosphere (at the nozzle outlet). The Joule-Thomson coefficient K can be determined experimentally. Table 3 presents the water jet temperature variation data resulting from water flow through the nozzle of the hydro-cutting jet-forming head at different operating pressures.

Table 3

Nozzle diameter d <sub>o</sub> , 10 <sup>-3</sup> m	Pressure ΔP, MPa	$t_2 - t_1 = \Delta T,$	K, °C/MPa
	500	46.7	0.0934
	300	27.9	0.0930
0.30	200	18.6	0.0930
	100	9.3	0.0930
	50	4.6	0.0920
	300	27.6	0.0920
0.60	200	18.4	0.0920
	100	9.24	0.0924

# Temperature of the water jet formed by the hydro-cutting jet forming head at different operating pressures

Note:  $V_n$  is a speed of movement of the jet of the working liquid relative to the sample surface.

The rise in the water jet temperature flowing through the nozzle of the jet-forming head results from the Joule-Thomson effect. This inference is drawn from a comparison of water jet temperature with the jet temperature of other liquids. The comparative study reveals that the temperature of the water jet increases largely when the hydro-cutting jet-forming head is employed with a working fluid having a lower heat capacity. The temperature of the technical oil jet, at a pressure of 500 MPa, reached 200 °C. It is evident that the nozzle diameter has a slight impact on the jet's temperature during throttling, with higher diameters causing a less significant increase. Table 1's experimental data enables the determination of the Joule-Thomson coefficient K (4) for water, which was discovered to be 0.0927 °C/MPa.

The experimental results under consideration hold crucial importance in designing and calculating equipment for implementing the water jet cutting process. It raises the question of how much pressure can be increased while developing adequate equipment. If water serves as a working fluid, it is evident that water jet cutting of food products will be feasible only if the temperature of the water jet does not cross 100 °C. It is evident from the above discussion that the highest attainable velocity of the water jet exiting the nozzle under high pressure is limited by the Joule-Thomson effect. This means that the water jet's temperature can rise to the point where it becomes vapour at a certain pressure value. It was shown that without special water jet cooling methods, the temperature of the water jet exceeds  $100 \,^{\circ}$ C at a pressure of 700 MPa for nozzles with orifice diameters from  $0.3 \times 10^{-3}$  to  $0.6 \times 10^{-3}$  m.

## Cutting under using water jet negative temperature

It is necessary to fully understand and improve the process of water jet cutting, particularly under reduced and negative temperature conditions. To achieve this understanding, it is important to consider the anomalous properties of water, specifically its liquid state, when using a water jet cutting food products at reduced temperatures. One distinguishing feature of water from other liquids is its decrease in crystallization temperature with increasing pressure (Yasutomi, 2021). The crystallization curve on the state diagram of water, increasing pressure up to 207 MPa, veers to the left up to - 22 °C. This characteristic on the water phase diagram permits the water jet's temperature to be lowered below freezing without transitioning into a solid state, such as ice. For instance, this can be achieved in the receiver of a hydraulic cutting unit at an operating pressure of 207 MPa where the pure water can be cooled down to -22 °C.

Thus, if the temperature of the working fluid (water) in the receiver is cooled to -15 °C, it is possible to obtain a temperature of -1.0 °C for the water jet at the nozzle outlet by applying a pressure of 150 MPa, according to equation (4) and the data presented in Table 3. It was experimentally determined that compressing water to 150 MPa and then throttling it through a  $0.3 \times 10^{-3}$  m diameter nozzle results in a temperature increase of 14 °C. By cooling the water in the receiver to -15 °C at 150 MPa, a water jet with a temperature of -1.0 °C can be obtained at the nozzle outlet. In this case, a super cooled water jet is produced, and upon exiting the nozzle, ice microcrystals should form due to the water transitioning to a crystalline state at temperatures below 0 °C and atmospheric pressure. These microcrystals can serve as abrasive additives.

An experimental study was conducted to investigate the influence of water jet temperature on the depth of cut for samples of beef meat and hake fish fillets. The tests were carried out at a temperature of minus 25 °C, with pressures of 50 and 150 MPa, a nozzle diameter of  $0.3 \times 10^{-3}$  m, and a speed of hydro jet movement relative to the frozen meat sample of  $15 \times 10^{-3}$  m/sec. The shearing distance from the nozzle to the surface of the frozen food product being cut was equal to the optimal value. Table 4 displays the data indicating the impact of water jet temperature on the depth of cut in beef samples chilled to - 25 °C.

Table 4

t, ⁰C	ΔP, MPa	h, 10 <sup>-3</sup> m
4	150	124
	50	27
-0.5	150	160
	50	41
-1.0	150	>200
	50	74

# Effect of water jet temperature on depth of cut in frozen beef meat $d_o = 0.3 \times 10^{-3} \text{ m}, V_n = 15 \times 10^{-3} \text{ m/s}, 1 = l_{opt} = 9 \times 10^{-3} \text{ m}, t_{meat} = -25 \text{ °C}$

Note: do- a nozzle diameter;

 $V_n - a$  speed of movement of the jet of the working liquid relative to the sample surface; l - distance between the nozzle cutter and the sample surface;  $t_{meat}$  - temperature of meat; t - temperature water jet;  $\Delta P - working$  pressure; h - depth of cut.

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It is evident that the depth of the cut, h, monotonically increases as the temperature of the water jet decreases to 0 °C. This increase in h with decreasing temperature of the water jet is primarily due to the increase in surface tension and viscosity of water, which enhances its compactness. A decrease in water temperature to -1.0 °C (Table 4) leads to a sudden increase up to 30 and 50% for pressures of 150 MPa and 50 MPa, respectively, in the depth of cut. The change in depth of cut of a frozen food product when the water jet temperature is at or below - 1.0 °C is a strong indication that microparticles – ice crystals – are formed in the water jet from the moment of its emergence from the air medium, and that these microparticles play the same role as the pre-prepared small ice particles introduced into the pure water jet.

Thus, the data confirm a significant increase in the efficiency of water jet cutting food products at reduced and especially negative (-1 °C and below) temperatures of the pure water jet.

# Water-nitrogen jet cutting

The water jet method of processing of food requires the hydraulic cutting unit to be equipped with a nitrogen vapour supply system to the collimator, which can be easily realized using liquid nitrogen vapour. The supply system for nitrogen vapour is straightforward – a Dewar container with liquid nitrogen from which vapour flows into the collimator via a throttling orifice. All parts of the liquid nitrogen supply system require a thermally insulating coating.

It was found under the specified conditions for cutting a sample of beef meat that the efficiency of the process for water jet cutting of food products using the water-nitrogen method significantly increased (Table 5).

Table 5

# Effect of pressure on the depth of cut in frozen of beef meat by water and water-nitrogen jets

 $d_o = 0.3 \times 10^{-3}$  m,  $V_n = 15 \times 10^{-3}$  m/s,  $1 = l_{opl} = 9 \times 10^{-3}$  m,  $t_{meat} = -25$  °C,  $C_{opt,nitrogen} = 20$  %

ΔP,	Depth of cut h, 10 <sup>-3</sup> m				
MPa	Water jet cutting a beef meat	Water-nitrogen jet cutting an ice samples at -30 °C as a model sample of meat at -25 °C			
50	1.3	80			
100	2.2	120			
150	3.5	>200			

Note: d<sub>o</sub> – nozzle diameter;

 $V_n$  – speed of movement of the jet of the working liquid relative to the sample surface;

1 -distance between the nozzle cutter and the sample surface;

 $t_{meat}-temperature\ meat;\ C_{opt,nitrogen}-optimal\ concentration\ of\ liquid\ nitrogen;$ 

 $\Delta P$  – working pressure; h – depth of cut.

It can be concluded that when dealing with deep-frozen beef meat, the depth of cut achieved by the water-nitrogen jet is several times greater than that achieved by the water jet, when all conditions are the same and the collimator receives an optimal concentration (20%) of liquid nitrogen. It was found that cutting a model meat (ice) sample at a temperature of -  $30 \, ^{\circ}$ C with a water-nitrogen jet resulted in a cut depth of over 0.2 m. The analysis of experimental results on the thermal effects of hydro-cutting reveals that the process can be

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intensified when a water-nitrogen jet, containing ice microcrystallites formed through cooling by liquid nitrogen vapours, is used as an abrasive. This technique shows high practical usefulness for cutting deeply frozen food products.

# Conclusions

- 1. Experimental verification indicated that using a water jet with ice particles to perform hydro-cutting on deep-frozen food products is possible. The method of intensifying of water jet cutting process in deep-frozen food products by introducing small ice particles into the water jet has proven to be highly efficient. However, due to the complex and costly process of obtaining an ice abrasive water jet, it is not currently feasible for use in the food industry.
- 2. It was shown that the temperature increase of the water jet formed by the flow of water through the nozzle of the hydro-cutting jet-forming head is due to the Joule-Thomson effect. Utilizing the calculated dependence of the water jet temperature increase from the throttling effect, we can estimate the threshold pressure value beyond which the process of cutting deep-frozen food products with a water jet is not feasible.
- 3. It was demonstrated that the implementation of water-nitrogen jets, in which ice microparticles formed during cooling of the pure water jet by liquid nitrogen vapour serve as the abrasive, yields a highly effective method for water jet cutting of frozen food products.

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# Intensification of drying process of shiitake mushroom (*Lentinula edodes*) using combined methods of dehydration

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# Abstract

**Introduction**. This study evaluated kinetics and value of swelling coefficient of cultivated shiitake mushrooms depending on different regimes of combined infrared and convective dehydration methods.

**Materials and methods**. Fresh cultivated shiitake mushrooms were sorted by shape, maturity, and size. The kinetics of the process was studied during convective two-stage drying (100 °C+60 °C) and combined infrared radiation and convective dehydration (100 W+60 °C) drying.

**Results and discussion**. The drying of shiitake mushrooms at a heat carrier temperature of 60 °C takes 156 min. and using the two-stage convective method at 100/60 °C takes 97 min. When using the combined infrared radiation and convective dehydration method (100 W+60 °C) the drying process takes 110 min. The use of the combined method (100 W+60 °C) and two-stage (100 °C/60 °C) reduces the duration of drying by 1.4–1.6 times compared to the traditional mode of 60 °C.

The efficiency of these processes is confirmed by a change in the Rebinder number, which depends on the heat carrier temperature and moisture content. During the period of changing the moisture content in shiitake mushrooms from 84 to 10%, the value of the Rebinder number is minimal. When the content of moisture in mushrooms reaches 10%, the Rebinder number increases sharply, which means that most of the energy is spent on heating of the material that leads to an increase in energy consumption for the process and a deterioration in the quality of the drying product. The determination of rehydration properties of mushrooms confirmed the high quality of the dried product.

The study provides valuable information on the optimization of drying processes, contributing to the production of high-quality dried mushrooms.

**Conclusions.** The difference between the duration of drying processes using combined infrared-convective (100 W + 60 °C) method and two-stage (100 °C/60 °C) method is 0.5%. It was shown that these two modes are the most effective to dry shiitake mushrooms.

# Introduction

Due to the high respiratory rate and moisture content close to 90%, cultivated shiitake mushrooms are vulnerable to microbial spoilage and deterioration in quality including texture, appearance, nutrients, and favor (Subramaniam et al., 2021; Yang et al., 2019). To extend the shelf life of mushrooms, thermal dehydration (drying), which prevents such spoilage as enzymatic darkening and microbial growth by reducing water activity, could be applied (Hu et al., 2021; Mierzwa et al., 2023). Drying significantly prolongs the shelf life of mushrooms, and dry mushrooms could be safely stored under proper conditions (Tian et al., 2016).

