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Editorial office address:
National University of Food Technologies
Volodymyrska str., 68
Kyiv 01601
Ukraine

E-mail:
Ukrfoodscience@meta.ua

Contents

Influence of rice flour on conformational changes in the dough during production of wheat bread

Anastasiia Shevchenko, Svitlana Litvynchuk

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Anastasiia Shevchenko
E-mail:
nastyusha8@ukr.net

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Abstract

Introduction. The aim of the work was to determine the influence of rice flour on the conformational transformations in the structure of the dough for the production of wheat bread, which contains lecithin.

Materials and methods. Chemical composition and fractional composition of proteins of rice flour were investigated. Conformational transformations of structural elements in dough and bread were investigated by infrared spectroscopy in the near-infrared region.

Results and discussion. In rice flour, the total protein content lower by 47%, and the dietary fiber content is 8.5 times lower than in premium wheat flour. The fractional composition of proteins showed a higher content of albumin, prolamin and insoluble proteins in wheat flour than in rice flour by 11, 90 and 75%, respectively. The content of globulins and glutelins is more in rice flour by 7 and 183%, respectively, but the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides. In rice flour, oryzenin is a representative of glutelins. The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: the extremes are observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection. The spectrum of sunflower lecithin differs significantly due to its different chemical composition. Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are visible. The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting α-helices and β-turns and contributed to the formation of disulfide bonds.

Conclusion. The conducted studies indicate the expediency of using rice flour in the technology of bakery products to replace wheat flour in order to minimize the fiber content in bread.

Introduction

The change in the environmental and economic situation in the world in recent years has significantly affected the structure of the population's diet and approaches to the production of food products (Ivanov et al., 2021). The increase in the incidence of alimentary and non-alimentary diseases, the rapid development of diseases of the gastrointestinal tract, including inflammatory bowel diseases (IBD), made it necessary to look for approaches to the development of dietary therapy for the prevention of these diseases. Need to investigate the possibility of using non-traditional raw materials increased in various branches of the food industry, in particular in the bakery industry, as one of the key ones (Forbes et al., 2017).

Most medical scientists prevent from using a large amount of dietary fiber in the diet of patients with IBD (Armstrong et al., 2021; Chiba et al., 2015), because they do not have the appropriate microorganisms to digest fiber (Armstrong et al.., 2022). For this purpose, rice processing products are promising raw materials, in particular rice flour with a low dietary fiber content (Drobot et al., 2021).

Rice germ contains lipophilic antioxidants (tocopherols, tocotrienols and γ-oryzanol) and phenolic compounds. These substances protect from free radicals and extend the shelf life of products (Esa et al., 2013; Min et al., 2011).

Water- and fat-absorbing capacities, emulsifying and foaming capacity allow the use of rice by-products in bakery and confectionery industries. Defatted rice bran can be used to replace up to 10-20% of wheat flour in recipes of biscuits without adversely affecting their quality (Ashraf et al., 2012).

Rice flour is basically used in the technology of gluten-free bread in a mixture with starches, in particular potato and corn starch (Mykhonik et al., 2017). It was established that it is advisable to add rice flour to the recipe of products in the amount of up to 30% to replace starch. At the same time, the content of aroma-forming substances in bread increases by 12.7% compared to bread made from a mixture of starches. The nutritional value of bread is also significantly increased, in particular, the content of proteins increases by 2.5 times, the content of potassium, magnesium, iron and vitamins increases too.

The influence of various rice by-products (flour, wholemeal flour, and bran) on the rheological parameters and quality of bread was studied (Genevois et al., 2021). A three-component mixture scheme was used. The optimal proportion of the mixture is 45:35:20. The specific volume of bread was 1.7 ± 0.1 cm^3/g, hardness – 0.23 ± 0.01, color intensity – 23.8 ± 0.4. The use of different rice fractions improved product quality and nutritional profile.

The use of phospholipids with a high content of phosphatidylcholine is important in the diet of patients with IBD, which prevents damage to the upper and lower parts of the gastrointestinal tract, participates in the formation of the protective layer of intestinal mucus (Stemmel et al., 2014). Soy lecithin became widely used in the food industry, but a promising non-GMO alternative raw material is sunflower lecithin (Guitto et al., 2015, Shevchenko et al., 2021).

It was determined that the dosage of lecithin in the amount of 1.0% to the mass of rice flour in gluten-free bread technology improves gas formation of the dough and increases the specific volume of bread by 16.2% and porosity by 12.7% (Medvid et al., 2018).

However, the influence of the mixture of lecithin and rice flour on the technological process and the quality and nutritional value of bakery products was not investigated, so this direction is relevant.

The aim of the work was to determine the influence of rice flour on the conformational transformations in the structure of the dough for the production of wheat bread, which contains lecithin.

Materials and methods

Materials

Premium wheat flour, sunflower lecithin and rice flour were used for research.

Dough samples were prepared with the addition of pressed baker's yeast (3% by weight of flour) and salt (1.5% by weight of flour). Lecithin was added in the amount of 3% to the mass of flour. This dosage was chosen based on the recommendations for the daily norm of lecithin for people with IBD (Partridge et al., 2019). Wheat flour was replaced with rice flour in the recipe in the amount of 10%, 20%, 30%, and 40%. The control was a sample without additional raw materials.

Total protein content

Kjeldahl method was used for determination followed by titration technique. 1 g of raw material must be hydrolyzed with 15 mL concentrated sulfuric acid for 2 h in a heat block at 420 ◦C with two copper catalyst tablets. After cooling before neutralization and titration, distilled H_2O was added to the hydrolysate. The amount of protein was calculated taking into account the nitrogen concentration in the product. Data was expressed as g proteins per 100 g of flour (Shevchenko et al., 2022).

Fiber

A collaborative study was conducted to determine the total dietary fiber (TDF) content in products, using enzymatic-gravimetric method. TDF was calculated as the weight of the residue minus the weight of protein and ash (Shevchenko et al., 2022).

Protein fractional composition

During the sample preparation, rice flour was sifted through a mesh sieve to obtain a fine powder, and kept at 4 °C until further use. The raw material was defatted with pentane. The flour/solvent slurry was mixed at a 1:10 *w/v* ratio for 24 h, and then the solvent was removed by centrifugation. The mixture was dried and stored in airtight bottles at 4 °C until subsequent use. According to the Osborne differential extraction procedure rice proteins were fractionated from pentane defatted meal (Horax et al., 2010). The meal-water suspensions (20 g of meal into 100 mL of deionized water) were mixed for 2 h at room temperature and centrifuged at 20,000 *g* for 30 min to separate the supernatant from the pellet, and obtain the deionized water extract (DWE). The same conditions for extraction/separation were kept for the next protein extraction steps. The water extract pellet was put into 100 mL of 1 M NaCl solution and mixed as mentioned above. The supernatant obtained after centrifugation, was extracted in 100 mL of deionized water at pH 11 with 0.5 M NaOH solution, leading to the alkaline extract (AE). Each extraction was made twice. Pellets were washed twice after each extraction using 20 mL of solvent. It was made to collect the residual protein entrapped in the insoluble residues. Obtained extracts were precipitated for isolation by adjusting the pH of the obtained supernatant to the pH corresponding to the minimum of solubility (pHms) determined from the turbidity experiment (Rezig et al., 2013). The pH was revised by 1 M HCl or 1 M NaOH solutions in acidic or alkaline pH, respectively. After centrifugation for 15 min at 15,000 g, the isolated protein precipitates were washed using deionized water twice at their respective pHms and recentrifuged. The resulting protein fractions were resolubilized by providing the pH to 7.0, freeze-dried, and stored at 4 °C until further analysis (Rezig et al., 2015).

Near-infrared reflection spectroscopy

Infrared spectrometer (Labor-Mim, Hungary) was used to research the reflection spectra from shredded samples and a smooth surface in near infrared range from 1330 to 2370 nm. Firstly, the spectrometer recorded the reflectance spectrum from reference I0, secondly a reflection spectrum from the researched sample. The spectra are represented as the reflectivity of R in relative units (the ratio of the intensities I/I0 = R), depending on the wavelength in nm (Litvynchuk et al., 2022; Niewitetzki et al., 2010; Yip et al., 2012). The intensity of reflection was measured in rice and wheat flours, in lecithin, in dough after kneading and after 3.5 hours of fermentation and in bread. The reflection intensity was expressed through the relative reflection coefficient.

Statistical analysis

The data represents the mean of a minimum three replicates ± standard deviation (S.D.). Graphical presentation of experimental data was performed using standard statistical processing programs – Microsoft Excel 2010.

Results and discussion

Protein and amino acid composition of wheat and rice flours

The chemical composition of raw materials, especially the content and composition of proteins, plays a decisive role in determination of the properties of dough for bakery products (Amjid et al., 2013).

The total protein content in rice flour is 47% lower than in premium wheat flour. The content of dietary fibers is 8.5 times lower, which makes it a valuable raw material for the production of bread for patients with IBD (Drobot et al., 2021).

Fractional composition of rice flour proteins differ from wheat flour (Table 1).

Table 1

Fractional composition of proteins of wheat flour and rice flour

Mass fraction of proteins (g/100g)	Wheat flour	Rice flour
Albumin	5.4±0.21	4.8±0.21
Globulin	9.9±0.34	10.6±0.36
Glutelin	27.1±0.78	76.7±1.67
Prolamin	43.1±1.12	4.3±0.18
Insoluble proteins	14.5±0.46	3.6±0.15

A higher content of albumin, prolamin and insoluble proteins was determined in wheat flour. The content of globulins and glutelins is higher in rice flour, but the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides (Huang et al., 2011). In rice flour, the representative of glutelins is orizenin, which does not have properties to form a dough frame (Jayaprakash et al., 2022).

The difference in the chemical composition of wheat flour, rice flour and lecithin should affect the change in the basic structural units of dough and bread with these components in the recipe. To identify and analyze these components, it is advisable to use the reflection spectrum in the near infrared region (Baslar et al., 2011).

The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: the extremes are observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection (Figure 1).

Wheat flour has a higher relative reflection coefficient, so its spectrum is placed higher than the spectrum of rice flour. Sunflower lecithin has different chemical composition in comparison with flours, due to which its reflection spectrum differs from the reflection spectra of flour and has different shape with the appearance of additional extreme (at a wavelength of 1720 nm, a clear minimum of reflection intensity is observed in the spectrum of sunflower lecithin, which was not previously observed in flour spectra). Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are noticeable. In particular, the reflection minimum of the sunflower lecithin sample compared to the flour samples shifted by 40 nm (from 2100 to 2140 nm). This indicates the absence of protein in its composition, which is present in flour samples (Beć et al., 2019).

Figure 1. Infrared reflection spectra of raw materials

After kneading the dough, the spectra of the samples obtained with different replacement of wheat flour by rice differed slightly, although all extremes were the same (Figure 2).

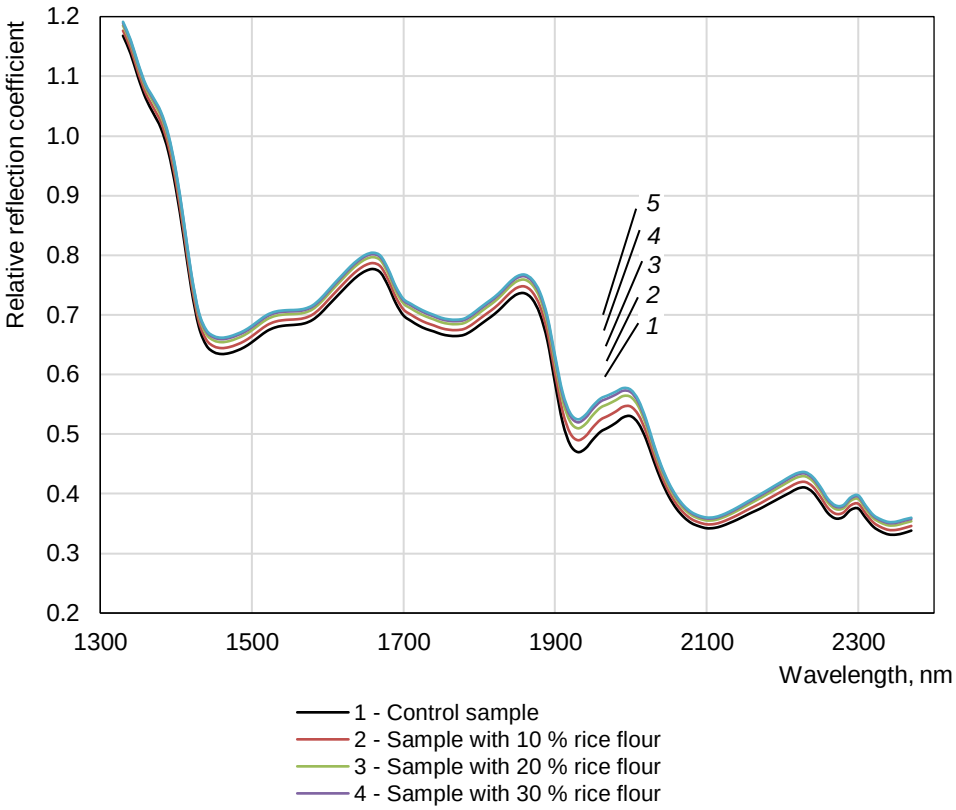

Figure 2. Infrared reflection spectra of dough samples with rice flour (10, 20, 30 and 40% replacement) after kneading

There is a direct regularity in the location of spectra of the samples: the control sample of the dough is the lowest, sample with 10% replacement is above it, samples with 20, 30, and 40% replacement are higher. The similarity of the obtained spectra indicated that the determining factor affecting the formation of dough properties is wheat flour, since its mass fraction remains the largest in the dough. However, the intensity of reflection of the dough with rice flour, regardless of the percentage content, tended to the spectrum of the control sample of dough, acquiring springy-elastic and visco-plastic properties, which in the control sample are provided by the content of glutenins and gliadins of wheat flour. The difference in the spectra near the wavelength of 1930 nm indicates the content of moisture in the samples (the control sample has the lowest intensity and a clear sequence is observed: the increase in the intensity of reflection by the samples with an increase in the replacement percentage). Since the dough samples were prepared for the experiment under the same conditions, it can be concluded that this trend is caused by the higher water absorption and moisture retention capacities of rice flour than wheat flour, which in turn is caused by the greater dispersion of its particles (Lapčíková et al., 2021).

After the end of fermentation and keeping of the dough (after 3.5 hours), the conformational changes of the structural substances of the dough were determined (Figure 3). All spectra of dough samples with different doses of rice flour changed their reflection intensity, but all extremes remained at the wavelength values observed in Figure 2.

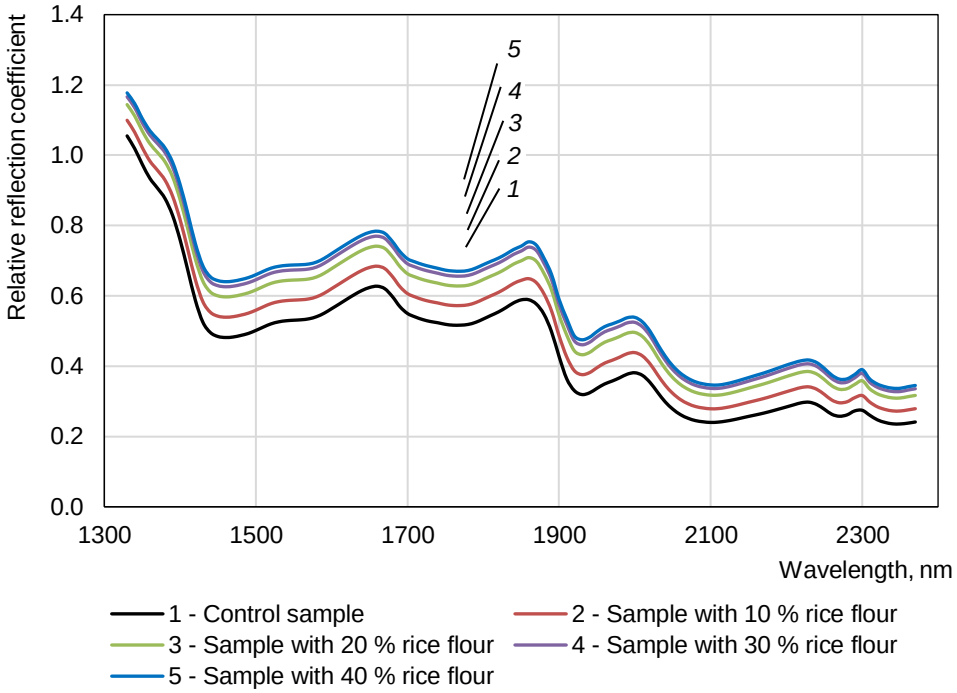

Figure 3. Infrared reflection spectra of dough samples with rice flour (10, 20, 30 and 40% replacement) after fermentation

In contrast to the spectra of the dough obtained after kneading, due to changes in the properties of the biopolymers of the raw material, in particular, the swelling of proteins and starch, during the fermentation process, the spectra changed the intensity of reflection and moved away from each other evenly, but kept their regularity of location. That is, with an increase in the percentage of replacement of wheat flour with rice, the reflection coefficient increased, and the spectrum was higher than the control sample of the dough. But in the dough samples with the replacement 30 and 40%, the spectra almost overlapped and their difference was barely noticeable. This indicates that structural changes are more active, although less, in samples with a lower percentage of replacement.

In the process of baking, under the influence of high temperatures, protein and other substances of the dough change, which affects the intensity of the reflection of the finished products. The analysis of the spectra of the control sample of dough and the dough samples with 40% replacement of wheat flour with rice flour (after kneading and after 3.5 hours of fermentation), as well as samples of bread (Figure 4), made it possible to conditionally divide them into two groups (three spectra in each group), since each of them has the same initial relative reflection coefficient.

The obtained spectra of the first group showed that the control sample of the dough after kneading and the sample of dough with 40% rice flour after 3.5 hours of fermentation are practically the same. The spectrum of the dough sample after kneading with 40% replacement with rice flour is close to them, but with a slightly higher value of the relative reflection coefficient.

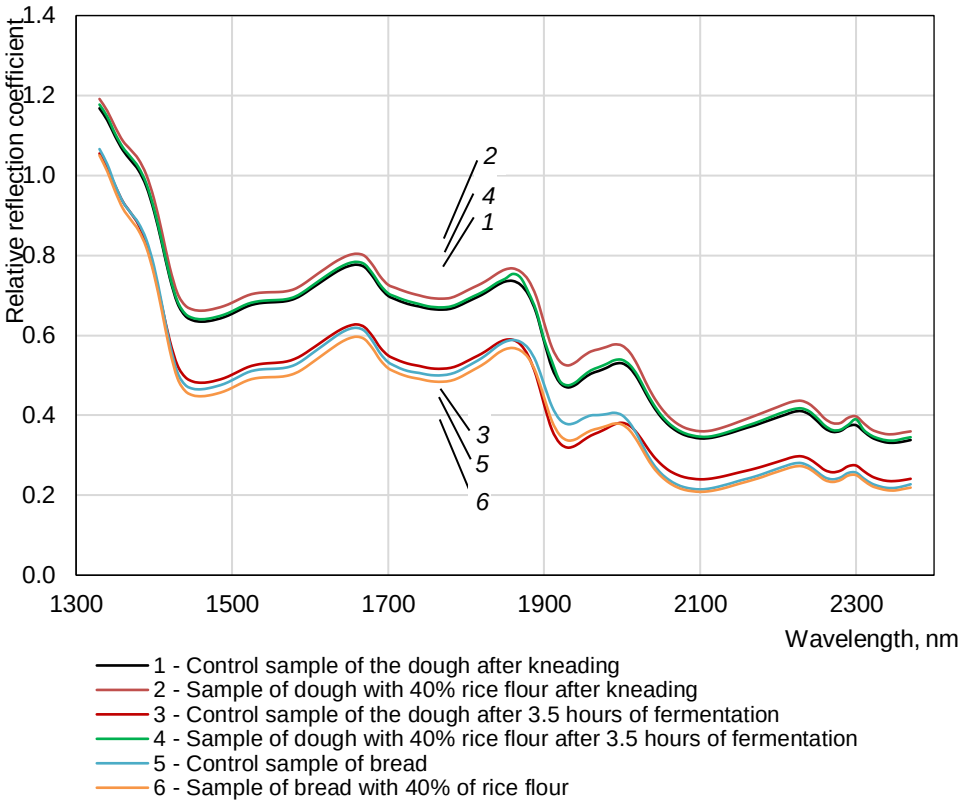

Figure 4. Infrared reflection spectra of dough and bread samples with rice flour (40% replacement)

The spectra of the control sample of the dough after 3.5 hours of fermentation, the control sample of bread and bread with 40% replacement, which are separated into the second group, also conditionally match and overlap each other. However, the initial relative reflection coefficient has a lower value than that of the first group. This indicates that the conformational transformations in finished products have a similar character to transformations in dough with wheat flour after fermentation and are determined to a greater extent by the properties of wheat flour (Chompoorat et al., 2022). The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting α-helices and β-turns and contributed to the formation of disulfide bonds (Xiang et al., 2020). This indicates that the deformation of gluten with a lower content of intermolecular β-sheets, antiparallel β-sheets and β-turns, but a higher content of α-helices compared to dough gluten (Verbauwhede et al., 2018) increases during the baking process.

The conducted studies indicate that in the technological process of breadmaking conformational changes of protein take place and the expediency of using rice flour in the technology of bakery products to replace wheat flour in order to minimize the fiber content in bread according to the recommendations of diet therapy for patients with IBD.

Conclusion

1. The total protein content in rice flour is 47% lower than in premium wheat flour, and the dietary fiber content is 8.5 times lower.
2. Wheat flour has a higher content of albumin, prolamin, and insoluble proteins than rice flour by 11, 90, and 75%, respectively. The content of globulins and glutelins is more in rice flour by 7 and 183%, respectively. But the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides. In rice flour, oryzenin is a representative of glutelins.
3. The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: extremes have been observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection. Sunflower lecithin has a minimum reflection intensity at a wavelength 1720 nm. Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are noticeable.
4. The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting α-helices and β-turns and contributed to the formation of disulfide bonds.

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Biological potential (phenolic complex and antioxidant activity) of white grapes and wines from varieties with different genetic origin, grown in the region of Central Northern Bulgaria

Dimitar Dimitrov, Tatyana Yoncheva

Agricultural Academy, Institute of Viticulture and Enology, Pleven, Bulgaria

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Corresponding author:

Dimitar Dimitrov
E-mail: dimitar_robertov@abv.bg

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Abstract

Introduction. The aim of the present study is to define the biological potential (based on phenolic complex and antioxidant activity) of white grapes (and their corresponding wines) grown under the influence of the terroir of Central Northern Bulgaria.

Materials and methods. The objects of the research were grapes and wine from the white varieties Chardonnay, Dimyat and Druzhba, harvest 2021, grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven. Chemical analysis, analysis of phenolic compounds and antioxidant activities of grape musts and wines were made.

Results and discussion.The highest amount of total phenolic compounds (TPC) was found in the grape must of the local variety Dimyat. The study of the presence of flavonoid phenolic compounds (FPC) in the grape musts showed that Chardonnay has the highest potential for FPC accumulation. The highest amount of non-flavonoid phenolic compounds (NPC) was found in the grape must of the control introduced variety Chardonnay. At titratable acid (TE) 600.00 mg/dm^3, the highest antioxidant activity was found in the grape must of the Druzhba hybrid. Dominance in the content of TPC was found in the wine form the local variety Dimyat (0.93±0.000 g/dm^3), while the Chardonnay showed the lowest amount of TPC (0.45±0.000 g/dm^3). In terms of FPC content, Chardonnay wine dominated (696.46±0.37 mg/dm^3), and Dimyat had the lowest content (439.38±3.35 mg/dm^3). The highest concentration of NPC was found in the wine of the Druzhba hybrid (130.47±0.59 mg/dm^3). The lowest result according to this indicator was found in the wine of the control variety Chardonnay (84.13±0.43 mg/dm^3). The Chardonnay wine showed significantly higher antioxidant activity compared to the other two studied varieties – 1.5 times higher radical-eliminating activity compared to Dimyat and compared to Druzhba – 2 times higher.

Conclusions. The research proved that the white varieties and wines grown in the region of Central Northern Bulgaria showed a balanced biological activity and potential, comparable to wines and grapes from other regions of the world.

Introduction

Modern food science places serious emphasis on the biological potential of various foods and beverages. In-depth research is being carried out, aiming to define the components of the composition of food and drink products with high biological activity, functional and health effects, all of which reflect in an overall improvement in the functions of the human organism.

Antioxidants are substances capable to neutralize reactive oxygen species (free radicals) through direct binding, which leads to blocking the possibility of oxidative stress in the human organism (Lobo et al., 2010; Young and Woodside, 2019). Oxidative stress, determined by the activity of free radicals in the body and their binding to cellular structures, leads to the initiation of various damages associated with the disruption of a number of functions and the formation of many diseases: the initiation of cancer forms, atherosclerosis, cardiovascular diseases, arthritis, accelerated aging, neurodegenerative and autoimmune diseases (Lea, 1966; Harman, 1992; Pham-Huy et al., 2008).

Phenolic compounds are secondary metabolites that underlie the antioxidant capacity and activities of various plant species (Arvanitoyannis et al., 2006). Their accumulation in the grapes of *Vitis vinifera* L species is dependent on a number of factors: grapevine variety, soil and climatic conditions in the area of cultivation, irrigation, plant protection measures, sun exposure and others (Burin et al., 2014). Their accumulation in the wine includes some other factors: degree of grapes ripeness (Andrade et al., 2001), vinification technology (Salacha et al., 2007), aging of the wines (Zafrilla et al., 2003).

Numerous studies confirm the inhibitory ability of wine and grapes phenolics against free radicals, reducing the risks of cancer, cardiovascular diseases, diabetes and others (Palma-Duran et al., 2017; Kerry and Abbey, 1997). The direct relationship between phenolic compounds and antioxidant activity has been demonstrated in various studies regarding the influence of maceration in the accumulation of phenolics and the increase in the antioxidant activity of must and red Syrah wines from Brazil (Alencar et al., 2017), a study of the phenolic composition and antioxidant capacity of table grapes from the region of Italy and Portugal (Di Lorenzo et al., 2019), a study on antioxidant activity and polyphenolic compounds in white and red wines from North Macedonia (Mitrevska et al., 2020), it was proved a direct relationship between phenolic compounds, antioxidant activity and antihypertensive capacity in Argentinian red wines from Malbec and Merlot varieties (Rodriguez-Vaquero et al., 2020) and others.

