Ukrainian Food Journal

Volume 11, Issue 1
2022

Kyiv　　　　　　　　　　**Київ**
2022

Editorial office address:

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Scientific Council of the National University of Food Technologies approved this issue for publication. Protocol № 10, 26.05.2022

Рекомендовано вченою радою Національного університету харчових технологій. Протокол № 10 від 26.05.2022

Ukrainian Food Journal is open access journal published by the National University of Food Technologies (Kyiv, Ukraine). The Journal publishes original research articles, short communications, review papers, news and literature reviews dealing with all aspects of the food science, technology, engeneering, nutrition, food chemistry, economics and management.

Studies must be novel, have a clear connection to food science, and be of general interest to the international scientific community.

Topic covered by the journal include:

- Food engineering
- Food chemistry
- Food microbiology
- Food quality and safety
- Food processes
- Automation of food processes
- Food packaging
- Economics
- Food nanotechnologies
- Economics and management

Please note that the Journal does not consider:
1. The articles with medical statements (this topic is not covered by the journal); the subject of research on humans and animals.
2. The articles with statements, that do not contain scientific value (solving the typical practical and engineering tasks).

Periodicity of the Journal
4 issues per year (March, June, September, December).

Reviewing a Manuscript for Publication
The editor in chief reviews the correspondence of the content of a newly submitted article to the Journal Profile, approves the article design, style and illustrative material, can provide suggestions how to improve them, and makes the decision whether to send it for peer-review.

Articles submitted for publication in "Ukrainian Food Journal" are double-blind peer-reviewed by at least two academics appointed by the Editors' Board: one from the Editorial Board and one, not affiliated to the Board and/or the Publisher.

For a **Complete Guide for Authors** please visit our website:

http://ufj.nuft.edu.ua

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Editorial

Ukrainian Food Journal is still a very young journal: in the spring of 2012 its first issue was published. However, the scientific and publishing traditions of the publisher, the National University of Food Technologies, date back to the middle of the 19th century. At the beginning, our Journal was aimed at creative youth, and until 2017, its description contained the sentence "The advantage in publication is given to PhD students and young scientists." Gradually, the Journal gained importance as a periodical publishing scientific research by leading scientists in the field of food and related branches of science, expanding the geography of authors and the editorial board. Some time later, the Ukrainian Food Journal began to be indexed by international scientometric databases, since 2018 – by Web of Science, and in 2022 a decision was made to include the Journal in the Scopus database. This indicates the proper rating and scientific significance of the materials published in it.

We are grateful to the international organization the Association of the Global Harmonization Initiative (GHI), which in 2022 helped us to invite leading scientists in the field of food technology, chemistry, processes and equipment, economics and management from different countries to participate in the work of the editorial board of our journal. At this time, new members entered the editorial board:

- Bảo Thy Vương (*Mekong University, Vietnam*)
- Cristina Luisa Miranda Silva (*Portuguese Catholic University – College of Biotechnology, Portugal*)
- Dora Marinova (*Curtin University Sustainability Policy (CUSP) Institute, Curtin University, Australia*)
- Eirin Marie Skjøndal Bar (*Norwegian University of Science and Technology, Trondheim, Norway*)
- Godwin D. Ndossi (*Hubert Kairuki Memorial University, Dar es Salaam, Tanzania*)
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- Yun-Hwa Peggy Hsieh (*Florida State University, USA*)

We welcome new members of our editorial team and sincerely thank the editorial board, which has been working fruitfully since 2012, as well as the authors who support the journal and publish their articles in it.

On February 24, the civilized world shuddered at the news of the russian invasion of Ukraine. Rockets flew to Ukraine, the bombing of our cities began, the occupying troops entered. Mass killings of civilians, robbery and looting, destruction of transport and energy infrastructure, food industry enterprises and food warehouses, farms, medical institutions, schools and universities, and research institutions began.

Ukrainian science is working in times of war now. Some scientists were forced to evacuate, the rest remained to work under rocket fire. We are grateful to colleagues from all over the world for their moral support. This gave us the strength to hold on and continue publishing our Journal.

Ukraine has always made a significant contribution to food supplies around the world. At present, when the rashists have blocked the supply of food raw materials from Ukraine, the world has understood the true global significance of our country in world food security. Wheat, sunflower, vegetables, fruits turned out to be more in demand than oil and gas. Ukraine needs global support to unlock supply chains, and the world is in danger of starvation without Ukrainian food.

There is no doubt that the truth will win this hateful war. Peace will reign again on Earth, research, innovation, and the work of educational institutions will resume. But we cannot just wait for victory. All ways to improve the Journal are now open. We have an excellent professional team, the world's leading scientists publish their results in our Journal. Let's join our efforts for the success of Ukrainian Food Journal!

Editor-in-Chief
Olena Stabnikova

Influence of drying temperature on the organoleptic properties, antioxidant activity and polyphenol content in dried leaves of *Allium ursinum* L. subsp. *ucrainicum*

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Abstract

Introduction. The short vegetative occurrence of *Allium ursinum* limits its availability. Therefore, drying seems to be an excellent method for year-round preservation. The aim of the present study was to determine the influence of drying temperature on antioxidant activity and polyphenol content in dried leaves of *Allium ursinum* L. subsp. *ucrainicum* and their organoleptic properties.

Materials and methods. The effect of three drying temperatures (40, 50 and 60 °C) on the organoleptic properties (colour, dehydration and rehydration ability), antioxidant activity and polyphenol content in the dried leaves of *A. ursinum* was evaluated. The colour of the samples was measured using the computer vision system. The total phenolic content was determined spectrophotometrically and the antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl method.

Results and discussion. Significant differences were found between the fresh, dehydrated and rehydrated *A. ursinum* samples for all the colour parameters analysed (dried leaves showed a much lower intensity of green colour than fresh). Drying at higher temperature results in greater colour change, which is more pronounced at higher drying temperatures (60 °C) due to chlorophyll degradation. The drying temperatures had a statistically significant effect on the dehydration and rehydration capacity of the dried samples. The higher drying temperature resulted in the higher degree of dehydration and rehydration (the pores of the dried food allowed water to re-enter the cells). Convection air-drying resulted in considerable moisture removal from the fresh leaves of *A. ursinum* (more than 91%), but the organoleptic quality of the *A. ursinum* leaves was maintained. The drying conditions tested had a significant effect on the total phenolic content and antioxidant activity of *A. ursinum* leaves. An increase of temperature drying decreased the total polyphenol content in the dried *A. ursinum* leaves. Across the range of measurements, the samples dried at lower temperatures had the higher antioxidant capacity, while the higher drying temperatures resulted in a greater decrease in the antioxidant activity of the dried plant material. *A. ursinum* is considered one of the functional foods for human consumption due to its high nutritional value and prophylactic or therapeutic effects in various diseases. To obtain a high quality dried product, the drying process should ensure a quality comparable to fresh vegetables.

Conclusions. Air drying showed a significant effect on the colour, drying properties, total polyphenol content and antioxidant activity of the leaves of *A. ursinum*. The losses were significantly dependent on the drying temperature and were more pronounced at higher process temperatures.

Introduction

Allium ursinum L. is known by many different names: wild garlic, leek, wood garlic, bear's garlic, ramsons, buckrams, broad-leaved garlic, gypsy onion and pig's garlic. It belongs to the large family *Amaryllidaceae*, which is represented all over the world with 59 genera and over 850 species. As a member of the genus *Allium*, wild garlic is closely related to herbs such as onion (*Allium cepa*), garlic (*Allium sativum*), leek (*Allium ampeloprasum*), and chives (*Allium schoenoprasum*) (Hanen et al., 2012). *Allium* species are considered a source of phytonutrients with diverse biological activities (Lachowicz et al., 2017; Gitin et al., 2012), such as antibacterial, antifungal (Parvu et al., 2011), antioxidant (Bozin et al., 2008), and therapeutic activities, which are associated with the presence of sulfur components (Gođevac et al., 2008). Due to the presence of sulfur compounds, which are otherwise rather characteristic components of *Allium* plants, *A. ursinum* has a distinctive garlic-like odour.

A. ursinum is a plant with a high potential for the prevention and treatment of cardiovascular, respiratory and digestive problems, as well as for the sterilisation of wounds (Sobolewska et al., 2013) and the prevention of carcinogenic diseases (Sengupta et al., 2004). These properties are due to many substances, including cysteine sulfoxides and thiosulfinates, ajoenes and dithiines, phenolic compounds, saponins and vitamins C, E and A (Lu et al., 2011; Roldan- Marin et al., 2009).

It grows mainly in moist deciduous forests throughout Europe and in parts of Asia and North Africa (Oborny et al., 2011; Rola 2012). *Allium ursinum* L. comprises two subspecies *Allium ursinum* subsp. *ursinum* and *Allium ursinum* subsp. *ucrainicum*. In Eastern and South Eastern Europe, and Croatia as well, *Allium ursinum* subsp. *ucrainicum* grows in continental and mountainous areas (Rola, 2012; Tutin 1957). Although all parts of this plant are edible (bulbs, leaves, buds, flower stalks, flowers and immature green cobs), leaves and bulbs are generally preferred for consumption. The fresh leaves or dried herb of *A. ursinum* is used in local cuisines of Europe. There are many products derived from garlic as a raw material: garlic powder, paste, extract, oil, macerated garlic, pickled garlic, dried garlic. The medicinal parts of the plant are the young spring leaves, harvested in April and May, and the underground bulbs, collected in the summer and autumn months. However, the short vegetative presence of *A. ursinum* limits its availability, so drying can be a solution for preserving it throughout the year.

Since agricultural products are highly seasonal and therefore abundant at certain times of the year, preserving fruits and vegetables through drying can both avoid major waste and ensure availability in the off-season. Drying is one of the thermal processes that agricultural products undergo in the post-harvest phase. The aim is to reduce the moisture content of the product in order to delay adverse biological (prevents the growth of microorganisms), chemical and enzymatic processes. Although drying is an alternative to extend the shelf life of food, it is a fact that the quality of dehydrated food is usually lower than that of the original food. Therefore, it is of interest to minimise chemical changes such as enzymatic and non-enzymatic browning and to maximise the retention of nutrients such as macronutrients (proteins, sugars, fibres), micronutrients (vitamins, minerals) or bioactive compounds (phenolic compounds, carotenoids, isoflavones) during drying. The drying process is considered to affect the content, activity and bioavailability of bioactive compounds (mainly polyphenols) in *A. ursinum* leaves. Therefore, the evaluation of the effects of drying on the naturally occurring antioxidants is a key issue in the choice of technological conditions that allow the preservation of their original activity and bioavailability. A lot of recent work has focused on studying the effects of drying on the phenolic compound content and antioxidant activities of dried vegetables (Kim et al., 2013; Ozgur et al., 2011; Sahoo et al., 2015; Telfser

et al., 2019). To achieve better results in terms of dried product quality, researchers have worked on optimising drying methods and different drying conditions (Arslan et at., 2010; Lim et al., 2007; Roshanak et al., 2015). The major quality problems associated with drying are loss of flavour (Ozkan-Karabaca et al., 2018), discolouration (Guine et al., 2012) and poor rehydration properties of the dried product (Aravindakshan et al., 2021; Lewicki, 1998).

The aim of the present study was to determine the influence of drying temperature on antioxidant activity and polyphenol content in dried leaves of *Allium ursinum* L. subsp. *ucrainicum* and their organoleptic properties.

Materials and methods

Materials

The plant material (fresh leaves) used in this study was collected from a natural population of wild garlic, *Allium ursinum* L. subsp. *ucrainicum*, before flowering (April 2021) in the Papuk Geopark (45°32'N 17°39'E) in the Slavonia region, Croatia. All plant samples were free from external damage and hand-picked. The leaves of *A. ursinum* were packed in linen bags and kept in the refrigerator for 24 hours until the start of the analysis of the plant material.

Drying

Drying was carried out in a drying cabinet with hot air, in which the fresh leaves of *A. ursinum* were placed in a thin layer on perforated stainless steel trays. The drying cabinet were equipped with a fan, a speed controller, a temperature controller, heating elements, a humidity, temperature, and air velocity meter. Fresh samples were dried at different drying temperatures of 40 °C, 50 °C, and 60 °C with a constant air velocity of 1.5 m/s and relative humidity of 35-45%. The drying process started when the drying conditions were reached. Weight loss was performed at a fixed time interval, and drying continued until a moisture content of approximately 12% (wet basis) was reached. Three independent dryings were performed for each drying temperature. The effect of temperatures on the quality of dried leaves of *A. ursinum* was determined by the colour characteristics, dehydratation and rehydration capabilities phenolic compounds, and antioxidant properties.

Determination of physicochemical characteristics

Dry matter content, ash, crude fat, pH and total acidity were determined in fresh *A. urisnum* samples. The analysis was performed in accordance with Association of Officiating Analytical Chemists standards (AOAC, 2000). The dry matter content of the leaves was determined by drying 5.0 g of the samples at 105 °C until constant weight. Ash content was determined by burning 5.0 g of the fresh samples at 550-600 °C until a homogeneous white ash without black spots was obtained. Crude fat was obtained by exhaustive extraction of 10.0 g of each sample in a Soxhlet apparatus using petroleum ether (boiling range 40-60 °C) as solvent (Dini et al., 2008). Tittratable acidity was determined by potentiometric titration and pH by a digital pH meter (Mettler Toledo, FiveEasy FE20, Switzerland).

Colour measurement

The colour of the fresh, dehydrated, and rehydrated leaves of *A. ursinum* samples was measured using the computer vision system. Samples were ground in a grinder (Retsh, Grindomix GM 200, Düsseldorf, Germany) to obtain a fine powder (Figure 1). For each sample (fresh, dehydrated, and rehydrated), colour parameters were measured three times directly on the product using a 2.2-megapixel digital SLR camera (EOS 1100D, Canon Ltd., Japan), calibrated with a calibration plate (Datacolor SpyderCheckr™, New Jersey, USA) just before imaging. The 24-bit colour images were captured in TIFF format and in the RGB colour model.Samples were photographed in a photochamber illuminated by four LED lamps with a diffuser.

Figure 1. Appearance of fresh (A) and dehydrated *A. ursinum* leaves at 40 °C (B), 50 °C (C), and 60 °C (D)

The colour parameters of the samples was determined using ImageJ™ image processing software (Wayne Rasband, National Institute of Health, Maryland, USA). The results were expressed as values for red (R), green (G), and blue (B) in the RGB colour system. The obtained colour values were then converted (Viscarra Rossel et al., 2006) and presented in the $CIELAB$ and $L^*C^*h^\circ$ colour system (Westland, 2016; Zhang et al., 2003), which is commonly used to evaluate dried foods. The three parameters L^* (lightness, from black $L^* = 0$ to white $L^* = 100$), a^* (a negative value of a^* represents green, while a positive value represents red colour) and b^* (a positive b^* represents yellow and a negative represents blue colour) were used for further calculation of hue angle, colour saturation, and total colour difference.

Hue angle (h°) is the attribute by which a colour is identified as green, yellow, red, etc. An angle of 0° or 360° represents red hue, whilst angles of 90°, 180° and 270° represent yellow, green and blue hues, respectively (Maskan, 2001). Hue angle is used to define the difference of a certain colour with reference to grey colour with the same lightness:

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Colour saturation or chroma (C^*), considered the quantitative attribute of colourfulness. The higher the chroma values, the higher is the colour intensity of samples perceived by humans. Chroma were calculated from the values of a^* and b^* (Lopez Camelo et al., 2004):

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Total colour difference (ΔE_{ab}) is colour change represents by distance vector between the initial colour values (fresh samples) and the dehydrated/reyhdrated colour coordinates (Roy Choudhury, 2015). Total colour difference were calculates as follows:

$$\Delta E_{ab} = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L_0^*, a_0^* and b_0^* are the colour parameters of fresh leaves of *A. ursinum* samples, and L^*, a^*, and b^* are dehydrated/rehydrated colour parameters.

Drying characteristics

The quality index of a dried product were observed and in this terms following parameters was calculated: dehydration ratio (DR), and rehydration ratio (RR). The DR is important parameter to show the bulk reduction in the weight of dried sample (higher the DR, better the quality of drying process). The RR is quality index for all dried product and higher the RR, better the quality of product. Dehydration ratio was calculated by taking the weights of sample before drying in gram (m_B) and weights of sample after drying in gram (m_D) (Kaur et al., 2008):

$$DR = \frac{m_B}{m_D}$$

To express ability of the dried material to absorb water the RR was used, and estimated according to method of Ranganna (2004). Approximately 5 g of dried samples of *A. ursinum* were placed in a 100 ml distilled water and bring to boil within 3 min. After 5 min of mild boiling, the mixture was cooled and then filtered under vacuum and weighed (mass of drained weight). Rehydration ratio was calculated by taking the drained weight (g) of rehydrated sample (m_R), and the weight (g) of dry sample used for rehydration (m_D) (Lewicki, 1998):

$$RR = \frac{m_R}{m_D}$$

Sample extract preparation

The extract from the leaves of *A. ursinum* was obtained by adding 2.5 g of fresh or dried leaf powder to 25 mL of absolute methanol and stirring with a magnetic stirrer for 30 minutes. The resulting mixture was stored in the dark at 4 °C for 24 hours and then filtered. The resulting extract was stored at 4 °C until further analysis (Dewanto et al., 2002).

Determination of total phenolic content

The total phenolic content (TPC) was determined spectrophotometrically according to the method Singleton et al. (1965) with gallic acid as standard. The 0.3 mL of the extract sample was mixed with diluted (1:10) Folin reagent (1.5 mL) and mixed vigorously for three min. Then 6.0% sodium carbonate solution (1.5 mL) was added and shaken. After standing for 90 minutes in dark at room temperature, the absorbance was measured at 760 nm using a UV-VIS spectrophotometer (Shimadzu, UV-1280, Germany). TPC of fresh and dried leaves was expressed using the calibration curve with gallic acid (0–500 μg/mL) as grammes of gallic acid equivalents (GAE) per 100 gramme of dry matter (g GAE /100 g d.b.).

Determination of antioxidant activity

Antioxidant activity (AOA) of the extracts was measured according the Brand-Williams et al. (1995) method based on using 2.2-diphenyl-1-picrylhydrazyl (DPPH). The reaction mixture was prepared using 0.1 mL of extract and 3.9 mL of DPPH methanol solution (0.1 mM). The mixture was shaken, left in the dark for 30 min, and absorbance was measured using the UV – VIS spectrophotometer (Shimadzu, UV-1280, Germany) at 517 nm. The AOA was expressed as the percentage inhibition of the DPPH radical.

Statistical analysis

Each drying test was performed in triplicate and all analyses were performed in at least five replicates, unless otherwise stated in a specific analysis. One-way analysis of variance (ANOVA) and multiple comparison post-hoc Fisher LSD (least significant-difference) test were used to evaluate the significant difference of the data at $p < 0.05$. Data were expressed as means ±standard deviation. Statistica 14 from StatSoft was used for statistical analysis.

Results and discussion

Determination of physicochemical characteristics

The results of the physicochemical properties of the leaves of *A. ursinum* are shown in Table 1. It can be seen that the average values of dry matter, crude fat content, total acidity and pH in the fresh leaf samples are 9.42, 8.84, 3.72, 0.90 and 5.50, respectively, and are in agreement with the results of other studies (Blazewicz-Wozniak et al., 2011; Dyduch et al., 2019).

Table 1

Physicochemical characteristics of fresh leaves of *A. ursinum* L.

Dry matter, %	Ash content,%	Crude fat content, %	Tittratable acidity, g acid/100 g plant d.b.	pH
9.42±0.48	8.84±0.52	3.72±0.29	0.90±0.03	5.50±0.09

Results are expressed as mean ± standard deviation.

Colour degradation

Product colour is an important quality parameter that must be maintained during drying. The leaves of the *A. ursinum* samples were dried at 40, 50, and 60 °C to the desired moisture content. The colour of the samples was measured before (fresh) and after drying (dehydrated and rehydrated). The effect of the different air temperatures on the colour characteristics of the *A. ursinum* samples is shown in Figures 2–7.

Figure 2. Effect of drying air temperature on lightness (L^*) of fresh, dehydrated and rehydrated *A. ursinum* leaves
The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

The colour of the leaves of *A. ursinum* was characterised by higher colour parameters L^*, a^*, and b^* of the dried plant material compared to fresh leaves. The value of hue angle ($h^°$) was lower in the dried material than in the raw or rehydrated material. Significant differences were observed in all analysed colour parameters between the fresh, dehydrated, and rehydrated leaves of *A. ursinum* dried at different air temperatures. Adverse changes in the colour of *A. ursinum* leaves are mainly due to the degradation of chlorophyll contained in them. Chlorophyll content decreases with increasing temperature, process duration (Lin et al., 2010; Krokida et al., 1998), and the presence of oxygen, leading to oxidation of the unsaturated colour compounds contained in the material (Negi et al., 2001), which contributes to unfavourable changes in the colour determinants of the dried material. Heating at higher temperatures caused the colour of *A. ursinum* leaves to change from green to olive brown, which is attributed to pheophytinization (Nido et al., 2003; Martins et al., 2002).

Figure 2 shows that the lightness (L^*) ranged from 22.59±0.06 to 36.89±0.14 regardless of drying temperature, with the lowest L^* values obtained for fresh samples (22.59±0.06) and the highest for samples dried at 60 °C (36.89±0.14). The drying temperature had a significant effect on the L^* values of the dehydrated and rehydrated samples. The L^* values increased proportionally with the drying temperature. The L^* values for the rehydrated samples were lower compared to the dehydrated samples. Rudy et al. (2020) also reported decrease in L^* values after convection drying. A negative a^* value represents a green colour, while a positive value represents a red colour.

Figure 3. Effect of drying air temperature on colour parameter a^* (redness – greenness) of fresh, dehydrated and rehydrated *A. ursinum* leaves

The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

The results of chromatic component redness – greenness (a^*) of *A. ursinum* leaves are presented in Figure 3 where can it be seen that a^* values ranged from -12.99±0.30 to -5.21±0.14 regardless of drying temperature, with the lowest a^* values obtained for fresh samples (- 12.99±0.30) and the highest (-5.21±0.14) for samples dried at 60 °C. The green colour is dominant in all samples, although the green hue is more pronounced in fresh and rehydrated samples. The drying temperature had a significant effect on the a^* values of the

dehydrated and rehydrated samples, and the a^* values for the dehydrated samples were lower compared to the rehydrated samples processed at the same temperature. Thus, the dehydrated leaves showed a much lower intensity of green colour than the fresh leaves. This effect is more pronounced at higher drying temperatures (60 °C) due to chlorophyll degradation (Guine et al., 2012).

The results of chromatic component yellowness – blueness (b^*) of *A. ursinum* leaves are presented in Figure 4 where can it be seen that b^* values ranged from 14.78±0.19 to 21.10±0.13 regardless of drying temperature, with the lowest b^* values obtained for fresh samples (14.78±0.19) and the highest for dehydrated samples (21.10±0.13) dried at 60 °C. The positive b^* value, representing the yellow colour, increases significantly after drying and rehydration. Arslan et al., (2010) reported similar results. There were statistically differences between dehydrated and rehydrated samples. The b^* values for the rehydrated samples were smaller compared to the dehydrated samples processed at the same temperature.
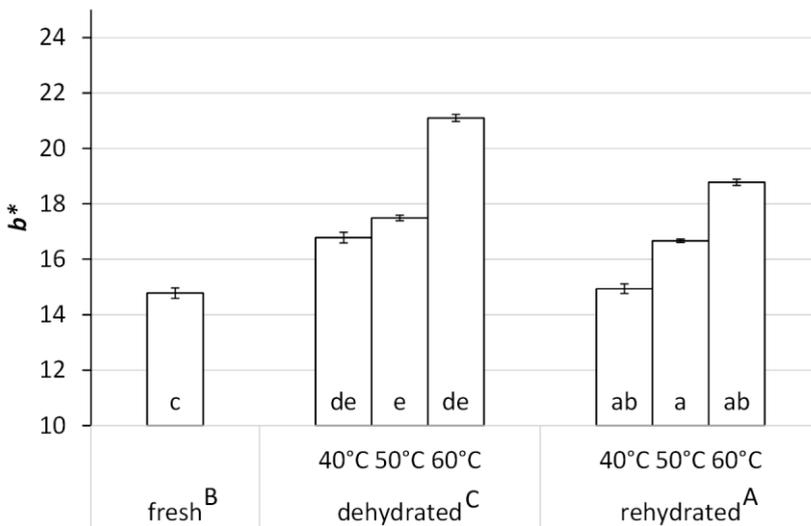
Figure 4. Effect of drying air temperature on colour parameter b^* (yellowness – blueness) of fresh, dehydrated and rehydrated *A. ursinum* leaves
The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

The hue angle ($h°$) values ranged from 103.87 ±0.31 to 131.30 ±0.99 regardless of drying temperature (Figure 5), with the lowest $h°$ values obtained for the samples dried at 60 °C (103.87 ±0.31) and the highest for the fresh samples (125.10 ±0.13). The dried leaves of *A. ursinum* had lower $h°$ values than the raw material. There were statistical differences between dehydrated and rehydrated samples, with drying resulting in a significant decrease in $h°$ values of the dried material. The $h°$ values of the dehydrated samples were smaller compared to the rehydrated samples processed at the same temperature. The hue angle of the dehydrated sample decreased with increasing heating temperature. The decrease in hue angle corresponds to a decrease in the intensity of the green and an increase in the yellow colour. The decrease in hue angle in this study is consistent with the results reported by Lau et al., (2000) that prolonged heating of green vegetables leads to deterioration of chlorophyll pigments and a change in colour from green to olive green.
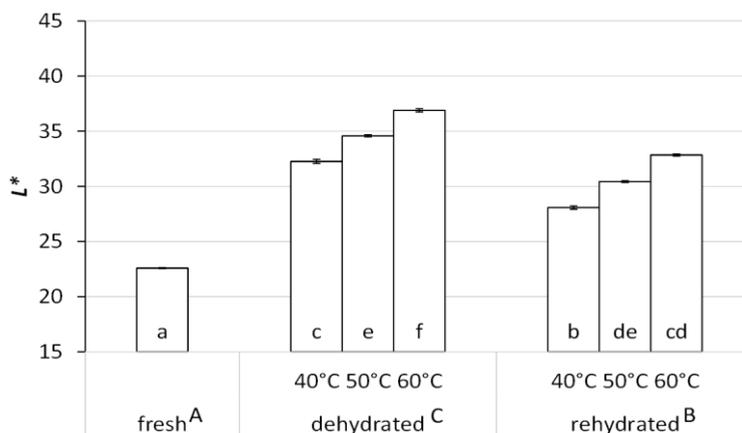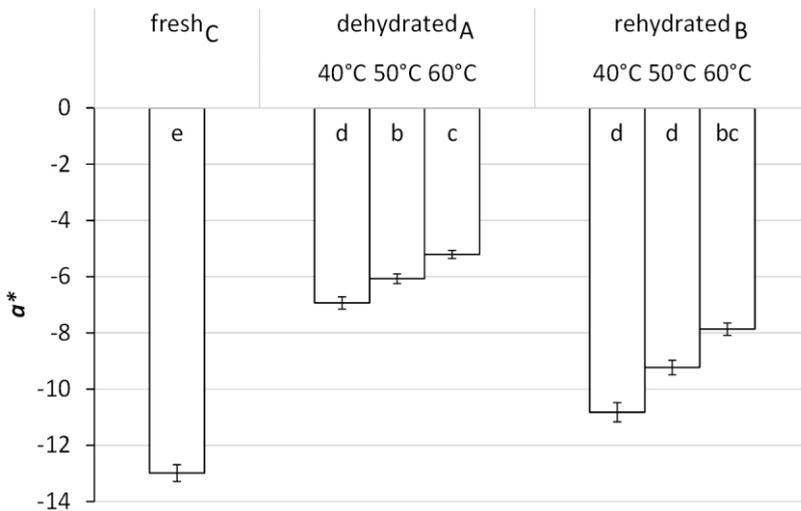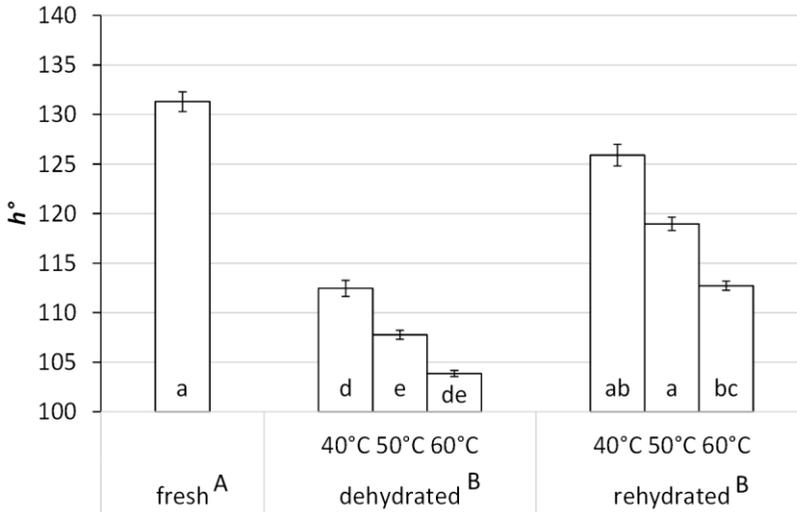
Figure 5. Effect of drying air temperature on hue angle (h°) of fresh, dehydrated and rehydrated *A. ursinum* leaves
The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

The results of colour saturation (C^*) of *A. ursinum* leaves are presented in Figure 6. It can be seen that C^* ranged from 18.12±0.18 to 21.72±0.15 regardless of drying temperature, with the lowest C^* values obtained for dehydrated samples dried at 40 °C (18.12±0.18) and the highest at 60 °C (21.72±0.15). Increasing the temperature of the drying air resulted in an increase in colour saturation during drying. There were no statistical differences between dehydrated and rehydrated samples. The C^* values for the rehydrated samples were smaller compared to the dehydrated samples processed at the same temperature.

Total colour difference (ΔE_{ab}) is a colorimetric parameter used to estimate the colour change of food during processing. Figure 7 shows that ΔE_{ab} values ranged from 5.91±0.10 to 17.47±0.19 regardless of drying temperature, with the lowest ΔE_{ab} values obtained for rehydrated samples dried at 40 °C (11.60±0.01) and the highest for dehydrated samples dried at 60 °C (17.47±0.19). There were statistical differences between dehydrated and rehydrated samples. The ΔE_{ab} values for the rehydrated samples were smaller compared to the dehydrated samples processed at the same temperature. It is evident that drying at higher temperature results in greater colour change (Kumar et al., 2004).

Total colour difference (ΔE_{ab}) is a colorimetric parameter used to estimate the colour change of food during processing. Figure 7 shows that ΔE_{ab} values ranged from 5.91±0.10 to 17.47±0.19 regardless of drying temperature, with the lowest ΔE_{ab} values obtained for rehydrated samples dried at 40 °C (11.60±0.01) and the highest for dehydrated samples dried at 60 °C (17.47±0.19). There were statistical differences between dehydrated and rehydrated samples. The ΔE_{ab} values for the rehydrated samples were smaller compared to the dehydrated samples processed at the same temperature. It is evident that drying at higher temperature results in greater colour change (Kumar et al., 2004).

Figure 6. Effect of drying air temperature on chroma (C^*) of fresh, dehydrated and rehydrated
***A. ursinum* leaves**
(The data are presented as the mean ±standard deviation. Bars with different letters are significantly
different ($p < 0.05$))
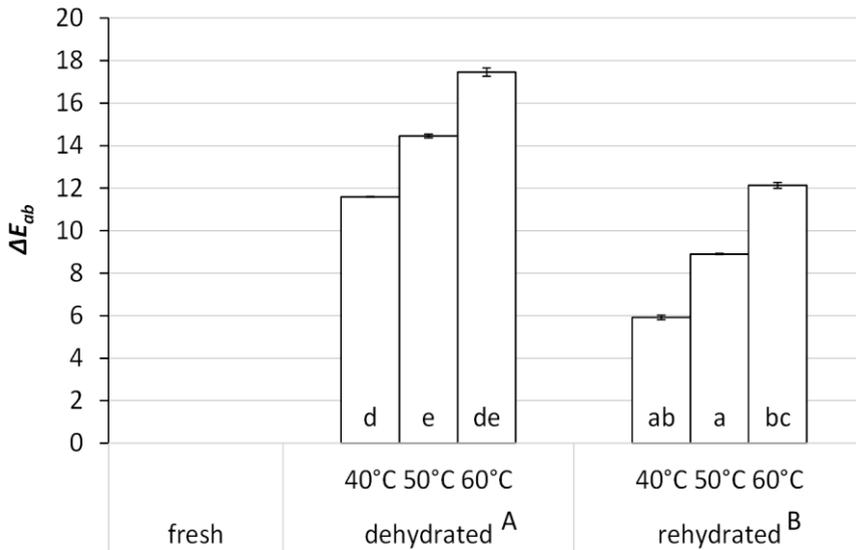
Figure 7. Effect of drying air temperature on colour difference (ΔE_{ab}) of fresh, dehydrated and
rehydrated *A. ursinum* leaves
The data are presented as the mean ± standard deviation. Bars with different letters are significantly
different ($p < 0.05$)

Dehydration and rehydration ability

The rehydration properties of a dry product are often used as an indicator of the quality of a dry product. Rehydration is a complex process that is influenced by both the physical and chemical changes associated with drying and the treatments that precede dehydration. Figure 8 shows the degree of dehydration and rehydration of *A. ursinum* leaves depending on the different temperatures of the drying air of the samples.

The dehydration ratio (DR) indicates the weight loss of the dried product, with high values indicating a better drying process. The values of DR at different drying air temperatures are shown in Figure 8. The values of DR differed significantly between the different drying air temperatures. It varies between 5.45 ±0.184 and 6.65 ±0.041 and increases with increasing drying air temperature from 40 to 60 °C.

Rehydration is a method of analysing dried products. The rehydration ratio (RR) indicates the physical and chemical changes during drying, which are influenced by the processing conditions and the composition of the samples. The RR values (Figure 8) differed significantly between the different drying air temperatures and ranged from 4.09 ±0.083 to 6.20 ±0.104. It was found that the RR of the samples dried at higher temperatures gave the highest rehydration. A high RR value means that the dried product is of good quality as the pores allow water to re-enter the cells.

Drying temperatures had a statistically significant effect on the dehydration and rehydration capacity of the dried *A. ursinum* leaves. The higher the drying temperature, the higher the degree of dehydration and rehydration of the *A. ursinum* leaves. Sahoo et al. (2015) and Ozgur et al. (2011) made similar observations. The drying process leads to changes in the permeability of the cell walls, loss of osmotic pressure and migration of solutes, which affects the rehydration ratio (Sharma et al., 2005). The less elastic cell walls and the reduced water binding capacity of proteins and starch reduce the rehydration ratio of the products, but this phenomenon is significantly reduced by optimising the drying process and the negative factors associated with cell rehydration are reduced (Kumar et al., 2004).
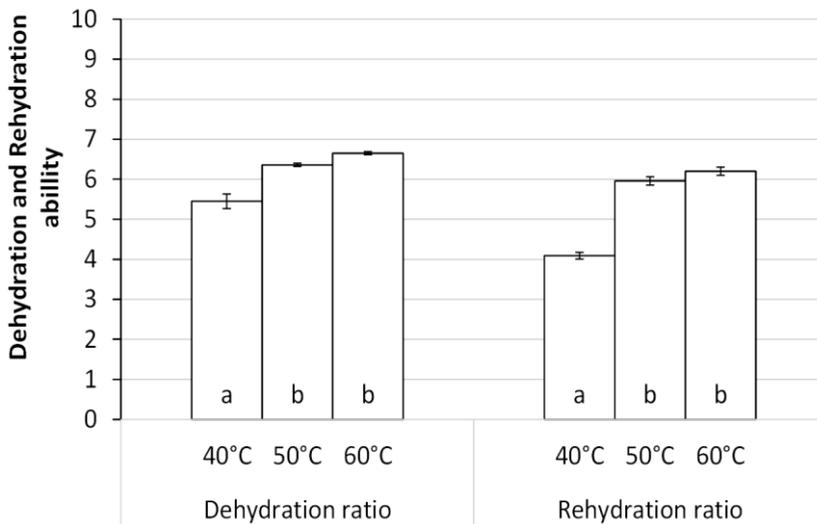
Figure 8. Effect of drying temperature on dehydration (DR) and rehydration (RR) ratio of dried leaves of *Allium ursinum*.
The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

Total phenolic content and antioxidant activity of *A. ursinum*

Drying is one of the oldest techniques for preserving food for later use. In this technique, water is removed to reduce water activity, which reduces bacterial activity in the dried food. In addition to the safety of food during preservation, many researchers have focused on the changes in phytochemicals during drying or dehydration. The degradation of phenolic compounds is mainly caused by oxidation, cleavage of covalent bonds or enhanced oxidation reactions due to thermal processing (Nicoli et al., 1999). Phytochemicals such as phenolic acid and flavonoids, which occur in fruits, vegetables and cereals in free and bound forms, are degraded or change their structural form during thermal and non-thermal processing. As processing progresses, naturally occurring antioxidants are degraded and new compounds with potential antioxidant activity are formed. Food processing involves heating with various energy transfer media such as water, air, oil and electromagnetic waves. Food processing involves various transformations of phenols that produce yellowish or brownish pigments (Clifford, 2000). The most important phenols in onions are quercetin, gallic acid, ferulic acid and their glycosides (Nitta et. al, 2007). Total phenolic content (TPC) was assessed in both fresh and dried leaves to compare the effects of different drying conditions on the change in TPC. The results are shown in Figure 9.
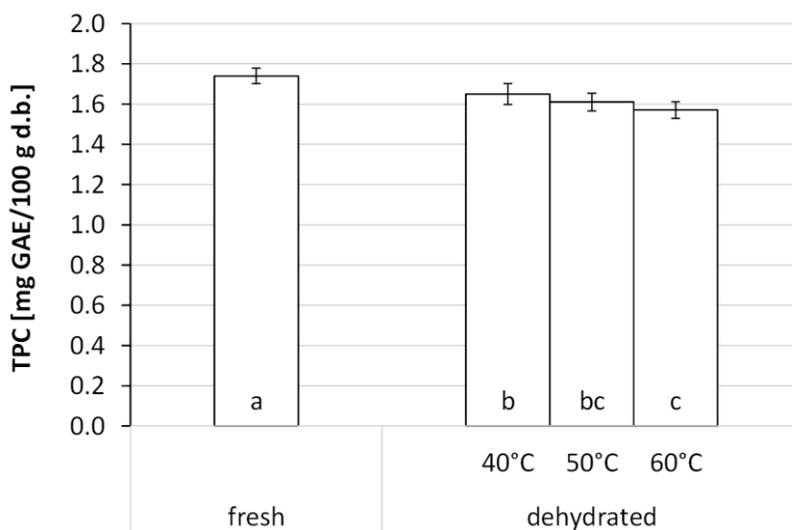
Figure 9. Total phenolic content of dried leaves of *Allium ursinum*.
The data are presented as the mean ± standard deviation. Bars with different letters are significantly different ($p < 0.05$)

The dried material of *A. ursinum* leaves obtained by convection drying was characterised by a decrease in TPC, which could be due to the degradation of phenolic compounds by drying (Lim et al., 2007). The TPC of the samples studied varied from 1.57 ±0.041 to 1.74 ±0.038 g GAE / 100 g dry weight. The highest contents of total polyphenols were found in the fresh samples and the lowest polyphenol contents in the samples dried at 60 °C. The dried material obtained after different drying air temperatures (40, 50 or 60 °C) differed

significantly in TPC content. Furthermore, the loss of this component was significantly different in the dried material compared to the raw material. The TPC content was lower at 60 °C than at the other two drying temperatures. The increase in drying air temperature during convection drying contributed to a decrease in TPC in the dried *A. ursinum* leaves. Physical and biological factors such as temperature increase and enzymatic activity can lead to the destruction of phenolic antioxidants such as phenolic acids and anthocyanins. According to Korus (2011), hot drying air promotes the oxidation of polyphenols by the oxygen absorbed by the convection drying air. The loss of polyphenol content is also attributed to their use as reactants in the Maillard reaction (Nicoli et al., 1999). Martin-Cabrejas et al. (2009) have reported that the reduction in TPC content could also be due to the binding of polyphenols to other compounds or to changes in their chemical structure after heat treatment. These changes prevent their extraction and determination with the methods used.

From the results of the antioxidant activity (AOA) of the dried plant material, it can be concluded that convection drying has an influence on the reduction of AOA (Figure 10). Drying of *A. ursinum* leaves resulted in a decrease in AOA of the dried material, regardless of the drying temperature used, compared to the raw plant material (88.4%). Across the range of measurements, the samples dried at lower temperatures had the higher value of AOA, while the higher drying temperatures resulted in a greater decrease in the antioxidant potential of the dried plant material (53.3, 45.3 and 40.7% at drying temperatures of 40, 50 and 60 °C, respectively).
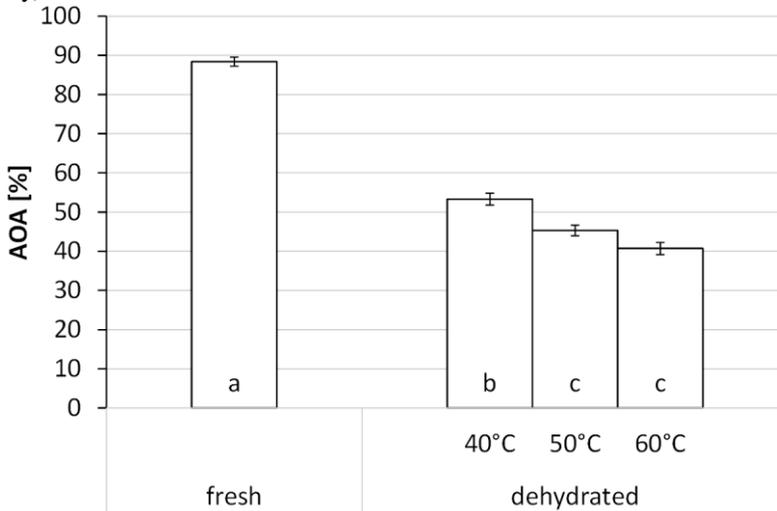
Figure 10. The antioxidant activity of leaves of *Allium ursinum*.
The data are presented as the mean ±standard deviation. Bars with different letters are significantly different ($p < 0.05$)

Antioxidant phytochemicals in plants can be broadly classified as carotenoids, phenols, alkaloids, nitrogenous compounds and organosulphur compounds (Liu, 2004). Antioxidant activity correlates with the presence of phytochemicals such as phenols, flavonoids and anthocyanins in food (Sun et al., 2002). Therefore, evaluating food processing operations that affect antioxidant activity in processed foods is critical to optimising conditions to increase or maintain their availability and functionality. Some authors report that antioxidant activity

increases or is maintained in processed foods, which may be due to the development of new compounds with potential antioxidant capacity, although the content of naturally occurring antioxidants has decreased significantly due to heat treatment. (Anese et al., 1999; Nicoli et al., 1997; 1999).

Conclusion

1. The colour of the samples was measured using a non-destructive method on fresh, dehydrated and rehydrated plant material, and significant differences were found in all the colour parameters analysed. It is evident that drying at a higher temperature leads to a greater change in colour. Thus, the dried leaves showed a much lower intensity of green colour than the fresh leaves. This effect is more pronounced at higher drying temperatures (60 °C) due to chlorophyll degradation.
2. Drying temperatures had a statistically significant effect on the dehydration and rehydration capacity of the dried *A. ursinum* leaves. The higher the drying temperature, the higher the degree of dehydration and rehydration (the pores of the dried food allow water to re-enter the cells). Convection air drying results in considerable moisture removal from the fresh leaves of *A. ursinum* (more than 91%), but the organoleptic quality of the *A. ursinum* leaves is maintained.
3. The drying conditions tested had a significant effect on the total phenolic content and antioxidant activity of *A. ursinum* leaves. An increase in temperature during drying decreased the total polyphenol content in the dried *A. ursinum* leaves.
4. Across the range of measurements, the samples dried at lower temperatures had the higher antioxidant capacity, while the higher drying temperatures resulted in a greater decrease in the antioxidant potential of the dried plant material.
5. *A. ursinum* is considered one of the functional foods for human consumption due to its high nutritional value and prophylactic or therapeutic effects on various diseases. To obtain a high quality dried product, the drying process should ensure a quality comparable to fresh vegetables.

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Aromatic profile of Macedonian and Bulgarian red wines from local variety Vranec and hybrid variety Kaylashki Rubin

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Abstract

Introduction. The aim of the present study was to define the aromatic profile of Bulgarian and Macedonian red wines obtained from the local variety Vranec and the hybrid variety Kaylashki Rubin.

Materials and methods. Gas chromatographic (GC-MS) study to define the aromatic profile of red wines from the local variety Vranec (grown in the Republic of Macedonia) and the hybrid variety Kaylashki Rubin (grown in the Republic of Bulgaria) was conducted.

Results and discussion. 1-pentanol was dominated in the fraction of higher alcohols in both wines. Other aroma compounds identified were 1-propanol, 2-propanol, 1-butanol, 1-hexanol, and 3-methylthio -1-propanol. The wine of the Vranec variety showed greater complexity in terms of this fraction, as in it 3-hexen-1-ol was identified, which was not present in the wine of Kaylashki Rubin. High amount of the aromatic alcohol – phenylethanol – was identified in both wines. This compound had great importance for their floral aroma. The ester fraction of the two wines was diverse, represented by isopentyl acetate, ethyl caprylate, ethyl hexanoate, ethyl decanoate and diethyl malate. The Vranec wine showed greater ester complexity, as in it two more ester representatives were identified – ethyl-2-hydrobutyrate and 2-hydroxy-3-methyl-diethyl ester. In both wines, one fatty acid was identified – heptanoic acid, in very low concentrations. According to the panelist both wines were very harmonious in their own way and had their typical notes as expected for the both varieties. In overall, the descriptive analyses confirmed the components determined by the GC-MS and gave a clear view about the aroma profile of both varieties.

Conclusions. Both wines showed a diverse, balanced aromatic profile, each of which, based on the peculiarities of its volatile composition. Meanwhile, each wine had individual aromatic properties.

Introduction

The aromatic profile of the wines is a descriptor for their quality. It is determined by the presence, concentrations, ratio and distribution of specific volatile compounds.

Vranec is the main variety for the production of red wines in R. N. Macedonia. It is also widespread in Montenegro, Serbia, Croatia and Bosnia and Herzegovina. The variety was brought to R. Macedonia in the distant 1950 by prof. Dragan Nastev in the experimental vineyard of the Institute of Agriculture in Skopje (Nastev, 1985). Nowadays, the Vranec variety is one of the main red grapevine varieties used for the production of quality red wines in the Republic of Macedonia. It occupies the largest share of vineyards in the Republic. Ivanova et al. (2013) studied the volatile composition of Macedonian and Hungarian wines. In this study the team found a total amount of volatile compounds of 41.318 ±56.30 µg/dm^3 in the red wine from the Vranec grapevine variety. The ester fraction of this wine had a total quantitative presence of 2631±21.90 µg/dm^3. The team did not establish the presence of terpenes in the studied wine. Bogoeva et al. (2018) studied the influence of different oenological practices on the aromatic composition of wines from Vranec. They identified 63 aromatic compounds from different volatile groups: esters, alcohols, fatty acids, aldehydes, ketones and sulfur compounds.

Kaylashki Rubin variety is an interspecific hybrid obtained by crossing of (Pamid x Hybrid VI 2/15) x (Game noir x *Vitis amurensis*). It was created by the scientists of the Institute of Viticulture and Enology, Bulgaria in 2009 and was patented in 2010 (Ivanov, 2016). It is characterized by high resistance to low winter temperatures. In Bulgaria, the aromatic profile of wines from this variety have been studied, mainly by the GC-FID method or classical chemical analysis, which provides information on components, in larger quantities (mg/dm^3). A study (Dimitrov et al., 2018) on the aromatic profile of red wines of several varieties grown in the region of Central Northern Bulgaria found high total final concentrations of volatile compounds in red wine of the variety Kaylashki Rubin (693.97 mg/dm^3). The study identified 4 higher alcohols, 5 esters, 1 aldehyde and 3 terpene alcohols in the wine of Kaylashki Rubin. Yoncheva et al. (2016) conducted a technological study of some varieties and clones of vines. The study also includes Kaylashki Rubin. It was concluded that the wines of Kaylashki Rubin are characterized by the highest concentration of total esters and aldehydes. Yoncheva et al. (2019) in a study on the chemical composition of Bulgarian wines of hybrid varieties found a total concentration of esters, aldehydes and higher alcohols in wines of Kaylashki Rubin respectively 228.80 mg/dm^3, 46.20 mg/dm^3 and 314.00 mg/dm^3. Dimitrov and Iliev (2021) studied the influence of different vine rootstocks on the volatile composition of wines from Kaylashki Rubin from three harvests (2017, 2018 and 2019). The team established a diverse volatile composition, represented mainly by 2-methyl-1-butanol, 3-methyl-1-butanol, 1-butanol, 1-hexanol, 4-methyl-2-pentanol, 1-propanol, 2-butanol (higher alcohols fraction), ethyl acetate (esters fraction), geraniol (terpenes fraction).The application of the GC-MS method in the present study provides a new information on the aromatic profile, identifying components of the aromatic composition in minor concentrations (µg/dm^3). This will enrich the scientific literature and provide new data on the potential of the variety to accumulate aromatic components in its wines, reflecting its qualities. Study was focused on identification and quantification of volatile compounds (quality wine descriptor) from the main aromatic groups, that were established in a lot of wine studies worldwide: esters, higher alcohols, aldehydes, terpenes, fatty acids (Bakharev et al., 2021; Itu et al., 2011; Kim et al., 2018; Manolache et al., 2018; Mateo et al., 2000; Meng et al., 2011; Nan et al., 2021; Rapp et al., 1986; Rusjan et al., 2008; Tardea, 2007; Tomasino et al., 2020; Yankov et al., 2000).

The aim of the present study was to define the aromatic profile of Bulgarian and Macedonian red wines obtained from the local variety Vranec and the hybrid variety Kaylashki Rubin. The significance of the purpose is based on the fact that the data obtained from the study will provide information on the characteristics of the qualities of regional wines (terroir influence) obtained from varieties with different genetic origin.

Materials and methods

Grapevine varieties

The study was conducted in 2017. The wines were obtained from two red grapevine varieties (Vranec and Kaylashki Rubin) form harvest 2016, different by their genetic origin and grown in two different locations – R. Macedonia and R. Bulgaria.

Climatic conditions of the area of cultivation

The vines of the Vranec variety used for this study were grown in the region of Veles. According to Nedelkovski (2017) this region is characterized by a typical continental climate with the following indicators: temperature sum during the vegetation period – 4626.5– 4942.6 °C; the average monthly temperature during the vegetation period is 18.1 °C; the min. temperature -12.9 °C and maximum temperature is 40.7 °C; duration of the vegetation period bud break to harvest 142–157 days; beginning of vegetation – 12.04 to 22.04; frequency of spring frosts up to 10%; annual precipitation amount – 355–663 mm/dm^3.

The experimental vines of the Kaylashki Rubin variety were grown in the Experimental Base of Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria. The region of the town of Pleven is characterized by a typical continental climate with the following indicators: temperature sum during the vegetation period – 3130–4003 °C; duration of the vegetation period – 190–210 days; duration of frost-free period – 178–223 days; beginning of vegetation – 02.04 to 14.04; frequency of spring frosts up to 20%; annual precipitation amount – 532– 753 mm/dm^3 (Katerov et al., 1990; Pandeliev et al., 2005).

Vinification

The Vranec grapes were harvested at technological grape maturity and processed in the experimental wine cellar of the Institute of Agriculture – Skopje. The production of the wines was carried out according to the classic scheme for production of red dry wines: Hand harvesting of the grapes → Crushing and destemming of the grapes → Adding 50 mg/dm^3 SO$_2$ → Inoculation of wine yeast (*Saccharomyces cerevisiae*) → Fermentation for 12 days at temperature 22±3 ºC → Raking → Wine filtration → Bottling → Storage.

The Kaylashki Rubin grapes were harvested after reaching of technological maturity, in the amount of 30 kg, and processed in the Experimental Wine Cellar of Institute of Viticulture and Enology – Pleven, in the conditions of microvinification, following the classic scheme for the red dry wines production: Crushing and destemming → Sulphitation (50 mg/kg SO$_2$) → Inoculation with pure culture dry yeasts *Saccharomyces cerevisiae* Siha Rubio Cru (EATON Begerow) –20 g/100 L→ Fermentation (temperature of fermentation – 28 °C) → Separation from solids → Further sulphitation → Storage (Yankov, 1992).

Chemicals and reagents

For the extraction of volatile components in the wine samples, dichloromethane was used, purchased by Sigma Aldrich (USA); Reference standard diethyl succinate, 2-phenyl ethanol, ethyl hexanoate, 1-hexanol, 1-heptanol purchased by Merck (Germany); Isoamyl acetate, purchased from Aldrich Chemicals (USA); The 1-octanol used as an internal standard was purchased from Sigma Aldrich (USA).