Turchyna et al. (2019) suggested that to improve the structuring and moistureconducting properties of the material during dehydration, the content of  $\beta$ -cyclodextrin in the mushroom suspension should be more than 10%, but less than 25%, and the temperature of the heat carrier should be at least 180 °C, but less than 200 °C. Due to this, greater strength and density of the dried particles is ensured and, thereby, the structural and mechanical characteristics of the powder are improved, and the increase in heat resistance of the material in combination with the achievement of the effect of microencapsulation of microparticles of thermolabile components of the mushroom during spray drying makes it possible to obtain high-quality mushroom powder.

The main modern methods of processing cultivated mushrooms were described by Avdieieva et al. (2021). The obtained results of the research by the spray method, which are presented in this paper, show that they have a favorable effect on the kinetics and content of nutrients, but lead to undesirable color changes and the use of  $\beta$ -cyclodextrin is not functional.

Convective drying is considered to be a low-cost and easy-to-control method, so it is widely used for drying cultivated mushrooms.

Argyropoulos et al. (2010) studied the convective drying of shiitake, the degree of redhydration, equilibrium moisture and color change. The time required to reduce the moisture of the sample was much shorter for the 70 °C – 190 min mode and too long for the 30 °C - 1400 min mode. Slight darkening (yellowness) was observed already at 50 °C, which was expressed more with higher temperatures of 60 and 70 °C. After drying, the degree of rehydration was determined – all samples could not absorb the same amount that they had in fresh form. Samples dried at 30 °C indicate slightly greater rehydration capacity compared to material dried at higher temperatures. Yang et al. (2019) determined the content of free amino acids by sublimation and convective methods of research, which showed that the total content of free amino acids in convective drying samples treated at 60 °C was the highest. That is, the sublimation method partially destroys amino acids. Microwave drying distributes energy unevenly creating problems associated with uneven heating (Zhang et al., 2013). Vacuum drying is especially suitable for heat sensitive products. However, the low heat transfer rate in vacuum drying causes an extended drying time (Zhang et al., 2006).

Another shiitake drying method that has been investigated is microwave vacuum drying (MVD) and microwave vacuum drying combined with infrared radiation (MVD+IR) (Kantrong et al., 2014). The application of infrared radiation in combination with microwave vacuum drying significantly improved the color of dried shiitake samples, as well as the rehydration coefficient and texture of rehydrated samples, reduced drying time and specific energy consumption compared to microwave-vacuum drying. The optimal conditions for drying shiitake mushrooms were drying in MVD+IR mode at 267 W MW in combination with 200 W IR under operating pressure at 18.66 kPa (Kantrong et al., 2014).

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Despite the large number of previous studies, scientific interest in the process of dehydration of cultivated shiitake mushrooms remains relevant, since the disadvantage of the main considered methods is the high cost, as it can be seen from Figure 1.



Figure 1. Specific energy consumption during drying of colloidal capillary-porous materials depending on the drying method

The highest level of the specific energy consumption indicator was registered during drying by sublimation (496.1 MJ/kg of water) and the combined vacuum-sublimation method (425 MJ/kg of water) in comparison with other drying methods (Wu et al., 2019). The reason for the high energy consumption of sublimation, which is the main disadvantage of this method, is that the process usually uses electric heating plates to provide the heat needed to sublimate the ice, with low thermal conductivity. With freeze drying, the basic energy required to remove 1 kg of water is almost twice as high as with conventional drying (Wu et al., 2019).

Previous studies (Petrova et al. 2018) have shown, that the problem of reducing energy consumption for the drying process remains relevant. Therefore, it is necessary to introduce modern energy-efficient heating technologies.

The aim of research was theoretical and experimental justification of the complex and effective processing of shiitake mushrooms taking into account the properties of the material and the development of energy-efficient drying modes due to combined and two-stage dehydration methods.

# **Materials and methods**

#### Materials

Fresh shiitake mushrooms were sorted by shape, maturity, and size. All mushrooms were stored at a temperature of  $4\pm0.5$  °C and a relative humidity 95% in a refrigerator before the experiment. The initial moisture was 86.4±1.4 %.

# Methods

# **Research of drying kinetics**

The study of the drying kinetics of shiitake mushrooms was carried out on an improved experimental installation, which consists of the following main parts: a drying chamber, a heating system and a fan supply of the heat carrier to the drying chamber, insulated air ducts, scales, speed regulators and resistance thermometers.

Figure 2 shows a diagram of an experimental convective drying installation. A change in a wide range of the speed of movement of the heat carrier and its temperature is achieved by adjusting the operation of the fan (3) on the control panel (5). The speed of the heat carrier is changed by changing the air supply by the centrifugal fan (3) using a frequency converter with manual adjustment (5). The ratio between exhaust and fresh air can be adjusted with the help of dampers on the nozzles (7) (Petrova et al., 2018).



Figure 2. Scheme of the experimental convective drying installation: 1 – drying chamber; 2 – electric heaters; 3 – fan; 4 – thermostat; 5 – control panel; 6 – resistance thermometers; 7 – nozzles with valves; 8 – psychrometer; 9 – special grids; 10 – weight bar; 11 – scales, 12 – IR source (infrared radiation)

Sliced shiitake mushrooms are placed in a mesh basket, which is placed on the scales (11) in the drying chamber (1). The camera has a transparent glass, through which you can observe the state of the material during the drying process. In particular, it is possible to note the moment when the color of the sample changes due to the influence of the heat carrier modes, the shrinkage process and change the mode if necessary. A block of infrared lamps is installed in the chamber with the ability to adjust the heat flow from 0 to 3800 W/m<sup>2</sup> (12). The power of the lamps is changed by a rheostat type regulator (Petrova et al., 2018).

Like the entire drying process, heat transfer is a non-stationary process. Another feature of this process is that (as indicated above) it is complicated by the transfer of matter (moisture), as it is due to the movement of heat (Petrova et al., 2018).

Experiments on the study of drying kinetics were carried out at the rate of the heat carrier v = 3 m/s (the rate was chosen based on the condition of more efficient drying of the same material in existing modern dryers); at a heat carrier temperature of 60 °C, 100 °C, 100 °C/60 °C and infrared radiation of 100 W when drying on a convective installation; as well

as the combined method of 100 W + 60 °C (infrared-convective). Dehydration processes continued up to a residual moisture content of 5.0% and within 60-160 minutes.

In connection with the automatic collection of information on the experimental convective drying installation, the temperature recording error does not exceed  $\pm 1$  °C and mass  $\pm 5$  mg.

## Study of rehydration properties

The samples of dried mushrooms were prepared in the form of a strip  $(25 \times 10 \times 3 \text{ mm})$ and powder with a dispersion of less than 0.5 mm. The coefficient of swelling was determined by the method described in (Sniezhkin et al., 2007). The essence of the method is that the material under study is weighed in prepared containers and filled with water (45 °C) in a ratio of 1:10. A certain period of time is maintained – 30 min, the liquid is drained, and the recovered sample is weighed and the swelling coefficient is calculated. The swelling is estimated by the coefficient of swelling  $C_{swell}$ , which shows the relative increase in mass of the product after swelling and determines the ability to restore the initial properties of the material upon dehydration. The coefficient of swelling is calculated according to the formula:

$$C_{swell} = \frac{G_2}{G_1}$$

where  $C_{swell}$  – the coefficient of swelling;  $G_1$  and  $G_2$  – mass of material before and after swelling, respectively, g.

# **Results and discussion**

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#### Heat and mass transfer during drying of shiitake mushrooms

Figure 3 shows the drying curves of changes in the average (integral) moisture content of material W over time and temperature curves. The heat carrier temperature is 60 °C, 100 °C and two-stage 100 °C /60 °C. Due to the small thickness of the material and the high intensity of evaporation, the analysis of all curves indicates the absence of the first drying period. Dehydration occurs in the second period. The change in moisture content (curve 1) of shiitake mushrooms at a heat carrier temperature of 60 °C occurs within 156 minutes. At the same time, as can be seen from the temperature curve 1', the material heats up evenly during the entire drying period and only at the end the temperature of the material is 55 °C.

Moisture content change curve 2 shows that the process intensifies at a heat carrier temperature of 100 °C and lasts only 60 minutes. Temperature curve 2' indicates intense heating of the material and at 55 minutes of the process, the temperature of the material rises sharply and is 90 °C for 60 minutes. This leads to the denaturation of proteins, the formation of melanoidin reactions, the destruction of vitamins and other undesirable processes, although at the same time the shortest duration of the process (Gou P. et al., 2005). Based on these studies, it is advisable at the beginning of the process, when the moisture content of the mushrooms is high, to increase the temperature of the heat carrier to 100 °C, without leading to high heating of the material. The curves of changes in moisture content 3 and temperature 3' show, that after 20 minutes the material temperature is 50 °C and it is advisable to reduce the heat carrier temperature to 60 °C. In this case, the process slows down a bit and its duration is 97 min, which is much longer than the mode heat carrier of 100 °C (curve 2, 2'), but much less, than at a temperature of 60 °C (curve 1, 1'). Based on these data, the drying study should be carried out in a two-stage mode at a heat carrier temperature of 100 °C/60 °C and a material temperature of 60 °C.

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Figure 3. Changes in the moisture content and temperature of shiitake mushrooms. 1, 2, 3 – moisture; 1', 2', 3' – temperature;  $\delta$  (material thickness) = 10 mm, v (heat carrier speed)= 3 m/s, d (moisture content of the air) = 10 g/kg dry air: Temperatures of the heat carrier: 1, 1' – 60 °C; 2, 2' – 100 °C; 3, 3' – 100 °C /60 °C





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The drying rate curves (Figure 4) show, that the drying rate is 3.5%/min both at a heat carrier temperature of 100 °C and at 100 °C /60 °C (curves 2.3). A temperature of 60 °C (curve 1) slows down the drying rate. The drying rate curve of the two-stage mode (curve 3), as well as the graph of the change in moisture content shows, that increases the drying rate compared to 60 °C (curve 1). As can be seen from figures 2-3, with the two-stage mode of 100 °C/60 °C, the drying process lasts 1.6 years faster, compared to the mode of 60 °C, and the drying rate is also higher and is 3.5%/min (Figure 4).

We investigated the change in the moisture content of cultivated shiitake mushrooms during convective drying and IR (infrared radiation when drying on a convective installation), as shown in Figure 5. The graph shows the comparative characteristics of the convective mode 60 °C (curves 1, 1'), the drying mode with infrared radiation when drying on a convective installation with an ambient temperature of 20 °C (hereinafter IR) – 100 W (curves 2, 2') and the combined (infrared-convective) mode 100 W+60 °C (curves 3, 3').

As can be seen from Figure 5, the duration of drying with IR (curves 2, 2') is 122 min, which is 19 % faster, than with the convective mode of 60 °C. Curve 3, 3' shows combined drying - at the beginning of the process, two types of heat carrier were supplied simultaneously (IR 100 W and convective 60 °C).



Figure 5. Change in moisture content of shiitake mushrooms during convective drying and IR (infrared radiation when drying on a convective installation).

1, 2, 3 – moisture; 1', 2', 3' – temperature;  $\delta$  (material thickness) = 10 mm, v (heat carrier speed) = 3 m/s, d (moisture content of the air) =10 g/kg of dry air: 1, 1' – 60 °C; 2, 2' – 100W (at 20 °C); 3, 3' – 100 W+60 °C

When the tested sample reached a temperature of 50 °C at the 20th minute of the experiment, one of the heat carriers (IR 100 W) was turned off. At the same time, a sharp drop in the temperature of the material to 39 °C with subsequent gradual heating is observed. Combined drying (curves 3, 3') lasts 110 minutes, and at the same time, the drying process is intensified by 1.4 times compared to the 60 °C mode. The drying rate at the convective mode of 60 °C is 2.0 %/min, at IR 100 W – 1.8 %/min, at the combined 100 W + 60 °C it is the highest – 3 %/min (Figure 6).



Figure 6. Influence of heat carrier temperature during convection and IR on drying rate of shiitake mushrooms.