The aim of the present study is to define the biological potential (based on phenolic complex and antioxidant activity) of white grapes (and their corresponding wines) grown under the influence of the terroir of Central Northern Bulgaria.

Materials and methods

The objects of the research were grapes and wines from the white varieties Chardonnay, Dimyat and Druzhba, harvest 2021, grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven.

Grapevine varieties. Three white grapevine varieties were selected – introduced, local and hybrid.

Introduced variety. It had a control role in the study. Chardonnay is a white grapevine variety, originating from the area of Burgundy and Champagne, France (Sweet, 2007). For the region of Pleven, it ripens around the middle of September. It has very good fertility. It

quickly accumulates sugars (20-24%) and retains relatively high titratable acidity (7.00-9.00 g/dm^3) (Radulov et al., 1992; Roychev, 2012).

Local variety. Dimyat – old local, Bulgarian, white grapevine variety, distributed in the Balkan Peninsula. Late-ripening, for the region of Pleven it ripens in the second half of September. It has high fertility and yield. Grapes have good sugar accumulation (19-21%) with titratable acids of 6.00-7.00 g/dm^3 (Radulov et al., 1992; Roychev, 2012).

Hybrid variety. Druzhba is a white grapevine variety created by complex interspecies hybridization (Muscat hamburgski x Save Villar 12 375) x (Zarya Severa x Muskat hamburgski) and approved in 1983. Included in the Official Varietal List of Bulgaria in 2012. The variety is medium-ripening, the grapes ripen at the end of August and the beginning of September. The vines have very good fertility. At technological maturity, the content of sugars is 19-21%, with titratable acids of 6.50-7.50 g/dm^3 (Radulov et al., 1992; Roychev, 2012).

Grape must chemical composition. The research was carried out according to the methods generally accepted in winemaking practice and included: Determination of sugar content (g/dm^3) using a Dujardin hydrometer; Determination of the content of titratable acids (TA, g/dm^3) by titration with 0.1n NaOH; Determination of pH was done potentiometrically, using a pH meter.

Vinification. The studied varieties were harvested when they reached technological maturity. The grapes, in the amount of 30 kg, of each variety were processed in the Experimental Wine Cellar of IVE – Pleven, in the conditions of microvinification, according to the classic scheme for the production of dry white wines:

- Crushing the grapes
- Destamming
- Pressing
- Sulphitation (50 mg/dm^3 SO_2)
- Clarification of the must and decanting
- Alcoholic fermentation (dry wine yeast *Saccharomyces cerevisiae* 20 g/hl; temperature 20°C)
- Racking
- Additional sulphitation
- Storage.

Chemical analysis of the obtained white wines. The analyzes were carried out according to the methods generally accepted in wine practice (Ivanov et al., 1979): The content of sugars (g/dm^3) by Schoorl's method; Alcohol content (vol.%) – distillation method using a Gibertini apparatus with a densimeter, by determining the density of a non-alcoholic sample; Titratable acids of the wine (TA, g/dm^3) by titration with 0.1n NaOH; Actual acidity (pH) potentiometrically with a pH meter; Total extract by densimeter (Gibertini).

Determination of the phenolic content of grape must and wines. Determination of total phenolic compounds (TPC) – according to the method of Singleton et Rossi; Determination of the content of flavonoid phenolic compounds (FPC); Determination of the content of non-flavonoid phenolic compounds (NPC);

Determination of antioxidant (DPPH•) activity of grape must and wines. Antioxidant activity was determined according to the method of Wang et al. (1996), as antiradical activity against the stable product DPPH• (2,2 – diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). The antiradical activity was calculated by the formula: AAR = $102(Ak - A0).Ak^{-1}$, % (1)

Statistical analysis. Statistical processing of the data was performed, including the determination of standard deviation (±SD), with triplicate replication for each analysis. The determination of the indicator was realized using the Excel 2007 program from the Microsoft Package (Microsoft Corporation, USA).

Results and discussion

The harvest for each variety was carried out at technological maturity. Analyzes of the grape must of each variety were carried out for the determination of three main technological indicators: sugar content, titratable acids (TA) and determination of pH of the must. The obtained results are presented in Table 1.

Table 1

Main technological indicators of grape must from the investigated varieties (harvest 2021)

Technological indicators	Grapevine varieties		
	Chardonnay	Dimyat	Druzhba
Sugars, g/dm^3	244.60±1.10	221.00±1.70	223.00±1.70
TA, g/dm^3	7.25±0.22	4.60±0.08	5.97±0.04
pH	3.26±0.005	3.44±0.005	3.42±0.005

The highest sugar accumulation in the grape must of the white varieties was found in the control introduced variety Chardonnay (244.60±1.10 g/dm3). The must of Dimyat and Druzhba showed a very close, almost similar content of sugars (221.00±1.70 g/dm^3 and 223.00±1.70 g/dm^3, respectively).

According to Radulov et al. (1995) and Roychev (2012), the studied varieties in the conditions of Bulgaria should accumulate sugars as follows: Chardonnay – 200.00 – 240.00 g/dm^3; Dimyat – 190.00 – 210.00 g/dm^3; Druzhba – 190.00 – 210.00 g/dm^3.

It can be seen that the established sugar accumulation in the must for the specific harvest (2021) showed slightly higher levels than the data presented by Radulov et al. (1995) and Roychev (2012), namely 4.60 g/dm^3 more sugars for Chardonnay, 11.00 g/dm^3 more sugars for Dimyat and, respectively, 13.00 g/dm^3 more sugars for Druzhba. The resulting higher sugar accumulation could be explained as a consequence of the climatic conditions of the year. It was characterized by a very hot and dry summer, with a long period without precipitation, which led to an increased synthesis of sugars in the grapes.

Regarding the established titratable acidity (TA), the highest content of this indicator (7.25±0.22 g/dm^3) was found in Chardonnay. Druzhba must showed higher content of titratable acids (5.97±0.04 g/dm^3) than Dimyat (4.60±0.08 g/dm^3).

According to Radulov et al. (1995) and Roychev (2012) the content of titratable acids in the grape must of the investigated varieties ranges as follows: Chardonnay – 7.00 – 9.00 g/dm^3; Dimyat – 6.00 – 7.00 g/dm^3; Druzhba – 6.50 – 7.50 g/dm^3.

The results obtained for Chardonnay and Dimyat must correlated with the data presented by the cited authors. Druzhba must showed a slightly lower titratable acidity. The lower content of titratable acids is explained by the higher sugar accumulation in these varieties, which is reflected in a slight decrease in their titratable acidity. In addition, the content of titratable acids is a highly variable factor depending on variety, geographical area and climate.

According to Abrasheva et al. (2008) in the conditions of the Republic of Bulgaria, the concentration presence of titratable acids in grapes varies in the wide range from 4.00 to 12.00 g/dm^3. The results of the conducted research correlated with this range, which confirmed the good accumulation of acids in the grapes in the conditions of the town of Pleven, Central Northern Bulgaria, and confirmed also the reach of grapes technological maturity for carrying out the "vinification" process.

The pH represents the relationship between the amount and the strength of acids. The normal pH of grape must should be in the range of 2.80 – 3.80 (Chobanova, 2012). In the grape must of the studied varieties, the lowest pH was found in the control introduced Chardonnay variety (3.26±0.005). Druzhba and Dimyat showed close values for this indicator. The obtained data were normal and correlated with those presented by Chobanova (2012).

The results for the three main technological indicators (sugars, TA, and pH) indicated that the grapes were harvested at the right time, at technological maturity, with a good balance between sugars and acids and good quality for undergoing the fermentation process.

The phenolic complex is a major factor for the biological value of grapes and wine, determining their antioxidant activities.

In the grape must of the investigated varieties, total phenolic compounds (TPC), flavonoid phenolic compounds (FPC) and non-flavonoid phenolic compounds (NPC) were determined.

The data for TPC in grape must of the studied varieties are presented in Figure 1.

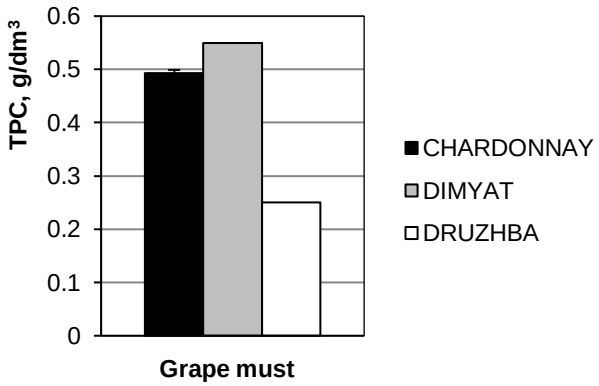

Figure 1. Total phenolic compounds (TPC) in grape must of the studied varieties

The research showed the lowest content of this indicator in the Druzhba hybrid (0.25±0.00 g/dm^3). It was two times lower, compared to the determined content of TPC in the introduced control variety Chardonnay (0.49±0.005 g/dm^3) and the local Dimyat (0.55±0.00 g/dm^3). When analyzing the results, it could be seen that the must of Dimyat was the richest of TPC.

The obtained data regarding the established presence of TPC in the grape must were in absolute correlation with the ranges of their presence (200.00 – 500.00 mg/dm^3), presented by Velkov et al. (1996) as well as with the study of Franco-Bañuelos et al. (2017), who found a variation in TPC content of 112.70 mg GAE. 100 g^{-1} to 218.00 mg GAE. 100 g^{-1} in a study of grapes from four white grapevine varieties (Sauvignon Vert, Palomino, Furmint and Semillon) from the Mexico region.

The obtained results regarding the content of FPC in grape must of the studied varieties are presented in figure 2.

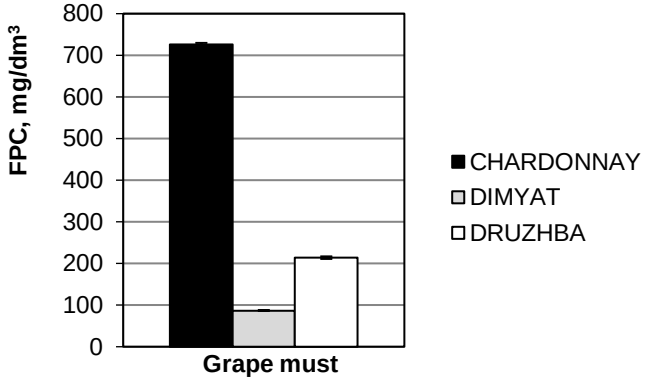

Figure 2. Flavonoid phenolic compounds (FPC) in grape must of the studied varieties

The grape must of the control introduced variety Chardonnay showed the highest quantitative presence of FPC (726.44±3.34 mg/dm^3), an amount that was more than eight times higher than that found in the grape must of the local variety Dimyat (87.07±1.16 mg/dm^3) and over three times higher than that found in the white hybrid variety Druzhba (213.97±3.04 mg/dm^3). It could be seen that the must of Dimyat showed the lowest levels on this indicator, and Chardonnay dominated the other two varieties.

The data on the content of NPC in grape must of the studied varieties are presented in Figure 3.

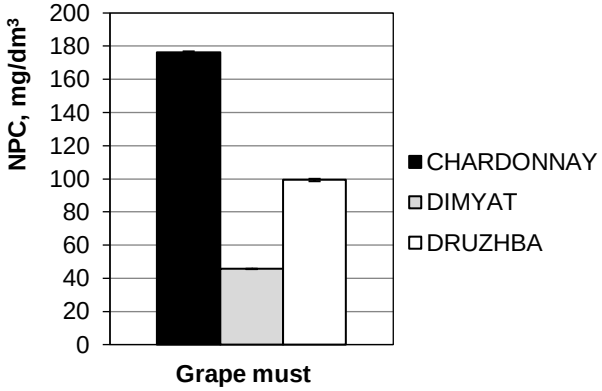

Figure 3. Non-flavonoid phenolic compounds (NPC) in grape must of the studied varieties

The highest NPC content was found in the control introduced variety Chardonnay (176.33±0.51 mg/dm^3). It was almost four times higher compared to that of the local variety Dimyat (45.65±0.11 mg/dm^3) and over one and a half times from that found in the must of the Druzhba hybrid (99.26±0.72 mg/dm^3). From the obtained results for NPC, it could be seen that Dimyat showed the lowest concentrations of the investigated indicator.

Some representatives of the NPC groups exhibit different biological activities (Chobanova, 2012), related to the antiseptic properties, bactericidal activity,

antihypercholesterolemic effect. The NPC group includes the so-called stilbenes characterized by high antioxidant activity, preventive effect against cardiovascular diseases, anticarcinogenic effect (Jang et al., 1997; Lu and Serrero, 1999).

The antioxidant activity is one of the main factors determining the biological potential of grapes and wines.

The obtained data on the antioxidant activity (DPPH•) of grape must from the introduced (Chardonnay), local (Dimyat) and hybrid (Druzhba) white grapevine varieties are presented in Figures 4, 5 and 6.

The data on the antioxidant activity of the must from the three investigated white varieties at TE = 600.00 mg/dm^3 revealed that the highest activity showed the must of the hybrid Druzhba variety. With a reaction time of 5 min, it registered a free radical elimination activity of 43.14±0.09 %. When the reaction time was increased to 15 min, an increased elimination of DPPH radicals was registered up to 48.73±0.15 %.

The grape must of the other two studied varieties – the introduced control Chardonnay and the local Dimyat at TE = 600.00 mg/dm^3 showed almost similar results with a very small difference in favor of Chardonnay. Its must at a reaction time of 5 min showed 34.72±0.28 % elimination of free radicals. When the reaction time increased to 15 min, an increased in the antiradical activity was recorded and it reached 37.22±0.14 %.

The grape must of the local Dimyat variety showed the lowest antiradical activity, but its difference with Chardonnay was small (2–3% higher activity in Chardonnay). Dimyat at TE=600.00 mg/dm^3 and reaction time 5 min demonstrated an antioxidant activity of 32.43±0.03 %. At 15 min of the reaction, in the same total extract, the activity increased slightly to 34.29±0.24 %.

At TE = 400.00 mg/dm^3, however, the grape must of the introduced control variety Chardonnay showed an increase in its antioxidant activity. Thus, 5 min from the moment of mixing the radical and the sample, an antioxidant activity of 43.50±0.47 % was recorded. At 15 min of the reaction, it marked a jump and reached 51.02±0.12 %.

Second according to this indicator at TE = 400.00 mg/dm^3 was the grape must of the Druzhba hybrid. At a reaction time of 5 min, it showed a free radical elimination capacity of 32.79±0.16 %. At 15 min of the reaction, a growth in the antioxidant activity was reported, and 36.12±0.58 % elimination of the DPPH radical was found.

The grape must of the local variety Dimyat showed the lowest antioxidant activity in this extract as well (400.00 mg/dm^3). At 5 min of the reaction, an antioxidant activity of 28.01±0.07 % was found. At 15 min, it rose and occupied a value of 34.99±0.04 %.

The grapes were vinified by microvinification. 30 kilograms of grapes, for each variety, were processed according to the classic scheme for the production of white dry wines (Yankov, 1992). The data regarding the main chemical parameters of the obtained wines are presented in Table 2.

Table 2

Chemical parameters of the experimental wines, harvest 2021

Wines	Alcohol content, vol. %	Total extract, g/dm^3	Sugars, g/dm^3	Titratable acids, g/dm^3	pH
Chardonnay	14.14±0.05	22.43±0.05	1.76±0.15	6.98±0.07	3.52±0.000
Dimyat	9.80±0.04	68.50±2.15	47.33±2.30	5.49±0.42	3.71±0.000
Druzhba	13.33±0.05	22.02±0.31	5.07±0.61	5.40±0.15	3.71±0.000

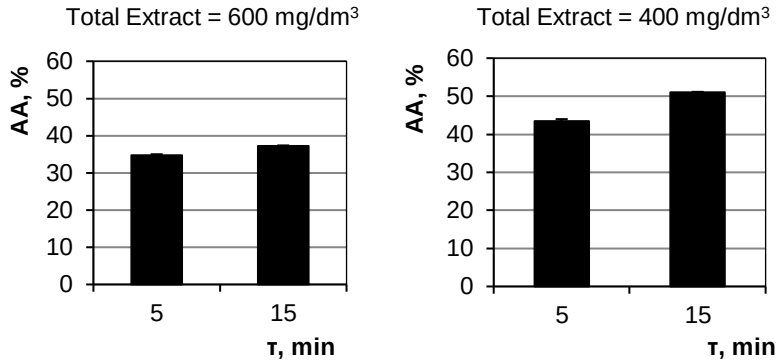

Figure 4. Antioxidant activity of grape must from white introduced variety – Chardonnay at TE = 600 mg/dm^3 and TE = 400 mg/dm^3

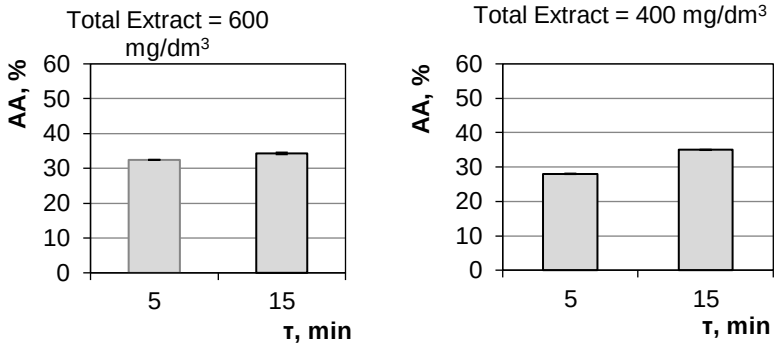

Figure 5. Antioxidant activity of grape must from white local variety – Dimyat at TE = 600.00 mg/dm^3 and TE = 400.00 mg/dm^3

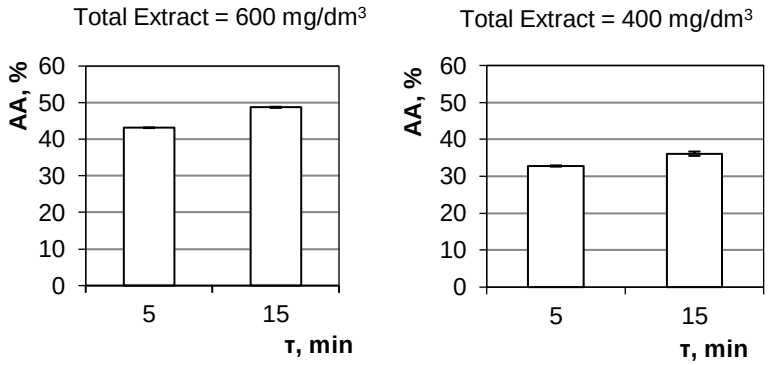

Figure 6. Antioxidant activity of grape must from white hybrid variety – Druzhba at TE = 600.00 mg/dm^3 and TE = 400.00 mg/dm^3

The highest alcohol content was found in Chardonnay (14.14±0.05 vol. %). Low levels of alcohol were found in the wine of the Dimyat variety (9.80±0.04 vol. %). Ethyl alcohol varies normally in wines in the range of 7.00 vol. % – 17.00 vol. % and accordingly it the analyzed wines could be categorized (by alcohol content) in the following order: Dimyat and Druzhba as dry (table), whose category (Chobanova, 2012) covers an alcohol range from 9.00 vol. % to 13.00 vol.%; Chardonnay – as strong, whose category is characterized by an ethanol content above 14.00 vol. %.

Almost all analyzed wines showed a normal total extract. An exception, however, was observed in the wine of the Dimyat variety. Its extract content was extremely high (68.50±2.15 g/dm^3). The reason for this was the high residual sugar of this wine (47.33±2.30 g/dm^3). The yeast microflora, only in this variant, did not complete the fermentation, which reflected in a lower alcohol content and directly on its higher total extract.

Examining the content of residual sugars in the analyzed white wines, their high presence in Dimyat wine were visible (47.33±2.30 g/dm^3). As interpreted above, this content, linked to the alcohol content, indicated incomplete fermentation biotransformation of grape sugars. According to the content of residual sugars, Chardonnay wine (1.76±0.15 g/dm^3) was categorized as dry (up to 4.00 g/dm^3 residual sugars), and that of the Druzhba hybrid (5.07±0.61 g/dm^3) as semi-dry (from 4.00 to 12.00 g/dm^3 residual sugars).

The highest titratable acidity was found in Chardonnay (6.98±0.07 g/dm^3). Dimyat (5.49±0.42 g/dm^3) and Druzhba (5.40±0.15 g/dm^3) showed close values for this indicator with a slight advantage for Dimyat. All established levels of titratable acids were in the optimum (5.00 to 9.00 g/dm^3) for this indicator (Chobanova, 2012).

Regarding the actual acidity (pH), a variation was found from 3.52±0.000 (Chardonnay) to 3.71±0.000 (Druzhba and Dimyat).

The data on the content of total phenolic compounds (TPC) in the investigated wines are presented in figure 7.

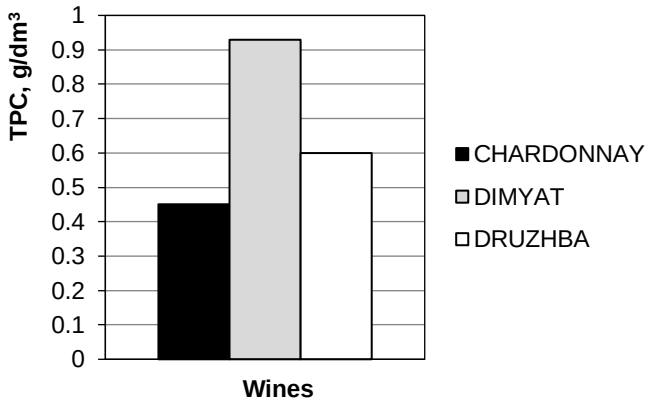

Figure 7. Content of total phenolic compounds (TPC) in white wines of the studied varieties

In the analysis of the TPC of white wines, the highest concentration presence was found in Dimyat (0.93±0.000 g/dm^3). The wine of the Druzhba hybrid variety also showed a high concentration of TPC (0.60±0.000 g/dm3). The lowest phenolic accumulation for the specific harvest (2021) was identified in the wine of the control introduced variety Chardonnay

(0.45±0.000 g/dm^3). In terms of concentration, the quantitative presence of TPC correlated with the results of other studies, which determined a range in the content of TPC in white wines from 50.00 to 2000.00 mg/dm^3 (0.05–2.00 g/dm^3) (Shadidi and Nazck, 1995). The data also correlated with the study of Radeka et al. (2022), who found total phenolic content of white wines from Croatian Malvasia and Pošip varieties ranged from 226.20 to 505.40 mg/dm^3 (0.26 to 0.50 g/dm^3).

The data on the detected flavonoid phenolic compounds (FPC) in white wines are presented in Figure 8.

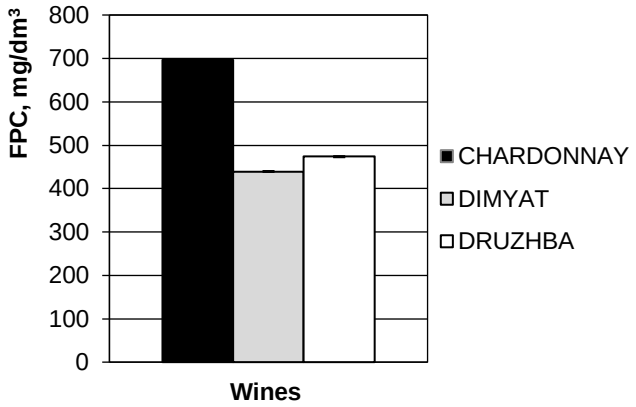

Figure 8. Content of flavonoid phenolic compounds (FPC) in white wines of the studied varieties

The highest content of FPC was found in the wine of the control introduced variety Chardonnay (696.46±0.37 mg/dm^3). The wines of the other two studied varieties demonstrated close values for this indicator – the wine of the Druzhba hybrid showed a slightly higher concentration of FPC (473.54±3.03 mg/dm^3), compared to the wine of the local Dimyat variety (439.38±3.35 mg/dm^3).

The main representatives of the flavonoid group of phenolic compounds in grapes and wine of *Vitis vinifera* L. varieties are anthocyanins, flavan-3-ols, tannins and their reaction products (Casassa, 2017). Mitrevska et al. (2020) investigated commercial Macedonian red and white wines and found that total flavonoid content in white wines ranged from 49.00±0.97 mg/dm^3 to 296.05±5.92 mg/dm^3. The FPC content found in our study was higher.

The data on the determined content of NPC in the investigated wines are presented in figure 9.

The highest content of NPC in white wines was found in the Druzhba hybrid variety (130.47±0.59 mg/dm^3). The wine of the local variety Dimyat ranked immediately after it (93.51±0.34 mg/dm^3), and the lowest content was recorded in the wine of the control introduced variety Chardonnay (84.13±0.43 mg/dm^3), as the difference between the wines of the local and the introduced variety according to this indicator was not great.

The non-flavonoid phenolic compounds (NPC) present in wines include representatives of phenolic acids and stilbenes (Fernandes et al., 2017). Woraratphoka et al. (2007) investigated the content of phenolic compounds in selected wines from northeastern Thailand. The team found a variation of NPC in the studied white wines from 159.60±68.40 mg/dm^3 to 275.20±35.40 mg/dm^3. The data in our study correlated with those established by the cited team.