Extraction procedure and gas chromatography (GC-MS) analysis

The volatile components were extracted by liquid-liquid extraction (Ivanova et al., 2012). We transfer 50 ml of the wine sample in 500 ml Erlenmeyer flask and add 50 ml of the extragent (dichloromethane), as internal standard 25 µl 1-octanol was added. The Erlenmayer flask was sealed and was placed on a magnetic stirrer for 1 hour. After one hour the mixture was centrifuged at 3000 rpm for 10 min. The separated dichloromethane phase was then evaporated under a stream of nitrogen until dryness. Then the evaporated sample was rehydrated with 100 µl of dichloromethane and it was injected into the GC-MS. The gas chromatograph used was Varian 3900 (Middelburg, The Netherlands). The mass spectrometer was Varian Saturn 2100T (Middelburg, The Netherlands). Parameters of gas chromatographic determination were: injector temperature – 240 °C, MS source – 230 °C, MS quad from 150 °C and 280 °C transfer line. The initial temperature was 40 °C for 3 min and then rises to 180 °C at a level of 3 °C/min. The temperature then rose further to 260 °C at 20 °C/min and hold at 260 °C for 10 min. The carrier gas was He with flow rate 1.5 ml/min.

Sensory evaluation

The sensory evaluation of both wines was performed by the descriptive method described by Mario Ubini (2004). The wine panelists (4 experts in the field of enology) first had to degustate both wines and then purpose descriptors that will describe both the aroma and taste of the analyzed wines. Four panelists were involved in the analysis. According to them 11 descriptors were proposed to describe these wines: red fruits, black fruits, flower aromas, herbal aromas, acidity, astringency, structure, harmony, typicity, bitterness and body.

Statistical analysis

Statistical analysis of the analyzed parameters between the two wine samples was performed with the computer statistical program SPSS 14.0. For the comparison of the results Pater Samples Statistic of T-Test was performed with significant differences of 0.05.

Results and discussion

The data on the quantitative presence of volatile compounds are presented in Table 1. The results were statistically analyzed with the statistical tool T-test guided by the fact that we wanted to make a comparison of each aromatic component between the examined wines. According to the statistical test statistical proven differences were found for aroma components like 1-propanol, 1,5-hexadien-3-ol, 1-pentanol, 2-propanol, 1-hexanol, 2,3-butanediol, ethyl decanoate, diethyl succinate, and 3-(methylthio)-1-propanol. For the phenylethanol in all samples statistical differences was not proven.

Identified volatile compounds in red wines of local variety Vranec and hybrid variety Kaylashki Rubin

Volatile compounds	Aromatic descriptor	Kaylashki Rubin, $\mu g/dm^3$	Vranec, $\mu g/dm^3$	T-Test	Sig.
1-Propanol		905.32 ±5.66	700.96 ±12.05	55.436	.000
1,5-hexadien-3-ol		9972.91 ±74.91	4747.42 ±59.59	590.592	.000
Isoamylacetate	Banana	105.33 ±13.76	120.49 ±9.41	-6.034	.026
1-Butanol	Medical, Alcohol	460.35 ±14.65	452.08 ±13.00	8.681	.013
1-Pentanol	Flowery	23519.08 ±288.9	11968.72 ±208.7	249.386	.000
ethyl hexanoate	Green apple, strawberry	178.38 ±13.35	185.41 ±14.00	-18.887	.003
2-Propanol		1078.90 ±78.91	668.48 ±30.35	14.639	.005
1-Hexanol	Green, Grassy	1076.30 ±66.54	1300.63 ±49.42	-22.693	.002
3-hexen-1-ol	Green, Flowery	ND	134.04 ±15.65		
Ethyl caprylate	Pineaple, Pear, flowery	202.95 ±14.42	233.48 ±13.81	-86.703	.000
Ethyl -2-hydroxibytirate		ND	124.16 ±13.08		
2,3-Butanediol	Butter, Creamy	1152.06 ±46.25	934.93 ±26.19	18.748	.003
1-Octanol (IS)		1397.86 ±30.25	1447.41 ±39.33		
Ethyl decanoate	Vegetable, Anise	174.28 ±25.19	45.65 ±8.37	13.246	.006
Diethyl succinate	Fruity	7623.03 ±52.97	1348.55 ±50.55	4500.085	.000
3-(methylthio)-1-propanol	Boiled potatoes, rubber	1149.80 ±59.91	749.63 ±26.32	8.039	.015
2 – phenyl ethyl acetate		ND	TRACES		
Vinyl butyrate		TRACES	ND		
Phenylethanol	Flower, pollen, perfume	17864.31 ±155.00	20076.12 ±88.02	-57.192	.000
Diethyl malate		55.00 ±5.00	63.12 ±6.12	-12.557	.006
Heptanoic acid		1209.76 ±51.25	1297.00 ±70.01	-8.055	.015
2-hydroxy-3-methyl-diethylester		ND	105.13 ±8.05		
Ethyl palmate		ND	TRACES		
Ethyl cinnamate		ND	259.01±11.22		

T-test with statistically significant difference ($p < 0.05$)

Identified alcohols

1-propanol is one of the main higher alcohols of the volatile wine fraction. In the wine of the local variety Vranec it was identified in an amount of 700.96±12.05 μg/dm^3. In Kaylashki Rubin this representative was found in a higher concentration – 905.32±5.66 μg/dm^3. The aromatic descriptor of 1-propanol is a flower bouquet and a ripe fruit. Characteristic of propanol is that it participates in transformational changes during the wine's aging, forming volatile esters with propionic, acetic and caprylic acids (Chobanova, 2012). A study of changes in the aromatic compounds of Cabernet Sauvignon red wines aged in stainless steel tanks (Meng et al., 2011) found a variation of this compound from 2554.87 μg/dm^3 to 5091.44 μg/dm^3. In the young wine (before the aging process) the team (Meng et al., 2011) found a concentration of this higher alcohol of 3058.80 μg/dm^3. The presence of 1-propanol in the studied wines of Vranec and Kaylashki Rubin was significantly lower. This could be attributed to the characteristic features where the grapes were grown and harvested. Both varieties were grown in different geographical locations under different soil and climatic conditions.

The highest concentration of higher alcohols in the studied wines was found for the 1-pentanol. In the wine of the Vranec variety it was identified in an amount of 11968.72±208.70 μg/dm^3, while in Kaylashki Rubin its concentration was almost twice as high – 23519.08±288.90 μg/dm^3. Its threshold of aromatic perception (with a characteristic aroma of flowers) is 30.00 μg/dm^3. In both wines it was found above this threshold, which was reflected in its special sensory expression. In a study on the volatile composition of Macedonian (Vranec, Merlot, Cabernet Sauvignon, Tamianka and Chardonnay) and Hungarian (Kefrankos and Tokaji) wines was found that in red wines 1-pentanol and 2-phenylethanol were the main components of the volatile fraction (Ivanova et al., 2013).

The data obtained in the present study correlate with the study of the above team. After 1-pentanol, 1,5-hexadien-3-ol was ranked by concentration. This compound was found in a higher amount in the wine of the Kaylashki Rubin variety (9972.91±74.91 μg/dm^3), compared to that of Vranec (4747.42±59.59 μg/dm^3).

3-hexen-1-ol was identified only in Vranec wine. It was available in an amount of 134.04±15.65 μg/dm^3. A characteristic aromatic nuance that this compound imparts is green, grassy (Newcomb et al., 2010). However, its threshold of aromatic perception is higher (400.00 μg/dm^3) than its established concentration. This was reflected in the lack of aromatic expression of 3-hexen-1-ol in its identified amount in the red wine of Vranec.

2,3-butanediol is a compound – a product of yeast metabolism. Its concentration is highly dependent on the type of yeasts (Romano et al., 1998; Ng et al., 2012). It was identified in both wines studied. In the wine of Kaylashki Rubin it was present in an amount of 1152.06±46.25 μg/dm^3, and in that of Vranec – 934.36±26.19 μg/dm^3. A characteristic aroma that gives this compound is butter, creamy. In both wines it was identified above its threshold of aromatic perception (120.00 μg/dm^3), which significantly determined the participation of its influence on the wine aromatic profile.

Another major representative of the higher alcohols fraction was 2-propanol (isopropyl alcohol). In the wine of Kaylashki Rubin it was identified in a higher concentration (1078.90±78.91 μg/dm^3), compared to that found in Vranec (668.48±30.35 μg/dm^3).

1-butanol was found in very similar amounts in the two wines studied. Its concentration in Kaylashki Rubin was 460.35±14.65 μg/dm^3, and in the red wine of Vranec it was contained in an amount of 452.08±13.00 μg/dm^3. A study on the volatile fraction of ten wines from north-western Spain obtained from varieties from *Vitis vinifera* (Vilanova et al., 2013) found a variation of 1-butanol from 8.96±1.23 μg/dm^3 (Riesling) to 76.98±9.13 μg/dm^3

(Gewürztraminer). On the other hand, Meng et al. (2011) in a study of Cabernet Sauvignon wines aged in stainless steel tanks found the content of 1-butanol in young wine – 3058.80 µg/dm^3. It could be seen that the concentration presence of 1-butanol varies between wines obtained from grapes grown in different geographical locations.

1-hexanol is a higher alcohol present in the volatile fraction of wine and imparting a characteristic grassy aroma (Abrasheva et al., 2008). It was identified in both studied wines, and in that of Vranec its quantity was higher (1300.63±49.42 µg/dm^3), in comparison with Kaylashki Rubin (1076.30±66.54 µg/dm^3). This component of the volatile fraction was also found in red wine from Cabernet Sauvignon (4017.70 µg/dm^3) from Xiangning County, China (Jiang et al., 2010). Another study (Tao et al., 2009) again on the volatile composition of Cabernet Sauvignon wine, Changli County region (China), identified it at a significantly higher concentration (average 17300.00 µg/dm^3). 1-hexanol has been identified as a major component of the higher alcohols volatile fraction in the study of the aromatic profile at the aging process (6 and 12 months; respectively in concentrations varying quantitatively from 139.04±3.25 µg/dm^3 – 529.77±0.39 µg/dm^3 and from 183.79±0.22 µg/dm^3 – 570.89±8.04 µg/dm^3) of red wines from Cabernet Sauvignon, Fetească neagră, Pinot Noir and Merlot from different regions of Romania (Manolache et al., 2018).

An aromatic alcohol – phenylethanol – was identified in the wines of Vranec and Kaylashki Rubin. It was identified in high concentration presence in the aromatic matrix of the two wines. In the wine of Vranec it was found in a higher amount (20076.12±88.02 µg/dm^3), compared to that of Kaylashki Rubin (17864.31±155.00 µg/dm^3). The characteristic aroma that this alcohol gives is floral, in particular rose (Etievant, 1991). Our data on the content of 2-phenylethanol were in agreement with the data of Manolache et al. (2018), which found this alcohol in high quantities by the GC-MS study of 4 red wines from the varieties Cabernet Sauvignon, Fetească neagră, Pinot Noir and Merlot from regions of Romania. This alcohol also has been found to be dominant quantitatively in the study of the volatile fraction of Italian red wines from the Negroamaro and Primitivo varieties (Tufarrielo et al., 2012; Capone et al., 2013). A study of the volatile composition of wine from two harvests of three varieties of *Vitis vinifera* grown in Spain (Vilanova et al., 2008) found a variation of phenylethanol on average for both harvests from 8321.20±5065.90 µg/dm^3 to 10116.90±3323.40 µg/dm^3. In Cabernet Sauvignon wines from China, phenylethanol was identified in an amount of 14504.80 µg/dm^3 (Jiang et al., 2010). The data obtained in our study for phenylethanol correlated with the results in the cited studies.

Other higher alcohol identified in the two wines studied was 3-methylthiol-1-propanol. It was found in a higher concentration in the wine from Kaylashki Rubin (1149.80±59.91 µg/dm^3), compared to Vranec (749.63±26.32 µg/dm^3). 3-methylthiol-1-propanol has an aromatic perception threshold of 500.00 µg/dm^3 and imparts a characteristic aroma of boiled potatoes. In both studied wines it was identified in concentrations above its threshold of aromatic perception.

Identified esters

Of the ester fraction, the highest quantitative presence in both wines was found for diethyl succinate ester. It gives a fruity aroma. It was found in a very high concentration in the wine of the Kaylashki Rubin variety (7623.03±52.97 µg/dm^3). It exceeded almost six times that found in Vranec (1348.55±50.55 µg/dm^3). According to Chobanova (2012), diethyl succinate is an important ester, the presence of which in wine is observed in the range of 20.00 – 400.00 mg/dm^3. The data in the present study confirmed the main presence of this ester in the two wines studied.

Isopentyl acetate was identified with a small quantitative difference between the two wines. In Vranec its amount was slightly higher (120.49 ± 9.41 µg/dm^3), compared to Kaylashki Rubin (105.33 ± 13.76 µg/dm^3). The ester is a major contributor to the fruity aroma of wines (Li et al., 2008), with its characteristic descriptor being the banana aroma (Vilanova et al., 2013). Data on the presence of isopentyl acetate in the wines of Vranec and Kaylashki Rubin were correlated with Vilanova et al. (2008), which identified it in Spanish red wine of the Seradelo variety in an average quantity for two harvests (2006 and 2007) of 301.00 ± 225.80 µg/dm^3. Our results for this ester were correlated with data of Ivanova et al. (2013), which established it in nine studied Hungarian and Macedonian wines, with concentrations ranging from 136.00 ± 1.89 µg/dm^3 to 1320.00 ± 0.35 µg/dm^3.

Ethyl caprylate was identified in close concentrations between the two wines. A slightly higher amount of this ester was found in Vranec wine (233.48 ± 13.81 µg/dm^3) compared to Kaylashki Rubin (202.95 ± 14.42 µg/dm^3). The ester gives a characteristic fruity aroma (pineapple and pear). Its threshold of aromatic perception is very low (2.00 µg/dm^3). In both wines it was identified in a concentration 100 times higher than the threshold, which determined its important influence on their aroma. This ester was identified in higher amounts (5107.90 µg/dm^3) in Cabernet Sauvignon wine from China (Jiang et al., 2010).

Ethyl hexanoate was identified in both wines studied. In the wine of Vranec it was present in an amount of 185.41 ± 14.00 µg/dm^3, and in that of Kaylashki Rubin it was present in a slightly lower concentration (178.38 ± 13.35 µg/dm^3). This ester is also one of the main ones present in the wine aromatic matrix. It was also found in another study in wines from Merlot (167.55 ± 1.05 µg/dm^3) and Cabernet Sauvignon (195.42 ± 8.72 µg/dm^3) (Vilanova et al., 2013). Our data were correlated with those established by this team. A characteristic aroma that gives ethyl hexanoate is of green apple, fruity, strawberry (Tao et al., 2009). Our data also correlated with the research of Manolache et al., (2018), which also found this ester in red wines aged for the periods of 6 months (86.44 ± 5.38 µg/dm^3 – 164.10 ± 1.92 µg/dm^3) and 12 months (124.30 ± 3.47 µg/dm^3 – 434.53 ± 6.82 µg/dm^3).

Ethyl decanoate was found in a higher concentration in the wine of Kaylashki Rubin (174.28 ± 25.19 µg/dm^3), compared to Vranec (45.65 ± 8.37 µg/dm^3). This ester belongs to the group of fatty acid ethyl esters, which is one of the important for this fraction (Francis et al., 2005). A typical descriptor of this compound is vegetable aroma.

Ethyl-2-hydrobutyrate and ethyl cinnamate were identified only in Vranec red wine in concentrations of 124.16 ± 13.08 µg/dm^3 and 259.01 ± 11.22 µg/dm^3, respectively. They were absent in the aromatic matrix of Kaylashki Rubin.

Diethyl malate was identified in both wines studied. In the wine of Vranec it was present in a slightly higher concentration (63.12 ± 6.12 µg/dm^3), compared to that of Kaylashki Rubin (55.00 ± 5.00 µg/dm^3). This ester is also one of the main representatives of the fraction, normally present in wines in concentrations of $10.00 – 100.00$ mg/dm^3 (Chobanova, 2012).

2-hydroxy-3-methyl-diethyl ester was identified only in Vranec wine (105.13 ± 8.05 µg/dm^3).

Identified fatty acids

Fatty acids originate from yeasts and bacterial biosynthesis and have an important contribution to wine aroma (Etievant, 1991). In the present study, only one fatty acid was identified – heptanoic acid. In Vranec wine it was found in a slightly higher concentration – 1297.00 ± 70.01 µg/dm^3. In the wine of Kaylashki Rubin it was available in an amount of 1209.76 ± 51.25 µg/dm^3. Jiang and Ziang (2010) found traces of heptanoic acid in red wines from Cabernet Sauvignon. Añón et al. (2014) investigated the influence of different

oenological practices on the fermentation aroma of Menica red wines and found the presence of heptanoic acid in the variants in the range from 2.00 to 20.00 μg/dm³. In the present study, this fatty acid was found in higher concentrations.

Sensory evaluation of red wines

The results obtained from the panelists (4 experts in the field of enology) were calculated and transferred into spider diagram (Figure 1) that showed us the two different wine profiles that the wines from these varieties had.
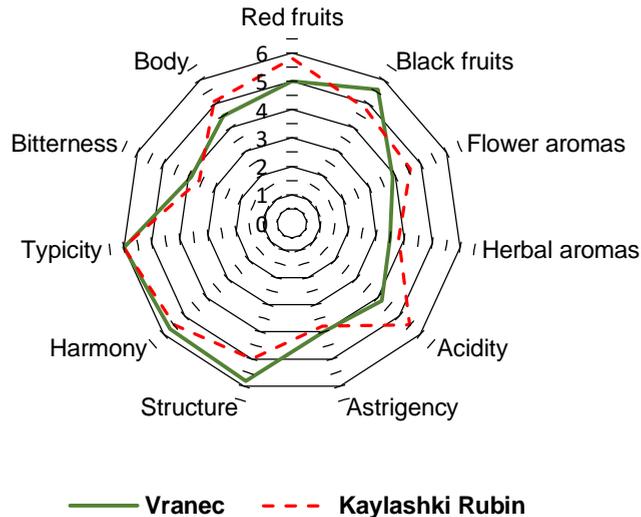
Figure 1. Sensory evaluation of red wines obtained from Vranec and Kaylashki Rubin varieties

According to this diagram we can see that the wine obtained from Vranec variety had more body and structure than Kaylashki Rubin, also the content of tannins was higher that could be noticed from the descriptor for bitterness and astringency. Vranec wine had less acidity and more black (dark) fruit aromas, lower freshness and less flowery notes in the wine. On the other hand, the wine from Kaylashki Rubin had higher level of acidity, more freshness which could be noticed from the descriptors flower and herbal aromas, also the wine had very intensive fresh red fruits aromas. According to the panelist both wines were very harmonious in their own way, both wines had their typical notes as expected for the both varieties. In overall the descriptive analyses confirm the analyzed components from the GC-MS analysis and gave us clear view about the aroma profile of both varieties.

The data regarding the sensory profile of the wine from Kaylashki Rubin were correlated with the research of Yoncheva et al. (2016, 2019), which defined the wine of this variety as harmonious, balanced and with pronounced varietal aroma, good color characteristics, dense and extractive. The data regarding the Vranec wine correlated with the study of Milanov et al. (2019), which determine the astrigenicity and bitterness as dominant sensory characteristics in the wine from this variety.

Conclusions

The following conclusions can be made from the study conducted to define the aromatic profile of red wines from the local variety Vranec and the hybrid variety Kaylashki Rubin:

1. The fraction of higher alcohols in both wines was consisted of 1-pentanol, 1-propanol, 2-propanol, 1-butanol, 1-hexanol, and 3-methylthio-1-propanol. 1-pentanol had the highest quantitative presence of this fraction. In the wine of the Vranec variety, 3-hexyl-1-ol was also identified, which was not present in that of Kaylashki Rubin.

2. One aromatic alcohol – phenylethanol – was identified. This compound was found in very high concentrations in both wines, with predominance in Vranec (20076.12±88.02 μg/dm^3), compared to Kaylashki Rubin (17864.31±155.0 μg/dm^3). Phenylethanol was an important component influencing the floral aroma of wines.

3. The main representative of the ester fraction in both wines was diethyl succinate. It occupied the highest concentration. Important ester compounds were identified – isopentyl acetate, ethyl caprylate, ethyl hexanoate, ethyl decanoate and diethyl malate. Ethyl-2-hydrobutyrate and 2-hydroxy-3-methyl-diethyl ester were identified only in Vranec wine. They were absent in the aromatic matrix of Kaylashki Rubin. This made the ester complexity of Vranec higher.

4. In the two studied red wines, only one fatty acid was identified, namely heptanoic acid in almost the same amounts.

5. The performed sensory evaluation showed that the Vranec wine had a better body and structure than that of Kaylashki Rubin. Vranec showed lower freshness and floral notes in the aroma, compared to Kaylashki Rubin. In Vranec the aromas of black fruits dominated, while Kaylashki Rubin showed a pronounced floral and herbal aromas, as well as fresh red fruits aroma. Both wines showed a diverse, balanced aromatic profile, each of which, based on the peculiarities of its volatile composition. Each wine has an individual aromatic capacity.

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Biological value of proteins of cultivated mushrooms

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Abstract

Introduction. The objectives of this research were to scientifically substantiate and experimentally prove the nutritional status of cultivated mushrooms as the probable source of easy-absorbed proteins, essential and dispensable amino acids, and other valuable biologically active components.

Materials and methods. Biochemical characteristics, such as the mass part of albumins, globulins, glutelins and prolamins, and the qualitative and quantitative composition of amino acids in free and constrained forms, of cultivated mushrooms, champignon (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*), and edible wild mushrooms, white mushrooms (*Boletus edulis*) and the brown-cap boletus (*Leccinum scabrum*), were determined.

Results and discussions. The biochemical composition of mushroom hats and legs is different in separate indices: the amount of dry substances in champignon hats is higher by 13–18%, the amount of proteins is higher by14.6–23.5%, meanwhile, the amount of cellulose is lower by 17–19% in comparison with legs. This shows the substantial nutritional advantage of hats, and it must be taken into consideration in the industrial procession of mushrooms: hats should be separated from legs, following the optimal parameters of the process for each anatomic part. The champignon proteins contain all the indispensable amino acids and, therefore, can be the important source of lysine (4.95 mg%), phenylalanine (7.04 mg%), leucine (9 mg%), and threonine (7.6 mg%). About 7.6% of amino acids are in free form, half of which are essential. This would help the human body effectively use the amino acids to synthesize its own proteins.

The amount of proteins in fresh champignons is 6–9% of their mass, in oyster mushrooms it is 4–5%, in wild mushrooms, it is 6–8.5%, which outlines the priority of champignons particularly by their protein component. Easy-soluble factions (albumins and globulins) at 70.3% present the champignon proteins; this index is slightly lower for oyster mushroom proteins (65%) and for brown-cap boletus, it decreased to 53.2%. Therefore, proteins of the cultivated mushrooms need the minimal amounts of energy to be dissociated to amino acids in the human body, and otherwise show the high grade of proteolysis (almost as milk proteins) under the influence of gut enzymes. These characteristics were achieved due to scientifically proven selection of raw materials, regarding their sensory characteristics that were estimated with the excellent grade. There were proposed criteria to select the cultivated mushrooms for culinary and industrial processing: the amount of proteins no less than 6–9%; cellulose 2–3.5%; carbohydrates 1–1.5%.

Conclusions. The cultivated mushrooms and the products of their procession with high content of proteins and other valuable components should become the essential constituent of diets in order to overcome the protein deficiency.

Introduction

Nutrient analysis and dietary quality for most people indicate a persistent protein deficiency, which should be exacerbated in the near future (Medek et al., 2017). Therefore, the search for potential new protein sources and production of high-protein foods are among the topical tasks for food technologies (Ivanov et al., 2021; Wu et al., 2014).

The National Center for Biotechnology Information (USA) highlighted that about 90 percent of adult people are aware of the advantages of high-protein foods (Chang & Wasser, 2012; Global Alternative, 2020). Due to ecological ideology and diffusion of vegetarianism, the production of proteinaceous foodstuffs from soybeans is the main stream in Asia, particularly in China, and, during the last years, in Europe (Elorinne & Kantola, 2016). The largest share belongs to champignons (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) (Martinez-Medina et al., 2021; Stabnikova et al., 2010; Stojkovic et al., 2014). There is an array of scientific research on using the mushroom raw materials as the meat substitute (Pasichny et al., 2009). In fact, this became one of the main tendencies of the food industry through the latest period, which is believed to be increasing significantly (Batraksas et al., 2021; Ferdousi et al., 2020; Mubiana et al., 2012).

The artificial cultivation of mushrooms becomes very important because the fruit bodies of forest mushrooms have the ability to accumulate heavy metals and radionuclides, thus becoming perilous for consumers' health and life (Struminska-Parulska et al., 2021). There is a point of view that in the nearest future about two thirds of protein needs for humans will be met through the consumption of mushrooms grown in industrial conditions. (Bolotskikh & Volfovsky, 2007). These mushrooms are ecologically clean, and their taste could be improved by addition of sodium glutamate (Chang, 2006). Mushrooms are widely used in production of therapeutic and preventive remedies with hepatoprotection, radioprotection, antidiabetic, anticancer, and immunoregulatory activities (Martinez-Medina et al., 2021; Sanket & Pravin, 2021; Valverde et al., 2015; Yaschenko, 2012). It was shown that consumption of mushrooms increased the immunity to inflectional and oncologic diseases (Krasnopolskaya et al., 2007; Meera et al., 2009; Wasser & Weis, 1999; Wasser et al., 2000); they get involved into metabolic processes and do not have cumulative ability (Cultivation, 2021; Yaschenko, 2012). Regular consumption of cultivated mushrooms can significantly increase the content of antioxidant markers and decrease the level of oxidative stress (Calvo et al., 2016; Glamočlija et al., 2015). Mushrooms can also become the only plentiful sources of vitamin D of non-animal origin (Bernas & Jaworska, 2017; Cardwell et al., 2018; Simon et al., 2013).

Therefore, the problem of increasing the volumes of consumption of cultivated mushrooms is scientifically proven and is actual for the population of over the world.

The protein content of mushrooms determines their biological value. In this case, the content of amino acids in the protein must meet the needs of the human body for the synthesis of its own proteins (Tagkouli et al., 2020). Moreover, proteins, upon being the most essential component of food, are responsible for growth, creation of the new tissues and restoration of those damaged (Malecki et al., 2021). Besides, all enzymes and certain hormones are proteins too. Decidedly, only the plentiful proteins provide the correlations of amino acids, which are compatible with human body needs.

Unfortunately, these problems are now studied sporadically. The majority of updated research are dedicated to the principles of mushroom cultivation (Royse, 2003; Zhang et al., 2014), their industrial production (Simakhina et al., 2014); elaboration of eco-friendly and wasteless cultivation technologies (Guan et al., 2016; Simon et al., 2011); improvement of the methods to process mushroom raw materials, including drying, fermentation and

freezing; mycelium preparation and so on. Only some studies deal with the general amount of proteins in oyster mushrooms, touching upon their amino acid content and the proportions between dispensable and indispensable amino acids, practically leaving aside the ways to increase the mushroom biological value and other related issues (Tolera & Abera, 2017).

Issues that have so far received insufficient attention include study of the fractional composition of proteins of cultivated mushrooms which is an essential index to predict the level of their absorption in human body; effectiveness of protein digestibility by proteolytic gut enzymes; elaboration of the criteria to select the sorts of cultivated mushrooms (starting from their sensory evaluation), compliance with which would guarantee obtaining the high-quality half and final products with increased biological and nutritional value.

The aim of the present research was scientifically substantiality and experimentally proven of the nutritional status of cultivated mushrooms as the probable source of easy-absorbed proteins, essential and dispensable amino acids and other valuable biologically active components for their use in the food industry. To achieve this goal it was necessary to examine the quantitative and qualitative content of the main nutrients in cultivated mushrooms, particularly, the fractional composition of proteins; to estimate the ratio between dispensable and indispensable amino acids; the grade of their digestibility by proteolytic gut enzymes; their sensory indices, and to formulate the criteria to select the champignons for both direct consumption and industrial procession.

Materials and methods

Mushrooms

Champignons (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus ostreatus*) became the object for the main part of research. For a comparative study, some experiments were conducted in parallel with wild white mushrooms (*Boletus edulis*) and brown-cap boletus (*Leccinum scabrum*). After having selection, washing, and removing the waste from the raw materials, the biochemical characteristics of mushrooms were evaluated, namely, fractional distribution of protein, content of amino acids, sensory characteristics, and the ratio of free and constrained amino acids, both dispensable and indispensable.

Determination of dry matter

The dry matter was determined using differential refractometry (Hernandez et al., 1998) using of IRF-454 B2M refractometer (Laboratorna tekhnika Ltd., Kharkiv).

Determination of protein and amino acid content

The general amount of proteins and the qualitative and quantitative content of amino acids were determined by the method described in (Redweik et al., 2012) with a usage of capillary electrophoresis. The ratio between dispensable and indispensable amino acids in free and constrained forms was determined by the method of Moore – Stein (Moore & Stein, 1972).

Determination of sugars content

The general amount of sugars was determined by ion analysis method using Bioscan 817 chromatographer (Metrohm IC). To prepare the sample for the analysis, mushrooms were powdered to homogenous mass and put into the automatic sample taker of the chromatograph.

Determination of cellulose content

Content of cellulose was determined by the method of direct weighing analysis, which combines oxidation, destruction, and solution of various chemicals, except for cellulose that, in process, was removed, dried and weighed (Kumar & Turner, 2015).

Fractionation of mushroom proteins

Fractionation of mushroom proteins was carried out according to (Table 1). The disintegrated samples of mushrooms (particle size 2-3 mm) were extracted, and then centrifuged for 15 min at 6000 rpm. The sediments were washed, and the volume of every extract was replenished to 150 ml by washing waters. The content of protein was determined in the extracts and sediments by the method (Redweik et al., 2012).

Table 1

Fractionation of mushroom proteins

Method	Fractions of mushroom proteins			
	Albumins	Globulins	Glutelins	Prolamins
Solvents	Water	1 M NaCl in 0.1 M phosphate buffer (pH 6.8)	0.1 N NaOH	70% ethyl alcohol
Weight ratio between mushroom mass and solvent	1 : 3	1 : 3	1 : 2.5	1 : 2.5

Evaluation of sensory characteristics of cultivated mushrooms

The selection of raw materials, primarily by the sensory characteristics, is the essential step to use the fresh mushrooms and, subsequently, obtaining the mushroom semi-finished products with suitable consumer properties and high biological value (Phat et al., 2016). Thus, high quality of mushroom semi-finished products and foods with their usage is guaranteed (Table 2).

Table 2

Methods to evaluate the main sensory characteristics of fresh mushrooms (champignons)

Characteristics	Description
Appearance	Mushrooms are clean, undamaged, elastic, fresh looking, without excessive external humidity, not frozen, not injured by harmful insects; legs are either cut or uncut. In the first case, the cut should be clean; in the second, the traces of greenhouse material are accessible. Insignificant surface damages are allowed if they do not affect the quality, storage terms and commercial appearance of the packed items.
Taste and smell	Typical for fresh champignons, without strange smells and smacks.
Color	The hat surface is white, cream-colored or brown with various hues typical to the cultivated sorts; the fresh cut of the hat is white with rosy hue.
Maturity grade	Mushrooms are of forms and colors typical for the certain botanical sort, homogenous in maturity grade, well shaped. The hats are open or closed but not flat. The plate color from the bottom side of the hat is pale rosy.

The sensory characteristics of mushrooms were evaluated according the 5-point scale proposed by the authors (Table 3).

Table 3

Scoring of sensory characteristics of fresh champignons

Index	Score points	Estimation of fresh champignons quality by score points
Appearance	5	Fresh, whole, without defects and microbial damages, homogenous.
	4	Fresh, whole, practically without defects.
	3	Whole, partly withered, slightly damaged.
	2	The significant share of withered and damaged mushrooms.
	1	Inhomogeneous, with defects and microbial damages.
Taste and smell	5	Typical for fresh champignons, without strange taste and smell.
	4	Slight strange taste and smell.
	3	Stable and obvious strange taste and / or smell.
	2	Stable and expressed, atypical strange taste and / or smell.
	1	Strong rotting stench and atypical taste.
Color	5	The hat surface is white or cream-colored; the fresh cut of the hat is white with rosy hue.
	4	The hat surface is white or cream-colored; the fresh cut of the hat is white.
	3	The hat surface is grayish as well as the fresh cut.
	2	The hat surface is grey with dark blots; the cut is grey.
	1	The hat surface is dark; the cut is rotten.
Maturity grade	5	Mushrooms are homogenous in maturity grade, well shaped. The hats are not flat. The plate color from the bottom side of the hat is pale pink.
	4	Mushrooms are sometimes inhomogeneous in maturity grade, well shaped. The hats are not flat. The plate color from the bottom side of the hat is pale.
	3	Mushrooms are slightly inhomogeneous in maturity grade, mostly well shaped. The hats are not flat. The color of the hat plates is grayish.
	2	Mushrooms are practically inhomogeneous in maturity grade, different in shape. The hats are mostly flat. The color of the hat plates is grey.
	1	Mushrooms are different in maturity grade, non-calibrated. The plate color from the bottom side of the hat is rotten brown.

Note: mushrooms with quality estimated as 1 or 2 points are not recommended for further procession.

Upon selection of mushroom raw material for technological purposes, the sensory evaluation should be complemented with the characteristics of mushroom biochemical compounds.

Results and discussion

Biochemical characteristics of mushroom fruit bodies

For the certain species of mushrooms, some essential biochemical characteristics of hats and legs were determined. The results are shown in Table 4.

Table 4

Biochemical characteristics of mushroom fruit bodies

Content,% to dry matter	Mushrooms species and anatomic parts					
	White mushrooms		Champignons		Oyster mushrooms	
	Legs	Hats	Legs	Hats	Legs	Hats
Water	87.0	85.5	86.0	84.2	91.0	90.5
Dry matter	13.0	14.5	14.0	15.8	9.0	9.5
Proteins	6.8	8.4	7.5	8.6	4.0	5.1
Carbohydrates	1.22	1.53	2.4	1.9	1.2	1.6
Cellulose	4.23	3.5	3.0	1.6	3.1	1.9

Analysis of the biochemical composition of separate fruit body parts of cultivated mushrooms showed the quantitative difference between hats and legs. The results show that hats and legs of champignons contain more proteins than any other studied mushroom species. The protein content in hats of champignons is 8.6%; in white mushrooms 8.4%; in oyster mushrooms 5.1%. The content of dry substances was slightly higher in hats, and the protein content was by 14.6-23.5% higher in hats than in legs, which is according to the results of other researchers (23, 24).

There is a problem of cellulose impact on nutritive value of cultivated mushrooms (Dubinina, 2009; Synytsia, 2009). Cellular membrane of mushrooms, due to the content of chitin (about 60% to dry matter), is able to reveal the antiviral and antibacterial action and absorb the heavy metals and radionuclides (Meera, 2009; Wasser, 2000). The large amount of cellulose represented by indigestible food fibers would retard the process of protein dissociation in the gut and their further absorption by the organs and tissues; it would mean that mushrooms are unsuitable for dietetic nutrition. Therefore, from our viewpoint, the amount of cellulose in cultivated mushrooms destined for obtaining the food products with increased nutritional and biological value should be within 3-3.5%.

Taking into account the difference between biochemical characteristics of hats and legs, we propose the notion of heterogeneity grade of anatomic parts of mushrooms by two main constituents – proteins and cellulose. This index should be evaluated with a coefficient:

CP = P / Cel, in which P is the protein content,% ;

Cel – cellulose amount,% .

For the studied types of mushrooms, the CP coefficient counts:

- White mushrooms: legs – 1.62; hats – 2.4;
- Champignons: legs – 1.87; hats – 2.15;
- Oyster mushrooms: legs – 1.29; hats – 2.6.

This discrepancy between the heterogeneity grades of different anatomic parts of mushrooms is evidence of their structural, mechanical properties and tissue firmness. Because mushroom hats and legs have different content of essential nutrients, we conclude that, upon elaboration of technology to produce mushroom semi-finished products, independently on the species, the hats should be separated from legs prior to procession, and then the optimal procession parameters should be determined for each of the anatomic parts.

Amino acid contents in fresh champignon proteins

Amino acids are the main structural elements of proteins. Twenty-six amino acids were observed in proteins, and the typical constituents of proteins are considered twenty of them. The latter are categorized into dispensable (total amount of twelve) and indispensable, or essential (total amount of eight) obtained only from foodstuffs. The results on the qualitative and quantitative content of fresh champignons to be later estimated as the ratio between dispensable and indispensable amino acids in free and constrained forms (as the characteristics of protein biological value) are shown in Table 5.

Table 5

Amino acid contents in fresh champignon proteins

Amino acid	Total amount,%	Free		Constrained	
		mg%	% to the total amino acid amount	mg%	% to the total amino acid amount
Lysine	4.98	0.38	0.26	4.60	3.21
Histidine	8.98	0.78	0.54	7.70	5.38
Phenylalanine	7.036	0.136	0.10	6.90	4.82
Tyrosine	2.51	0.05	0.03	2.46	1.72
Leucine	9.0	0.5	0.34	8.50	5.94
Isoleucine	2.94	0.64	0	2.30	1.60
Valine	5.08	0.7	0.48	4.38	3.06
Methionine	1.71	0.01	0.01	1.71	1.18
Alanine	7.4	1.3	0.90	6.10	4.26
Glycine	17.17	0.27	0.18	16.91	11.81
Proline	2.31	0.01	0.01	2.32	1.60
Serine	9.00	0.40	0.27	8.60	6.01
Threonine	7.63	0.53	0.37	7.11	4.96
Asparagine acid	21.72	0.38	0.26	21.34	14.92
Cystine	0.31	0.02	0.01	0.29	0.20
Arginine	-	-	-	-	-
Tryptophan	1.05	-	-	1.05	0.73
Glutamine acid	34.7	1.5	1.04	33.2	33.2
Total	143.021	7.6	-	135.4	-

Fractional composition of fresh champignon proteins

Biological value of proteins in any foodstuffs determines not only by the total amount or the amino acid content, but also by fractional composition. The proteins are classified into four classes, namely, albumins, globulins, prolamins, and glutelins (Garidel, 2013). Albumins, water-soluble proteins, are characterized by the highest biological and nutritional value; globulins, salt-soluble proteins, have also high biological value but are poor in sulfur-containing amino acids. The last two, prolamins, alcohol-soluble, and glutelins, alkali-

soluble, have no some indispensable amino acids in their compositions, harder digested by proteolytic enzymes and thus have lower the biological value.

The literary data about fractional composition of proteins of cultivated mushroom are still limited. Therefore, fractional composition of mushrooms proteins were studied in the present research, and compared (Table 6).

Table 6

Fractional composition of mushroom proteins

Protein fractions	Ratio of fractioned proteins,% of total protein amount		
	Brown-cap boletus	Champignons	Oyster mushrooms
Water-soluble (albumins and easy-soluble globulins)	30.8	46.4	39.8
Salt-soluble (hard-soluble globulins)	22.4	23.9	25.2
Alkali-soluble (glutelins)	12.6	8.06	10.6
Alcohol-soluble (prolamins)	11.5	5.6	3.6
Unsolved remnant	22.7	16.24	20.8

According to results, cultivated mushrooms have higher biological value, because protein substances are mostly presented by easy-soluble factions – 70.3% in champignons and 65% in oyster mushrooms. These proteins are alleged to dissociate in human body to amino acids, which are necessary for synthesis of the native proteins, with minimal energy losses.

Fractional composition of champignon proteins is slightly better than of oyster mushrooms, however, both kinds of mushrooms are suitable for direct usage and industrial procession into proteinaceous semi-finished products as ecologically clean, useful and safe enough raw materials.

Wild mushroom proteins contain less albumins and globulins (53.2%), therefore, they are worse soluble in water and neutral salt solutions. Human body worse absorbs such proteins, and their biological availability and value are lower in comparison to cultivated mushrooms.

Because of high content of albumins and globulins, proteins of cultivated mushrooms will be far easily hydrolyzed in the gut by proteolytic enzymes, and proteins of wild mushroom have lower proteolysis degree because they contain much more cellulose, which may block the enzyme access to protein substances.

Sensory characteristics of champignons

Therefore, conducted research and obtained results showed the perspectives for using of cultivated mushrooms, particularly hats of champignons, as the reserve of native proteins, well-balanced proportion of essential and dispensable amino acids, prevalent content of easy-soluble factions, and higher grade of digestibility by proteolytic enzymes. Regarding to the fact that appearance is considered the complex index to include the shape, size, maturity grade, freshness, and color, the maximal figure of quality coefficient will be 0.35 (Table 7).

Scoring the sensory characteristics of fresh champignons

Properties	Coefficient	Score points	Characteristics
Appearance	0.35	5	Mushrooms are whole, clean, elastic, fresh, without excessive external humidity, non-frozen and non-damaged by agricultural pests. Cut champignons should have their cuts clean; surface damages are not allowed.
Taste and smell	0.25	5	Typical for fresh champignons, without strange smell and smack.
Color	0.15	5	Hat surface is white or cream-colored, with different hues typical for certain sorts; hat pulp on the cut is white with pink hues; leg pulp is slightly darker due to higher cellulose content.
Maturity grade	0.25	5	Mushrooms are typical in appearance and color for the certain botanical species, homogenous in maturity, well shaped. Hats are closed or opened but not flat. The color of under-cup plates is pale pink. Legs of non-cut mushrooms may carry the traces of greenhouse soil materials.

**Criteria for selection of champignons for direct consumption
and processing in semi-finished products**

No.	Criterion	Criterion characteristics
1	High protein content (6-9% and more)	The significant reserve of food proteins; their validity in terms of correlation between dispensable and indispensable amino acids; the important additional source of lysine, phenylalanine, asparagine and glutamine amino acids
2	High biological value	The presence of all the indispensable amino acids; correspondence of amino acid composition to human needs for synthesis of the native proteins; protein digestibility equal to the one of milk proteins
3	Optimal cellulose content (2-3.5%)	Positive impact on gut functions; adsorption of heavy metals and radionuclides; prebiotic properties
4	Sufficient carbohydrate content (1-1.5%)	The ability to stimulate anti-body synthesis and thus to increase the immune protection; cancer-protecting properties due to presence of polysaccharides
5	Relative initial humidity (no more than 80-84%)	Quite an intensive drying process should be maintained; circa 90% of moisture are represented by free faction to be removed easily
6	The absence of toxic substances, heavy metals and carcinogens	Environmental friendliness of production and procession; safety for consumers in both fresh and processed forms
7	Sensory characteristics	Appearance, taste and smell, color and maturity grade

Moreover, since mushrooms are discrepant in appearance to the requirements proposed, the usage of all the other criteria appears to be inexpedient. In case when mushrooms are discrepant in appearance to the requirements proposed, the usage of all the other criteria appears to be inexpedient. The studied champignons by all the sensory characteristics scored the maximal five points, confirming their status of a reliable source of proteins, amino acids and food cellulose that are the main nutrients in human diets.

Upon taking into account, the results of studying the biochemical composition of champignons and scoring their sensory characteristics, the criteria to select mushrooms for either cookery or industrial procession were established (Table 8).

Conclusions

1. Proteins as macronutrients are essential in growth, creation of the new tissues and recovery of the damaged ones. They take part in regulation of the majority of vital processes in human body, enhance the biological influence of other nutrients, and provides the transport of oxygen, hormones and trace elements. Insufficient supply of proteins or separate amino acids with foodstuffs would lead to protein deficiency, causing serious damages in the body due to misbalance between protein anabolism and catabolism. This is why the searches for new untraditional sources of proteins are relevant today. One of the ways to solve this problem is the usage of cultivated mushrooms that contain about 50% of proteins (in terms of dry matter) and other value biocomponents.

2. It was shown that cultivated champignons and oyster mushrooms have in their fractional composition a high content of easily digestible proteins (more than 70%), which facilitates their digestibility by proteolytic enzymes. At the same time, they contain all the essential amino acids that confirms their nutritional value. Organoleptic criteria have been proposed for the selection of cultivated mushrooms in order to use them in obtaining high-quality food products.

3. The advantages of cultivated mushrooms over the wild ones in terms of ecological friendliness and safety were demonstrated.

4. The optimal ration between proteins and cellulose in champignons (3 : 1) will provide the high grade of protein digestion by proteolytic enzymes and allow using the detoxifying properties of cellulose as the natural sorbent. Therefore, the further studies on cultivated mushrooms, the search of new high-protein species, and the design of effective methods to process the mushroom raw materials into semi-finished and final products are tasks targeted at overcoming the protein deficiency and ameliorating the human health.

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Fermentation of apple juice using selected autochthonous lactic acid bacteria

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Abstract

Introduction. Dairy products are the most common probiotic food. However, due to lack of enzyme lactase, their ingestion for lactose intolerant people is a challenge. Fruit juices are rich in nutrients and have proved to be effective carriers or growth media for probiotics, specifically for lactic acid bacteria; they are also lactose-free and can be taken by lactose intolerant individuals.

Materials and methods. Self-made apple juice and selected lactic acid bacteria were used in the study. The number of viable bacterial cells was determined by a serial dilution method; titratable acidity was determined by automatic titrator; sugars and organic acids concentrations were measured using High-Performance Liquid Chromatography; total phenolic compound content was determined by the Folin-Chiocaltus method; and the antioxidant activity was determined by FRAP (ferric-reducing antioxidant power) assay.

Results and discussion. Selected *Lactiplantibacillus plantarum* strains were used to ferment apple juice. The optimal conditions for the fermentation were an initial pH 4.5 and 24 h duration, with maximum bacterial cells viability 8.23±0.17 log CFU/mL and 8.55±0.19 log CFU/mL for *L. plantarum* 74 and *L. plantarum* 76, respectively. Characteristics of apple juice were changed during fermentation, particularly, after 48 hours of fermentation, an increase in the titratable acidity caused the pH decrease and gradual decrease of the sugar contents was also observed. The highest production of lactic and malic acids were observed during 48 h of fermentation with the strain *L. plantarum* 74. The fermented juice with *L. plantarum* 52, *L. plantarum* 74, and *L. plantarum* 76 had concentration of total phenolic compounds 532.9±26.7 mg GAE/L, 587.3±29.4 mg GAE/L, 488.4±24.4 mg GAE/L and antioxidant activity 281.6±14.1 mg AAE/L, 300.6±15.0 mg AAE/L, 172.8±8.6 mg AAE/L, respectively after 72 h of fermentation.

Conclusion. Apple juice fermented with selected strains of *Lactiplantibacillus plantarum* was enriched with lactic acid bacteria and can be used as a probiotic product that people with lactose intolerance can consume.

Introducton

The most common and affordable food products that are sources of beneficial probiotic bacteria are fermented dairy products. However, some people, including children, are unable to fully digest lactose, a sugar present in milk and milk products. On average, 65% of the world's population is lactose intolerant (Bayless et al., 2017). Fruit juices are healthy products that are rich in important nutrients such as vitamins, minerals and antioxidants (Hussein et al., 2022), and they can serve as a medium for the growth of lactic acid bacteria. At the same time, they do not contain lactose. Thus, fruit juices enriched with lactic acid bacteria might be used as probiotic food products that even persons with lactose intolerance could consume (Gomes et al., 2021; Katoch et al., 2021; Lillo-Pérez et al., 2021, Tkesheliadze et al., 2021). Fermentation of fruit juices using lactic acid bacteria (LAB) could be used to preserve sensorial and nutritional properties and to extend the shelf life of the final products (Garcia-Gonzalez et al., 2021; Plessas, 2022). In addition, plant-based diets become increasingly popular, so fermentation of plant products and the selection of microorganisms to be used as starter culture is an issue of high demand (Cichońska and Ziarno, 2022). During the fermentation of the juice caused by lactic acid bacteria, changes in its chemical and microbiological properties occur and the final product can be enriched with microbial metabolites such as organic acids, phenolic compounds, exopolysaccharides and bacteriocins (Khubber et al., 2022). Due to these beneficial properties, fruit juices fermented by LAB increases the availability of probiotic non-dairy products on the market. For this purpose, the application of autochthonous starters is preferred (Garcia et al., 2020).

One of the most promising types of lactic acid bacteria used for the fermentation of fruit juices are strains of *Lactiplantibacillus plantarum* whose cells have high gastrointestinal adaptability and adhesion ability. These LAB also possess antimicrobial, antioxidant and anti-inflammatory properties (Garcia-Gonzalez et al. 2021; Rocchetti et al., 2021; Won et al., 2021). Application of *L. plantarum* strains to ferment of vegetable and fruit juices is extremely appealing since they can improve their sensorial properties (Plessas, 2022). From a variety of fruit beverages, apple juice is considered as a good food substrate for enrichment with lactic acid bacteria (Wu et al., 2020). This advantage is facilitated by the fact that the fermentation process is thought increases the bioavailability of bioactive compounds found in the plant material, namely in apples, which leads to a change in the substrate composition and, as a result, affects the sensory properties of the final product (Guiné et al., 2021).

The aim of the present study was to investigate change of the selected technological characteristics of apple juice fermented with the strains of *Lactiplantibacillus plantarum.*

Materials and methods

The apple fruits from different regions of Georgia were used in the study. Apples were washed with tap water, cutting, crushed using a food processor and filtered through a paper filter, 12.5 cm diameter, and then through the Millex-GS Syringe Filter Unit with a 0.22 μm pore size mixed cellulose esters membrane (Millipore).

For the fermentation of apple juice three probiotic strains of autochthonous lactic acid bacteria *Lactiplantibacillus plantarum* 52, *L. plantarum* 74, *L. plantarum* 76 from the collection of microorganisms at Durmishidze Institute of Biochemistry and Biotechnology of the Agricultural University of Georgia, specifically were used. The bacterial strains stored at -80 °C were transferred to MRS broth and incubated at 37 °C for 48 hours.

Apple juice was inoculated with lactic acid bacteria and incubated at 37 °C. Cell viability, pH values, titratable acidity, content of sugars, organic acids and total phenolic compounds as well as antioxidant activity were evaluated prior to the fermentation process and after 24 h, 48 h, and 72 h of fermentation.

The total number of viable cells of *L. plantarum* 52, *L. plantarum* 74, *L. plantarum* 76 on MRS agar was determined using the serial dilution method. Aliquots (0.1 mL) of diluted fermented juice were plated in triplicate onto MRS agar and incubated at 37 °C for 72 h in an anaerobic incubator (Hashemi et al., 2017, ISO/TS 19036, 2006). The results were expressed as log CFU/mL.

The pH was measured using a digital pH meter. Titratable acidity (TA) was determined by titrating with 0.1 M NaOH to a pH end-point of 8.2 using an automatic titrator (ZDJ-4A, NASA Scientific Instrument Co., Ltd, Anting Shanghai, China). The results were expressed as gram of malic acid equivalents per 100 mL of juice (Wlodarska et al., 2017).

HPLC was used to determine the **concentrations of sugars** such as sucrose, fructose, and glucose, as well as the content of organic acids, in fermented and unfermented apple juices. The data were recalculated in grams per liter.

Total phenolic content in apple juice was determined using the Folin-Chiocaltheus method (Bond et al., 2003). 5 mL of diluted 10 times Folin-Chicolteo reagent was added to 1 mL of the test sample and left at room temperature for 8 minutes. As a control, 1 mL of distilled water was used. Then 4 mL of sodium carbonate was added and thoroughly mixed. The samples and standards were kept at room temperature for 1 hour. A spectrophotometer was used to determine the **sample absorption rate** at a wavelength of 765 nm. The results were expressed in mg gallic acid equivalents (GAE) per litre of juice.

The total antioxidant concentration was determined by the FRAP method (Benzie and Strain, 1996). A spectrophotometer was used to measure the change in absorption intensity. Initially, a working solution for determining the sample was prepared using a mixture of three solutions: 300 mM acetate buffer (pH 3.6); TPTZ (2.4.6-tripiridyl-5-triazine), and trivalent iron chloride, in the ratio 10:1:1. The obtained working solution was submerged in a 37 °C water bath for 15 minutes. The working solution, 3 mL, was added to the apple juice sample, 100 µl, and the absorbance was measured using a spectrophotometer at 593 nm; the absorption was recorded after 4 minutes. The working solution was used as a control, while ascorbic acid was applied as a comparator. The data were measured in mg of ascorbic acid equivalent (AAE) in 1L of the juice.

All experiments were performed in triplicate. The data are expressed as the mean ±standard deviation. Statistical analysis was carried out using one-way ANOVA and Tukey's HSD tests. One-way analysis of variance (ANOVA) was done to analyze the variation of the means between the experimental samples. Tukey's HSD test was used to differentiate between the mean values. All the analyses were done using XLSTAT (free trial version 2022, Addinsoft, Inc., Brooklyn, NY, USA). p value < 0.05 was considered statistically significant.

Results and discussion

Determination of the viable cell number

An important parameter for evaluating the fermentation process is the quantitative change in the number of bacteria (Janiszewska-Turak et al., 2022). Changes of viable cell number of *Lactiplantibacillus plantarum* 52, 74, and 76 during the fermentation of apple juice are shown in Table. 1.