 $\begin{aligned} &\delta \text{ (material thickness)} = 10 \text{ mm, } \text{v (heat carrier speed)} = 3 \text{ m/s,} \\ &d \text{ (moisture content of the air)} = 10 \text{ g/kg dry air:} \\ &1 - 60 \text{ }^\circ\text{C}\text{; } 2 - 100 \text{ W (at 20 }^\circ\text{C}\text{)}\text{; } 3 - 100 \text{ W+60 }^\circ\text{C} \end{aligned}$ 

A comparative characterization of the change in moisture content of cultivated shiitake mushrooms (Figure 7) was carried out in the combined infrared-convective 100 W + 60 °C (curve 1, 1') and two-stage 100/60 °C (curve 2, 2') drying regimes. The use of an IR lamp at the beginning of the process made it possible to reduce the drying time, as indicated above.

Curve 2, 2' (Figure 7) shows the characteristics of two-stage drying in comparison with combined drying, which shows that at the beginning of the process, the heat carrier temperature was 100 °C, then when the material temperature reached 50 °C (for about 20 min), the heat carrier temperature was reduced up to 60 °C, however, the material temperature continues to increase by inertia to 55 °C, since the decrease heat carrier temperature occurs after a certain period of time gradually. Further, the temperature of the material begins to gradually decrease from 35 min to reach 48 °C at 75 min, and then increases to 50 °C. The drying rate for the combined method is 3%/min and for the stepwise method is 3.5%/min (Figure 8).

These two modes are the most effective, the difference in drying time between them is 8-10 min and the difference in drying rate is 0.5%/min with an advantage for the two-stage mode (Figure 8). This happens, because the temperature of the material during drying by the combined method drops to 40 °C, and with two-stage drying – to 50 °C.

For comparison, the duration of drying shiitake mushrooms, which was described by Argyropoulos et al. (2010), was 190 min at 70 °C, and 1400 min at 30 °C. The duration of the combined drying method described in the present study was 110 minutes and using the two-stage method it was 97 minutes.

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Figure 7. Comparative characteristics of changes in moisture content of shiitake mushrooms in effective drying modes.

 $\begin{array}{c} 1,\ 2-\text{moisture; 1', 2'-temperature;}\\ \delta \ (\text{material thickness}) = 10 \ \text{mm, v} \ (\text{heat carrier speed}) = 3 \ \text{m/s,}\\ d \ (\text{moisture content of the air}) = 10 \ \text{g/kg} \ \text{dry air:}\\ 1,\ 1'-100 \ \text{W+60 °C; 2, 2'-100 °C /60 °C} \end{array}$ 



Figure 8. Comparative characteristics of drying rate of shiitake mushrooms in effective drying modes.

 $\begin{aligned} \delta \text{ (material thickness)} &= 10 \text{ mm, v (heat carrier speed)} = 3 \text{ m/s,} \\ d \text{ (moisture content of the air)} &= 10 \text{ g/kg dry air:} \\ 1 - 100 \text{ W+60 °C; } 2 - 100 \text{ °C /60 °C} \end{aligned}$ 

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Mierzwa et al. (2023) showed, that the duration of the process decreases with the use of hybrid drying. This effect was also found when drying leafy cabbage using microwave drying (Lichtenthaler et al., 2001) and hybrid microwave-convective drying of onion pieces (Maftoonazad et al., 2022). Our use of the hybrid infrared-convective method in drying Shiitake mushrooms in this work also confirmed these studies.

## Heat and moisture exchange in the case of drying shiitake mushrooms

One of the main characteristics of the kinetics of the dehydration process is the Rebinder number (Rb) – a drying optimization criterion that determines the ratio of the amount of heat spent on heating the material and on the evaporation of significant moisture in an infinitesimally small period of time (Petrova et al., 2018):

$$Rb = b\frac{\bar{c}}{r} = \frac{\bar{c}}{r} \left(\frac{d\bar{t}}{dW}\right)$$

where Rb - Rebinder number,

 $b\frac{c}{r}$  – integral characteristic of the kinetics of the drying process,

 $\frac{dt}{dW}$  – the change in the average temperature of the material being dried per unit of change in its average moisture over an infinitesimally small period of time is called the temperature coefficient of drying.



Figure 9. Change in Rebinder's number depending on heat carrier temperature.  $\delta$  (material thickness) = 10 mm, v (heat carrier speed) = 3 m/s, d (moisture content of the air) =10 g/kg dry air: 1-60 °C; 2-100 °C

In Figure 9 it is shown the change in the Rebinder number depending on the heat carrier temperature. At the beginning of the drying process to a moisture content of 84%, the material is heated with a decrease in its moisture content, while the Rb number decreases.

The change in moisture content occurs from 84% to 10%, as seen in Figure 9. During the period of change in moisture of cultivated shiitake mushrooms from 84-10% to Rebinder number is minimal. At this time, most of the heat is spent on removing moisture from the material (Petrova et al, 2018). After the moisture content point of 12-10%, the Rebinder number increases sharply, which means that most of the heat is spent on heating the material that leads to additional energy costs for the process and deterioration of the quality of the dried material. The change in Reminder number proves the effectiveness of the introduction of two-stage drying regimes.

The value of the Rebinder number depends on the temperature coefficient of drying, the specific heat capacity of the wet material, the specific heat of evaporation, and, accordingly, on the form of moisture connection with the material (Petrova et al., 2018). Therefore, it makes no sense to maintain a high temperature of the heat carrier in the last period of the dehydration process.

Mierzwa et al. (2023) found that hybrid processes such as convective drying with microwave or in combination with microwave and ultrasound are more energy efficient than the convective method and ultrasonic convection. These findings are consistent with previous studies of microwave convective drying of kale (Lichtenthaler et al., 2001) and a hybrid microwave hot air drying of onion slices (Maftoonazad et al., 2022).

#### Quality characteristics (swelling coefficient) of dried shiitake mushrooms

For dried agricultural products, rehydration characteristics are often used as an index of structural quality. These characteristics largely depend on the drying conditions used (Sniezhkin et al., 2007). In Figure 10 the dependence of the swelling coefficient on the drying modes is shown.



Figure 10. Dependence of the swelling coefficient on the modes of convective drying at the heat carrier temperature 60 °C, 100 °C, 100 °C /60 °C; with IR radiation 100 W and with the combined infrared-convective method 60 °C/100 W

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The shiitaki (Fig, 11a) was studied in the form of dry strips (Figure 11b) or in the form of a powder (Figure 11c).





**Figure 11. Shiitake mushrooms:** a – fresh; b – dried in form strips; c – dried in form of a powder.

The highest value of the swelling coefficient  $C_{swell} - 6.02 \% - \text{is}$  for the sample (piece) dried at the temperature of the heat carrier 60 °C. At a temperature of 100 °C,  $C_{swell}$  decreases by 40 % and is the lowest. When drying mushrooms under infrared radiation with a lamp power of 100 W –  $C_{swell}$  is 25 % less. With effective two-stage and combined modes by 7–11 % and 5-7 %, respectively. That is, these two modes are not only energy efficient, but also preserve the physical properties of the material as much as possible.

As can be seen from Figure 10, the coefficient of swelling  $C_{swell}$  of the samples that were presented in the form of a piece, for each mode, is 9-12 % more meaning, than for the samples in the form of powders. In our opinion, in a piece, the structural skeleton is not physically disturbed, and in the powdery state, the skeleton of the material is already disturbed, which becomes finely dispersed. During the experiment, dry substances are released from the powder into the water, so the weight and recoverability are lower, and in a plate they remain unchanged.

# Conclusions

- 1. The most effective modes of drying of the cultivated shiitake mushrooms were determined: two-stage 100 °C/60 °C and combined (infrared-convective) 100 W + 60 °C.
- 2. Using of proposed methods allow to reduce the duration of drying for shiitake mushrooms by 1.4–1.6 times compared to the 60 °C mode, which is the key to the light color of the dry product.
- 3. The change in Rebinder number depending on the temperature of the heat carrier proves the effectiveness of the introduction of stepwise drying modes.
- 4. The coefficient of swelling for each mode and form of samples (strips or powder) has maximum value in the combined 100 BT+60 °C and two-stage 100 °C/60 °C modes are 7 and 11%.

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# Synergetic antimicrobial activity of a mixture of essential oils and *Acinetobacter calcoaceticus* IMV B-7241 surfactants synthesized in the presence of the eukaryotic inducer

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	Abstract
Keywords:	Introduction. The research was devoted to study the synergetic
-	antimicrobial effect and role in the destruction of biofilms under the
Co-cultivation	action of a mixture of tea tree or cinnamon essential oil with surfactant
Biological	Acinetobacter calcoaceticus IMV B-7241, synthesized in the presence of
inducer	a yeast inducer.
inducer	Materials and methods. Cultivation of A. calcoaceticus IMV B-
Synergism	7241 was carried out in a liquid mineral medium with purified or crude
Antimicrobial	glycerol. The yeast Saccharomyces cerevisiae BTM-1 was used as the
activity	inducer. The concentration of the surfactant was determined after
Biofilms	extraction from the supernatant by the gravimetric method. The
Destruction	antimicrobial activity of essential oils, surfactants and their mixtures was
	analysed according to the minimum inhibitory concentration (MIC).
	<b>Results and discussion.</b> Surfactants was produced by the A.
	calcoaceticus IMV B-7241 strain cultivated on glycerol of various
	qualities in the presence of the eukaryotic inducer. The antibacterial
	activity against <i>Staphylococcus aureus</i> BMS-1, <i>Enterobacter cloacae</i> C-
	8, Proteus vulgaris PA-12, Bacillus subtilis 51-2; antifungal activity
Article history:	against Candida albicans D-6 and C. tropicalis PE-2, and the ability to
Dereined	destruct the biofilms by a mixture of microbial surfactants and tea tree or
27 02 2022	cinnamon essential oil was studied. MIC of a mixture of tea tree or
Paceived in	MV P 7241 strain both on purified and strade glucorel in the presence of
revised form	autory b-7241 strain both on purmed and crude giver of in the presence of
14 08 2023	2.2.40 times lower respectively than the MICs of individual essential
Accepted	oils and surfactants. Using a mixture of tea tree essential oil and
29 09 2023	surfactants synthesized by IMV B-7241 strain on purified glycerol in the
29.09.2023	presence of live veast cells increased the degree of bacterial biofilm
	destruction by 1.1–4.2 times, respectively, compared with individual
Corresponding	compounds. Using a complex of tea tree essential oil and surfactants
author:	obtained in medium with crude glycerol with a veast inducer increased
	the degree of bacterial biofilms destruction by 1.2–4.1 times compared
Liliia Kliuchka	with individual compounds. Destruction of yeast biofilms under the
E-mail:	action of a mixture of cinnamon essential oil and surfactants, synthesized
KluchkaLV@	in the presence of the inducer in a medium with glycerol of different
nuft.edu.ua	quality, was 1.2-2.1 and 1.1-2.4 times higher, respectively, than this
	indicator established for individual compounds.
	Conclusions. The results showed the synergistic effect biological
DOI:	activity including the destruction of biofilms of mixture of tea tree or
10.24263/2304-	cinnamon essential oil with surfactants A. calcoaceticus IMV B-7241,
974X-2023-12-3-	obtained in the presence of live cells S. cerevisiae BTM-1 in producer

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culture medium.

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# Introduction

Every year, the availability of antibiotic therapy provokes an increase in the incidence of resistance to commercial antibiotics and, as a result, leads to the emergence of multiresistant microorganisms (Huemeret al., 2020). Difficulties in treating infections caused by multidrug-resistant pathogens force researchers to search for new antimicrobial substances with different mechanisms of action, and, if possible, with fewer side effects on human health (Gan et al., 2023). Essential oils are a source of new natural antimicrobial molecules with a wide spectrum of effects on gram-positive and gram-negative bacteria, fungi, and viruses. The use of such natural compounds complicates or eliminates the possibility of resistance in microorganisms, which is due to the absence of corresponding mutations to all active molecules of various chemical natures simultaneously present in essential oils (Feudjieu et al., 2023).