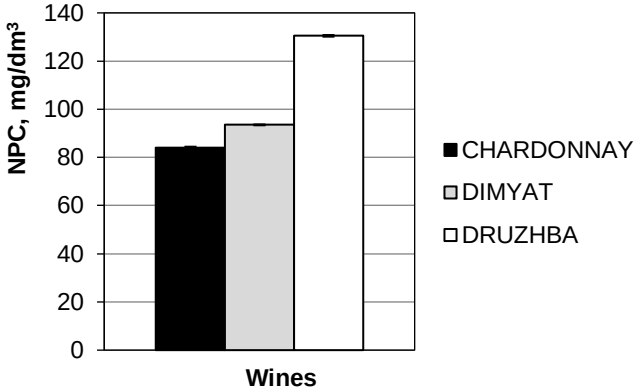

Figure 9. Content of non-flavonoid phenolic compounds (NPC) in white wines of the studied varieties

The data on the antioxidant activity of the white wines from the studied varieties are presented in Figures 10, 11 and 12.

The highest antioxidant activity in these wines was found in the wine of the control introduced variety Chardonnay. At TE = 600.00 mg/dm^3 at 5 min of the reaction with added DPPH radical, the elimination of free radicals was found to be 62.37±0.20 %. After 15 min of interaction with the radical, a slight decrease in activity was recorded, reaching 54.26±0.11 %. At TE = 400.00 mg/dm^3 at 5 min of the reaction in the wine of this variety, a radical-scavenging activity of 58.30±0.04 % was found. A slight decrease in this extract was observed after 15 min of the reaction with a generated antioxidant activity of 52.00±0.13 %.

Second, in terms of its ability to capture free radicals, was the wine of the local Dimyat variety. At TE = 600.00 mg/dm^3 and a reaction time of 5 min, a radical-scavenging activity of 38.47±0.31 % was generated. A slight decrease was observed at 15 min of the reaction reaching 35.15±0.19 % DPPH capture. At TE = 400.00 mg/dm^3, after 5 minutes of the reaction, an activity of 41.55±0.45 % was found. At 15 min in the same extract, a decrease in antioxidant activity was found, reaching 30.79±0.00 %.

Among the investigated white wines, the Druzhba hybrid wine has the lowest antioxidant activity. At TE = 600.00 mg/dm^3 and at the 5 min from the moment of mixing of the radical with the wine, a radical-scavenging activity of 31.05±0.24 % was found. At 15 minutes of reaction, the antioxidant activity of the wine of this variety had low change – a negligible decrease was observed with a reported antioxidant activity of 30.44±0.03 %. At TE = 400.00 mg/dm^3, a decrease in activity was observed, and at 5 min it was 25.40±0.20 %. At 15 min, in this extract, as with the previous one, a very slight decrease was found with generated antioxidant activity of 24.51±0.02 %.

The trend in the established antioxidant activity of white wines could be directly related to the accumulation of FPC. In terms of both indicators (antioxidant activity and FPC), the Chardonnay wine is significantly different from the wines of the other two studied varieties, which showed similar concentrations of FPC and, accordingly, similar percentages in the capture of free radicals. Compared to the wine of the Dimyat variety, Chardonnay showed more than 1.5 times higher radical-eliminating activity, and compared to Druzhba – 2 times higher. This, for the specific harvest (2021), proved that the control introduced Chardonnay variety was characterized by the highest biological potential.

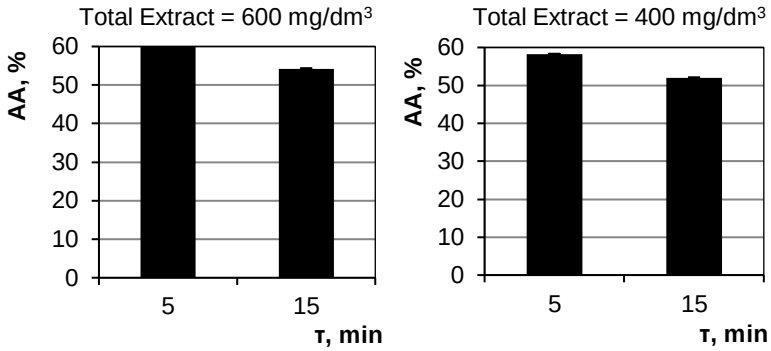

Figure 10. Antioxidant activity of white wine from control introduced variety Chardonnay at TE = 600 mg/dm^3 and TE = 400 mg/dm^3

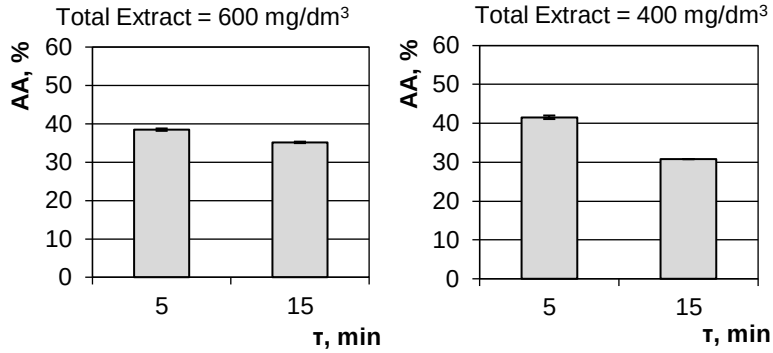

Figure 11. Antioxidant activity of white wine from the local variety Dimyat at TE = 600 mg/dm^3 and TE = 400 mg/dm^3

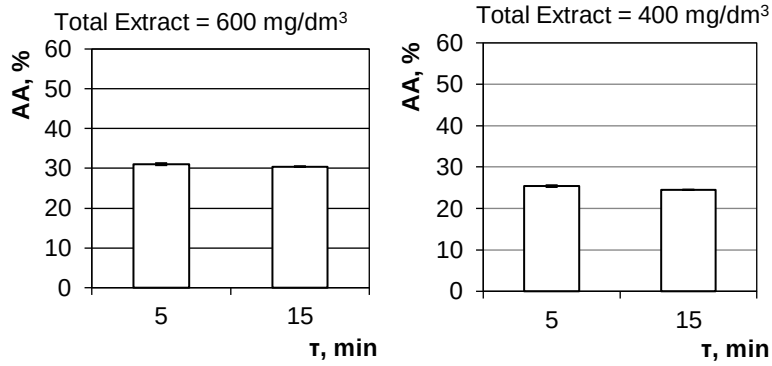

Figure 12. Antioxidant activity of white wine from Druzhba hybrid variety at TE = 600 mg/dm^3 and TE = 400 mg/dm^3

Paixão et al. (2007) studied white wines from the region of the island of Madeira and found a variation in the percentage of eliminated DPPH radicals from 13.47±2.10 % to 68.69±5.40 %. The results of our research were in complete agreement with the cited team. The results were also in agreement with the study of Marković et al. (2015), who found antioxidant activity in white wines of the local Croatian white variety Žilavka, which varied between the eighteen investigated samples from different regions of Croatia in the range of 23.40±1.40 % to 83.10±1.60 %. Our data also correlated with the study by Kharadze et al. (2018), who determined the antioxidant activity of white wines from five varieties grown in the region of western Georgia. The team examined 14 white wines and found a variation in their antioxidant activity from 27.00 % to 45.25

Conclusions

The following conclusions could be made from the conducted research:

- The results obtained regarding the three main technological indicators (sugars, titratable acids and pH) of the grape must indicated that the grapes were harvested at the right time, at technological maturity, with a good balance between sugars and acids and good quality for undergoing fermentation process.
- The highest presence of TPC was proved in the grape must of the local variety Dimyat.
- The study of the presence of FPC in the grape must of the varieties showed that Chardonnay has the highest potential for FPC accumulation, reflecting in a significantly higher amount of this component of the phenolic composition, compared to the other two varieties.
- The highest presence of NPC was found in the grape must of the control introduced variety Chardonnay. It was almost four times higher, compared to that of the local variety Dimyat and more than one and a half times, compared to the Druzhba hybrid.
- At TE=600.00 mg/dm^3, the highest antioxidant activity was found in the grape must of the Druzhba hybrid variety. The musts of the introduced Chardonnay and local Dimyat showed similar antioxidant activity in this extract.
- When the extract was reduced (400.00 mg/dm^3), however, Chardonnay demonstrated the highest antioxidant activity, followed by Druzhba, and it was lowest in Dimyat.
- The chemical parameters of the analyzed wines were optimal. The only exception was the wine of Dimyat, in which low alcohol (9.80±0.04 vol. %), very high extract (68.50±2.15 g/dm^3) and high residual sugars (47.33±2.30 g/dm^3) were found. The reason was a disturbance in the alcoholic fermentation, which led to its incomplete progress.
- Dominance in the content of TPC was found in the wine form the local variety Dimyat (0.93±0.000 g/dm^3), while the Chardonnay control showed the lowest presence of TPC (0.45±0.000 g/dm^3).
- In terms of FPC content, Chardonnay wine dominated (696.46±0.37 mg/dm^3), and Dimyat had the lowest content (439.38±3.35 mg/dm^3).
- The highest concentration of NPC was found in the wine of the Druzhba hybrid variety (130.47±0.59 mg/dm^3). The lowest result according to this indicator was found in the wine of the control introduced variety Chardonnay (84.13±0.43 mg/dm^3).
- The trend in the established antioxidant activity of the white wines was directly related to the accumulation of FPC. In terms of both indicators (antioxidant activity and FPC), the Chardonnay wine was significantly different from the wines of the other two

studied varieties, which showed similar concentrations of FPC and, accordingly, similar percentages in the capture of free radicals. Compared to the wine of the Dimyat variety, Chardonnay showed more than 1.5 times higher radical-eliminating activity, and compared to Druzhba – 2 times higher.

The research proved that the white varieties and wines grown in the region of Central Northern Bulgaria showed a balanced biological capacity and potential, comparable to wines and grapes from other regions of the world.

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Reduction of acrylamide formation in molded potato chips of increased nutrition value

Alina Kovtun, Volodymyr Kovbasa, Oleg Bortnichuk, Oleksandr Shevchenko, Oleksandr Duboriezov

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Alina Kovtun
E-mail: kovtunav@ukr.net

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Abstract

Introduction. The aim of the study is to determine the optimal temperature regimes for baking and drying potato dough to reduce formation of acrylamide in molded potato chips of increased nutritional value.

Materials and methods. The following raw materials were selected: potato grits, rye and barley bran, pumpkin seed meal, broccoli and red beet cryopowders. The amounts of aspartic acid and reducing sugars in raw materials, as well as acrylamide formed during the temperature treatment of the dough potato mass were determined.

Results and discussion. In order to increase the nutritional value of molded potato chips, the traditional raw material base has been expanded using various cereal bran, meal, dietary fibers, and vegetable powders. The recommended parameters for processing potato dough are: temperature of 125°C and a duration of 4.5 minutes without vegetable oil addition, in contrast to the traditional methods of molded potato chips production. The amount of aspartic acid in potato grain is 190.5 mg/g of protein and reducing sugars is 0.6%. The amount of aspartic acid in rye and barley bran is 77.5 and 72.6 mg/g of protein, respectively, in pumpkin seed meal – 80.5 mg/g of protein, in cryopowders of broccoli and red beet – 72.5 and 72.9 mg/g of protein. The content of reducing sugars in rye and barley bran is 0.74 and 0.8%, respectively, in pumpkin seed meal – 0.5%, in cryopowders of broccoli and red beet – 0.3 and 0.5%, respectively. After addition of non-traditional materials to the recipe, products were obtained in which no acrylamide was detected due to changes in the classical technology and parameters of molded potato chips production. It was investigated that when applying the classical technology of molded potato chips, the amount of acrylamide in the finished products is 61 μg in 100 g of the product. In molded potato chips without the addition of bran, meal and cryopowders, which were baked and dried, the amount of acrylamide is 9.35 μg in 100 g of the product.

Conclusions. The formation of acrylamide depends on the chemical composition of raw materials, the duration and temperature of baking-drying, and the technology of molded potato chips production.

Introduction

The modern rhythm of life and the lack of time for a full meal lead to the habit of "quick snacking". Therefore, snack products are becoming popular in the diets of many peoples in the world. Nowadays one of the most popular food products are chips, which can quickly saturate the human body with energy and satisfy hunger (Ivanov et al., 2021). Along with this, it was found that excessive consumption of chips can provoke various diseases due to the accumulation of toxic substances such as methylpropanesulfonic acid and acryloyloxyethyltrimethylammonium chloride in the human body (Howard et al., 2010; Majzoobi et. al., 2013).

In recent years, the use of non-traditional raw material based on pumpkin seeds, which after extracting the oil, are crushed to form a food meal, has become popular. Pumpkin seed meal fiber contains a balanced composition of amino acids and carotenoids, as well as a large amount of organic salts and has good functional properties (Shevchenko, 2022). Some vegetables (carrots, zucchini, cabbage, beets, peppers, and broccoli) are rich in insoluble dietary fibers, pectin, vitamins, and minerals. Modern equipment allows the production of vegetable powders due to various methods of drying without loss of useful nutrients (WHO, 2002). It was shown that bran, meal and cryopowders contain 4 times less starch than potato grits and 7 times more fiber. Cryopowders contain 2.5 times more mono- and disaccharides compared to potato grits, which affects further chemical processes in molded potato chips. In addition, the amount of fat in the cryopowders of broccoli and red beet is 0.2-0.3% of dry matter (DM). Therefore, these raw materials meet the requirements for initial parameters in the production of molded potato chips (Kovtun et al., 2018).

Scientists are concerned with the issue of reducing the amount of acrylamide formation in snack products. The toxicity of acrylamide is due to easy penetration into the human body through the gastrointestinal tract (Hunko et al., 2010).

Acrylamide is formed spontaneously in the process of cooking the product at a temperature above 120°C, especially during frying (WHO, 2005). According to the conclusions of experts of the FAO/WHO committee, the largest amount of acrylamide is found in such products as French fries (16-30%), chips (6-46%), coffee (13- 39%), confectionery and biscuits (10-20%), bakery products and bread fried in toasters (10-30%). Therefore, these products require a detailed study and justification of their production technology. Vacuum drying and infrared treatment are applied, addition of enzyme asparaginase, which is able to inhibit the reaction of melanoid formation and the formation of acrylamide, are implemented in the technology of preparing foods for decrease content of acrylamide in them (Wenzl et al., 2003).

Research on the addition of asparaginase enzyme was carried out in the production of ginger cookies and crispbreads. It was established that when asparaginase was added to the dough for bread production, the acrylamide content decreased from 910 μg/kg to 740 μg/kg, and the amount of acrylamide in ready-made loaves was reduced by 50%. For ginger cookies, the amount of acrylamide was 530 μg/kg, when asparaginase was added, the amount of acrylamide decreased to 60 μg/kg. As a result, it was possible to reduce the amount of acrylamide up to 90% (Sharma et al., 2017).

The use of antioxidants in combination with a mixture of vegetable oils is proposed to reduce acrylamide in chips from potato slices due to balance of the ratio of ω-3 and ω-6 fatty acids in them. It was established that when frying chips from potato slices of the 'Kimmeria' variety at a temperature of 140-180°C, the content of acrylamide in chips fried in palm oil was 194-252 μg/kg, meanwhile in a mixture of deep-fried (corn + rapeseed oil) its content was 198-320 μg/kg. To reduce the amount of acrylamide, the optimal ratio (1:1) of blends of

corn oil + rapeseed oil, which was used for frying, was selected due to experimental studies. These types of oil are balanced by polyunsaturated ω-3 and ω-6 fatty acids. In addition, the optimal thickness of potato chips was selected for frying (with a specific surface of 10.7 - 14.0 cm^{-1}), which allows to reduce the passage of the Maillard reaction. When eating 75 g of such chips, 184-188 μg of acrylamide is consumed by person, which does not exceed the permissible value according to WHO data. According to the FAO/WHO committee, the average intake of acrylamide with food is 1-4 μg/kg of body weight per day. However, there is no research on the amount of acrylamide in molded potato chips that are made from dry mashed potatoes.

Therefore, the aim of the present research was to study the influence of the parameters of molded potato chips production on the amount of acrylamide formation.

Materials and methods

Materials

Potato grits ('Bikrampur', Germany), traditional raw materials – rye and barley bran, pumpkin seed meal ('Agrosilprom', Ukraine), as well as non-traditional raw materials (cryopowders of broccoli and red beet) ('Gammy', Ukraine) were chosen for the research. This choice of raw materials is due to their high nutraceutical composition, namely the increased amount of dietary fibers, cellulose, hemicellulose, pectin, minerals, fat-soluble vitamins in these raw materials.

Amino acid composition

The determination of amino acid composition of studied materials was carried out on an automatic amino acid analyzer T 339 (Mikrotechna Praha a.s., Praha, Czech Republic). The efluent from the container is driven through the chromatographic column using a dosing pump. The area of the peaks on the chromatogram is calculated and compared with the area of the peaks of amino acids with a known concentration. From the comparison of these areas, the absolute amount of amino acid in the analyzed sample is calculated.

On the chromatogram, the peak area of each amino acid (peak height) is calculated. Hydrolysis is carried out as follows: a weighted sample with a dry protein content of about 2 mg is placed at the bottom of the test tube. 0.5 ml of distilled water and 0.5 ml of concentrated hydrochloric acid are added to a dry weight of protein in a test tube. An equal amount of concentrated hydrochloric acid is added to the aqueous protein solution. The tube is cooled in a mixture of dry ice with acetone or liquid nitrogen. After the content of the tube freeze, air is pumped out of it using a vacuum pump to prevent oxidation of amino acids. Then the test tube is sealed and placed for 24 hours in a thermostat with a constant temperature of +106°C. At the end of hydrolysis, the test tube is opened, previously cooled to room temperature. The contents are quantitatively transferred into a glass beaker and placed in a vacuum-desiccator over granulated caustic soda. Then air is removed from the desiccator using a water jet pump. After drying the sample, 3-4 ml of deionized water is added to the buxes and the drying procedure is repeated. The sample prepared in this way is dissolved in 0.3-normal lithium citrate buffer (pH=2.2) and applied to the ion exchange column of the amino acid analyzer (Litvynchuk et. al., 2022).

Mass fraction of sugars

Determined was provided by the accelerated iodometric method. The procedure for conducting the analysis consists of the following main stages: preparation of a water extract; hydrolysis of sucrose in the obtained extract and quantitative determination of sugar by its reducing properties.

30 cm^3 of the obtained filtrate, 10 cm^3 of a 6.9% solution of copper sulfate and 10 cm^3 of an alkaline solution of potassium-sodium tartaric acid are added to a 50 cm^3 conical flask and the content is brought up to the mark with distilled water. The flask is heated on a gas burner for up to 3 minutes and boiled for 2 minutes from the moment of boiling start and cooled. Then 10 cm^3 of a 30% potassium iodide, 25% sulfuric acid solution are added to the flask and the released iodine is titrated with a 0.1 mol/dm^3 solution of sodium thiosulfate to a light yellow color. Under these conditions, a control experiment is also conducted (Shevchenko et al., 2022).

Determination of the amount of acrylamide

Determination was performed on an Agilent 7890A gas chromatograph (USA) with a Supelcowax chromatographic column (60 m, 0.53 mm, 1 μm). The gas chromatograph was equipped with electron capture (Agilent) and mass-selective (Finnigan Trace DSQ II) (USA) detectors, which worked alternately. Centrifugation of samples was carried out on centrifuges: 'Rotina 38' (Germany) and 'Eppendorf 5418' (Germany), shaking – in a shaker 'Biosan OS-10' (USA).

About 50 mg of acrylamide was weighed into a 50 ml volumetric flask. 20 ml of distilled water was added and stirred until complete dissolution. After that, the volume was brought up to the mark with distilled water and mixed (basic solution, concentration 1 μg/ml), 1 ml of the basic solution was transferred to a 100 ml volumetric flask, brought up to the mark with distilled water and mixed (working solution, concentration 10 μg/ml) then this solution was transferred to a gas chromatograph. For the control sample, dry milk was selected (preliminarily tested for the absence of acrylamide) and acrylamide was added in the amount of 0.005 to 5.0 mg/kg. The next step was extraction to remove proteins and fats, followed by bromination of the solutions and purification of the ethyl acetate extract. Chromatographic separation and detection conditions are: 140°C – 1 min, then 220°C – 5 min, isotherm – 33 min, nitrogen carrier gas 4 ml/min. Operating parameters of the electron capture detector are: temperature 300°C, blowing flow – 30 ml/min (Ghiasvand et. al., 2016).

Results and discussion

Considering the fact that in cereal products, unlike potato, the content of asparagine plays a decisive role in the formation of acrylamide, it was established that the highest content of it is in bran with a grain size of more than 560 μm (Table 1). Therefore, additional grinding of this raw material to sizes commensurate with potato grits and cryopowders of 30-40 microns was carried out. Cryopowders have asymmetric proportions in their chemical composition relative to bran and meal. Obviously, this is explained by the fact that all these types of raw materials contain dietary fibers that are able to retain asparagine even under the conditions of its extraction.

Table 1

Content of aspartic acid in raw materials

Raw material	Amount of amino acid mg/g of protein	Amount of amino acid mg/100 g of product
Potato grits	190.5 ±2.0	769.0±3.6
Rye bran	77.5±1.0	340.5±3.2
Barley bran	72.6 ±1.0	298.3±3.2
Pumpkin seed meal	80.5 ±1.5	354.7±3.2
Broccoli cryopowder	72.9 ±1.5	292.6±3.0
Red beetroot cryopowder	72.5 ±1.5	287.6±3.0

* Results given as: M ± SD (mean ± standard deviation) of triplicate trials.

Potato grits contain 2.5 times more aspartic acid than bran, meal and cryopowders. According to the proposed technology, a part of potato grits was mixed in a ratio of 4:1 with raw materials – bran, meal or cryopowder. Potato grits contain 2.5 times more aspartic acid than bran, meal and cryopowders, which contributes to the formation of acrylamide. According to the proposed technology, part of the potato grits was replaced by rye and barley bran, pumpkin seed meal, broccoli and red beetroot cryopowders in a prescription ratio of 4:1. At other ratios (1:1, 2:1, 3:1), which were chosen for the experiment, significant deviations of the organoleptic parameters of the finished chips (appearance, taste, smell, consistency) were observed, and the chips production technology did not meet regulatory requirements. The process of frying molded potato chips in frying oil was replaced by baking-drying. which will llow further prediction of changes in the amino acid composition of the finished molded potato chips and reduce the formation of acrylamide.

One of the main indicators affecting the quality of molded potato chips is the amount of reducing sugars in raw materials, which, in addition to color, affect the process of formation of complex substances (melanoidins) and the accumulation of acrylamide in finished products (Sharma et al., 2017). Therefore, it is advisable to determine the mass fraction of reducing sugars in the studied raw materials (Table 2)

Table 2

Mass fraction of reducing sugars in potato grits, bran, meal and cryopowders

Raw material	Mass fraction of reducing sugars, % ofDM
Potato grits	0.60±0.01
Rye bran	0.80±0.03
Barley bran	0.74±0.03
Pumpkin seed meal	0.50±0.02
Broccoli cryopowder	0.35±0.01
Red beetroot cryopowder	0.30±0.01

* Results given as: M ± SD (mean ± standard deviation) of triplicate trials.

The content of reducing sugars in potato grits is lower compared to rye bran by 33.3%, barley bran – by 23.3%. Scientists have researched that the content of reducing sugars in fresh potato tubers from which chips are made is more than 1%, therefore, such potatoes are washed well after cutting, and also blanched in order to decrease the content of reducing sugars and inactivate some enzymes, then freezed and dried (Burtron, 2013). On the other hand, the content of reducing sugars in the pumpkin seed meal is lower than in potato grits

by 23.3%, this is due to the fact that the pumpkin seeds from which meal is obtained belong to oil crops and chemical composition of this raw material differs from potato grits (Drobot et al. , 2021). Pumpkin seed meal is produced mechanically (by crushing the husk), which does not affect the quantitative content of reducing sugars. The amount of reducing sugars in cryopowders is 42-50% less than in potato grits. During the hydrothermal processing of vegetables and their drying in a hot stream of air, part of the reducing sugars may partially evaporate, thereby reducing the content of reducing sugars, which is a positive factor in the production of molded potato chips. Such values are related to the features of technological processing of raw materials and their chemical composition. Based on the obtained experimental data all the presented samples of raw materials are suitable for the further production of molded potato chips from the point of view of safety.

To reduce the amount of acrylamide in molded potato chips, part of the potato grits was replaced in a ratio of 4:1 with one of the studied raw materials: rye or barley bran, pumpkin seed meal, broccoli or red beet cryopowders. Pre-prepared raw materials with a size range (raw material coarseness up to 40 μm) were mixed in a dry form, gradually (in equal parts) a salt solution of water with a temperature of 20°C was added, until the moisture content of the potato dough was 42%. The obtained mass was mixed until a homogeneous consistency, formed and left for 20 minutes for the passage of physico-chemical processes. One sample of molded potato chips without the addition of secondary raw materials was fried in oil according to a standard technology, other samples of molded potato chips were baked and dried. The results of studies on the amount of acrylamide formation are shown in Figures 1–7.

A small amount of acrylamide – 9.35 μg per 100 g of product, was found in molded potato chips (control) on the 2.8 min of heating (Figure 1).

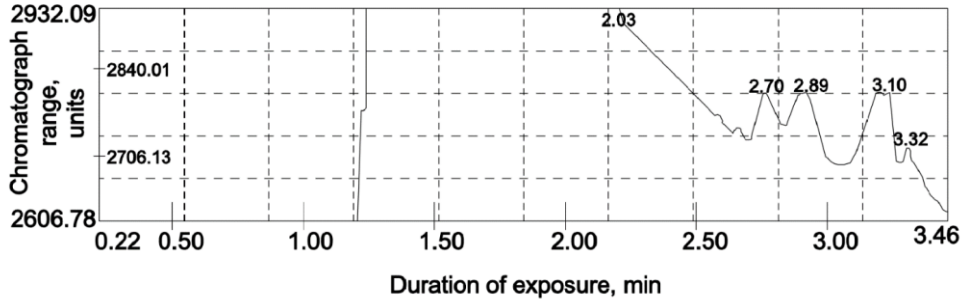

Figure 1. Amount of acrylamide in molded potato chips without oil (control sample)

Chips were baked and dried without oil at a temperature of 125°C for 4.5 min. With further heating up to 3.3 min, the amount of pre-fixed acrylamide did not increase. A small amount of acrylamide is formed due to free asparagine, which is contained in potato grain. In addition, potato grits contain amylose and amylopectin. Intact starch grains, which are insoluble in water, can absorb water initially in a limited amount, and when the temperature rises above 100°C, they actively absorb moisture in an unlimited amount. Baking-drying was carried out at a temperature of 125°C without the use of additional raw materials, which accelerates the release of moisture, therefore reducing sugars react with amino acids and the Maillard reaction occurs, which leads to the formation of acrylamide.