Changes of viable cell number in apple juice fermented with strains *Lactiplantibacillus plantarum*

Time of fermentation, h	*L. plantarum* 52	*L. plantarum* 74	*L. plantarum* 76
	log of colony forming units (CFU)/mL		
0	8.1±0.1	8.2±0.2	8.3±0.2
24	9.0±0.4	8.2±0.2	8.6±0.2
48	6.6±0.2	7.4±0.3	7.4±0.3
72	9.1±0.3	7.4±0.1	7.8±0.3
192	7.8±0.1	7.5±0.4	8.5±0.3
288	7.0±0.4	7.3±0.2	7.8±0.2

All strains of the lactic acid bacteria revealed different growth (Table 1). In the case of *L. plantarum* 76, the initial cell number of 8.3±0.2 log CFU/mL increased to 7.8±0.3 log CFU/mL after 72 h, whereas in *L. plantarum* 52, an increase approximately 1 log CFU/mL of bacterial cells was observed within 24 h of fermentation, followed by decrease to 2.45 log CFU/mL, which could be caused by sugar consumption in fermented juices. Similar results were observed in the research of Janiszewska-Turak with co-authors (2022). Li with co-authors (2019) in their study found that during the first 24 h of fermentation, the viability of *L. plantarum* cells increased due to adequate nutrient content and suitable growth conditions, and after 24 h their growth slowed down. Wang with co-authors (2021) studied the viability of bacteria in apple juice fermented with *Lactobacillus plantarum*. As the results showed, in the case of additional ultrasound processing of juice, in the early stage of fermentation, in particular after sonication for 0.5 h, the number of the bacteria increased from 7.5 log CFU/mL to 7.9 log CFU/mL and 7.8 log CFU/mL, whereas the number of *Lactobacillus plantarum* cells did not change when this treatment was not used. The viable cell count of *L. plantarum* decreased during the storage at 25 °C of fermented dairy substrates from 8.89 log CFU/mL to 8.6 log CFU/mL after 240 h and the decrease was more pronounced after 1440 h in fermented transition milk stored at 25 °C, showing a viable cell count 5.7 log CFU/mL (Fonseca et al., 2020). According to Ostlie with co-authors (2003), viable cell counts for the probiotic strains in fermented milk increased from 8.7 to 9.2 log CFU/mL after 6–16 h of incubation.

Determination of pH

The pH changes in apple juice fermented using different strains of *L. plantarum* are shown in Table 2.

The initial pH of control sample was 4.5±0.2. The juice pH reduced after 48 h of fermentation from 4.5 to 3.6 and a modest decline in the cell viability was observed after 48 h of fermentation (Table 1). The pH of apple juice increased from 3.8 to 4.0 on the 192 h of fermentation because of *L. plantarum* 76 activity, and the number of viable cells increased as well (Table 1). Meanwhile, the pH of the juice fermented with *L. plantarum* 74 increased to 4.0, and at the end of fermentation decreased to pH 3.8. According to the literary data, a similar drop was shown in fermented dairy product as well. After 2 h of fermentation, the pH of the milk began to decrease rapidly, from 6.8 reached ~4.5 in less than 6 h (Dan et al., 2019).

Changes of the pH of apple juice fermented with the strains *Lactiplantibacillus plantarum*

Time of fermentation, h	*L. plantarum* 52	*L. plantarum* 74	*L. plantarum* 76
48	3.6±0.2	3.6±0.2	3.6±0.2
72	3.8±0.2	3.8±0.2	3.8±0.2
192	3.9±0.2	4.0±0.3	4.0±0.1
288	3.8±0.2	3.8±0.4	3.9±0.1

It should be mentioned that the pH of MRS broth was 6.4 at the start of the experiment and ranged between 3.8 and 4.0 by the 288 h as a result of the action of all three strains, whereas the pH of apple juice was 4.5. The value reduced on the 288 day of fermentation by *L. plantarum* strains.

The pH changes, in turn, impacted the number of bacteria present during apple juice fermentation. In particular, on the 288 h of fermentation, the initial number of bacteria *L. plantarum* 52, *L. plantarum* 74, *L. plantarum* 76 decreased from 8.1±0.1 log CFU/mL, 8.2±0.2 log CFU/mL and 8.3±0.2 log CFU/mL to 7.0±0.4 log CFU/mL, 7.3±0.2 log CFU/mL and 7.8±0.2 log CFU/mL, respectively, while no reduction in the number of cells of every culture was observed in MRS broth.

The establishment of various conditions, particularly when the pH of MRS broth and apple juice do not match, may be one of the causes of the decrease in viable cell number (Mousavi et al., 2013). Given that pH is one of the most significant factors determining the probiotic survival, the pH value of the juice at the start of our experiment may have resulted in a steady decline in microbial growth. According to Dimitrovski with co-authors (2015), a low pH value does not promote LAB growth, and pH low than 4.4 could inhibit the growth or slow down the growth rate (Saeed et al., 2013) and thus the initial pH influences the fermentation process (Peng et al., 2021).

Determination of titratable acidity

The changes of the titratable acidity (TA) of apple juice fermented with strains of *Lactiplantibacillus plantarum* are shown in Table 3.

Changes of the titratable acidity of apple juice fermented with the strains *Lactiplantibacillus plantarum*

Time of fermentation, h	*L. plantarum* 52	*L. plantarum* 74	*L. plantarum* 76
	g /100mL		
48	2.07±0.10	2.54±0.11	2.40±0.23
72	1.08±0.05	1.14±0.19	1.07±0.07
192	1.25±0.06	0.97±0.09	1.06±0.06
288	1.37±0.15	1.01±0.16	1.14±0.14

The initial titratable acidity of control sample was 1.25±0.13 g/100mL. For the first 48 h, it increased, when compared to controls in all three samples. Our findings are consistent with those of Mashayekh with co-authors (2015) who found that the acidity of a mixture of pineapple, apple and mango juice significantly increased during fermentation with *Lactobacillus casei* 1608 due to production of organic acids. As seen in Tables 2 and 3, there is a correlation between the pH value and the titratable acidity, with the pH decreasing sharply from the start of fermentation to 48 h, while the titratable acidity increases significantly during this time of fermentation. In the case of milk fermentation by *L. plantarum* P9, the TA increased after 13.5 h of fermentation. It was accompanied by a rapidly dropping in the pH value and reaching the fermentation endpoint pH of 4.5 from 6.47. At this incubation period, the viable counts of *L. plantarum* P9 increased from 7.35 to 8.44 log CFU/mL (Zha et al., 2021).

Determination of sugars

Changes of glucose, fructose and sucrose concentrations in apple juice fermented with strains of *Lactiplantibacillus plantarum* are shown in Table 4.

Table 4

Changes of the glucose, fructose and sucrose concentrations in apple juice fermented with the strains *Lactiplantibacillus plantarum*

Apple juice fermented with	Concentration of sugars, g/L, at time of fermentation, h					
	Sucrose		Glucose		Fructose	
	24	48	24	48	24	48
Apple juice (control)	2.4±0.1	2.4±0.1	39.0±2.0	39±2.0	105±5.3	105±5.3
L. plantarum 52	2.3±0.1	1.9±0.1	32.5±1.6	26.8±1.3	93.5±4.7	84.3±4.2
L. plantarum 74	2.2±0.1	2.0±0.1	33.6±1.7	28.5±1.4	95.5±4.8	87.8±4.4
L. plantarum 76	2.2±0.2	2.0±0.1	33.9±1.7	28.5±1.3	100±5.0	88±4.4

The strain of *L. plantarum* 52 fermented 0.16 g/L of sucrose during 24 h and 0.41 g/L for 48 h. Fermentation of glucose and fructose was going more intensive during the first 24 h. 6.5 g/L of glucose and 11.5 g/L of fructose were fermented on the first day, while 5.7 g/L of glucose and 9.2 g/L of fructose were converted on the second day. Fermentation using the strain of *L. plantarum* 74 was similar. In the case of *L. plantarum* 76 use, fructose fermentation increased on the second day. According to the literary data, different bacterial strains ferment sugars in different ways. Some strains of lactic acid bacteria ferment glucose at a rate higher than the rate of fructose assimilation (Mousavi et al., 2013), while others do the opposite (Peng et al., 2021).

Determination of organic acids

Apple juice used in the study contained 6.5±0.3 g/L of malic and 1.2±0.1 g/L of lactic acids. During 48 h fermentation in samples with *L. plantarum* 52, 74, 76, bacteria consumed malic acid and produced lactic acid; the concentration of lactic acid reached 6.9±0.3, 7.0±0.3, 6.9±0.5 g/L, respectively, while concentration of malic acid reduced to 3.3±0.2, 3.5±0.2, 3.3±0.1 g/L (Figure 1).
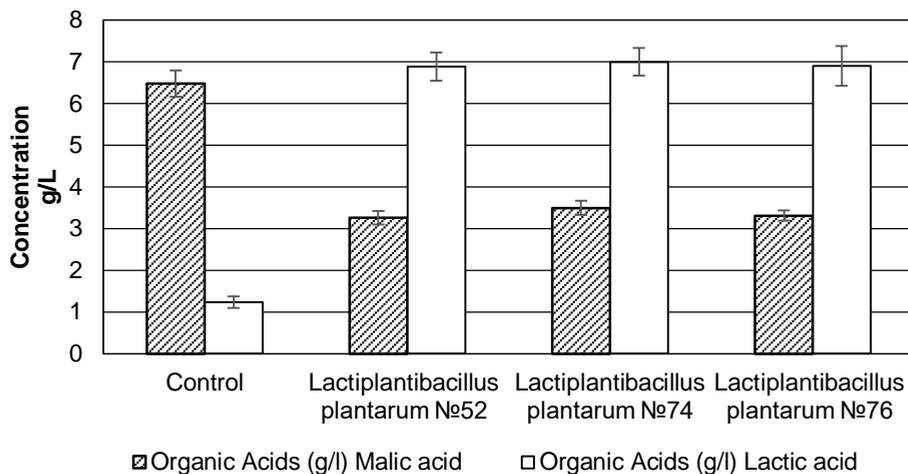
Figure 1. Changes in organic acid concentrations in apple juice fermented with the strains
Lactiplantibacillus plantarum

The mail product of sugar fermentation by LAB is lactic acid, and the breakdown of malic acid is going due to their activity (Chen et al., 2019; Fonseca et al., 2021; Mousavi et al., 2013; Ricci et al., 2019). For example, concentration of lactic acid in the soymilk increased after 56 h of fermentation from 0.5 g/L to 2.04 g/L (Shu et al., 2022).

Determination of total phenolic content

The concentration of polyphenols in apple juice varies depending on the apple cultivars (Wlodarska et al., 2017). Phenolic compounds enrich the fermented juice with flavor, inhibit microbial spoilage, and regulate fermentation rate (Ye et al., 2014). Phenolic compounds frequently occur as complex molecules associated with sugars or proteins. LAB can break down polyphenols into simpler components through decarboxylation, reduction, de-esterification, and deglycosylation reactions (Lee and Paik, 2017). The total amount of phenols in the fermented product may raise or decrease because of fermentation (Crespo et al., 2021).

After fermentation with *L. plantarum* 74, the total phenol concentration in the fermented apple juice increased from 405.0±20.3 to 414.8±20.8 mg GAE/L in 24 h and 443.4±22.2 mg GAE/L in 48 h. From 24 to 72 hours of fermentation, all samples showed a tendency to increase total phenols content, although various tendencies were seen from the third day of fermentation (Table 5).

Lactic acid fermentation of chokeberry juice with *L. paracasei* SP5 resulted in an increase in total phenolics content and antioxidant activity in fermented chokeberry juice compared with non-fermented juice after storage at 4 °C for 4 weeks (Bontsidis et al., 2021). Hashemi with co-authors (2017) also found significant changes in the common phenolic content during the fermentation of sweet lemon juice with *L. plantarum*.

Table 5

Changes of the total phenol contents in apple juice fermented with the strains *Lactiplantibacillus plantarum*

Apple juice fermented with	Concentration of the total phenol contents, mg GAE/L, at time of fermentation, h					
	0	24	48	72	192	288
L. plantarum 52	406.0± 20.3[ef]	397.2± 19.9[ef]	479.6± 24.0[bcd]	532.9± 26.7[ab]	404.3± 20.2[ef]	400.5± 20.0[ef]
L. plantarum 74	406.0± 20.3[ef]	414.8± 20.7[def]	443.4± 22.2[cdef]	587.3± 29.4[a]	391.2± 19.6[ef]	403.8± 20.12[ef]
L. plantarum 76	406.0± 20.3[ef]	387.9± 19.4[f]	457.6± 22.9[cde]	488.4± 24.4[bc]	393.4± 19.7[ef]	414.8± 20.7[def]

Note: means ±standard deviation (SD) in the table with different alphabet letters indicate the significant difference at $p < 0.05$.

Determination of antioxidant activity

The changes in the total antioxidant activities in apple juices fermented with the three selected *Lactiplantibacillus plantarum* strains are shown (Table 6).

Table 6

Changes of the total antioxidant activities of the apple juice fermented with the strains *Lactiplantibacillus plantarum*

Apple juice fermented with	Concentration of the total phenol contents, mg AAE/L, at time of fermentation, h					
	0	24	48	72	192	288
L. plantarum 52	173.1± 8.7[cd]	117.2± 5.9[f]	274.4± 13.7[ab]	281.6± 14.1[ab]	183.5± 9.2[c]	155.0± 7.8[cde]
L. plantarum 74	173.1± 8.7[cd]	269.6± 13.5[b]	282.8± 14.1[ab]	300.6± 15.0[a]	132.2± 6.6[ef]	177.3± 8.9[c]
L. plantarum 76	173.1± 8.7[cd]	140.3± 7.0[ef]	144.8± 7.2[def]	172.8± 8.6[cd]	153.8± 7.7[cde]	138.2± 6.9[ef]

Note: means ±standard deviation (SD) in the table with different alphabet letters indicate the significant difference at $p < 0.05$.

Maximum antioxidant activities were found in apple juice fermented by all strains of *L. plantarum* after 72 h of fermentation and were 274.4, 282.6 and 172.8 mg AAE/L for *L. plantarum* 52, 74 and 76, respectively. The influence of the unique properties of various strains as well as composition of fermented juice on the increase of the antioxidant activity is shown (Multari et al., 2020; Nguyen, 2019). The antioxidant activity of orange juice fermented with different strains of *Lactobacillus brevis* POM and *Lactobacillus plantarum* varied significantly (de la Fuente et al., 2021). According to Shakya and co-authors (2021), the rise in total phenol content in the plant extracts fermented with *Lactobacillus brevis* 174A may be associated with an increase in antioxidant activity. The increase in the total phenol

content was observed in the present study as well: for example, in the case of *L. plantarum* 74 application, the total phenolic content increased from 406. 0±20.3 mg GAE/L to 587.3±29.4 mg GAE/L, while the antioxidant activity increased from 173.1±8.7 mg AAE/L to 300.6±15.0 mg AAE/L after 72 h of fermentation (Tables 5 and 6).

Conclusions

The apple juice was used to be fermented by autochthonous *Lactiplantibacillus plantarum* strains. Fermented apple juice possessed higher content of the phenolic compounds and increased antioxidant activity. The number of alive lactic bacteria cells increased to the end of fermentation, so apple juice is a good substrate for maintaining the viability of probiotic lactic acid bacteria. Apple juice fermented by lactic acid bacteria could be considered as a probiotic product consumption of which   will have a beneficial effect on human health, especially for people with lactose intolerance.

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Quality assessment of sponge cake with reduced sucrose addition made from composite wheat and barley malt flour

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Abstract

Introduction. The aim of this study was to investigate the effects of replacing part of the wheat flour (WF) with brewer's barley malt flour (BMF), while reducing the sucrose in the recipe, on the quality characteristics of sponge cakes.

Materials and methods. For the production of sponge cake samples, WF and three different types of brewer's BMF (*Pilsen*, *Amber* and *Black*) were used in different ratios with simultaneous reduction of sucrose addition. The content of reducing sugars in WF and BMF was determined, as well as the moisture content and water activity in sponge cake samples. Determination of specific volume, colour in CIE$L*a*b*$ space, texture profile analysis (TPA) and sensory analysis using the nine-point hedonic scale were also performed.

Results and discussion. The contents of reducing sugars were 0.43, 7.75, 17.05 and 61.02 g/100 g in WF, *Amber*, *Pilsen* and *Black* BMF, respectively. Since sucrose is known to be an excellent ingredient for lowering water activity, both moisture content and water activity in the sponge cake samples increased significantly when the addition of sucrose was reduced. The specific volume decreased from 1.99 cm^3/g in the control WF sample to 1.79 cm^3/g in the WF sample with reduction of sucrose content by 50.0%. Reducing the sucrose addition significantly increased the hardness and chewiness, while the resilience and cohesiveness of the cake decreased ($p < 0.05$). Addition of 20% BMF and reduction of sucrose to 83.3% of the original recipe mitigated these effects and there were no statistically significant differences between these samples and the control WF sample in terms of specific volume and texture parameters. The addition of BMF significantly affected all colour parameters of the sponge cake crumb ($p < 0.05$). *Amber* BMF:WF (20:80) sponge cake with reduced sucrose addition (83.3%) had the highest sensory scores for colour, appearance and overall acceptability. *Pilsen* BMF:WF (20:80) with reduced added sucrose (83.3%) had the best odour and the best taste was the WF control sample.

Conclusion. By replacing WF with BMF in the production of sponge cakes, a very wide range of sponge cake products with different quality characteristics, improved nutritional and functional properties can be obtained. BMF has significant amounts of its own sugars, which can minimize the effect of the reduction of sucrose content in the sponge cake recipe.

Introduction

Sponge cakes are considered to be food with low nutritional value because they usually contain high amounts of refined wheat flour (WF) and sucrose. Consumption of so-called sweetened grain products, which include sponge cakes, significantly increases the intake of sugar and decreases the intake of fibre (Frary et al., 2004). This has a negative impact on the quality of the human diet and increases the risk of various diseases such as diabetes, dental decay and obesity, and thus also hypertension and cardiovascular diseases. Therefore, all attempts to increase the nutritional value of these types of products are welcome. One way to achieve this goal is to use non-wheat flour in a sponge cake recipe. For fermented products, such as bread, the use of non-wheat flour is limited as a significant amount of gluten is desirable to produce quality products (Ho et al., 2018). However, this is not the case for products such as sponge cakes. Sponge cakes are foam-like products and their structure depends mainly on the incorporation of air bubbles into the foam during the mixing phase and on the functionality of sucrose and eggs in the recipe (Godefroidt et al., 2019). Since gluten is not so important for making sponge cake, many recipes have been developed with flours from other grains and even legumes (Sobhy et al., 2015). Barley flour has long been used as a substitute for WF in the manufacture of various cereal-based products, including different types of cakes (Gupta et al., 2009; Khalek, 2020; Sangeeta and Chopra, 2013).

Barley grain is considered more nutritious than wheat due to a higher content of β-glucan, insoluble fibre, vitamins, minerals and phenolic substances (Farag et al., 2022). However, as far as we know, no attempt has yet been made to use barley malt flour (BMF) for making sponge cake. Barley malt is normally used in the production of beer and other barley malt-based beverages and in small quantities in the production of bakery products to optimise amylolytic activity (diastatic malt) and in confectionery to improve colour and flavour (non-diastatic malt) (Pyler and Gorton, 2008). The malting process consists of four steps: steeping, germination, kilning and/or roasting, and cleaning the malted grains from rootlets and impurities. During the malting process, barley undergoes numerous changes in its composition and its functional and nutritional properties. During germination, there is an intensive synthesis of hydrolysing enzymes (β-glucanase, amylases and proteases) and a moderate change in the main components of the barley grain (starch, proteins, β-glucan) (Celus et al., 2006; Gupta et al., 2010; Šimić et al., 2015). Elevated temperatures during kilning and/or roasting step abort these modifications and contribute to the development of the colour and flavour of the malt (Hertrich, 2013). Malting is considered a process that improves the nutritional value of barley by increasing the digestibility of protein and the bioavailability of vitamins B and C and minerals (copper, calcium, zinc and manganese) (Baranwal, 2017), increasing antioxidant activity through the release of bound phenolic compounds and the generation of Maillard reaction products (Carvalho et al., 2016).

Although the use of non-wheat flour is widespread and does not cause major problems in the production of sponge cakes, the situation is quite different when sucrose is reduced because sucrose is not only a sweetener but an ingredient that significantly affects the technological quality of sponge cakes (Godefroidt et al., 2019). Sucrose plays a multiple role in creating the structure of the sponge cake. It facilitates the incorporation of air and improves the stability of the foam (Goranova et al., 2020), delays the development of gluten and the gelatinisation of starch, so that the cake can expand better before it sets and the texture becomes softer (Godefroidt et al., 2019; Paton et al., 1981).

The aim of this study was to investigate the effects of replacing part of the WF with three different types of brewer's BMF with a simultaneous reduction of sucrose in the recipe on the physical and sensory properties of sponge cakes.

Materials and methods

Materials

Commercial plain WF (Tena-Žito Ltd., Đakovo, Croatia) and three different types of brewer's BMF were used for this study: *Pilsen* (enzymatically active), *Amber* (low-enzymatically active) and *Black* malt (non-enzymatically active). (Slavonija slad d.o.o., Nova Gradiška, Croatia; Boortmalt, Antwerp, Belgium). Protein content was 10.6, 11.2, 11.1 and 10.8% in WF, *Pilsen*, *Amber*, and *Black* BMF respectively. Shortening (Zvijezda d.d., Zagreb, Croatia), sucrose, eggs, milk, and sodium bicarbonate ($NaHCO_3$) were purchased from a local market.

Reducing sugar content in flour

The content of reducing sugars in WF and BMF was determined using AACC International Method 80-68.01 (Schoorl method) (AACC, 2010). Since maltose is the dominant reducing sugar in malt, the results of reducing sugar content were expressed on a maltose basis. The measurements were carried out in triplicate for each sample.

Sponge cake production

The sponge cakes were prepared according to the procedure of Velioğlu et al. (2017) with slight modifications. The quantities of raw materials (100 g flour base) are given in Table 1. First, the total amount of eggs and sugar was added to the bowl of an electronic mixer (Gorenje MMC800W, Slovenia) and the mixture was stirred with a wire attachment for 4 minutes at maximum speed until a voluminous foam was formed. The other raw materials were then added and mixing continued at a lower speed for a further 4 minutes. The accurately weighed sponge cake mixture (175 g) was distributed into moulds, which were placed in the oven (Wiesheu Minimat Zibo, Wiesheu GmbH, Germany). Baking was carried out at 180 °C for 20 minutes in triplicate batches.

Moisture content and water activity (a_w)

Moisture content was determined according to AACC International Method 44-15.02 (AACC, 2010) and water activity with the Hygropalm AW1 indicator (Rotronic, USA).

Physical analysis

The specific volume (cm^3/g) of the sponge cakes was measured using the VolScan Profiler (Stable Micro Systems, UK).

Texture profile analysis (TPA) was performed using the TA.XT2i Texture Analyzer (Stable Microsystems Ltd., Surrey, UK). The sponge cake samples were cut into cubes (30x30x30 mm) and subjected to double compression at 40% with 5 s delay between compressions and a test speed of 1 mm/s. An aluminium plate with a diameter of 75 mm was used. Hardness (N), cohesiveness, resilience, and chewiness (N) were determined from the TPA curves.

The colour of the cross-section of sponge cakes was measured using the CR -400 chromameter (Konica Minolta, Japan) and expressed in a CIE*L*a*b** colour model. The *L** value ranges from 0 (black) to 100 (white) and represents the lightness or luminance of the sample. The *a** and *b** values range from -128 to 127 and represent the green-red (*a**) and blue-yellow (*b**) axes of the colour space. The total colour difference (ΔE) between the control and sample sponge cakes was calculated according to the CIE76 colour difference equation (Mokrzycki and Tatol, 2011).

Formulation of sponge cakes made from composite flours containing wheat flour (WF) and barley malt flour (BMF)

	WF (g)	BMF (g)	Sucrose [1] (g)	Shortening (g)	Sunflower oil (mL)	Milk (mL)	Egg (g)	NaHCO$_3$ (g)
WF (Control)	100	-	79.5 (100%)	28.4	22.7	45.5	40.0	2.4
WF	100	-	66.2 (83.3%)	28.4	22.7	45.5	40.0	2.4
	100	-	53.0 (66.6%)	28.4	22.7	45.5	40.0	2.4
	100	-	39.8 (50.0%)	28.4	22.7	45.5	40.0	2.4
PILSEN:WF	80	20	66.2 (83.3%)	28.4	22.7	45.5	40.0	2.4
	60	40	53.0 (66.6%)	28.4	22.7	45.5	40.0	2.4
	40	60	39.8 (50.0%)	28.4	22.7	45.5	40.0	2.4
AMBER:WF	80	20	66.2 (83.3%)	28.4	22.7	45.5	40.0	2.4
	60	40	53.0 (66.6%)	28.4	22.7	45.5	40.0	2.4
	40	60	39.8 (50.0%)	28.4	22.7	45.5	40.0	2.4
BLACK:WF	80	20	66.2 (83.3%)	28.4	22.7	45.5	40.0	2.4
	60	40	53.0 (66.6%)	28.4	22.7	45.5	40.0	2.4
	40	60	39.8 (50.0%)	28.4	22.7	45.5	40.0	2.4

[1] Percentage of added sucrose compared to standard recipe.

Sensory analysis

The sensory assessment of the sponge cakes was conducted by a panel of eleven semi-trained evaluators with previous experience in sensory analysis. The nine-point hedonic scale was used to asses individual sensory characteristics: colour, appearance, odour, taste, and overall acceptance. The scores were: like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (3), dislike very much (2), and dislike extremely (1).

Statistical analysis

Analysis of variance (ANOVA) and multiple comparison post-hoc Fisher Least Significant Difference (LSD) test were performed ($p < 0.05$) using XLSTAT software (Addinsoft, New York, USA).

Results and discussion

In this study, WF was partially replaced by brewer's BMF (*Pilsen*, *Amber*, and *Black* BMF). The ratios of the prepared BMF:WF composite flours were 20:80, 40:60 and 60:40, respectively. The addition of sucrose was reduced to 83.3, 66.6 and 50.0% (66.2, 53.0 and 39.8 g/100 g flour base), compared to the original sponge cake recipe in the ratios 20:80, 40:60 and 60:40 BMF:WF, respectively. The sponge cake made from 100% WF and 100% (75 g/100 g flour base) added sucrose served as the control sample. In order to evaluate only the influence of sucrose on the quality characteristics of the sponge cake, WF sponge cakes with reduced sucrose addition were also prepared.

Reducing sugar content

The reducing sugars content in WF and BMF was 0.43, 7.75, 17.05 and 61.02 g/100 g in WF, *Amber*, *Pilsen* and *Black* BMF, respectively (Table 2). The content of reducing sugars in BMF samples is significantly higher ($p < 0.05$) than in WF. This is due to the enzymatic hydrolysis of starch and thermal dextrinization in the kilning/roasting stage of malt production, where a certain proportion of starch is broken down into various dextrins and short-chain sugars, many of which have a reducing potential.

Table 2

Reducing sugars content in wheat flour (WF) and barley malt flours (BMF)

Flour	Reducing sugar content (g/100 g)[1]
WF	0.43±0.09[d]
Pilsen BMF	7.75±0.21[c]
Amber BMF	17.05±0.19[b]
Black BMF	61.02±0.32[a]

[1] The values are Mean±SD ($n = 3$). Different letters (a–d) indicate statistically significant differences ($p < 0.05$)

Maltose is the dominant sugar in malt, followed by maltotriose and glucose. Moreover, the dextrin content is proportional to the applied temperature in the final malting step, which is evident from the results obtained, where the reducing sugar content was highest in *Black* BMF and lowest in *Pilsen* BMF (Duke and Henson, 2008; Koljonen et al., 1995). This was to be expected as the kilning temperature for *Pilsen* malt is in the range of 50–85 °C, roasting of *Amber* malt is done at 100–150 °C and for *Black* malt at 230 °C. The higher the temperature and the longer the roasting, the more dark dextrins are formed. Thus, the roasting of *Black* malt produces a very dark, almost black colour, which comes from the pyrodextrins (Srivastava et al., 1970). Since starch hydrolysis during malting produces maltodextrins together with various simple sugars, the exact molecular composition is not known. Therefore, the results obtained should be understood as the total reduction potential and not the exact content of reducing sugars.

Moisture content and water activity (a_w) of BMF:WF sponge cakes

Sucrose has many functions in the production of sponge cakes. One of the most important functions of sucrose is its moistening (hygroscopic) effect and its influence on water activity. It is very important to keep water activity as low as possible to prevent spoilage and staling of products. Therefore, the reduction of sucrose in the sponge cake recipe is a very difficult task.

Moisture content and water activity (a_w) of sponge cakes

BMF:WF	Sucrose (%) [1]	Moisture content (%)	Water activity
WF (Control)			
0:100	100	21.4±0.1[j 2]	0.851±0.016[h]
WF			
0:100	83.3	23.7±0.2[de]	0.924±0.005[bc]
0:100	66.6	24.8±0.0[b]	0.945±0.003[a]
0:100	50.0	25.9±0.3 [a]	0.946±0.015[a]
PILSEN:WF			
20:80	83.3	21.6±0.2[ij]	0.875±0.012[g]
40:60	66.6	22.5±0.0[gh]	0.901±0.003[de]
60:40	50.0	23.9±0.1[cd]	0.924±0.009[bc]
AMBER:WF			
20:80	83.3	22.0±0.3[hi]	0.881±0.001[fg]
40:60	66.6	23.0±0.1[fg]	0.900±0.006[def]
60:40	50.0	24.2±0.1[cd]	0.914±0.006[cde]
BLACK:WF			
20:80	83.3	22.2±0.1[hi]	0.850±0.008[h]
40:60	66.6	23.3±0.4[ef]	0.883±0.009[fg]
60:40	50.0	24.4±0.1[bc]	0.916±0.002[cde]

[1] Percentage of added sucrose compared to original recipe.
[2] The values are Mean±SD ($n = 3$). Different letters (a–j) indicate statistically significant differences ($p < 0.05$)

The results of moisture content and water activity are summarised in Table 3. The moisture content and water activity in the control WF sponge cake sample were 21.4% and 0.851 respectively. When the addition of sucrose was reduced, both the moisture content and water activity increased significantly. The highest moisture content and water activity were obtained in sponge cake with 50% added sucrose. In this sponge cake sample, the moisture content was 25.9 and the water activity was 0.946. This was to be expected as sucrose is known to be an excellent ingredient for lowering water activity in various products. Similar results were obtained in the study by Milner et al. (2020), when cakes with sucrose had a significantly lower moisture content and water activity than samples in which sucrose was partially replaced by whey permeate, apple pomace, polydextrose and oligofructose.

When BMF was used as a partial substitute for WF in the ratio 20:80 and sucrose was reduced to 83.3% of the original recipe, moisture content increased slightly but water activity remained well below 0.900. Water activity was 0.875, 0.881 and 0.850 for *Pilsen*, *Amber* and *Black* BMF:WF (20:80), respectively. This was much lower than the WF sample with the same degree of sucrose reduction (83.3%), where a water activity of 0.924 was measured. This can be explained by the fact that BMF has significant amounts of its own sugar, which can weaken the effect of the reduced sucrose addition in the sponge cake recipe. A similar attenuating effect of BMF was observed in the production of biscuits with reduced sucrose content (Jukić et al., 2022).

Further reducing the amount of sucrose added (66.6% of the original recipe) and increasing the amount of BMF (40%) increased the water activity, but it was still within an

acceptable range (bellow 0.883-0.901) and significantly lower than samples with the same amount of sucrose. In samples with a BMF:WF ratio of 60:40, the water activity remained similar or even lower than in WF samples with a sucrose addition of 83.3% of the original formulation, even when the sucrose addition was reduced to 50%.

Physical properties of BMF:WF sponge cakes

The formation of the cake structure during baking is controlled by the occurrence of three phase transitions: water evaporation, gelatinisation of starch and thermosetting of egg white and gluten proteins.

Table 4

Specific volume and texture properties of sponge cakes

BMF:WF	Sucrose (%) [1]	Specific volume (cm³/g)	Hardness (N)	Cohesiveness	Resilience	Chewiness (N)
WF (Control)						
0:100	100	1.99±0.03[b 2]	23.3±3.2[de]	0.68±0.02[a]	0.88±0.03[ab]	15.5±1.3[gh]
WF						
0:100	83.3	1.94±0.01[bc]	27.6±0.3[cd]	0.68±0.00[a]	0.89±0.01[a]	18.6±0.0[fg]
0:100	66.6	1.78±0.03[d]	42.6±3.4[b]	0.63±0.00[bcd]	0.86±0.00[abc]	26.5±2.1[bc]
0:100	50.0	1.79±0.05[d]	50.1±0.9[b]	0.57±0.00[ef]	0.85±0.01[bcd]	28.3±1.0[b]
PILSEN:WF						
20:80	83.3	2.11±0.02[a]	20.2±0.3[de]	0.65±0.00[ab]	0.89±0.02[a]	13.3±0.4[h]
40:60	66.6	1.89±0.04[c]	32.5±2.1[c]	0.63±0.01[bc]	0.85±0.00[bcd]	19.8±1.0[def]
60:40	50.0	1.77±0.03[de]	44.0±3.4[b]	0.55±0.00[f]	0.80±0.00[ef]	22.7±1.7[cde]
AMBER:WF						
20:80	83.3	1.91±0.03[c]	27.7±2.5[cd]	0.64±0.00[bc]	0.88±0.01[ab]	17.6±1.3[fg]
40:60	66.6	1.71±0.02[f]	42.3±2.3[b]	0.61±0.03[cd]	0.85±0.01[bcd]	25.1±0.3[bc]
60:40	50.0	1.47±0.02[g]	77.3±1.8[a]	0.57±0.00[ef]	0.81±0.01[def]	42.6±1.1[a]
BLACK:WF						
20:80	83.3	2.05±0.01[a]	18.6±4.6[e]	0.66±0.02[ab]	0.86±0.01[abc]	11.7±2.4[h]
40:60	66.6	1.81±0.05[d]	32.2±2.0[c]	0.59±0.01[de]	0.83±0.01[cde]	18.9±1.5[efg]
60:40	50.0	1.72±0.01[ef]	46.4±2.9[b]	0.54±0.00[f]	0.78±0.03[f]	23.0±0.4[cd]

[1] Percentage of added sucrose compared to original recipe.
[2] The values are Mean±SD ($n = 3$). Different letters (a–g) indicate statistically significant differences ($p < 0.05$)

The addition of sucrose affects all three factors by regulating water activity, raising the gelatinisation temperature of starch and increasing the denaturation temperature of proteins. In this way, the thermal stability of the proteins is increased and they form a network with the incorporated swollen and gelatinised starch granules (Godefroidt et al., 2019; van der Sman and Renzetti, 2021).

One of the most important indicators of the quality of sponge cakes is the formation of a porous structure, which forms when air is incorporated during batter mixing and the volume increases during baking. Evaluation of the specific volume of a sponge cake serves as an excellent tool to assess the porosity of a product (Psimouli and Oreopoulou, 2012). The results showed that the addition of sucrose plays a decisive role in the porosity of the sponge cakes (Table 4). A higher specific volume indicates greater porosity. The specific volume decreased from 1.99 cm^3/g in the control WF sample to 1.79 cm^3/g in the WF sample with 50.0% reduced sucrose addition. This is consistent with the study by Sangeeta and Chopra (2013), where a 10% reduction in sugar content reduced cake volume by almost 8%. Specific volume is directly related to the texture of sponge cakes. A high specific volume means a low cake density and consequently a softer texture of the product. Therefore, by reducing the addition of sucrose, the hardness and chewiness increased significantly, while the resilience (elasticity) and cohesiveness of the cake decreased. The use of BMF in a 20:80 ratio and the reduction of sucrose to 83.3% of the original recipe mitigated this deterioration and there were no statistically significant differences between these samples and the control WF sample in terms of specific volume and texture parameters. Further reduction of sucrose and addition of higher amounts of BMF significantly deteriorated the specific volume and textural properties of sponge cakes. This is particularly evident in sponge cakes made with *Pilsen* and *Black* BMF. The probable reason for this negative effect is that although these BMFs contain significant amounts of reducing sugars, the starch content is considerably reduced due to intensive starch hydrolysis during malting of barley, and starch is one of the key factors in the formation of sponge cake structure. This lower starch content should be taken into account in future studies when BMF is used as a substitute for WF. A similar effect on the textural properties of sponge cake was found by Gupta et al. (2009) in their study on the effects of partial replacement of WF with barley flour. Their results showed that adding barley flour up to 40% increased the hardness and chewiness and reduced the volume, cohesiveness and elasticity of the cake.

The interdependence between sucrose addition, water activity, specific volume and texture parameters of sponge cake was demonstrated and described by correlation analysis (Table 5).

Table 5

Correlation matrix of data for specific volume and texture properties of sponge cakes

Variables	Water activity	Specific volume (cm^3/g)	Hardness (N)	Cohesiveness	Resilience	Chewiness (N)
Sucrose (%)	-0.729**	0.781**	-0.808**	0.934**	0.834**	-0.720**
Water activity	-	-0.573*	0.629*	-0.571*	-0.361	0.633*
Spec. volume (cm^3/g)	-	-	-0.950**	0.725**	0.696**	-0.928**
Hardness (N)	-	-	-	-0.722**	-0.636*	0.982**
Cohesiveness	-	-	-	-	0.890**	-0.599*
Resilience	-	-	-	-	-	-0.491

*$p < 0.05$; **$p < 0.01$

The results showed that there were significant correlations ($p < 0.01$) between sucrose addition and water activity ($r = -0.729$), specific volume ($r = 0.781$), hardness ($r = -0.808$), cohesiveness ($r = 0.934$), resilience ($r = 0.834$) and chewiness ($r = -0.720$). The highest correlation ($r = -0.950$) was between the specific volume and the hardness of sponge cake.

In this study, the colour of the sponge cake crumb was measured using the CIE*L*a*b** colour, as this model approximates human vision. The *L** value stands for the lightness or luminance of the sample, and the *a** and *b** values stand for the "green-red" and "blue-yellow" axes of the colour space.

Table 6

Colour of sponge cakes

BMF:WF	Sucrose (%) [1]	*L**	*a**	*b**	ΔE
WF (Control)					
0:100	100	76.4±0.8[a]	-2.8±0.1[i]	23.6±0.5[de]	-
WF					
0:100	83.3	76.4±0.8[a]	-2.7±0.1[hi]	23.1±0.3[ef]	0.5
0:100	66.6	76.5±2.2[a]	-2.6±0.2[hi]	23.0±0.8[f]	0.7
0:100	50.0	76.4±2.5[a]	-2.5±0.1[h]	22.8±0.7[fg]	0.9
PILSEN:WF					
20:80	83.3	69.5±1.3[b]	0.2±0.2[g]	22.4±0.4[gh]	7.6
40:60	66.6	65.2±1.9[c]	1.5±0.2[f]	23.9±0.4[d]	12.0
60:40	50.0	59.2±1.3[d]	2.7±0.1[e]	24.9±0.7[c]	18.1
AMBER:WF					
20:80	83.3	56.0±0.9[e]	6.7±0.3[c]	30.4±0.6[b]	23.5
40:60	66.6	47.6±0.7[f]	9.9±0.4[b]	31.4±0.5[a]	32.4
60:40	50.0	38.2±2.1[g]	11.2±0.4[a]	31.9±1.3[a]	41.5
BLACK:WF					
20:80	83.3	24.3±0.7[h]	6.6±0.2[c]	14.3±0.4[i]	53.8
40:60	66.6	15.0±0.8[h]	3.5±0.4[d]	6.6±0.7[j]	64.0
60:40	50.0	14.1±1.0[h]	1.7±0.2[f]	3.8±0.3[k]	65.6

[1] Percentage of added sucrose compared to original recipe.
[2] The values are Mean±SD ($n = 3$). Different letters (a–j) indicate statistically significant differences ($p < 0.05$)

Unlike biscuits, where the colour is largely determined by the amount of sucrose added in the recipe, the colour of sponge cakes is more strongly influenced by added eggs and other coloured ingredients. From the results of the colour determination shown in Table 6, it can be seen that the addition of sucrose had no effect on the colour of the sponge cake crumb. There were no statistically significant differences between the control WF sample and the WF samples with reduced sucrose addition in the *L**, *a** and *b** values and the (ΔE) total colour difference was very small (0.5–0.9).
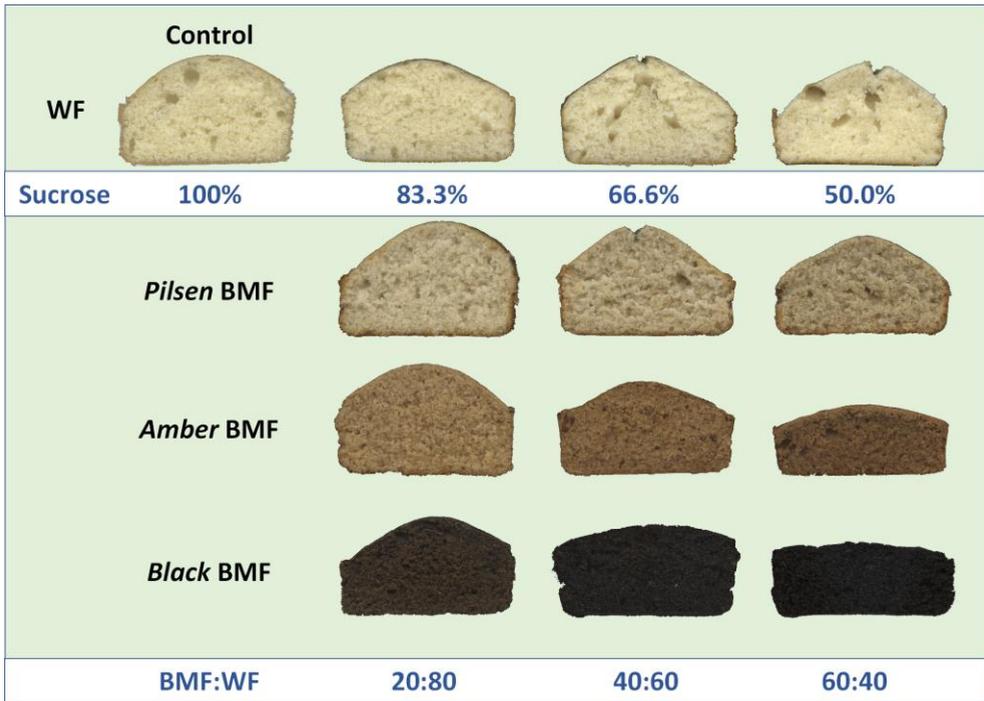
Figure 1. Sponge cakes made from composite flours containing wheat flour (WF) and barley malt flour (BMF)

The addition of BMF significantly influenced all colour parameters of the sponge cake crumb ($p < 0.05$). The colour changes depended on the type of BMF used and its amount in the recipe. The colour of BMF comes from the process of barley malting and the intensity of the colour is directly proportional to the temperature during kilning and/or roasting (Hertrich, 2013). The lightest BMF was the *Pilsen* BMF, slightly darker with an intense amber hue was the *Amber* BMF, while the *Black* BMF was the darkest, almost black. Consequently, the sponge cakes with *Pilsen* BMF were the lightest among the three composite sponge cakes, and the cakes with *Black* BMF were the darkest. The addition of the *Amber* BMF caused the highest increase in $a*$ and $b*$ values, resulting in a reddish amber colour of the sponge cakes. The lowest $L*a*b*$ values were observed in the *Black* BMF:WF cakes, as these samples had a very dark colour even at a BMF:WF ratio of 20:80. These samples also had the largest colour difference (ΔE) compared to the WF control sample. The total colour difference (ΔE) between the control WF sample and the *Pilsen* BMF:WF cakes was the smallest, but even with a BMF:WF ratio of 20:80, this difference was > 5, which is the smallest difference that can be easily perceived by the consumer (Mokrzycki and Tatol, 2011). Since many types of BMFs can be found on the market, it can be concluded that by using them in the production of sponge cakes, it is very easy to influence the colour of the product and achieve a more attractive appearance of the cakes (Figure 1).

Sensory evaluation

The results of the sensory evaluation, which was carried out using the nine-point hedonic scale, are shown in Table 7. The sensory evaluation showed that by reducing the sugar content in the sponge cake samples, all sensory ratings decreased, confirming the importance of adding sucrose in the production of sponge cake and its multifunctionality. *Black* BMF:WF sponge cakes had the lowest sensory scores, followed by WF cakes with reduced added sucrose.

Table 7

Sensory evaluation of sponge cakes

BMF:WF	Sucrose (%) [1]	Colour	Appearance	Odour	Taste	Overall acceptance
WF (Control)						
0:100	100	7.4±1.4[ab]	7.6±1.2[a]	7.1±1.8[ab]	7.6±1.8[a]	7.4±1.2[ab]
WF						
0:100	83.3	6.8±1.3[abc]	6.6±0.5[abc]	7.1±1.2[ab]	6.9±1.6[ab]	6.9±1.1[ab]
0:100	66.6	6.7±1.4[abc]	6.1±1.1[abcd]	6.6±1.6[ab]	6.1±1.6[abc]	6.4±1.7[ab]
0:100	50.0	6.0±1.9[abcd]	5.1±1.4[cd]	6.2±2.1[ab]	5.9±2.5[abc]	5.8±1.7[abcd]
PILSEN:WF						
20:80	83.3	7.5±1.1[ab]	7.1±1.4[ab]	7.6±1.0[a]	7.0±1.9[ab]	7.3±1.1[ab]
40:60	66.6	7.3±1.7[ab]	7.2±1.4[ab]	7.3±1.2[a]	6.4±1.9[abc]	7.1±1.3[ab]
60:40	50.0	7.0±1.9[abc]	6.0±1.7[abcd]	6.7±1.7[ab]	6.2±2.1[abc]	6.5±1.8[abc]
AMBER:WF						
20:80	83.3	7.9±0.8[a]	7.8±1.1[a]	7.3±0.9[a]	7.0±1.3[ab]	7.5±1.0[a]
40:60	66.6	7.4±1.1[ab]	6.6±1.0[abc]	6.9±1.1[ab]	6.3±1.6[abc]	6.8±1.2[ab]
60:40	50.0	6.9±1.1[abc]	6.3±1.3[abc]	6.5±1.4[ab]	6.0±2.5[abc]	6.4±2.1[abc]
BLACK:WF						
20:80	83.3	4.5±3.0[d]	5.9±2.1[bcd]	4.2±3.1[cd]	4.5±2.8[cd]	4.8±2.7[cd]
40:60	66.6	3.2±1.9[e]	3.4±2.0[ef]	3.0±3.1[cd]	3.0±2.8[de]	3.2±2.3[de]
60:40	50.0	2.3±1.5[e]	2.5±2.5[f]	2.5±3.3[d]	2.3±1.8[e]	2.4±1.8[e]

[1] Percentage of added sucrose compared to original recipe.
[2] The values are Mean±SD (*n* = 3). Different letters (a–e) indicate statistically significant differences ($p < 0.05$)

Amber BMF:WF (20:80) sponge cake with reduced sucrose addition of 83.3% had the highest sensory scores for colour, 7.9, and appearance, 7.8, resulting in the highest overall acceptability score of 7.5, placing this sample between "like moderately" and "like very much" on the nine-point hedonic scale. *Pilsen* BMF:WF (20:80) sponge cake with reduced sucrose addition, 83.3%, had the best odour, 7.6, and WF control sample had the best taste, 7.6. The panellists emphasised the pleasant aroma and rich flavour of *Pilsen* and *Amber* BMF:WF composite sponge cakes, and the attractive colour and caramel-like taste of *Amber*

BMF:WF cakes. This is consistent with the research of Gupta et al. (2009) where the sponge cake samples with 20% barley flour achieved the best sensory results. Further increasing the addition of *Pilsen* and *Amber* BMF and reducing the sucrose content lowered the liking scores of the sponge cakes, but there were no significant differences (p <0.05) compared to the control sample, even with a BMF:WF ratio of 60:40 and a 50% reduction in sucrose addition.

The low sensory rating of the *Black* BMF:WF composite sponge cakes was to be expected as the *Black* BMF had a distinct "roasted" flavour, but for comparison it was used in this study in the same quantities as *Pilsen* and *Amber* BMFs. The samples with *Black* BMF added resulted in the highest level of disagreement among the panellists (highest standard deviation). Most rated these samples as inferior and considered them undesirable, with a bitter taste and too dark a colour. Nevertheless, some panellists found these samples interesting precisely because of their special taste and chocolate-like appearance. It can be concluded that Black BMF should be used in much smaller quantities (e.g. < 10%) as an effective colouring agent. Apart from the detrimental effect on flavour and taste, the use of this type of BMF in large quantities should also be considered from a safety point of view, as highly roasted BMF can contain significant amounts of acrylamide and therefore the amount of its addition should be kept to a minimum in order to comply with health regulations.

Conclusion

1. By replacing WF with BMF in the production of sponge cakes, a very wide range of sponge cake products with different quality characteristics and improved nutritional and functional properties can be obtained, as many types of brewing malts can be found on the market.
2. BMF has significant amounts of its own sugars, which can minimize the effect of the reduced sucrose addition in the sponge cake recipe.
3. By substituting WF with up to 40% *Pilsner* or *Amber* BMF while reducing the addition of sucrose to 66.6% of the original recipe, the sponge cakes retained similar qualitative characteristics to the control WF sponge cake samples.
4. The *Black* BMF:WF composite sponge cakes had a pronounced "roasted" flavour and taste, and poorer technological properties compared to control WF samples. Therefore, it can be concluded that *Black* BMF should only be used in much smaller quantities (e.g. < 10%) as an effective colouring agent.

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Mineral composition of flours produced from modern and ancient wheat varieties cultivated in Romania

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Abstract

Introduction. The aim of the present research was to study the mineral composition of flours produced from different wheat varieties from the collection of the Plant Genetic Resources Bank "Mihai Cristea" Suceava, Romania cultivated under the same conditions.

Materials and methods. Twenty four samples of whole wheat flour produced from different wheat varieties namely fifteen from common wheat (*Triticum aestivum* L.), five from einkorn wheat (*Triticum monococcum* L.) and four from spelt wheat (*Triticum spelta* L.) were analyzed to determine their mineral composition using an Energy Dispersive X-ray Analysis. The statistical analysis of the results was made using the hierarchical cluster analysis technique with a WARD method as a grouping algorithm.

Results and discussion. Generally, the ancient species of wheat were characterized by higher total mineral content than the modern ones, especially einkorn varieties. For all samples of flours significant differences were found in the amount of potassium (K), phosphorus (P), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu). However, all wheat varieties had high potassium and low copper amounts comparative to the other determined elements. Some of the most important microminerals for human nutrition, for example, Fe and Zn, were found in high amounts in flours from different wheat varieties but the samples from ancient wheat were characterized with bigger amounts of these elements than the modern ones. Meanwhile, in some modern wheat varieties these minerals were also present in sufficient quantities. The content of minerals depended more on the agronomic yield than on whether wheat varieties belonged to ancient or modern species.

Conclusions. The results show high variation in the mineral amount between different varieties. The knowledge of this variation can be useful in further breeding studies which aim to improve the nutritional quality of wheat grain and to develop micronutrient biofortification strategies. Both spelt and common wheat varieties showed overall a high mineral content. It seems that the agronomic yield has a significant impact on the mineral nutrients amount in wheat.

Introduction

Many valuable nutritional and sensorial qualities of some wheat varieties have been lost over time due to the need to increase wheat productivity, which is currently being carried out for modern commercial varieties (Velimirovic et al., 2021). So, the requirement for high-yielding wheat often leads to a decrease in its mineral content and modifications of its composition (Magallanes-López et al., 2017). Nowadays, consumers are more and more concerned about their health and demand food products of high quality (Codină et al., 2019). Thus, it is expected that significant breeding efforts will be made in the coming decades to improve the nutritional quality of the modern wheat varieties (Alvarez & Guzmán, 2018). It has been reported that the healthier alternatives to modern varieties of wheat are the ancient ones which are still being explored nowadays (Arzani & Ashraf, 2017). They are mainly used for human consumption in the bread making of various types of fermented bread and unleavened bread, but are also used as animal feed (Shewry, 2018). The ancient wheat term refers only to wheat varieties from *Triticum* genus which were not subjected to intensive genetic improvement programs and characterized as an origin prior to 1961. This includes *Triticum spelta*, *Triticum monococcum* and *Triticum dicoccum* as ancient wheat varieties (Cappelli & Cini, 2021). Grains of old wheat varieties are used for salads and for different products after processing; for example, spelt wheat is mainly used for bakery products, whereas einkorn and emmer grains are mainly used for pasta production (de Sousa et al., 2021; Mastrangelo & Cattivelli, 2021; Witczak & Gałkowska, 2021).

Production of microelements-enriched food is considered as one of the trends for the manufacturing of the breaking edge food in the next decade and wheat flour with a high content of mineral elements is of particular interest as a widespread consumer product (Ivanov et al., 2021). Ancient wheat varieties are used for food preparation because consumption trends are constantly changing and there is a growing demand for sustainable, regional and artisanal products that promote food diversity (Geisslitz & Scherf, 2020). However, the reasons why ancient wheat varieties are more valuable for health and have a higher nutritional value than modern ones are still unclear for now (Csákvári et al., 2021). For a healthy diet, it is recommended to use wheat flour, which is obtained by milling of the whole wheat grain (Gómez et al., 2020). This means that all the component parts of the wheat (bran, germ and endosperm) remain in the flour and nothing is lost in the milling process of the wheat grains (Šramková et al., 2008). Mineral substances are mainly located in the peripheral parts of the grain, so the whole meal flour has higher contents of minerals than the refined flour (Gómez et al., 2020). In wheat grains, the main minerals are potassium, phosphorus, magnesium and calcium, followed by zinc, manganese and iron in smaller amounts. Also, the presence of copper and selenium as oligominerals has been reported (Anglani, 1998).