Microbial surfactants with controlled biological activity (antimicrobial, antiadhesive) are also effective natural antimicrobial agents. An effective mechanism for regulating the activity of such compounds is the introduction of competitive microorganisms (inducers) into the cultivation medium of their producers. Thus, in the literature, the majority of studies are devoted to the use of bacterial inducers to increase the concentration of surfactants (Alves et al., 2019; Chen et al., 2022) and/or their biological activity (Alves et al., 2019; Gómezet al., 2016; Hamza et al., 2018). At the same time, the inducer bacteria used by most researchers are pathogenic or opportunistic, while there is no information on the use of non-pathogenic representatives, in particular yeast of the genus *Saccharomyces*, as inducers for regulating the biological activity of surfactants.

It should be noted that the use of a mixture of two or more natural antimicrobial agents opens up promising opportunities for their use in the treatment of infectious diseases, since combinations of synergistic compounds can reduce the likelihood of resistance while showing effective pharmacological results (Cheesman et al., 2017).

In recent years, a fairly large number of studies have appeared in the literature on the synergistic biological activity of essential oils with antibiotics (Asadi et al., 2023; Iseppi et al., 2023a; Parker et al., 2022) and surfactants (Haba et al., 2014), while at the same time there is no data on the use of essential oils with surface-active compounds synthesized by bacterial producer in the presence of inducers, in particular yeast, in the cultivation medium.

Therefore, the aim of this study is to investigate the synergistic antibacterial and antifungal effects and ability to destroy biofilms by a complex of essential oils with surfactants *A. calcoaceticus* IMV B-7241, synthesized in the presence of *Saccharomyces cerevisiae* BTM-1.

# Materials and methods

#### **Objects of research**

The main object of research was oil oxidizing bacteria strain *Acinetobacter* calcoaceticus IMV B-7241 from Microorganisms Depositary of Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine (Pirog et al., 2022). Yeast Saccharomyces cerevisiae BTM-1 was used as a biological inducer.

To determine the antimicrobial and antiadhesive activity of surfactants, as well as their ability to destroy microbial biofilms, bacteria (*Staphylococcus aureus* BMS-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2) and yeast (*Candida albicans* D-6 and *C. tropicalis* PE-2) from the collection of live cultures of microorganisms of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used as test cultures.

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#### Cultivation of the producer of surfactants

The strain *Acinetobacter calcoaceticus* IMV B-7241 was grown in a liquid mineral medium of the following composition (g/l):  $(NH_2)_2CO$ , 0.35, NaCl, 1.0, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 0.6, KH<sub>2</sub>PO<sub>4</sub>, 0.14, MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1, distilled water up to 1 litre, pH 6.8–7.0. Yeast autolysate, 0.5% (v/v), and microelement solution, 0.1% (v/v), containing (g/100 ml): ZnSO<sub>4</sub>·7H<sub>2</sub>O, 1.1; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.6; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.1; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.004; CoSO<sub>4</sub>·7H<sub>2</sub>O, 0.03; H<sub>3</sub>BO<sub>3</sub>, 0.006; KI, 0.0001; EDTA (Trilon B), 0.5. Purified glycerol, 3% (v/v) and crude glycerol, which is a waste of biodiesel production, 5% (v/v) were used as a carbon source. The concentration of carbon in medium with pure glycerol was the same as in medium with crude glycerol (Pirog et al., 2023). Inoculum was produced by the cultivation of the strain in the basic medium with 0.5% of the appropriate substrate. The cultural liquid with a concentration of bacterial cells from 10<sup>4</sup> to 10<sup>5</sup> cells/ml was taken from the exponential growth phase and added to the medium for the strain cultivation, 5% (v/v). Cultivation was carried out in 750 ml Erlenmeyer flasks with 100 ml of medium for 120 hours in a shaking incubator at 30 °C and 320 rpm.

#### Cultivation of eukaryotic inducer

The yeast *Saccharomyces cerevisiae* BTM-1 was grown in a liquid mineral medium containing glucose (0.5%) as a carbon source for 24 hours in a shaking incubator at 30 °C and 320 rpm. After 24 hours, the liquid culture was poured into sterile eppendorf tubes and centrifuged at 10,000 g for 10 min using Eppendorf 5417R Refrigerated Centrifuge. The biomass was resuspended in sterile tap water to the volume taken for centrifugation. Resuspended biomass (live inductor cells) was added in the amount of 2.5 ml of suspension per 100 ml of surfactant producer culture medium at the beginning of the cultivation process. Cultivation of strains was carried out in 750 ml flasks with 100 ml of medium for 7 days in a shaking incubator at 30 °C and 320 rpm.

# Determination of extracellular surfactants concentration

The surfactant concentration was determined by the Blay and Dyer method (Pirog et al., 2020a) in our modification. Since *A. calcoaceticus* IMV B-7241 synthesizes a complex of polar and non-polar lipids, and the well-known Blay and Dyer method used to isolate surfactants allows the isolation of mainly non-polar lipids, we modified the classical solvent system (Folch mixture) by adding 1 M HCl (chloroform - methanol - water = 4:3:2). This system allows to fully isolate both polar and non-polar lipids.

The supernatant, 25 ml (to obtain a supernatant, the culture broth was centrifuged at 5000 g for 20 minutes) was placed in a 100 ml cylindrical separating funnel and surfactants were extracted according to the advanced procedure described below. Firstly, 5 ml of 1M HCl was added and shaken for 5 min, then 20 ml of a modified Folch mixture (16 ml of Folch reagent and 4 ml of 1M HCl) was added immediately and shaken again for 5 min. The mixture obtained after extraction was left in a separating funnel to separate the phases, then the lower fraction was drained (organic extract 1) and the aqueous phase was re-extracted. After re-extraction, 25 ml of the modified Folch mixture was added to the aqueous phase again (but at once 16 ml of Folch reagent and 9 ml of 1M HCl) and extracted with shaking for 5 min. After phase separation, the lower fraction was poured off to obtain organic extract 2. The extraction was repeated once more using a standard Folch mixture (chloroform: methanol = 2:1), and organic extract 3 was obtained. The extracts 1-3 were combined and evaporated on an IP1-M2 rotary evaporator at 50 °C and an absolute pressure of 0.4 atm to constant weight.

#### Determination of antimicrobial activity

The antimicrobial effect of essential oil, surfactants and their mixtures was analysed using the minimum inhibitory concentration (MIC) as described in (Pirog et al., 2020b). Essential oils of tea tree and cinnamon were dissolved in 5% ethyl alcohol to a concentration of 500  $\mu$ g/ml. MIC determination was carried out by the method of two-fold serial dilutions in meat-peptone broth (MPB) for bacteria and liquid wort for yeast.

Under sterile conditions, 1 ml of the medium was added to 10 tubes, 1 ml of an antimicrobial substance (surfactant or essential oils) of a certain concentration was added to the first tube, after which it was mixed, 1 ml was taken and transferred to the next tube. Similarly, the dilution was carried out for the next nine tubes. 1 ml was taken from the last tube. Thus, the final volume in each test tube was 1 ml, and the concentration of surfactants, antifungal substances, or essential oils in each subsequent tube decreased by 2 times. As a control, 1 ml of meat-peptone broth or liquid wort without the addition of a solution of antimicrobial substances was used. Then, 0.1 ml of the test culture suspension ( $10^5-10^6$  CFU/ml) was added to each of the tubes and mixed. The tubes were incubated for 24 hours at 24–26°C. The results were evaluated visually by the turbidity of the medium: (+) - test tubes in which the turbidity of the medium was observed (growth of the test culture), (-) - there was no turbidity (no growth), The minimum inhibitory concentration of antimicrobial substances in the first test tube, where there was no growth.

When determining the MIC of a mixture of drugs, their ratio was 1:1, while in one of the options the concentration of surfactants remained unchanged, and the concentration of essential oil was reduced by the method of successive two-fold dilutions, in the other, the concentration of essential oil remained unchanged, and the concentration of surfactant reduced.

Synergistic effect of surfactants with essential oils was evaluated by indicator of fractional inhibitory concentration (FIC), which is the sum of the ratio of the concentration of each substance in a mixture with their minimum inhibitory concentration (Hallander et al., 1982). FIC is calculated by the formula

 $FIC = (C_A/MIC_A) + (C_B/MIC_B),$ 

where C<sub>A</sub> and C<sub>B</sub> are the concentrations of the antimicrobial substance in the mixture;

MIC<sub>A</sub> and MIC<sub>B</sub> are minimum inhibitory concentrations of antimicrobial substance.

# Determination of the degree of biofilms destruction

The study of the effect of surfactant, essential oil and their mixture on biofilm destruction was carried out as described in (Pirog et al., 2020b). To form a biofilm, 180  $\mu$ l of meat-peptone broth or liquid wort and 20  $\mu$ l of a suspension of a one-day test culture were added to polystyrene microplates and incubated for 24 hours at the temperature optimal for the test culture. After this, the culture liquid was drained and 180  $\mu$ l of fresh MPB (liquid wort) and 20  $\mu$ l of test culture suspension were added and re-incubated for the next 24 hours.

After 48 hours, the culture liquid was drained, and 200  $\mu$ l of biocidal preparations (surfactant, essential oil and their mixture) of different concentrations (15.6–500  $\mu$ g/ml) were added to the wells of the microplate (with the biofilm of the test culture previously formed on them). To study synergism, use surfactants and essential oil in a 1:1 ratio. Instead of drugs, sterile tap water (200  $\mu$ l) was added to the control wells. After 24 hours of exposure, the wells were washed three times with 200  $\mu$ l of distilled water and the number of adhered cells was determined by the spectrophotometric method. The degree of biofilm destruction (%) was

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determined as the difference between cell adhesion in untreated and treated surfactant, essential oil or their mixture wells of a polystyrene tablet.

# **Results and discussion**

# Antimicrobial and antifungal activity of a complex of surfactants and essential oils

At the first stage, the effect of live inducer cells on the synergism of the antimicrobial activity of surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain on glycerol of various qualities was studied (Table 1).