In a sample of molded potato chips, which were fried under standard conditions (temperature – 160°C, duration – 1.5-2 minutes) using oil (Figure 2), an increased concentration of acrylamide begins to be recorded on the chromatogram starting from 2.2 minutes, the point at which the maximum value of acrylamide accumulation wasafter 2.5 min of heating – 61 μg in 100 g of product. Starting from 2.76 min, the peak of acrylamide formation falls and the amount of acrylamide does not increase within 1 min.

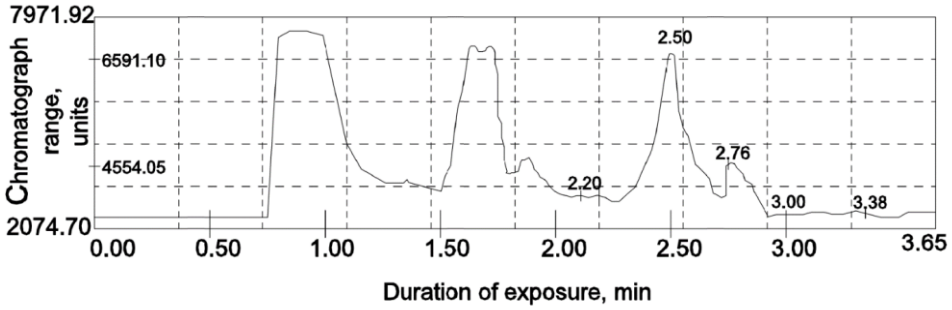

Figure 2. Amount of acrylamide in molded potato chips fried in oil

The heating time of the studied sample of molded potato chips with rye bran is 12.32 minutes (Figure 3).

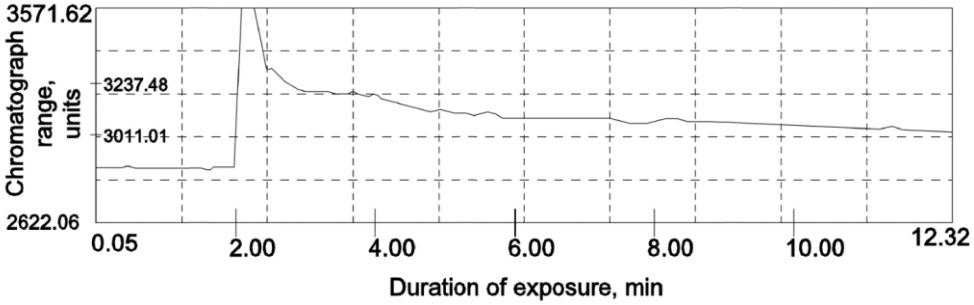

Figure 3. Amount of acrylamide in molded potato chips with rye bran

The oscillation range of the chromatograph starts from 0.05 min, while there is a sharp jump in the bromination of the extract of the studied mixture, but the peak of acrylamide formation in this sample was not recorded. It is due to the presence of fiber in the secondary raw material (47.6% by weight of the DM) because cellulose, hemicellulose, lignin, which speeds up the removal of moisture, are included in its composition.

The heating of the sample of molded potato chips with barley bran occurs immediately (Figure 4). At 2 min, a jump on chromatogram is observed, which does not record the formation of acrylamide. The final stage of completion of the reaction takes place during 10 minutes of heating the test sample. The process of completing the research is 1.2 times faster than for the sample with rye bran. This is due to the fact that the dietary fibers of barley bran are more easilybrominated and therefore the process of completing the experiment is shortened.

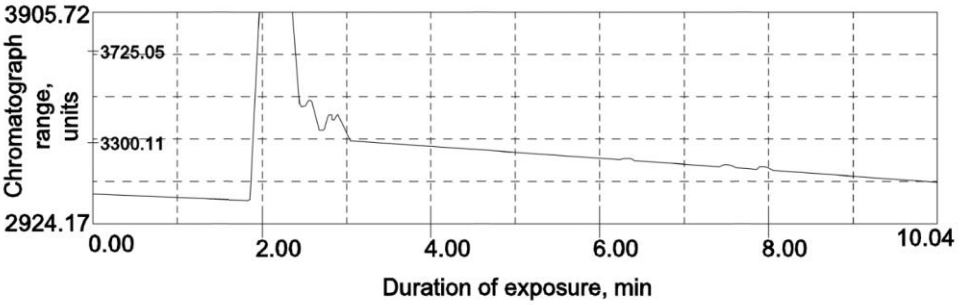

Figure 4. Amount of acrylamide in molded potato chips with barley bran

For sample with pumpkin seed mealcloser to 2 min, the peak of the reaction occurs with a gradual decline starting from 2.7 min to 3.3 min (Figure 5). Then the system stabilizes and the bromination process stops. A slight peak is observed at 12 min, which leads to the completion of the reaction. These data are explained by the fact that the pumpkin seed meal contains a small amount of oil, which can provoke sudden fluctuations in the system.

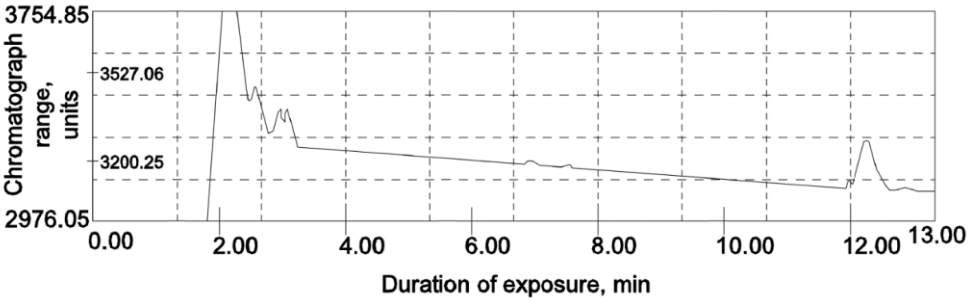

Figure 5. Amount of acrylamide in molded potato chips with pumpkin seed meal

The process of heating the samples with cryopowders begins at 0.01-0.02 min (Figures 6, 7).

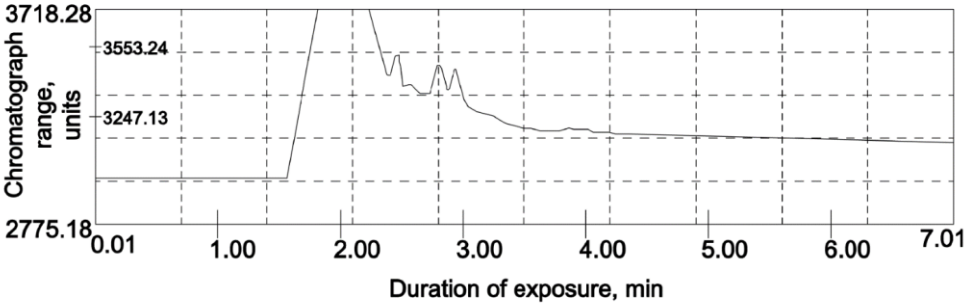

Figure 6. Amount of acrylamide in molded potato chips with broccoli cryopowder

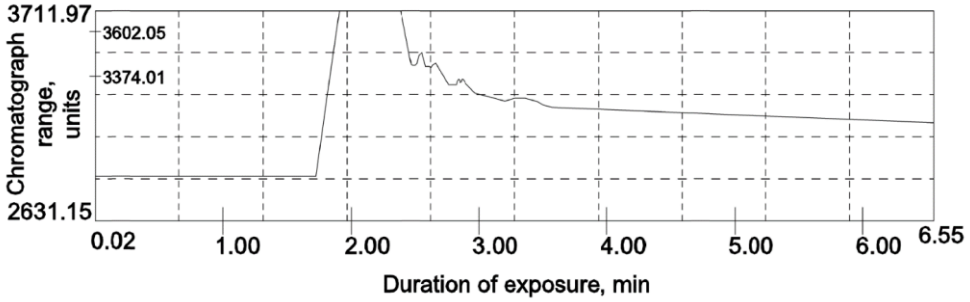

Figure 7. Amount of acrylamide in molded potato chips with red beet cryopowder

When approaching 2 min, a reaction peak is observed, which gradually stabilizes to 6.55 min and 7.01 min in samples with broccoli cryopowder and red beet cryopowder, respectively. Compared to the samples of molded potato chips with bran and pumpkin seed meal, the reaction time for molded potato chips with cryopowders is 2 times shorter. This is obviously due to a decrease in the pH level due to the content of organic acids and a reduced amount of aspartic acid.

Analyzing the obtained data of chromatograms of samples of molded potato chips minor peaks were found on all chromatograms that did not reach the critical point at which acrylamide formation occurs. This is due to the fact that the potato dough has a high moisture content – more than 42%, and the baking-drying process itself takes place at a relatively low temperature of 125 ºC for up to 4.5 minutes.Therefore, part of the acrylamide can evaporate from the product. It is important that acrylamide begins to form only when the temperature in the middle of the product reaches above 120ºC due to the chemical composition of the studied raw materials, namely the content of dietary fibers. During the keeping of the potato dough, there is a uniform redistribution of moisture due to which, at a baking-drying temperature of 125ºC, the temperature in the middle of the formed potato chips fluctuates is about 115ºC (Sharma et al., 2017).

Therefore, it is proposed to produce molded potato chips according to improved technology and to changed the temperature treatment parameters.It's advisable to remove the vegetable oil used in frying, and to carry out baking-drying of molded potato chips without the use of oil under the following parameters: the potato mass of the semi-finished product of one sample of molded potato chips is no more than 20 g, processing time – up to 4.5 min, baking-drying temperature – no more than 125ºC.

Since the rye and barley bran, and pumpkin seed meal are secondary raw materials and are made from rough fruit shells of plants, they contain a small amount of aspartic acid and sugars. Potato grits, broccoli and red beet cryopowders are produced by cryogenic drying at high pressure and multi-stage cryo-shredding. At the same time, it is possible to significantly reduce the amount of aspartic acid and reducing sugars in chips when these additives are added to the recipe.

Conclusions

1. Traditional and non-traditional raw materials were selected for the production of molded potato chips, and the frying process was replaced by baking-drying at a temperature of 125 °C to reduce formation of acrylamide.

2. It was established that the bran, meal and cryopowders included in the formulation of molded potato chips have a small amount of reducing sugars (content does not exceed 1%), as well as 2.5 times less aspartic acid compared to the potato grits.
3. The presence of acrylamide in chips with rye and barley bran, pumpkin seed meal, broccoli and red beet cryopowders was not detected during chromatographic studies. It is due to the use of raw materials with a reduced amount of aspartic acid and reducing sugars, as well as a temperature processing method without frying in oil and a reduced baking-drying time of up to 4.5 minutes.

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Influence of alternating impulses of pressure on sensory characteristics in fermentation technology

Iryna Dubovkina

Institute of Engineering Thermophysics of National Academy of Sciences of Ukraine, Kyiv, Ukraine

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Corresponding author:

Iryna Dubovkina
E-mail: dubovkinai@ukr.net

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Abstract

Introduction. The aim of this research was to study the influence of the application of alternating impulses of pressure all through processing in foodstuff production. It was done by the quantitative sensory analysis and tasting evaluation of samples of the wine, fortified wine and associated liquid aqueous systems and solutions.

Materials and methods. Wine, fortified wine and associated liquid aqueous systems and solutions were used for investigations and analysing the change of their physical and chemical parameters and properties during the processing by the different methods, approaches, and technological modes.

Electrochemical methods were used for the results analysing. A quantitative sensory analysis and tasting evaluation of the experimental liquid samples of wine, fortified wine and associated liquid aqueous systems and solutions was also conducted.

Results and discussion. In general, an effect of the treatment in the conditions of alternating impulses of pressure during uninterrupted mode gave the opportunity to increase the general score of a quantitative sensory analysis of wine and fortified wine by 7.3%.

During treatment in uninterrupted mode of alternating impulses of pressure with flow shear speed $3.0 \times 10^5\ s^{-1}$ and flow shear stress 300 Pa, the general score reached its lowest value 8.4. The verification of the cavitation number from 0.1 to 0.5 gave the possibility to receive the highest general score 8.8 from 10.

It substantiates the technology of obtaining the fortified wine involving grape comminution, crest separation, must infusion on the seeds and skins, pressing, must fermentation, blending, and alcoholization. Wine alcoholization is performed under conditions of hydrodynamic cavitation with cavitation number of 0.3, flow shear rate of $2.6 \cdot 10^5\ s^{-1}$ and flow shear stress of 260 Pa.

Conclusions. The total score of wine and fortified wine samples obtained under the conditions of alternating impulses of pressure had increased quality indicators compared to control samples, which were obtained by the traditional technology. This has a positive effect on the quality of the finished product.

Introduction

Sensory evaluation is an essential step of food product development. However, when using traditional methods to evaluate the quality of products that have very different sensory characteristics, but do not have defects, they can receive the same quality score. Therefore, such an evaluation does not record differences in sensory characteristics that may be very important for consumer acceptance (Paul et al., 2022).

The use of alternative types of raw materials and innovative technologies lead to a change in taste properties, consistency and other organoleptic indicators of food quality. Organoleptic evaluation tests can be used in product development, research, quality control and shelf life studies (Marjorie et al., 1990).

There are intensively developing novel food processing technologies that are environmentally-friendly and energy-saving ones (Ivanov et al., 2021). The methods of controlled energy impact can influence on structural and energy transformation in multifarious liquid media on micro- and nano- level and give opportunity to begin physical and chemical alteration in these media (Dolinskij et al., 2005).

Consuming of innovative technologies gives the possibilities to reduce the energy and recourses consumption of foodstuff production. The method of the alternating impulses of pressure may be appropriate for technology of the associated liquid aqueous systems and solutions processing for food and foodstuff production. Therefore, further investigations of the influence of alternating impulses of pressure on wine are needed.

The purpose of this research was to study the influence of the application of alternating impulses of pressure all through processing in foodstuff production by the quantitative sensory analysis and tasting evaluation of samples of the wine, fortified wine and associated liquid aqueous systems and solutions.

Materials and methods

Materials

Objects of research are different kinds of processing methods and modes of wine, fortified wine and associated liquid aqueous systems and solutions for the application in the food industry.

Subjects of study are the changing of the physical and chemical parameters and properties of wine, fortified wine and associated liquid aqueous systems and solutions (Dubovkina, 2017).

Wine, fortified wine and associated liquid aqueous systems and solutions were used to study the change of their physical and chemical parameters and properties during the processing by the alternating impulses of pressure with verification of the technological modes (Dubovkina et al., 2019).

Experimental installation

Research was carried out on the experimental unit shown in Figure 1. The main part of this unit is rotary-pulsed apparatus. That’s why the object of this study was rotary-pulsed

apparatus in which liquid solutions were processed by alternating impulses of pressure and hydrodynamic effects such as the speeds of shift of a stream and pressures of shift of a stream.

The work of the experimental unit was carried out as follows. At first wine or associated liquid aqueous systems and solutions entered the tank 1. After that through the flowmeter 2 wine or associated liquid aqueous systems and solutions followed into rotary-pulsed apparatus 5 for the processing by alternating impulses of pressure. There were two ways of the processing. According to the first, the processing was carried out in a continuous mode from the very beginning, and according to the second, the processing was carried out in a recirculating mode with a variable processing duration. After treatment wine or associated liquid aqueous systems and solutions followed into tank for treatment liquid 9 through the valve 8.

During the treatment temperature by the temperature sensing device 6 and pressure by pressure sensing device 7 were controlled. Other parameters were controlled by analogue-digital transmitter 10 and results were transmitted on personal computer 11.

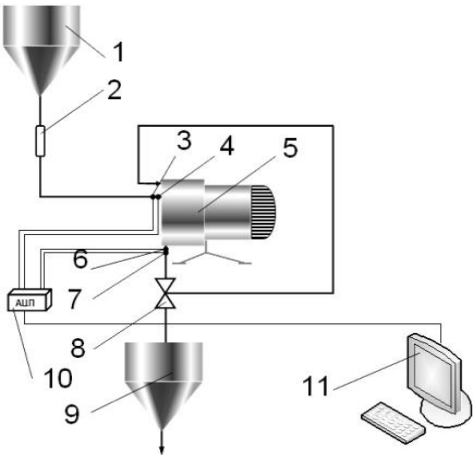

Figure 1. The scheme of experimental unit:
1 – tank for liquid; 2 – flowmeter; 3 – temperature sensing device;
4 – pressure sensing device; 5 – rotary-pulsed apparatus; 6 – temperature sensing device;
7 – pressure sensing device; 8 – valve; 9 – tank for treatment liquid;
10 – analogue-digital transmitter; 11 – personal computer.

The rotary-pulsed apparatus was the cylindrical type with the working chamber with two rotors and one stator (Shurchkova et al., 2015). Technical parameters of the experimental equipment such as alternating impulses of pressure:

- ΔP = 370 kPa near an external surface of an internal rotor;
- ΔP = 240 kPa near an external surface stator;
- ΔP = 155 kPa near an internal surface stator;
- ΔP = 190 kPa near an internal surface of an external rotor.

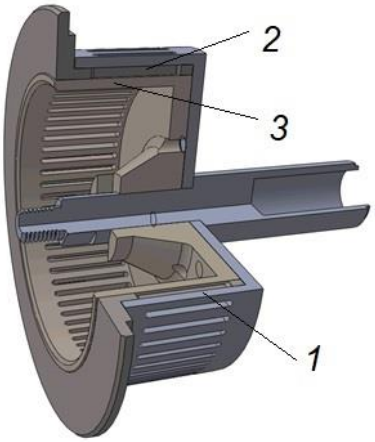

Figure 2. Scheme of working chamber:
1 – external rotor; 2 – stator; 3 – internal rotor.

Methods

General methods and special methods, such as electrochemical analysis were used for the determination of the results of study (Singh V. P. et al., 2018). For the interpretation of physical and chemical parameters of liquid samples of solutions t al., which obtained through the experimental treatment, chemical methods described in (Sullivan et al., 2005) were used.

The tasting evaluation and quantitative sensory analysis of the experimental liquid samples of wine, fortified wine and associated liquid aqueous systems and solutions was performed. The wines were presented to the panellists for tasting evaluation at 8 to 10 °C in standard wine-tasting glasses (AWWA, 2014) (ISO 3591, 1977).

To obtain related data, liquid samples of wine, fortified wine and associated liquid aqueous systems and solutions were analysed in triplicates with the following statistical procedure (Kenneth, 2007).

Results and discussion

Recently, researchers, technologists and engineers have turned their interest to employment of the original non-traditional technologies and methods in processing of the liquid mediums, solutions, systems, which consists of the alcohol, water or water solutions.

The physical and chemical properties and parameters of associated liquid aqueous systems, solutions and mediums, such as oxidation-reduction potential, pH value and dissolved oxygen may be changed by the physical methods, particularly by hydrodynamic treatment.

The method of alternating impulses of pressure is one of the methods to control energy impact with many hydrodynamic effects, such as power of pressure of shift, cavitation, the effect of explosive boiling, collective effects in assembly of vials, crossness of an interphase surface in gas-liquid bubbly medium, action of hydrodynamic oscillations, alternating

impulses of pressure, effects which associated with acceleration of movement of a continuous phase.

The most important effects of the alternating impulses of pressure are associated with increase of velocity of association of a continuous phase of medium. The fundamental nature of alternating impulses of pressure comprises in that preliminary permanently entered and any rank the energy distributed in working volume to accumulate in locally disconnected discrete points of system and further pulse to realise for achievement of necessary physical effects:

- forcing and dumping of pressure,
- adiabatic boiling,
- hydraulic blow,
- shock waves of pressure or depression,
- pressure of shift, local turbulence,
- cavitation and many other effects (Dolinskij and Basok, 2005).

Three-dimensional and period concentration of energy gives the possibility to receive the big capacity of pulsation power accomplishment, to release internal energy of substance, to create active energetic processes which take place at microlevel and also at nanolevel.

The development of different microliquid devices for some last decades has caused growth of interest to microscale streams. Rotary pulse apparatus is characterised by small enough sizes of width of channels which gives the possibility to consider them as microchannels with effects of slippage of a watercourse on walls and surfaces. A number of heat and mass technological processes (structuring, crushing, dispersion, emulsification, homogenization, and mixing) are spent in rotary pulse apparatus of cylindrical type, which realise principles of alternating impulses of pressure.

Wine is a multicomponent system consisting of volatile components such as higher alcohols, aldehydes, ethers, acetals, ketones, terpenes and various acids. Monoterpenes are isoprene compounds currently found in aromatic plants such as hydrocarbons, aldehydes, alcohols, acids or esters (Marcon et al., 2019). Hydration processes in such complex systems differ in their essence from hydration in water-alcohol mixtures, so it is impossible to apply the same processing methods for water-alcohol mixtures, wine and wine materials, since its quality depends on the bouquet and aroma of the wine. The study of sensory space can also demonstrate the influence on the perceived sensory characteristics of products, helping to characterize the tasting learning in oenology (Caissie et al., 2021).

Technological factors will define the complexity of wine aroma but numerous viticulture, ethnological techniques significantly influence the types and concentrations of volatile compounds (Zhou et al., 1996). Cavitation is used to intensify the process of assimilation of wine with alcohol in the process of distilling wine materials.

Since water is an ampholyte, preliminary activation of water systems is suggested to increase the degree of hydration, i.e. to initiate the formation of hydrogen bonds and proton transfer in the system, during the processing of wine materials and wine, preliminary treatment is not desirable, because during the intensive action of cavitation a decrease in the amount of aldehydes may occur, which in turn leads to a deterioration of the bouquet and taste of the wine. In order to intensify the assimilation of wine with alcohol, it is proposed to use not only cavitation, but also flow shear stresses and flow shear velocities, which together allow to improve the quality of wine.

Sensory evaluation is a key method to assess the flavor quality of foods because it measures what consumers really perceive; however, it is a subjective method. Several factors must be controlled in conducting a sensory evaluation test to minimize experimental error in the data. Sensory tests may be divided into three groups based on the type of information that they provide. The three types are discrimination, descriptive, and affective. Descriptive sensory analysis of wines related to methodology and vocabulary, tasting room and taster selection and training (ISO-11035, 2007).

Therefore, descriptive sensory evaluation methods give an additional tool for application in research, product development, and marketing. In addition, descriptive sensory evaluation methods use a panel of assessors, rather than one expert, and therefore the result obtained represents a consensus that is less subjective, and less susceptible to bias, than the result obtained when one expert performs the evaluation. To avoid errors in the sensory evaluation in daily quality control, it is necessary to follow well-defined grading systems or guidelines and standards (Martinsdóttir, 2010).

The techniques of descriptive sensory analysis allow obtaining an objective description of the wine based on its attributes of aroma and perceived taste and can be both qualitative and quantitative (Sánchez-Palomo et al., 2018).

Wine critics are clarity, tone, pure aroma, aroma concentration, aroma quality, pure taste, taste concentration, lasting taste, taste quality, overall assessment (Hou et al., 2020).

*The control example of the **red wine** obtained by traditional technology:*

- alcohol content is 14,2%;
- characteristics of the sample: transparent, deep ruby colour, the aroma is vaguely expressed, the perceptible smell of ethyl alcohol, the taste is harmonious with sharp notes, incomplete;
- tasting ball is 8.2.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.5:

- alcohol content is 14,2 %;
- characteristics of the sample: transparent, rich dark ruby colour, simple aroma, harmonious taste, but noticeable astringent tones;
- tasting ball is 8.35.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.4:

- alcohol content is 14.1 %;
- characteristics of the sample: transparent, dark ruby colour, rich, the aroma is complex, the taste is harmonious, but astringent tones are felt;
- tasting ball is 8.4.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.3:

- alcohol content is 14.1 %;
- characteristics of the sample: transparent, dark ruby, velvety, the aroma is complex, the taste is full-rounded, velvet;
- tasting ball is 8.8.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.2:

- alcohol content is 14.0 %;
- characteristics of the sample: weak opalescence, the colour is dark with a ruby-new velvet shade complex aroma with a side tone, the taste is full, not harmonious enough;
- tasting ball is 8.4.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.1:

- alcohol content is 14.0 %;
- characteristics of the sample: pronounced opalescence, dark velvet colour, complex aroma with a side tone, the taste is full and not harmonious enough with astringent tones;
- tasting ball is 8.25.

The general score (tasting ball) of control samples was 8.2 and the general score of wine which was processed by alternating impulses of pressure was 8.8.

The results of the tasting evaluation are given in the Figure 3.

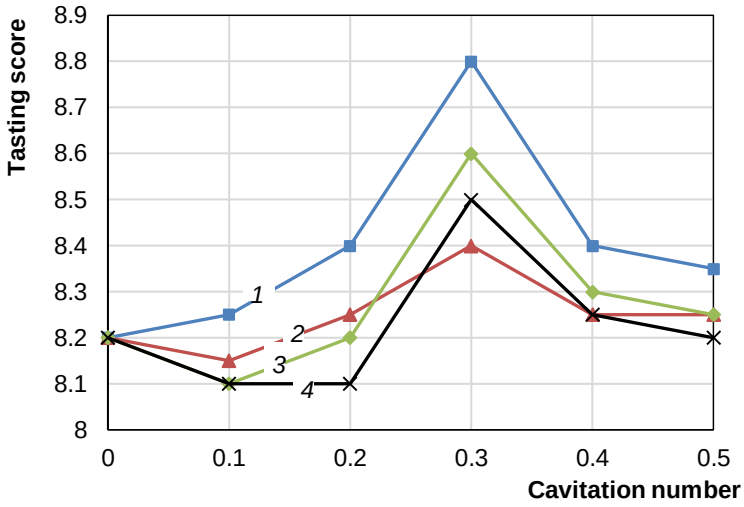

Figure 3. Dependence of the tasting score of red wine on the number of cavitation number the influence of different flow shear stresses and flow shear rates:
1 – flow shear rate 2.6×10^5 s^{-1}, flow shear stress 260 Pa;
2 – flow shear rate 2.5×10^5 s^{-1}, flow shear stress 250 Pa;
3 – flow shear rate 2.8×10^5 s^{-1}, flow shear stress 280 Pa;
4 – flow shear rate 3.0×10^5 s^{-1}, flow shear stress 300 Pa.