The content of oligominerals in wheat grains depends on wheat variety, genetic predisposition, agricultural practices, type of soil and climatic conditions, as well as the technological and culinary practices that have been applied (Fan et al., 2008). The nutritional value of wheat products can be improved by the general use of less refined flour and by the selection of wheat varieties with high content of minerals (Cubadda et al., 2009).

Due to the widespread use of wheat flour in human nutrition, it is an effective way to supply the human diet with the required amounts of micronutrients thus preventing their deficiencies (Zhao et al., 2009). The content of minerals in bakery products is determined by their amount in the grains. Although their content can be increased by post-harvest processing, such as fermentation and micro-milling, it seems that modern breeding approaches may be the only way to achieve the significant increases in the amounts of mineral elements in wheat (Balk et al., 2019).

The significant differences in the mineral contents in different wheat varieties were reported (Magallanes-López, 2017; Zhao et al., 2009). However, these studies were performed for the wheat varieties grown in different geographical areas. Romania is one of the most important wheat producers within the European, being on the 4th rank for wheat production (Ionescu et al., 2020). In the present work, the amount of different minerals in various varieties of wheat stored in the current collection of BRGV and cultivated under the same conditions was studied. Evaluation of the potassium (K), phosphorus (P), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) present in ancient (einkorn, spelt) and modern wheat varieties was made.

Materials and methods

Materials

Twenty four samples of wheat grains from the active collection of BRGV Suceava were used to determine their mineral amount (Table 1).

The wheat samples were of various origins namely Romanian, German, French, Austrian and Russian. From the analyzed samples, fifteen samples were of common wheat (*Triticum aestivum* L.), five samples of einkorn (*Triticum monococcum* L.) and four samples of spelt (*Triticum spelta* L.). All varieties of wheat were cultivated in the experimental field of the BRGV Suceava, under the same growing conditions. All samples were collected in 2021, when the wheat grains reached the optimum stage of maturation. The mature whole grains were milled using the laboratory disc mill 3100 (Perten Instruments, Hägersten, Sweden). The wholemeal flours obtained were kept at a temperature of 4 ºC, until be analyzed for mineral amount.

Mineral composition analysis

The total mineral content of the wheat flours were determined by using the ICC standard method 104/1. To determine the mineral composition of the flour from wheat varieties a non-destructive method using an EDX system (Energy Dispersive X-ray Analysis), was used (Atudorei et al., 2020; Golea et al., 2021; Mironeasa et al., 2016). For this purpose a spectrometer Shimadzu EDX-900HS (Shimadzu Corporation, Kyoto, Japan) was used. The method is an analytical one, which exploits the emission of X-rays (of a certain wavelength), generated by an electron beam, accelerated incident on the sample.

The method of X-ray spectral analysis is based on a fundamental principle, which states that each chemical element has a unique atomic structure. According to the theory, the X-ray emission is made in high vacuum tubes, which contain a heat-emitting cathode and a metal anode, which emits high-energy electrons, acquired by acceleration at direct voltage, tens or hundreds of volts. Thus, in order to stimulate the characteristic X-ray emission of the samples, an energy-charged beam, such as electrons or protons, or cannon X-rays, is directed to the sample to be analyzed. The photons emitted by the sample are captured by a detector, a silicon-doped silicon semiconductor or SDD (silicon drift detector), cooled by the Peltier effect. Photons of different wavelengths reach the detector, which turns them into pulses proportional to their energy (Golea et al., 2021; Mironeasa et al., 2016). The working parameters used were: collimator diameter – 10 mm, atmosphere in the sample chamber – air, concentration unit – % , the measuring channel used was Na-U, the measurement time of the flour samples was 100 seconds.

Wheat samples

Variety	Sample number	Accession name	Biological status	Origin
Triticum aestivum L.	1	Izvor	Modern [1]	Romania
	2	Glosa	Modern [1]	Romania
	3	Miranda	Modern [1]	Romania
	4	Andrada	Modern [1]	Romania
	5	Dumbrava	Modern [1]	Romania
	6	Aurelius	Modern [1]	Austria
	7	Sofru	Modern [1]	France
	8	Sosthene	Modern [1]	France
	9	Amicus	Modern [1]	Austria
	10	Sothys	Modern [1]	France
	11	Flavor	Modern [1]	France
	12	Solindo	Modern [1]	France
	13	Izalco	Modern [1]	France
	14	Tonnage	Modern [1]	Austria
	15	Sophie	Modern [1]	France
Triticum monoccocum L.	16	SVGB-11842	Landrace [2]	Romania
	17	SVGB-11861	Breeding line [3]	Romania
	18	SVGB-11865	Breeding line [3]	Romania
	19	SVGB-11887	Breeding line [3]	Romania
	20	SVGB-11886	Breeding line [3]	Romania
Triticum spelta L.	21	Ebners Rotkorn	Modern [1]	Austria
	22	Frankenkorn	Modern [1]	Austria
	23	Alkoran	Modern [1]	Russia
	24	Oberkulmer Rotkorn	Modern [1]	Germania

Currently cultivated variety, which has a high production potential and uniformity compared to a primitive variety and which constitutes a major part of working collections and is extensively used as a parent in the breeding program.

[2]Local variety of a plant species that has distinctive characteristics arising from development and adaptation over time to conditions of a localized geographic region.

[3]Biological material developed by breeders to be used in modern scientific plant breeding.

Statistical analysis

Data were expressed as means ±standard deviations for triplicate determination. Statistical analysis was performed using the Statistical Package for Social Science (free trial, SPSS, Chicago, IL, USA). The data were subjected to the hierarchical cluster analysis (HCA) technique, using the WARD method of the program as a grouping algorithm (Golea et al., 2021).

Results and discussion

Mineral content of the flour from different wheat varieties

The composition of minerals in the studied wheat samples is shown in Table 2.

The samples from 1 to 15 are flours obtained from common wheat (*Triticum aestivum* L.), the samples from 16 to 20 are (*Triticum monococcum* L.) flours from einkorn wheat, and the samples from 21 to 24 are flours from spelt wheat (*Triticum spelta* L.). The macrominerals analyzed were: potassium (K), phosphorus (P) and calcium (Ca). The microminerals analyzed were: iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu). The wheat flour samples with the highest amounts of mineral elements were: sample 4 (common wheat, Romanian variety Andrada) with 57.62% K; sample 9 (common wheat, Austrian variety Amicus) with 1.53% Fe; sample 13 (common wheat, French variety Izalco) with 44.39% P and 1.80% Mn. A high amount of mineral elements 8.29% Ca, 0.08% Cu and 0.97 Zn% was observed in einkorn (sample 18).

The lowest amount of mineral elements were obtained in sample 5 (common wheat, Romanian variety Dumbrava): 0.92% Ca, 0.39% Mn and 0.29% Fe; in sample 8 (common wheat, French variety Sosthene) with 0.01% Cu and 0.15% Zn; in sample 20 (spelt wheat) with 28.12% K, and in sample 23 (spelt wheat) with 27.74% P.

According to our data, the descending order of the macroelements was K >P >Ca for all wheat varieties analyzed which is in the agreement with those reported by Biel with co-authors (2021). Literature data (Arzani &Ashraf, 2017; Shewry, 2018) indicate the higher levels of mineral content for ancient wheat species which is partially confirmed by our study (an einkorn sample showed the highest Ca amount from all wheat samples). According to Fan and co-authors (2008) this variation could be due to a mineral dilution caused by the increased yields and that newer wheat cultivars have lower amounts of minerals in the grains.

Sample 24 contained the lowest amount of calcium, and sample 23 had the highest amount of calcium. Both samples belong to the species *Triticum spelta*, which indicates a high intraspecific variability in the amount of Ca. This variation is in agreement with the results obtained by Suchowilska and co-authors (2012) and Biel with co-authors (2021), who observed a large intraspecific variability in the amount of Ca as well as in common wheat varieties (*Triticum aestivum*). In the data obtained for the analyzed wheat samples, a large variation was found for all macromineral elements, confirming the results obtained by Krochmal-Marczak & Sawicka (2016). According to Simsek and co-authors (2019), these variations are related to the age of the wheat variety (year of introduction in cultivation) and a possible reduction in bran content in relation to grain yield.

A great variation was found for all macromineral elements, confirming the results obtained by Krochmal-Marczak & Sawicka (2016). According to Simsek and co-authors (2019), these variations are related to the age of the wheat variety (the year of introduction into the crop) and a possible reduction in the content of bran in relation to the yield of cereals. However, from all the macroelements, calcium is the least associated with the launch year, indicating that this element has changed the least in the historical period. Studies by Morgounov with co-authors (2013) showed that old wheat varieties had higher amounts of calcium than modern varieties. In our study, the amount of calcium did not show higher values in the samples of ancient wheat compared to modern ones. This aspect is important for wheat varieties, because calcium plays an essential role in human health, helping to strengthen the immune system, and to regulate the heartbeat (Pravina et al., 2013).

Content of potassium (K), phosphorus (P), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) in wheat varieties,% of total mineral composition

Sample	K	Ca	P	Mn	Fe	Cu	Zn	Total mineral content, % of dry matter
1	55.19 ±0.11	4.25 ±0.08	37.89 ±0.23	1.18 ±1.18	0.82 ±0.01	0.04 ±0.01	0.42 ±0.01	1.64 ±0.08
2	55.25 ±0.09	2.50 ±0.06	39.46 ±0.20	1.26 ±0.01	0.85 ±0.01	0.05 ±0.01	0.41 ±0.01	1.74 ±0.07
3	55.75 ±0.09	3.35 ±0.06	37.38 ±0.19	1.21 ±0.01	0.68 ±0.01	0.04 ±0.01	0.36 ±0.01	1.63 ±0.06
4	57.62 ±0.10	1.21 ±0.05	37.20 ±0.21	1.08 ±0.01	0.91 ±0.01	0.06 ±0.01	0.50 ±0.01	1.83 ±0.06
5	29.92 ±0.05	0.93 ±0.02	32.38 ±0.16	0.39 ±0.02	0.29 ±0.01	0.01 ±0.00	0.18 ±0.01	1.85 ±0.07
6	53.61 ±0.08	8.11 ±0.05	35.31 ±0.18	1.52 ±0.01	0.90 ±0.01	0.04 ±0.00	0.49 ±0.01	1.83 ±0.06
7	57.38 ±0.08	7.48 ±0.05	32.37 ±0.16	0.92 ±0.01	0.58 ±0.01	0.02 ±0.00	0.31 ±0.01	1.83 ±0.05
8	29.37 ±0.05	2.09 ±0.02	32.56 ±0.16	0.57 ±0.01	0.29 ±0.01	0.01 ±0.00	0.14 ±0.01	1.85 ±0.06
9	52.53 ±0.09	3.75 ±0.06	39.89 ±0.21	1.56 ±0.01	1.53 ±0.01	0.04 ±0.00	0.57 ±0.01	2.25 ±0.07
10	56.85 ±0.09	3.69 ±0.05	35.62 ±0.19	1.34 ±0.01	0.96 ±0.01	0.04 ±0.00	0.47 ±0.01	1.63 ±0.08
11	29.78 ±0.05	1.62 ±0.02	32.44 ±0.16	0.52 ±0.01	0.26 ±0.01	0.01 ±0.000	0.16 ±0.01	1.78 ±0.07
12	55.80 ±0.09	3.55 ±0.06	38.14 ±0.20	1.28 ±0.01	0.64 ±0.01	0.03 ±0.00	0.36 ±0.01	1.69 ±0.07
13	48.17 ±0.10	3.73 ±0.06	44.39 ±0.24	1.80 ±0.01	1.00 ±0.01	0.05 ±0.00	0.69 ±0.01	1.68 ±0.08
14	57.48 ±0.09	3.84 ±0.06	34.58 ±0.19	1.14 ±0.01	0.79 ±0.01	0.03 ±0.01	0.42 ±0.01	1.68 ±0.07
15	56.01 ±0.09	3.97 ±0.05	37.39 ±0.19	1.20 ±0.01	0.88 ±0.01	0.04 ±0.01	0.44 ±0.01	1.72 ±0.11
16	38.91 ±0.07	2.44 ±0.29	28.27 ±0.23	0.71 ±0.01	0.94 ±0.01	0.07 ±0.00	0.53 ±0.01	2.61 ±0.10
17	35.82 ±0.08	2.23 ±0.28	31.05 ±0.23	0.67 ±0.01	1.05 ±0.01	0.05 ±0.01	0.44 ±0.01	2.47 ±0.07
18	49.57 ±0.08	8.29 ±0.05	38.19 ±0.20	1.57 ±0.01	1.31 ±0.01	0.08 ±0.01	0.97 ±0.01	2.34 ±0.07
19	51.247 ±0.098	3.50 ±0.05	41.66 ±0.21	1.47 ±0.01	1.12 ±0.01	0.04 ±0.01	0.83 ±0.01	2.56 ±0.07
20	28.119 ±0.051	1.19 ±0.02	33.49 ±0.16	0.58 ±0.01	0.52 ±0.01	0.03 ±0.00	0.35 ±0.01	2.37 ±0.11
21	36.36 ±0.09	2.23 ±0.25	27.86 ±0.33	0.54 ±0.01	1.12 ±0.01	0.04 ±0.01	0.50 ±0.01	1.83 ±0.11
22	35.79 ±0.08	2.92 ±0.04	30.76 ±0.23	0.51 ±0.01	0.75 ±0.01	0.05 ±0.00	0.45 ±0.01	1.96 ±0.10
23	46.26 ±0.08	6.24 ±0.04	27.73 ±0.18	0.58 ±0.01	0.82 ±0.01	0.05 ±0.01	0.40 ±0.00	1.82 ±0.11
24	47.80 ±0.08	0.97 ±0.05	30.84 ±0.21	0.58 ±0.01	0.94 ±0.01	0.06 ±0.00	0.49 ±0.01	2.54 ±0.09

The potassium amount varied between 28.119 and 57.617%, which indicates that this mineral element did not show significant differences compared to the rest of the analyzed samples, which showed a rather large variance interval. Ca amount ranged from 0.92 to 8.29% in wheat flour samples analyzed in this study. Also, the amount of Ca in the flour of wheat varieties showed significant differences between all the analyzed samples. The phosphorus amounts ranged from 27.74 to 44.39%, which was overall higher in modern wheat varieties compared to the ancient ones in agreement with the results reported by Biel with co-authors (2021).

Regarding the analyzed microelements, all of them varied between grain samples no matter of their variety. These results were unexpected since wheat varieties have been cultivated in the same conditions and no fertilization has been made. It seems that wheat lines producing higher grain yields may lead to lower contents of trace elements. That way, depending on grain yields the trace elements may vary. This is a consequence of concentration-dilution by the dry matter accumulated in wheat grains. Interestingly, it was reported that increasing grain yield by nitrogen fertilization did not influence the micronutrient content of the grains and that only the grain yield has a major impact on trace elements (McGrath, 1985; McDonald et al., 2008). This may explain the high variability between microlements which was obtained between our wheat variety samples. According to our data the Mn amount of the analyzed wheat varieties varied between 0.39 and 1.80%, observing significant differences between samples. The Fe amount ranged from 0.29 to 1.53%, resulting in significant differences between samples with similar data reported also by others (Zhao et al., 2009). Regarding the Cu amount, significant differences were observed between the analyzed samples, except the spelt wheat. The variance range of Cu amount was between 0.011 and 0.076%. It presented the lowest content from all wheat samples, this data are in agreement with those reported by Biel with co-authors (2021). Also, the Zn amount determined for the samples in our study varied between 0.145 and 0.966%, indicating significant differences between samples. Generally, it may be seen that the modern varieties presented a lower value of Zn than ancient ones, being in agreement with those reported by Zhao et al. (2009) which concluded that this may be due to the genetic improvement of wheat which seems to dilute.

According to our study, it can be seen that all the samples of ancient wheat have a high amount of Zn and Fe, except for sample 20. These results are relevant because studies have shown that these two microelements are deficient among children and women in developing countries (Zhao et al., 2009). Also, it was estimated that one-third of the world's population present deficiencies in these minerals (Hotz & Brown, 2004) and therefore their content in wheat grains is very important.

This concludes that ancient wheat is richer in these minerals, a result which is important regarding wheat grains cultivation. However, there are some modern wheat varieties which have high levels of Fe and Zn, but among these are some samples with a very low amount in these minerals (for example samples 5, 8, 11). A possible reason for the low amount of minerals, respectively Zn and Fe microelements, could be the fact that plant improvement is oriented towards a high agronomic yield (Biel, 2021).

Cluster analysis of wheat varieties flours based upon its mineral amount

In order to analyze in a more complex way the mineral amount determined for the twenty-four wheat varieties grown in Romania, in the experimental field of BRGV Suceava the hierarchical analysis of clusters (HCA) was used. The WARD method was used as a grouping algorithm, which determines for each cluster, the average of each variable, and the

distance between the clusters, is determined as the average of the distances from the middle element to all the elements of the other cluster.

By applying HCA, the similarities between wheat genotypes were analyzed, based on their mineral constituents and quality traits. Depending on the selected parameters, namely the geometric mean given by the concentrations of the seven determined mineral elements, the wheat samples were delimited into three groups called clusters, presented in the form of a dendrogram in Figure 1.
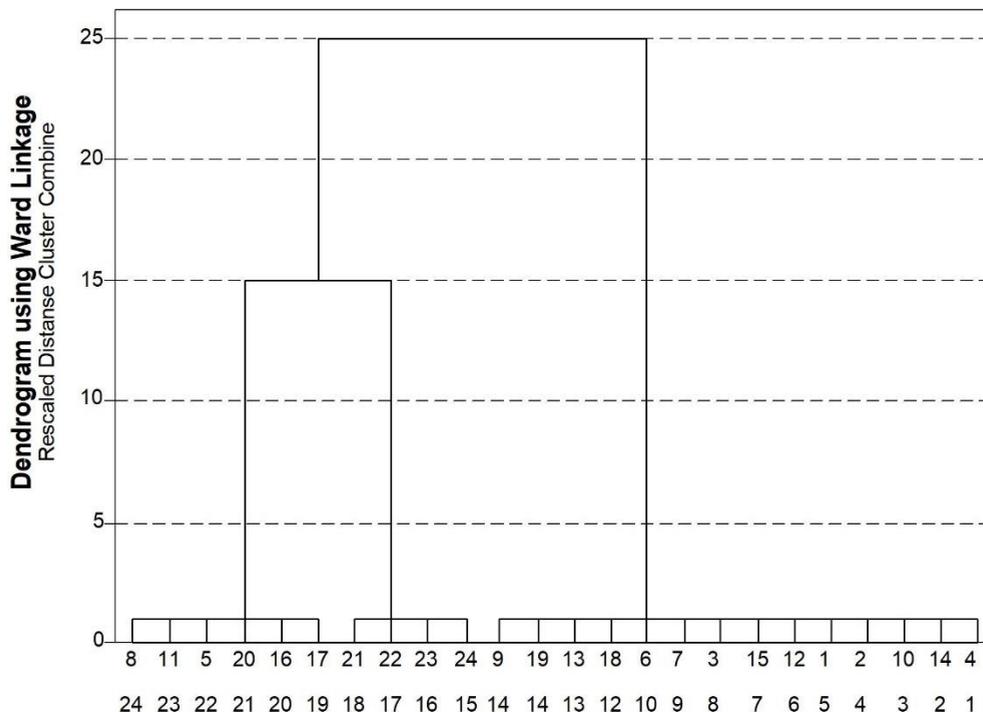
Figure 1. Dendrogram for wheat flour samples showing single linkage with Euclidean distances based on mineral amount

Thus, the use of HCA was effective for the classification of wheat varieties, based on the similarity of mineral compositions, resulting in three groups (clusters) of the twenty four samples of wheat studied as it may be seen from Table 3.

Table 3

Clusters grouped of the wheat varieties

Clusters	Wheat varieties
Cluster 1	Sosthene (8), Flavor (11), Dumbrava (5), T.monococcum (20), T.monococcum (16), T.monococcum (17)
Cluster 2	Ebners Rotkorn (21), Frankenkorn (22), Alkoran (23), Oberkulmer Rotkorn (24)
Cluster 3	Amicus (9), T. monococcum (19), Izalco (13), T .monococcum (18), Aurelius (6), Sofru (7), Miranda (3), Sophie(15), Solindo (12), Izvor (1), Glosa(2), Sothys(10), Tonnage (14), Andrada (4)

The first group includes six wheat samples (8, 11, 5, 20, 16, 17), of which 3 belong to the species *Triticum aestivum* (8, 11, 5) and the other three to the species *Triticum monoccocum* (20, 16, 17). It could be seen that in this group the wheat samples that had the lowest amounts of: K (sample 20); Ca, Mn and Fe (sample 5), Cu and Zn (sample 8) are present.

The second cluster comprises all four *Triticum spelta* samples studied in this experiment and includes sample 23, which had the lowest amounts values of P content. In this group we can highlight that there are no significant differences between the samples, except for the values of Ca amounts, at which a considerable range of variance is observed.

In terms of the number of samples, the third group is the largest, comprising 14 samples, of which 12 were *Triticum aestivum* and two were *Triticum monoccocum* (samples 18 and 19). In this cluster were found the samples with the largest amounts of minerals, determined in this study. Thus, sample 9 had the highest amount of Fe, sample 13 had the highest amount of P and Mn, also sample 18 had the highest amounts of Ca, Cu and Zn, and sample 4 had the highest amount of K.

All the wheat samples presented a large variability of the mineral compositions. The einkorn and modern wheat varieties are grouped in a mixed way in clusters 1 and 3 indicating no significant differences between these wheat flours. However, all the spelt grains flours are grouped together only in one cluster indicating some homogeneity between samples.

Mineral average amount for each cluster obtained for wheat flour varieties

The averages of the amounts of the seven mineral elements, corresponding to each of the 3 clusters formed, are presented in Table 4.

Table 4

Potassium (K), phosphorus (P), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) average amount for each cluster obtained

Clusters	Mineral contents,% of total mineral composition							
	K	P	Ca	Mn	Fe	Zn	Cu	Total mineral content,% of DM
Cluster 1	31.99 ±4.32	31.17 ±1.85	1.73 ±0.60	0.57 ±0,11	0.56 ±0.35	0.30 ±0.16	0.03 ±0.02	2.03 ±0.29
Cluster 2	41.55 ±6.36	29.30 ±1.73	3.09 ±2.25	0.55 ±0.04	0.91 ±0.16	0.46 ±0.05	0.05 ±0.01	1.84 ±0.28
Cluster 3	54.46 ±3.02	37.82 ±3.00	4.37 ±2.08	1.32 ±0.23	0.97 ±0.26	0.52 ±0.18	0.04 ±0.01	1.97 ±0.37

It may be seen that all three groups have a higher amount of micronutrients, as well as an improved nutritional value of wheat samples. Also, the genotypes in the same group are similar to each other and differ from the genotypes in the other groups. From all the analyzed samples, spelt grains presented the highest homogeneity. These results were similar with those reported by Gomez-Becerra with co-authors (2010) who concluded that spelt grains had a good broad adaptation, stability across various environments and high heritability values. It presents a genomic affinity and comparable yields with common wheats being a valuable source of mineral nutrients.

Conclusion

Mineral contents of twenty four samples of wheat flour from modern and ancient varieties were determined. A high variability has been recorded among the samples with no significant differences between modern and ancient ones. From all the wheat flour samples the highest homogeneity were obtained for spelt wheat flours which had higher mineral amounts compared to the einkorn and common wheat flours ones. Among all the analyzed mineral elements, potassium was in the largest amount followed by phosphorus and calcium. Copper was in the lowest amounts in all wheat flour samples. Among the minerals analyzed the highest interest presented calcium, zinc and iron which have the most important role in human health. Calcium is a structural component in the human body which prevents osteoporosis and maintains bone health, whereas iron combats anemia and zinc activates body enzymes essential for cell division. The calcium amount significantly varied among the samples. However, from all analyzed varieties einkorn ones presented the highest homogeneity with high amounts in this mineral. All the ancient samples had high amounts of iron and zinc. Also, many samples of modern wheat varieties also showed high amounts of minerals including calcium, zinc and iron. These are more due to the high agronomic yield of the grains that lead to a lower density in minerals. That way, the mineral amount profile in wheat, especially for the most relevant ones for the human body, should be a breeding target, aiming to improve the consumption of these important nutritional components in the diet.

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Use of pumpkin seed flour in preparation of bakery products

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Abstract

Introduction. The aim of the study was to determine the effect of pumpkin seed flour on the technological indicators of bakery products.

Materials and methods. The flour from seeds of the large-fruited, hard-skinned variety "Pink Banana" pumpkin was used in the study. Microbiological processes in the dough were characterized by the gas-forming ability of the dough and the dynamics of gas formation. Biochemical processes in the dough were investigated by kinetics of sugars in the dough. Indicators of the quality of finished products were also studied.

Results and discussion. "Pink Banana" pumpkin seed flour contains 3.8 times more protein and 3.5 times more fiber than wheat flour. The particle size of pumpkin seed flour is much larger than wheat wholemeal flour, so its application should affect the structural and mechanical properties of dough semi-finished products and finished bakery products. The water absorption capacity of pumpkin seed flour exceeds the corresponding value for wheat flour by 1.5 times. With an increase in the dosage of this additive the gas-forming capacity of the dough for bakery products decreased by 1.9–7.4% compared to the control sample without pumpkin seed flour and the amount of formed sugars decreased by 7.6–16.2%, but the amount of fermented sugars increased by 16.9–20.3%. The acidity of the crumb of products increased slightly, its specific volume decreased by 3.6–38.4% and porosity – by 1.4–4.1%. In finished bakery products, the protein content increased by 13.9–55.5% depending on the dosage of pumpkin seed flour, fiber content – by 12.07–48.7%, which indicates the ability of this raw material to significantly increase the nutritional value of products when it is included in the recipe.

Conclusions. Replacing part of wheat flour in the recipes of bakery products by pumpkin seed flour can increase the protein and fiber contents in these products, which will improve their nutritional value.

Introduction

Products for mass consumption often have insufficient nutritional value due to the low content of complete proteins, dietary fiber, vitamins and minerals (French et al., 2019; Ivanov et al., 2021). To improve the nutritional quality of foods, addition of different plant additives, including pumpkin seed products, is recommended (Stabnikova et al., 2021).

Pumpkin itself as well as its parts and processing products could be the source of proteins with high content of tryptophan, carotenoids, minerals, and unsaturated fatty acids (Kalyna et al., 2021). To enrich food products with physiological and functional ingredients, it is recommended to use pumpkin pulp, seeds (Dotto and Chacha, 2020), protein isolates and hydrolysates, flour, fiber (Vinayashre et al., 2021), and pumpkin oil (Gedi et al., 2022). However, the impact of these ingredients on the physico-chemical and technological properties of food products, including bakery, remains insufficiently studied.

Flour from seeds of gymnosperm pumpkin contains all main nutrients, % of dry matter: proteins, 43.2; fats, 17.3%; carbohydrates, 20.9% (including monosaccharides, 0.63%, sucrose, 1.84%, starch, 4.21%, fiber, 14.28%) (Jurgita et al., 2014). Pumpkin seeds and shells are potentially good raw materials for enriching food products due to the presence of antioxidant compounds such as polyphenols and high antioxidant activity (Saavedra et al., 2015). However, there is no data on the impact of the studied additives on the technological process of manufacturing different groups of food products.

The total carotene content in wheat bread increased with the addition of pumpkin products (Kampuse et al., 2015; Rakcejeva et al., 2011). However, the volume of bread decreased with the increase of dosage of pumpkin pomace and pumpkin powder. The addition of semi-finished products such as juice and puree from different varieties of pumpkin improved the sensory bread properties, but physico-chemical characteristics of bread have not been studied (Barabolia et al., 2018). Bread from a composite mixture of pumpkin and spelled flour had potassium and calcium contents 1.5 times higher than wheat, in terms of contents of phosphorus, magnesium and zinc 2–3 times higher. Altogether, prolongation of freshness of finished products was also observed (Mykolenko et al., 2017). However, there are no studies of the processes which occur in semi-finished bakery products added with pumpkin products in the manufacture of bread, as well as indicators of quality of finished products. Partial replacement of wheat flour up to 15% with pumpkin seed flour helped to improve nutritional and sensory values of cookies (Alshehry, 2020).

Pumpkin has a special attention for food product enhancement for its health promoting values. Pumpkin seeds contain biologically active substances having antidiabetic, antidepressant, antioxidant, antitumor and cytoprotective activities (Dotto and Chacha, 2020). It is considered that consumption of pumpkin products reduces the risk of gastrointestinal inflammation (Gad et al., 2019) and they are recommended in nutrient therapy for persons suffering from intestinal diseases (Dar et al., 2017). Pumpkin seeds have especially high beta-carotene content, so, consumption of food enriched with pumpkin products helps to prevent skin diseases and support vision (Lyu et al., 2021). As beta-carotene is a fat-soluble vitamin, its bioaccessibility increases in the presence of lipids. So nutritionists recommend to include in recipes of food products enriched with pumpkin seeds lipid components, in particular phospholipids, for example lecithin.

The aim of the present study was to determine the effect of pumpkin seed flour addition on the technological characteristics of bakery products manufactured from wheat flour.

Materials and methods

Preparation of dough samples

Dough samples were prepared from premium wheat flour, pressed baker's yeast, salt, sunflower lecithin as a source of phosphatidylcholine, in the amount of 3% by weight of flour (this dosage was chosen based on the recommendations for the daily intake of lecithin) (Partridge et al., 2019), pumpkin seed flour in the amount of 5, 10, 15, 20% to replace wheat flour. A sample without pumpkin seed flour and lecithin was used as a control sample.

Methods

Size of the flour particles. The size of the flour particles was determined by sieving on sieves. Sieves of different sieve fabric and different hole sizes were used: No 33/36 (35) (220 μm), No 27 (260 μm), No 067 (670), No 49/52 PA (43) (132 μm), No 41/43 (38) (160 μm) (Patwa et al., 2014).

Gas-forming ability of the dough. The indicator of gas-forming ability is the amount of cm^3 of carbon dioxide (CO_2) emitted during the fermentation and keeping of the dough from 100 g of flour at a temperature of 30 °C. This indicator was determined by the volumetric method, namely the volume of CO_2 emitted at constant temperature and pressure (Munteanu et al., 2019; Verheyen et al., 2015).

Kinetics of sugar accumulation in the dough. The amount of sugars formed during the fermentation of the dough was determined by the difference between their content in the dough without yeast immediately after kneading and after 180 minutes of fermentation. The amount of fermented sugars was determined by the difference between the sum of the amount of sugars at the beginning of fermentation of yeast dough and the amount of sugars formed in yeast-free dough and the amount of sugars contained in yeast dough after 180 minutes of fermentation. The kinetics of sugar accumulation in the dough was determined by the accelerated iodometric method (Manual of methods of analysis of food, beverages, sugar and confectionery product, 2012)

Titrated acidity of the dough. Titrated acidity in semi-finished products (dough) was determined by titration (Manual of methods of analysis of food, beverages, sugar and confectionery products, 2012).

Moisture. The moisture content was determined using the SuperPoint grain moisture meter. To measure the grain humidity, the appliance was switched on, the name of the scale of the corresponding measuring crop or product was selected on the LCD screen, the necessary sample was selected, which falls into the device, the pressure cover of the pressurizes to the level until the pressure indicator was set to the level with the upper surface of the lid. After tightening the button "TEST" was pressed and after 10 seconds the result of the measurements of humidity in% was received. Measurement was carried out with an accuracy of 0.5% with a range of humidity measurement from 8 to 45% (Manual of methods of analysis of food, beverages, sugar and confectionery products, 2012).

Total protein. A product was digested with a strong acid so that it released nitrogen which could be determined by a suitable titration technique. The amount of protein present was then calculated from the nitrogen concentration of the product.

1 g of raw material was hydrolyzed with 15 mL concentrated sulfuric acid containing two copper catalyst tablets in a heat block at 420 °C for 2 h. After cooling, H_2O was added to the hydrolysates before neutralization and titration (Mæhre et al., 2018).

Fat. The sample is placed in a thimble; once the flask is heated, the solvent is evaporated and moved up to the condenser, where it is converted into a liquid and collected into the extraction chamber containing the sample. When the solvent passes through the sample, it extracts the fats and carries them into the flask. This extraction process typically lasts several hours (6–24 h). After completion of the extraction, the solvent is evaporated, and the mass of lipid remaining is measured and used to analyze (López-Bascón et al., 2020).

Fiber. A collaborative study was conducted to determine the total dietary fiber (TDF) content in products, using enzymatic-gravimetric method (McCleary et al., 2012). TDF was calculated as the weight of the residue minus the weight of protein and ash.

Specific volume of bread. The grain was filled with the excess, which was raked with the edge of the ruler into the receiving container and removed through the hole. After that, the curtains of the main capacity with grain were opened manually and put through the hole into the bucket. This grain was used for determination. A small amount of grain was put into the main container, bread was put on it carefully, without passing the grain, and the rest of the grain was put in excess of the capacity. Grain was raked with the edge of the ruler and put into the receiving container, and then, after opening the latch – into the measuring cylinder. The volume of grain in a cylinder (cm^3) was equal to the volume of bread. Measurements were performed twice, deviations between parallel determinations should not exceed 5%. The specific volume of bread was determined by dividing the volume of bread by its weight and expressed to the nearest 0.01 cm^3/g (Zhu et al., 2016).

Porosity of bread. The porosity of bread reflects the volume of the pores in a certain volume of the crumb, expressed as a percentage to the total volume (Verheyen et al., 2015).

Statistical analysis. The statistical processing of the result values was performed by sequential regression analysis using the Microsoft Excel XP and OriginPro 8 software calculating correlation coefficients (Hinkle et al., 2003).

Results and discussions

Physico-chemical and technological characteristics of pumpkin seed flour

The chemical composition of raw materials is a major determinant in the development of new product formulations (Jurgita et al., 2014). The chemical composition of pumpkin seed flour compared to premium grade wheat flour is presented in Table 1.

Table 1

Chemical composition of wheat flour and pumpkin seed flour

Indicator	Premium grade wheat flour	Pumpkin seed flour
Moisture,% weight	11.5	15.6
Proteins,%	10.3	40.0
Fats,%	1.1	9.0
Carbohydrates,%	69.8	23.6
Cellulose,%	3.5	12.2
Ash,%	0.75	4.7

It was shown that pumpkin seed flour contains 3.8 times more protein, 3.5 times more dietary fiber, 6.3 times more ash than wheat flour. Thus, partial replacement of wheat flour with pumpkin seed flour may lead to enhancement of nutritional value of bakery products.

Microbiological and biochemical processes in the dough, its structural and mechanical properties are important in the manufacture of bakery products (Lisowska et al., 2016). They are significantly influenced by the size of the components of the recipe (Table 2) and their water absorption capacity (Figure 1).

Table 2

Size of the particles in pumpkin seed flour in comparison with wheat flour

Size indicators, No of sieve	Size of the hole, µm	Wheat flour, variety			Pumpkin seed flour
		First	Second	Whole-meal	
The residue on the sieve% , no more:					
No 33/36 (35)	220	2	-	-	28.1
No 27	260	-	2	-	63.18
No 067	670	-	-	2	6.92
Passage through a sieve,% no less:					
No 49/52 PA (43)	132	80	-	-	-
No 41/43 (38)	160	-	65	35	0.18

Estimation of the particle size distribution of pumpkin seed flour showed that it is much larger than wheat wholemeal flour, as the residue on the sieve No 067 exceeds the maximum standard value for wholemeal flour by 3.5 times.

The water absorption capacity of raw materials depends on the composition of its biopolymers, particle size, the state of their surface (Zykova et al., 2015). The water absorption capacity of pumpkin seed flour is 1.5 times higher than of wheat flour.
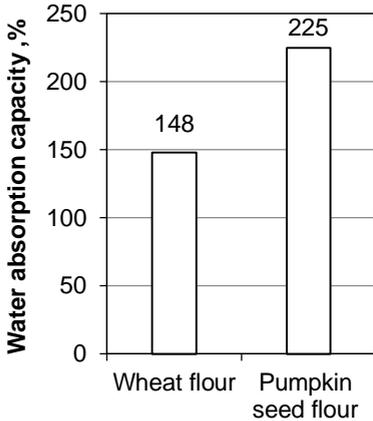
Figure 1. Water absorption capacity,%

Influence of pumpkin seed flour on microbiological processes in the dough

The intensity of dough fermentation, which was determined by the amount of carbon dioxide emitted during dough fermentation and keeping of dough pieces, is determined by the interaction of dough microflora and products of enzymatic hydrolysis of flour biopolymers and other components of the recipe. Samples with lecithin as emulsifier and a source of phosphatidylcholine and samples with different dosage of pumpkin seed flour were studied.

The gas-forming capacity of the dough (Figure 2) increased slightly with the addition of lecithin, which can be explained by the presence of choline in lecithin, which improves the enzymatic ability of yeast (Medvid et al., 2018). However, with increasing dosage of pumpkin seed flour, the gas-forming capacity decreased by 1.9–7.4% compared to the control sample. This can obviously be explained by the formation of protein complexes of pumpkin seed flour with wheat flour starch, which reduces its availability to amylolysis.
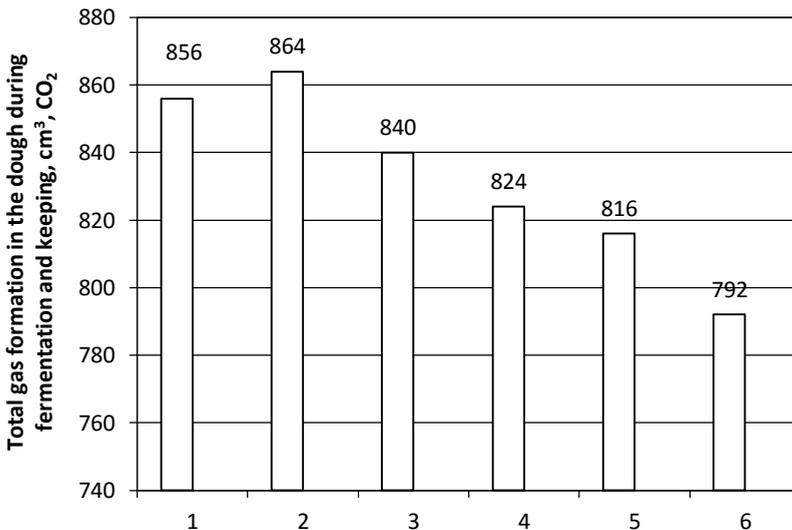
Figure 2. Total gas formation in the dough during fermentation and keeping, cm^3, CO$_2$:
1 – control sample;
2 – sample with lecithin;
3 – sample with lecithin and 5% pumpkin seed flour to replace wheat flour;
4 – sample with lecithin and 10% pumpkin seed flour to replace wheat flour;
5 – sample with lecithin and 15% pumpkin seed flour to replace wheat flour;
6 – sample with lecithin and 20% pumpkin seed flour to replace wheat flour

Decreased fermentation activity of yeast affects the dynamics of carbon dioxide emission during the fermentation of the dough and the keeping of the dough pieces (Figure 3).
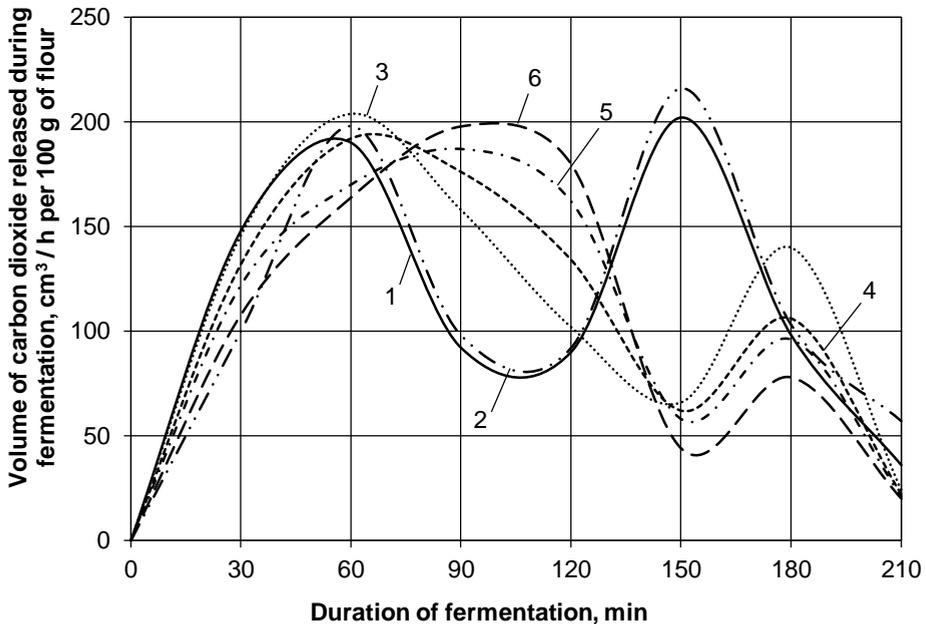
Figure 3. Dynamics of gas formation in the dough with different dosage of pumpkin seed flour:
1 – control sample;
2 – sample with lecithin;
3 – sample with lecithin and 5% pumpkin seed flour to replace wheat flour;
4 – sample with lecithin and 10% pumpkin seed flour to replace wheat flour;
5 – sample with lecithin and 15% pumpkin seed flour to replace wheat flour;
6 – sample with lecithin and 20% pumpkin seed flour to replace wheat flour

It was found that in the dough with flour from pumpkin seeds gas formation was less intense, because there was a delay in fermentation by reducing the availability of nutrients. The graph of the dynamics of carbon dioxide emissions shows that the first peak of gas formation in the dough with the replacement of 5% wheat flour by pumpkin seed flour was observed after 60 minutes, when replacing 10% – after 70 minutes, 15% – after 100 minutes, 20% – after 115 minutes of fermentation, and for wheat flour – in 50 minutes. Then the amount of carbon dioxide emitted in the dough with pumpkin seed flour decreased sharply and the second peak of gas formation was observed after 180 minutes, while in the control sample – after 150 minutes. This is due to the fact that amylolytic enzymes of pumpkin seed flour are less active than of wheat flour, which is due to the poor susceptibility of the starch of this flour to amylolysis.

Influence of pumpkin seed flour on biochemical processes in the dough

The process of gas formation in the dough is due to the sugar-forming ability (Drobot et al., 2014), which in turn is provided by the susceptibility of starch to amylolysis and amylase activity. The sugar content depends on the relationship between the intensity of sugars accumulation in the dough and their fermentation by microorganisms (Drobot et al., 2014). The depth of this process was characterized by the kinetics of accumulation and fermentation of sugars (Table 3).

Table 3

Accumulation and fermentation of sugars during the fermentation of the dough (in terms of maltose),% to dry matter

Indicators	Control sample	Sample with lecithin	Pumpkin seed flour to replace wheat flour,%			
			5	10	15	20
Yeast-free dough						
After kneading	2.10±0.10	2.10±0.10	2.10±0.10	2.00±0.10	2.00±0.10	1.99±0.09
After 3 hours of fermentation	3.15±0.13	3.39±0.17	3.32±0.15	3.21±0.14	3.20±0.14	3.12±0.12
Formed sugars	1.05±0.01	1.29±0.03	1.22±0.03	1.21±0.03	1.20±0.03	1.13±0.02
Yeast dough						
After kneading	2.12±0.10	2.15±0.12	2.13±0.11	2.08±0.10	2.02±0.10	1.98±0.09
After 3 hours of fermentation	1.69±0.06	1.78±0.08	1.62±0.06	1.54±0.05	1.46±0.05	1.33±0.04
Fermented sugars	1.48±0.05	1.66±0.06	1.73±0.08	1.75±0.08	1.76±0.08	1.78±0.08

It was found that with increasing the dosage of pumpkin seed flour, the amount of formed sugars decreased by 7.6-16.2%. This can be explained by the fact that pumpkin seed flour proteins form complexes with wheat starch and therefore impair the access of enzymes to starch grains. However, the fermentation of sugars increased by 16.9–20.3%, due to the depolymerization of carbohydrate additives (Teri et al., 2014).

The quality indicators of the finished products (Table 4) indicated an increase in the acidity of the crumb of the products with the replacement of part of the wheat flour with pumpkin seed flour due to the higher acidity of the added raw material. The shape stability of bread did not change significantly.

However, its specific volume decreased by 3.6–38.4% and porosity decreased by 1.4–4.1%, which can be explained by the specifics of swelling of pumpkin components, including fiber (Pereira et al., 2018). At the same time, the organoleptic characteristics of the products improved, in particular the taste and smell, which acquired a pleasant pumpkin hue. The bread crumb was elastic, well fluffed.

Quality indicators of finished bread with pumpkin flour

Indicators	Control sample	Sample with lecithin	Samples with pumpkin flour,% to replace wheat flour			
			5	**10**	**15**	**20**
Acidity of the crumb, degrees	2.0	2.0	2.5	2.8	3.1	3.4
Specific volume of bread, cm³/100 g	224	234	220	218	178	146
Shape stability of bread, H/D	0.56	0.61	0.60	0.58	0.55	0.53
Porosity of the crumb,%	73	75	67	65	62	54

To meet the body's needs in essential nutrients, it was important to determine the effect of different dosages of pumpkin seed flour on the nutritional value of the product (Table 5).

Nutritional value of bread with the replacement of part of wheat flour with pumpkin seed flour

Indicators	Control sample	Sample with lecithin	Pumpkin seed flour to replace wheat flour,%			
			5	**10**	**15**	**20**
Proteins,%	8.10	8.10	9.23	10.35	11.48	12.60
Fats,%	1.09	3.06	3.36	3.66	3.96	4.26
Carbohydrates,%	52.97	52.97	51.20	49.43	47.65	45.88
Cellulose,%	2.65	2.65	2.97	3.30	3.62	3.94

Analysis of the chemical composition of bread prepared with partial replacement of wheat flour with pumpkin flour showed an increase in protein content by 13.9–55.5%, fiber – by 12.07-48.7% depending on its dosage in comparison with control sample made from wheat flour only that indicates enhancement of the nutritional value of products.

Conclusions

1. Pumpkin seed flour is high in protein and fiber. The use of this raw material for partial replacement of wheat flour in the bread recipe will make it possible to enrich products with protein and fiber and increase their nutritional value.
2. The gas-forming capacity of the dough with increasing dosage of pumpkin seed flour from 5% to 20% to replace wheat flour decreased by 1.9–7.4% compared to the control sample without pumpkin seed flour.
3. With the increase in the dosage of pumpkin seed flour, the amount of formed sugars decreased by 7.6-16.2%. This can be explained by the fact that pumpkin seed flour proteins form complexes with wheat starch and therefore impair the access of enzymes

to starch grains. However, the amount of fermented sugars increased by 16.9–20.3%, due to the depolymerization of carbohydrate additives.

4. The specific volume and porosity of bread decreased with increasing percentage of replacement of wheat flour by pumpkin seed flour. Therefore, from a technological point of view, it is rational to replace no more than 10% of wheat flour with this raw material.

5. The protein content in finished products with the replacement of 5–20% of wheat flour with pumpkin seed flour increased by 13.9–55.5% and fiber content increased by 12.07–48.7% that indicates enhancement of the nutritional value of the products.

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Effect of *Spirulina platensis* and kelp biomass addition on the fatty acid composition of wheat bread

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Abstract

Introduction. The aim of the present study was to study the effect of biomass of some edible algae – *Spirulina platensis* and kelp – addition on the content of saturated and unsaturated fatty acids in wheat bread.

Materials and methods. Bread was obtained from wheat flour with the addition of biomass of kelp and *Spirulina platensis* in the form of powder in the amount of 2 or 4% by the weight of flour. The extraction of total lipids was performed by the conventional method, the methyl esters of fatty acids were analyzed using a gas chromatograph equipped with a flame ionization detector.

Results and discussion. It was found that enrichment with biomass of kelp and *Spirulina platensis* added in the amount of 2 and 4% by the weight of wheat flour changes the content of saturated and unsaturated fatty acids in bread. As the different algal species have a different fatty acid profile, the addition of two aquacultures to the wheat flour had different effects. In terms of saturated fatty acids, the incorporation of kelp biomass in the bread recipe caused an increase in the content of stearic, arachidonic and heneicosanoic acids, while enrichment with biomass of *Spirulina* led to an increase in the content of caproic, palmitic, arachidonic acids and, especially, of heneicosanoic acid. In the control bread, the amount of heneicosanoic acid was 0.17 g/100 g of fat. In the bread enriched with 2 and 4% of kelp, the amount of heneicosanoic acid was in 2.2 and 3.5 times higher than in control, respectively; in the bread enriched with with 2 and 4% of *Spirulina platensis* – in 3.4 and 3.1 times higher than in control, respectively. Seaweed addition also affects the content of unsaturated fatty acids in wheat bread. When kelp was included in the bread recipe, there was an increased content of oleic and α-linolenic acids, while in the case of paulinic acid, enrichment with *Spirulina platensis* was more efficient.

Conclusions. Fortification of wheat bread with biomass of edible algae kelp and *Spirulina platensis* is an effective way for increasing the content of some fatty acids in it. The effect of biomass of *Spirulina platensis* addition is more pronounced.

Introduction

Fatty acids are a major structural component of lipids (de Carvalho et al., 2018). They are a source of energy for cell growth, especially in childhood (Shahidi et al., 2008). It is well known that polyunsaturated fatty acids are of great physiological importance (Simopoulos, 1999). The total lipid intake and the consumption ratio of saturated fatty acids (SFA) has increased significantly in the Western diet in recent decades (Simopoulos, 2016). In order to reduce the saturated fat content of processed foods, the food industry is facing a challenge to replace animal fat by vegetable fat, which has a high content of unsaturated fats (Tavella et al., 2000). On the other hand, there has been a growing public awareness of the benefits of essential fatty acids (Kaur et al., 2014). However, most of them are either not synthesized at all or are synthesized in insufficient quantities by the human body, which necessitates their intake as supplements or enriched food products (Kaur et al., 2014). Predominance (over 50%) of omega-6 linoleic acid in grain cereals is a major reason for the imbalanced omega-3/omega-6 ratio consumption in western diets (Fradique et al., 2013). This limitation can be overcome by enrichment of food products with sources of omega-3 polyunsaturated fatty acids (Barrow et al., 2009). Therefore, among the main tasks of the food industry is the development of product formulations with better nutritional characteristics (Osuna et al., 2014). According to Petrovna et al. (2022) enrichment of daily consumed food products with essential fatty acids is an innovative approach, which is most advantageous for people that do not require major changes to their dietary habits.

Incorporating bioactive ingredients, rich in different valuable compounds, into popular foods such as bread, have grown rapidly due to the increased consumer health awareness (Ibrahim et al., 2016). It is considered that bread prepared from refined flour has lower nutritional value than whole grain bread and does not adequately meet the requirements for many macro- or micro-nutrients (al-Kanhal et al., 1999; Škrbić et al., 2008). Due to its relatively low cost, availability and widespread consumption, bread is a suitable product for incorporation of functional ingredients, including omega-3 fatty acids (Dziki et al., 2014). In recent decades, different research teams have worked on fortifying bread with natural compounds due to the demands for healthier food (Melilli et al., 2019; 2020; Sillitti et al., 2016).

Incorporation of edible seaweeds to increase nutritional value of different food products including wheat bread are presently very popular (Stabnikova et al., 2021). Despite the quantitative differences in chemical composition, seaweed is a sustainable and almost inexhaustible source of polyunsaturated fatty acids. They are characterized by an optimal ratio about 1.0 of omega-6: omega-3 fatty acids. According to the recommendations of the World Health Organization, to prevent inflammatory, cardiovascular problems and diseases of the nervous system this ratio should be less than 10. Prabhasankar et al. (2009) prepared pasta, incorporating wakame (*Undaria pinnatifida*) as an ingredient at different ratios of semolina to wakame (100:0; 95:5.0; 90:10; 80:20 and 70:30). Authors reported that compared to the control (1:15.2), the ratio of omega-3 to omega-6 fatty acid in wakame enriched pasta was 1:3.4. The importance of algae lipids lies in their potential as an alternative source for the production of functional foods with increasing content of essential fatty acids, such as eicosapentaenoic acid, docosahexaenoic acid, and their precursor α-linolenic acid (Ferreira et al., 2019).

Nutritional characterization of seaweed and their application in food products preparation are well studied (Caporgno et al., 2018; Lafarga, 2019; Sanjari et al., 2018). The total lipid content and the fatty acid of different algae species is well known (Gosch et al., 2012; Jay et al., 2018; Rodrigues et al., 2015). For example, *Spirulina* has lipid content 5.6 –

7.0% including linoleic and γ-linoleic fatty acids (Othes et al., 2001). *Chlorella vulgaris* contains approximately 35–40% lipids, with up to 27% α-linolenic and 24% linoleic acid (Freitas, 2017). *Spirulina* sp. has been frequently claimed as the cheapest source of γ-linoleic acid (Choopano et al., 2016). Nevertheless, knowledge about the effect of supplementation of wheat bread with some edible algae on the fatty acid composition of bread are limited. Due to their valuable chemical composition, microalgae and brown algae are among the most widely used as a food additive, also in the bakery industry. Most often, enriching bread with these algae aims to increase its protein, mineral and fiber content (Ak et al., 2016; Saharan et al., 2017; Yaiche et al., 2014). The effect of this aquaculture on the fatty acid profile of wheat bread has been less studied.