Table 1

	Chaoral	Drosonao of	MIC (µg/ml) of						
Test culture	as substrate	inductor in the medium	Surfactants	*Surfactants mixed with essential oil **Essential oil mixed with surfactants **   0 25 16.87 ()   0 25 16.87 ()   0 2.1 31.25 ()   0 2.9 3.90 ()   0 2.5 31.25 ()   0 25 31.25 ()   0 25 31.25 ()   0 25 31.25 ()   0 5.7 15.63 ()   0 5.7 15.63 ()   1.2 15.63 () ()   0 3.1 31.25 ()   0 3.1 31.25 ()   0 3.1 31.25 ()   0 3.1 31.25 ()   0 3.1 31.25 ()   0 3.1 56.3 ()   0 1.5 15.63 ()	***FIC				
	munified	_	100.0	25	16.87	0.38			
Enterobacter	purmed	+	16.8	2.1	31.25	0.37			
cloacae C-8	orudo	_	185.0	2.9	3.90	0.05			
	crude	+	157.5	2.4	3.90	0.05			
Proteus vulgaris PA-12	purified	_	200.0	25	31.25	0.62			
		+	33.7	4.2	15.63	0.37			
		_	185.0	5.7	15.63	0.28			
	crude	+	78.7	1.2	iixed with sential oil mixed with surfactants ***FIC   25 16.87 0.38   2.1 31.25 0.37   2.9 3.90 0.05   2.4 3.90 0.05   2.5 31.25 0.62   4.2 15.63 0.37   5.7 15.63 0.28   1.2 15.63 0.26   3.1 31.25 0.15   0.53 15.63 0.07   1.2 15.63 0.07   1.2 15.63 0.07   0.78 31.25 0.50   0.26 31.25 0.52   1.44 15.63 0.27   1.23 15.63 0.27				
	nurified	_	100.0	3.1	its **Essential oil mixed with surfactants   16.87   31.25   3.90   3.90   31.25   15.63	0.15			
Bacillus subtilis	purmed	+	67.5	Surfactants Essential oil surfactants   25 16.87 0.38   2.1 31.25 0.37   2.9 3.90 0.02   2.4 3.90 0.02   2.5 31.25 0.62   4.2 15.63 0.37   5.7 15.63 0.26   1.2 15.63 0.26   3.1 31.25 0.15   0.53 15.63 0.07   11.5 15.63 0.07   0.78 31.25 0.57   0.26 31.25 0.57   1.23 15.63 0.07   1.23 15.63 0.07   1.23 15.63 0.27	0.07				
BT-2		_	315.0	11.5	15.63	0.10			
	crude	+	315.0	1.23	15.63	0.07			
	munified	_	25.0	0.78	31.25	0.50			
Staphylococcus aureus BMS -1	purmed	+	16.8	0.26	31.25	0.52			
	a ma d a	_	92.5	1.44	15.63	0.27			
	crude	+	78.7	1.23	15.63	0.27			

Minimum inhibitory concentrations of surfactants *A. calcoaceticus* IMV B-7241 synthesized on glycerol of different quality in the presence of live cells of *S. cerevisiae* BTM-1, tea tree essential oil and their mixture

Notes: The MIC of tea tree essential oil against *B. subtilis* BT-2 and *E. cloacae* C-8 was 250 and 125  $\mu$ g/ml, respectively, against *P. vulgaris* PA-12 and *S. aureus* BMS-1–62.5  $\mu$ g/ml;

<sup>\*</sup> the concentration of tea tree essential oil was unchanged and equalled their  $\frac{1}{2}$  MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 200–0.19 and 268–0.26 µg/ml for surfactants synthesized on purified glycerol by presence and without inductor respectively; 315–0.30 and 370–0.36 µg/ml for surfactants synthesized on crude glycerol by presence and without inductor respectively.

\*\*the concentration of surfactants remained unchanged and equalled their  $\frac{1}{2}$  MIC, and the concentration of tea tree essential oil was reduced by sequential double dilutions in the concentration range of 500–0.48 µg/ml.

\*\*\* - FIC  $\leq 0.5$  indicates synergism

It was found that surfactants synthesized in the presence of *S. cerevisiae* BTM-1 cells in the cultivation medium of *A. calcoaceticus* IMV B-7241 with both purified glycerol and crude glycerol showed a synergistic antibacterial effect with tea tree essential oil. For example, the MIC of surfactants synthesized in the presence of inducer cells in medium with purified glycerol against *P. vulgaris* PA-12 and *S. aureus* BMS-1 was 33.7 and 16.8 µg/ml, with tea tree essential oil it was 62.5 µg/ml, and with their mixtures it was 15.6 and 31.2 µg/ml, respectively. The use of surfactants obtained after cultivation of *A. calcoaceticus* IMV B-7241 in the presence of live cells of *S. cerevisiae* BTM-1 on crude glycerol in combination with an essential oil makes it possible to reduce the MIC of the latter against *P. vulgaris* PA-12 and *S. aureus* BMS-1 from 62.5 µg/ml to 15.6 µg/ml (Table 1).

It is worth noting that the index of the fractional inhibitory concentration did not exceed 0.5 (with the exception of the FIC for the mixture of surfactants synthesized on purified glycerol without an inducer with tea tree oil for strain PA-12), which indicates the synergism of the antimicrobial action of such compounds.

Table 2 presents the results the antifungal action of surfactants synthesized in the presence of live *S. cerevisiae* BTM-1 cells, cinnamon essential oil, and their mixture.

Table 2 Antifungal activity of surfactants *A. calcoaceticus* IMV B-7241 synthesized on glycerol of different quality in the presence of live cells of *S. cerevisiae* BTM-1, cinnamon essential oil and their mixture

		Presence	MIC (µg/ml) of						
Test culture	Glycerol as substrate	of inductor in the medium	Surfactants	*Surfactants mixed with essential oil	**Essential oil mixed with surfactants	***FIC			
Candida	purified	_	12.5	0.39	62.5	0.25			
albicans D-6		+	8.4	0.26	31.2	0.12			
	crude	_	185	1.44	62.5	0.25			
		+	157.5	0.62	31.2	0.12			
Candida tropicalis PE-2	purified	_	12.5	0.39	62.5	0.25			
		+	8.4	0.26	15.6	0.07			
	orudo	_	46.2	1.44	62.5	0.25			
	crude	+	39.4	0.15	15.6	0.07			

Notes: The MIC of cinnamon essential oil against *Candida albicans* D-6 and *Candida tropicalis* PE-2 was 250 µg/ml.

\*the concentration of cinnamon essential oil was unchanged and equaled their  $\frac{1}{2}$  MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 200–0.19 and 268–0.26 µg/ml for surfactants synthesized on purified glycerol by presence and without inductor respectively; 315–0.30 and 370–0.36 µg/ml for surfactants synthesized on crude glycerol by presence and without inductor respectively. \*\*the concentration of surfactants remained unchanged and equaled their  $\frac{1}{2}$  MIC, and the concentration

<sup>\*\*</sup>the concentration of surfactants remained unchanged and equaled their  $\frac{1}{2}$  MIC, and the concentration of cinnamon essential oil was reduced by sequential double dilutions in the concentration range of 500–0.48 µg/ml.

\*\*\* FIC  $\leq 0.5$  indicates synergism.

It was found that the minimum inhibitory concentrations of surfactants obtained by introducing live yeast cells into the medium of A. calcoaceticus IMV B-7241 with purified glycerol against C. albicans D-6 and C. tropicalis PE-2 were 8.4  $\mu$ g/ml, with cinnamon essential oil it was 250  $\mu$ g/ml, and with their mixtures it was 31.2 and 15.6  $\mu$ g/ml,

respectively. In particular, using surfactants in combination with essential oil, the MIC of the latter was reduced to  $0.26 \ \mu g/ml$ . Similar patterns were observed in the case of the use of surfactants synthesized in a medium containing crude glycerol in the presence of a eukaryotic inducer. The MIC values of such surfactants in a mixture with essential oil against yeast of the genus *Candida* were by 2.5–5 times lower than the minimum inhibitory concentrations of monosurfactant preparations.

The FIC index relative to yeast test cultures did not exceed 0.5, which indicates the synergism of the antifungal effect of surfactants synthesized by introducing a eukaryotic inducer into the environment of *A. calcoaceticus* IMV B-7241 with cinnamon essential oil against yeast of the genus *Candida*.

As noted above, there are no data in literature on the synergistic effect of essential oils with microbial secondary metabolites synthesized in the presence of biological inducers. However, there is work on the synergistic antimicrobial effects of essential oils or surfactants with other antimicrobial secondary metabolites such as antibiotics (Aleksic Sabo et al., 2021; Asadi et al., 2023; El Atki et al., 2019; Gan et al., 2023; Iseppi et al., 2023b; Özel et al., 2022;) and antifungal agents (Angiolella, 2021; Essid et al., 2017; Parkeret al., 2022; Sharifzadeh et al., 2017). For example, in a study (Asadi et al., 2023), it was found that a mixture of carvacrol, a component of cumin essential oil, and cefixime have a synergistic antimicrobial effect against *E. coli*, which was confirmed by the FIC index ( $\leq 0.5$ ).

The study (Özelet al., 2022) showed a synergistic effect of the components of cumin essential oil with tigecycline, linezolid, gentamicin and tetracycline against bacterial test cultures. Thus, for the complex of tigecycline with carvacrol, a synergistic effect was established against both *E. coli* and *P. aeruginosa* (FIC value did not exceed 0.25). When using a mixture of linezolid with both thymol and eugenol relative to *E. coli*, the fractional inhibitory concentration value was 0.375. Complex preparations of carvacrol with gentamicin and eugenol with tigecycline demonstrated a synergistic effect against *P. aeruginosa*, the FIC value was 0.375.

It was shown the synergistic antimicrobial activity of tea tree and eucalyptus essential oils with oxacillin against various strains of *S. aureus* (Iseppi et al., 2023b). Thus, under the action of a complex of tea tree essential oil and oxacillin against *S. aureus* 13 and *S. aureus* 20, the FIC was 0.19. At that time, the value of the fractional inhibitory concentration of a mixture of both oxacillin with tea tree oil and eucalyptus oil relative to *S. aureus* 32 did not exceed 0.5.

The work (Gan et al., 2023) recorded the synergism of antimicrobial activity (FIC 0.375) of a mixture of thymol essential oil with both streptomycin and gentamicin against *S. aureus*, as well as this ester with chloramphenicol against *Acinetobacter baumannii*. The authors also noted that the MIC of antibiotics decreased from 62.5 to 7.8  $\mu$ g/ml.

El Atki et al. (2019) showed a synergistic antibacterial effect of cinnamon essential oil with antibiotics against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Thus, the FIC value for a mixture of cinnamon essential oil with chloramphenicol was 0.5 for each test culture. The complex of this essential oil with ampicillin demonstrated a synergistic effect against *S. aureus* ATCC 25923, with an inhibitory fractional concentration of 0.38. In addition, the minimum inhibitory concentrations of each antimicrobial compound in the mixture were 2-4 times lower compared to MICs for both the essential oil and antibiotics used alone.

The synergistic effect of essential oils with antifungal agents was studied in the numerous research (Angiolella, 2021; Essid et al., 2017; Parker et al., 2022; Sharifzadeh et al., 2017). Thus, in the work (Angiolella, 2021) it was shown that when using a mixture of pelargonium essential oil with fluconazole, a synergistic antifungal effect was observed against all tested strains of *C. albicans* with FIC values of 0.5. It was also reported that the

MIC of each antimicrobial compound in such a mixture decreased to 390 mg /ml and 0.0625 mg/ml, respectively.

Another study (Essid et al., 2017) also investigated the synergistic effect of various combinations of essential oils with fluconazole on *Candida* yeasts. It was found that when using complexes of fluconazole with essential oils of *Pelargonium graveolens* and *Cinnamomum verum*, the FIC value was 0.37 for each complex, respectively. Such combinations made it possible to reduce the effective concentrations of selected biocides by 4-8 times.

Parker et al. (2022) found that using a mixture of clove or lemongrass essential oils with antibiotics flucytosine, fluconazole and micafungin can achieve a synergistic antifungal effect against yeast test cultures. A complex preparation of clove essential oil with fluconazole demonstrated synergistic antifungal activity against *C. auris* AR0381, *C. lusitaniae* AR0398 with FIC values of 0.28; 0.0625 respectively.

Sharifzadeh with co-authors (2017) showed that a mixture of menthol essential oil combined with itraconazole or nystatin showed a synergistic effect against all *Candida* species tested. FIC values for combinations of menthol with itraconazole and nystatin ranged from 0.250 to 0.561 and 0.139 to 0.623 against *C. glabrata* strains and from 0.182 to 0.750 and 0.188 to 0.760 against *C. krusei*, respectively.

Only a small number of research are devoted to the study of the synergistic antimicrobial action of the surfactant complex with essential oils (Haba et al., 2014; Pirog et al., 2020b) or antibiotics (Olfa et al., 2015; Pirog et al., 2020c). Olfa et al. (2015) studied the antimicrobial activity of the complex of bacillomycin D, synthesized by *B. subtilis* B38, with amphotericin B against *Candida* yeasts. It was found that under the action of a mixture with an antibiotic, it was possible to reduce the MIC of surfactants by 8-25 times against all tested yeast strains. The FIC of bacillomycin D with amphotericin B was from 0.27 to 0.5, which indicates the synergism of the compounds.