*The control example of the **white wine** obtained by traditional technology:*

- alcohol content is 16.3 %;
- characteristics of the sample: transparent, light-golden colour, aroma thin, vaguely pronounced, noticeable smell of ethyl alcohol, the taste is bitter with sharp notes, incomplete;
- tasting ball is 8.15.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.5:

- alcohol content is 16.2 %;
- characteristics of the sample: transparent, light golden colour, weak aroma developed, taste developed, but bitter-cotton tones are noticeable;
- tasting ball is 8.30.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.4:

- alcohol content is 16.2 %;
- characteristics of the sample: transparent, light golden colour, subtle aroma, noticeable floral notes the taste is harmonious and slightly velvety;
- tasting ball is 8.45.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.3:

- alcohol content is 16.0 %;
- characteristics of the sample: transparent, golden colour, the aroma is subtle, complex, with floral notes, the taste is light, full and soft;
- tasting ball is 8.75.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.2:

- alcohol content is 16.0 %;
- characteristics of the sample: pronounced opalescence, rich golden colour, the aroma is complex with an extraneous tone, the taste is light and not harmonious enough;
- tasting ball is 8.5.

The example of the wine obtained by alternating impulses of pressure with cavitation number 0.1:

- alcohol content is 15.9 %;
- characteristics of the sample: strong opalescence with shimmer, golden-straw color, the aroma is complex with a herbal tone, the taste is full of roughness;
- tasting ball is 8.5.

The general score (tasting ball) of control samples of white wine was 8.15 and the general score of wine which was processed by alternating impulses of pressure was 8.75.

The results of the tasting evaluation are given on Figure 4.

A quantitative sensory general score (tasting ball) of red wine which was processed by alternating impulses of pressure depending on the processing time or the duration and from the cavitation number. This sensory approach provides a global sensory image of a product and can give an overall assessment of a sensory concept (Barbe et al., 2021).

Two main descriptive methodologies are generally considered according to the way data are gathered (generation of marks on a scale or determination of descriptor citation frequencies), but modified approaches have also been developed. It is a very useful technique to establish a solid knowledge for the identification of the wines based on evidence and facts of their aromatic characteristics (Magalios et al., 2019).

Control liquid samples of wine and fortified wine were used for qualified analysis. These were samples of wine which were obtained according to the traditional standard methodology. In general, an effect of the treatment in the conditions of alternating impulses of pressure during uninterrupted mode gave the opportunity to increase the general score of a quantitative sensory analysis of wine and fortified wine by 7.3%.

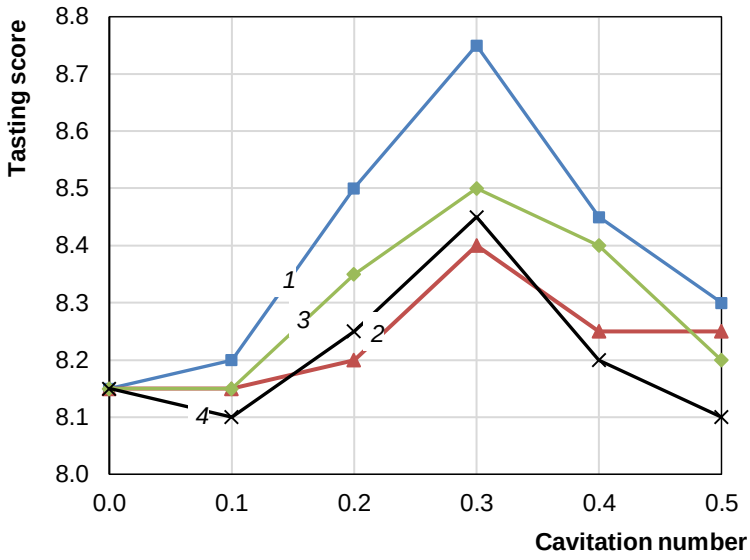

Figure 4. Dependence of the tasting score of white wine on the number of cavitation under the action of different flow shear stresses and flow shear rates:
1 – flow shear rate $2.6x10^5 s^{-1}$, flow shear stress 260 Pa;
2 – flow shear rate $2.5x10^5 s^{-1}$, flow shear stress 250 Pa;
3 – flow shear rate $2.8x10^5 s^{-1}$, flow shear stress 280 Pa;
4 – flow shear rate $3.0x10^5 s^{-1}$, flow shear stress 300 Pa.

As a result of study the new technology was created. The invention relates to a process for fortified wine producing involving grape comminution, crest separation, must infusion on the seeds and skins, pressing, must fermentation, blending, and alcoholization. Wine alcoholization was performed under conditions of hydrodynamic cavitation with cavitation number of 0.3, flow shear rate of $2.6 \cdot 10^5$ s^{-1} and flow shear stress of 260 Pa.

Conclusions

At the present time one of original and innovative methods and equipment that were used for the advancement of processing by samples of wine, fortified wine and associated liquid aqueous systems and solutions is the method of alternating impulses of pressure.

New experimental and theoretical studies and modeling have shown that the method of alternating impulses of pressure may possibly be suitable for processing in food industry and foodstuff production, where hydrodynamic effects are found to be an substitute to traditional methods and modes in technological lines, equipment and processes of wine, fortified wine and associated liquid aqueous systems and solutions.

According to the general results of sensory analysis and tasting evaluation, it was established that wine and fortified wine has a higher general tasting score and improved parameters, which has a positive effect on the quality of the finished product, as compared to the control samples, which were obtained by the traditional technology.

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Industrial wastes as substrates for synthesis of surfactants with antiadhesive activity by *Rhodococcus erythropolis* IMV Ac-5017

Tetiana Pirog[1,2], Igor Kliuchka[1], Liliia Kliuchka[1]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – Institute of Microbiology and Virology of National Academy of Sciences of Ukraine Kyiv, Ukraine

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Corresponding author:

Liliia Kliuchka
E-mail:
liya.nikityuk@ukr.net

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Abstract

Introduction. Microbial surfactants can affect the degree of microorganism's adhesion to different surfaces and lead to biodegradation of the already formed biofilms. They are also non-toxic to the environment. These properties allow to use the microbial surfactants in medicine and food industry.

Material and methods. Surfactants were obtained by cultivation of the *Rhodococcus erythropolis* IMV Ac-5017 on waste from biodiesel production, used sunflower oil after frying meat, potatoes, onions, and cheese. The surfactants were extracted from the supernatant of cultural liquid by a Folch mixture. The anti-adhesive activity and the degree of destruction of biofilms were determined by spectrophotometric method.

Results and discussion. It was found that surfactants obtained by cultivation of *R. erythropolis* IMV Ac-5017 on waste mixed sunflower oil reduce the adhesion of yeast *Candida albicans* D-6, *Candida utilis* BVS-65, *Candida tropicalis* PE-2 and bacterial strains *Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Pseudomonas* sp. MI-2 to abiotic surfaces (tiles, steel and glass) by 51–73% and 57–86%, respectively. The effectiveness of the anti-adhesive activity of surfactants, obtained on waste from biodiesel production as a substrate against the studied yeast and bacterial cultures was lower (44-77%). The ability of surfactants to destroy of bacterial biofilms by 44–73% was achieved at low concentrations of surfactants (8−15 μg/ml) synthesized on different waste oil. Surfactants were equally effective in destroying biofilms (72–80%) of *Candida* genus and their activities were not depended on the nature of carbon source in the culture medium of *R. erythropolis* IMV Ac-5017.

Conclusion. The obtained results show the possibility of using surfactants of *R. erythropolis* IMV Ac-5017, gained on industrial waste, as effective anti-adhesive and antibiofilm agents.

Introduction

There is an increasing interest to microbial surfactants as alternative to synthetic analogues due to such advantages as biodegradability, lack of toxicity, stable physical and chemical properties in a wide range of temperatures and pH (De Almeida, 2016) and unique biological properties (Naughton et al., 2019; Vecino et al., 2018). Thus, the possibility of using surfactants of microbial origin in the oil and mining, chemical, food industries, agriculture, and environmental technologies has already been proved.

Since the 90s of the XX century surfactants of microbial origin are actively studied as alternative agents for the destruction of biofilms formed on various materials used in medicine and the food industry (Velraeds et al., 1996). It is known that the colonization of abiotic surfaces by microorganisms is a very dangerous phenomenon, which causes not only spoilage of products, but also the spread of infectious diseases. Many studies supported the use of inorganic compounds, anti-adhesive chemicals, antibiotics and bacteriophage therapy to prevent various infections. However, the increasing resistance of microorganisms to antibiotics and other biocides, high cost of current methods for preventing of formation and further destruction of biofilms leads to search for new substances with appropriate properties – and such substances are surfactants of microbial origin.

Although at present the effectiveness of biotechnology methods for obtaining surfactants is low due to the high cost of the products of microbial synthesis. A possible solution is to use industrial waste (that are available in large quantities) as substrates (Singh et al., 2019).

In our previous studies (Pirog et al., 2013; 2015; 2021) a possibility of synthesis of surfactants by *Rhodococcus erythropolis* IMV Ac-5017 on waste from biodiesel production and refried sunflower oil was shown. However, to date, an anti-adhesive activity of surfactants synthesized on these toxic wastes has not been studied.

Therefore, the aim of this work is to study surfactants, synthesized by *R. erythropolis* IMV Ac-5017 on waste from biodiesel production and refried sunflower oil, influence on the attachment of microorganisms to abiotic surfaces and destruction of biofilms.

Material and methods

Study objects

Strain *R. erythropolis* IMV Ac-5017 is the main object of research, isolated from oil-contaminated soil samples and registered in the Depository of Microorganisms of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Bacterial strains (*Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Pseudomonas* sp. MI-2) and yeast (*Candida albicans* D-6, *Candida utilis* BVS-65, *Candida tropicalis* PE-2) from the collection of living cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies (Kyiv, Ukraine) were used as a test cultures for the determination of biological activity of the surfactants.

Cultivation conditions

R. erythropolis IMV Ac-5017 was grown in a liquid mineral medium of the following composition (g/l): $NaNO_3$ – 1.3; $MgSO_4 \times 7H_2O$ – 0.1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; $FeSO_4 \times 7H_2O$ – 0.01; pH 6.8–7.0.

The following compounds were used as a carbon source (2% volume fraction): refined glycerol; waste from biodiesel production (Komsomol Biofuel Plant, Poltava region, Ukraine), refined sunflower oil (TM «Oleyna», Ukraine), waste mixed sunflower oil after frying meat, potatoes, onions, cheese (from RocketPub fast food restaurants, Kyiv, Ukraine).

A culture from the end of the exponential growth phase (48 h), grown in the media as described above with a suitable carbon source (0.5%, volume fraction) was used as the inoculum. The amount of inoculum was 5% of the volume of the culture medium.

Bacteria were cultured in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30 °C for 120 hours.

Determination of the concentration of surfactants

The surfactant concentration in the culture fluid (g/l) was determined by the weight method after their extraction from the supernatant with a modified Folch mixture as described in our previous work (Pirog et al., 2020)

Determination of the anti-adhesive activity

The study of anti-adhesive properties of surfactants was carried out as described previously (Pirog et al., 2014). Purified plates of studied materials (tiles, steel and glass) of equal size were sterilized at 112 °C for 40 min. One-day test-cultures of bacteria and yeast were suspended in 100 ml of sterile tap water. Pre-treated with preparations of surfactants with different concentration and untreated (control) materials were added to the suspension, kept in a thermostat for 2 h at 30 °C, and then rinsed with 10 ml of sterile tap water to remove unattached cells.

Further the degree of cell adhesion was determined using a spectrophotometric method. For this purpose, the plates of materials were treated with methanol (99%) for 15 min to fix the adherent cells and dried at room temperature; then placed in 1% solution of gentian violet for 5 min and rinsed with tap water. After drying, the materials were treated with 10 ml of 33% acetic acid solution, and optical density of the resulting cell suspension was measured. The optical density of the suspensions was measured by photoelectric colorimeter at a wavelength of 540 nm. The number of adhered cells (adhesion) was defined as the ratio of the optical density of the suspension obtained from the treated surfactant samples to the optical density of the control samples and expressed as a percentage (Pirog et al., 2014).

Determination of the ability to destroy microbial biofilms

Study on the effect of surfactants on the destruction of biofilms was carried out as described in (Gomes et al., 2012). For biofilm formation, 180 µl of meat-peptone broth or liquid wort and 20 µl of one-day test culture suspension were added to the polystyrene microplates and incubated for 24 h at the optimum temperature for the culture test. Then the culture fluid was drained and 180 µl of fresh meat-peptone broth (or liquid wort) and 20 µl of test culture suspension was added for further incubation for the next 24 hours. After 48 h, the culture fluid was drained, and 200 µl of surfactants of different concentrations (0.0078–1 mg/ml) were added to the wells of the microplate (with the test film biofilm previously formed on them).

Sterile tap water (200 µl) was added into the control variants (wells) instead of surfactants. After 24 h of exposure, the wells were washed three times with 200 µl of distilled

water and the number of adherent cells was determined by spectrophotometric method as described above.

All experiments were performed in 3 repeats, the number of parallel measurements in the experiments was 3-5. Statistical processing of experimental data was performed as described previously (Pirog et al., 2013; Pirog et al., 2015). Difference in the average values was considered reliable at $p < 0.05$ significance level.

Results and discussion

Anti-adhesive activity of surfactants synthesized by *R. erythropolis* IMV Ac-5017 grown on industrial waste

The data given in Table 1, show that the adhesion of bacteria and yeast to abiotic surfaces treated with solutions of surfactants of *R. erythropolis* IMV Ac-5017, depends on the nature of the carbon source in the culture medium, concentration of a surfactant and a type of test cultures.

Table 1

Influence of surfactants of *R. erythropolis* IMV Ac-5017 on attachment of test cultures to abiotic surfaces

Substrate for surfactant synthesis	Abiotic surface	Adhesion,%				
		Escherichia coli IEM-1	*Bacillus subtilis* BT-2	*Pseudomonas* sp. MI-2	*Candida utilis* BVS-65	*Candida albicans* D-6
Refined glycerol	Tile	62	75	83	54	68
	Steel	78	60	60	77	80
	Glass	67	56	58	86	78
Waste from biodiesel production	Tile	45	63	78	45	49
	Steel	66	52	50	68	65
	Glass	58	46	48	77	60
Refined sunflower oil	Tile	25	50	50	65	81
	Steel	47	62	42	48	51
	Glass	24	74	65	68	52
Waste refried sunflower oil	Tile	14	41	33	49	27
	Steel	26	42	40	41	33
	Glass	14	43	40	40	34

Notes: The effective surfactant concentration, which ensured minimal adhesion of bacterial and yeast test cultures, was 6–12 and 24–48 μg/ml, respectively.

Thus, the most effective anti-adhesive agents were surfactants synthesized on the oil waste: after treatment with such agents of the studied surfaces, the adhesion of microorganisms on them was only 14–49%. At the same time for the surfactants synthesized

on refined oil the adhesion increased to 24–81%. Replacement of refined glycerol in the culture medium of *R. erythropolis* IMV Ac-5017 with waste from biodiesel production was accompanied by the formation of surfactants with higher antiadhesive activity: the number of attached bacteria and yeast cells on abiotic materials was by 5–19% lower comparing to those from purified substrates. In general, surfactants synthesized on refined and waste oils reduced the adhesion of test cultures more effectively than those formed on refined glycerol and waste from biodiesel production.

In order to ensure minimal adhesion of yeast cells a higher concentration of surfactants is required compared to that of bacteria (24−48 and 6−12 μg/ml, respectively).

In order to ensure minimal adhesion of yeast cells a higher concentration of surfactants is required compared to that of bacteria (24−48 and 6−12 μg/ml, respectively).

Analysis of the literature showed that information on the anti-adhesive activity of surfactants of the representatives of genus *Rhodococcus* is limited; available data is related to surfactants synthesized only on hexadecane (Janek et al., 2018; Kuyukina et al., 2016). In addition, an effective reduction in adhesion was achieved by treating surfaces with such surfactants in high concentrations (100–1000 μg/ml). In the study (Janek et al., 2018) it was found that the adhesion of *E coli* 17-2, *E. coli* ATCC 10536, *Staphylococcus epidermidis* KCTC 1917 and *C. albicans* ATCC 10231 did not exceed 28% after treatment of the wells of polystyrene tablets and silicone surfaces with surfactants of *Rhodococcus fascians* BD8 at a concentration of 500 μg/ml. Number of attached cells of *B. subtilis* ATCC 6613 and *E. coli* K-12 to the wells of polystyrene tablets treated with surfactants of *Rhodococcus ruber* IEGM 231 (100–1000 μg/ml) reduced to 20% (Kuyukina et al., 2016).

It should be noted that there are no publications regarding to anti-adhesive properties of microbial surfactants synthesized on waste from biodiesel production; there is also a limited information regarding to substrates containing oil. The study (Rufino et al., 2011) reported that Rufisan synthesized by *C. lipolytica* UCP 0988 on waste soybean oil reduced the adhesion of bacteria *Streptococcus* and *Lactobacillus* on polystyrene plates. Even at the minimum studied surfactant concentration (0.75 mg/l) the degree of adhesion of test cultures was 61–91%. As the surfactant concentration in solution increased to 12 mg/l, Rufisan reduced the number of attached *E. coli* and *C. albicans* cells by 21–51%.

Probiotic strain *Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674 under conditions of growth on various substrates, including sunflower oil waste, synthesized surfactant, which at a concentration of 10 mg/ml reduced the number of attached cells of *E. coli* to the plate by 13% and for *Staphylococcus aureus* by 37% (Hajfarajollah et al., 2014).

Data listed in the Table 1 shows that surfactants of *R. erythropolis* IMV Ac-5017, synthesized on either refined or waste oil, are more effective anti-adhesive agents than Rufisan and surfactants of *P. freudenreichii* subsp. *freudenreichii* PTCC 1674. They reduce the adhesion of test cultures by 19–86% at a concentration of only 6–12 μg/ml.

Destruction of bacterial and yeast biofilms under the action of surfactants synthesized by strain *R. erythropolis* IMV As-5017 grown on industrial waste

In the Tables 2 and 3, the data on effect of surfactants of *R. erythropolis* IMV Ac-5017 on the destruction of bacterial and yeast biofilms is shown.

Table 2

Destruction of bacterial biofilms under the action of *R. erythropolis* IMV Ac-5017 surfactants

Substrate for surfactant synthesis	Biofilm destruction,%		
	***Escherichia coli* IEM-1**	***Bacillus subtilis* BT-2**	***Pseudomonas* sp. MI-2**
Refined glycerol *	48	71	72
Waste from biodiesel production*	44	73	69
Refined sunflower oil**	42	60	44
Waste refried sunflower oil**	65	76	63

Notes: * – The concentration of surfactant is 8 mg/ml, ** – concentration of surfactant is 15 mg/ml.

Table 3

Influence of surfactants synthesized by *R. erythropolis* IMV Ac-5017 on industrial waste, on destruction of yeast biofilms

Test culture	Destruction of biofilms (%) under the action of surfactants synthesized on			
	Refined glycerol	**Waste from biodiesel production**	**Refined sunflower oil**	**Waste refried sunflower oil**
Candida albicans D-6	72	74	79	80
Candida utilis BVS-65	76	73	72	77
Candida tropicalis PE-2	75	78	75	78

Note: The surfactant concentration is 30 μg/ml.

The ability of surfactants to destroy the biofilms, as well as their anti-adhesive activity, depended on the nature of the growth substrate, the concentration of surfactants and the type of test culture.

The degree of destruction of bacterial biofilms by surfactants synthesized on both refined glycerol and waste from biodiesel production was almost the same. While surfactants synthesized on waste oil destroyed such biofilms more effective than those obtained on refined oil (see Table 2). It should be noted that the destruction of bacterial biofilms by 42−76% was achieved at low concentrations of surfactants (8−15 μg/ml) synthesized on all studied substrates. Surfactants were equally effective in destroying biofilms (72–80%) of *Candida* (see Table 3) and not depended on the nature of carbon source in the culture medium of *R. erythropolis* IMV Ac-5017. The concentrations of surfactants were 2–4 times higher than bacterial biofilms.

In (Janek *et al.*, 2018) it was found that the destruction of bacterial biofilms was achieved at a surfactant of *R. fascians* BD8 concentration 250 μg/ml, which is up in the order of magnitude greater than the surfactant of the IMV strain Ac-5017 (see Table 2). In addition,

the authors of (Janek et al., 2018) analyzed an efficiency of destruction of biofilms by surfactants only visually using confocal laser scanning microscopy.

Das et al. (Das et al., 2014) showed that rhamnolipids of *Pseudomonas aeruginosa* IMP67 synthesized on glycerol destroyed the biofilms of *B. subtilis*, *E. coli* and *Staphylococcus aureus* by 50% at a concentration of 64–128 μg/ml, which is higher in comparison with surfactants of *R. erythropolis* IMV Ac-5017.

In addition, there is virtually no published data regarding to the destruction of biofilms in the presence of surfactants synthesized on oil-containing substrates. In 2017, there was a publication (Kiran et al., 2017) regarding to the effect of lipopeptide synthesized by actinobacteria *Nesterenkonia* sp. MSA31 (isolated from the sea sponge *Fasciospongia cavernosa*) on destroying biofilms of *S. aureus* strain MSA31 was grown on a medium with 10% olive oil, the lipopeptide was extracted with organic solvents (ethyl acetate, methanol, petroleum ether, dichloromethane) from the supernatant, previously pre-acidified to pH 2.0. To determine the role of lipopeptide in the destruction of the biofilm, surfactant solutions in the concentration range of 25–150 μg/ml were used. It was established that the maximum degree of destruction of a biofilm of *S. aureus* (90%) was achieved in the presence of lipopeptide at a concentration of 125 μg/ml (Kiran et al., 2017).

It should be noted that he destruction of yeast biofilms of the genus *Candida* is an urgent problem today, because most modern biocides, including some surfactants, are not effective enough (Gulati and Nobile, 2016). For example, surfactants synthesized by *Lactobacillus jensenii* P6A and *Lactobacillus gasseri* P65 at a concentration of 180 μg/ml destroyed yeast biofilms only by 25–35% (Morais et al., 2017).

C. albicans biofilms are resistant to most known antifungal drugs that complicates the control of infections caused by these yeasts. In (Shinde et al., 2012) it was shown that these yeasts easily colonize the surfaces of prostheses (laryngeal, knee, heart valves), implants (especially thoracic), endotracheal tubes, which causes the spread of infection throughout the body. Azoles and polyenes are not effective against biofilms of *C. albicans.* This reduces the number of potential agents for treating these infections and underlines the need for new effective agents (Gulati and Nobile, 2016).

Conclusions

As a result of this work it was found that surfactants synthesized by *R. erythropolis* IMV Ac-5017 on toxic industrial waste are characterized by high anti-adhesive activity and able to destroy bacterial and yeast biofilms. This set of biological properties makes the surfactant strain IMV Ac-5017 promising for practical use. Also, bioconversion of waste from biodiesel production and waste oil into microbial surfactants will help solving several problems: reduce the cost of surfactants production by using cheap raw materials as a substrate; increase the profitability of biodiesel production by disposing of a by-product – glycerol; protect the environment from uncontrolled emissions of toxic waste.

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Effect of nanoparticles of double divalent and trivalent iron oxide to protect of flax seeds from microbial spoilage

Mykola Riabchykov[1], Iryna Tsykhanovska[2], Oleksandr Aleksandrov[2], Denis Kovylev[2]

1 – Lutsk National Technical University, Lutsk, Ukraine
2 – Ukrainian Engineer-Pedagogic Academy, Kharkiv, Ukraine

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Corresponding author:

Iryna Tsykhanovska
E-mail: cikhanovskaja@gmail.com

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Abstract

Introduction. The effect of nanoparticles (NPs) of double divalent and trivalent iron oxide ($FeO \times Fe_2O_3$ – nanomagnetite) on protection of flax seeds against fungal infections was studied, and the dependence of this effect on the amount of nanomagnetite nanoparticles was determined.

Materials and methods. Microscopic observation of morphological and cultural features of micromycetes, yeast *Saccharomyces cerevisiae*, and mycelial fungi, *Mucor racemosus*, grown on an agar nutrient medium was done. Experimental samples of micromycetes were obtained by sowing a standard microbial preparation in the form of a suspension (initial dilution of the microbial suspension 1:100) in Petri dishes.

Results and discussion. The ability of nanoparticles of double divalent and trivalent iron oxide to protect the flax seeds was shown: addition of 0.1%; 0.15%; 0.2% of nanomagnetite to flax seeds inhibits by 8 –20 times the development of micromycetes on them.

A decrease by 8–10 times of the cell number of micromycetes yeast *Saccharomyces cerevisiae* and mycelia fungi *Mucor racemosus* as well as diminishing of the colonies size by 10–20 times on the surface of the treated flax seeds compared to control was found.

The recommended dosage of nanoparticles of double divalent and trivalent iron oxide was determined as 0.15% from the weight of the flax seeds. The proposed mathematical model makes it possible to predict the effectiveness of NPs $FeO \times Fe_2O_3$ – nanomagnetite application to inhibit the growth of spoilage microorganisms on the flax seeds.

Conclusions. For the first time, the ability of nanoparticles of double divalent and trivalent iron oxide (NPs $FeO \times Fe_2O_3$ – nanomagnetite) to protect flax seeds from fungal spoilage was shown.

Introduction

Flaxseed (*Linum usitatissimum* L.) has high contents of protein and fiber, low contents of carbohydrate and fat, contains such useful compounds as α-linolenic acid (omega-3), phenolic acids, phytic acid, vitamins, minerals especially Ca, K, P, Mg, and are recommended to be used in preparation of functional food with potential health benefits (Stabnikova et al., 2021). The main problem of application of flaxseed, especially if it will be used for manufacturing of food products, is preservation of their high quality during the storage (Brigante et al., 2022; Bruno et al., 2020; Rollemberg et al., 2022).