The present study focuses on the impact of some of the most commonly used as supplements in the food industry algae *Spirulina platensis* and kelp on the fatty acid amount in wheat bread. The optimal amount of algae added was determined by preliminary experimental studies. It was found that the biomass of algae mentioned above in the amount of 2 and 4% by the weight of wheat flour has a clear positive effect on the nutritional value of bread, without compromising its sensory characteristics and consumer acceptance. The aim of this study was to investigate the effect of addition of *Spirulina platensis* and kelp biomass in amounts of 2 and 4% by the weight of wheat flour on the content of saturated and unsaturated fatty acids in bread.

Materials and methods

Materials

For the preparation of the bread samples, the following materials were used:
- Commercial wheat flour type 500 with the following properties: moisture content – 12.8% ; gluten content – 27.07% ; release of gluten – 6 mm; titratable acidity – 2 °H;
- Water – according to ISO 6107-1:2004;
- Commercial yeast (Lesafmaya);
- Salt – according to Codex Standard for Food Grade Salt CX STAN 150-1985;
- *Spirulina platensis* powder (average chemical composition: protein 64 g/100 g; fat 8.2 g/100 g of which saturated 3.42 g; carbohydrates 16.1 g/100 g, of which sugars 0.52 g, fiber 7 g/100 g).
- Kelp powder (average chemical composition: protein 5.3 g/100 g; fat 4.2 g/100 g of which saturated 0.9 g; carbohydrates 12.0 g/100 g, of which sugars 0.5 g, fiber 1.25 g/100 g).

Methods

Dough and bread composition

The composition of the bread samples is presented in Table 1.

Table 1

Bread samples composition

Ingredients	Control sample	Sample S2 – with 2% *Spirulina platensis*	Sample S4 – with 4% *Spirulina platensis*	Sample K2 – with 2% kelp	Sample K4 – with 4% kelp
Wheat flour, g	250	245	240	245	240
Water, cm^3	140	145	155	145	155
Yeast, g	3.37	3.37	3.37	3.37	3.37
Salt, g	3.25	3.25	3.25	3.25	3.25
S. platensis, g	–	5	10	–	–
Kelp, g	–	–	–	5	10

Bread preparation

Bread was prepared from type 500 wheat flour by a two-phase method. Initially, knead the yeast, flour and water dough in a 1:1 ratio in a kneading machine (Labomix 1000, Hungary). Pre-mixed *Spirulina platensis* and kelp algae (powder) in the amount of 2% or 4% by the weight of flour are added to the mixing water (combinations K2 and K4, for the breads prepared with kelp and combinations S2 and S4, for the breads prepared with *Spirulina platensis*, respectively). The control sample was prepared only with wheat flour. The dough thus prepared matures for 4 hours at 33 °C and then mixes the dough to obtain a homogeneous mass by adding the remainder of the flour according to the formulation and salt (1.3 kg/100 kg flour). The bread dough divides into pieces by 440 g and forms, matures for 55 minutes at 38 °C (Tecnopast CRN 45–12, Novacel ROVIMPEX Novaledo, Italy). After the end fermentation, the pieces of dough were put into an electric oven (Salva E-25, Spain) pre-heated to 200–220 °C. The baking time was 24 min, until the temperature in the center of the bread crumb reached 96-98 °C. After baking, the bread was allowed to cool down for 3 h at room temperature.

Determination of fatty acid composition

The extraction of total lipids was performed by the conventional method, as the methyl esters of fatty acids were analyzed using a gas chromatograph "Shimadzu GC-17A" equipped with an automatic injector (AOC 2), a Restek (19091N-213) column (100 m length × 0.32 mm inside diameter, and 0.5 μm film thickness), and a flame ionization detector (FID).

The tested sample was placed in a suitable flask and 4 ml of methanolic NaOH solution and boiling aid were added. A Graham condenser was connected to the flask. If the fatty acids contain more than two double bonds, the air from the flask was removed by blowing with dry nitrogen for a few minutes. The sample is boiled for 5 to 10 minutes, shaking the flask periodically. Then 5 ml of boron trifluoride methanol solution through the upper end of the condenser were added. Boiling lasts 3 minutes. 1 to 3 ml of isooctane are added to the boiling mixture through the upper end of the condenser. When the heating of the flask is completed 20 ml of NaCl solution are added immediately. The flask should be closed and shaken vigorously for at least 15 s. Saturated NaCl solution is added so that the liquid level is up to

the neck of the flask. The two phases are separated in a separating funnel. 1-2 ml of the upper isooctane layer are placed in a 4 ml vial and anhydrous sodium sulphate is added to remove all traces of water. The isooctane solution thus obtained can be injected (ISO 5508:1990). The temperature of the injector and detector was kept at 250 °C. The injection volume was 1 µl. Fatty acids were identified by comparison of their retention times with those of authentic standards and reported as g/100 g fat.

Results and discussion

Effect of *Spirulina platensis* and kelp addition on the content of saturated fatty acids in wheat bread

The results on the influence of *Spirulina platensis* and kelp algae addition on the amount of saturated fatty acids (SFA) in the different samples of bread are presented in Table 2.

Table 2

Influence of *Spirulina platensis* and kelp on the content of saturated fatty acids in wheat bread

| *Saturated fatty acids* | Content of saturated fatty acids in the bread samples, g/100 g fat | | | | |
	Control	**K2 with 2% of kelp**	**K4 with 4% of kelp**	**S2 with 2% of *S. platensis***	**S4 with 4% of *S. platensis***
Caproic acid C 6:0	0.19	0.06	0.12	0.27	0.41
Capric acid C 10:0	0.21	0.08	0.10	0.11	0.21
Lauric acid C 12:0	0.45	0.25	0.21	0.24	0.19
Myristic acid C 4:0	0.14	0.04	0.02	0.07	0.13
Palmitic acid C 6:0	8.46	9.11	8.19	8.54	8.79
Stearic acid C 18:0	3.01	3.84	3.16	3.07	2.84
Arachidic acid C 20:0	0.15	0.20	0.19	0.19	0.25
Heneicosylic acid C 21:0	0.17	0.38	0.61	0.58	0.52

As it can be seen from the results, the amount of saturated fatty acids in the control sample ranged from 0.14 g/100 g fat (for myristic acid) to 8.46 g/100 g fat (for palmitic acid). Palmitic and stearic acids were predominant in quantity, and for all other fatty acids the reported amounts were below 1 g/100 g of fat. The addition of algae in the bread recipe has an effect on the amount of saturated fatty acids, and in this case the influence of both the species and the amount of aquaculture is clearly seen. It's known that the fatty acid composition depends on the species – the alga *Porphyra spp.* have the lowest content of saturated fatty acids (17.4% of the total fatty acids), while *Plocamium brasiliense* have the highest – 74% of the total (Gressler et al., 2011).

The content of caproic acid in the control sample was low – 0.19 g/100 g fat. There was a twofold increase in its content when enriching wheat bread with 4% alga *S. platensis*. This fatty acid acts as a membrane stabilizer – it supports the formation and maintains the stability of cell membranes. Enriching bread with seaweed did not increase the amount of capric, lauric and myristic acids. Other authors showed results that do not match ours. Fradique et al. (2013) found that when enriching pasta with two types of marine microalgae from the class *Haptophyceae*, the amount of myristic acid (14:0) increased from 0.14% in the control sample to 2.14% in the sample enriched with algae. However, it depends on the spice of algae used.

In the control sample of bread the highest content of palmitic acid – 8.46 g/100g fat was obtained. Similar results for the content of palmitic acid in bread were published by Lazova-Borisova et al. (2019) – 10.40 g/100 g fat. Of all the tested samples with a maximum content of palmitic acid, the one prepared with the addition of 2% kelp algae (9.11 g/100 g fat) stands out. For the other samples, the quantities are comparable to those in the control sample. In their study, Rodrigues et al. (2015) found that seaweed *S. muticum*, *S. polyschides* and *C. tomentosum* are characterized by low fat content (0.6–3.6%), combined with a specific fatty acid profile, with a predominance of palmitic and arachidonic acid. Another study (Jay et al., 2018) also focuses on the fact that palmitic acid is predominant in the fatty acid profile of different algae species – in *Chlorella vulgaris* its amount reaches 22.8%, and in *Nannochloropsis gaditana* – 53.4%. Fradique et al. (2013) study the effect of *Isochrysis galbana* and *Diacronema vlkianum* on the fatty acid composition of pasta. They point out that both types of algae are rich in palmitic acid (2711 mg/100 g DW for *Isochrysis galbana* and 1320 mg/100 g DW for *Diacronema vlkianum* respectively). That's why after the incorporation of aquacultures palmitic acid (16:0) is the main SFA present in raw pastas. In the control sample, palmitic acid represents 21.75% of the total fatty acids, while in the enriched sample – 23.67%. For a long time, high intake of palmitic acid has been associated with harmful health effects. In fact, it is the most common saturated fatty acid, representing 20–30% of the total fatty acids in the human body and can be obtained through food or synthesized endogenously. To maintain the balance of membrane phospholipids, the optimal intake of palmitic acid in a certain ratio with unsaturated fatty acids, especially omega-6 and omega-3 (Carta et al., 2017) is crucial.

The amount of stearic acid increases when enriching bread with kelp seaweed, and when using *S. platensis*, 2%, the reported result was similar and lower in the sample with 4%. Probably the reason is that kelp brown algae is rich in this fatty acid, unlike *Spirulina platensis*. This makes sense because algae of different species have different fatty acid contents, and those of the same species can vary greatly depending on different growing conditions, techniques and cultivation environment. Relatively low content of stearic acid, which does not affect its amount in fortified wheat products, found other authors too (Fradique et al., 2013).

The content of archaic acid was influenced (but not significantly) by the inclusion of algae in the bread recipe. In contrast, in the case of heneicosanoic acid, there was a clear difference in the results for the tested samples. In the control sample, the amount was 0.17 g/100 g fat. The addition of both types of aquacultures led to an increase in the content of this fatty acid. In the enriched samples the quantities were as follows: for bread with 2% of kelp in 2.2 times higher than in the control, for bread with 4% of kelp in 3.53 times, for bread with 2% of *S. platensis* in 3,4 times, for bread with 4% of *S. platensis* in 3.05 times higher.

Other authors have also studied the fatty acid profile of different species of algae. According to Gosch et al. (2012) the highest relative share of C16:0 (palmitic acid) is in relation to the total fatty acid content of red algae, followed by green and brown. Another

study found that *Spirulina platensis* contained 33.68 – 66.75% saturated fatty acids and 28.20 – 47.78% polyunsaturated fatty acids. Eicosapentaenoic acid and docosahexaenoic acid were found only in individual samples (Diraman et al., 2009).

Until recently, it was thought that high saturated fatty acid intake was associated with some negative health effects (Kromhout et al., 2000). However, more recent studies show that this view is not true (Mozaffarian et al., 2004). A number of scientific studies have proven the ability of saturated fatty acids to be transformed into unsaturated fatty acids in the human body. Each of the saturated fatty acids from C12:0 (lauric acid) to C18:0 (stearic acid) is converted to the corresponding monounsaturated acid under the action of the enzyme Δ9-desaturase (stearoyl-CoA-desaturase), but with different efficiency. Evidence has been presented that palmitic acid (C16:0) can also be desaturated from the enzyme Δ6-desaturase (Guillou et al., 2003) to sapienic acid (C16:1n-10) (Ge et al., 2003).

Effect of *Spirulina platensis* and kelp algae addition on the content of unsaturated fatty acids in wheat bread

According to Polat et al. (2013), the content of monounsaturated fatty acids (MUFA) in different algae species varies from 12.52% to 32.94%, with the highest content found in *Dasya rigidula* algae harvested in autumn. Oleic acid is a monounsaturated omega-9 fatty acid found in various foods of animal and plant origin. The content of oleic acid in algae varies greatly depending on the species, region and season of extraction. Thus, in green algae *Ulva lactuca,* harvested off the coast of northern California in November, the oleic acid content was 1% (Khotimchenko et al., 2002), while in *U. lactuca*, obtained from the shores of the North Sea in September/October, the amount reached 20% (van Ginneken et al., 2011).

The results on the effect of *Spirulina platensis* and kelp algae on the oleic acid content of wheat bread are presented in Figure 1.
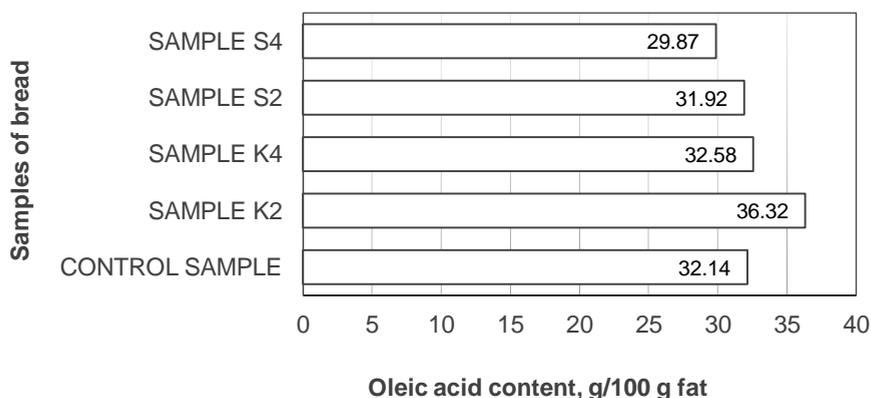
Figure 1. Effect of addition of algae *Spirulina platensis* and kelp on the oleic acid content in wheat bread
Source: author's research

The amount of oleic acid reported in the control sample is 32.14 g/100 g fat and it is comparable to the results found for samples K4, S2 and S4. Giaretta et al. (2018) found the content of oleic acid in wheat bread 24.53 mg/100 g of total lipids, indicating that it is the predominant monounsaturated fatty acid in bread. The most pronounced influence on the

content of this fatty acid in wheat bread had the enrichment with 2% of kelp seaweed – there was an increase of 13%. This result is supported by data published by Matanjun et al. (2008). According to the authors, brown algae, which includes kelp, is rich in oleic acid. Silva et al. (2013) studied the fatty acid profile of ten brown macroalgae and pointed out that oleic acid was in general the most abundant monounsaturated fatty acid, representing 2.3–12.1% of total content. Khotimchenko et al. (2002) also found this acid to be one of the major MUFA in other brown algae species. That is why enriching bread with kelp is more effective in increasing the oleic acid content. Another study evaluated the partial replacement of pea flour by *Chlorella sorokiniana* biomass powder to increase the nutritional quality of gluten free bread. The oleic acid was the primary fatty acid found in the bread samples, ranging from 46.4 to 50.6% of the total fatty acids (Diprat et al., 2020).

Paulinic acid is also monounsaturated, but contains 20 carbon atoms and is an omega-7 fatty acid. It is involved in the metabolism of lipids and fatty acids, and is also needed for the formation and maintenance of cell membranes. The results obtained in determining the content of paulinic acid in the tested bread samples are presented in Figure 2.
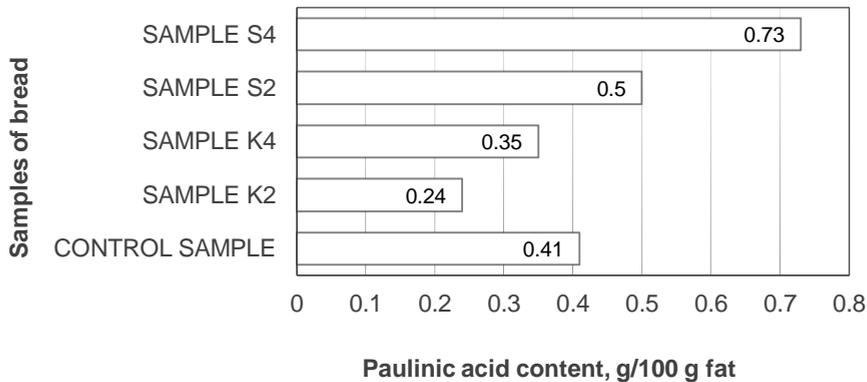
Figure 2. Effect of algae *Spirulina platensis* and kelp addition on the paullinic acid content in wheat bread
Source: author's research

Experimental results show that the inclusion of kelp algae in the recipe is accompanied by a reduction in the amount of paullinic acid in wheat bread. In its enrichment with *Spirulina platensis* an increase in the available amount was reported, and it is weaker when the aquaculture is added in the amount of 2% by the weight of flour – by 18%. In the sample prepared with 4% of *Spirulina platensis*, the amount of paulinic acid was 1.78 times higher than in the control sample, 3 times higher than in sample K2 and 2.08 times higher than in sample K4. There is no specific literature data on the content of paullinic acid in *Spirulina platensis*, but the results presented in this study give a reason to believe that this kind of algae is rich in paullinic acid.

The linoleic acid content of the control bread sample was 54.37 g / 100 g fat. Very close to this are the results published by Lazova-Borisova et al. (2019) – 54.09 g/100 g fat (although in rye bread). Another study points out that the linoleic acid and the α-linolenic acid were the only unsaturated fatty acids in bread, representing around 23 and 5.5% of total fatty acids, respectively (Diprat et al., 2020). When kelp seaweed was included in the composition of wheat bread, the amount of linoleic acid decreased insignificantly compared to that in the control sample. Enrichment with 4% of algae *Spirulina platensis* led to an increase in the

content of this fatty acid by 2.35%, while at lower doses the effect was less significant. Diraman et al. (2009) published research, according to which *Spirulina platensis* is a rich source of γ-linolenic acid, which represents 4.07 – 22.51% of fatty acids. The results for the composition of 10 strains of *Spirulina* show the highest content of γ-linolenic acid and linoleic acid, if it grown at 20 °C (Mühling et al., 2005), which proves the influence of environmental factors. The data are also confirmed by other authors, according to which *Spirulina* algae are rich in essential fatty acids (Belay et al., 1993; Duda-Chodak, 2013).

Until now, fish oil was considered the main source of omega-3 and omega-6 long-chain polyunsaturated fatty acids. It should be noted, however, that they are not synthesized in the body of fish, but in seaweed and phytoplankton, which are their main food source (Nordy et al., 1989). It is therefore of interest to determine the effect of addition of kelp and *Spirulina platensis* biomass on the α-linolenic acid content in wheat bread. The experimentally obtained results are presented in Figure 3.

Figure 3. Effect of algae *Spirulina platensis* and kelp addition on the content of α-linolenic acid in wheat bread
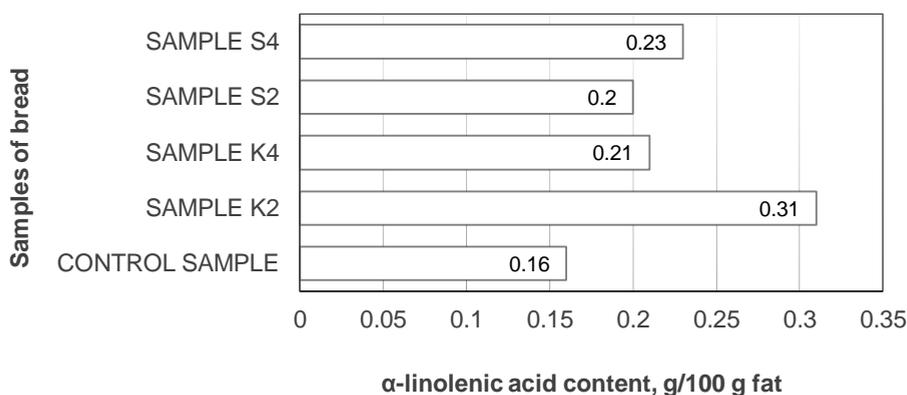*Source: author's research*

As it can be seen from the figure, the lowest content of α-linolenic acid was determined in the control sample – 0.16g/100g fat. Enrichment with both types of aquacultures led to an increase in the amount of this essential fatty acid. The values reported for samples K4, S2 and S4 vary within very narrow limits, while sample K2 had the highest content – 0.31 g / 100 g of fat, which was almost twice as high as in unenriched bread. This is due to the fact that seaweeds, and more precisely brown algae (including kelp), produce polyunsaturated fatty acids, especially long chain fatty acids of the ω-3 series (Colombo et al., 2006). Kumari et al. (2010) emphasized that the availability of linoleic acid, α-linoleic acid, γ-linoleic acid and other polyunsaturated fatty acids with proven nutraceutical effect, indicates the potential of brown macroalgae to be included in functional foods. In terms of the other type seaweed used in the study Shabana Ali and Arabi Saleh reported that the content of α-linolenic acid in *Spirulina* powder was about 8.87% (Ali et al., 2012). Fatty acid profile of *S. platensis* includes saturated fatty acids (46.9%), monounsaturated (7.8%) and polyunsaturated fatty acids (42.8%) with γ-linolenic acid as the most abundant PUFA (Sahbazizadeh et al., 2015). The effect of *Spirulina* on the amount of fatty acids in other bakery products has been studied. Cookies contain γ-linolenic acid of 2.54; 2.78; 2.80 and 2.73% at 0, 0.5, 1 and 1.5% of *S. platensis* microalgal biomass incorporation, respectively. The levels of γ-linolenic acid were increased in fortified cookies, even after baking, whereas all the other fatty acids, mainly

provided by shortening, showed large variations. The authors suggested that the microalgae cells could resist thermal treatment, encapsulating the fatty acid molecules, thus protecting them from oxidation (Prabhasankar et al., 2009).

Different species of algae have a different fatty acid profile, and there is no consensus among the authors which factor is crucial – genetic characteristics or environmental conditions. Some authors believe that more important are the genetic features (Matanjun et al., 2008; van Ginneken et al., 2011), while others consider the influence of the environment to be decisive, including: temperature (Colombo et al., 2006), harvesting season of aquaculture (Denis et al., 2010), salinity of sea water (Floreto et al., 1998) and its mineral content (Sanina et al., 2004). Japanese researchers have found that in *Spirulina platensis* a higher content of polyunsaturated fatty acids (and in particular – γ-linolenic acid) can be achieved by culturing the algae in direct light, and then leaving them in the dark for a week (Hirano et al., 1990).

Different authors highlight that algae have been reported overall to have a low lipid content, but their fatty acid composition is superior to those of the terrestrial vegetables (Darcy-Vrillon, 1993; Susanto et al., 2016). They are rich in polyunsaturated fatty acids with nutritional value and thus have to be studied extensively for biotechnological and food applications (Chandini et al., 2008).

Conclusions

1. The enrichment of wheat bread with seaweed has an impact on the content of fatty acids. As different species of algae have a different fatty acid profile, the two aquacultures used in the study (*Spirulina platensis* and kelp) affect the amount of individual fatty acids differently.
2. The inclusion of kelp in bread recipes leads to an increase in the content of some saturated fatty acids – stearic, arachidonic and heneicosanoic acids. When *Spirulina platensis* (at the amount of 2 or 4% on the basis of flour) is added to the raw materials for bread making, the amount of caproic, palmitic, arachic and heneicosanoic acids increases.
3. The amount of unsaturated fatty acids is also affected by the enrichment of wheat bread with algae. When kelp is included in the bread recipe, a higher content of oleic and α-linolenic acids is measured, while in the case of paulinic acid, enrichment with *Spirulina platensis* is more efficient.

These findings confirm the importance of algae incorporation in traditional foods (such as wheat bread) as an easily accessible way to enhance the nutritional value. It can be definitely said that with appropriate selection of the types and quantities of added aquaculture, the desired impact on the fatty acid profile of bread and the resulting healthy effect for consumers can be achieved.

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Oenological characterisation of white wines produced from some Georgian grape varieties using Kakhetian winemaking methods

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Abstract

Introduction. The aim of the study was to highlight the quality parameters (oenological characterisation and content of bioactive compounds) of wines based on their varietal origin and winemaking methods without environmental, soil, viticulture, and industrial environment.

Materials and methods. Four white grape varieties Rkatsiteli, Mtsvane Kakhuri, Kisi, and Khikhvi were used to make twelve wine samples. "Kakhetian" winemaking technique was applied by spontaneous must fermentation with grape's skins, seeds, peduncles, pips, and stalk. The content of organic acids was determined by the HPLC method. The total tannin content was analysed by the titration method. Spectrometric methods were used to measure the total phenolic content and total antioxidant activity.

Results and discussion. The research demonstrated that the quality characteristics of Kisi and Khikhvi wines were better than those of Rkatsiteli and Mtsvane Kakhuri. However, Khikhvi showed higher results related to the technological parameters: the content of alcohol ranged between 13.6 and 13.7%; the content of reducing sugars was between 3.7 and 4.0 g/L. Those values were predictable due to the high sugar concentration in Khikhvi and Kisi's grape juice. Concentrations of volatile acids (VA) depend on intracellular metabolism during vinification and can cause differences in their values, which varied from 0.40 to 0.46 g/L. In addition, concentrations of malic, citric, and succinic acids varied from 1.72 to 1.85 g/L, from 0.007 to 0.72 g/L, and from 1.05 to 1.5 g/L, respectively. Mtsvane Kakhuri differed by the composition of the organic acids and revealed the highest tartaric acid content, 1.42–1.95 g/L, within the studied wine samples. Both grape variety and yeast strain can cause variations in the content of organic acids during spontaneous fermentation. The content of bioactive compounds was higher in the Kisi wine samples than in the rest of the analysed white wines. Total tannin content ranged from 0.123 to 0.155%, total phenolic content varied from 636.4–743.7 mg/L gallic acid equivalents and possessed a total antioxidant activity of 651.2–2629.8 mg/L in the Kisi samples. Therefore, it seems possible that the grape cultivar also played a significant role in the content of phenolic compounds and tannins. Furthermore, a high positive correlation was found between total tannins content and antioxidant activity (R^2 =0.8871), which was stronger than the correlation between total phenolic content and antioxidant activity with R^2=0.8324. This could be explained by the different chemical structures of bioactive compounds, particularly the quantitative content of the OH group.

Conclusion. The "Kakhetian" winemaking method is advantageous by enhancing wine with oenological and bioactive compounds and ensures obtaining high-quality wine. Additionally, the quality of the wines is highly correlated with the grape cultivar.

Introduction

Wine contains various chemical compounds, including plant secondary metabolic products, which affect the nutritional quality of food, wine, and other beverages (Morata and Loira, 2016). The quality parameters of wine correlates with its chemical composition and content of bioactive compounds, which get into the wine from different parts of the grape such as skin, pulp, seeds, pips, and stalks (Bora et al., 2016). The composition and quantity of bioactive compounds depend on the grape variety, winemaking and post-fermentation methods (Luna-Guevara et al., 2018). "Kakhetian" technique is an oenological practice based on fermenting and maturing the grape must with all solid parts of the grape: skins, seeds, peduncles, pips, and stalk (Glonti, 2020). In contrast, the classical white winemaking method is described by must fermentation without solid parts of the grape (Ribéreau-Gayon et al., 2006). Characterising Georgian varieties and winemaking methods is vital due to the increasing popularity in the domestic and international market (Kharaishvili et al., 2014; Rytkönen et al., 2021). Rkatsiteli (R), Mtsvane Kakhuri (MK), Kisi (K), and Khikhvi (KH) are one of the autochthonous and widely planted grapevines in Georgia. These grape wines have some of the most commercial relevance for Georgian white wine production (National Statistics Office of Georgia, 2016). Numerous studies have been conducted on the correlation between chemical and bioactive compounds and the antioxidant properties of wine. However, access is limited to the most commercially available Georgian white grape varieties fermented by the traditional winemaking method. Especially white wines according to potential chemical descriptions without the influence of terroirs and viticulture methods (Gurgenidze et al., 2019; Sordia, 2020; Tauchen et al., 2015).

Therefore, the research aims to assess the wine quality based on grape varieties and winemaking methods. For this purpose, the following objectives were established: (1) to determine the major chemical component and organic acids composition (i.e., tartaric, malic, citric, succinic, fumaric acids), total phenolic and total tannin content and (2) to find a correlation, if any, between content of bioactive compounds and antioxidant activity in studied wines (Rkatsiteli, Mtsvane Kakhuri, Kisi, and Khikhvi varieties).

Materials and methods

Wine samples

Four individual white grape varieties were selected to make wine, which were as follows- Rkatsiteli (R), Mtsvane Kakhuri (MK), Kisi (K), and Khikhvi (KH). For each grape variety, three simultaneous micro-vinification was carried out. As a result, twelve wine samples were collected and used for further analysis (Table 1).

The grapes were obtained from the experimental base of perennial crops at Georgian Scientific Research Centre of Agriculture, Jigaura, Mtskheta. All-wine samples were produced by "Kakhetian" methods, which included spontaneous must fermentation with skins, seeds, peduncles, pips, and stalks of the grape. Each wine was stirred several times a day. Vinification was carried out approximately at 20 ºC. After completed fermentation, maceration was extended for about five months. Later wine samples were filtered and bottled into 750 ml glass vessels, and samples were stored at 4 ºC before analyses.

Wine samples of each micro-vinification

Grape variety	Wine sample	Grape variety	Wine sample
Rkatsiteli	R1 R2 R3	Kisi	K1 K2 K3
Mtsvane Kakhuri	MK1 MK2 MK3	Khikhvi	KH1 KH2 KH3

Grape juice sugar concentration was determined refractometrically. The main chemical wine indicators, alcohol content (AC), pH, residual sugars (RS), total acidity (TA) and volatile acidity (VA), were assessed according to the methods of the International Organization of Vine and Wine (2009, 2015, 2011).

Total tannins content by titration method

Total tannin content (TTC) was determined by the potassium permanganate titration method (Khudbudin al., 2016). Concisely, back (A) and blank (B) titration was conducted for the wine samples. Consumed quantity for the back (A) and blank (B) titration were recoding for later calculations. The back titration: 5 mL wine with 10 mL distilled water was heated until the volume was decreased to 5 mL. Another 10 mL of water with Indigo carmine (2 mL, 0.5%) was added, and titration was carried out by Potassium permanganate (0.004 M) until the golden yellow colour was noted. For blank (B) titration – charcoal (1g) was mixed with a 25 mL wine sample and was kept at room temperature for a quarter of an hour. For titration, the back titration producer was carried out. The presence of tannin in wine can be calculated using the following equation:

$$TTC = 0.01664 \cdot (A - B) \tag{1}$$

where A is a volume of 0.004 M $KMnO_4$ consumed by the back sample in mL;

B is a volume of 0.004 M $KMnO_4$ consumed by the blank sample in mL;

The standard tannin solution for which 1 mL of 0.004 M $KMnO_4$ = 0.0832 mg of tannin, therefore, percent of tannins in wine is $0.01664 \cdot (A - B)$

Total phenolic content

Total phenolic content (TPC) was measured by methods (Singleton et al., 1999) with slight modification. Concisely, the total phenolic content was determined using the Folin-Ciocalteu reagent and spectrophotometric method and expressed as mg/L gallic acid equivalent. First, 1 mL of 20 times diluted wine sample was mixed with ten times diluted 5 mL Folin-Ciocalteu reagent and was kept at room temperature. Then, about 8 minutes later solution was mixed with sodium carbonate (4 mL) and was measured at 765 nm wavelength after an hour delay again at room temperature.

The correlation of gallic acid standard solution concentration (10–50 µg/mL) and absorbance was used to calculate TPC (R^2=0.983). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per litre of wine.

Total antioxidant activity

A quantitative equivalent of ascorbic acid was used to measure total antioxidant activity (AOA) (Benzie and Strain, 1996) with minor variation. For AOA determination ferric reducing ability of plasma (FRAP) solution was prepared by the concentration 1:1:10–0.01M 2,4,6-tripyridyl-s-triazine (TPTZ) diluted with 0.04 M hydrochloric acid, 0.02 M Iron (III) chloride, and 0.3 M acetate buffer (pH 3.6). The resulting solution and 1mM ascorbic acid were placed in a water bath at 37°C for a quarter of an hour. 1000 μmol/L solution of $FeSO_4 \cdot 7H_2O$ was used to calibrate the spectrophotometer. First, the 100 μL wine sample was added to 3 mL of working solution; later, the absorbance was determined by 593 nm wavelength. The result was fixed after 4 minutes. A working solution was used for control, and ascorbic acid was used for comparison.

Determination of organic acid by HPLC

Organic acid determination was carried out according to the International Organization of Vine and Wine method (OIV, 2020). Concisely, the sample was filtered by nylon membrane (0.45 mm), and a volume of 10 μL was injected into the C18 column (4 X 250:5mm) of HPLC (Varian Prostar 500, Walnut Creek, California, USA). The organic acid was detected at 210 nm. The eluent was KH_2PO_4, and an ammonium sulphate solution with pH 2.1. Identification was performed by calculating pure compound retention times. Calibration curves were applied to determine quantification.

Statistical analysis

Three replicates were performed for all data and were expressed as mean values ±Standard Deviation (SD). Pearson correlation between two variables was determined. In addition, the ANOVA was used to represent variance among chemical data in individual wines produced by different grapevines. All statistical analyses were performed with Microsoft Excel (Microsoft 365, 2021).

Results and discussion

Oenological composition

The physicochemical composition of the studied wines is presented in Table 2. Regarding the alcoholic content, no significant differences were observed between the analysed samples. The average alcohol content among samples was 13.3% (12.6–13.7%). All studied samples had higher residual sugar concentrations than Georgian dry wines, which were predictable due to the sugar content in grape juice. Residual sugar concentration (1.6–4.0 g/L) indicated complications of the fermentation process. Sugar content in grape juice was as follows: Rkatsiteli – 21.2 g/100 mL; Mtsvane Kakhuri – 21.8 g/100 mL; Kisi – 24.0 g/100 mL; Khikhvi – 28.7 g/100 mL. For white wines maximum limit of volatile acids is1.0 g/L and total acidity should be not less than 4 g/L (Resolution No. 3 of 2014 of Georgian Government on General Rules for Production of Grape Wines). All sample studied herein corresponded to the local regulations and had the content ranged: TA from 5.03±0.03 to 6.08±0.00g/L, and VA from 0.26±0.01 to 0.46±0.05g/L. Khikhvi wine exhibited the highest content of VA 0.46±0.05 g/L and pH 4.2 and the lowest content of TA 5.03±0.06 g/L.

Physicochemical composition of studied wines

Wine sample	Reducing sugar, g/L	Alcoholic content,% vol.	pH	Volatile acidity, g/L	Total acidity, g/L
R1	1.63±0.01	13.00±0.09	4.1	0.26±0.01	5.55±0.05
R2	1.72±0.01	13.20±0.01	4.1	0.33±0.01	5.70±0.05
R3	1.72±0.02	12.60±0.01	4.1	0.33±0.00	5.40±0.05
MK1	1.82±0.01	13.00±0.03	4.0	0.33±0.00	5.18±0.07
MK2	1.58±0.05	13.30±0.00	4.0	0.33±0.03	5.33±0.05
MK3	1.92±0.09	13.20±0.00	4.0	0.33±0.01	5.25±0.03
K1	3.78±0.17	13.20±0.00	4.1	0.33±0.04	5.93±0.02
K2	2.54±0.23	13.50±0.00	4.1	0.40±0.01	6.08±0.00
K3	3.99±0.16	13.00±0.03	4.1	0.33±0.00	6.00±0.01
KH1	3.99±0.14	13.60±0.05	4.2	0.46±0.05	5.18±0.05
KH2	3.68±0.2	13.70±0.00	4.2	0.40±0.07	5.03±0.06
KH3	3.17±0.07	13.70±0.05	4.2	0.40±0.02	5.18±0.04
Mean	2.63	13.25	4.1	0.35	5.48
Max	3.99	13.7	4.2	0.46	6.08
Min	1.58	12.6	4.0	0.26	5.03

Organic acid composition

The content of tartaric, malic, citric, succinic, and fumaric acids were determined in studied white wines (Table 3).

Organic acids concentration of the studied wines

Wine sample	Tartaric acid, g/L	Malic acid, g/L	Citric acid, g/L	Succinic acid, g/L	Fumaric acid, g/L
R1	1.38±0.02	0.29±0.05	0.085±0.01	N/A*	N/A
R2	1.63±0.07	0.3±0.29	0.058±0.02	N/A	N/A
R3	1.44±0.01	0.32±0.12	0.052±0.01	N/A	N/A
MK1	1.95±0.03	0.09±0.04	0.001±0.01	N/A	1.3±0.23
MK2	1.48±0.02	0.33±0.09	0.66±0.12	0.46±0.04	N/A
MK3	1.42±0.09	0.13±0.12	0.65±0.10	0.9±0.09	N/A
K1	1.51±0.04	0.6±0.04	0.006±0.00	N/A	N/A
K2	1.63±0.06	0.94±0.14	0.007±0.00	N/A	N/A
K3	1.49±0.02	1.18±0.08	0.092±0.01	N/A	N/A
KH1	1.37±0.01	1.78±0.07	0.095±0.01	1.23±0.16	0.1±0.01
KH2	1.18±0.03	1.72±0.05	0.72±0.2	1.05±0.18	N/A
KH3	1.38±0.08	1.85±0.13	0.007±0.01	1.5±0.25	N/A
Mean	1.488	0.794	0.203	1.328	0.655
Min	1.180	0.090	0.001	0.460	0.100
Max	1.950	1.850	0.720	1.500	1.300

*Not determined.

The tartaric acid concentration varied from 1.18 g/L to 1.95 g/L with a mean of 1.49 g/L. Tartaric acid has an essential role in wine sensory perception (Volschenk et al., 2006). Thus, tartaric acid content in Mtsvane Kakhuri wines was in a range from 1.95±0.03 to 1.42±0.09 g/L with mean 1.62 g/L, in Kisi wines varied from 1.49±0.02 to 1.63±0.06 g/L with mean 1.54 g/L, in Rkatsiteli wines the tartaric acid ranged from 1.38±0.02 to 1.63±0.07 g/L with mean 1.48 g/L, and in Khikhvi wine its content varied from 1.18±0.03 to 1.38±0.08 g/L with mean 1.31 g/L. According to Waterhouse et al. (2016), tartaric acid concentration changes during ageing through the different physicochemical processes.

Different concentration variations were reported for malic acid, g/L, based on the average value. They were as followed: in Khikhvi wine it was 1.72±0.05 – 1.85±0.13 g/L (mean 1.78 g/L), Kisi wines contained 0.60±0.04 – 1.18±0.08 g/L (mean 0.90 1/L), in Rkatsiteli wines this acid ranged from 0.29±0.05 to 0.32±0.12 g/L (mean 0.30 g/L), in Mtsvane Kakhuri wines it was in a range from 0.09±0.0 to 40.33±0.09 g/L (mean 0.18 g/L).

Similar results were reported by Whiting (1976). Furthermore, according to his study, malic acid content can vary due to spontaneous fermentation.

Presence of citric acid has an essential effect on the sensory profile of the wine and its stability (Mendes Ferreira and Mendes-Faia, 2020). The highest concentration of citric acid was observed in KH2, 0.72±0.2 g/L, and the lowest was found in MK1, 0.001±0.01 g/L. The average content of citric acid among the studied samples was 0.203 g/L.

The succinic acid was detected only in the second and third samples of Mtsvane Kakhuri: 0.46±0.04 and 0.9±0.09 g/L, respectively, and in all samples of Khikhvi wines – 1.05±0.18 – 1.5±0.25 g/L, with an average mean of 1.328 g/L. According to Chidi et al. (2018), succinic acid concentration depends on several factors, especially from yeast strain.

The fumaric acid was detected only in KH1, 0.1±0.01 g/L, and in MK1, 1.3±0.23 g/L. According to Ough and Kunkee (1974), fumaric acid may be metabolised by yeast and therefore were not represented in other samples.

The organic acid composition differs based on the terroirs, grape variety, microbial diversity, and winemaking methods (Chidi et al., 2018). In our study, grape variety strongly effect succinic acid and fumaric acid content. All organic acids were found only in the first sample of Khikhvi wine, and as mentioned above, fumaric acid was observed in MK1 and KH1. Khikhvi wine samples contained a higher average content of all organic acids. However, it is worth underlining that tartaric acid content was lowest compared to the other wine sample. The results of total acid contents, tartaric, malic, and citric acids, were compared to other white wines reported in publications. The average content of tartaric, citric, and succinic acids was similar to results reported by Chahine et al. (2019), meanwhile, the fumaric acid content was higher and malic acid lower in the studied Georgian wine than reported in the above research.

Total tannin and total phenolic contents

Bioactive components are present in much smaller quantities in white wines than in red wines (Pérez-Navarro et al., 2020). Even though, the content of bioactive compounds is low in white wines, studied wines contained relatively high values of total tannin content (TTC) ranged from 0.067±0.001% to 0.155±0.002% with a mean of 0.098% . The highest value of tannins was observed in Kisi, 0.123±0.002 – 0.155±0.002%, followed by Khikhvi, 0.093±0.00 – 0.108±0.001%, and Rkatsiteli, 0.077±0.002 – 0.083±0.002%. The lowest TTC content was found in Mtsvane Kakhuri, 0.067±0.001 – 0.078±0.001% (Table 4). This difference is due to the varietal properties, and in the same wine, the variation may be caused by grape skins and seeds tannins ability to bind with proteins and cell wall material (Watrelot and Norton, 2020).

Bioactive components and antioxidant activity of the studied wines

Wine sample	Total tannin content,%	Total phenolic content, mg GAE /L	Antioxidant activity, mg AAE /L,
R1	0.083±0.002	233.171±8.31	661.247±63.455
R2	0.077±0.002	225.041±7.424	777.155±83.681
R3	0.087±0.002	241.301±3.415	725.852±89.452
MK1	0.067±0.001	174.634±3.032	418.030±13.764
MK2	0.078±0.001	176.260±3.031	317.323±27.925
MK3	0.072±0.001	203.902±6.163	321.123±23.705
K1	0.123±0.002	636.423±31.621	1651.217±19.489
K2	0.155±0.002	743.740±35.111	2460.675±148.209
K3	0.135±0.002	670.569±28.455	2629.787±129.095
KH1	0.103±0.001	504.715±1.849	1548.610±93.637
KH2	0.093±0.002	478.699±8.31	765.754±81.132
KH3	0.108±0.001	486.829±7.424	965.269±79.420
Mean	0.098	397.940	1103.504
Min	0.067	174.634	317.323
Max	0.155	743.740	2629.787

Total phenolic content in the studied wine samples showed the same varietal characteristics as was observed in the case of total tannin quantity: Kisi, 636.423±31.621 – 743.740±35.111 mg GAE/L, followed by Khikhvi, 504.715±1.849 – 478.699±8.31 mg GAE/L, and Rkatsiteli, 225.041±7.424 – 241.301±3.415 mg GAE /L. The lowest content of TP was showed by Mtsvane Kakhuri, 174.634±3.032 – 203.902±6.163 mg GAE/L. According to Sordia (2020) the fermentation and maturation during winemaking by the Kakhetian method ensure phenolic compounds' migration into wine. The same results were shown by Shalashvili et al. (2010) while studying white wine obtained by the Kakhetian method.

The total antioxidant activity presents one of the essential attributes of wine quality and may determine consumer buying behaviour (Droli et al., 2019). In this research, all wines showed relatively high level of AOA ranging between 317.323 – 2629.787 mg AAE/L with average: 721.418 mg AAC/L for Rkatsiteli, 352.1587 mg AAC /L for Mtsvane Kakhuri, 2247.226 mg AAE/L for Kisi and 1093.211 mg AAE/L for Khikhvi. The highest total antioxidant activity, 2629.787±129.095 mg AAE/L, was observed in the third sample of Kisi, which was almost eight time higher than the lowest antioxidant activity in the MK2 sample, 317.323±27.925 mg AAC /L.

High total tannin content and antioxidant activity were found in samples R2 and R3: the total antioxidant activity was 77.16–725.85 mg AAC/L and the content of tannin was 0.08–0.09%. It was similar to KH2: the total antioxidant activity was 765.75 mg AAC/L and the content of tannin was 0.09%. This may be explained by spontaneous fermentation (Vejarano et al., 2019). As a result, in Rkatsiteli, wine bioactive components were ultimately transformed from the grape into wine, while a minimum of them was migrated into the Khikhvi wine sample. The MK1 sample had the lowest total tannin and total phenolic contents. However, the highest total antioxidant activity was found for MK1 among the Mtsvane Kakhuri wine samples. The MK3 showed higher total phenolic content than the MK

2 sample, however content of total tannin showed the opposite; KH1 had lower total tannin content, and higher total antioxidant activity compared to the KH3 sample, having higher TTC and lower AOA. The R2 sample had a lower TTC and TPC than the R3 sample, but AOA was higher for the R2 sample. Similarities were observed between the K2 sample (higher TTC and TPC) and the K3 sample (higher AOA).

Pearson correlation was used to quantify the correlation between bioactive parameters and antioxidant activity. Among all samples, a strong relationship was observed between the TPC-AOA and TTC-AOA. The correlation coefficient for antioxidant activity and total tannin content was 0.832 (Figure 1) and for antioxidant activity and total phenolic content was equal 0.8871 (Figure 2).
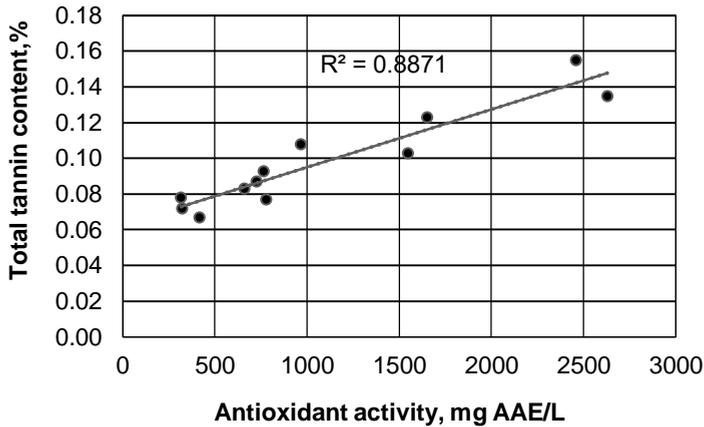

Figure 1. Correlation between the total tannin content and antioxidant activity of Kakhetian style wine samples
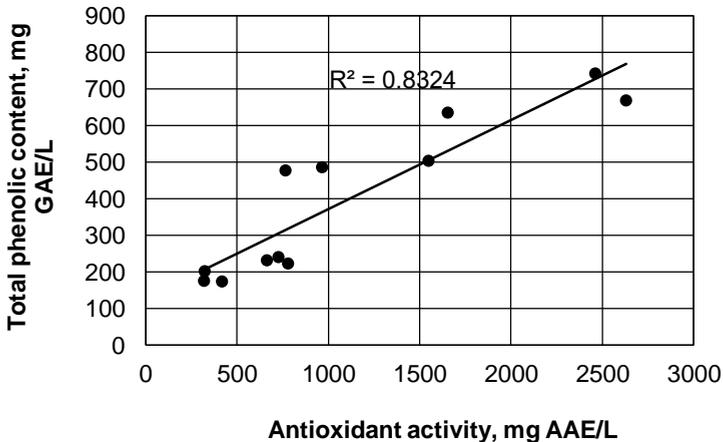

Figure 2. Correlation between the antioxidant activity and total phenolic content of Kakhetian style wine samples

An incomplete correlation and some exemption between TTC-AOA and TPC-AOA can be caused by different chemical structures of bioactive compounds, particularly the quantitative content of the OH group (Bendary et al., 2013). The high correlation between TPC and AOA within Kakhetian style Georgian white wines was described by Khatchapuridze et al., (2021). It is worth mentioning that the present study was focused on micro-vinification, and further studies are needed to observe the same parameters in a large-scale wine production.

Conclusions

1. The technological parameters of the wine produced by the "Kakhetian" method using the Khikhvi grape variety had the highest concentration of the studied technological indicators compared to the rest of the studied white wines. Kisi had the highest content of some bioactive compounds, and the Mtsvane Kakhuri was distinguished by tartaric acid content.
2. The studied wines were rich in bioactive compounds and possessed high antioxidant activity.
3. A high positive correlation was found between total phenolic content and antioxidant activity and between total tannins content and antioxidant activity. It should be mentioned that antioxidant activity and total tannin content were better correlated than antioxidant activity and total phenolic content.

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China and changing food trends: A sustainability transition perspective

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Abstract

Introduction. Global population has witnessed significant changes in the way food is produced and consumed. Although this has benefitted population health, it has also contributed to climate change and unsustainable use of natural resources.

Materials and methods. Comprehensive literature review.

Results and discussion. The characteristics of four transition theories related to food are outlined to help explain population behaviour, namely demographic, nutrition/protein, food and sustainability transition. This is followed by a further desktop analysis of the changes occurring in China, the world's largest demography, and this country's contribution to a most-needed global sustainability transition.

The theoretical framework of transition theories used since the mid-20th century outlines changes in population behaviour impacting relationships between people and more recently with the natural environment. As a multidisciplinary field describing fundamental shifts in human societies, transition theories are very insightful in relation to food and nutrition. The demographic transition links industrialisation with fertility and mortality rates but also with food availability. During the nutrition transition, a change occurs in people's calorie intakes from different food groups. While the share of protein remains relatively stable, the initial transition from plant- to animal-based foods now changes in reverse with increasing ecological and health awareness. This nutrition/protein transition can result in a better dietary behaviour with reduction in over-consumption, losses and waste. The food transition explains the transformations on the supply side – how food is produced, processed and distributed, reflecting changes in agricultural methods, use of land, soil, water, fertilisers and chemicals, supply and distribution chains. More sustainable farming methods are currently being introduced in response to ecologically threatening trends as a result of land-use changes and use of chemicals. As distinct from the other concepts, sustainability transition does not describe an evolutionary pattern of changes but only the current most necessary transformation in development. It requires radical transformation and action towards reduced environmental footprints of all human activities, including food.

China's development has experienced similar transitions although with unique features. Its demographic transition has been influenced by the "one child policy" while the nutrition/protein transition has been fuelled by increasing income levels. Industrialisation of food production with application of chemicals is widespread but more recently, organic methods of farming are gaining momentum. Food security and production are recognised as a challenge and opportunity in China's sustainability transition with state-driven dietary efforts to contain domestic meat consumption.

Conclusion. China has the opportunity to play a prominent role in the global transition to improved food choices, as required by the current environment and climate emergency, by shifting its own eating habits and also contributing to the burgeoning field of new alternatives to livestock products.

Introduction

With a population nearing 1.5 billion (Worldometer, 2022), China is the world's largest demography. Its economic power has consistently risen since the opening up and reforming of the country's economy in 1978, which was followed by an extended period of Gross Domestic Product (GDP) annual growth of 10% and 800 million people lifted out of poverty (World Bank, 2022). China has also become a powerful player in manufacturing and technology development, resulting in being the world's top exporter of goods and services estimated at US$2.72 trillion in 2020 (World Population Review, 2022).

These pronounced changes over a relatively short period of time have significantly improved many aspects of the quality of life of Chinese citizens. One of them is availability and access to food. The Chinese staple diets where rice and other coarse crops occupied a prominent place have gradually been replaced with a variety of foods, including many that are processed and animal-based (Chang et al., 2018). This transition is one of several demographic and socio-economic changes experienced across the globe. The latest and most needed transition is that towards more sustainable ways of living, where environmental, social and economic priorities co-exist in an integrated way that looks after the well-being of people but also the planet.

There is ample evidence (Marinova et al., 2022, Tirado et al., 2018; Willett et al., 2019;) that changes in the way we produce food and in our eating preferences need to be part and a main driver in such a sustainability transition. What this paper explores is China's role in the global transition posing the question how this country can influence its own and planetary future. It uses the theoretical framework of transition theories to first outline the concepts. This is followed by evidence from China. The last section explores opportunities to impact the global sustainability transition through changing food preferences and novel alternative proteins which reduce the presence of livestock-sourced foodstuffs.

Materials and Methods

Material

Food is a basic necessity for human survival (Maslow, 1943). Throughout the centuries, the ways people have satisfied their demand for food has evolved. Globally, starvation and hunger have become less dominant while overweight and obesity have been radically on the increase, particularly among richer sections of society (FAO, 2019). While humanity has been successful in producing more food, this has largely come at the expense of the health of the natural environment and our production and consumption practices are threatening the well-being of all species on the planet (Marinova & Bogueva, 2022). How did we get to this situation? What changes have occurred in the process and most importantly, what transformations are needed to shift the downward spiral trends?

The paper explores the transitions in human behaviour as consumers and producers of food. It particularly analyses the changes in China, the world's largest demography, and links them to the global developments in search of sustainability transition.

Methods

This study is based on desktop analysis of existing literature and secondary data. It covers published and online sources related to major societal shifts in food consumption and production. This analysis allows to synthesise and describe historical trends in human

behaviour as well as outline the importance of future changes towards sustainable development in response to climate change, depletion in soil fertility, biodiversity loss, freshwater use and other major environmental challenges. By systematically integrating insights from a wide range of sources, the study provides a transdisciplinary overview of existing evidence and synthesis of areas in which research has been previously dispersed and disparate (Snyder, 2019).

Results and discussion

Theoretical framework and method

Transition theories are a multidisciplinary field that tries to explain human behaviour and following from that, fundamental shifts in human societies. The theoretical frameworks of transition studies vastly vary – from system thinking (Zolfagharian et al., 2019) to psychology and counselling (Bailey-Taylor, 2009), education (Jindal-Snape, 2021), economics (Topalli & Ivanaj, 2016)*,* innovation (Twomey & Gaziulusoy, 2014), technology (Paredis, 2011), sustainable development (Geels, 2011) and politics (Avelino et al., 2016), offering different perspectives ranging from individual conducts of people and organisations to multi-level societal and governance developments to technological elements and new knowledge generation.