Pirog et al. (2020b) studied the synergistic effect of cinnamon and lemongrass essential oils and the surfactant of the *Nocardia vaccinii* IMV B-7405 against yeast of the *Candida* genus. It was found that surfactants obtained in a medium with refined or waste sunflower oil have a synergistic antimicrobial effect when mixed with essential oils of cinnamon and lemongrass. The use of such a mixture against *C. albicans* D-6 and *C. tropicalis* RE-2 led to a decrease not only in the MIC of surfactants (from 16–78 to 4–19  $\mu$ g/ml), but also in a decrease in the MIC values of essential oils in 8–130 times.

Consequently, the results obtained indicate that surfactants synthesized by introducing live *S. cerevisiae* BTM-1 cells into the cultivation medium of *A. calcoaceticus* IMV B-7241 with glycerol of various qualities demonstrate a synergistic antimicrobial effect in combination with essential oils. The use of a mixture of such surfactants with these biocidal compounds makes it possible to reduce the MIC of the surfactants themselves and essential oils by 2–47 and 2.5–250 times, respectively.

# Destruction of microbial biofilms under the action of a complex of surfactants with essential oils

The mechanisms of biofilm destruction under the action of microbial surfactants are based on the antimicrobial action of such compounds (Sharma et al., 2019). Therefore, at the next stage of research, the ability to destroy microbial biofilms through the action of a mixture of essential oils with surfactants of *A. calcoaceticus* IMV B-7241, synthesized by introducing live cells of *S. cerevisiae* BTM-1 into a medium with glycerol of various degrees of purification was studied (Tables 3–4).

#### Table 3

Destruction of biofilms of bacterial test cultures under the action of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the presence of live cells of *S. cerevisiae* BTM-1, tea tree essential oil and their mixture

Test culture	Anti- microbial	Glycerol as substrate		resence of concentration of antimicrobial compounds (μg/ml)					
	compound		mauctor	500	250	125	62.5	31.2	15.6
		munified	_	80	60	50	41	36	28
	annefa atamta	puilled	+	86	71	62	54	41	22
	surfactants	amada	—	63	44	30	34	26	15
Staphylococcus		crude	+	100	83	51	38	41	21
aureus BMS -1	mixture of surfactant and tea tree	purified	—	84	50	47	34	30	24
			+	100	88	70	59	46	40
		crude	_	86	60	52	38	33	17
	essential oil		+	92	89	60	45	42	22
		purified	_	100	100	94	72	60	50
			+	68	78	80	90	100	100
	surfactants	1	_	75	72	70	69	55	42
<i>Bacillus subtilis</i> BT-2		crude	+	72	75	72	100	100	32
	mixture of	munified	_	100	92	88	85	57	60
	surfactant	puilled	+	44	63	69	78	100	100
	and tea tree	amida	_	100	86	72	53	40	32
	essential oil	oil crude	+	47	63	72	75	94	98

Note: The degree of *S. aureus* BMS-1 and *B. subtilis* BT-2 biofilms destruction under the action tea tree essential oil in the range of studied concentrations (500–15.6  $\mu$ g/ml) was 13–59 and 24–72%, respectively.

It was found that when using a mixture of surfactants with essential oils of tea tree and cinnamon, it was possible to increase the degree of destruction of both bacterial and yeast biofilms of most of the test cultures studied compared to the effect of single preparations of surfactants and essential oils. For example, the degree of destruction of biofilms under the action of a tea tree essential oil complex from surfactants synthesized by adding an inducer to a medium with purified glycerol against to the bacteria S. aureus BMS-1 was 1.1 to 3.1 times higher compared to the use of monopreparations surfactants and essential oil (Table 3).

Similar patterns were observed when using a mixture of essential oil and surfactant obtained on crude glycerol in the presence of live cells of S. cerevisiae BTM-1. At the same time, the degree of destruction of the bacterial biofilm when using such a mixture was higher by 1.1 and 1.3–1.7 times for the indicators established separately for the surfactant and essential oil, respectively (Table 3).

It should be noted that with a decrease in the concentration of surfactants synthesized by adding live yeast cells to a medium with glycerol of varying degrees of quality, and used both separately and in mixture with tea tree oil, the degree of B. subtilis BT-2 biofilms destruction gradually increased (Table 3). Thus, under the influence of surfactants obtained during growth on a medium with purified glycerol in the presence of S. cerevisiae BTM-1, in a mixture with essential oil (concentration 15.6–31.25  $\mu$ g/ml), the degree of destruction of the biofilm of strain BT-2 reached 100%, which was 2.4–4.2 times higher than that established for monopreparations surfactants and essential oils.

Similar patterns were observed when using a complex of essential oil and surfactants obtained during the cultivation of the producer on crude glycerol with the addition of live inducer cells. With such a mixture in the concentration range of 15.6–31.2  $\mu$ g/ml, the degree of *B. subtilis* BT-2 biofilm destruction was 94–98%.

It was found that with increasing concentration of the tested antimicrobial compounds, both in the mixture and separately, the degree of destruction of *Candida* yeast biofilms increases (Table 4).

Table 4

Destruction of Candida biofilms under the action of surfactants synthesized by A. calcoaceticus
IMV B-7241 in the presence of live S. cerevisiae BTM-1 cells, cinnamon essential oil and their
mixture

Test culture	Antimicrobial compound	Glycerol as	erol Presence of		estruc) actio imicro	ction o on, con obial co	f biofili centrat ompour	m (%) tion of nds (μg	by /ml)
		substi ate	muuctor	500	250	125	62.5	31.2	15.6
		munified		82	68	58	45	34	33
	surfactants	puilled	+	92	84	80	83	73	68
	Surfactants	crude		77	71	69	57	46	34
Candida,		glycerol	+	100	100	92	69	60	51
albicans	mixture of	nurified		86	70	62	50	41	34
D-6	surfactant and cinnamon essential oil	puimea	+	100	100	100	94	80	76
		crude glycerol		70	60	50	30	19	14
			+	78	62	53	32	27	18
	surfactants	purified	_	35	37	31	25	14	15
			+	46	39	35	35	33	28
		crude	_	43	38	27	22	16	15
Candida		glycerol	+	55	41	33	23	25	17
tropicalis	mixture of	munified	_	54	48	42	35	26	19
PE-2	surfactant and	puilled	+	58	65	67	56	50	40
	cinnamon	crude	_	60	50	47	36	21	18
	essential oil	glycerol	+	61	65	66	59	47	40

Note: The degree of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms destruction under the action of cinnamon essential oil in the range of studied concentrations ( $500-5.6 \mu g/ml$ ) was 57–5 and 24–2%, respectively.

Thus, under the action of a complex of cinnamon essential oil and surfactants, synthesized in the presence of an inducer in the producer's cultivation medium with purified glycerol, at a concentration of 125–500 µg/ml, it was possible to achieve 100% destruction of C. albicans D-6 biofilms. However, under the influence of a complex of such essential oil from surfactants (15.6–500 µg/ml) formed on crude glycerol when adding live yeast cells, the degree of destruction of the *C. albicans* D-6 biofilm decreased by 1.3–2.8 and 1.2–3.2 times compared to the use of monosurfactants and essential oils, respectively (Table 4). It was found that under the influence of a mixture of surfactants synthesized in the presence of the BTM-1 strain in a medium with purified glycerol, with cinnamon essential oil, it was possible to increase the degree of *C. tropicalis* PE-2 biofilms destruction by 1.2–2.1 times, compared using each of the drugs separately (Table 4).

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Similar results were obtained using surfactants synthesized on crude glycerol in the presence of a eukaryotic inducer. Thus, the degree of destruction of biofilms of the corresponding test culture was higher when using a complex preparation of such surfactants and cinnamon essential oil (concentration 500–15.6  $\mu$ g/ml), compared with the indicators established for monopreparations of surfactants and oil in 1.1–2.5 and 1.3–2.2 times, respectively (Table 4).

Previously (Pirog et al., 2020b) synergism in the destruction of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms under the action of a mixture of surfactants synthesized by *Nocardia vaccinii* IMV B-7405 on refined and waste sunflower oil and cinnamon, lemongrass essential oils was observed. The degree of destruction of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms when using a mixture of these surfactants with essential oil was 1.5–2 times higher than the values established for individual compounds.

There are no studies in the available literature on the synergistic effect of essential oils with microbial secondary metabolites synthesized with the participation of biological inducers in the destruction of biofilms, including the ability of a mixture of surfactants with essential oils to destroy microbial biofilms. However, there are studies on the destruction of biofilms under the influence of essential oils mixed with antibiotics (Iseppi et al., 2023a; Miladi et al., 2017; Rosato et al., 2020).

Rosato et al. (2020) showed a synergistic effect of using a mixture of essential oils of cinnamon, peppermint and thymol in combination with antibiotics in the destruction of bacterial biofilms. Thus, the degree of *S. aureus* ATCC 29213 biofilms destruction under the action of oxacillin with peppermint or cinnamon essential oil was 68 and 73%, respectively, and was 16.8 or 19.2% higher than for the use of monopreparations.

Iseppi with co-authors (2023b) examined the ability to destroy bacterial biofilms under the influence of tea tree essential oil, the antibiotics cefotaxime, vancomycin, oxacillin and their mixture. It was found that when using a mixture of tea tree essential oil ( $32 \mu g/ml$ ) and cefotaxime ( $16 \mu g/ml$ ) or vancomycin ( $32 \mu g/ml$ ), the degree of *E. coli* 22BT or *E. faecalis* VAN3 biofilms destruction increases by 40 or 47% compared with the use of monopreparations of these antibiotics. It is also noted that when exposed to a mixture of such essential oil with oxacillin, the degree of *S. aureus* biofilm destruction was 50% higher than when using only the antibiotic.

Miladi et al. (2017) found that thymol, eugenol and carvacrol (the main antimicrobial components of cumin essential oil) showed a synergistic effect with tetracycline in the destruction of *S. aureus* strains biofilms. Thus, the degree of *S. aureus* B193 biofilm destruction under the action of a mixture of tetracycline (12  $\mu$ g/ml), as well as eugenol (250  $\mu$ g/ml), carvacol (79  $\mu$ g/ml) and thymol (85  $\mu$ g/ml), was 50% and was greater compared to the use of single-agent essential oils and antibiotics.

# Conclusions

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The results obtained show the synergistic positive effect of using mixtures of microbial surfactants, synthesized by the *Acinetobacter calcoaceticus* IMV B-7241 strain on glycerol of various qualities in the presence of a eukaryotic inducer, and tea tree or cinnamon essential oils on their antimicrobial and antifungal activities and ability to destroy microbial biofilms.

Minimum inhibitory concentrations of a mixture of tea tree and cinnamon essential oils and surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain both on purified and crude glycerol in the presence of eukaryotic inducer against bacteria *S. aureus* BMS-1, *E. cloacae* C-8, *P. vulgaris* PA-12, *B. subtilis* БТ-2 and yeast *C. albicans* D-6 and *C. tropicalis*  PE-2 were by 15–960 and 2.2–40 times lower, respectively, than the MICs of monopreparations of essential oils and surfactants.

Under the action of a mixture of tea tree essential oil and surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain on glycerol of different quality in the presence of live yeast cells, it was possible to increase the degree of bacterial biofilms destruction by 1.2–4.2 times, compared to the indicators established for individual antimicrobial compounds. The degree of yeast biofilms destruction under the action of a mixture of cinnamon essential oil and bacterial surfactants was by 1.2–2.4 times higher compared to monopreparations of essential oil and surfactants.

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# Анотації

# Харчові технології

# Споживчі властивості біодеградабельних їстівних стаканів для гарячих напоїв

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**Вступ.** Визначення споживчих властивостей біодеградабельного їстівного стакана дасть змогу знайти екологічну альтернативу одноразовим стаканам для гарячих напоїв.