Some methods for determining flax seed quality indicators, including those related to damage, were proposed but were not sufficiently developed to be used in practice (Podio et al., 2022). A serious problem in the preservation of flaxseed and the manufacture of products from it is that it is susceptible to infection by fungi, which significantly affect the nutritional properties of food and endanger the health of the consumers (Choudhry et al., 2021; Farag et al., 2021; Gu et al., 2021). This problem is described well, but real ways of solving it are not defined. Unfortunately, the undeniable harmfulness of flax seed diseases is not considered in terms of creating bacteriostatic storage conditions.

Fungal infections of flax seeds in common (Pal et al., 2022). More often the fungi of the genera *Alternaria* (from 20.0 to 42.5% of infected seed) and *Fusarium* (up to 50.0% of infected seed) caused flax seed microbial spoilage. Among others fungi that caused flax damage the representatives of the genera *Colletotrichum*, *Sydowia*, and *Septoria* are reported (Gruzdevienė et al., 2006). Six genera and eight species of fungi were found in the flax seeds (Rollemberg et al, 2022). The representatives of genus *Aspergillus* were the most abundant including *A. cibarius*, *A. appendiculatus*, and *A. amstelodami*. The second most abundant genus was *Wallemia*, with the species *W. muriae*, some strains of this genus is known can produce toxins

The norms of damage and contamination of flax seeds are described in (Asad, 2022; Soureshjani et al., 2019; Xie et al., 2019). Different ways of solving the problem were considered at the stage when the danger has already been identified (Singh et al., 2021). In our opinion, the direction of creating conditions for inhibiting flaxseed diseases at the initial stages is more promising.

Recent advances in plant and food protection have often been linked to nanotechnology and nanomaterials (Fursik at el., 2019). In particular, the effect of iron oxides is considered positive (Tsykhanovska et al., 2020). These studies considered the bacteriostatic properties of nanomaterials for dairy products subject to fungal infections. Considering the research data on the danger of contamination of flax seeds with fungal infections, we consider it relevant to check the effect on these products. Tsykhanovska with co-authors (2021) describe the use of magnetite nanoparticles with bactericidal and bacteriostatic properties in the food industry. These results make it possible to predict a positive effect in the protection of flax seeds from fungal spoilage.

We propose the creation of preventive methods that act at the initial stages of the occurrence of infection diseases.

Analysis of publications on flaxseed diseases showed the following:

1. The main problem for flax seeds is fungal infections, the effectiveness of combating those remains low.
2. Methods of combating fungal infections involve the treatment of infected products, it is desirable to develop preventive methods.
3. Preliminary studies of magnetic nanomaterials testify their potential bacteriostatic properties. It is desirable to study these properties towards flax seeds.

The aim of this work is to investigate the effect of magnetite nanoparticles on the resistance of flax seeds to fungal diseases at the stage of their development.

Materials and methods

The method of determining harmful fungi is based on seeding the product or product homogenate and (or) their dilutions in nutrient media, determining the affiliation of isolated microorganisms to fungi by the characteristic growth on nutrient media and cell morphology.

The method is designed to establish the compliance of microbiological indicators of food quality to the requirements of regulatory and technical documentation, to clarify the causes of product defects (Chow et al., 2019; te Giffel and Zwietering, 2009).

Sowings of the product or its corresponding dilutions are carried out on Petri dishes, spend two parallel determinations. At the bottom of a sterile Petri dish make 1 cm^3 of product or its dilution and sterile pour 15–20 cm^3 of nutrient medium Saburo, or agar medium to identify harmful fungi. Petri dishes with crops are placed to solidify on a horizontal surface, then the Petri dishes are turned upside down and placed in a thermostat with a temperature of (24±1) °C for 5 days or at a temperature of (30±1) °C for 3–5 days. The crops are incubated at a temperature of (24±1) °C for 5 days (Chow et al., 2019; te Giffel and Zwietering, 2009). After 3 days, a preliminary count of typical colonies or the appearance of characteristic signs of growth on liquid nutrient media.

If in crops on dense media there are flour, very fast-growing fungi, the removal of preliminary results should be carried out very carefully, not allowing the spores of these fungi to crumble and give rise to secondary colonies. In 5 days the final account of results of thermostating of crops is carried out. Mushroom colonies are divided visually.

Colonies of fungi on flax seeds are shown in Figure 1.

Figure 2. Colonies of fungi on flax seeds

The development of harmful fungi on nutrient media is accompanied by the appearance of mycelia of different colours.

For quantitative counting, cups are selected on which 5 to 50 colonies of harmful fungi have grown.

If it is necessary, microscopic studies are carried out to separate colonies of harmful fungi. For this aim, preparations are prepared from individual colonies or from cultures on a liquid medium using the crushed drop method. A drop of sterile tap water is applied to the

slide. Then a part of the colony is introduced into this drop with a heated needle or a drop of culture liquid is applied with a loop. The resulting suspension is covered with a cover glass.

Microscopy results are evaluated using the characteristics of harmful fungi.

If the growth of harmful fungi is detected when testing the product on nutrient media and their presence is confirmed by microscopy, then a conclusion is drawn about the presence of these microorganisms in the product.

Each cup is placed upside down and counted using a magnifying glass to count the number of colonies that have grown, counting the colonies of harmful fungi separately. Each counted colony is marked at the bottom of the cup.

The following method of counting the number of harmful fungi colonies is proposed in the work:

In the graphic editor, a circle is created, the diameter of which is equal to the real diameter of the Petri dish (95 mm) and a system of squares with a size of 1×1 mm. Rows of squares are placed in mutually perpendicular directions.

The photo of the drug is placed on the same sheet, its size is adapted to the size of the circle. A system of squares is superimposed on top (Figure 2).

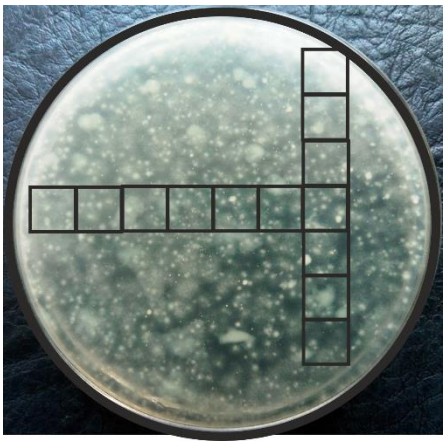

Figure 2. Example of calculation of number of colonies in graphic editor

The number of colonies of harmful fungi is counted separately in each square. The number of colonies is recalculated according to the proportionality of the area of the circle and the area of the squares:

$$N_p = \frac{n\pi r^2}{z},$$

n – number of colonies counted in all squares;
r –the radius of the Petri dish;
z – the number of squares in which the calculation was carried out (in our case, 13).

For convenience when counting, you can increase or decrease the size of the image. The number of harmful fungi N, Q/g of product was calculated according to the formula:

$$N = \frac{N_p}{m}10^p,$$

where: m is the weight of the sample taken for sowing; p is the number of 10-fold dilutions.

If the test of the product on nutrient media revealed the growth of fungi and their presence is confirmed by microscopy, it is concluded that the presence of these microorganisms in the product.

It is possible to slow down the development of the microflora and, accordingly, to extend the shelf life of the product by introducing additives that have a bacteriostatic effect into the product.

We offer as this additive a nanomaterial in the form of double oxide of ferrous and trivalent iron (NPs $FeO \times Fe_2O_3$), described in our previous works (Tovma, 2020, Tsykhanovska at el., 2021; Riabchykov at el, 2022) .

To determine the properties of the environment where the fungi grew, nanomagnetite powder (NPs $FeO \times Fe_2O_3$) was added to the flax seeds.

Results and discussion

Effect of magnetite nanoparticles on the growth of harmful fungi

The main component of magnetite is nanoparticles of mixed oxide of divalent and trivalent iron with the general formula Fe_3O_4 or $FeO \times Fe_2O_3$ with a nanoparticle size of 30–78 nm.

Nano-objects, which include nanomagnetite (NPs $FeO \times Fe_2O_3$), have enormous potential and carry many important fundamental discoveries, new functional and technological properties and promising technological applications. It should be noted that most nanomaterials used in food products occupy an intermediate position between nano- and microstructures. Thus, the diameter of DNA is 12 nm, liposomes 30–10000 nm, amylopectin 44–200 nm, cubosomes 500 nm, nanosensors < 1000 nm (Tovma, 2020)

Nanoparticles of the multifunctional food additive of complex action magnetite (NPs $FeO \times Fe_2O_3$) have a huge potential and high bioaffinity to biopolymers, in particular, proteins, carbohydrates. Therefore, they carry many important fundamental discoveries, new functional and technological properties and promising technological applications. Noncovalent adsorption of polymer molecules, H_2O dipoles occurs on the surface of magnetic nanoparticles (NPs $FeO \times Fe_2O_3$). The process of adsorption of biopolymeric food ingredients and water is mainly determined by ionic, vanderwaals, hydrogen and hydrophobic types of interactions. These interactions occur between the surface of nanoparticles (NPs $FeO \times Fe_2O_3$) and adsorbing molecules and entail a change in the Gibbs free energy. The result is the formation of supramolecular ensembles, which significantly affect the functional and technological properties of raw components and semi-finished products, as well as the quality of finished products (Tsykhanovska at el., 2021).

In previous studies (Tovma, 2020, Tsykhanovska at el., 2021)it was established that the addition of nanomagnetite (NPs $FeO \times Fe_2O_3$) to food products leads to a comprehensive improvement of their food, consumer and technological properties. So, in particular, nanoparticles NPs $FeO \times Fe_2O_3$ have bacteriostatic, bactericidal, and antioxidant properties, promotes better digestion of protein components of food, exhibits a moisture-, fat-retaining, and fat-emulsifying effect, is a source of easily digestible iron, and has a beneficial effect on metabolic processes (Riabchykov at el, 2022).

To determine the degree of development of harmful fungi, a microscopic examination of drugs derived from fungal colonies was performed. Images of the corresponding drugs were obtained by microimaging (Figure 3–4).

Figure 3. Micrograph of the drug from sample No 0 from a colony of fungi (×400 times magnification)

Figure 4. Micrograph of the drug from sample No 1 from a colony of fungi (×1000 times magnification)

From the given micrographs it is visible that in the sample which does not contain nanomagnetite (NPs $FeO \times Fe_2O_3$) there is an intensive development of microflora. So in a preparation which is made of a colony of fungi, even at rather small increase (400 times) the developed mycelium is visible (Figure 3).

Effect of nanomagnetite on the quantitative composition of harmful fungal colonies on flax seeds.

In a sample containing nanomagnetite (NPs $FeO \times Fe_2O_3$) in a relatively small amount (0.1%, sample №1) there is inhibition of microflora development. Only at a magnification of 1000 times in the colonies of fungi can be found small and sluggish fragments of mycelium (Figure 4).

In preparations made from sample №3 (content of nanomagnetite – NPs $FeO \times Fe_2O_3$ 0.2%) from colonies of fungi externally similar to colonies, even at 1000-fold magnification it was not possible to establish the presence of signs of mycelium.

More informative were the studies of samples conducted by a similar method after five days of exposure. Selected samples were diluted three times for seeding. The appearance of the drugs after five days of incubation is shown in Figure 5.

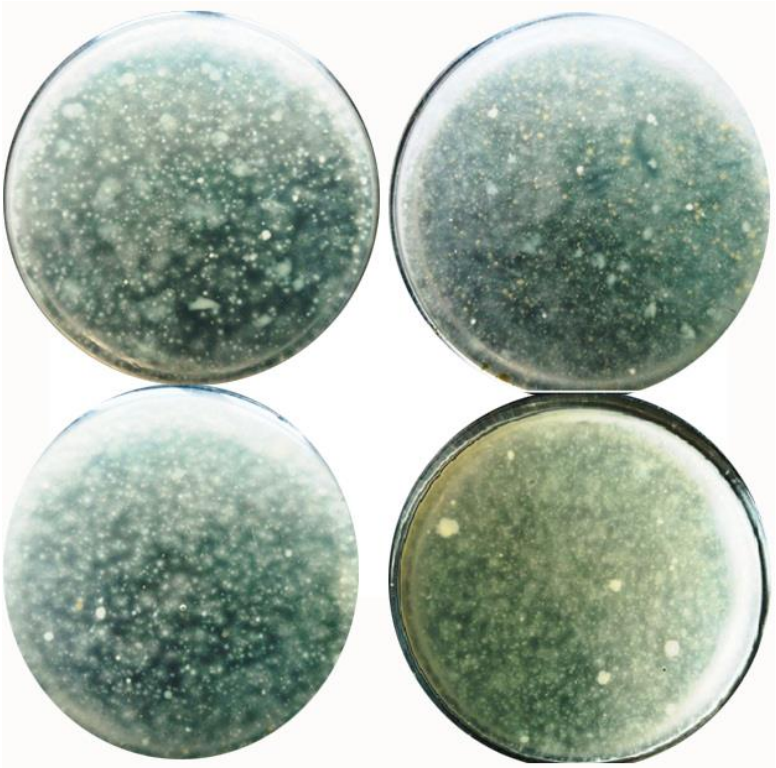

Figure 5. Micrograph of the drug from sample No 1 from a colony of fungi (×1000 times magnification)

On the photomicrograph of the preparation from the fungi colony of sample No 2 (Figure 6), weak mycelium without reproductive organs can be observed only at maximum magnification. Colonies of this sample contain a large number of cells that do not show vital activity.

Yeast colonies of sample No 3 (Figure 7) also contain non-viable cells, and in the fungal colonies of this sample, microscopy did not even reveal signs of the presence of mycelium. It is likely that the fungal colonies counted on the petri dishes were false.

Figure 6. Photomicrograph of the preparation from sample No 2 from the fungi colony (×1000 times magnification)

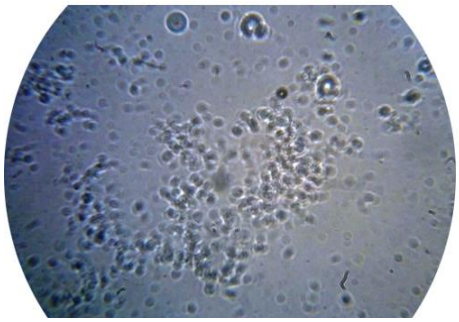

Fig 7. Micrograph of the drug from sample No 3 (×1000 times magnification)

From the obtained data it is seen that the addition of nanomagnetic powder (NPs $FeO \times Fe_2O_3$) significantly inhibits the development of microflora in flax seed samples (Table 1)

Table 1

Microbiological indicators of samples

Sample	**0**	**1**	**2**	**3**
The number of harmful fungi in 1 gram of product, Q	45×10^4	37×10^4	220×10^3	65×10^3

The addition of nanomagnetite powder (NPs $FeO \times Fe_2O_3$) has a bacteriostatic effect on harmful fungi in flax seeds (inhibits their development) and the concentration of 0.15% (sample No 2) is sufficient to implement this function.

Mathematical model of the influence of the composition of nanomagnetite on the growth efficiency of harmful fungi in flax seeds

Analysis of the dependence of the growth of the number of harmful fungi on the composition of nanomagnetite allows us to determine the following properties.

1. The general dependence demonstrates a continuous decrease in the number of harmful elements with an increase in nanomagnetite
2. At the initial stage, with a small amount of magnetite, the bacteriostatic efficiency decreases slightly, which is mathematically proven by the zero value of the derivative.
3. With a significant composition of magnetite, the bacteriostatic efficiency significantly decreases compared to the initial stage and gradually turns into an asymptotic dependence.

A function with a negative exponent whose argument has the form of a power function can correspond to similar properties.

If we determine the number of harmful fungi N, the composition of nanomagnetite u, the proposed function can look like this

$$N = N_0 e^{\left(\frac{u}{a}\right)^2}.$$

The unknown coefficients of this dependence can be determined by the method of least squares. For our case, the searched dependency looks like this:

$$N = 4.5 \cdot 10^5 e^{\left(\frac{u}{0.192}\right)^2}$$

The graph of this dependence is shown in Figure 8.

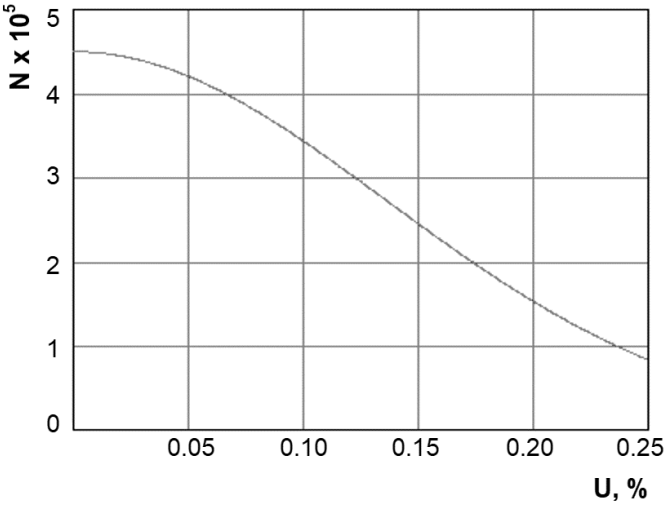

Figure 8. Dependence of the number of cells of harmful fungi on the amount of nanomagnetite.

The proposed dependence makes it possible to predict the effectiveness of the growth of the number of harmful fungi depending on the content of nanomagnetite and to determine the amount of nanomagnetite necessary to ensure bacteriostatic properties.

Conclusion

The main harmful substances in the storage of flax seeds intended for food use are harmful fungal microorganisms. The use of nanomagnetic powder (NPs $FeO \times Fe_2O_3$) containing of double oxide of ferrous and trivalent iron (NPs $FeO \times Fe_2O_3$) significantly reduces the growth rate of such microorganisms on the seeds of flax. These results allow us to recommend the addition of nanoparticles of magnetic powders during storage of flax seeds.

1. The addition of nanomagnetite to flax seeds infected with harmful fungi significantly reduces their ability to grow. The size of harmful fungi is reduced by 3–5 times, with a higher concentration, the size of harmful fungi is reduced by 10–20 times.
2. The number of colonies of harmful fungi decreases by 1.5–2 times, by 8–10 times with the addition of 0.2% nanomagnetite
3. The proposed mathematical function allows predicting the number and size of harmful fungi when nanomagnetite is added. The composition of nanomagnetite 0.15% is sufficient to significantly suppress the development of harmful fungi

4. The proposed methods of protecting flax seeds with the help of nanomagnetite allow to increase significantly their protective properties and significantly slow down their development even at the stage of infection.

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Antibacterial and anti-biofilm activity of cinnamon and clove oils against uropathogens

Neha Mishra, Ena Gupta, Priyanka Singh

Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India

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Corresponding author:

Priyanka Singh
E-mail:
priyanka.singh.29@
gmail.com

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Abstract

Introduction. The aim of the study is to explore *in vitro* antibiofilm activity of cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum*) essential oils and their main phytoconstituents cinnamaldehyde and eugenol against clinical bacteria isolates of patients with urinary tract infection (UTI).

Materials and methods. Antibacterial activities of cinnamon and clove essential oils and their active constituents were determined using disc diffusion and minimal inhibition concentration methods.

Result and discussion: In the present research, *Escherichia coli* was studied as the main bacterial uropathogen accounting for 70% of bacterial isolates identified among twenty patients with UTI. The results showed that eugenol had the highest antimicrobial activity against *E. coli* strains, followed by cinnamaldehyde and clove oils. Further, fourteen *E. coli* isolates obtained from the urine samples were screened for their biofilm-forming activity on polystyrene surfaces. Our finding reported that 28.5% isolates were not able to form biofilm as was determined by crystal violet staining assay. Eugenol and cinnamaldehyde exhibited high biofilm inhibition activity with a percentage of inhibition ranged from 80.54 to 95.2 and 73 to 89.0%, respectively. The minimum inhibitory concentration values of cinnamon oil, clove oil, cinnamaldehyde, and eugenol for *E. coli* strains range from 0.68–0.823 mg/ml, 0.75–0.923 mg/ml, 0.55–0.72 mg/ml and 0.44–0.56 mg/ml, respectively. A low minimum inhibitory concentration value may be an indicator of the high efficacy of an antibacterial agent in inhibiting the visible growth of microorganisms. Eugenol showed the highest antibacterial activity with minimum inhibitory concentration of 0.44 mg/ml for 71.4% of isolates and minimum inhibitory concentration of 0.56 mg/ml for 24.8% of isolates.

Conclusion. The tested cinnamon and clove essential oils exhibited very strong antibacterial and antibiofilm activities against *Escherichia coli* isolates obtained from UTI patients and hence can be used as a promising alternative for antibiotics substitution.

Introduction

Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Proteus species, Acinetobacter species, Enterobacter species, Klebsiella species,* and *Citrobacter species* are the most common bacterial species associated with urinary tract infection (UTI). These uropathogens are found to be drug-resistant and biofilm forming bacteria, which is a major factor in the pathogenesis of recurrent urinary infections.

Biofilm is the structure made by colonies of microorganisms that adhere and grow on the surface of the cell and non-cellular materials, which is eventually enclosed in an extracellular polymeric substance matrix (Hall et al., 2017; Jamal et al., 2018). This extracellular matrix is mainly composed of polysaccharides and can to adhere to both biotic and abiotic surfaces. Biofilm is the colony of microbes that are irreversibly attached to a range of surfaces in the food industry in just a minute. Recently, biofilm formation in food processing surfaces and equipment has become a great concern in the food industry because they are responsible for the corrosion of metal surfaces, equipment damage, and cross-contamination of processed food (Abdallah et al., 2014; Colagiorgi et al., 2017). The formation of biofilm is a self-protecting barrier for the growth of bacteria against environmental stresses, antimicrobial drugs, and the host human defense. Extensive research has been done to prevent and inhibit biofilm formation through the implementation of several synthetic and natural drugs. However, complete success in the detachment or destruction of biofilm has not yet been developed (Basavaruju et al., 2016). Synthetic drugs contain so many side effects like nausea, diarrhea, dizziness, lightheadedness, headache, or trouble sleeping. There is a need to search for natural sources which can heal the problem without causing any side effects to the patients. However, plant-based natural products have received much attention in preventing antibiofilm activity in the last decades. Hence it is a matter of much concern, and the research was done to acquire a new source of antimicrobials and biofilm-eradicating agents to combat the infections associated with biofilm-causing drug-resistant bacteria.

The aim of the present study is to evaluate the antimicrobial activity of natural antimicrobial agents (cinnamon and clove essential oils and their main phytoconstituents cinnamaldehyde and eugenol) against clinical bacteria isolates of UTI patients.

Materials and methods

Sample preparation and extraction of essential oils

The spices cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum*) were thoroughly cleaned and then dried in an oven at 55 °C. After drying, barks and buds were grounded into a coarse powder and placed inside a thimble made from thick filter paper, which was then subjected to solvent extraction technique by a soxhlet extractor. The solvent used for extraction was ethanol. The solvent was heated to reflux at a temperature above 100 °C for 5 and 10 hours. After the extraction, the products were collected and purified using a rotary evaporator at a fixed temperature of 50 °C (Wong et al., 2014).

Isolation, identification of bacterial strains

The bacterial strains were isolated from urine samples of twenty UTI patients from Janhit the hospital, Prayagraj, and were streaked on nutrient agar for single colony separation. Three single colonies were selected, streaked separately in MacConkey agar and Eosin

methylene blue (EMB) agar (HiMedia, Mumbai, India). The morphological (Gram's reaction, cell shape, and arrangement) and biochemical characterization (indole test, motility test, Vogues Proskeur test, methyl red test, catalase test, nitrate reduction test, triple sugar iron test, urease test)of recovered uropathogens were carried out according to Harley and Prescott (1997), and the results were compared with the pure maintained cultures. The most probable species was decided on the basis of PIBWN software.

Antibiotic assay

The disk diffusion technique did the antibiotic susceptibility test of *Escherichia coli* isolates. The antibiotics used were amoxicillin (30 µg/disc), amoxicillin/clavulanic acid (10 µg), piperacillin + tazobactam (10 µg), ticarcillin + clavulanic acid (10 µg), Cefepime, cefuroxime (30 µg) and ceftriaxone (CT, 30 µg). The isolates were inoculated into 10 ml of sterile nutrient broth and incubated at 37±1.0 ºC overnight. 100 µL inoculum was swabbed on the surface of agar plates, and then antibiotic-impregnated discs were immersed in it after cooling. The plates were then kept for incubation at 37 °C for 24 hours. An inhibition zone on the plates showed antimicrobial activity.

Antibacterial assay

The antimicrobial activity was evaluated by the agar well diffusion method **(**Bagamboula et al., 2004). Bacterial inoculums containing 10^6 CFU/ml were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 3–5 minutes. A sterilized borer (10 mm) impregnated by the tested essential oils (10 µl /disc) was used to prepare agar wells and then incubated in an upright position at 37±10 ºC for 24 hrs. Sterile blank paper discs impregnated with only sterile dimethyl sulfoxide (DMSO) served as the negative control, and antibiotic ciprofloxacin (5 µg/disc) was used as a positive control. The zone of inhibition was measured around each disc to evaluate the antibacterial activity and expressed in millimeters (mm).

Assessment of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by the microdilution method proposed by (Jorgensen and Turnidge, 1999) with minor modifications. Different concentrations of samples ranging from 0.05 to 2.5% (v/v) were prepared in DMSO and transferred into 96 well microliter plate (100 µl/well. The test was performed in triplicates alongside a blank sample (without bacterial inoculum). The concentration at which no visible growth was observed was recorded as MIC value**.**

Biofilm production assay

100 µl of overnight grown bacterial suspension were poured into the wells of a 96-well microtiter plate and allowed to incubate for 24 h to 48 h at 37° C. Then, the supernatant culture was discarded, and wells were washed twice with phosphate-buffered saline to remove the loosely adhered. The adhered cell was stained with 200 µl 1% of crystal violet for 10 minutes. The excess strain was discarded, and the wells were washed with distilled water and air-dried. The plate was then read spectrophotometrically at 570 nm in an ELISA reader **(**Sa et al., 2012).