Another distinctive area of interest in transition studies is population health and nutrition (Santosa et al., 2014). In fact, the oldest transition theory, namely the demographic transition (from high to low birth and death rates) developed in 1945 by Notestein (Diggs, 2020), originated in population studies. Furthermore, epidemiological transition analyses disease and mortality patterns across human populations (McKeown, 2009) while in the field of food, nutrition transition describes major dietary changes (Popkin, 2006), including the occurrence of obesity (Poulain, 2009).

Transition theory frameworks are also applied to area-based studies, such as related to Eastern Europe (Genov, 2021), China (Hong, 2016), Sub-Saharan Africa (Leshabari, 2021), Latin America (Juri et al., 2021), the European Union (European Environment Agency, 2017) or USA (Gersten, 2021). It is interesting to note the specific features of each transition as they relate to the geo-political, cultural and historical background of the place as well as access to technologies and knowledge.

A common feature of all transition theories' approaches is the difficulties to support the conceptual frameworks with reliable statistical data, quantitatively verifiable models and other hard evidence. There is also ambiguity in definitions, opacity of the way boundaries are established and how stability and change are conceptualised (Zolfagharian et al., 2019). Despite these challenges, transition theories continue to experience substantial interest from researchers and growth in conceptual, empirical and methodological insights. This contributes to building a broader and pluralistic body of knowledge that can guide and inform fundamental shifts in socio-technical systems (Zolfagharian et al., 2019) as well as the policy arena.

The work presented in this paper is based on literature review. We bring together four transition theories that are related to food production and consumer choices, covering in a historical order demographic transition, nutrition/protein transition, food transition and finishing with sustainability transition. Linking this analysis to the role of China in the last section is important on two levels: first, the size of the Chinese economy, and second, its access to traditional knowledge and production capacities.

Transition theories

Transition theories received a lot of attention since mid-20th century and are now increasingly becoming a burgeoning area of research. A Google Scholar search with the keyword "transition theory" produces 1.79 million hits between 1945 and 2009, and 2.26 million between 2010 and 2022. The focus here however is only on transition theories that are connected to development and food. These transitions are not happening separately or in a purely consecutive way. They are interlinked and, in many ways, synergistically reinforce each other. Another important conceptual observation is that whilst the demographic, nutrition/protein and food transitions have already occurred, transitioning to sustainability is currently emerging as a global priority, driven particularly by the nature of our food systems and dietary choices (Willett et al., 2019). Let's look at the explanatory patterns that these theories offer.

Demographic transition

Conceptualised in the 1940s by the demographer Notestein, this transition model describes stages in population growth based on fertility and mortality rates (see Figure 1) during which societies transition from a relatively stable low to relatively stable high population size.
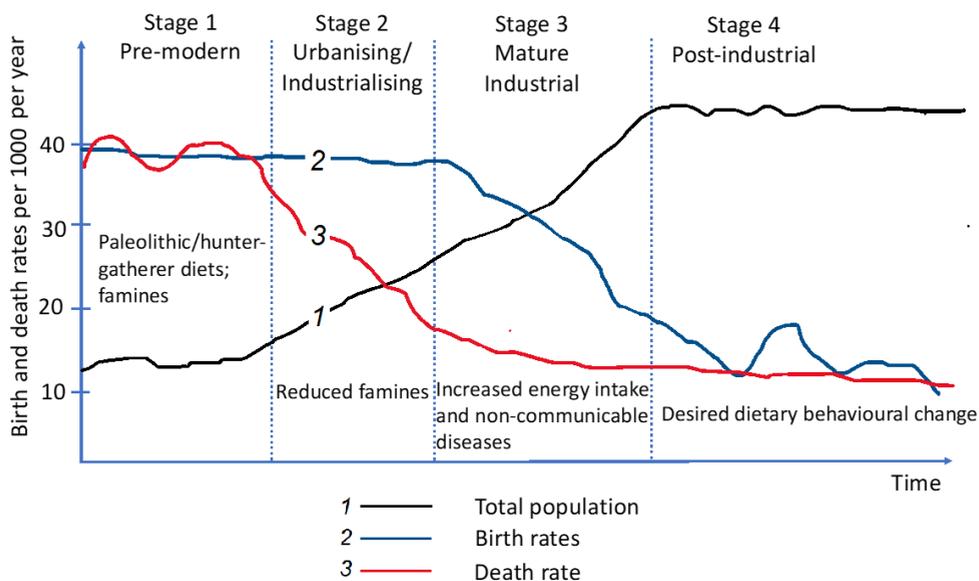
Figure 1. Demographic and nutritional transition model

These stages are also linked to urbanisation and industrialisation (GeogSpace, 2015). At Stage 1 or pre-transition, birth and death rates in so-called traditional societies are high but they cancel each other out, people's life expectancies are low and population numbers remain stable. This is followed by Stage 2 or the first phase of transition during which death rates significantly drop due to improvements in health care, medicine, hygiene, sanitation as

well as improved production, transportation and distribution of food. Societies also commence to modernise through industrialisation and become more urbanised. Birth rates however take longer to decrease as they are defined mainly by social factors, such as how society values children, their contribution to the household and old-age security of parents. As a result from the balance between birth and death rates, population numbers sharply increase. At Stage 3 or the second phase of transition, fertility rates also drop because of the changing social status of women, improved standards of living, increased mechanisation and less demand for workers as well as availability of family planning and contraceptives. Consequently, the combination between low death and birth rates results in steady populations at higher numbers than the original starting point. This leads to Stage 4 or post-transition, described also as post-industrial societies (GeogSpace, 2015).

Being a model, the demographic transition has its limitations in describing universal patterns in population changes. It has been a benchmark theoretical framework for western countries, particularly USA and Europe, while its applicability in other parts of the world, such as Africa and Asia, has remained unclear. The demographic transition concept is very much aligned with Adam Smith's "invisible hand" and liberal ideology which excludes the role of government in influencing demographic trends (Poulain, 2021). Different theories have been put forward, and in some cases statistically tested (Ranganathan et al., 2015), to explain the demographic transition, such as links between fertility, mortality and GDP or the role of education for women.

What happens at the post-transition stage is also unclear and subject to interpretation. Evidence from many European societies, such as Bulgaria, Germany, Italy or Sweden, as well as from highly industrialised countries, such as Japan or South Korea, shows birth rates falling behind death rates. Without migration, the populations of such countries would shrink. A shift in attitudes and norms within society from altruistic behaviour towards greater individual freedom and self-actualisation, manifested through delayed age of giving birth, the deinstitutionalising of the marriage and diversity in union types, results in decline in fertility rates below replacement levels described by some as the second demographic transition (Lesthaeghe, 2014; Zaidi & Morgan, 2017). Again, the role of government policies in influencing people's fertility behaviours is rarely discussed.

From the perspective of the demographic transition, China is very unique. Between 1980 and 2015, China had the "one-child policy" which restricted the number of children born in the majority of families to one. Access to adequate food and nutrition for China's large population was part of the state policy considerations. The "one-child policy" rapidly pushed the country into a post-transitional society where fast industrialisation was accompanied with increased life expectancies, improved living standards and government-encouraged below-replacement level of fertility (Feng & Mason, 2007). China is seen as an "overachiever" in its demographic transition which happened at an unparallel pace with the exceptional role of the state (Feng, 2011).

After the abolition of the "one-child policy", the post-transition demographic stage in China is yet to be fully understood, particularly with the impacts of the COVID-19 pandemic. What some describe as an "economic demographic transition" (Johnston, 2020) and "economic miracles" (Yuan & Gao, 2020) is now challenged by China's need to reduce its environmental impacts, including those related to food production and consumption.

Nutrition/protein transition

The term nutrition transition was used for the first time by Popkin in 1993 (Popkin, 1993). It describes a link between GDP per capita and structural changes in people's calorie intakes from different food groups with a shift from plant- to animal-based products. There is also wider availability of food. Increase in the overall calorie or energy intake is observed with the nutrition transition leading to obesity, including childhood obesity, and non-communicable diseases (Drewnowski & Popkin, 1997; Popkin, 2016). The evolution of diets loosely follows the demographic transition (see Figure 1). According to Popkin (2002, 2006) and Poulain (2021; Drenowski & Poulain, 2019), the following nutritional changes occur:

− Paleolithic and hunter-gatherer diets included a lot of fibre and carbohydrates from wild plants and low-in-fat meat from wild animals;
− Increased population numbers, establishment of settlements and the development of agricultural practices made cereals the main source of food but nutritional deficiencies and famines became a regular occurrence;
− The start of industrialisation and urbanisation reduced the exposure to famines and diets were based mainly on starchy foods, low in fat and with a lot of fibre; iron deficiencies were common;
− Technological progress, the use of fertilisers, mechanised equipment, irrigation and livestock husbandry, improved food security; this was accompanied with development of food processing, storage and distribution methods; overall the preferences for animal-based products, fats, sugar and processed foods increased contributing to larger energy intakes and leading to obesity and non-communicable diseases;
− Due to concerns related to health, climate change, safeguarding of the natural environment and animal welfare, people who live in societies with ample availability and choice of foods and who are sustainability aware, are making a conscientious decision to change their behavioural practices towards increased intake of vegetables, fruits, legumes, nuts and other plant-based options and reduced consumption of animal-sourced products.

A subset of the nutrition transition is the protein transition which highlights specifically the changes in relation to the sources of proteins. While the actual share of protein in the human diet remains relatively constant between 8% and 16% (Carpenter et al., 2021), the initial changes during the transition are from plant- to animal-based foods and more recently, in reverse – from animal- to plant-based foods, because of increased ecological and health awareness (Drewnowski & Poulain, 2019; Poulain, 2021; Tziva et al., 2020). Aiking and de Boer (2020) explain that the next protein transition is from primarily animal towards plant protein products, including analogues and whole foods such as beans and nuts, combined with reduction in over-consumption and of losses and waste during the supply chain and in the household. This will lead overall to better dietary behaviour.

Similar nutritional/protein transition has been observed in China. During the country's accelerated demographic transition, many measures were taken to reduce malnutrition and provide adequate access to food for all sections of society. This however happened with a shift towards increased animal-sourced foods as manifested through higher levels of consumption of meat, mainly pork (see Figure 2), and eggs (Popkin et al., 2012). With 90% of the Chinese population being lactose intolerant (Yang et al., 2013), dairy-based products have not experienced such a growth. However, the intake of processed foods increased while that of legumes, particularly soy, vegetables and fruits decreased. The prevalence of hypertension and diabetes in China has also been linked to increased energy intake combined with reduced physical activity and sedentary lifestyle (Popkin et al., 2012). China's "one-child policy" further contributed to overindulging children in energy-rich foods whose taste they like (Dearth-Wesley et al., 2011). Only disease outbreaks, such as the African swine fever in 2018–2019, have slowed down China's appetite for meat (see Figure 2).
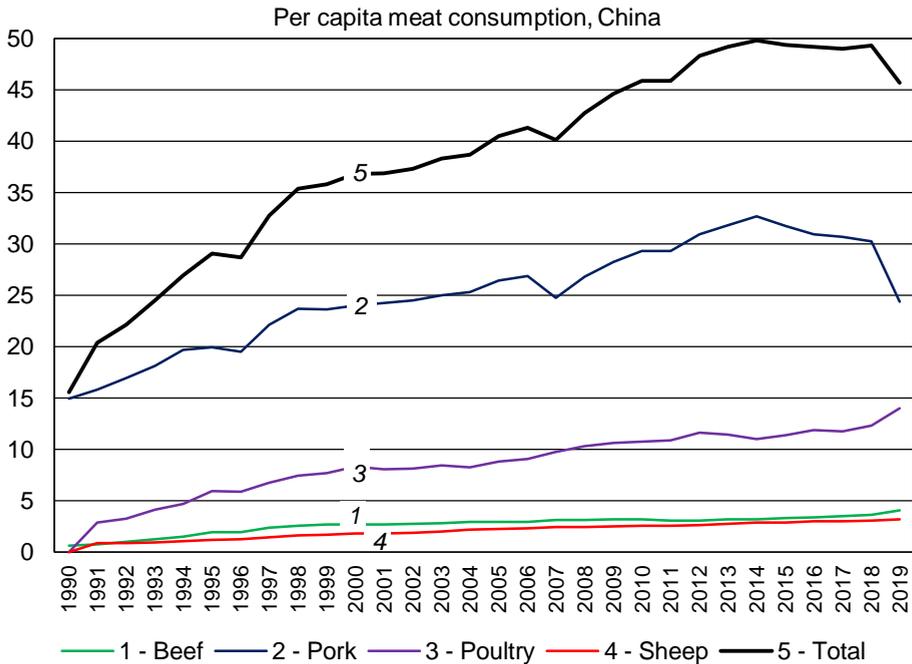
Per capita meat consumption, China

Figure 2. Annual per capita meat consumption, China, 1990–2019 (kg/person)

As in other parts of the world, the analysis of China's dietary patterns shows the significant benefits of whole-food, plant-based options and the surging interest in such choices (Campbell & Campbell, 2017). Educating Chinese citizens is revealed to be the most important factor in creating awareness about the benefits of a dietary balance and healthy staple diets (Chang et al., 2018) which is likely to result in desired behavioural changes.

Food transition

Food transition relates to transformations in the ways food is produced, processed and distributed. Although this area is more loosely defined compared to the other transitions, it reflects changes in agricultural methods – from subsistence to broad-acre farming, use of resources, such as land, soil, water, fertilisers and chemicals, supply and distribution chains and globalisation of food production. In economic terms, it describes the supply side of food, rather than demand represented through consumer needs and preferences as described in the nutrition/protein transition, although the two are interrelated.

Parallels can also be drawn with the other transitions as food production and supply were defining characteristics of historical periods in socio-economic development and the search for improved quality of life. The following four types of agricultural systems describe the food transition in modern times:

− Subsistence agriculture – localised on small plots of land surrounding the place of abode, small-scale to satisfy the needs of a family unit, diverse species of crops are

grown in season with primitive technology used, it is labour-intensive and with relatively small yields, livestock are raised but animal-based products are sparsely consumed; it is focussed on survival with little surplus for marketing or community sharing, crop failure or livestock dying expose the household at the risk of starvation; subsistence agriculture however is attuned to nature's cycles and less exploitative of natural resources, such as soil and water; it continues to be practised by rural communities in less industrialised parts of the world, such as in Africa (Mbatha et al., 2021) or Asia (Holmelin, 2021).

- Farming – this is mainly commercial food production on specially designated land cleared of native vegetation; in some cases, individual farms may be organised in village-level, community systems or farmers' cooperatives. It is estimated that 75% of the world's agricultural land is operated by family farms (Lowder et al., 2016). The types of crops produced or livestock raised vary. Farms have different levels of mechanisation and some may continue to provide only subsistence food for their families while others may be specialised and operate highly mechanised equipment.
- Industrialised agriculture – this represents intensification of agriculture to grow monocultures for commercial purposes on vast areas of land with the aim to achieve high productivity and crop yields through the application of fertilisers, insecticides, fungicides and herbicides as well as technology and irrigation equipment when needed. Industrialised agriculture (also referred as intensive agriculture) requires significant investment in machinery for planting, cultivation and harvesting followed by storage and transportation expenses (Britannica, 2017). The intensive cultivation of the land on an annual basis depletes the soil of its nutrients, particularly when crop rotation and fallow periods are not practised (Gupta, n.d.). In some cases, genetically modified organisms are used to increase yields and improve crop resistance to pests and climatic conditions. Livestock is also subject to industrial methods of intensification with the establishment of factory farming (Safran Foer, 2009) where animals are contained in small areas and exploited for their meat, milk or eggs. Antibiotics are given preventatively to animals in crowded conditions, particularly to chickens in broiler facilities and aquaculture fish, to avoid the spread of infections. These intensively produced agricultural commodities are traded on the global markets. The industrialised methods of food production cause significant environmental damage and contribute to deforestation, biodiversity loss, soil degradation, pollution and climate change as well as the exploitation of sentient animal beings. Nitrogen-induced soil acidification (Tian & Niu, 2015) and soil pollution with chemicals, plastics and other substances at higher-than-normal concentration (Rodríguez-Eugenio et al., 2018) have become a global problem threatening the health of this non-renewable resource essential for food production.
- More sustainable farming methods – in response to the threatening trends of transgressing the planetary boundaries because of land-use changes for food production (Steffen et al., 2015), there are many calls for transitioning to better farming methods and human diets (Willett et al., 2019). Examples include regenerative agriculture (Massy, 2018), agroforestry (Rosati et al., 2021), organic farming and seminatural habitat (Tscharntke et al., 2021), urban agriculture (Follmann et al., 2021; Puigdueta et al., 2021), circular agriculture (Marinova & Bogueva, 2022) as well as lab-grown meat (McClements, 2019). Application of artificial intelligence (AI), machine learning, drones and other smart technologies for precision farming are also helping reduce the environmental footprint of food while fresh and nutritious products are delivered to the consumers (Aggarwal & Singh, 2021; Choudhury et al., 2021; Jerhamre et al., 2022).

China's food transition mirrors the global trends but displays its own characteristics. In pre-modern times up to 8th century, China had equal-field distribution of agricultural land to peasants which allowed for self-sustenance and slowed down accumulation of wealth but gradually declined with population growth (Britannica, 2016). Soring population numbers resulted in severe food shortages and inefficient distribution caused famines. Chinese farmers developed many classical farming practices to maintain and manage the productivity of the land, including application of organic manure, crop rotations, intercropping and multiple cropping combined with engineering solutions through reservoirs, levelling the ground and building terraces (Gong et al., 2001). These techniques are now considered pre-cursors of sustainable farming methods.

Although subsistence agriculture is still practised in villages and some rural parts of the country, since the 1980s agricultural mechanisation is widely used making farming very productive (Xinhua, 2019). Industrialised agriculture is now vastly spread in China and increased productivity is achieved through the application of pesticides and synthetic fertilisers (Scott & Si, 2020). As in other parts of the world, the run-off from the applied synthetic nitrogen fertilisers causes the formation of nitrous oxide, a greenhouse gas 260 times more powerful than carbon dioxide (IPCC, 2014). Research evidence from China shows that these types of fertilisers reduce the microbiological diversity in the soil making it more susceptible to pathological strains (Zhou et al., 2017). The nutritional shift to more meat-based proteins is reinforced through fast-food chains contributing to the westernisation of the Chinese diets (Wang Y. et al., 2016). Furthermore, China's changing dietary preferences and demand for meat are triggering massive land-use changes around the world for the expansion of livestock grazing and intensive feed production at the expense of native vegetation (Stoll-Kleemann & Schmidt, 2017).

More recently, there has been a major swing towards organic food production and sustainable agricultural practices (Scott & Si, 2020). Such changes are encouraged and supported by the Chinese state through national sustainable agriculture policies and plan with the view to support population health, achieve ecological protection and economic benefits. The food challenge for China is complex (GAP Report, 2018) but positive changes are emerging as a top-down approach by the state but also with the bottom-up efforts of individual producers and community groups (Scott & Si, 2020). There is also most rapid development and investment in AI to support more sustainable farming methods (Galaz et al., 2021).

According to Warnaar and Methorst (2017), the stage we are in the food transition means that the human population needs to start producing and consuming food in a completely different way. Reduction of food loss and waste is one such aspect, with industrialised Asia losing 28% of food in the supply chain from (and inclusive of) on-farm harvesting through to the final consumer waste (Our World in Data, n.d.). Online shopping and home delivery with environmentally friendlier packaging are also becoming increasingly common in China and all over the world in the search for healthier and better food practices.

Sustainability transition

Compared to the other transition frameworks, the sustainability transition does not describe an evolutionary pattern of changes but only the current and most necessary transformation we need to see within the concept of development. In other words, sustainability transition indicates the latest stage of development with the fundamental changes occurring in human history driven by social and environmental imperatives, including climate change, environmental deterioration, biodiversity loss and soil depletion. From a demographic transition perspective, the sustainability transition can be aligned with the latest phase of mature industrial society and progress to post-industrial ways of

development when the size of human population stabilises. Sustainability transition, however, is not so much about the demographic dimensions of human population but about the way people live on Earth (Dovers & Butler, 2015). Its main focus is not on how many people are there, but on their consumption patterns, technological choices, the pollution they generate in the air, waters, soil and land, the governance models which define socio-political and economic pathways, and what is fair and just for current and future generations as well as for other species with whom we inhabit the same planet. Technological advancements are likely to shape the sustainability transition overall and in specific areas, such as energy, transport, buildings, industry as well as agriculture, sometimes referred to as separate sustainability transitions (e.g. by the European Commission, 2020). According to the EAT-Lancet Commission (Willett et al., 2019), food will define the 21st century.

A sustainability transition is defined as a "radical transformation towards a sustainable society, as a response to a number of persistent problems confronting contemporary modern societies" (Grin et al., 2010, p. 1). Ultimately a sustainability transition delivers sustainable development which can be described as a process of navigating between two sets of boundaries – those of the planet and the social foundation of basic needs (EEA & Eionet, 2016). Food is an essential part of basic needs but food systems have encroached and transgressed planetary boundaries. An extension of sustainability is regeneration as the harm caused to the planet's ecosystems needs to be reversed. This includes the damage caused by food production which has been the single largest driver of environmental degradation (Willett et al., 2019). Regeneration requires bringing science and practice together integrated with spirituality in a holistic way that reflects fundamental shifts in people's behaviour based on increased awareness, education, leadership and empowerment (Gibbons, 2020).

Most of the literature about sustainability transition takes a multi-level perspective, where:
− The landscape (macro level) is defined by external structures;
− The regimes (meso level) are relatively stable configurations which determine what is normal; and
− Niches (micro level) are protected spaces where innovations can develop without the pressures from the regimes within the existing landscape (European Commission, 2020).

This perspective is justifiable for many sectors in the economy but in the case of food, people's values and behaviour are manifested with each meal they take and each person is potentially a "niche" for innovation and behaviour change. Such a way of conceptualising food's place in a sustainability transition is empowering allowing for leadership to be demonstrated until what are considered "niche" behaviours become the new norm. New production and consumption practices need to be mainstreamed and old preferences which exploit nature's biophysical systems and farm animals need to be phased out (see Figure 3). In the theory of societal transitions, this is described as the X-curve framework (Loorbach, 2014; Hebinck et al., 2022).
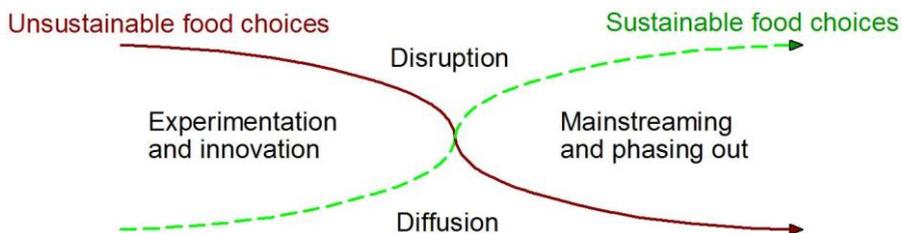
Figure 3. Sustainability transition in food
Source: Based on European Commission (2020)

Flexitarianism or voluntary reduction in the consumption of animal-based proteins (Raphaely & Marinova, 2014) is an example of a niche behaviour in a country like Australia which has one of the world's highest levels of meat supply on a per capita basis. There is mounting evidence that meat, and particularly red meat, is detrimental to the natural environment (Poore & Nemecek, 2018), has much higher greenhouse gas emissions compared to plant-based options (Clark & Tilman, 2017), takes up disproportionately more land (Ritchie & Roser, 2019a), represents an inefficient energy and protein conversion in the supply of food (Eshel et al., 2014; Ritchie & Roser, 2019b) and is potentially carcinogenic (WHO, 2015). Flexitarianism acts as a disruptor to the current unsustainable food preferences and the innovations in the alternative proteins market aim to ease its diffusion, that is broader adoption. Despite representing the best nutritional choice, the appeal of traditional legumes, nuts, vegetables, grains, fruits and other plant-based foods has gradually declined. The development, experimentation and innovation in novel plant-based analogues of familiar animal-sourced foods, such as sausages, mince, nuggets, mayonnaise, cheese and cultured meat, aspire to attract consumers. Such products are seen as a way to progressively shift food preferences by imitating the taste and look of familiar animal-sourced products. When decreased consumption of animal-based foodstuffs is achieved, and in the case of Australia this reduction should be by 80-90%, sustainable food choices will be mainstreamed with old addiction to livestock-based products significantly reduced or phased out. The interest in plant-based milks is particularly strong in western societies because of their better environmental performance (Marinova & Bogueva, 2020) and cow's milk is gradually being displaced.

Food's contribution to the sustainability transition is not only through the types of products consumed. The status quo can be disrupted with new production ways, including for the humble fruits, vegetables, nuts, grains, legumes and tubers. There are numerous new technologies that are gaining momentum (McClements, 2019) and we are witnessing rapid advancements in areas, such as vertical farming, hydro-, aero- and aquaponics (Marinova & Bogueva, 2022). Agroecology (FAO, 2018) is another field that links food production with the ecology to help transition to sustainable food and agricultural systems and in response to the global 2030 Sustainable Development Goals (SDGs). It covers principles and approaches for human society to live in harmony with nature (Franzluebbers et al., 2020), allow it to regenerate and heal.

Sustainable food choices are essential for a sustainability transition and any progress made in other areas, such as electricity, transport or buildings, will be defeated by the burden human diets pose on planetary health. Conservatively estimated, food systems are responsible for 34% of the global greenhouse emissions (Crippa et al., 2021). It is only academic whether we describe the shift to sustainable development as one or many sustainability transitions and lament the methodological complexities and challenges in studying these transformations (Geels, 2011; Zolfagharian et al., 2019). Embracing this latest transition framework is much more about practice than theory.

The economic development transition that China has undergone since 1978 has been characterised as a "socialist market economy" in which the state is coordinating the building of a harmonious society based on prosperity (Hong, 2016). Four decades of exceptional economic growth have accelerated urbanisation and taken hundreds of millions of people out of poverty. However, this has come at a high environmental cost with substantial levels of pollution and greenhouse gas emissions. China's economy is now "in a transition period from rapid development to high-quality development" (Cheshmehzangi & Chen, 2021, p. 56) with targets to reach a carbon peak before 2030 and decarbonise by 2060 through top-down socio-

economic development plans and bottom-up economic incentives and technology development (Liu et al., 2022).

Food security and production are recognised as a challenge in China's sustainability transition and the country has a National Plan for Sustainable Agricultural Development 2015–2030 (Cheshmehzangi & Chen, 2021). Its focus is on improving people's livelihoods and building resilience to disasters while safeguarding the natural environment. Furthermore, there is convincing evidence that it is more energy-efficient and financially beneficial to focus on the production of vegetables and legumes, such as soy, than to produce or import meat given China's farmland, water and other resource restrictions (Cheshmehzangi & Dawodu, 2019). This is supported by government policies but some argue that dietary changes for reducing meat intake have to be a gradual and slow process (Cheshmehzangi & Chen, 2021). In the next section, we look at how China can specifically contribute to a global sustainability transition.

China's contribution to a global sustainability transition

Because of the size of its population and economy, China can influence all aspects of the global sustainability transition – from energy to industrial production, buildings and technology. With the focus of this article specifically on food, we discuss two particular aspects, namely the state-driven dietary changes to contain domestic meat consumption and China's knowledge and expertise in alternative proteins.

Domestic transition

China's latest 2016 dietary guidelines, namely the Balanced Diet Pagoda, limit the intake of meat and poultry to 40–70 g per day to "help promote healthy lifestyles and physical strength" aimed at reducing risk for many chronic diseases and mortality (Wang, S.-S. et al., 2016, p. 649). The message to the consumer also is that meat, poultry, fish and eggs should be eaten in moderation which is in contrast to the encouragement to eat plenty of vegetables, fruits, tubers and bean products, including soybeans. An analysis by the Global Panel on Agriculture and Food Systems for Nutrition (2016) shows that 880 projected deaths per million in 2050 can be avoided in China by reducing red meat consumption.

The Chinese state is responding proactively to the scientific evidence not only by adjusting its dietary guidelines towards less red meat consumption compared to the 2007 Food Pagoda (Wang, S.-S., 2016), but also through social marketing campaigns. What is particularly interesting is that the social marketing campaigns, such as Less Meat Less Heat More Life (2016) by the Chinese Nutrition Society in collaboration with WildAid China, explicitly link meat consumption with climate change, habitat loss and other environmental deterioration (Table Debates, n.d.). The promotion advertisements and video used in the campaign that reached millions of Chinese citizens included China's most famous actress Li Bingbing, the very popular Hollywood actor Arnold Schwarzenegger and was directed by James Cameron. It endorses the efforts of the Chinese Government to decrease the actual consumption in China by 50%. In reality, this reduction is more than the one included in the Diet Pagoda, and represents the combined effort to restore the public good of health and environmental well-being. James Cameron explicitly draws attention to hypocrisy in the global environmental movement saying: "How can I call myself an environmentalist when I'm contributing to environmental degradation by what I eat?" (Shoard, 2016, para. 4).

Although on a per capita basis, Chinese eat around half of the daily meat consumed by Australians or Americans, there is still a well-defined trend to increase the intake of animal

proteins as population wealth improves (Whitton et al., 2021). Given the size of China's population, this consumption results in more than twice the amount consumed in USA and requires substantial imports of feed and meat products. The efforts by the state aim at reversing the trend of increasing meat consumption and also promote preservation of healthier traditional diets. Compared to other government positions, the Chinese state is showing strong leadership in an area that is considered complex and difficult to navigate.

The empirical evidence confirms the complexity of the efforts to speed up the nutrition/protein and food transition in China. In recent years, the cultivation of staple crops, such as sorghum, millet and rye, has decreased to give space to high-yield and economically more profitable potato and rice (Chang et al., 2018). Overall, Chinese diets have moved away from the traditional healthy balanced and nutritious composition towards foods that are high in fat, nutritionally poor and energy dense as exemplified by choices of animal-sourced products (Chang et al., 2018).

Added to this is food waste, with Chinese households wasting 64 kg per person per year (UNEP, 2021). Although this is not at the extreme end of the spectrum on a per capita basis – Australians waste 102 kg per person per year, it amounts to a staggering 91 million kg per year. The state again is using social marketing to promote a 40% reduction through the "clean plate" (*Guang Pan*) campaign launched by the president Xi Jinping (Sheldon, 2020). The higher the animal-based content, the higher the environmental footprint of food waste.

The current trends in increased meat consumption (see Figure 2) need to be seen also within the context of China's history in producing nutritious food. Relatively recently, a negative (from a sustainability point of view) X-curve transition has occurred to introduce meat and western types of diets disrupting traditional food practices. Soybean, considered the "miracle crop" (Guo et al., 2021), was domesticated thousands of years ago. Different methods of processing soybeans into tofu, yuba (dried tofu skin) and soymilk were developed first in China and then spread all over the world (Marinova & Bogueva, 2022). Soy-based products contain all nine essential amino acids for humans and are also a good source of vitamin B1 and important micronutrients, such as iron, calcium, manganese, phosphorus, magnesium, copper and zinc. From a food production point, soybeans are a legume and help maintain soil fertility through nitrogen fixation, making them suitable for crop rotation.

With the nutrition/protein transition, soybeans however have become a popular crop for making animal feed. It is estimated that 85% of the soybeans consumed in China are eaten by livestock (Nepstad, 2021). China's raising consumption of meat, and pork in particular, has fuelled the global demand for and imports of soybeans, including from Brazil, USA and Argentina, where this has led to deforestation, loss of biodiversity and the use of genetically-modified varieties to increase yields. Similar to beef, the protein conversion of pork is extremely inefficient – 91.5% of the proteins fed to the animal are lost in the process of producing meat for human consumption (Alexander et al., 2016). Soybeans can easily provide these proteins directly to create a diet nutritious and healthy for the planet.

China's 14th Five-Year Plan focussed on high-quality and sustainable development provides a roadmap for a sustainability transition. It also emphasises the "greening" of food production through the use of organic methods. At the moment, China is the 4th largest provider in the world of organic soybeans (Nepstad, 2021) for direct human consumption with farmers given incentives to switch to this crop. If meat demand is curbed, this would have immediate and long-term benefits for people's well-being and ecological health. In 2014, the state-owned enterprise Sinograin became the first to implement an international standard for responsibly produced soybeans, which includes zero deforestation and no land conversion for agricultural purposes as well as no application of synthetic fertilisers. This is driven by a top-down approach and bottom-up demand.

Consumers' preferences in China are also changing, particularly among young people and those who are more environmentally oriented. A 2019 survey conducted by the Swiss Federal Institute of Technology found that 55% of Chinese citizens supported reduction in meat consumption and a range of policy initiatives that could facilitate such a change (Meat Atlas, 2021). They include higher taxes on meat products (30% higher), 75% vegetarian options in public cafeterias, elimination of subsidies for meat producers, reducing the prices of plant-based alternatives (by 30%) as well as frequent information campaigns and supporting low-income households. Another 2019 study by the European Investment Bank shows that 78% of the Chinese respondents have already reduced the size of their red meat portions and another 14% are intending to do this to fight climate change (Meat Atlas, 2021). This was also the highest population share from all 30 surveyed countries.

Although less than 5% of the Chinese population identify as vegetarian or vegan, 87% have tried plant-based meat alternatives (Global Food Institute, 2018). In fact, China is one of the fastest-growing markets for plant-based alternatives with demand predicted to increase by 200% within five years driven by consumer interest in health and sustainability but also in taste (DuPont Nutrition & Biosciences, 2020). Variety and novelty of food products are particularly important for young consumers but taste and texture are critical for the success of these new and more sustainable protein options. According to market research (DuPont Nutrition & Biosciences, 2020), the vast majority of consumers, namely 78%, believe that plant-based alternatives are going to become a mainstream option. What is of particular interest is that in China people with higher attachment to meat are more likely to buy the plant-based alternatives (compared to vegan and vegetarians) while the opposite is the case in USA (Bryant et al., 2019). China can build on its millennia-old traditions in consuming and processing soy and other plants to create novel foods that satisfy consumer expectations and disrupt the current meat tends facilitating a sustainability transition encouraged and supported by the Chinese government.

Global opportunities

According to Alexander et al. (2016), if the average Indian diet (which contains around 5 kg of meat per person per year) is adopted globally, only half of the current arable land would be needed to feed the world. They also stress that the types of food commodities are more important than the quantities because of the large land and environmental footprint of meat and other animal-based foods. China can play an important part in a global transition to more sustainable food choices.

The plant-based alternatives have had a long history as an industry supplying Buddhist temples and prestigious restaurants to showcase culinary skills (Global Food Institute, 2018). Many of the companies are small-scale but they have already ventured on a global scale providing vegetarian duck, chicken, beef, seafood and sausages to countries, such as Australia, Canada, New Zealand, USA and the rest of Asia, including large supermarket chains, fast-food and specialised restaurants (Global Food Institute, 2018). China is a dominant supplier of soy and pea protein to the world processing 79% of the global soy protein isolate, 50% of the global textured soy protein and 23% of the global soy protein concentrate (Siu, 2019). Technology advancements in extruders, 3D printing and locally grown quality whole foods, including soybeans, will further strengthen China's position in the global opportunities in the sustainability transition.

Conclusions

The first three transition frameworks related to development and food, namely demographic, nutrition/protein and food transitions, describe major shifts in population behaviour. They are associated with improved standards of living and food security. However, the current food systems are not delivering the best outcomes for humanity. Malnutrition – from undernourishment to obesity and associated non-communicable diseases – is contributing to shorter life spans while food production is one of the major causes for the deterioration of the natural environment (Lindgren et al., 2018). The fourth transition, namely a sustainability transition, is required to respond to the major challenges and persistent problems triggered by human activities on the planet. Food is a defining aspect of the sustainability transition and the current moment in history.

As countries go through different phases of development towards a stage when population numbers are expected to stabilise, we also see dietary changes with reduced famines and increased energy intake, driven among others by animal-based foods, and meat in particular. The current stage of this protein transition represents not only an inefficient use of resources but also threatens human well-being and the health of the biophysical systems on the planet, including contributing to climate change, destruction of natural habitats through land-use conversion, biodiversity loss and soil degradation. To counteract this destruction, a conscientious desired transition towards predominantly plant-based foods is at the core of the sustainability transition which will trigger innovation, diffusion and mainstreaming of new dietary products known as alternative proteins together with resurrection of the importance of the humble vegetables, fruits, legumes, whole grains and nuts.

There is already evidence, particularly from China, that people are embracing reduced consumption of animal-sourced products with 92% of the country's citizens willing to do this to fight climate change (Meat Atlas, 2021). Supported by social marketing and endorsed by government initiatives, China can become a significant contributor towards the changing food trends domestically and globally. It can facilitate a faster sustainability transition by shifting its own eating habits and also contributing globally to the burgeoning field of new alternatives to livestock products. From a defining characteristic of the 21st century (EAT-Lancet Commission, 2019), food can become a message of hope and regeneration empowered by a new holistic awareness and spirituality (Gibbons, 2020) redefining the meaning of being human.

Following in the vein of previous research on food transition and sustainability transition, this study is the first to conceptually link the two. It also positions food, and plant-rich choices in particular, as a defining characteristic of the urgently required sustainability transition making it a priority global agenda. The potential of China to be part and positively influence such changes has not been previously explored and the insights provided here bring hope and optimism.

This qualitative conceptual analysis can be expanded in the future with quantitative evidence about the adoption of plant-rich diets, including plant-based analogues, across the world and specifically in China. Another interesting direction of future research is to understand the driving motivations behind any responses by consumers and producers to these new opportunities. Young people in particular have been very active in the area of climate change and it will be worthy to investigate how they react to imbedding food choices in the regeneration agenda for the planet. New research will also need to bridge our understanding of the place of food choices in exacerbating global inequalities.

From a transition, sustainability will have to become the normal way of human existence. With this, food will no longer defy life on Earth but instead will provide support and future answers.

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Intelligent automatic control of sugar factory evaporator operation using behavior prediction subsystem

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Abstract

Introduction. The aim of the presented research was to substantiate the intelligent automatic control of the sugar juice evaporation with the subsystem for behavior prediction, which allows to determine the behavior of the automatic system.

Materials and methods. The operation of the evaporator unit with system behavior prediction to regulate the sugar juice level was investigated. Capacitive level gauges were used as a sensor in the automation scheme of sugar juice level control. Pneumatic seat valves with a built-in throttle and an electro-pneumatic converter were used as actuators.

Results and discussion. The use of neuro-fuzzy regulators occurs only in some specific cases of intelligent control of the evaporation process. There is no data comparing the use of intelligent regulators with classical ones and the possibility of combining several types of intelligent regulators, as well as clear means of predicting their work. Therefore, in the present study, a prediction method was used to compare methods to regulate the level of sugar juice in the evaporator. This made it possible to predict the behavior of the system during the formation of the control action and display the finished forecast on the operator's screen, which made it possible to increase the efficiency of the evaporative station. Statistical data on the behavior of the automation system contours in various operating modes were collected using intelligent and classical controllers, and a model was built to determine the operation of the evaporator using the local trend method and the modified algorithm of prediction. The advantage of this method is its easy and fast implementation, which does not require large economic and energy costs. The accuracy of the prediction model was 98% for the PID-controller, 95% for the fuzzy-controller and 96% for the neural network. The obtained model of the system prediction is stable because the absolute error does not change when dividing the time series into intervals.

Conclusions. The proposed system of intelligent automated control of the evaporation of sugar juice with a modified prediction method based on local trends has an insignificant delay, while prediction is performed with high accuracy and stability.

Introduction

The evaporation process is one of the main operations in the sugar factory. However, the high temperature conditions of evaporation results in unwanted sucrose losses. High-quality automatic control of the evaporator operation is of the highest importance in the sugar production because it ensures adherence to the temperature regime, prevents overheating of the sugar juice, and increases the overall efficiency of the sugar factory.

System of automatic control of the evaporating plant can be described as a one requiring the intervention of the operator who makes adjustments to the tasks for regulators responsible for maintaining temperature and material flows.

 Such adjustments are required because of the instability of the technological and quality indicators of sugar juice at the inlet to the evaporation plant, as well as of the need to change them at the outlet (Hrama et al., 2019a). When making changes to the automated control system, the operator must take into account both the impact of the work of adjacent sections on the process of sugar juice concentration in the evaporation station, and the impact of changes in sugar juice indicators on the activity of subsequent equipment (Hrama et al., 2019b).

To ensure an effective automation system, the use of modern software and hardware is needed. However, the use of intelligent systems in automating the sugar evaporation process provides a large number of options, some of which can lead to extraordinary and emergency situations. It is very important to prevent their occurrence in time (Chantasiriwan, 2017). To predict the possibility of insufficient situations, it is proposed to introduce a forecasting module into automation systems. This will allow predicting the state of the system and making operational decisions (Verma et al., 2018).

Improving the evaporation process is rather an important task. Chantasiriwan (2017) proposed a model of the evaporation process that takes into account the balance of mass and energy. However, the issues related to the occurrence of nonlinearity and the problem of fluid flow deviation remain unresolved. In addition, the possibility of using intelligent regulators in the evaporation process was not considered in this exploration. The reason for this may be the difficulties that arise due to the need to use special software.

Verma with co-authors (2018) studied the process of linearization of a nonlinear model of an evaporator plant consisting of 14 first order nonlinear differential equations. The change of the product concentration from the deviation of the liquid flow rate was found y for the first time, but intelligent controllers were not used. This may be due to the difficulty of developing rule bases for neural fuzzy regulators or the lack of an appropriate neural network training model.

The need to upgrade existing control systems was shown (Sidletskyi et al., 2016). The authors presented some approaches that are used for the distributed level of process control, but application of intelligent controllers in the evaporation process were also not disclosed.

The main objective of the present research was to study the possibility of using intelligent methods for regulating the level of sugar juice in an evaporator with a prediction subsystem, which will allow foreseeing the behavior of the system and getting a ready forecast, and thus improve the efficiency of the evaporator station.

Materials and methods

A five-corps sugar evaporator was used. The scheme of automation of sugar juice level control is shown in Figure 1.
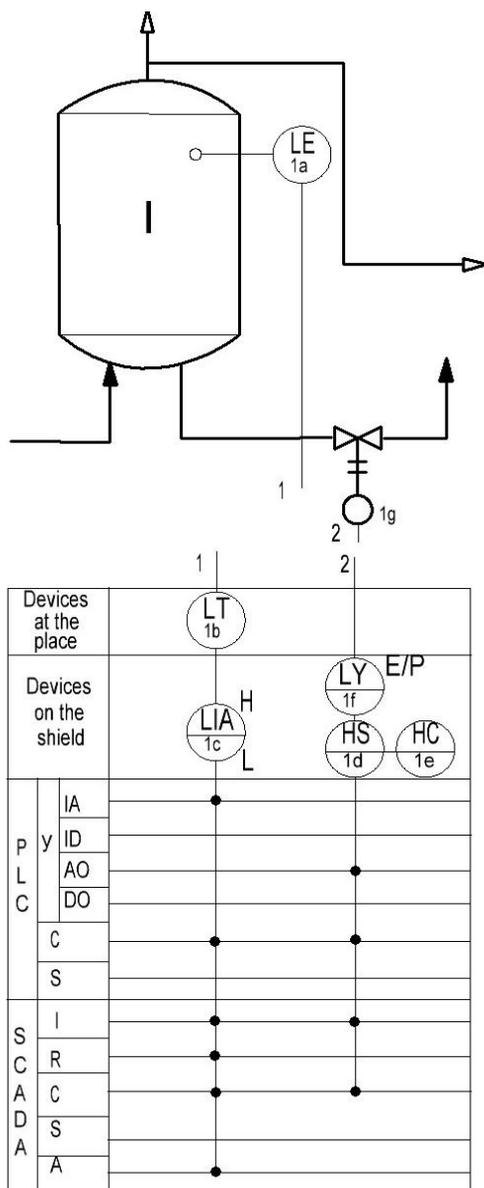
Figure. 1 Automatic liquid level control in evaporation station

Capacitive equalizers (LE 1a, LT 1b) are used as sensors in sugar juice level control circuits. Single channel microprocessor-based indicator ITM-110 (Mikrol, LLC, Kharkiv) was used as a secondary indicating device (LIA 1c). The signal is sent to the regulator (PLC) on the control unit (intersection with C), as well as to the human-machine interface (SCADA), which displays the level of sugar juice value on the screen of the automated operator's workplace (computer) (intersection with I). The obtained data is stored in memory (R). This data (actual level of sugar juice values) is used for conducting the experiment. In

case the level of sugar juice value exceeds the set limits, an alarm signal (A) is generated. The control signal, which is output by the regulator (AO), is sent to an electro-pneumatic converter (LY 1f), which converts an analog unified electrical signal. In turn, the actuator (e.g. 9f) changes the position of the control valves. The operator can control the position of the regulator in remote (manual) mode (intersection with C – remote control of the SCADA operator). БРУ-17 manual control units are used for switching the "Manual/Automatic" mode (HS 1d, HC 1e). Modicon M340 is used as a regulator. Pneumatic seat valves (1g) J4SPG1805 KRAFTt-AIR, with integrated choke and electro-pneumatic converter (Hrama et al., 2019b) are used as actuator.

Description of prediction using the method of local tendencies

Prediction the operation of the evaporating station using the method of local tendencies can be carried out using fuzzy time series models (Jolly et al., 2000). A fuzzy time series model is generated to obtain the forecasted local tendency (Lahtinen, 2001). To do this, the model of fuzzy dynamic process with fuzzy increment is used (Fig. 1). This increment looks in the following way: $X_t (t = 1, 2, ...) \subset R^1$ – a universal set for which fuzzy sets $\tilde{x}_t^i, (i = 1, 2, ...), \tilde{v}_t^j, (j = 1, 2, ...), \tilde{a}_t^s, (s = 1, 2, ...)$ are defined (Dong et al., 2017). Next, there is a need to set the values of the parameters of the time series model of the first order (Lei et al., 2016) and calculate the sum of the intensities of fuzzy elementary tendencies for each interval by creating an algorithm for fuzzy local tendencies for this case (Dong et al., 2017). The algorithm, which first converts the initial time series to a fuzzy time series, was used to forecast the operation of the automated evaporating station. The next step is to convert the obtained fuzzy time series into a time series of fuzzy elementary tendencies and to perform defuzzification using the method of the center of gravity of intensity of each fuzzy elementary tendency for each time series

$$a_t = DeFuzzy(\tilde{a}_t) \quad \text{(Anghinoni et al., 2018).}$$

The analysis of the stability of the prediction model is as follows. The automation system of the five-hull evaporator station is launched (Fig. 1), after which the SCADA system is removed from the graphs of the transition process and the predicted values during the operation of the installation (Dong et al., 2017). They are shown in Fig. 6. Next, the graphs are divided into any number of equal time intervals (González-Potes et al., 2016). Each time interval is separated from the next by a dot called a Latin letter. The value that corresponds to the transition process at a given time is the actual meaning, and the number that corresponds to the graph with the predicted meanings at this time is the predicted value. All these numbers are entered in Table 1. Also calculated and entered in Table 1 the meanings of absolute and relative prediction error for each point (Dong et al., 2017).

The value of the absolute error (A) is calculated by formula (1):

$$A = \left| Z(t) - \tilde{Z}(t) \right| , \tag{1}$$

where $Z(t)$ is the actual value of the time series, $\tilde{Z}(t)$ is forecasted value of the time series (Lei et al., 2016).

The value of the relative error (V) for each value of the time series point is calculated by the following formula (2):

$$V = \frac{\left| Z(t) - \tilde{Z}(t) \right|}{Z(t)} \times 100\% \tag{2}$$

For stability analysis, this compares the dependence of the relative error when the noise level changes (i.e. the change or the absolute error). To do this, a graph of the dependence of the relative error on the absolute (González-Potes et al., 2016). This graph shows the points in Table 1 at the same time intervals into which the transition process was divided (Dong et al., 2017). Graphs of changes in relative and absolute errors over time are built on these points. The values of absolute and relative errors are also taken from Table 1. If the values of relative error do not change when the absolute error does not change, the prediction system is stable (Zhang et al., 2011).

The next step is to assess the accuracy of the system. The automation system of the five-hull evaporator station is launched (Figure 1), the type of control is selected, after which the SCADA system is removed from the graphs of the transition process and the predicted values during the operation of the installation. They are shown in Fig. 6. Next, the graphs are divided into a free number of equal time intervals (González-Potes et al., 2016). Each hour interval is separated from the next dot by a Latin letter. The value that corresponds to the transition process at a given time is the actual value, and the value that corresponds to the graph with the predicted values at that time is the predicted value. All these values are entered in Table 2. The values of absolute errors are calculated by formula (1). The average error (SP) calculated by formula (3) (Dong et al., 2017):

$$SP = \frac{1}{n}\sum_{t=1}^{n}(Z(t) - \tilde{Z}(t)), \tag{3}$$

where SP is the mean error of the forecasted value of the time series, n is the number of time series intervals, $Z(t)$ is the actual value of the time series, $\tilde{Z}(t)$ is forecasted value of the time series (Lei et al., 2016).

The average absolute error (SAP) was calculated by formula (4):

$$SAP = \frac{1}{n}\sum_{t=1}^{n}\left|Z(t) - \tilde{Z}(t)\right| \tag{4}$$

The mean relative error of prediction (SVP) was calculated by formula (5) (Lei et al., 2016):

$$SVP = \frac{1}{n}\sum_{t=1}^{n}\frac{\left|Z(t) - \tilde{Z}(t)\right|}{Z(t)}\times 100\% \tag{5}$$

The mean standard error (SKP) was calculated by formula (6) (Chowdhury et al., 2015):

$$SKP = \frac{1}{n}\sum_{t=1}^{n}(Z(t) - \tilde{Z}(t))^2 \tag{6}$$

The square root of mean standard error (SQSKP) was calculated by formula (7):

$$SQSKP = \sqrt{SKP} \tag{7}$$

The standard deviation (SV) was calculated by formula (8):

$$SV = \sqrt{\frac{1}{n}\sum_{t=1}^{n}(Z(t) - SKP)^2} \tag{8}$$

Accuracy of prediction model (T) was calculated by formula (9):

$$T = 100\% - \frac{1}{n}\sum_{t=1}^{n}V \tag{9}$$

The closer to 100% the accuracy of the model (T), the more accurate the model (Lei et al., 2016).

Results and discussion

Synthesis of the algorithm of local tendencies

Let's consider the operation of the algorithm of prediction based on high-order fuzzy time series (Chen, 2002) and the operation evaporating station. A description of control of several evaporating stations with full integration of fuzzy control and the use of wireless network sensors and actuators was shown (González-Potes et al., 2016). Though, the comparison of the use of neural fuzzy regulators with other types of intelligent control was not included. There is also no justification for the feasibility or unreasonableness of using this type of control in case of the possibility of implementing a system with another type of intelligent control. In addition, neural fuzzy control is not used in all control circuits. The reason for this may be the high complexity of such study. The authors faced similar problems (Zhang et al., 2011). This research contains a consideration of the control of evaporator overheating using a fuzzy slider mode regulator. In addition, this paper does not address the use of fuzzy control for other control circuits of the evaporating station.

The paper (Lavarack et al., 2004) features the consideration of the methodology of increasing the efficiency of steam use. However, modern types of control are not used in it. That is why there is a high probability that the use of intelligent regulators can further increase the efficiency of steam. This work also contains the consideration of options for improving the evaporation process (Srivastava et al., 2013). These studies also feature complex calculations. In the exploring, the authors prove that the rate of evaporation decreases noticeably over time (Roger et al., 2018). They perform a calculation and demonstrate that diffusion in the liquid phase is a step, which limits the rate for this system, in contrast to the evaporation of pure water. A generalized stationary mathematical model for modeling a multichannel evaporator system was developed in the paper (Srivastava et al., 2013). Patan with co-authors (2005) have considered the problems of detection of malfunctions of industrial processes using dynamic neural networks on the example of an evaporating station. The considered neural network had a multilevel feed structure. In the exploration, Merino with co-authors (2018) investigated the application of real-time optimization in the evaporation section of a sugar refinery using methods that reduce the time for developing models. Polupan with co-authors (2018) proposed to use of genetic algorithms in sugar production. The paper (Sidletskyi et al., 2019) features the consideration of the development of the structure of an automated control system using tensor methods in sugar production. However, the authors of these studies also did not use intelligent control. This may so due to the high complexity of the calculations or the lack of necessary hardware or software.

It was claimed that by using intelligent control it is possible to provide a faster decrease in housing temperature and achieve more stable control of overheating in the first evaporator tank (Jolly et al., 2000. Though, this examine also does not disclose the use of intelligent regulators for regulating other parameters (e.g., pressure, level of beet juice, consumption). In addition, only the possibility of using intelligent regulators in other housings than the first one is considered in this study. This may be so due to the high complexity of the calculations and the need of using the specific software. The paper (Lahtinen, 2001) features the consideration of the problem of control of other parameters of the evaporation process. In this exploration, it is proved that the control of evaporation can be implemented by recirculation of liquid in the evaporation section or by feeding only liquid to the evaporator. However, this article also does not address the use of intelligent regulators during the evaporation process.

It is necessary to improve the model of prediction the operation of the evaporator station using the method of local tendency and prediction algorithm and determine the impact of the algorithm on the accuracy and stability of the obtained prediction model.