Матеріали і методи. Виготовляли здобне печиво, яке формували у вигляді стаканів, з подальшим нанесенням водонепроникного шару на основі пектину або полівінілового спирту. Розроблено 10-балову шкалу оцінювання стаканів за окремими показниками, використано функцію Харрінгтона.

обговорення. Результати i Біолегралабельний їстівний стакан за органолептичними, ергономічними й геометричними параметрами повністю відповідає разовим стаканам, які нині використовуються. Їстівний стакан з гарячим напоєм за рахунок більшої товщини стінок і властивостей матеріалу (печива) можна тримати в руках без додаткового прошарку, що важливо з економічної та екологічної точки зору. Їстівний стакан утримує напій всередині від 20 хв до 1 год. Зовнішній вигляд стаканів не змінювався під впливом напоїв різної температурою: 10-15 °C (глясе, фрапе, холодний американо, айскава), теплими – 60–65 °С (лате, капучино, мокачино, макіато, флет вайт, раф) і гарячими – 85–90 °С (американо, еспресо та їх різновиди). Біодеградабельний їстівний стакан стійкий до дії оцтової кислоти та етилового спирту, що є важливим, оскільки деякі види напоїв на основі кави передбачають додавання алкогольних напоїв, зокрема, ірландської кави, фарисею, карску, кавового пуншу. Стакан може використовуватися для широкого асортименту напоїв з температурою від 10 до 90 °С. Має високі ергономічні показники, екологічно чистий, оскільки розкладається в навколишньому середовищі, як і будь-який інший кондитерський виріб.

**Висновки**. Критерій якості біодеградабельного їстівного стакана має більше значення порівняно з полімерним стаканом, тому такий виріб може замінити існуючі одноразові стакани.

**Ключові слова:** одноразовий стакан, їстівний, екологічний, біодеградабельний, критерії якості.

## Вплив продовженого протеолізу на біохімічний склад солодового сусла

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Вступ. Зміна режимів затирання солоду збільшує кількість екстрагованих речовин. Визначено біохімічний склад солодового сусла з пролонгованою дією протеолітичних ферментів у процесі затирання.

Матеріали і методи. Основним матеріалом була суміш трьох видів солоду: світлого солоду, меланоїлинового солоду смаженого солоду. Аналіз та вуглеводного амінокислотного, ліпідного профілю модифікованому й в протеолітичному суслі проводили хроматографічними методами.

Результати і обговорення. В аналізованому суслі виявлено 19 амінокислот, 10 з яких незамінні. Загальний вміст амінокислот у контрольному суслі становив 1349 мг/дм<sup>3</sup>. Загальний вміст амінокислот у протеолітичному суслі, модифікованому за першим режимом затирання, збільшився на 55% – до 2096 мг/дм<sup>3</sup>. Загальний вміст амінокислот у протеолітичному суслі, модифікованому за апершим режимом затирання, збільшився на 55% – до 2096 мг/дм<sup>3</sup>. Загальний вміст амінокислот у протеолітичному суслі, модифікованому за другими режимом затирання, збільшився на 90% – до 2572 мг/дм<sup>3</sup>. Вміст окремих амінокислот у модифікованому протеолітичному суслі зростав у широкому діапазоні. Вміст валіну та фенілаланіну в модифікованому протеолітичному суслі підвищився на 101-102%, в той же час кількість гліцину зросла лише на 18%.

Хроматографічний аналіз показав, що тривалий протеоліз не впливає на вуглеводний і ліпідний склад сусла. Загальний вміст жирних кислот коливався від 5,6 до 11,4 мг/дм<sup>3</sup>. Загальна кількість жирних кислот у зразках модифікованого протеолітичного сусла була різною, без вираженої залежності. Різниця загального вмісту жирних кислот між зразками модифікованого протеолітичного сусла становила більше 30%. Аналіз сусла виявив 11 жирних кислот. Пальмітинова та лінолева кислоти мали найвищий вміст серед жирних кислот у модифікованому протеолітичному суслі. Вміст пальмітинової кислоти в суслі становив 38–48%, вміст лінолевої кислоти (омега-6) становив 30–37%.

Вуглеводний аналіз модифікованого солодового сусла показав, що вуглеводний профіль сусла представлений мальтозою (53,1%), декстринами (23,4%), мальтотріозою (15,2%) та глюкозою (8,3%). Вуглеводний профіль модифікованого сусла не відрізнявся від звичайного солодового сусла.

Висновки. Модифіковане протеолітичне сусло після тривалого протеолізу під час затирання є гарним джерелом амінокислот. Тривала білкова пауза значно підвищує вміст амінокислот у солодовому суслі та може бути використана в технології БАДів або функціональних напоїв.

Ключові слова: солод, сусло, амінокислота, протеоліз, затирання.
# Вплив позакореневого внесення добрив на леткі сполуки червоних вин

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Вступ. Мета досліджень – визначити впливу різних позакореневих добрив (у чотирьох врожаях – 2019, 2020, 2021 і 2022 років) на склад летких та ароматичних сполук червоних вин болгарського гібридного сорту Сторгозія.

**Матеріали і методи**. Для визначення летких сполук у червоних винах використоно метод газової хроматографії з полум'яно-іонізаційним виявленням.

Результати і обговорення. Отримані результати щодо загального вмісту летких сполук в аналізованих винах підтвердили, що оброблення виноградної лози добривом MaxGrow NS (склад: N 20%, S 7%, Mg 14%, P 5%, Mn 1%, Zn 0,01%, Cu 0,05%, Fe 0,05%) сприяло підвищенню рівня летких і ароматичних сполук у вині трьох із чотирьох досліджених урожаїв (2019, 2020 та 2021). Як окремі представники з групи вищих спиртів 2-метил-1-бутанол і 3-метил-1-бутанол домінували у всіх чотирьох зборах. Два спирти є основними метаболітами дріжджової мікрофлори. Застосування різних позакореневих добрив не вплинуло негативно на вміст ацетальдегіду в отриманих винах. Його присутність відповідала видимості позитивного впливу. В урожаях 2020 та 2022 років позакореневе підживлення азотом і мінеральними джерелами вплинуло на вміст естерів. У двох інших зборах окремі варіанти показали більш високе накопичення складного ефіру, ніж необроблений контроль, але інші продемонстрували нижчі рівні. У результаті неможливо було зробити жодного конкретного висновку щодо впливу позакореневого підживлення на кінцеві концентрації ефірів вина. Основним естером цієї фракції був етилацетат. Позакореневе підживлення впливає на синтез В-цитронеллолу, що призводить до підвищення рівня цього терпену. І навпаки, спостерігалося зниження концентрації гераніолу у винах, отриманих після застосування позакореневого підживлення.

**Висновки**. Застосування позакореневих добрив призводило до зміни у складі фітонцидів досліджуваних червоних вин і впливало як на різні групи фітонцидів, так і на окремі сполуки.

Ключові слова: виноград, добриво, червоне вино, леткі сполуки.

# Економіка і управління

# Шляхи скорочення м'ясних відходів у домашньому господарюванні мексиканських сімей

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Вступ. Побутові харчові відходи є основною проблемою в усьому світі, однак у країнах, що розвиваються, кількісної інформації з цього питання мало. Це дослідження мало на меті отримати базову інформацію про споживчу поведінку, практики, переконання і ставлення, пов'язані з відходами яловичого фаршу в мексиканських домогосподарствах.

Матеріали і методи. Дані отримано в результаті опитування 740 споживачів із двох великих міст Мексики. Опитування щодо обізнаності й утилізації яловичого фаршу вдома було проведено серед споживачів, які мали вибрати відповідь серед п'яти фіксованих варіантів. Статистичний аналіз проводили за допомогою ксі-квадрат тесту.

Результати і обговорення. Споживачів просили вибрати з опитування найбільш близький на їхню думку варіант. Більше 75% респондентів мали домогосподарства з 3-5 членами, які складалися переважно з дітей віком ≤10 років і меншою часткою людей віком 70 років і старшими. Лише 33% купують м'ясний фарш, упакований і виставлений у відкритих холодильних шафах у супермаркетах, тоді як решта, 67%, купують його в місцевих м'ясників. Термін придатності вважався важливим параметром, оскільки йдеться про рівень свіжості м'яса і його позитивний вплив на здоров'я. I, навпаки, для >50% респондентів м'ясні відходи асоціюються з прибиранням. Викидання фаршу в мексиканських домогосподарствах може статися протягом кількох днів після покупки, що пов'язано з неадекватними методами зберігання й охолодження. Основні причини обізнаності споживачів про побутові харчові відходи були пов'язані з почуттям провини (73,0%) і шкодою для навколишнього середовища (71,6%), тоді як тухле м'ясо і недоїдені залишки пов'язували з викиданням фаршу. Несправності побутових холодильників і купівля надлишків іноді впливали на швидкопсувні харчові відходи. Існують значні зв'язки між харчовими втратами та різними видами діяльності, такими як купівельна практика, методи приготування, ставлення і спосіб життя, харчові звички. Причини, які можуть бути пов'язані з відходами – це тривале зберігання сирого м'яса, закінчення терміну придатності та огида. Крім того, інші пов'язані аспекти зосереджені на нав'язливих покупках у поєднанні з потребою в плануванні та визначенні пріоритетів при покупці великих пакетів.

**Висновок**. Будь-яка програма, пов'язана зі зменшенням відходів від фаршу в домогосподарствах, повинна включати розробку урядової та неурядової політики, яка впливає на всі ланки ланцюга постачання, особливо на кінцевого споживача.

Ключові слова: харчування, відходи, Мексика, яловичий фарш, побут.

#### Уявлення про рослинне харчування на основі вибірки угорських жінок

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**Вступ.** Мета дослідження – вивчити переконання і хибні уявлення про рослинну дієту угорських жінок і з'ясувати, як вони залежать від способу життя.

Матеріали і методи. Дані, зібрані за допомогою онлайн-опитування в соціальних мережах, оброблені за допомогою одномірної статистики (загальний опис вибірки), дослідницького факторного аналізу (визначення здорового способу життя), кластерного аналізу (з метою сегментації), статистики Хі-квадрат тесту (профілювання кластерів), F-статистики (порівняння ставлення до рослинної дієти) та перехресної табуляції (знання та уявлення про рослинну дієту).

**Результати і обговорення.** Було визначено чотири виміри способу життя, пов'язані зі здоров'ям (здорове харчування, уважність, відмова від вуглеводів, відмова від червоного м'яса), та виділено чотири сегменти (ті, хто обирає здорову їжу, ті, хто відмовляється від червоного м'яса, жінки, які не піддаються стресам, ті, хто відмовляється від їжі). Ті, хто обирає здорову їжу (40,9%), надають перевагу здоровому харчуванню, уникають солодких закусок і стежать за споживанням вуглеводів. Ті, хто уникає червоного м'яса (27,9%), ставляться до здорового харчування нейтрально, але уникають червоного м'яса та перероблених продуктів, не зосереджуються на вуглеводах. Жінки, які живуть без стресу (20,8%), цінують уважність, релаксацію та фізичну активність на свіжому повітрі для життя без стресу. Відмовники (10,4%) негативно ставляться до здорового харчування, вуглеводів і червоного м'яса. Прихильники червоного м'яса живуть у сслах і їдять фрукти та овочі кожні 4–5 днів на тиждень або не їдять фрукти та овочі більше разів на день. Стресостійкі люди їдять фрукти та овочі кожні 2–3 дні на тиждень.