Biofilm Inhibition Assay

100 µl of the final concentration of essential oils (equivalent to MIC) was poured into the 96-well microtiter plate consisting of fresh 50 µl of bacterial culture (10^8 CFU/ mL) in each well and incubated at 37 °C for 24 h. The formation of biofilm was quantified by the crystal violet method as described earlier. The well with sterile water was considered a control. Inhibition of biofilm was assessed by the formula described by (Jadhav et al., 2013):

% Inhibition = 100 - (OD570 sample/OD570 control) x 100 Inhibition of biofilm

Statistical analysis

The experiments were done in triplicates, and their mean values were represented. All statistical analyses were done using analysis of variance (ANOVA) in SPSS Version 17.0. Differences were considered significant when $p< 0.05$. Graphs were prepared in Sigma.

Ethical consideration. Patient consent was obtained, and anonymity was preserved by assigning an identification number.

Results and discussion

Isolation and identification of bacteria

Twenty urine samples of UTIs patients were collected from Janhit hospital, Prayagraj. The result of present study revealed that 70% of bacterial isolates belonged to *E. coli*, and 30% belonged to *Klebsiella spp.* The bacterial species were identified on the basis of their morphological and biochemical characteristics (Table 1, 2). The results recorded from biochemical tests were subjected to PIBWIN software to identify the most probable organism. It was found that *E. coli* was the most prevalent UTI pathogen, followed by *Klebsiella spp*. Earlier studies also have recorded similar findings (Bitew et al., 2017; Gupta et al., 2011).

Table 1

Identification of clinical isolates

Features	Nutrient Agar		EMB Agar	
	Isolates 1, 3, 4, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	Isolates 2, 6, 7, 8, 9, 10	Isolates 1, 3, 4, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	Isolates 2, 6, 7, 8, 9, 10
Gram reaction	Gram –ve	Gram -ve	Gram –ve	Gram –ve
Shape	Circular	Circular	Circular	Circular
Color	Creamy white, shiny	Grayish white	Green metallic sheen	Pink to purple
Surface	Mucoid	Mucoid	Mucoid	Mucoid
Elevation	Flat	Dome-shaped	Convex	Convex
Opacity	opaque	Translucent- opaque	opaque	Translucent- opaque

Table 2

Biochemical characteristics of culture isolates

Biochemical tests	Isolates 1, 3, 4, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	Isolates 2, 6, 7, 8, 9, 10
Catalase test	Positive	Negative
Lysine test	Positive	Negative
Nitrate test	Negative	Negative
Citrate test	Negative	Negative
Methyl test	Positive	Negative
Voge's test	Positive	Negative
Indole test	Positive	Negative
Urease test	Positive	Positive
Peptone test	Positive	Negative
Motility test	Negative	Negative
Tryptophan test	Positive	Positive

Antibiotic sensitivity test of *E. coli* isolates

In the present work, the bacterial isolates *E. coli* were selected for further studies because of the high prevalence in urine samples among UTI patients. Results of the disc dilution test for antibiotic sensitivity of isolates to seven different antibiotics are represented in Table 3.

Table 3

Antibiotic resistant pattern of the *E. coli* isolated strains

Isolates	***AM***	**AMC**	**PTB**	**TCA**	**Cefepime**	**Cefuroxime**	**Ceftriaxone**	**Biofilm activity**
E1	R	R	S	R	R	R	R	M
E2	R	R	S	R	R	R	R	S
E3	R	R	S	R	R	R	R	M
E4	R	R	S	R	R	R	R	S
E5	R	R	S	R	S	S	S	N
E6	R	S	S	S	S	S	S	N
E7	R	S	S	S	S	S	S	N
E8	R	S	S	S	S	S	S	N
E9	R	R	S	R	S	S	S	M
E10	R	R	S	R	S	S	S	M
E 11	R	R	S	R	S	R	S	M
E12	R	R	S	R	S	R	S	M
E13	R	R	S	R	R	R	R	M
E14	R	R	S	R	S	R	S	M

Amoxicillin (AM, 30 μg/disc), amoxicillin/clavulanic acid (AMC, 10 μg/disc), piperacillin + tazobactam (PTB, 10 μg/disc), ticarcillin + clavulanic acid (TCA, 10 μg/disc), cefepime (30 μg/disc), cefuroxime (30 μg/disc g), ceftriaxone (CT, 30 μg/disc). R-resistant, S-sensitivity.

In this study, common antibiotics used to treat UTIs were selected. Our finding revealed that all the bacterial isolates were resistant to amoxicillin, and most of the bacterial isolates (78.57%) were resistant to amoxicillin/clavulanic acid and ticarcillin + clavulanic acid. This is attributed to the ability of strains to produce beta-lactamase. Previous studies also reported the resistance of these strains to aminopenicillins (Iroha et al., 2012). Similarly, Dibua et al. (2014) revealed that amoxicillin was not active against any Gram-negative strains of the urine specimens. Our finding observed that only piperacillin associated with tazobactam presented very good activity against *E. coli* isolates, consistent with previous studies that reported antibiotics combination increased sensitivity (Sagna, 2019). Inconsistent with other studies found that tested multidrug-resistant strains isolated from clinical samples were resistant to the majority of commonly used antibiotics in developing countries.

Antibacterial assay

Several studies reported the antibacterial activity of medicinal plants for the management and cure of UTIs. In the present study, the effect of essential oil and their phytocompounds were performed after 24 h of the incubation period of bacterial culture in Mueller Hinton broth (Sigma Aldrich, SA). Figure 2 depicts the antibacterial activity of spice oil and its active compound against *E. coli* clinical isolates, expressed as diameters of inhibition. The result of preliminary antibacterial activity revealed that cinnamon oil, clove oil, cinnamaldehyde, and eugenol were active against all the tested *E. coli* isolates with a zone of inhibition >12 mm. Eugenol has a high percentage of inhibitory action on 85.70% of isolates (n-10) with the inhibition zone of 27mm, followed by cinnamaldehyde with the inhibition zone of 22 mm within only two isolates. Clove oil exhibited a zone of inhibition of 19 mm for 50% of the isolate, and cinnamon oil showed a zone of inhibition of 15 mm for 42.8% of the isolates (Figure 2). The result of the study was consistent with the work done by Prabuseenivasan et al. (2006).

After preliminary screening, the antimicrobial agents were subjected to minimum inhibitory assay. The minimum inhibitory concentration (MIC) is the minimum concentration of the antibacterial agent that could inhibit 50% of the bacteria after 24 h incubation. Figure 3 illustrated the MIC values of cinnamon oil, clove oil, cinnamldehyde and eugenol for *E. coli* ranges 0.68–0.823 mg/ml, 0.75–0.923 mg/ml, 0.55–0.72 mg/ml, and 0.44–0.56 mg/ml, respectively. A low MIC value may be an indication of the high efficacy of the antibacterial agents in inhibiting the visible growth of microorganisms. Eugenol showed the highest antibacterial activity, with a MIC 0.44 mg/ml in 71.4% of isolates and a MIC of 0.56 mg/ml in 24.8% of isolates. Our finding reported that individual phytochemical compounds eugenol followed by cinnamaldehyde had highest antibacterial activity than their respective essential oil. This finding concurs with other studies which reported cinnamon and clove oil exhibited the lowest MIC values for *E. coli*. Another study by Millezi et al. (2019) showed the antibacterial and antibiofilm activity of cinnamon essential oil and eugenol against uropathogens.

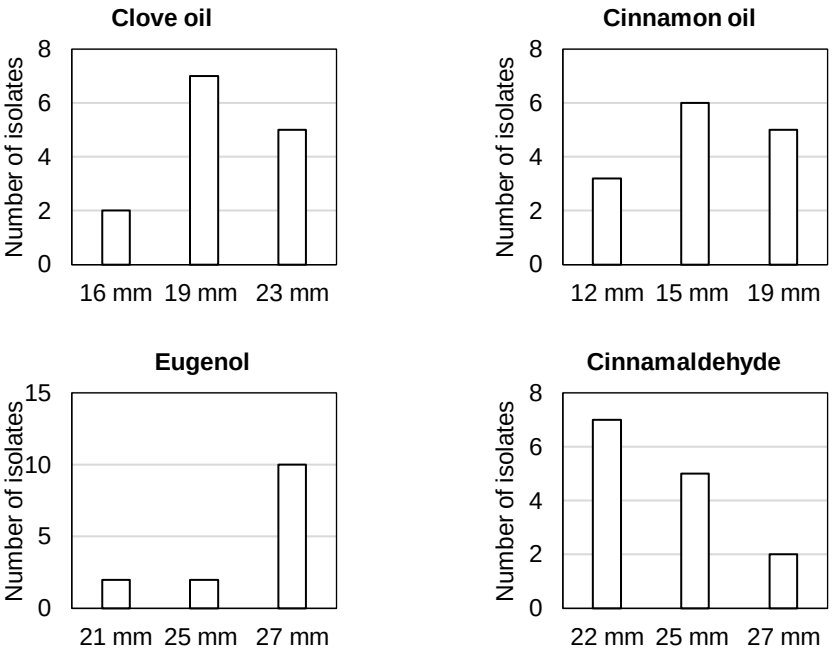

Figure 2. Zone of inhibition (mm) of essential oils against *E. coli* isolates

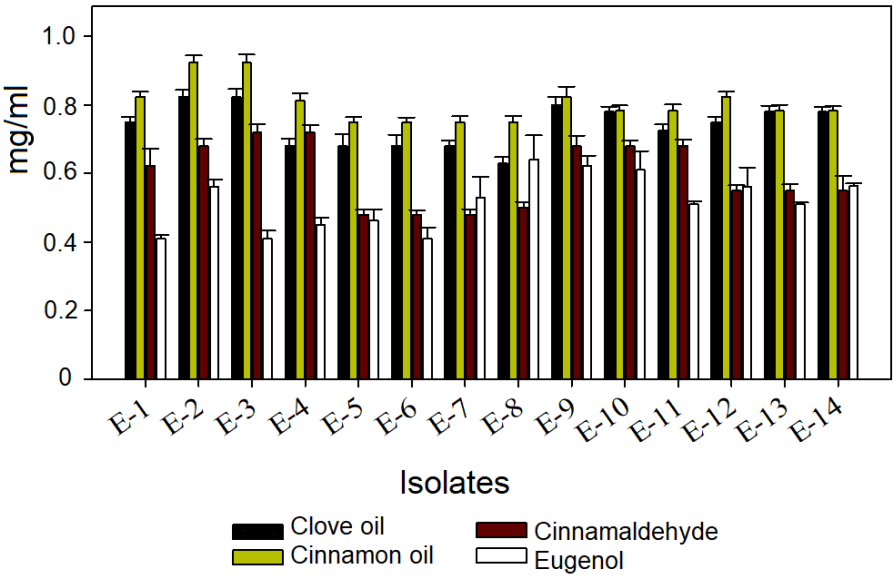

Figure 3. Minimal inhibition concentration (MIC) values (mg/mL) of essential oils against *E. coli* isolates tested with micro-dilution assay

Biofilm formation capacity of isolates

Previous studies reported that different isolates have different capacities to form biofilm. In this study, fourteen *E. coli* isolated from UTI patients were examined for their biofilm-forming abilities on polystyrene surfaces. Our results suggested that 57.8% (n-8) of the isolates have moderate biofilm formation ability, and only 14.2% (n-2) form strong biofilm formation ability with optical density 570 (OD570) values ranging from 0.146–0.18 and 0.26–0.29 respectively whereas 28.5% (n-4) were not able to form biofilm as determined by crystal violet staining assay. These strains were divided as strong (OD570 > 1), moderate (0.1 > OD570 < 1), and negative (OD570 < 0.1) based on their absorbance in crystal violet assay (Table 3). This result agrees with the researchers that showed each isolate have own capacity to form thickness due to the difference in the ability to produce autoinducers (Quorum sensing signaling molecules). Our finding reported that out of 14 isolates 10 isolates were able to form biofilm.

Anti-biofilm activity

The *in vitro* biofilm activity of essential oils and main active constituents on the prevention or reduction of biofilm development in 24 and 48 h preformed biofilms are presented in Figure 4.

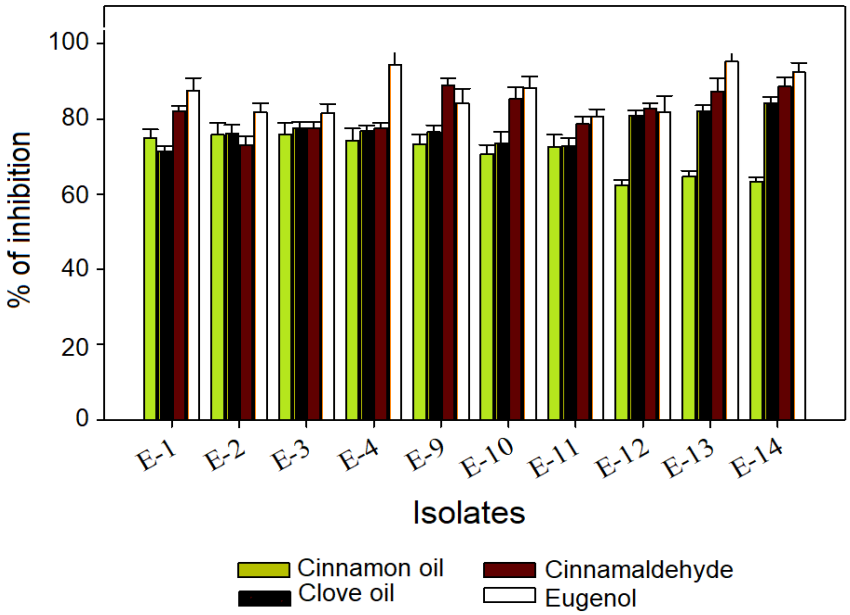

Figure 4. Antibiofilm activity (% of inhibition) of essential oils against *E. coli* isolates

Eugenol, followed by cinnamaldehyde, showed the highest antibiofilm activity, with the percentage of inhibition varying from 80.54% to 95.2% and 73% to 89.0%, respectively. The action of eugenol resulted in no biofilm formation by 90% of isolates; however, cinnamaldehyde provided inhibition for 80% of isolates, whereas the application of clove oil

and cinnamon oil resulted in weak biofilm formation by 60% to 70% of isolates. Cinnamon oil exhibited the minimum antibiofilm activity that ranges 62.4%-75.9%. The outcomes of the present work showed that eugenol and cinnamldehyde had highest antibiofilm effect followed by the clove oil and cinnamon oil. Amalaradjou et al. (2011) showed the ability of trans-cinnamaldehyde to inhibit uropathogenic *Escherichia coli* biofilm formation isolated from catheters and also demonstrated that trans-cinnamaldehyde prevented virulence factors of uroepithelial cells by down-regulating genes in the pathogen. Clove oil at sub-MIC levels was reported to repress QS-associated characters in reporter strains (Khan et al., 2000)

Conclusion

A urinary tract infection is the second most common infection that occurs in the human body and *Esherichia coli* is found to be the most prominent bacterial strain responsible for the disease. Most of the uropathogenic bacterial strains are found to be drug-resistant and have biofilm-forming capacity. The present study was done to discover the natural antibacterial and anti-biofilm agents from a huge reservoir of Indian medicinal plants. The study revealed that *E. coli* strains isolated from clinical samples were resistant to the majority of commonly used antibiotics. Eugenol, followed by cinnamaldehyde, has highest antibacterial and antibiofilm activity than their respective essential oils. In conclusion, our study suggested that eugenol and cinnamaldehyde can be used as plant-derived antibiotics for biofilm-associated urinary tract infections.

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Automated methods of controlling the flow of syrup in the evaporation station with subsystems of decision support and forecasting

Mykhailo Hrama, Viktor Sidletskyi

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Mykhailo Hrama
E-mail: mpgmay6@gmail.com

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Abstract

Introduction. The purpose of the presented study is to substantiate the methods of regulating the consumption of syrup in the evaporation station with a forecasting subsystem, which will allow to predict the behavior of the system and the decision-making subsystem, which will reduce the influence of the human factor on the course of the evaporation process.

Materials and methods. The work of the evaporation station with the subsystem of forecasting and decision support when regulating the consumption of syrup was researched. In the automation scheme for regulating the flow rate of syrup, induction flow meters are used as a sensor. Pneumatic saddle valves with a built-in throttle and an electro-pneumatic converter were used as actuators.

Results and discussion. The use of neural sensors occurs only in certain specific cases of intelligent control of the evaporation process, there is no data comparing the use of intelligent regulators with classical ones, the possibility of combining the work of several types of intelligent regulators, as well as clear means of predicting their work and supporting decision-making. Therefore, in this paper, a decision-making subsystem has been justified, which made it possible to assess the priorities of user requests when using a human-machine interface. The highest priority was given to the request to display information on possible changes to the adjustment parameters of other control circuits. The forecasting method was also used to compare the methods of regulating the flow rate of syrup in the apparatus, which 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 and, thus, increase the efficiency of the evaporation station. Statistical data on the behavior of the contours of the automation system in different modes of operation using intelligent and classical regulators were collected, a model for predicting the operation of an evaporation station by the method of local tendency was built and a forecasting algorithm was developed. The accuracy of the obtained forecasting model is also evaluated. The accuracy of the forecasting model was 98% for the PID controller, 95% for the neural fuzzy regulator and 96% for the neural network.

Conclusions. The model for predicting the operation of the evaporation station is characterized by high accuracy in general, but during the occurrence of oscillations in the transition process, there is an insignificant delay in predicting these fluctuations. The most important in the output of information by the decision-making subsystem is the function of displaying information about the possible changes to the parameters of regulation of other control circuits.

Introduction

The system of automatic control of the evaporation station can be described as a system that requires the intervention of an operator-technologist, who in the course of his work makes adjustments to the tasks of regulators responsible for temperature and material flows. Such adjustments can be explained both by a change in the technological and quality indicators of the components at the inlet of the evaporation station, and by the need to change them at the exit of the section. When making changes to the operation of the automation system, the operator must take into account how adjacent sections affect the operation of the evaporation station, as well as the impact of the evaporation station on the operation of adjacent sections of the plant (Hrama et al., 2019).

Perfection of the evaporation process is quite an important task. In the study (Chantasiriwan, 2021), the author considers a model of the evaporation process that takes into account the balance of mass and energy in the stages of the evaporation process. However, this study did not consider the possibility of using intelligent regulators in the evaporation process. The reason for this may be the difficulties arising from the need to use special software. Research (Verma et al., 2018) makes it possible to overcome the problem of its occurrence. This paper explores the process of linearization of a nonlinear model consisting of 14 nonlinear levels of primary order, which is a dynamic model of the evaporator. This study for the first time revealed the function of changing the concentration of the product from the deviation of the flow rate of a liquid (Garcés et al., 2021). However, no research has been conducted on the use of intelligent regulators in this study. This may be due to difficulties in developing rule bases for neural fuzzy regulators or the lack of an appropriate neural network training model (Said et al., 2021).

Evaporation stations for the sugar industry are equipped, as a rule, with stations with natural circulation (Petrenko et al., 2022). In which, in case of non-compliance with the optimal mode in the evaporation process, there is a decrease in alkalinity due to the decomposition and caramelization of sucrose, which leads to the decomposition of amides such as aspargin (Hrama et al., 2019). Juices of condensate (ammonia water) and vapors from the evaporation station contain carbon dioxide, carbon monoxide and ammonia. Sugar juice contains glucose ($C_6H_{12}O_6$), the above factors cause a change in its properties. When the glucose temperature reaches 160 °C and leaves it unchanged for a long time, one of the two water molecules is cleaved, that is, glucose anhydride is formed ($C_6H_{10}O_5$), from which the formation of crystallized sugar is impossible. With a further increase in temperature to 220 °C, tasteless caramel or bitter assamar (a substance formed when heating products of animal and vegetable origin) is formed from sugar juice, which are not capable of fermentation. Therefore, the formation of sugar from such substances is impossible (Hrama et al., 2019). Therefore, in order to prevent overexposure and overheating of the sugar syrup, it is necessary to ensure the best quality control parameters.

The need to update existing control systems is indicated in the work (Sidletskyi et al., 2020). The paper presents some approaches used for a distributed level of control of technological processes. But this work does not reveal the issue of using intelligent regulators in the evaporation process. Perhaps this is due to the complexity of the calculations.

In the works (Garcés et al., 2021) the author claims that with the help of intelligent control, it is possible to ensure a faster decrease in tank temperature and achieve more stable overheating control in the first evaporator tank. But in this paper there is also no disclosure of the issue of using intelligent regulators to regulate other parameters (for example, pressure, syrup level, flow rate). In addition, this paper considers only the possibility of using intelligent regulators in buildings other than the first. The problem of controlling other

parameters of the evaporation process is considered in the work (Verma et al., 2018). In this paper, it is proved that evaporation control can be implemented by recirculation of fluid in the evaporation section or by supplying only liquid to the evaporator. But this paper also does not address the use of intelligent regulators in the evaporation process. In the paper (Cao et al., 2020), the authors research decision-making subsystems and argue that their use in automation systems can improve the quality of automation processes by reducing the human factor, but for the correct operation of the decision-making subsystem, it is also necessary to develop a forecasting subsystem. However, the work does not consider the evaporation process.

The paper explores the use of methods for regulating the consumption of syrup in an evaporator with a subsystem of forecasting and decision support, which will allow to predict the behavior of the system and derive a ready-made forecast, which will thus increase the efficiency of the evaporation station.

The aim of the work is to substantiate the methods of regulating the consumption of syrup in the evaporator with the forecasting subsystem and the decision-making subsystem. This will make 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, increasing the efficiency of the evaporation station by reducing the influence of the human factor on the process.

Materials and methods

Object and subjects

As the station on which the study was conducted, a five-corps evaporation station of the sugar factory was taken. Figure 1 shows the scheme of automation of the contours for regulating the flow rate of the syrup. In the syrup flow control circuits, induction flow meters COMACCAL FLOW 28 are used as a sensor (FE 14a, FIT 14b). Induction flow meters are used for instantaneous and total measurement of water and conductive fluids in filled pipelines. The principle of their operation is based on the phenomenon of electromagnetic induction. With the help of electrodes isolated from the pipe and recessed into a level with an insulating layer, the electromotive force is removed, which in the measuring unit is amplified and converted into a unified current signal of 0 – 5 mA. Measurement error ±1.5%. As secondary display devices (FIS 14c) selected KD140M manufactured by "LPZ Lviv Instrument-Making Plant". These devices are designed to work complete with non-interchangeable primary transducers (sensors) that convert the measured non-electric quantities (pressure, flow rate, level, vacuum) into an AC output signal (340 ± 30) mV (at a current of 250 mA) by 1 mm of movement of the sensor plunger. The signal goes to the controller (PLC) to the control unit (intersection with C), as well as to the human-machine interface (SCADA) in which the syrup flow value is displayed on the screen of the automated workplace of the operator (computer) (intersection with I). The resulting data is stored in memory (R). These data (the actual values of the syrup consumption) are used to conduct this experiment. If the syrup consumption value exceeds the set limits, then an alarm (A) is generated. The control signal output by the controller (AO) goes to the electropneumatic converter (LY 14e), which converts an analog unified electrical signal. In turn, the actuator (for example, 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 from the SCADA operator). To switch the "Manual/Automatic" mode (HS 14d, HC 14d), the

BRU-17 manual control units were used. It has one analog input with support for a unified signal of 0-5 mA, 0-20 mA or 0-10 V and one analog output with support for a unified signal of 0-5 mA, 0-20 mA or 0-10 V. Supports interfaces and protocols of the RS-485 and ModBus network. Modicon M340 is used as a controller. Modicon M340 is an industrial logic controller for machine manufacturers, small and medium-sized automation systems. Supports 4 MB of memory for saving programs and 256 KB for data storage. It is equipped with built-in communications such as the CANopen bus, supports TCP/IP Ethernet network, RTU serial interface, and ASCII character interface. This controller uses the BMX AMI 0810 input module and the BMX AMO 0410 output module The pneumatic saddle valves (14f) Danfoss VFG33, with a built-in throttle and an electro-pneumatic converter, were used as actuators (Hrama et al., 2019).

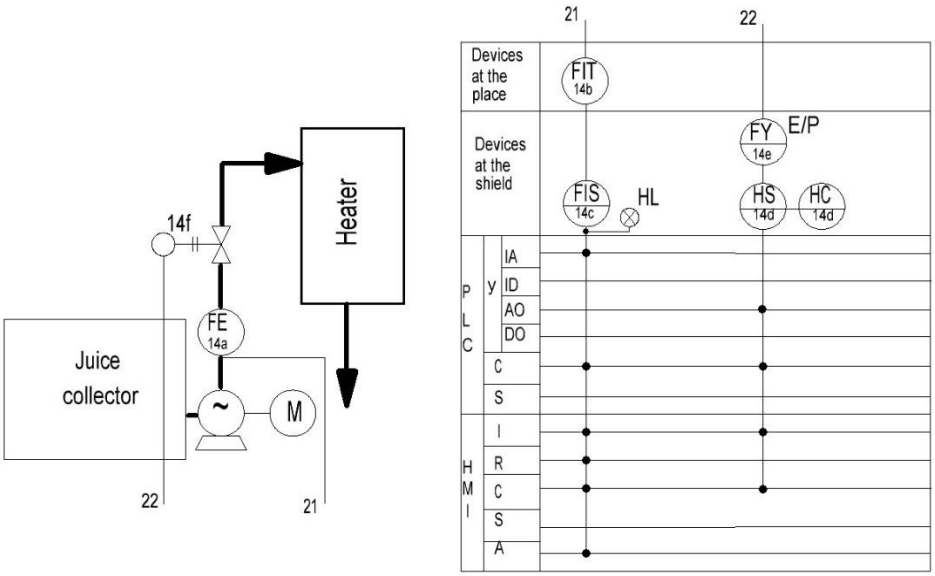

Figure. 1. Automation of the control circuit of the flow rate of the evaporation station syrup

Description of the methodology for developing a decision-making subsystem

In this paper, a method of calculating and forecasting user priorities for the development of a decision-making subsystem was chosen.

To work with this technique, it is necessary to obtain information about users (U), their requests (Q) and their requests for modification (D). Obtaining this data allows you to form time series of changes in priority estimates (1) (Piazzoni et al., 2020):

$$X = \{x_t, t = t', ...N\} \quad (1)$$

where t is the observation number, , $t \in (1, ...N)$

t' – the number of observation in which the user's request was first recorded,

x_t – the value of the priority of the user's request at the time of the start of iteration with the number t,

N – the number of iterations during which monitoring and prioritizationof user requests is carried out.