The following dependences of parameters was used to work with the algorithm of local tendencies (Lei et al., 2016):

$$\tilde{x}_i = Fuzzy(x_i),$$
$$\tilde{\upsilon}_i = TTend(\tilde{x}_i, \tilde{x}_{i-1}),$$
$$\tilde{\upsilon}_{i+1} = \tilde{f}_{\tilde{\upsilon}}(\tilde{\upsilon}_i),$$
$$\tilde{a}_i = RTend(\tilde{a}_i, \tilde{a}_{i-1}),$$
$$\tilde{a}_{i+1} = \tilde{f}_{\tilde{a}}(\tilde{a}_i),$$
$$\tilde{x}_{i+1} = Comp(\tilde{x}_{i+1}, \tilde{\upsilon}_{i+1}, \tilde{a}+1),$$
$$x_{i+1} = DeFuzzy(\tilde{x}_{i+1}) + \varepsilon_{i+1},$$

where Fuzzy is scale fuzzification operation, TTend is operation to determine the type of difference, RTend is operation to detect the difference intensity, Comp is operation to calculate a new fuzzy estimate, DeFuzzy is scale defuzzification operation. $\tilde{f}_{\tilde{\upsilon}}, \tilde{f}_{\tilde{a}}$ is fuzzy dependencies are presented in the form of a composite rule of implication, $x_{i+1}, \varepsilon_{i+1}$ − numerical evaluation and error of the forecasted level of the time series.

In this model, the definition of the absolute fuzzy estimate $\tilde{x}_i$ is determined using the fuzzification of a scale according to the value of the estimated object x_i. Next goes the operation to determine the type of differences. The process of determining the intensity of differences would be the next step. It is followed by the calculation of a new absolute fuzzy estimate (Xu et al., 2020). The last step is defuzzification of the scale according to the definition of the evaluated object x_i by an absolutely fuzzy estimate $\tilde{x}_i$.

A two-stage algorithm for selecting a time series prediction model has been developed. Let's calculate the amount of the intensities of fuzzy elementary tendencies for each interval by the following way (Dong et al., 2017):

$$if \ P_{up}(\tau_t) = true \ then \ ST_{up} = ST_{up} + a_t,$$
$$if \ P_{down}(\tau_t) = true \ then \ ST_{down} = ST_{down} + a_t,$$
$$if \ ST_{up} = 0 \ and \ ST_{down} = 0 \ then$$
$$\tilde{\upsilon} = "Stable", a=0,$$
$$if \ ST_{up} \geq 2 \cdot ST_{down} \ then$$
$$\tilde{\upsilon} = "Up", a=abs(ST_{up} - ST_{down}),$$
$$if \ ST_{down} \geq 2 \cdot ST_{up} \ then$$
$$\tilde{\upsilon} = "Down", a=ads(ST_{up} - ST_{down}),$$
$$if \ 0,9 \cdot ST_{up} \leq ST_{down} \leq 1,2 \cdot ST_{up}$$
$$or \ 0,9 \cdot ST_{down} \leq ST_{up} \leq 1,2 \cdot ST_{down}$$
$$then \ \tilde{\upsilon} = "Regular", a=(ST_{up} + ST_{down})/2$$
$$else \ \tilde{\upsilon} = "Chaos", a=abs(ST_{up} - ST_{down}),$$
$$\tilde{a} = Fuzzy(a).$$

where P is a final set of points in the n interval (final set of tendencies), ST is a time interval of the fuzzy tendency length.

With the developed algorithm, local tendencies are assessed. The next step is to use language and numerical forms in the algorithm (Anghinoni et al., 2018). For the operation of this algorithm it is necessary to convert the initial time series into a fuzzy time series (Mehmood et al., 2020) using the model shown in Figure 2. The next step in this algorithm is to divide the obtained time series into a number of intervals. The amount of the intensities of the same type of fuzzy elementary tendencies is calculated at each interval. Next, the type of local trend ("Stable", "Ascending", etc.) can be selected by comparing the time intervals during the increase (ST_{up}) and decrease (ST_{down}) of time intervals of the fuzzy tendency length (Xu et al., 2020).

This algorithm does not require additional user interpretation. This algorithm has a disadvantage due to the limitation of its operation by the number of predefined time intervals. Therefore, the number of identified local tendencies will be equal to the number of intervals specified by the developer (Anghinoni et al., 2018). This algorithm allows obtaining time series that can be used in the future to forecast local tendencies. The advantage of this algorithm is the ability to reduce the knowledge base, which can be represented as a set of rules that are generated over a fuzzy time series (Dong et al., 2017).

Analysis and synthesis of control action using prediction methods in the evaporating station control system

It is suggested using the flow chart of control (Tang et al., 2001), modifying it so as to include the possibility of prediction (Lei et al., 2016), and changing the type of control (Lapin et al., 2016) (Figure 2).

Flow chart of control is shown in Figure 2, where $Y_z(t)$ is a task signal, $e(t)$ is a mismatch between task signal and feedback, $u(t)$ is control signal, $v(t)$ is external disturbance, $Y(t)$ is output signal, and $Y_M(t)$ is output signal from the object model.

The paper (Tang et al., 2001) contains a more detailed consideration of the work of intelligent regulators, on the example of fuzzy regulators. In this exploration, the fuzzy PID-regulator is investigated as a discrete version of an ordinary PID-regulator. Therefore, it retains the same structure but has an independently adjustable control factor. It is proven that it is possible to improve the classic PID-regulator with a certain adaptive control ability. Though this regulator cannot be considered to be a full-fledged neural fuzzy regulator. In addition, the use of other types of intelligent regulators is not considered in this study. The cost of research may be a possible reason for this. The article (Carvajal, 2000) contains a more detailed consideration of the issue of using neural fuzzy regulators. This analysis presents a new PID-regulator of fuzzy logic. This regulator is a fuzzy PID-regulator with a computable efficient analytical circuit. The author proves that the regulator is stable with limited input/limited output. However, it is very difficult to implement this regulator, and this paper does not provide the possibility of using other types of intelligent regulators. In addition, it is not possible to use this type of regulator for some control parameters. Also, none of the above-mentioned studies justify the need for upgrading existing evaporating station automation systems. The cost of research may also be a possible reason for this. The paper (Sidletskyi et al., 2019) features a consideration of the issue of using neural fuzzy regulators. This exploration states that the addition of fuzzy and neural fuzzy logic is one of the advanced methods of improving control systems. Methods of dynamic power control were analyzed using fuzzy logic and adaptive neural networks. The use of fuzzy inferences (so-called fuzzy systems) may be one of the possible options for power control. The control action is formed by checking the coordination of fuzzy rules with the actual parameters of the system. Rules are created according to the experience of the operator, which reflects his/her actions when changing technological parameters. Though this paper does not contain the consideration of the use of neural fuzzy regulators in the evaporation process. In addition, it also does not address other types of intelligent regulation.
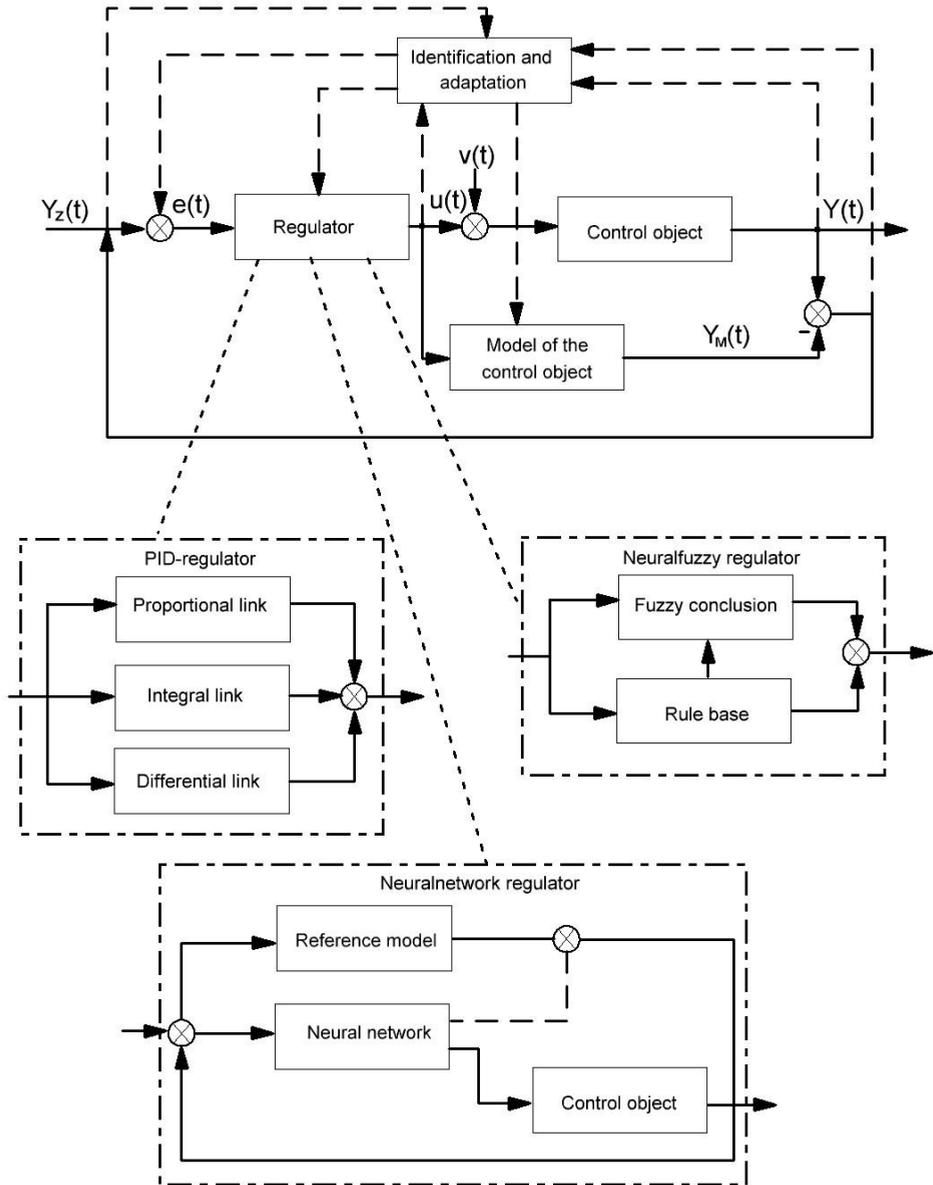
Figure 2. Flow chart of control

Analysis of the prediction model stability

The results of the study of the prediction model stability were shown (Table 1).

Absolute and relative errors of the evaporating station level of sugar juice prediction model

a	b	c	d	e
A	0	0	0	0
B	28	26	2	7.7
C	24	26	2	7.7
D	27	25	2	8
E	26	25	1	4
F	26	25	1	4
G	26	25	1	4
H	26	25	1	4
I	26	25	1	4
J	26	25	1	4
K	26	25	1	4

Note: *a is the point name; b is the forecasted value,% ; c is the actual value,% ; d is the absolute error (1), %; e is the relative error (2), % .

The dependence of the relative error on the absolute errors is shown in Figure 3.
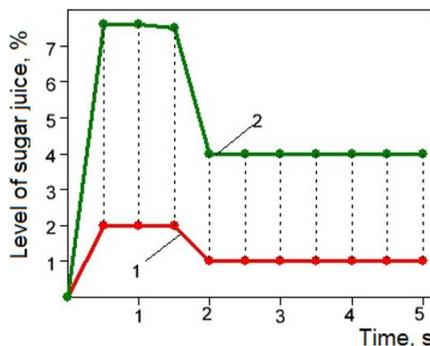
Figure 3. The dependence of relative error on absolute errors:
1 (——) – absolute error variation, 2 (——) – relative error variation.

This graph indicates that the relative error does not exceed 8%. In addition, it is apparent that in the case of dividing the time series into intervals, the accuracy of measurements remains stable if the absolute error does not change. This fact allows asserting that the obtained model of system prediction is stable and can be used to predict the operation of the evaporating station (Lei et al., 2016). Problems of the complexity of calculations are widely revealed in research (Xiao-Yang, 2007). The research (Liu et al., 2013) features development of a mathematical model of control of overheating of the electronic evaporator system of the expansion valve with the investigated control strategy. The authors of this article conducted the and modeling of the electronic expansion valve of the evaporator with fuzzy regulation were carried out in the exploration. The model is identified by the least-squares algorithm

based on the minimized sum of square residues. The research (Zhong et al., 2007) features a consideration of fuzzy control for evaporator overheating. The lack of development of intelligent regulators for the system as a whole is a common problem of these studies. Such problems can also arise due to the high complexity of calculations, lack of necessary hardware and software, and high cost of research. The analysis of the robust controller use in the evaporation process was done (Normey-Rico et al., 2005). The author conducted a comparative analysis of this type of controller with the PID-regulator and concluded that the suggested controller provides better performance. However, the comparison of the use of classical regulators and intelligent regulators was not conducted in this study. The cost of research may be the reason for this.

Analysis of the level of sugar juice prediction algorithm operation

The result of the implementation of the prediction algorithm for the level of beet juice in the first case of the evaporating station using neural fuzzy control is shown in Fig. 3. Table 2 indicates the results of calculations for the level of sugar juice in the first case of the evaporating station using PID, fuzzy, and neural network regulators. In Table 2: a is a point name, b is forecasted value,% , c is actual value,% , d is absolute error,% , e is mean error (SP) (formula 3), f is mean absolute error (SAP) (formula 4), g is mean relative prediction error (SVP) (formula 5), h is mean standard error (SKP) (formula 6), i is square root of the mean standard error (SQSKP) (formula 7), j is standard deviation (SV) (formula 8).

Based on the results shown in the table, it can be concluded that, since the value of SP is negative, the forecast was overestimated relative to the actual data (Lei et al., 2016). This is true because the forecast shows a small absolute error of 1% when using fuzzy control. Though it is absent in the actual use of this type of control. However, such an overestimation is insignificant, as can be seen from the mean relative prediction error (Dong et al., 2017).

Theoretically, when using the mean relative error in estimating the accuracy of the evaporation process prediction model, the value of the accuracy of the forecast can reach 100% (Lei et al., 2016). This will mean that the selected prediction model describes the process with absolute accuracy (Anghinoni et al., 2018). In practical terms, such a phenomenon is almost impossible, because the forecast cannot take into account all the factors that affect the automation system (Xu et al., 2020). In case when the value of the forecast accuracy is close to 0%, this model does not describe the forecasted process.
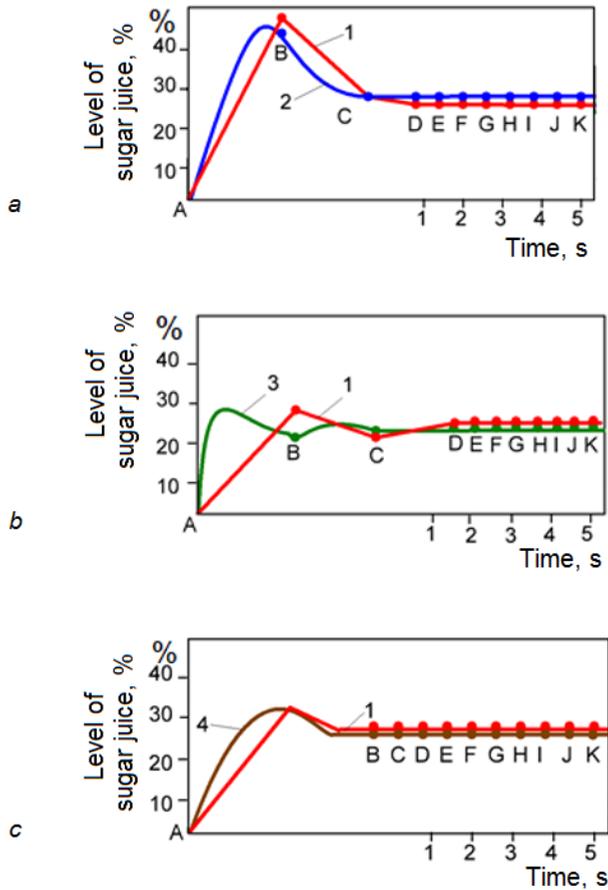
The forecast accuracy indicator is also used in order to select the optimal prediction model. The model with the accuracy closest to 100% (Lei et al., 2016) is considered optimal because it is more likely to make a more accurate forecast.

So far as in our case, the value of the mean relative error is 5%, consequently, the accuracy of the model is 95%. This is a very high assessment of the quality of our prediction system. Since the accuracy of the prediction model is very close to 100%, it can be considered optimal (Dong et al., 2017). In order to correctly understand how much one can trust the obtained evaporation process prediction algorithm, it is also necessary to evaluate the accuracy of the obtained forecast (Lei et al., 2016). Figure 4 shows a comparison of the forecasted value of the level of sugar juice change in the first case of the evaporating station using PID, fuzzy and neural network regulators, and the actual level of sugar juice change.

Table 2

Prediction error estimation indicators for the first evaporating station body

a	b	c	d	e	f	g	h	i	j
PID-regulator									
A	0	0	0						
B	49	50	1						
C	28	28	0						
D	27	28	1						
E	27	28	1						
F	27	28	1	0.8	0.8	2	0.8	0.89	24.18
G	27	28	1						
H	27	28	1						
I	27	28	1						
J	27	28	1						
K	27	28	1						
Accuracy of prediction model (9):								98%	
Neural fuzzy regulator									
A	0	0	0						
B	28	25	2						
C	24	25	2						
D	27	25	2						
E	26	25	1						
F	26	25	1	-0.9	0.9	5	0.002	0.045	24.02
G	26	25	1						
H	26	25	1						
I	26	25	1						
J	26	25	1						
K	26	25	1						
Accuracy of prediction model (9):								95%	
Neural network regulator									
A	0	0	0						
B	26	25	1						
C	26	25	1						
D	26	25	1						
E	26	25	1						
F	26	25	1	-0.9	1.27	4	0.9	0.94	21.87
G	26	25	1						
H	26	25	1						
I	26	25	1						
J	26	25	1						
K	26	25	1						
Accuracy of prediction model (9):								96%	

Figure 4. Comparison of transients of forecasted and actual levels of sugar juice in the first body of the evaporating station using:
a – PID-regulator,
b – neural fuzzy regulator and
c – neural network regulator
1 (—) – forecasted level of sugar juice value,
2 (—) – actual level of sugar juice value (PID-regulator),
3 (—) – actual level of sugar juice value (neural fuzzy regulator),
4 (—) – actual level of sugar juice value (neural network regulator),
AB, BC, CD, …, JK – time series intervals

In other studies, most of the problems of intelligent control in the evaporation process remain unresolved (Chantasiriwan, 2021; Lahtinen, 2001; Sidletskyi et al., 2016; Verma et al., 2018.) The use of neural fuzzy regulators takes place only in some specific cases. In addition, there is no comparison of the use of intelligent regulators with the use of classic regulators. There is also no explanation of the possibility of combining the work of several types of intelligent controllers if necessary. In addition, there is no clear means of prediction the operation of intelligent regulators.

In this study, the prediction method was used to compare the methods of level of sugar juice control in the device. This allows prediction the behavior of the system during the formation of the control action and displaying the finished forecast on the operator's screen, thus, increasing the efficiency of the evaporating station. This method has an advantage due to its easy and fast implementation, which does not require large economic and energy costs. The disadvantages of this method are the need to divide the transition process into separate time intervals of the numerical series manually and the direct dependence of the accuracy of the model on the number of elements of the time series.

Conclusions

1. According to the conducted studies of literary sources, it was determined that there is an unresolved part of the problems of intelligent control in the evaporation process – this is the use of neuro-fuzzy controllers in some specific cases.
2. Statistical data on the behavior of the automation system circuits in transient operating modes were collected using intelligent and classical controllers, and a model was built to predict the operation of the evaporator plant using the local trend method and a prediction algorithm was developed. The advantage of this method is its easy and fast implementation, which does not require large economic and energy costs. The disadvantage of this method is the need to divide the transient process into separate intervals of the time series manually and the direct dependence of the model accuracy on the number of elements of the time series.
3. In the work, a modification of the forecasting model by the local trend method was performed and an algorithm for predicting the operation of the evaporator plant was developed. The accuracy of the prediction model was 98% for the PID controller, 95% for the neuro-fuzzy controller and 96% for the neural network, which are high rates. the resulting system prediction model is stable and can be used to predict the operation of the evaporative plant
4. Analysis of the study data indicated that when fluctuations occur in the transient, an insignificant delay occurs, while the advantage of the model is its high accuracy and stability, which satisfies its use.

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Application of surface-active substances produced by *Rhodococcus erythropolis* IMB Ac-5017 for post-harvest treatment of sweet cherry

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Abstract

Introduction. The aim of the present study was testing of the supernatant of *Rhodococcus erythropolis* IMB Ac-5017 with different concentration of surface-active substances (SAS) for treatment of sweet cherry for shelf-life extension.

Materials and methods. *R. erythropolis* IMB Ac-5017 were grown in the medium with ethanol. Supernatant with concentration of SAS from 0.1 to 0.5 g/L was used for the treatment of sweet cherry fruit. Concentration of SAS in supernatant was determined by weight method. The total number of heterotrophic bacteria and fungi were determined by the plate dilution method.

Results and discussion. The treatment of sweet cherries with a supernatant containing 0.5 g/L SAS diminished the numbers of bacteria and fungi on the fruit's surface by 10 and 5 times, respectively, in comparison with cherries washed with water. The treatment of sweet cherries with supernatant containing 0.2 g/L SAS diminished the numbers of bacteria and fungi on the fruit's surface by 5 and 3 times, respectively; treatment with supernatant containing 0.1 g/L diminished the numbers of bacteria and fungi by 2 times in comparison with cherries washed with water. The treatment with supernatant with concentration SAS 0.5 g/L was most effective. Treated with supernatant sweet cherries fruits did not show signs of decay even on 7th day of storage, while untreated or washed with water fruits lost moisture, fruit's skin became wrinkled, cracks and decayed areas appeared on it.

Content of fungal cells on the surface of sweet cherry pretreated with supernatant with concentration of SAS from 0.1 to 0.5 g/L and after that contaminated with spore's suspension of *Aspergillus niger* P-3 were by 2 – 11 times lower than on the surface of fruits washed with water after 5 days of incubation.

The possibility of multiple usage of supernatant was shown. Application of supernatant with concentration of 0.5 g/L resulted in decrease of bacterial concentration after first usage by 10 times, after second usage it was diminished by 5 times and after third usage it was diminished by 3 times, meanwhile concentration of fungi decreased by 9, 5 and 4 times after I, II, and III usage of supernatant.

Conclusion. Surface-active substances synthesized by *Rhodococcus erythropolis* IMB Ac-5017 could be used for treatment of sweet cherry to extend their shelf life.

Introduction

The harvest season of sweet cherries is short; these fruits are extremely perishable and spoil easily after harvest due to physical damage during harvesting, transportation, water loss during the storage and rapid microbiological deterioration of the stored fruits. Therefore, even a short extension of shelf life due to postharvest treatments will be profitable for the fresh sweet cherries market. Different methods for sweet cherry fruit preservation have been developed. Traditional methods for maintenance of fresh-cut cherry quality include regulation of temperature and humidity (Chockchaisawasdee et al., 2016). Treatments of different fruits and vegetables with chemicals are widely used to prolong their postharvest storage life. To coat fruits and vegetables with chemicals, immersion or sprinklings are usually used (Golding, 2017; Suslow, 2005). Among the chemicals, chlorination is effective and relatively inexpensive method for reduction of the incidence of postharvest diseases. To decrease the quantity of microbial cells on the surface of fruits and vegetables, they can be immersed in water with added chlorine-containing substances – salts (calcium hypochlorite or sodium hypochlorite) or gases (chlorine gas or chlorine dioxide). It is known that this method is applied for the treatment of sweet cherries, melons, apples, pears, tomatoes, peppers, potatoes, and salads (Suslow, 2005). Treatment with fungicides is also provided by immersion of fruits or vegetables in their solutions, but if the amount of harvested fruits or vegetables is not too big, a sprinkling is used (Golding, 2017). In spite of the effectiveness of chemicals, their application is not appreciated by consumers because of health concerns. So, alternative safe methods have to be developed. Different biological methods for post harvested treatment of fruits are intensively studied. They include application of edible coating made of natural polysaccharide chitosan (Pasquariello et al., 2015; Romanazzi et al., 2018), natural biocides such as plant essential oils and methyl jasmonate (Maghenzani et al., 2018), microbial antagonists (Dukare et al., 2019; Lastochkina et al., 2019), and also combination of different biological agents (Guo et al., 2014; de Oliveira et al., 2017).

In recent years, several studies have been carried out to establish the possibility of biosurfactants – microbial surface-active substances (SAS) – application to extend shelf-life for fresh-cut fruits (Adetunji et al., 2018; Toral et al., 2018). The aim of the present study was testing of the supernatant of *Rhodococcus erythropoli*s IMB Ac-5017 with different concentration of SAS for the treatment of sweet cherry for shelf-life extension.

Materials and methods

Microorganisms

The strain *Rhodococcus erythropolis* was isolated from the oil-polluted soil and was deposited in the Collection of Microorganisms of Institute of Microbiology and Virology, National Academy of Science, Ukraine as *Rhodococcus erythropolis* IMB Ac-5017 (Pirog et al., 2020). This strain produces extracellular surface-active substances, which contain glycolipids (trehalose mono- and di-mycolates), neutral lipids and phospholipids (Pirog et al., 2013).

To determine antifungal activity of surface-active substances produced by *R. erythropolis* IMB Ac-5017, the fungal strain *Aspergillus niger* P-3 from the Collection of Microorganisms of the Department of Biotechnology and Microbiology, National University of Food Technologies, Ukraine, was used as a test culture.

Cultivation of *R. erythropolis* IMB Ac-5017

The liquid mineral medium with the following composition, g/L: $NaNO_3$, 1.3; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.1; Na_2HPO_4, 0.16; KH_2PO_4, 0.14; $CaCl_2$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.001; distilled water up to 1L, pH 6.8−7.0 was used for cultivation of the bacterial strain *R. erythropolis* IMV Ac-5017. Ethanol, 2% (v/v), was a source of the carbon and energy.

Inoculum was produced by the cultivation of bacterial strain in the liquid mineral medium of the same composition as shown above with 0.5% (v/v) of ethanol. Inoculum with the concentration of the cells of 10^4–10^5 cells/mL was taken from the exponential phase of growth and added to the medium for *R. erythropolis* IMV Ac-5017 cultivation in quantity of 10% (v/v).

Cultivation of *R. erythropolis* IMB Ac-5017 was conducted in the 750 mL flasks with the 100 mL of medium under shaking 320 rpm at 30 ºC during 120 hours.

Determination of surface-active substances concentration

The amount of surface-active substances (SAS) synthesized by *R. erythropolis* IMB Ac-5017 was determined by weight method. The culture liquid was centrifuged at 5000×g for 45 minutes (laboratory centrifuge LP–8, Kiev, Ukraine). The Folch solution (chloroform and methanol in volume ratio 2:1) was used for extraction of surface-active substances as it was described earlier (Pirog et al., 2019).

Preparation of SAS-containing supernatant

The cultural liquid after cultivation of *R. erythropoli*s IMB Ac-5017 was centrifuged at 5000×g for 25 minutes. Supernatant was separated from bacterial biomass and sterilized for 30 min at 112 °C. Fruits were treated with supernatant with concentration of SAS from 0.1 to 0.5 g/L. To achieve the desired concentration, supernatant was diluted by the addition of sterile tap water.

Fruits treatment

Fruits of the sweet cherry cultivar "Regina" were picked by hand from the trees cultivated without pesticides in the Experimental station, Gvozdev, Kyiv Oblast, Ukraine, GPS 50°14'53.5"N 30°28'41.3"E. The harvested fruits were ripe, without visible damages and infections. Selected fruits were divided into three groups with 10 – 30 pieces in each. The fruits from the first group were not treated at all, fruits of the second group were washed with tap water, and fruits of the third group were washed with supernatants with concentration of SAS from 0.1 to 0.5 g/L.

Fruits from second and third groups were placed in the glass cylinder, 250 mL of tap water or supernatant was added, treatment lasted for 5 min, and after that fruits were taken off and supernatant was reused to treat new group of fruits. The procedure was repeated and the third group of fruits was treated with the same supernatant. So, one solution of supernatant was used to tread three different groups of fruits. Untreated and treated fruits were placed on the plates and left at room temperature for observation. Microbiological analysis was done before the beginning of the fruit's storage.

Microbiological analysis

Some fruits from each group were taken aseptically   and then were homogenized for 3 min using dispersing instrument T 10 basic ULTRA-TURRAX. Homogenized mixture, 1 g, was placed in the tube with 9 mL of sterile water and was shaken vigorously. The quantity of microbial cells (colony-forming units, CFU) was determined by the plate dilution method. The quantity of heterotrophic bacteria was determined by their growth on the meat-and-peptone agar at 30 ºC for 24 hours, and the quantity of fungi was determined by their growth on the wort agar-agar at 24ºC for 48 hours.

Evaluation of antifungal activity of SAS containing supernatant of *R. erythropolis* IMB Ac-5017 against fungi *Aspergillus niger* P-3

Antifungal activity of SAS containing supernatant of *R. erythropolis* IMB Ac-5017 was determined by the following method described in (Matei e al., 2016). Selected fruits were divided into three groups with 10 – 30 pieces. Half of the fruits were bruised with a sterile lancet, and then fruits were washed with water or supernatant with different SAS concentration as it was described above. After 30 min, all sweet cherry fruits were sprayed with spore suspension (10^6 spores/mL) of fungi *Aspergillus niger*, which is one of the most common infectious agents of post-harvest spoilage of sweet cherry. After incubation, some sweet cherries from each group were taken with sterile pincette, were homogenized and microbiological analysis were performed.

Evaluation of fruits quality

Evaluation of sweet cherry fruits quality was done by viewing during the storage time. The experiment was finished when the signs of deterioration (usually on the seventh day) such as decay, changes of color and texture, the presence of the cracks and wrinkling were evident on all fruits.

Statistical analysis

The experiments were carried out in triplicates and the number of the parallel determinations varied from 3 to 5. Statistical analysis was done using computer program Statistix 10.0 for Windows version 11.5. The average means and standard deviations were calculated for the experimental results.

Results and discussion

Effect of concentration of SAS in supernatant of *R. erythropolis* IMB Ac-5017 and method for the treatment of sweet cherries on numbers of heterotrophic bacteria and fungi on the fruit's surface

Quality of fresh-cut sweet cherries are usually evaluated by their appearance, texture, colors, firmness, chemical composition, level of physiological activity and microbial characteristics such as a percent of fungal infections and the level of mesophilic bacteria on the surface of fruits (Asghari, 2019; Maghenzani 2018). Microbial load on the surface of

fresh-picked vegetables is not obviously negatively correlated with time of their storage, but this linkage becomes critical in case of fruits storage (Fan and Song, 2008). This is associated with high content of sugar in fruits which may cause rapid microbial spoilage. In comparison with cherries which have high content of organic acids, 1.5–1.8%, and 8−20% of sugar, content of sugars in sweet cherries is higher, 13–25% , and organic acids significantly lower, 0.4–1.5% (Chockchaisawasdee et al., 2016) that is one of the reasons of their susceptibility to microbial spoilage.

Decrease of microbial contamination on the surface of sweet cherries could increase the time of their storage, so first step in our investigation was determination of influence of the treatment of sweet cherries with supernatant with different concentration of SAS on the microbial numbers on the fruits surface (Fig. 1).

The numbers of bacteria and fungi on the surface of fruits washed with water were $5.4 \cdot 10^3$ colony forming units (CFU)/mL and $2 \cdot 10^3$ CFU/mL, respectively.

Treatment of sweet cherries with a supernatant containing 0.5 g/L SAS diminished the numbers of bacteria and fungi on the fruit's surface by 10 and 5 times, respectively, in comparison with cherries washed with water. The treatment of sweet cherries with supernatant containing 0.2 g/L SAS diminished the numbers of bacteria and fungi on the fruit's surface by 5 and 3 times, respectively; treatment with supernatant containing 0.1 g/L diminished the numbers of bacteria and fungi by 2 times in comparison with cherries washed with water. The treatment with supernatant with concentration SAS 0.5 g/L was most effective (Figure 1). Treated with supernatant sweet cherries fruits did not show signs of decay even on 7th day of storage, while untreated or washed with water fruits lost moisture, fruit's skin became wrinkled, cracks and decayed areas appeared on it (Figure 2).
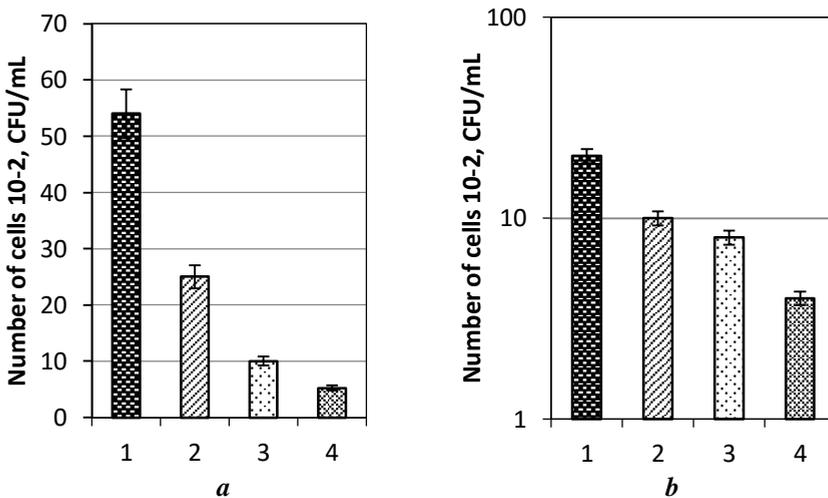
**Figure 1. The total number of heterotrophic bacteria (A) and fungi (B) depends on the method of sweet cherries treatment: washing with water (1);
the treatment with supernatant of *R. erythropolis* IMB Ac-5017 with SAS concentration:
0.1 g/L (2); 0.2 g/L (3); 0.5 g/L (4).**

On day		1st	7th
Control			
Washing with water			
SAS concentration, g/L	0.1		
	0.2		
	0.5		
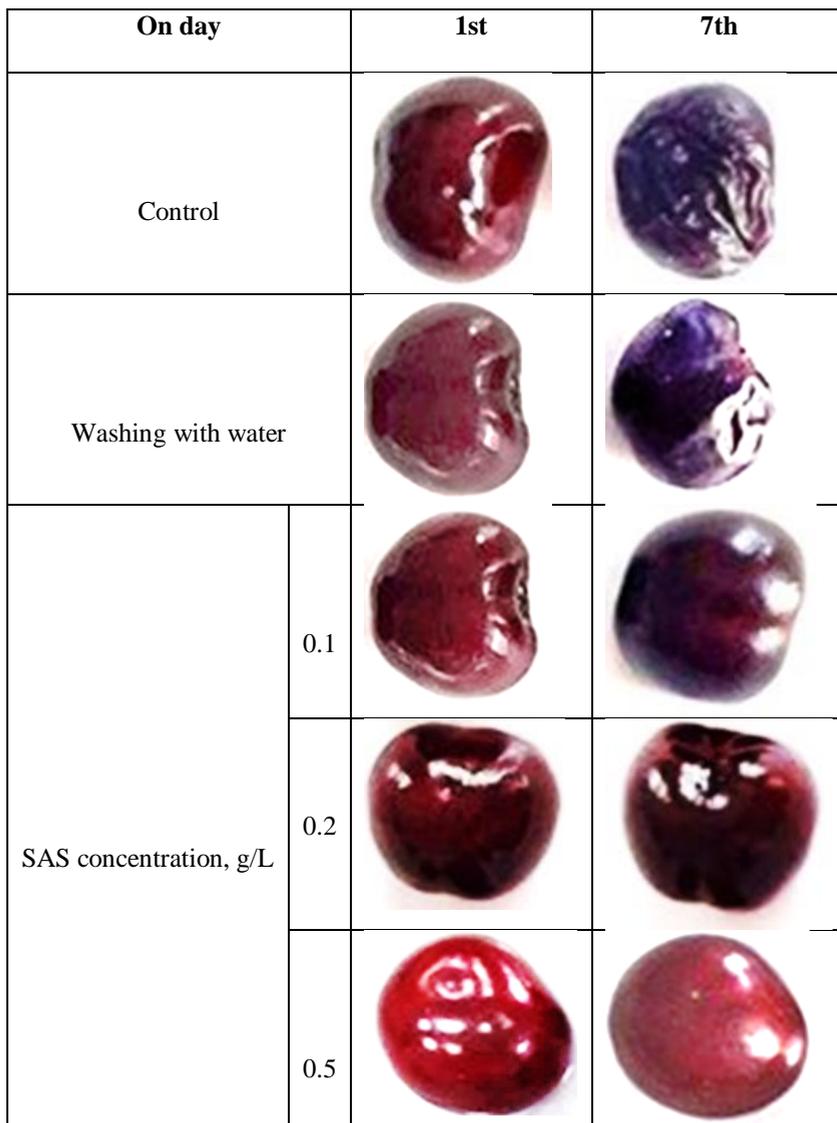
Figure 2. Effect of the treatment of the sweet cherries with SAS-containing supernatants produced by *R. erythropolis* IMB Ac-5017 on their storage.

There are some publications related to applications of biological methods to treat post-harvested sweet cherry fruits, but only a few ones studied application of microbial SAS for the treatment of fruits (Dengle-Pulate et al., 2015; Jing and Bingbing, 2010). Thus, it was shown that post-harvested treatment of sweet cherry cultivar "Regina" with solution of rhamnolipids was more effective in comparison with washing with water (Golding, 2017). It was also shown that solutions of these SAS had antifungal activity *in vivo* on the causative agent of brown rot (*Monilinia fructicola*) and gray rot (*Botrytis cinerea*) in ripe fruits.

Application of solution of rhamnolipids synthesized by *Pseudomonas aeruginosa* LBI (1.0 g/L) for the treatment of Surinam cherry decreased the number of fungi cells on the fruit's surface by 6 times and bacterial cells by 33 times in comparison with washing with water (Dilarri et al., 2016). Concentrations of microbial SAS solutions used for the treatment of fruits and vegetables usually ranged from 1 to 3 g/L (Dilarri et al., 2016; Jing and Bingbing, 2010). Application of microbial SAS gave possibility to extend shelf-life of different fruits. The lemons treated with germicidal composition containing 2.5% sophorolipids synthesized by *Candida bombicola* ATCC 22214, sodium silicate as water softener, 1%, sodium carbonate as absorbing material, 1.5%, and polyethylene glycol as an antifoamer, 1%, did not show any signs of microbial spoilage after 7 days of storage (Dengle-Pulate et al., 2015). Spiking with the solution of sophorolipids, produced by strain *Wickerhamiella domercqiae* Y2A, with concentration of 3 g/L was proposed to prolong the preservation life of apples, pears, citrus fruits, and apricots at room temperature (Jing and Bingbing, 2010).

Combined usage of coating containing rhamnolipids (2% w/v) and chitosan (2% w/v) of sweet oranges extended the shelf life of fruits. Addition of chitosan increased the antimicrobial effect of rhamnolipids application against spoilage microorganisms on ripe oranges (Adetunji et al., 2015). The treatment with solution of lipopeptides (8 g/L), produced by *Bacillus methylotrophicus* XT1 CECT 8661, of grapes, strawberries and tomatoes infected with a common plant pathogen *Botrytis cinerea*, resulted in disease reductions by 100, 12 and 50%, respectively, after 6 days of incubation at 25 °C and 70% humidity (Toral et al., 2018). According to our results, SAS produced by *R. erythropolis* IMB Ac-5017 showed effective antimicrobial properties in concentrations 0.1 – 0.5 g/L (Figs. 1 and 2) that is lower than described in literature.

Microbial number on the surface of treated with supernatant sweet cherries depending on multipleness of the usage of SAS-containing supernatant of *R. erythropolis* IMB Ac- 5017

When sweet cherries are harvested, they pass a few successive stages before realization: hydrocooling shortly after harvest (if transporting in remote points will be necessary), sorting, washing, and packing (Quero-García et al., 2017). Solutions of mineral or organic substances to treat sweet cherries by immersion or sprinklings usually were used just one time (Chockchaisawasdee et al., 2016; Dilarri et al., 2016). For large scale treatment, sweet cherries more often are immersed in recirculated solution of sodium hypochlorite (Quero-García et al., 2017). Sodium hydrochloride has high antimicrobial activity and its usage is economically reliable, however its effectiveness is decreased during recirculation process due to the presence of organic impurities in water (Golding, 2017; Suslow, 2005). In our research, we studied the possibility of multiple usage of supernatant: the same SAS containing supernatant of *R.erythropolis* IMB Ac-5017 was used to treat three different groups of sweet cherries. The numbers of bacteria and fungi on the surface of fruits washed with water were $4.0 \cdot 10^3$ CFU/mL and $2.6 \cdot 10^3$ CFU/mL, respectively. It was shown that the amount of bacteria and fungi were higher when the same supernatant was used in the second and third time. After the third time of supernatant usage, the numbers of bacteria and fungi were almost the same as in the case when fruits were washed with water (Fig. 3). Application of supernatant with concentration of 0.5 g/L showed the best results: concentration of bacteria diminished after first usage of this supernatant by 10 times, after second usage it was diminished by 5 times and after third usage it was diminished by 3 times, meanwhile concentration of fungi diminished by 9, 5 and 4 times after I, II, and III usage of supernatant.
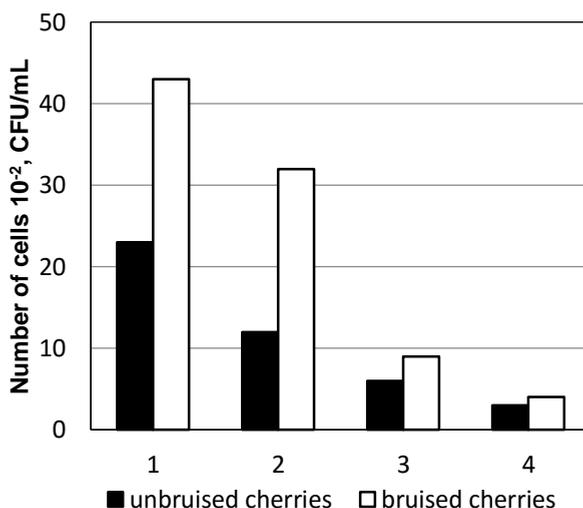
a

b
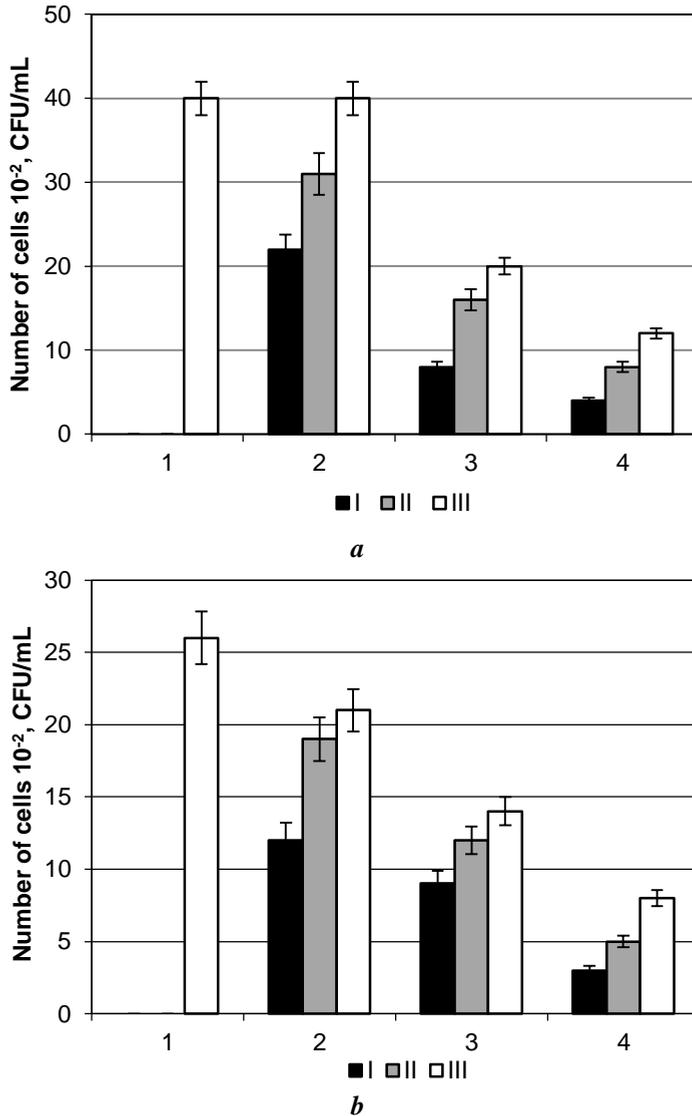
Figure 3. The total number of heterotrophic bacteria (A) and fungi (B) on the surface of sweet cherries washing with water (1); treated with supernatant of *R. erythropolis* IMB Ac-5017 with SAS concentration:
0.1 g/L (2); 0.2 g/L (3); 0.5 g/L (4) and different time of usage (I, II, III).

Antifungal activity of SAS-containing supernatant of *R. erythro*polis IMB Ac- 5017 on *Aspergillus niger,* infectious agents of postharvest spoilage of sweet cherry

One of the reasons for postharvest decay of sweet cherries is their contamination by fungi such as *Botrytis cinerea*, *Monilinia* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus*

stolonifer, *Cladosporium* spp. and *Aspergillus niger*. Application of synthetic fungicides for postharvest treatment of sweet cherries allows managing post-harvest decay caused by these pathogens. However, their use is limited by fungicide regulatory issues in some countries (Project CY17000, 2017). For example, iprodione, which is active against brown rot of sweet cherries, is permitted to be used in Australia, but its usage is prohibited in the European Union countries because of its toxicity (Karabulut et al., 2001; Project CY17000, 2017). So, searching other substances with antimicrobial activity against certain plant pathogens is a subject of many studies (Sharma et al., 2018; Yan et al, 2016).

Antifungal activity of supernatant of *R. erythropolis* IMB Ac-5017 against *Aspergillus niger* is shown in Figure 4.

Figure 4. The number of cells *Aspergillus niger* P-3 on the surface of sweet cherry washing with water (1);
treated with supernatant of *R. erythropolis* IMB Ac-5017 with SAS concentration:
0.1 g/L (2); 0.2 g/L (3); 0.5 g/L (4).

Sweet cherries in one group were bruised because cracks and fractures on the fruit's skins are possible entry sites for fungal infections. The number of fungi on the surface of untreated fruits was $8.6 \cdot 10^3$ CFU/mL. The number of cells of *A. niger* P-3 on the surface of unbruised and bruised sweet cherries washed with water diminished by 4 and 2 times, respectively. The number of cells of *A. niger* P-3 on the surface of unbruised and bruised sweet cherries treated with supernatant with SAS concentration 0.1%, 0.2 and 0.5% diminished in comparison with cherries washed with water by 2 and 1; 4 and 5; 7 and 11 times, respectively.

There are known a few researches concerning the application of microbial surface-active substances, most often solutions of rhamnolipids, in concentrations from 0.5 to 1.5 g/L, to treat artificially contaminated citrus fruits, potatoes and tomatoes (Sharma et al., 2016; Yan et al., 2014). Suppression of causative agents of potato rot *Fusarium solani* and tomatoes rot *Curvularia* sp. was observed after the treatment of vegetables with the solution containing 1 g/L of SAS produced by *Pseudomonas* sp. (Sharma et al., 2018). Effectiveness of application of solution of rhamnolipids (1.5 g/L) synthesized by *Pseudomonas aeruginosa*

JS29 to treat cherry tomatoes infected with *Alternaria alternata* was comparable with activity of synthetic fungicide carbendasim (Yan et al., 2016). There is information about application of rhamnolipids produced by P*seudomonas aeruginosa* to suppress growth of *Alternaria alternata* in lower concentration (0.5 g/L), but in combination with antagonistic of phytopathogens yeasts *Rhodotorula glutinis* (Yan et al., 2014). Treatment of tomatoes with solution of rhamnolipids (0.5 g/L) and suspension of yeasts *Rhodotorula glutinis* (1×10^8 cells/mL) decreased fungal contamination of tomatoes by *Alternaria alternata* by 60%.

According to our results, surface-active substances produced by *R. erythropolis* IMB Ac-5017 had high antifungal activity against *Aspergillus niger,* which is a causal agent of sweet cherries post-harvest spoilage, and could be used in concentrations lower than rhamnolipids described in literature.

Conclusion

Surface-active substances synthesized by *Rhodococcus erythropolis* IMB Ac-5017 have high antimicrobial activity in concentration lower then known microbial SAS and could be used as supernatant without extraction and purification for treatment of sweet cherry to extend their shelf-life. SAS – containing supernatant was effective even in the case of its reuse.

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Antimicrobial activity of a mixture of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 with antifungal drugs and essential oils

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Abstract

Introduction. The aim of the work was to study the effect of a mixture of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 under various cultivation conditions with antifungal drugs (clotrimazole and fluconazole) and essential oils (cinnamon and lemongrass) on yeast of genus *Candida*.

Material and methods. The cultivation of *A. calcoaceticus* IMV B-7241 was carried out in a basic medium that did not contain NaCl (medium 1), contained NaCl, 2.0 g/l (medium 2), contained NaCl, 2.0 g/l, and KCl, 1.0 g/l (medium 3). The surfactants were extracted from supernatant of cultural liquid by modified Folch mixture. Antimicrobial properties of the surfactants, antifungal drugs and essential oils were determined by index of the minimum inhibitory concentration (MIC). To assess the synergistic effect of a mixture of surfactants with antifungal drugs or essential oils the fractional inhibitory concentration index was used.

Results and discussion. Surfactants synthesized by *A. calcoaceticus* IMV B-7241 on the basic medium were the most effective antimicrobial agents against the yeasts strains *Candida albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 with MIC 22.5–45 µg/ml that were 2.6–17 times lower than the values determined for surfactants synthesized on modified media. At the same time, regardless of the strain cultivation in different media, all surfactants showed synergism of antifungal activity with clotrimazole, fluconazole, cinnamon or lemongrass essential oils. Thus, in the presence of surfactants synthesized on basic and modified media in a mixture with antifungal drugs, MIC of clotrimazole and fluconazole against the studied yeast test cultures decreased by 4–32 times. The use of a mixture of essential oils with surfactants synthesized by *A. calcoaceticus* IMV B-7241 growing in different media made it possible to reduce MIC of cinnamon and lemongrass oils against yeasts of *Candida* genus 4–18 and 8–32 times, respectively. At the same time, the index of fractional inhibitory concentration did not exceed 0.5, which indicates the synergism of anifungal activity between the studied compounds.

Conclusion. The results confirm the possibility to reduce the minimum inhibitory concentrations of antifungal drugs or essential oils against members of genus *Candida* by their mixture with microbial surfactants.

Introduction

The number of publications devoted to the study of yeast of genus *Candida,* which are causative agents of nosocomial infectious diseases, is increasing every year. This is due, first of all, to the spread of their resistant forms, arising against the background of prolonged use of broad-spectrum antibiotics, immunosuppressive therapy, and prolonged catheterization of patients (Singh et al., 2020).

Compared to antibacterial, the amount of antifungal agents is much less. This is mainly due to the fact that fungi are eukaryotes, so the development of new drugs for the selective control of such pathogens without toxic effects on humans is long-term and problematic (Pappas et al., 2016), So, at present, there are only five classes of drugs available for the treatment of fungal infections: azoles (fluconazole, miconazole, clotrimazole), polyenes (amphotericin, nystatin), echinocandins (micafungin, aspofungin, anidulafungin), allimines (terbinafine) and pyrimidine analogs (flucytosine) (Tsui et al., 2016), Compared to antibacterial agents, the number of antifungal agents is much smaller, and most clinical isolates of the genus *Candida* (in particular *C. albicans, C. tropicalis* and *C. glabrata*) are resistant to azoles, which are currently the most common medicine to treat fungal infections (Bhattacharya et al., 2020),

One of the approaches to increase the effectiveness of the use of existing antifungal compounds is the application of several drugs at once (for example, caspofungin and mycofungun) (Cui et al., 2015), zinc oxide nanoparticles and nystatin (Hosseini et al., 2020) and combination of antifungal drugs with essential oils or plant extracts (Jafri and Ahmad, 2020), At the same time, the concentration of such natural components should be minimal, which is associated with the ability of essential oils, when ingested, to cause severe damage to the central nervous system and aspiration pneumonia. This led to the search for methods to reduce the concentration of essential oils while maintaining their properties, in particular, their use in a mixture with other natural compounds, which can be microbial surfactants.