Учасники опитування відрізнялися рівнем знань, ставленням і сприйняттям рослинної дієти. 72,1% прихильників здорового харчування, 84,8% тих, хто уникає червоного м'яса, 75,8% людей, які не піддаються стресам, і 71.9% тих, хто відмовляється від нього, вважають, що рослинне харчування схоже на веганське і вегетаріанське. Позиції варнюються від «може мати переваги для здоров'я при певних захворюваннях» як позиції з найвищим середнім рівнем згоди (4.26), особливо серед тих, хто уникає червоного м'яса, до «заохочує щоденне споживання» як позиції з найнижчим середнім рівнем згоди (1,69), особливо серед тих, хто уникає червоного м'яса. Жінки, які уникають червоного м'яса, обирають здорову їжу, не схильні до стресу мали більш позитивне ставлення до рослинної дієти, ніж ті, хто відмовляється від неї. Більшість жінок думали про те, щоб спробувати рослинну дісту. Жінки, які уникають червоного м'яса, дотримуються здорового харчування та не схильні до стресу, більш позитивно ставляться до рослинної дієти, ніж ті, хто її відкидає. Прихильники здорового харчування сприймали рослдинну дієту як здорову їжу. Ті, хто не вживає червоного м'яса, вважали рослинну дісту здоровою, безпечною, різноманітною, цікавою, екологічно чистою та повноцінною. Жінки, які не страждають від стресу, вважали рослинну дісту нездоровою та екологічно недружньою. Негативно налаштовані жінки характеризували рослинну дієту як нездорову, небезпечну, одноманітну, нудну, екологічно несприятливу. Така їжа важко перетравлюється і призводить до недоїдання.

Висновки. Результати дослідження доповнюють літературну дані, додаючи емпіричні докази нових тенденцій (рослинна діста, веганські, вегетаріанські дісти), а також генерують пропозиції для фахівців з харчування і дістології та уряду, оскільки можна планувати цільові маркетингові програми, спрямовані на зміну поведінки в харчуванні.

Ключові слова: здоров'я, спосіб життя, рослинна дієта, веган, вегетаріанська дієта, стійкість.

# Процеси і обладнання

# Визначення коефіцієнта ежекції рідинно-газового ежектора з комбінованою камерою змішування

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Вступ. Метою досліджень була розробка методики визначення фактичного коефіцієнта ежекції рідинно-газового ежектора з комбінованою камерою змішування.

Матеріали і методи. Використовували теоретичні методи розрахунку (рівняння балансу маси й енергії у формі рівняння Бернуллі та закони гідродинаміки), експериментальні методи (коефіцієнти ежекції ежектора з комбінованою камерою змішування, що визначались експериментально на гідравлічному стенді з метою визначення константи ежекції). Для порівняння коефіцієнтів ежекції використовували графоаналітичний метод Соколова-Зінгера.

Результати і обговорення. Характерною особливістю ежектора з комбінованою камерою змішування є наявність початкової конічної та наступної циліндричної частин камери змішування. Кут розкриття конічної частини на 3–8° менший від кута факелу розпалення рідини з сопла. Така конструкція знижує гідравлічний опір надходженню рідини і запобігає утворенню зворотних циркуляційних потоків. Коефіцієнт ежекції струминного апарата з комбінованою камерою змішування на 15– 55 % вищий, ніж у ежектора з циліндричною камерою змішування.

Спільне розв'язування рівнянь балансу для конічної та циліндричної частин камери змішування з урахуванням втрат енергії в кожній дає змогу визначити теоретичні витрати фаз в ежекторі для різних режимів роботи.

Коефіцієнт k враховує вплив перерозподілу енергії між фазами при ежекції та конструкцію камери змішування. При підвищенні тиску від 0,05 до 0,25 МПа коефіцієнт k зростає від 3,6 до 4,8 у раціональній показниковій функції.

Ефективний коефіцієнт ежекції визначається як добуток теоретичного коефіцієнта викиду на експериментальну постійну, при цьому похибка не перевищує 5%.

**Висновки**. Запропонована методика розрахунку дає змогу визначити ефективний коефіцієнт ежекції рідинно-газового ежектора з комбінованою камерою змішування.

Ключові слова: ежектор, ежекція, рідинно-газовий, змішування.

# Інтенсифікація процесів сушіння культивованих грибів шиїтаке *(Lentinula edodes)* за рахунок комбінованих методів зневоднення

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Вступ. Це дослідження оцінювало різні режими комбінованих методів зневоднення інфрачервоного випромінювання та конвективного на кінетику і коефіцієнт набухання культивованих грибів шиїтаке.

Матеріали і методи. Свіжі культивовані гриби шиїтаке були сортовані за формою, зрілістю і розміром. Кінетику сушіння було досліджено конвективним двостадійним 100/60 °C та комбінованим 100 Вт+60 °C методами. Якісну характеристику висушених грибів було досліджено методом регідратації.

Результати і обговорення. Зміна вологості грибів шиїтаке при температурі теплоносія 60 °С відбувається протягом 156 хв, а при двостадійному методі 100/60 °С (конвективне) вона становить 97 хв. При використанні комбінованого методу 100 Вт+60 °С вологість змінюється за 110 хв. Застосування комбінованого методу 100 Вт+60 °С та двостадійного 100/60 °С зменшує тривалість сушіння в 1,4–1,6 раза порівняно з традиційним режимом 60 °С.

Ефективність цих процесів підтверджена визначенням зміни числа Ребіндера, який залежить від температури теплоносія та вологовмісту. В період зміни вологості культивованих грибів шиїтаке від 84–10% значення числа Ребіндера *Rb* мінімальне. Після досягнення вологості матеріалу 10% число Ребіндера різко збільшується, а отже, більша частина теплоти витрачається на нагрівання матеріалу, що призводить до збільшення енерговитрат на процес і погіршення якості матеріалу. Визначення регідратаційних властивостей підтвердили високу якість зразків при цих режимах, які зберігають фізичні властивості матеріалу.

Дослідження дає цінну інформацію щодо оптимізації процесів сушіння, сприяючи виробництву високоякісних сушених грибів.

Висновки. Різниця між тривалістю процесів сушіння комбінованим інфрачервоно-конвективним 100 Вт+60 °С та двостадійним 100 °С /60 °С становить 0,5 %. Отже, ці два режими є найбільш ефективними і можуть бути рекомендовані до використання.

Ключові слова: гриби, шиїтаке. сушіння, набухання, регідратація.

# Інтенсифікація процесу гідрорізання харчових продуктів глибокого заморожування

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Вступ. Проведено дослідження процесу різання харчових продуктів глибокого заморожування водяним, водольодовим і водоазотним струменями з метою інтенсифікації процесу гідрорізання шляхом модифікації робочої рідини.

Матеріали і методи. Матеріали: філе риби хека і яловиче м'ясо, а також зразки льоду, які мали температуру -30 °С як модельний зразок м'яса при температурі -25 °С. *Експериментальні методи* дослідження: гідрорізальна установка з робочим тиском до 500МПа. Досліди проводилися за температур харчових продуктів від -3°С до -25 °С, зміни тиску від 50 МПа до 500 МПа, діаметра сопла від 0,2 10<sup>-3</sup> до 0,8 10<sup>-3</sup> м та швидкості переміщення струменя робочої рідини відносно зразка харчового продукту від 0,01 до 0,07 м/с.

Результати і обговорення. Збільшення тиску з 200 до 500 МПа викликає зростання глибини розрізу для сопел з діаметром  $0.2 \cdot 10^{-3}$  і  $0.4 \cdot 10^{-3}$  м майже в 21 і 6 разів відповідно. Збільшення діаметра отвору сопла призводить до збільшення глибини розрізу в замороженому харчовому продукті. При цьому максимальна глибина розрізу в харчовому продукті, що має температуру  $-11^{\circ}$ С, не перевищувала  $85 \cdot 10^{-3}$  м при тиску 500 МПа. Зниження температури харчового продукту до  $-11^{\circ}$ С і нижче виключає можливість використання гідрорізання при тисках 250–300 МПа. Проведено експериментальну перевірку можливості використання водяного струменя з введеними в нього малими частинками льоду для розширення технологічних можливостей гідрорізання. Інтенсифікація процесу гідрорізання харчових продуктів глибокого заморожування шляхом додавання до водяного струменя мікрочастинок льоду є високоефективною, але занадто економічно затратною. Використання водозотних струменів, в яких роль абразиву відіграють мікрочастинки льоду, що утворюються в процесі охолодження водяного струменя парами рідкого азоту, дає змогу суттєво інтенсифікувати процес гідрорізання харчових продуктів.

Висновки. Для суттєвої інтенсифікації процесу гідрорізання харчових продуктів глибокого заморожування найдоцільніше використовувати як робочу рідину водоазотний струмінь, в якому абразивом є мікрочастинки льоду, що утворюються в процесі охолодження водяного струменя парами рідкого азоту, які подаються в коліматор.

Ключові слова: харчові продукти, струмінь, тиск, температура, гідрорізання.

# Біотехнологія, мікробіологія

# Синергізм біологічної активності суміші ефірних олій і поверхнево-активних речовин Acinetobacter calcoaceticus IMB B-7241, синтезованих у присутності еукаріотичного індуктора

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Вступ. Дослідження присвячено вивченню синергічної антимікробної, антифунгальної дії та ролі у руйнуванні біоплівок за дії комплексу ефірних олій чайного дерева та кориці з поверхнево-активними речовинами *Acinetobacter calcoaceticus* IMB B-7241, синтезованими за наявності дріжджового індуктора.

Матеріали і методи. Культивування A. calcoaceticus IMB B-7241 здійснювали у рідкому мінеральному середовищі з очищеним або неочищеним гліцерином. Дріжджі Saccharomyces cerevisiae БТМ-1 використовували як індуктор. Концентрацію ПАР визначали після екстракції з супернатанту ваговим методом. Антимікробну активність ефірних олій, поверхнево-активних речовин та їх суміші аналізували за показником мінімальної інгібуючої концентрації (МІК).

Результати і обговорення. Поверхнево-активні речовини одержані культивуванням штаму *A. calcoaceticus* IMB B-7241 на гліцерині різної якості в присутності еукаріотичного індуктора. Встановлено, антибактеріальну активність щодо *Staphylococcus aureus* БМС-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* ПА-12, *Bacillus subtilis* БТ-2 і антифунгальну активність щодо *Candida albicans* Д-6 і *Candida tropicalis* PE-2, а також здатність до руйнування біоплівки за дії комплексу мікробних поверхнево-активних речовин та ефірної олії чайного дерева або кориці. МІК суміші ефірної олії чайного дерева або кориці та поверхнево-активних речовин, синтезованих штамом *A. calcoaceticus* IMB B-7241 як на очищеному, так і неочищеному гліцерині в присутності еукаріотичного індуктора щодо досліджуваних бактерій і дріжджів, була у 15–960 та 2,2–40 разів нижчою відповідно, ніж мінімальна інгібуюча концентрація ефірних олій та ПАР окремо.

Використання суміші ефірної олії чайного дерева та поверхнево-активних речовин, синтезованих штамом IMB B-7241 на очищеному гліцерині в присутності живих дріжджових клітин, підвищувало ступінь руйнування бактеріальних біоплівок в 1,1– 4,2 раза, порівняно з індивідуальними сполуками. Використання комплексу ефірної олії чайного дерева та поверхнево-активних речовин, отриманих на середовищі з неочищеним гліцерином з дріжджовим індуктором, підвищувало ступінь руйнування бактеріальних біоплівок у 1,2–4,1 раза порівняно з індивідуальними сполуками. Деструкція дріжджових біоплівок під дією суміші ефірної олії кориці та ПАР, синтезованих у присутності індуктора в середовищі з гліцерином різної якості, була відповідно в 1,2–2,1 та в 1,1– 2,4 раза вищою за встановлений показник для окремих сполук.

Висновки. Результати показали синергічний вплив на біологічну активність, зокрема на руйнування біоплівок суміші ефірної олії чайного дерева або кориці з поверхневоактивними речовинами *A. calcoaceticus* IMB B-7241, отриманими за присутності у середовищі культивування продуцента живих клітин *S. cerevisiae* BTM-1.

**Ключові слова:** комбіноване культивування, біологічний індуктор, синергізм, антимікробна активність, руйнування біоплівок.

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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

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