Interest in surfactants as antimicrobial agents is due to the unique mechanism of their action, which consists in violating the integrity of the cytoplasmic membrane and, due to this, practically excludes the possibility of the emergence of microorganisms resistant forms (Singh and Cameotra, 2004), Meanwhile the biological activity of microbial surfactants can be changed under different cultivation conditions, which should be taken into account when developing a technology for obtaining such metabolites. It was previously shown that the strain *Acinetobacter calcoaceticus* IMV B-7241 synthesizes surfactants having antimicrobial and antifungal activity (Pirog et al., 2021a; 2022), and it is possible to regulate their biological activity changing the potassium and sodium cations concentrations in the medium for cultivation (Pirog et al., 2016). These monovalent cations at high 50 and 100 mM concentrations are inhibitors of $NADP^+$-dependent glutamatedehydrogenase, a key enzyme in the biosynthesis of lipopeptides, which are the main antimicrobial agents, which ultimately resulted in low antimicrobial and antifungal activity of surfactants (Pirog et al., 2021). It was also found that surfactants synthesized by *Nocardia vaccinii* IMV B-7405 possessed synergistic antimicrobial activity against a wide range of yeasts and bacteria in mixure with antifungals drugs (nystatin and fluconazole) (Pirog et al., 2017) and essential oils (Pirog et al., 2020). So, it was assumed that it is possible to enhance the antifungal activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the presence of potassium and sodium cations in a mixture with antifungal agents and essential oils. This will simultaneously increase the efficiency of using not only surfactants as antimicrobial agents, but also antifungal drugs or essential oils, as well as reduce the concentration of components in the mixture.

The purpose of the present study was to investigate the possibility of synergistic action on the yeast of Candida genus of a mixture of surfactants, synthesized by Acinetobacter calcoaceticus IMV B-7241 in a medium with different contents of monovalent cations, with antifungal drugs and essential oils.

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.

Yeast *Candida albicans* D-6, *Candida utilis* BVS-65 and *Candida tropicalis* RE-2 from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technology were used as test cultures in determining the antimicrobial activity of surfactants, antifungal drugs or essential oils.

Clotrimazole and fluconazole, synthetic drugs belonging to the broad-spectrum azole class; essential oils of lemongrass (manufacturer Aromatika LLC, Ukraine) and cinnamon (manufacturer RosKosmetika LLC, Ukraine) were used as antifungal drugs.

Composition of the nutrient medium and cultivation conditions

The strain *A. 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, $Na_2HPO_4 \cdot 12H_2O$ – 0.6, KH_2PO_4 – 0.14, $MgSO_4 \cdot 7H_2O$ – 0.1, distilled water – up to 1 liter, pH 6.8–7.0. Yeast autolysate, 0.5% (v/v), and microelement solution, 0.1% (v/v), containing (g/100 ml): $ZnSO_4 \cdot 7H_2O$ – 1.1; $MnSO_4 \cdot H_2O$ – 0.6; $FeSO_4 \cdot 7H_2O$ – 0.1; $CuSO_4 \cdot 5H_2O$ – 0.004; $CoSO_4 \cdot 7H_2O$ – 0.03; H_3BO_3 – 0.006; KI – 0.0001; EDTA (Trilon B) – 0.5, were also added to the medium (basic medium). Cultivation of the strain IMV B-7241 was carried out in a basic medium that did not contain NaCl (medium 1), contained NaCl, 2.0 g/l (medium 2), contained NaCl, 2.0 g/l, and KCl, 1.0 g/l (medium 3), Used sunflower oil after frying potatoes at a concentration of 2% (v/v) was a source of carbon and energy**.** Seed material was a culture in the middle of the exponential growth phase, grown in base medium with 0.5% (v/v) used oil. The amount of inoculum was 5% of the medium volume (10^4–10^5 cells/mL), Cultivation was *carried out* in *flasks* (*750 ml*) with *100 ml* of *medium* in under rotation with *320 rpm* at 30 ºC for *120 hours.*

Determination of extracellular surfactants concentration

The surfactant concentration was determined by the Blay and Dyer method (Bligh and Dyer, 1959) 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.

25 ml of the supernatant (to obtain a supernatant, the culture broth was centrifuged at 5000 g for 20 minutes) was placed in a 100 ml cylindrical separatory funnel and extracted surfactant according to the advanced procedure 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 (Russia) at 50 °C and an absolute pressure of 0.4 atm to constant weight.

Determination of antimicrobial activity

The antimicrobial activity of surfactants, antifungal drags, essential oils and their mixtures on yeast was determined by index of the minimum inhibitory concentration (MIC) (Andrews, 2001), Determination of MIC was carried out by the method of two-fold serial dilutions in liquid wort. Under sterile conditions, 1 ml of the medium was added to 10 tubes, 1 ml of an antimicrobial substance (surfactant, antifungal substances 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 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 was determined as the value of the concentration of the studied 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 antifungal drugs or oil was reduced by the method of successive two-fold dilutions, in the other, the concentration of essential oil or antifungal drugs remained unchanged, and the concentration of surfactant reduced.

Determination of synergy of antifungal activity

Synergistic effect of surfactants with antifungal drugs or essential oils and essential oils was evaluated by indicator of fractional inhibitory concentration (FIC) – 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_A or C_B are the concentrations of the antimicrobial substance in the mixture;

MIC_A or MIC_B are minimum inhibitory concentrations of antimicrobial substance.

Results and discussion

Determination of synergistic antifungal action of a mixture of surfactants produced by *A. calcoaceticus* IMV B-7241 and antifungal agents

Synergistic antifungal action of a mixture of surfactants produced by *A. calcoaceticus* IMV B-7241 mixed with clotrimazole (Table 1) or flucanazole (Table 2) was studied. These drugs were chosen due to the availability of data on the spread of resistant yeast of *Candida* genus against the background of the widespread use of azoles (Bhattacharya et al., 2020; Cui et al., 2015) and the possibility of combining antifungal drugs with natural compounds (Carbone et al., 2019; Kumar et al., 2013; Tabbene et al., 2015) including those of microbial origin (Tabbene et al., 2015),

It has been found that surfactants synthesized by *A. calcoaceticus* IMV B-7241 on the basic medium were the most effective antifungal agents, and the values of the minimum inhibitory concentrations in relation to the test yeast cultures were 22.5–45 µg/ml, which were lower than MIC of surfactants synthesized using modified media 1–3 (Table 1 and 2).

Table 1

Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, clotrimazole and their mixtures

Media for surfactants synthesis	Test culture – yeast of genus *Candida*	MIC (µg/ml) of			[***]FIC
		Surfactants	[*]Surfactants mixed with clotrimazole	[**]Clotrimazole mixed with surfactants	
Basic	*C. albicans* D-6	22.5	5.6	1.9	0.28
	C. tropicalis RE-2	22.5	5.6	3.9	0.30
	C. utilis BVS-65	45	11.2	1.9	0.30
Medium 1	*C. albicans* D-6	608	38	3.9	0.06
	C. tropicalis RE-2	304	19	3.9	0.12
	C. utilis BVS-65	304	38	3.9	0.18
Medium 2	*C. albicans* D-6	118	29.5	15.6	0.49
	C. tropicalis RE-2	118	14.7	15.6	0.36
	C. utilis БМС-65	59	14.7	7.8	0.49
Medium 3	*C. albicans* D-6	769	96.1	3.9	0.18
	C. tropicalis RE-2	384	48	7.8	0.24
	C. utilis BVS-65	384	48	7.8	0.24

Notes:
[*]the concentration of clotrimazole was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 180 – 0.17 µg/ml for surfactants synthesized on the basic medium; 608–1.1 µg/ml on medium 1; 236 – 0 .92 µg/ml on medium 2, and 768 – 1.5 µg/ml on medium 3.
[**]the concentration of surfactants was unchanged and equaled their ½ MIC, and the concentration of clotrimazole was reduced by sequential double dilutions in the concentration range of 250 – 0.9 µg/ml.
[***] FIC ≤ 0.5 indicates synergism.

The minimum inhibitory concentration of clotrimazole against *C. albicans* D-6 and *C. tropicalis* RE-2 was 62.5 µg/ml, against *C. utilis* BVS-65 it was 31.2 µg/ml. Addition of all surfactants synthesized by *A. calcoaceticus* IMV B-7241 to the solution of clotrimazole reduced the MIC of this drug against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 by 4-32 times. The value of the fractional inhibitory concentration did not exceed 0.5, which indicates synergism between the compounds.

Surfactants synthesized by *A. calcoaceticus* IMV B-7241 on different media also showed synergism of antifungal activity in mixture with fluconazole (Table 2).

Table 2

Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, fluconazole and their mixtures

Media for surfactants synthesis	Test culture – yeast of genus *Candida*	MIC (µg/ml) of			***FIC
		Surfactants	*Surfactants mixed with fluconazole	**Fluconazole mixed with surfactants	
Basic	*C. albicans* D-6	22.5	5.6	4.6	0.36
	C. tropicalis RE-2	22.5	5.6	1.1	0.30
	C. utilis BVS-65	45	5.6	1.1	0.18
Medium 1	*C. albicans* D-6	608	76	4.6	0.24
	C. tropicalis RE-2	304	38	2.3	0.18
	C. utilis BVS-65	304	38	2.3	0.18
Medium 2	*C. albicans* D-6	118	14.7	9.3	0.38
	C. tropicalis RE-2	118	7.3	9.3	0.32
	C. utilis BVS-65	59	7.3	4.6	0.24
Medium 3	*C. albicans* D-6	769	192.2	9.3	0.51
	C. tropicalis RE-2	384	192.2	9.3	0.76
	C. utilis BVS-65	384	96.1	9.3	0.71

Notes:
*the concentration of fluconazole was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 180 – 0.17 µg/ml for surfactants synthesized on the base medium; 608 – 1.1 µg/ml on medium 1; 236 – 0.92 µg/ml on medium 2, and 768 – 1.5 µg/ml on medium 3.
** the concentration of surfactants was unchanged and equaled their ½ MIC, and the concentration fluconazole was reduced by sequential double dilutions in the concentration range of 284 – 0.55 µg/ml.
*** FIC ≤ 0.5 indicates synergism.

The minimum inhibitory concentration of fluconazole against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BMS-65 was 35.5 µg/ml. Addition of surfactants synthesized by *A. calcoaceticus* IMV B-7241 to the solution of fluconazole reduced the MIC of this drug against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 35.5 µg/ml to 1.1–9.3 µg/ml. Despite the higher FIC values of the mixture of surfactants synthesized on medium 3 with fluconazole (FIC 0.51-0.76), the minimum inhibitory concentrations of the latter were reduced by almost 4 times (from 35.5 to 9.3 µg/ml) (Table 2). Only a few reports were published on the synergism of antifungal compounds with microbial surfactants (Tabbene et

al., 2015) and other natural compounds (Carbone et al., 2019; Kumar et al., 2013; 2015). Thus, surface-active lipopeptides synthesized by *Bacillus subtilis* B38 showed synergistic antifungal activity with amphotericin B against *C. albicans* ATCC 10231 and clinical isolates of *C. albicans* and *C. tropicalis* (strains not specified) (Tabbene et al., 2015). At the same time, the minimum inhibitory concentrations of monopreparations of lipopeptides and amphotericin B were in the range of 12.5–25 µg/ml and 0.25–1 µg/ml, respectively. The use of the mixture made it possible to reduce both the concentration of lipopeptides to 0.39 µg/ml against *C. albicans* ATCC 10231 and to 0.78–1.56 µg/ml for clinical isolates and amphotericin B to 0.06 µg/ml against strain ATCC 10231 and 0.25 µg/ml against clinical isolates.

It was established in (Kumar et al., 2013) that diketopiperazines are cyclic dipeptides synthesized by *Bacillus* sp. N, in combination with clotrimazole, showed synergism of antimicrobial activity against *C. albicans* MTCC 277. Thus, the minimum inhibitory concentrations of the Cyclo-(L-Pro-L-Leu) dipeptide and clotrimazole against the MTCC 277 strain were 64 and 8 mg/ml, and in the mixture decreased to 2 and 1 µg/ml, respectively. The value of the fractional inhibitory concentration did not exceed 0.15, which indicates synergism. Similar results were observed when using a mixture of Cyclo(D-Pro-L-Leu) and Cyclo(L-Pro-L-Tyr) dipeptides with clotrimazole (Kumar et al., 2013). The MICs of these mixtures against *C. albicans* MTCC 277 were 2–4 µg/ml, respectively, while the minimum inhibitory concentrations of dipeptides were in the range of 16–32 µg/ml, and the MIC of clotrimazole was 8 µg/ml. Note that the cytotoxic effect of diketopiperazines in relation to fibroblast and epithelial cell lines was observed at a concentration of more than 200 µg/ml, which indicates the safety of using such a natural metabolite.

There is information in the literature about the use of essential oils (Carbone et al., 2019) and extracts (Kumar et al., 2015) of plant extracts in combination with clotrimazole. In the article (Carbone et al., 2019) it was found that the MIC of clotrimazole against *C. albicans* ATCC 10231 was 128 µg/ml, but in a mixtures with lavender or rosemary essential oils (ratio 1:1, concentration of essential oils 0.5–2%, v/v) decreased to 78 and 62.5 µg/ml, respectively. It should be noted that a mixture of clotrimazole with essential oils was used in the form of nanostructured lipid carriers, which made it possible to reduce the cytotoxicity of essential oils. It was shown that α- and β-asarans, the main active components of calamus (*Acorus calamus*), and showed antifungal activity against representatives of the *Candida* genus not only in the form of monodrugs, but also in combination with azoles (clotrimazole, fluconazole) (Kumar et al., 2015). When using their mixture with clotrimazole and fluconazole, there was a decrease in the minimum inhibitory concentrations against *C. tropicalis* MTCC 184 of both antifungal drugs (from 1 and 4 µg/ml to 0.06 and 0.25 µg/ml, respectively) and natural compounds (with 500 and 8 µg/ml to 64 and 2 µg/ml for α- and β-assarones, respectively).

Our results (see Tables 1 and 2) showed that the MICs of clotrimazole and fluconazole mixed with surfactants synthesized by *A. calcoaceticus* IMV B-7241 on basic or modified media are comparable and in some cases even lower than described in the literature (Kumar et al., 2013; Carbone et al., 2019; Kumar et al., 2013; 2015; Tabbene et al., 2015).

Determination of synergistic antifungal action of a mixture of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 and essential oils

The synergism of the antifungal activity of a mixture of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on different media and cinnamon and lemongrass essential oils was studied. The choice of essential oils was due to the following reasons: (a) the main

component of cinnamon essential oil, cinnamaldehyde, prevents the synthesis of ergosterol by binding to enzymes involved in the formation of the cytoplasmic membrane in yeast cells (da Nóbrega Alves et al., 2020); (b) geraniol, citral, citronellal and citronellol, which are the main components of lemongrass essential oil, inhibit the formation of hyphae as one of the virulence factors in the members of *Candida* genus (de Toledo et al., 2020).

The minimum inhibitory concentration of cinnamon essential oil against all test cultures was 156 μg/m. It has been found that the use of a mixture of cinnamon essential oil with surfactants synthesized by *A. calcoaceticus* IMV B-7241 grown on different media made it possible to reduce the minimum inhibitory concentrations of the essential oil against studied yeast of *Candida* genus by 4–18 times, from 156 to 8.5–39 μg/ml (Table 3).

Table 3

Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, cinnamon essential oil and their mixture

Media for surfactants synthesis	Test culture – yeast of genus *Candida*	MIC (μg/ml) of			***FIC
		Surfactants	*Surfactants mixed with essential oil	**Essential oil mixed with surfactants	
Basic	*C. albicans* D-6	22.5	1.4	8.5	0.11
	C. tropicalis RE-2	22.5	2.8	39	0.34
	C. utilis BVS-65	45	1.4	17	0.11
Medium 1	*C. albicans* D-6	608	19	17	0.04
	C. tropicalis RE-2	304	9.5	39	0.28
	C. utilis BVS-65	304	9.5	17	0.13
Medium 2	*C. albicans* D-6	118	14.7	39	0.37
	C. tropicalis RE-2	118	14.7	39	0.37
	C. utilis BVS-65	59	7.3	39	0.37
Medium 3	*C. albicans* D-6	769	24	17	0.13
	C. tropicalis RE-2	384	12	17	0.13
	C. utilis BVS-65	384	12	17	0.13

Notes:
*the concentration of cinnamon essential oil was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 180 – 0.17 μg/ml for surfactants synthesized on the base medium; 608 – 1.1 μg/ml on medium 1; 236 – 0.92 μg/ml on edimum 2, and 768 – 1.5 μg/ml on medium 3.
**the concentration of surfactants remained unchanged and equaled their ½ MIC, and the concentration of cinnamon essential oil was reduced by sequential double dilutions in the concentration range of 624 – 1.2 μg/ml.
*** FIC ≤ 0.5 indicates synergism.

The FIC index did not exceed 0.5, which indicates synergism between the compounds. Similar patterns were observed when using a mixture of lemongrass essential oil and *A. calcoaceticus* IMV B-7241 surfactant. For example, the minimum inhibitory concentrations against *C. albicans* D-6 of surfactants synthesized in all media were in diapason from 22.5 to 769 μg/ml, lemongrass essential oil was 312 μg/ml, and their mixtures were only 9.7–39 μg/ml. At the same time, the value of the fractional inhibitory concentration did not exceed 0.5, which indicates the synergism of the antifungal action of surfactants and lemongrass

essential oil. It was shown that the minimum inhibitory concentration of a mixture of *Nocardia vaccinii* IMV B-7405 surfactants with cinnamon and lemongrass essential oils against yeast of *Candida* genus was 4 – 19.5 µg/ml that was significantly lower than the MIC of single surfactants, 16–76 µg/ml, or essential oils, 156 µg/ml (Pirog et al., 2020). At the same time, surfactants synthesized by strain *N. vaccinii* IMV B-7405 under different cultivation conditions were effectively being mixed with essential oils and reduced the MIC of the latter.

There are only single reports on the synergism of the antifungal activity of microbial surfactants mixed with essential oils (Pirog et al., 2020). At the same time, there is information on the use of various essential oils and plant extracts in combination with antifungal drugs (fluconazole, nisin, ketoconazole, amphotericin B) against drug-resistant strains of *Candida* genus (Herman and Herman, 2021). However, the authors do not give the values of the minimum inhibitory concentrations of monodrugs and their mixtures.

Conclusions

The results confirm the possibility of using a mixture of microbial surfactants and antifungal drugs or essential oils to reduce the minimum inhibitory concentrations of the latter against members of *Candida* genus.

Surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 cultivated on different media showed synergism of antifungal activity of their mixture with antifungal drugs or essential oils. In the presence of surfactants synthesized on basic medium and modified media in a mixture with antifungal drugs and essential oils, made it possible to reduce minimum inhibitory concentrations of clotrimazole, fluconazole and cinnamon, lemongrass essential oils against yeasts of *Candida* genus by 4–32, 4–18 and 8–32 times, respectively. Nevertheless, the possible influence of the composition of the nutrient medium on surfactant antifungal ability should be taken into account when developing technologies for these products manufacturing.

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Анотації

Харчові технології

Вплив температури сушіння на органолептичні властивості, антиоксидантну активність і вміст поліфенолів у висушеному листі *Allium ursinum* L. subsp. *ucrainicum*

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Вступ. Коротка вегетативність *Allium ursinum* обмежує його доступність, тому сушіння забезпечує його цілорічне збереження. Метою пропонованого дослідження було визначення впливу температури сушіння на антиоксидантну активність і вміст поліфенолів у висушеному листі *Allium ursinum* L. subsp. *ucrainicum* та його органолептичні властивості.

Матеріали і методи. Оцінено вплив трьох температур сушіння (40, 50 і 60 °C) на органолептичні властивості (колір, здатність до дегідратації та регідратації), антиоксидантну активність і вміст поліфенолів у висушеному листі *A. ursinum*. Колір зразків вимірювали за допомогою системи комп'ютерного зору. Загальний вміст фенолів визначали спектрофотометрично, а антиоксидантну активність – методом DPPH.

Результати і обговорення. Виявлено значні відмінності між свіжими, зневодненими та регідратованими зразками *A. ursinum* за всіма проаналізованими параметрами кольору (висушене листя показало набагато нижчу інтенсивність зеленого кольору, ніж свіже). Висушування за вищих температур призводить до зміни кольору, яка більш виражена за вищих температурах сушіння (60 °C) через деградацію хлорофілу. Температури сушіння мали статистично значущий вплив на дегідратацію і регідратаційну здатність висушених зразків. Вища температура сушіння призводила до вищого ступеня зневоднення та регідратації (через пори висушеного продукту вода знову надходила в клітини). Конвекційне повітряне сушіння призводило до значного видалення вологи зі свіжого листя *A. ursinum* (більше 91%), але органолептичні якості листя *A. ursinum* зберігалися. Випробувані умови сушіння мали значний вплив на загальний вміст фенолів і антиоксидантну активність листя *A. ursinum*. Підвищення температури під час сушіння знижувало загальний вміст поліфенолів у висушеному листі *A. ursinum*. У всьому діапазоні вимірювань зразки, висушені за нижчих температур, мали вищу антиоксидантну здатність, тоді як більш високі температури сушіння призводили до більшого зниження антиоксидантного потенціалу висушеного рослинного матеріалу. *A. ursinum* вважається одним із функціональних харчових продуктів для споживання людиною завдяки високій харчовій цінності та профілактичному чи лікувальному впливу під час різних захворювань. Для отримання високоякісного сушеного продукту процес сушіння повинен забезпечувати якість, порівнянну зі свіжими овочами.

Ключові слова: *Allium ursinum L. subsp. ucrainicum, сушіння, аналіз зображень, фенол, антиоксидант.*

Ароматичний профіль македонських і болгарських червоних вин сорту Вранець та гібриду Кайласький рубін

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Вступ. Метою цього дослідження було визначення ароматичного профілю болгарських і македонських червоних вин, отриманих з місцевого сорту Вранець та гібриду сорту Кайласький рубін.

Матеріали і методи. Проведено газохроматографічне (GC-MS) дослідження для визначення ароматичного профілю червоних вин місцевого сорту Вранець (вирощується в Республіці Македонія) та гібридного сорту Кайласький рубін (вирощується в Республіці Болгарія).

Результати і обговорення. У фракції вищих спиртів в обох винах переважав 1-пентанол. Іншими спиртами були 1-пропанол, 2-пропанол, 1-бутанол, 1-гексанол, 3-метилтіол-1-пропанол. Вино сорту Вранець виявило більшу складність щодо цієї фракції, оскільки в ньому виявлено 3-гексен-1-ол, якого не було у вині Кайласький рубін. В обох винах виявлено велику кількість ароматичного спирту – фенілетанолу. Ця сполука мала велике значення для їх квіткового аромату. Складноефірна фракція двох вин була різноманітною, представленою ізопентилацетатом, етилкаприлатом, етилгексаноатом, етилдеканоатом та діетил малатом. Вино із сорту Вранець мало більшу складність ефіру, оскільки в ньому виявлено ще два ефірні представники – етил-2-гідробутират і 2-гідрокси-3-метил-діетиловий ефір. В обох винах була виявлена одна жирна кислота – гептанова, у дуже близьких концентраціях. За визначенням учасника дискусії, «обидва вина по-своєму були дуже гармонійними та мали характерні для обох сортів ноти». Загалом, описовий аналіз підтверджує компоненти, визначені за допомогою GC-MS, і дає чітке уявлення про профіль аромату обох сортів.

Висновки. Обидва вина демонстрували різноманітний, збалансований ароматичний профіль, виходячи з особливостей його леткого складу. Кожне вино виявляло індивідуальний ароматичний профіль.

Ключові слова: *Червоне вино, Вранець, Кайласький рубін, виноград, складний ефір, вищий спирт.*

Біологічна цінність білків культивованих грибів

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Вступ. Мета дослідження – науково обґрунтувати та експериментально підтвердити харчовий статус культивованих грибів як джерела легкозасвоюваних білків, есенціальних і замінних амінокислот, інших цінних біокомпонентів і перспектив їх використання у харчових технологіях.

Матеріали і методи. Досліджено два види культивованих грибів – печерицю двоспорову (*Agaricus bisporus*) та гливу звичайну (*Pleurotus ostreatus*), один вид дикорослих – підберезники (*Leccinum scabrum*): за біохімічними характеристиками,

масовою часткою альбумінів, глобулінів, глютелінів, проламінів; якісним і кількісним складом амінокислот у вільній і зв'язаній формах.

Результати і обговорення. Біохімічний склад шапок і ніжок грибів відрізняється за окремими показниками: вміст сухих речовин у шапках печериць на 13–18% більший, вміст білків – на 14,6–23,5%. Вміст клітковини – на 17–19% менший, що є істотною перевагою шапок. Це потрібно враховувати при промисловому переробленні грибів, попередньо відділивши ніжки від шапок, з дотриманням оптимальних параметрів процесу для кожної анатомічної частини. Білки печериць містять усі незамінні амінокислоти і можуть бути важливим джерелом лізину (4,95 мг%), фенілаланіну (7,04 мг%), лейцину (9 мг%), треоніну (7,6 мг%). 7,6% амінокислот міститься у вільному вигляді, серед них незамінних амінокислот майже половина. Це забезпечує ефективне використання амінокислот організмом людини для синтезу власних білків.

Вміст білків у свіжих печерицях становить 6–9% за їхньою масою, у гливі – 4–5%, у білих грибах – 6–8,5%, що підкреслює пріоритетність за білковою складовою саме печериць. Білки печериць на 70,3% представлено легкорозчинними фракціями – альбумінами і глобулінами, дещо менше їх у білках гливи (65%), а в білках підберезників цей показник зменшується до 53,2%. І тому білки культивованих грибів з мінімальними витратами енергії розкладаються в організмі до амінокислот, а також відзначаються високим ступенем протеолізу (майже на рівні білків молока) під дією ферментів шлунково-кишкового тракту. Високих результатів досягнуто завдяки науково обґрунтованому виборові досліджуваної сировини, в тому числі з урахуванням її органолептичних характеристик, кожну з яких оцінено на відмінно. Запропоновано й охарактеризовано критерії вибору печериць для домашньої кулінарії та промислового перероблення: вміст білка не менш ніж 6–9%, клітковини 2–3,5%; вуглеводів 1–1,5%.

Висновки. Культивовані гриби та продукти їх перероблення з високим вмістом білків та інших цінних компонентів мають стати неодмінною складовою харчових раціонів для подолання білкового дефіциту.

Ключові слова: *гриби, білки, амінокислоти, безпека, фракціонування.*

Ферментація яблучного соку за допомогою відбірних автохтонних молочнокислих бактерій

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Вступ. Через брак ферменту лактази вживання молочних продуктів є проблемою для людей з непереносимістю лактози. Фруктові соки мають високий вміст поживних речовин, тому виявилися ефективними носіями або живильними середовищами для пробіотиків, зокрема для молочнокислих бактерій; вони не містять лактози і можуть вживатися особами з непереносимістю лактози.

Матеріали і методи. У дослідженні використовували власноруч виготовлений яблучний сік і відбірні молочнокислі бактерії. Кількість життєздатних бактеріальних клітин визначали методом серійного розведення; титрувальну кислотність визначали автоматичним титром; концентрації цукрів і органічних кислот вимірювали за допомогою високоефективної рідинної хроматографії; загальний вміст фенольних

сполук визначали методом Фоліна-Хіокальтуса; антиоксидантну активність визначали за допомогою аналізу FRAP (залізо-знижувальна антиоксидантна здатність).

Результати і обговорення. Вибрані штами *Lactiplantibacillus plantarum* використовували для ферментації яблучного соку. Оптимальні умови для ферментації: початковий рН 4,5, тривалість 24 год, максимальна життєздатність бактеріальних клітин 8,23±0,17 log КУО/мл і 8,55±0,19 log КУО/мл для L. plantarum 74 і *L. plantarum* 76 відповідно. Характеристики яблучного соку в процесі бродіння змінювалися. Так, через 48 год ферментації підвищення титрованої кислотності викликало зниження рН, крім того, спостерігалося поступове зниження вмісту цукру. Найбільшу продуктивність молочної та яблучної кислот спостерігали протягом 48 год ферментації зі штамом *L. plantarum* 74. Ферментований сік з L. plantarum 52, L. plantarum 74 і *L. plantarum* 76 мав концентрацію загальних фенольних сполук 532,9±26,7 мг ЕГК/л, 587,3±29,4 мг ЕГК/л, 488,4±24,4 мг ЕГК/л і антиоксидантну активність 281,6±14,1 мг ЕАК/л, 300,6±15,0 мг ЕАК/л, 172,8±8,6 мг ЕАК/л відповідно після 72 год бродіння.

Висновок. Яблучний сік, ферментований відбірними штамами *Lactiplantibacillus plantarum* і збагачений молочнокислими бактеріями, може використовуватися як пробіотичний продукт, який можуть вживати особи з непереносимістю лактози.

Ключові слова: *яблуко, сік, ферментація, Lactiplantibacillus plantarum, антиоксидант, фенол, пробіотик*

Оцінка якості бісквіту зі зниженим вмістом сахарози з ячмінного солоду і пшеничного композитного борошна

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Вступ. Мета дослідження – визначити вплив заміни частини пшеничного борошна (WF) борошном пивного ячмінного солоду (BMF) зі зниженим вмістом сахарози в рецептурі на якісні характеристики бісквітних коржів.

Матеріали і методи. Для виробництва бісквітних зразків використовували WF і три різні типи пивоварного BMF (Plsen, Amber і Black) у різних співвідношеннях зі зниженим вмістом сахарози. Визначали вміст редукувальних цукрів у WF та BMF, а також вологість та активність води у зразках бісквіту. Також визначено питомий об'єм, колір за показниками CIEL*a*b*, проведено аналіз профілю текстури (TPA) та органолептичний аналіз за дев'ятибальною гедонічною шкалою.

Результати і обговорення. Вміст редукувальних цукрів становив 0,43, 7,75, 17,05 та 61,02 г/100 г у WF, Amber, Pilsen та Black BMF відповідно. Оскільки сахароза, як відомо, є чудовим інгредієнтом для зниження активності води, то вміст вологи та активність води у зразках бісквітного тіста значно збільшувалися зі зменшенням додавання сахарози. Питомий об'єм знизився з 1,99 см³/г у контрольній пробі WF до 1,79 см³/г у зразку WF зі зниженням вмісту сахарози на 50%. Зниження вмісту сахарози значно підвищило твердість і жувальну здатність, в той час як пружність і згуртованість бісквітних коржів зменшилася (p < 0,05). Додавання 20% BMF і зниження сахарози до 83,3% від вихідної рецептури пом'якшили ці ефекти, тому статистично значущих відмінностей між цими зразками та контрольним зразком WF з

точки зору питомого об'єму і параметрів текстури не було. Додавання BMF суттєво вплинуло на всі параметри кольору м'якушки бісквіту (p < 0,05). Бурштиновий бісквіт BMF:WF (20:80) зі зниженим додаванням сахарози (83,3%) мав найвищі органолептичні оцінки за кольором, зовнішнім виглядом і загальною прийнятністю. Pilsen BMF:WF (20:80) зі зниженим додаванням сахарози (83,3%) мав найкращий запах, а найкращий смак – контрольний зразок WF.

Висновок. Заміна WF на BMF у виробництві бісквітних тістечок надає можливість отримати широкий асортимент бісквітних виробів з різними якісними характеристиками, покращеними харчовими та функціональними властивостями. BMF має значну кількість власних цукрів, що може мінімізувати ефект зниження вмісту сахарози в рецептурі бісквіту.

Ключові слова: *бісквіт, ячмінний солод, борошно, сахароза, функціональність.*

Мінеральний склад борошна із сучасних і румунських сортів пшениці

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Вступ. Метою цього дослідження було вивчення мінерального складу борошна, отриманого з різних сортів пшениці колекції Банку генетичних ресурсів рослин «Mihai Cristea» міста Сучава, Румунія, вирощених в однакових умовах.

Матеріали і методи. Двадцять чотири зразки цільнозернового борошна, отриманого з різних сортів пшениці, зокрема п'ятнадцять з м'якої пшениці (*Triticum aestivum* L.), п'ять з однозернянки (*Triticum monococcit* L.) та чотири з пшениці спельти (*Triticum spelta* L.), були проаналізовані для визначення їхнього мінерального складу за допомогою рентгенівського енергодисперсійного аналізу. Статистичний аналіз результатів проведено за допомогою методики ієрархічного кластерного аналізу за методом WARD як алгоритму групування.

Результати і обговорення. Загалом, старі види пшениці характеризувались вищим вмістом мінеральних речовин, ніж сучасні, особливо сорти однозернянки. Для всіх зразків борошна виявлено суттєві відмінності у кількості калію (K), фосфору (P), кальцію (Ca), марганцю (Mn), заліза (Fe), цинку (Zn) та міді (Cu). Однак усі сорти пшениці мали високий вміст калію та низький вміст міді порівняно з іншими елементами, що визначалися. Деякі найбільш важливі для харчування людини мікромінерали, наприклад, Fe і Zn, у великій кількості виявлялися в борошні з різних сортів пшениці, але зразки давньої пшениці характеризувалися більшим вмістом цих елементів, ніж сучасні. У деяких сучасних сортах пшениці ці мінерали також були у достатній кількості. Вміст мінеральних речовин залежав від агрономічної врожайності, а не від приналежності сортів пшениці до давніх чи сучасних видів.

Висновки. Результати дослідження підтвердили велику відмінність у кількості мінералів між різними сортами. Інформація про цю варіацію може бути корисною у подальших селекційних дослідженнях, спрямованих на покращення поживної якості зерна пшениці та розробку стратегій біозбагачення мікроелементами. Як спельта, так і звичайна пшениця загалом показали високий вміст мінеральних речовин. Звісно ж, агрономічна врожайність значно впливає на кількість мінеральних речовин у пшениці.

Ключові слова: *Triticit sp., пшениця, зернові, мінерал, ієрархічний, кластерний аналіз.*

Використання борошна з насіння гарбуза при виготовленні хлібобулочних виробів

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Вступ. Метою дослідження було визначення впливу додавання борошна з насіння гарбуза на технологічні показники хлібобулочних виробів з пшеничного борошна.

Матеріали і методи. Досліджували борошно з насіння гарбуза великоплідного, твердокорого сорту «Рожевий банан» і вплив його додання на мікробіологічні (газоутворювальна здатність тіста та динаміка газоутворення) та біохімічні (кінетика цукрів у тісті) показники технологічного процесу виготовлення хліба з пшеничного борошна та якість готових виробів.

Результати і обговорення. Борошно з гарбуза сорту «Рожевий банан» має високий вміст білка (40%) та клітковини (12,2%), що у 3,8 та 3,5 раза більше, ніж у пшеничному борошні. За гранулометричним складом борошно з насіння гарбуза значно крупніше за пшеничне обойне борошно, що має позначитись на структурно-механічних властивостях тістових напівфабрикатів і хлібобулочних виробів при його внесенні. Водопоглинальна здатність цього гарбуза переважає відповідне значення для пшеничного борошна у 1,5 раза. Газоутворювальна здатність тіста для хлібобулочних виробів зі збільшенням дозування борошна з насіння гарбуза зменшилась на 1,9–7,4% порівняно з контролем, також на 7,6–16,2% зменшилась кількість утворених цукрів, однак збродження цукрів збільшується на 16,9–20,3%. Дещо збільшується кислотність м'якушки виробів, на 3,6–38,4% знижується питомий об'єм, на 1,4–4,1% – пористість. Проте споживчі властивості хліба покращуються. У готових хлібобулочних виробах вміст білка збільшується на 13,9–55,5% залежно від дозування гарбузового борошна, клітковини – на 12,07–48,7%, що свідчить про здатність цієї сировини значно підвищити харчову цінність виробів при її внесенні в рецептури.

Висновки. Використання борошна з насіння гарбуза для заміни частини борошна пшеничного в рецептурах хлібобулочних виробів дає змогу підвищити вміст білка та клітковини в цих виробах, а також їхню харчову цінність.

Ключові слова: *гарбуз, борошно, хліб, тісто, газоутворення.*

Вплив *Spirulina platensis* та *Kelp* на жирнокислотний склад пшеничного хліба

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Вступ. Метою цього дослідження було вивчення впливу деяких їстівних водоростей – *Spirulina platensis* та ламінарії, на вміст насичених і ненасичених жирних кислот у пшеничному хлібі.

Матеріали і методи. Хліб отримували з пшеничного борошна з додаванням ламінарії *Kelp* та спіруліни *Spirulina platensis* (порошок) у кількості 2 або 4% від маси борошна. Екстракцію загальних ліпідів проводили загальноприйнятим методом, а метилові ефіри жирних кислот аналізували за допомогою газового хроматографа з полум'яно-іонізаційним детектором.

Результати і обговорення. Збагачення ламінарією *Kelp* та спіруліною *Spirulina platensis* (у кількості 2% та 4% від маси борошна) впливає на вміст насичених і ненасичених жирних кислот у пшеничному хлібі. Оскільки різні види водоростей мають різний профіль жирних кислот, дві аквакультури давали різний ефект. За насиченими жирними кислотами включення ламінарії *Kelp* в рецептуру хліба викликало збільшення вмісту стеаринової, арахідонової та генейкозанової кислот, а збагачення спіруліною *Spirulina platensis* призводило до збільшення вмісту капронової, пальмітинової, арахідонової кислот і, особливо, генейкозанової кислоти. У контрольному хлібі кількість генейкозанової кислоти становить 0,17 г на 100 г жирів. У хлібі, збагаченому 2% і 4% ламінарії *Kelp*, кількість генейкозанової кислоти була в 2,2 та 3,5 раза вищою, ніж у контролі, а в хлібі з 2% та 4% *Spirulina platensis* – у 3,4 та 3,1 раза вище, ніж у контролі відповідно. Додавання морських водоростей також впливає на вміст ненасичених жирних кислот у пшеничному хлібі. При включенні ламінарії *Kelp* до рецептури хліба спостерігався підвищений вміст олеїнової та α-ліноленової кислот, тоді як при додаванні паулінової кислоти збагачення *Spirulina platensis* було ефективнішим.

Висновки. Збагачення пшеничного хліба їстівними водоростями *Kelp* та *Spirulina platensis* є ефективним способом підвищення вмісту в ньому деяких жирних кислот. При цьому ефект від додавання *Spirulina platensis* більш виражений.

Ключові слова: *білий хліб, Spirulina platensis, Kelp, жирні кислоти*.

Енологічна характеристика білих вин, виготовлених з деяких грузинських сортів винограду за кахетинськими методами виноробства

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Вступ. Метою дослідження було визначення показників якості (енологічна характеристика та вміст біологічно-активних сполук) вин з урахуванням їх сортового походження та методів виноробства без урахування екологічних, ґрунтових, виноградарських і виробничих умов.

Матеріали і методи. Для виготовлення дванадцяти зразків вина використовувалися чотири білі сорти винограду Ркацителі, Мцване Кахурі, Кісі та Хіхві. Застосовувалася «кахетинська» технологія виноробства шляхом спонтанного бродіння шкірок, кісточок і плодоніжок. Вміст органічних кислот визначали методом ВЕРХ. Загальний зміст танінів визначали методом титрування. Спектрометричні методи використовувалися для вимірювання загального вмісту фенолів та загальної антиоксидантної активності.

Результати і обговорення. Дослідження показали, що якісні характеристики вин Кісі та Хіхві кращі, ніж у Ркацителі та Мцване Кахурі. Однак Хіхві показало вищі результати за технологічними параметрами: вміст спирту коливався в межах 13,6–13,7%; вміст редукуючих цукрів становив від 3,7 до 4,0 г/л. Ці значення були передбачувані через високу концентрацію цукру у виноградному соку Хіхві та Кісі. Концентрації летких кислот (ЛК) залежать від внутрішньоклітинного метаболізму при вініфікації та можуть обумовлювати відмінності у їх значеннях, які варіювали від 0,40 до 0,46 г/л. Крім того, концентрації яблучної, лимонної та бурштинової кислот варіювали від 1,72 до 1,85 г/л, від 0,007 до 0,72 г/л та від 1,05 до 1,5 г/л відповідно. Мцване Кахурі відрізнявся складом органічних кислот і виявив найвищий вміст винної

кислоти, (1,42–1,95 г/л) серед досліджених зразків вин. І сорт винограду, і штам дріжджів можуть викликати коливання вмісту органічних кислот під час мимовільного бродіння. Вміст біоактивних сполук у зразках вина Кісі був вищим, ніж в інших проаналізованих білих винах. Сумарний вміст танінів коливався від 0,123 до 0,155%, загальний вміст фенолів варіював від 636,4–743,7 мг/л еквівалента галової кислоти та володів загальною антиоксидантною активністю 651,2–2629,8 мг/л у зразках Кісі. Тому можливо, що сорт винограду також відіграв значну роль у вмісті фенольних сполук і дубильних речовин. Крім того, була виявлена висока позитивна кореляція між загальним вмістом дубильних речовин та антиоксидантною активністю ($R^2 = 0,8871$), яка була сильнішою, ніж кореляція між загальним вмістом фенолів та антиоксидантною активністю з $R^2 = 0,8324$. Це можна пояснити різною хімічною будовою біоактивних сполук, особливо кількісним вмістом OH-групи.

Висновок. «Кахетинський» спосіб виноробства вигідний завдяки наповненню вина енологічними та біоактивними сполуками, що забезпечує отримання високоякісного напою. Крім того, якість вин сильно корелює з сортом винограду.

Ключові слова: *біле вино, кахетинське виноробство, біоактивний, антиоксидант.*

Економіка і управління

Китай і зміни харчових тенденцій: перспектива переходу до сталого розвитку

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Вступ. Населення світу стало свідком значних змін у способах виробництва і споживання їжі. Хоча це принесло користь здоров'ю населення, воно також сприяло зміні клімату та нестабільному використанню природних ресурсів.

Матеріали і методи. Комплексний огляд літератури.

Результати і обговорення. Окреслено характеристики чотирьох теорій переходу, пов'язаних із харчуванням, щоб допомогти пояснити поведінку населення, а саме: демографічного, харчового/білкового, харчового і стійкого переходу. Після них описано подальший настільний аналіз змін, що відбуваються в Китаї, найбільшій у світі демографії, і внесок цієї країни в найбільш необхідний глобальний перехід до сталого розвитку.

Теоретична основа теорій переходу, що використовуються з середини 20-го століття, окреслює зміни в поведінці населення, які впливають на відносини між людьми, а останнім часом і з природним середовищем. Будучи мультидисциплінарною галуззю, яка описує фундаментальні зрушення в людських суспільствах, теорії переходу є дуже проникливими стосовно їжі та харчування. Демографічний перехід пов'язує індустріалізацію з народжуваністю і смертністю, а також із доступністю їжі. Під час зміни харчування відбувається зміна у споживанні людьми калорій з різних груп їжі. Хоча частка білка залишається відносно стабільною, початковий перехід від рослинної їжі до тваринної зараз змінюється у зворотному порядку із зростанням обізнаності про екологію та охорону здоров'я. Перехід «харчування/білок» може призвести до кращої дієтичної поведінки зі зменшенням надмірного споживання, втрат і відходів. Перехід до харчових продуктів пояснює зміни на стороні пропозиції

– як виробляється, обробляється та розподіляється їжа, відображаючи зміни в методах сільського господарства, використання землі, ґрунту, води, добрив і хімікатів, ланцюгів постачання і розподілу. Більш стійкі методи ведення сільського господарства в даний час впроваджуються у відповідь на екологічно загрозливі тенденції в результаті змін у землекористуванні та використання хімічних речовин. На відміну від інших концепцій, перехід до сталості описує не еволюційну модель змін, а лише поточну найбільш необхідну трансформацію в розвитку. Це вимагає радикальної трансформації та дій, спрямованих на зменшення впливу на навколишнє середовище всієї діяльності людини, включаючи харчування.

Розвиток Китаю зазнав подібних змін, але з унікальними особливостями. Його демографічний перехід відбувся під впливом «політики однієї дитини», тоді як перехід «харчування/білок» був спричинений підвищенням рівня доходів. Індустріалізація виробництва харчових продуктів із застосуванням хімікатів широко поширена, але останнім часом набирають обертів органічні методи землеробства. Продовольча безпека та виробництво визнаються як виклик і можливість у переході Китаю до сталого розвитку з державними дієтичними зусиллями щодо обмеження внутрішнього споживання м'яса.

Висновок. Китай має можливість відігравати важливу роль у глобальному переході до покращеного вибору їжі, як того вимагають нинішнє середовище і надзвичайна кліматична ситуація, змінюючи власні харчові звички, а також вносячи внесок у розвиток нових альтернатив продуктів тваринництва.

Ключові слова: *Китай, харчування, білок, теорія переходу, сталість.*

Процеси і обладнання

Інтелектуальне автоматизоване керування випаровуванням цукрового соку з підсистемою прогнозування

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Вступ. Метою статті було дослідження інтелектуального автоматизованого керування рівня цукрового соку у випарному апараті з підсистемою прогнозування, що дасть змогу визначити поведінку системи автоматизації.

Матеріали і методи. Досліджували інтелектуальне автоматизовання керування рівня цукрового соку у випарній установці з підсистемою прогнозування. В схемі автоматизації регулювання рівня цукрового соку як датчик використовуються ємнісні рівнеміри. Як виконавчі механізми використано пневматичні сідельні клапани з вбудованим дроселем та електропневмоперетворювачем.

Результати і обговорення. Використання нейронечітких регуляторів відбувається лише в окремих специфічних випадках інтелектуального керування процесом випарювання. При цьому відсутні дані порівняння застосування інтелектуальних регуляторів з класичними, можливості комбінування роботи кількох типів інтелектуальних регуляторів, а також чітких засобів прогнозування їх роботи. Тому в пропонованому дослідженні було використано метод прогнозування для порівняння методів регулювання рівня цукрового соку в апараті. Це дало змогу спрогнозувати поведінку системи при формуванні управляючого діяння та вивести

готовий прогноз на екран оператора, що підвищило ефективність роботи випарної станції. Було зібрано статистичні дані поведінки контурів системи автоматизації в різних режимах роботи з використанням інтелектуальних та класичних регуляторів і побудовано модель прогнозування роботи випарної станції методом локальної тенденції та модифіковано алгоритм прогнозування. Перевагою цього методу є легка і швидка його реалізація, яка не потребує великих економічних та енергетичних затрат. Точність моделі прогнозування склала 98% для ПІД-регулятора, 95% – для нейронечіткого регулятора та 96% – для нейромережевого. Отримана модель прогнозування системи є стабільною, оскільки під час квантування часового ряду на інтервали абсолютна похибка залишиться сталою, то точність вимірювань відповідно буде незмінною.

Висновки. Запропонована система інтелектуального автоматизованого керування випаровуванням цукрового соку з модифікованим методом прогнозування на основі локальних тенденцій. Незважаючи на те, що запропонована система має несуттєве запізнення, прогнозування виконується з високою точністю і стабільністю.

Ключові слова: *цукор, випарний апарат, нейронечіткий регулятор, інтелектуальне керування, прогнозування.*

Біотехнологія, мікробіологія

Перспективи використання поверхнево-активних речовин *Rhodococcus erythropolis* ІМВ Ас-5017 для післяврожайної обробки черешні

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Вступ. Метою статті було дослідження можливості використання супернатанту *Rhodococcus erythropolis* ІМВ Ас-5017 з різною концентрацією біосурфактантів для обробки черешні з метою подовження терміну зберігання.

Матеріали і методи. *R. erythropolis* ІМВ Ас-5017 вирощували у середовищі з етанолом. Для обробки черешні використовували супернатант з концентрацією ПАР 0,1−0,5 г/л. Концентрацію ПАР у супернатанті визначали ваговим методом після екстракції сумішшю Фолча. Загальну чисельність гетеротрофних бактерій і грибів визначали за методом серійних розведень.

Результати і обговорення. Обробка плодів черешні супернатантом, який містив 0,5 г/л ПАР, зменшувала число бактерій і грибів на поверхні плодів в 10 та 5 разів відповідно, порівняно з їх кількістю на митих водою черешнях. Обробка плодів черешні супернатантом, який містив 0,2 г/л ПАР, зменшувала число бактерій і грибів на поверхні плодів в 5 та 3 рази відповідно, а обробка супернатантом, який містив 0,1 г/л ПАР, зменшувала число бактерій і грибів на поверхні плодів в 2 рази порівняно з їх кількістю на митих водою черешнях.

Черешня, яка була оброблена супернатантом з концентрацією ПАР 0,5 г/л , не мала ознак гниття навіть на сьому добу зберігання, в той час як необроблена або мита водою черешня втрачала вологість, шкірка починала зморщуватися, тріскатись і з'являлися плями гниття.

Вміст клітин грибів на поверхні черешні, яка була оброблена ПАР з концентрацією 0,1−0,5 г/л, а потім інфікована суспензією спор *Aspergillus niger* Р-3,

був у 2–11 разів нижче, ніжув контролі, де черешня милась водою, після п'яти діб інкубації. Необроблені або миті водою фрукти швидше піддавалися гниттю порівняно з обробленими ПАР-вмісними супернатантами.

Показана можливість багаторазового використання розчину ПАР *R.erythropolis* IMB Ac-5017 для обробки черешні. Найкращі результати було отримано при концентрації ПАР 0,5 г/л: концентрація бактерій зменшилася після першого використання в 10 разів, після другого використання – в 5 разів, а після третього використання – в 3 рази, в той час як концентрація грибів зменшилася в 9, 5 та 4 разів після I, II, та III використання супернатанту.

Висновок. Поверхнево-активні речовини, що синтезуються бактеріями *Rhodococcus erythropolis* IMB Ac-5017, можуть бути використовувані для обробки фруктів з метою подовження терміну їх зберігання.

Ключові слова: *черешня, бактерія, Rhodococcus erythropolis, біосурфактант, зберігання.*

Антимікробна активність суміші поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241 з антифунгальними препаратами та ефірними оліями

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Вступ. Метою статті було дослідження дії суміші поверхнево-активних речовин (ПАР), синтезованих у різних умовах культивування *Acinetobacter calcoaceticus* IMB B-7241, з протигрибковими препаратами (клотримазол і флуконазол) та ефірними оліями (кориці та лемонграсу) на дріжджі роду *Candida*.

Матеріали і методи. Культивування *A. calcoaceticus* IMB B-7241 здійснювали у базовому рідкому мінеральному середовищі з відпрацьованою соняшниковою олією, а також базовому у середовищі, що не містило NaCl (середовище 1), в якому концентрація NaCl становила 2,0 г/л (середовище 2), в яке додатково вносили 1,0 г/л KCl (середовище 3). Концентрацію ПАР визначали ваговим методом після екстракції супернатанту культуральної рідини модифікованою сумішшю Фолча. Антимікробну активність поверхнево-активних речовин, антифунгальних препаратів, ефірних олій та їх суміші аналізували за показником мінімальної інгібуючої концентрації (МІК). Для оцінки синергічної дії суміші поверхнево-активних речовин з антифунгальними препаратами чи ефірними оліями використовували показник фракційної інгібуючої концентрації (ФІК).

Результати і обговорення. Встановлено, що найбільш ефективними з досліджуваних поверхнево-активних речовин антимікробними агентами виявилися ПАР, утворені *A. calcoaceticus* IMB B-7241 на базовому середовищі: мінімальні інгібуючі концентрації щодо *Candida albicans* Д-6, *Candida tropicalis* PE-2 та *Candida utilis* БМС-65 становили 22,5-45 мкг/мл і були у 2,6-17 разів нижчими порівняно з показниками, визначеними для препаратів, синтезованих на модифікованих середовищах 1−3. Водночас, незалежно від культивування штаму на різних середовищах, усі ПАР проявляли синергізм антифунгальної активності з клотримазолом, флуконазолом, ефірними оліями кориці та лемонграсу. Так, за наявності в суміші з антибіотиками поверхнево-активних речовин, синтезованих у

базовому і модифікованих середовищах, мінімальні інгібуючі концентрації клотримазолу і флуконазолу щодо досліджуваних дріжджових тест-культур знижувалися у 4–32 рази. Використання суміші ефірних олій з ПАР, синтезованих *A. calcoaceticus* ІМВ В-7241 на різних середовищах, дало змогу знизити мінімальні інгібуючі концентрації щодо досліджуваних дріжджів роду *Candida* олії кориці та лемонграсу у 4–18 та 8–32 рази відповідно. При цьому показник фракційної інгібуючої концентрації не перевищував значення 0,5, що вказує на синергізм анифунгальної активності між досліджуваними сполуками.

Висновки. Наведені результати підтверджують дані щодо можливості використання суміші мікробних ПАР та антифунгальних лікарських засобів чи ефірних олій для зниження мінімальних інгібуючих концентрацій останніх щодо представників роду *Candida*.

Ключові слова: *ПАР, Acinetobacter calcoaceticus ІМВ В-7241, синергізм, протигрибковий, ефірна олія, антимікробний.*

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Book

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

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2 і більше авторів	(Bazopol et al., 2021)

Приклад тексту із цитуванням: It is known (Bazopol et al., 2006; Kuievda, 2020), the product yield depends on temperature, but, there are some exceptions (Arych, 2019).

У цитуваннях необхідно вказувати одне джерело, звідки взято інформацію.
Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:
Автори А.А. (рік видання), Назва статті, *Назва журналу (курсивом)*, Том (номер), сторінки.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
1. Приклад:
   Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (Juglans regia L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108.

2. Посилання на книгу:
Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
  Приклад:
2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.
   Приклади:
   (2013), *Svitovi naukovometrychni bazy*, Available at:
   http://www.nas.gov.ua/publications/q_a /Pages/scopus.aspx
   Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт.
   Зручний сайт для транслітерації з української мови: http://translit.kh.ua/#lat/passport

Стаття надсилається за електронною адресою: ufj_nuft@meta.ua

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

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Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

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InfoBase Index (2015)
Chemical Abstracts Service Source Index (CASSI) (2016)
Emerging Sourses Citaton Index (2018)

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Члени міжнародної редакційної колегії:

Наукове видання

Ukrainian Food Journal

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