The result of the chosen methodology is to obtain recommendations P_j in the form of estimates of requests for changes in the human-machine interface (2) (Zgurovsky et al., 2018), ranked by the degree of importance:

$$P_j = F(x_{N+1,j}, B(X_j)), j = 1,...,L \quad (2)$$

where x_{N+1} is the projected priority score of the user's request,

j – user request identifier,

L – the number of user requests to make changes to the human-machine interface,

$B(X_j)$ – assessment of the trend of changing the priority of the user request, obtained by the time series X_j,

F – the procedure for integrating point predictive $x_{N+1,j}$ and temporal $B(X_j)$ estimates of the priority of user requests for changes to the human-machine interface.

This technique of prioritizing user requests to make changes to the human-machine interface consists of several of the following steps (Rajan et al., 2017):

1. Stage of data extraction and transformation. The provided stage is the removal of data from the database of records of user requests for changes. Converting these hits to a modification of the human-machine interface. At this stage, information is generated about users (U), their requests (Q) and their requests for modification (D). Next, a matrix of relations of user requests (R) is formed. After that, point and temporal estimates of the priorities of modification requests (X_j and $B(X_j)$) are calculated.
2. At the second stage, there is a modeling and forecasting of estimates of priorities for the time series X_j. First you need to build models of fuzzy time series using linguistic variables. Next, the forecasting of point estimates of priorities based on fuzzy models is carried out.
3. At the third stage, recommendations are formed for the decision-making subsystem. The basis for this is the linguistic summary of temporal and predictive estimates of the priorities of user requests.

For the correct development of the decision-making subsystem, it is necessary to collect data (Lin et al., 2019). Input data are the key characteristics of human-machine interface users and information about their actions. Data on the main characteristics of users can be recorded in the following form (3) (Talebi et al., 2019):

$$U = \{u_i, i = 1,...,M\} \quad (3)$$

where u_i is the key characteristic of the user; i – user ID; M – number of users.

Only those data that are necessary for the study and correspond to the declared period during which the data will be processed are uploaded from the database. To do this, the human-machine interface must contain the ability to identify users (Lin et al., 2008). The list of requests (Q) to make changes to the human-machine interface can be written as follows (Cao et al., 2020) (4):

$$\begin{aligned} Q &= \{q_k, k = 1,...,K\} \\ q_k &= \{i, j, t\} \end{aligned} \quad (4)$$

where q_k is a description of user requests for changes in the human-machine interface.

k – hit index,

K – number of hits,

i – user ID,

j – user hover identifier,

t – iteration number of the development.

It is also necessary to classify user appeals in order to separate changes to change the type of regulation (Talebi et al., 2019). The list of requests for changes to the human-machine interface can be written as follows (Lakhno et al., 2017) (5):

$$D = \{d_j, j = 1,...,L\} \quad (5)$$

where d_j – description of the user's request; j – user hover identifier; L – the number of user requests to make changes to the human-machine interface.

Another important parameter is the date and time when the user makes changes to the human-machine interface (Piazzoni et al., 2020). This parameter is necessary for the formation of time series of estimates of the priorities of requests for changes to the human-machine interface (Zgurovsky et al., 2018).

The priority of a human-machine interface change request can be calculated as follows (6) (Talebi et al., 2019):

$$R = \{r_{i,j}\}$$
$$r_{i,j} = \begin{cases} t', \text{ if } \{i,j,t\} \in Q, \\ 0 \end{cases} \quad (6)$$

where R is the relationship matrix, $r_{i,j}$ – iteration number of development t'.

Next, it is necessary to calculate the point and temporal estimates of the priorities of modification requests for each problem, which are received from users in the form of time series (Rajan et al., 2017). The calculation of these estimates allows you to display information on requests for changes in the human-machine interface in more detail (Piazzoni et al., 2020). After that, the user's priority score is calculated and a temporal assessment is formed, which is considered as a fuzzy trend (Talebi et al., 2019). To this end, it is necessary to develop a special algorithm for assessing the priority of user requests based on the data obtained according to the methodology described above (Zgurovsky et al., 2018).

On the basis of this method of developing a decision-making subsystem, the basic requirements for the functioning of the software for the operation of the evaporation station are formed (Piazzoni et al., 2020). Several iterations of software development were carried out. The development involved 20 users who made approximately 100 requests. The results of the survey after processing according to the algorithm (Figure 3) were entered in Table 4. Fragments of data received from users are given in Tables 1–3:

Table 1

Description of user requests for software modification

a	b
1	The possibility of changing the regulation regime.
2	Revision of regulation forecasting using different types of regulators.
3	Derivation of recommendations for changing the regulatory regime.
4	Display information on possible changes to the adjustment parameters of other control circuits.

where a is the user request ID, j. Set in random order (Piazzoni et al., 2020); b – description of user requests, d.

Table 2

Description of key user characteristics

a	b
1	Director
2	Engineer
3	Engineer
4	Operator
...	...
20	Operator

where a is the user request index, i. Set in order from the highest position to the lowest (Piazzoni et al., 2020); b – key characteristic of the user.

Table 3

Description of user requests

a	b	c
2	1	1
3	1	1
4	2	3
5	4	2
...	...	...
1	3	4

where a is the user request index, i; b – user request identifier, j; c is the iteration number of software development, t. Installed in the order of software development from the first iteration to the last (Piazzoni et al., 2020).

Description of forecasting by local trends

Forecasting the operation of an evaporation station using the method of local trends occurs using fuzzy time series models (Jolly et al., 2000).

To obtain a predictive local trend, a fuzzy time series model is generated (Lahtinen., 2001). For this purpose, a model (Figure 4) of a fuzzy dynamic process with a fuzzy increment is used, which looks like this: – a universal set for which fuzzy sets are defined $X_t(t=1,2,...) \subset R^1\ \tilde{x}_t^i,(i=1,2,...),\ \tilde{v}_t^j,(j=1,2,...),\ \tilde{a}_t^s,(s=1,2,...)$ (Dong et al., 2017).

Next, the parameter value of the first-order time series model is set (Lei et al., 2016) and the sum of the intensities of fuzzy elementary trends for each interval is calculated by creating an algorithm of fuzzy local trends for this case (Dong et al., 2017).

To predict the operation of an automated evaporation station, the following algorithm was used: first, it is necessary to convert the initial time series into a fuzzy time series. The next step is to convert the resulting fuzzy time series into a time series of fuzzy elementary tendency and dephase the intensity center of gravity method of each fuzzy elementary trend for each time series $a_t = DeFuzzy(\tilde{a}_t)$ (Anghinoni et al., 2019).

Analysis of the stability of the forecasting model is as follows. The automation system of the five-corps evaporation station is launched (Figure 1), after which the transient graphs

and projected values during the operation of the station are removed from the SCADA system (Dong et al., 2017). They are shown in Figure 6. Further, the graphs are divided into an arbitrary number of equal time intervals (González-Potes et al., 2016). Each time interval is separated from the next by a point with the name in Latin letter. The value to which the transient corresponds to at a given time is the actual value, and the value that corresponds to the graph with the projected values at a given time is the predicted value. All these values are recorded in Table 1. The values of the absolute and relative forecasting error for each point are also calculated and recorded in Table 1 (Dong et al., 2017).

The value of absolute error (A) is calculated by the formula (7).

$$A = \left| Z(t) - \tilde{Z}(t) \right| \tag{7}$$

where $Z(t)$ is the actual value of the time series,

$\tilde{Z}(t)$ – forecast value of the time series

The relative error value (V) for each time series point value is calculated using the following formula (8):

$$V = \frac{\left| Z(t) - \tilde{Z}(t) \right|}{Z(t)} \times 100\% \tag{8}$$

The next step is to assess the accuracy of the system. The automation system of the five-corps evaporation station is launched (Figure 1), the type of regulation is selected, after which the transient graphs and predicted values during the operation of the station are removed from the SCADA of the system. They are shown in Figure 6. Further, the graphs are divided into an arbitrary number of equal time intervals (González-Potes et al., 2016). Each time interval is separated from the next by a point with the name in Latin letter. The value to which the transient corresponds to at a given time is the actual value, and the value that corresponds to the graph with the projected values at a given time is the predicted value. All these values are recorded in Table 1. The values of absolute errors are calculated using the formula (7). The average error (SP) is calculated by the formula (9) (Dong et al., 2017):

$$SP = \frac{1}{n} \sum_{t=1}^{n} (Z(t) - \tilde{Z}(t)) \tag{9}$$

where SP is the average error of the forecast value of the time series,

n – the number of intervals of the time series, $Z(t)$ – the actual value of the time series,

$\tilde{Z}(t)$ – forecast value of the time series (Lei et al., 2016).

The average absolute error (SAP) is calculated by the formula (10).

$$SAP = \frac{1}{n} \sum_{t=1}^{n} \left| Z(t) - \tilde{Z}(t) \right| \tag{10}$$

The average relative forecasting error (SVP) is calculated by the formula (11) (Lei et al., 2016).

$$SVP = \frac{1}{n} \sum_{t=1}^{n} \frac{\left| Z(t) - \tilde{Z}(t) \right|}{Z(t)} \times 100\% \tag{11}$$

The accuracy of the forecasting model (T) is calculated by the formula (12)

$$T = 100\% - \frac{1}{n} \sum_{t=1}^{n} V \tag{12}$$

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

Results and discussion

Analysis and synthesis of control action using forecasting methods in the control system of the evaporation station

We propose to use a block diagram of regulation (Hrama et al., 2022), modifying it in such a way as to include the possibility of forecasting (Lei et al., 2016) and decision-making subsystems (Piazzoni et al., 2020).

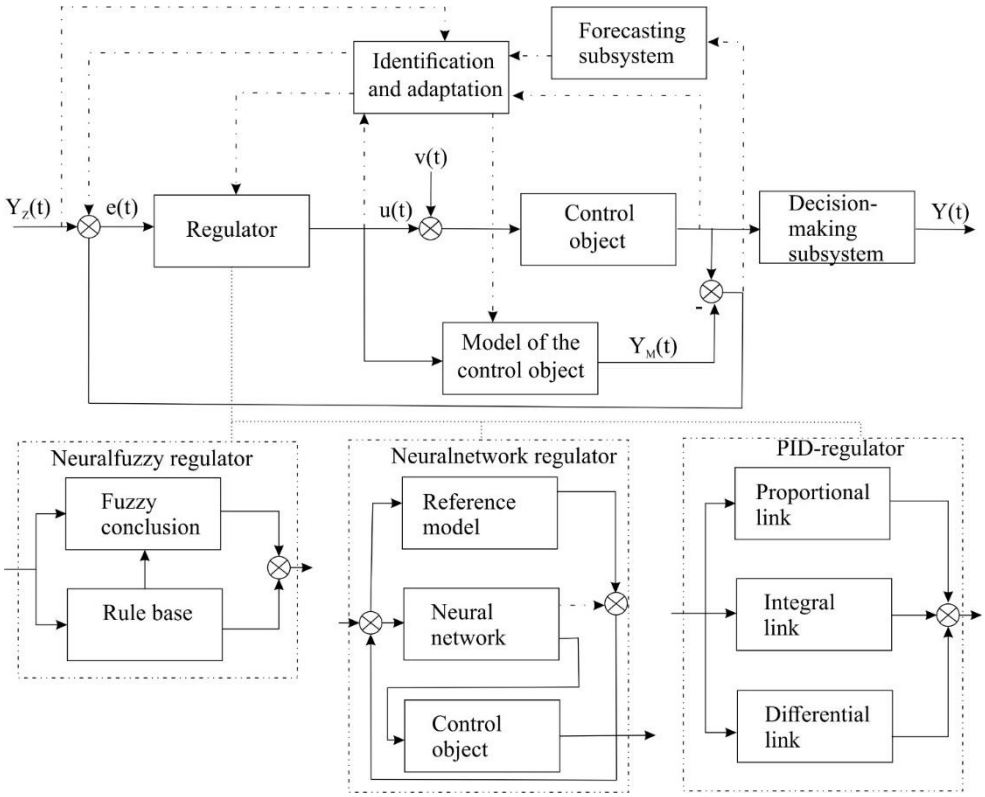

Figure. 2. Figure of the structural scheme of regulation

The block diagram of the regulation is shown in Figure 2, where $Y_z(t)$ is the signal of the task, e(t) is the disagreement between the task signal and the feedback, u(t) is the control signal, v(t) is the external perturbation, Y(t) is the output signal, $Y_M(t)$ is the output signal from the object model.

In more detail, the work of intelligent regulators, on the example of fuzzy ones, is discussed in the work (Tang et al., 2001). In this work, a fuzzy PID controller was researched as a discrete version of a conventional PID controller, so it retains the same structure, but has an independent adjustable control factor. It is proved that it is possible to improve the classic PID controller with a certain adaptive control ability. But this regulator cannot be considered a full-fledged neural fuzzy regulator. In addition, this paper also does not consider the use of

other types of intelligent regulators. A possible reason for this may be the costly part in conducting research. The problems of using neural obfuscive regulators are discussed in more detail in the work (Chantasiriwan, 2017). This work presents a new PID controller of fuzzy logic. This regulator is a fuzzy PID controller with a computational efficient analytical scheme. The author proves that the controller is stable with limited input / limited output However, this regulator is very difficult to implement, and this paper does not provide the possibility of using other types of intelligent regulators. In addition, there is no possibility of using this regulator for some adjustment parameters. Also, none of the above works has any justification for updating existing automation systems for the evaporation station. A possible reason for this may also be the costly part in conducting research. The use of neural sensors is considered in the work (Sidletskyi et al., 2019). The data says that one of the advanced methods of improving control systems is the addition of fuzzy and neural fuzzy logic. Methods of dynamic power control were analyzed using fuzzy logic and adaptive neural networks. One of the possible options for regulating power is the use of fuzzy conclusions (the so-called fuzzy system). The control action is formed by checking the consistency of fuzzy rules for the actual parameters of the system. Rules are created in accordance with the experience of the operator, which reflects his / her actions when changing technological parameters. But this work does not consider the use of neural regulators in the evaporation process. In addition, it also does not address other types of intellectual regulation.

Synthesis of the algorithm of the decision-making subsystem

To assess the priority of user requests to make changes to the human-machine interface and the formation of time series with their help, a special algorithm was developed (Figure 3).

The first step of this algorithm is to determine the iteration number of the development of a human-machine interface. To do this, determine the value of $t^{'}$, at which the value of the vector $\{r_i\}$ will be minimal, but not zero (Zgurovsky et al., 2018). A zero value means that users of a human-machine interface do not address a given function or problem (Rajan et al., 2017).

The next step is to determine the estimate of the priority x_t of some user request to make changes to the human-machine interface (Talebi et al., 2019). This calculation occurs according to the formula (6). In this algorithm, the intensities of fuzzy trends of various types are grouped, followed by the formation of linguistic rules of the main trend of time series. The implementation of this algorithm allows you to form a set of estimates of the priorities of requests. In this study the following estimates are used: "Increase in request priorities", "Decrease in request priorities", "Priorities remain stable", "Uncertainty of changes in priorities", "Fluctuations in changes in priorities", "Increase in request priorities with fluctuation", "Decrease in query priorities with fluctuation". If you apply this algorithm for each user request to make changes to the human-machine interface of the evaporation station and attribute each request to its corresponding time series, you can determine the linguistic term that carries information about changes in priorities (Rajan et al., 2017). This information allows you to form recommendations of the decision-making subsystem (Piazzoni et al., 2020).

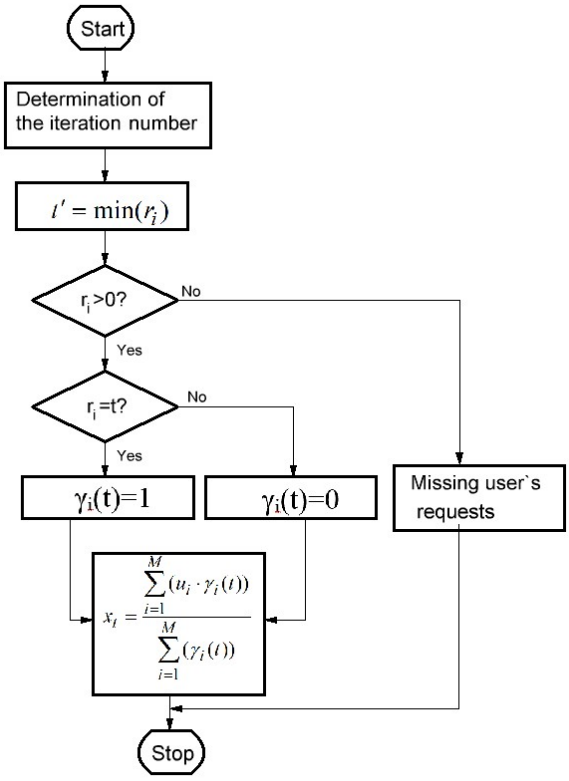

Figure. 3. Special algorithm for assessing the priority of user requests

The next step, according to formula (1), we form time series for changing priority estimates (Talebi et al., 2019). The following is the time series for displaying information about possible changes to the adjustment parameters of other control circuits (13):

$$X_1 = \{3,1;2,2;1,3;1,4\} \quad (13)$$

So, from the time series X_1 we can say that at the first observation, the function of displaying information about the possible changes to the parameters for regulating other control circuits among users had a third priority, but with further observations, the priority of this function increased and after the third observation took first place (Zgurovsky et al., 2018). From this we can conclude that the projected priority of users of this function will be high (Piazzoni et al., 2020).

Therefore, according to the formula (2), you can get recommendations P and in the form of ranked estimates of queries (Talebi et al., 2019). For the function of displaying information about the possible changes to the parameters of regulation of other control circuits, the following series has the form (14):

$$P_1 = F(1, \text{"Very important"}) \quad (14)$$

According to the formula (3), data on the main characteristics of users can be written in the following form (15):

$$U = \{\text{"director"},1;\ \text{"engineer"},2;\ \text{"engineer"},3;...;\text{"operator"},20\} \quad (15)$$

Next, the formula (4) creates a list of requests for changes to the human-machine interface:

$$q_1 = \{1, 5, 2\} \quad (16)$$

According to formula (5), it is necessary to classify user appeals in order to separate the introduction of changes to change the type of regulation (Lin et al., 2008):

$$D = \{\text{"change of regulation type"}, 5\} \quad (17)$$

Next, using the formulas (1) and (2), the relationship matrix is filled in to determine the priorities of the queries. The formula (6) calculates the determination of priority estimates (Piazzoni et al., 2020). The results are listed in Table 4.

Table 4

Determination of priority assessments

a	b	c	d	e	f
X_1	2	1	Important	Oscillation	Very important
X_2	3	2	Important	Height	Very important
X_3	4	3	Important	Height	Important
X_4	1	4	Important	Height	Very important

where a is the time series (1); b – user request identifier (table 1); c – assessment of the priority of the request (13); d – linguistic evaluation of the request (6); e – tendency to change the priority of the request (2); f – Recommendation for creating functional requirements (14).

According to Table 4, it can be concluded that the most important in the output of information by the decision-making subsystem is the function of displaying information about the possible changes to the parameters of regulation of other regulatory circuits, since it has the highest priority rating (Zgurovsky et al., 2018). Also very important is the availability of the function of reviewing the forecasting of regulation using different types of regulators (Rajan et al., 2017). Therefore, during the operation of the decision-making subsystem, these functions will have the highest priority.

Synthesis of the algorithm of local trends

We will develop an algorithm for local trends of the evaporation station. The paper (González-Potes et al., 2016) describes the management of several evaporation stations with full integration of fuzzy control and the use of wireless network sensors and actuators. But in this paper there is no comparison of the use of neural sensors with other types of intelligent regulation and there is no justification for the expediency or inexpediency of using this type of regulation in case of the possibility of introducing a system with another type intelligent control. In addition, neural fuzzy regulation in this study does not apply to all regulatory circuits. The reason for this may be the high complexity and cost of conducting such a study. The authors of the study also meet with similar problems (Tang et al., 2001). In this paper, the control of evaporator overheating using a fuzzy sliding mode controller is considered. In addition, this study does not disclose the use of fuzzy regulation for other circuits of regulation of the evaporation station.

It is necessary to improve the model of forecasting the operation of the evaporation station by the method of local tendency and the forecasting algorithm and determine the influence of the algorithm on the accuracy and stability of the obtained forecasting model.

To work with the algorithm of local trends, we set the following parameter dependencies (Lei et al., 2016):

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

Figure. 4. First-order time series model

Where Fuzzy is the operation of the phase of the scale, TTend is the operation to determine the type of difference, RTend is the operation to identify the intensity of the difference, Comp is the operation to calculate the new fuzzy assessment, DeFuzzy is the operation of defasification of the scale. $\tilde{f}_{\tilde{\upsilon}}, \tilde{f}_{\tilde{a}}$ – fuzzy dependencies are presented in the form of a composite implication rule, $x_{i+1}, \varepsilon_{i+1}$ – numerical estimate and error of the predicted level of the time series.

In this model, an absolute fuzzy estimate $\tilde{x}_i$ is determined by phasifying the scale according to the value of the object being evaluated x_i. Next, an operation takes place to determine the type of differences and the next step is the process of determining the intensity of the differences. After that, a new absolute fuzzy estimate is calculated (Xu et al., 2020). The final step is the defasification of the scale according to the definition of the object x_i being evaluated according to an absolute fuzzy estimate $\tilde{x}_i$.

A two-stage algorithm for selecting a time series forecasting model has been developed (Dong et al., 2017). It is calculated the sum of the intensities of fuzzy elementary trends for each interval (Figure 5).

Using the algorithm (Figure 5), it is possible to evaluate local trends using linguistic and numerical forms (Anghinoni et al., 2019). To work this algorithm, it is necessary to convert the initial time series to a fuzzy time series (Mehmood et al., 2021) using the model shown in Figure 1. The next step in the implementation of this algorithm is to divide the resulting time series into a certain number of intervals. At each interval, the sum of the intensities of the same type of fuzzy elementary trends is calculated. Further, comparing the time intervals with growth (ST_{up}) and decrease (ST_{down}) of the time intervals of the length of a fuzzy trend, the type of local trend ("Stable", "Growing", etc.) is chosen (Xu et al., 2020).

This algorithm does not require additional interpretation by the user. The disadvantage of this algorithm is the limitation of its operation by the number of predetermined time intervals, which is why the number of identified local trends will be equal to the number of intervals specified by the developer (Anghinoni et al., 2019). This algorithm allows you to get time series, which can be used in the future to predict local trends. 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 by a fuzzy time series (Dong et al., 2017).

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

Figure. 5. Algorithm of local trends for research
P – the finite set of points on the interval n (the finite set of tendencies);
ST – the time interval of the length of a fuzzy trend.

Result of the syrup flow forecasting algorithm with the decision-making subsystem

The result of the algorithm execution (Figure 5.) Forecasting of an automated syrup consumption system using intelligent regulation and a decision-making subsystem is shown in Figure 5. Forecasting of an automated syrup consumption system using intelligent regulation and a decision-making subsystem is shown in Figure 5. Forecasting of an automated syrup consumption system using intelligent regulation 6. Table 5 shows the results of calculations for the consumption of syrup using PID, neural fuzzy, and neural network regulators. Based on the results presented in the table, we can conclude that, since the value of SP is negative, the forecast was overestimated relative to the actual data (Lei et al., 2016). This is true, since the forecast shows a small absolute error of 1% when using neurone-fuzzy regulation. And with the actual use of this type of regulation, it is absent. However, this overestimation is insignificant, as can be seen from the indicator of the average relative error of forecasting (Dong et al., 2017).

In theory, when applying the average relative error in assessing the accuracy of the model for predicting the evaporation process, the value of forecast accuracy can reach 100% (Lei et al., 2016). This will mean that the selected forecasting model describes the process with absolute accuracy (Anghinoni et al., 2019). In practice, such a phenomenon is almost impossible, since the forecast cannot take into account absolutely all the factors that affect the automation system (Xu et al., 2020). In the case when the value of forecast accuracy approaches 0%, then this model does not describe the predicted process at all (Butt et al., 2020).

Table 5

Indicators for assessing the error of forecasting the consumption of syrup

№	a	b	c	d	e	f	g
PID regulator							
1	A	0	0	0	-0,8	0,8	2
2	B	222	223	1			
3	C	217	215	2			
4	D	215	214	1			
5	E	215	214	1			
6	F	215	214	1			
7	G	215	214	1			
Accuracy of the forecasting model (12):						98%	
Neural obscure regulator							
1	A	0	0	0	-0,9	0,9	5
2	B	214	216	2			
3	C	215	214	2			
4	D	215	214	2			
5	E	215	214	1			
6	F	215	214	1			
7	G	215	214	1			
Accuracy of the forecasting model (12):						95%	
Neural network regulator							
1	A	0	0	0	-0,9	1,27	4
2	B	217	216	1			
3	C	215	214	1			
4	D	215	214	1			
5	E	215	214	1			
6	F	215	214	1			
7	G	215	214	1			
Accuracy of the forecasting model (12):						96%	

where a – Point name, b – Actual value, m^3/h, c – Predicted value, m^3/h, d – Absolute error, %, e – mean error (SP) (9), f – mean absolute error (SAP) (10), g – average relative forecasting error (SVP) (11).

The forecast accuracy indicator is also used to select the optimal forecasting model. The optimal model is the model whose accuracy is closest to 100% (Lei et al., 2016), since it is more likely to make a more accurate forecast.

Since, in our case, the value of the average relative error is 5%, it follows that the accuracy of the model is 95%, which is a very high assessment of the quality of our forecasting system. Since the accuracy of the forecasting model is very close to 100%, it can be considered optimal (Dong et al., 2017).

In order to correctly understand how much, you can trust the obtained algorithm for predicting the evaporation process, it is also necessary to assess the accuracy of the forecast obtained (Lei et al., 2016). In Figure 4 shows a comparison of the predicted value of the change in the flow rate of syrup using PID, neurone-fuzzy and neural network regulators and the actual change in syrup consumption.

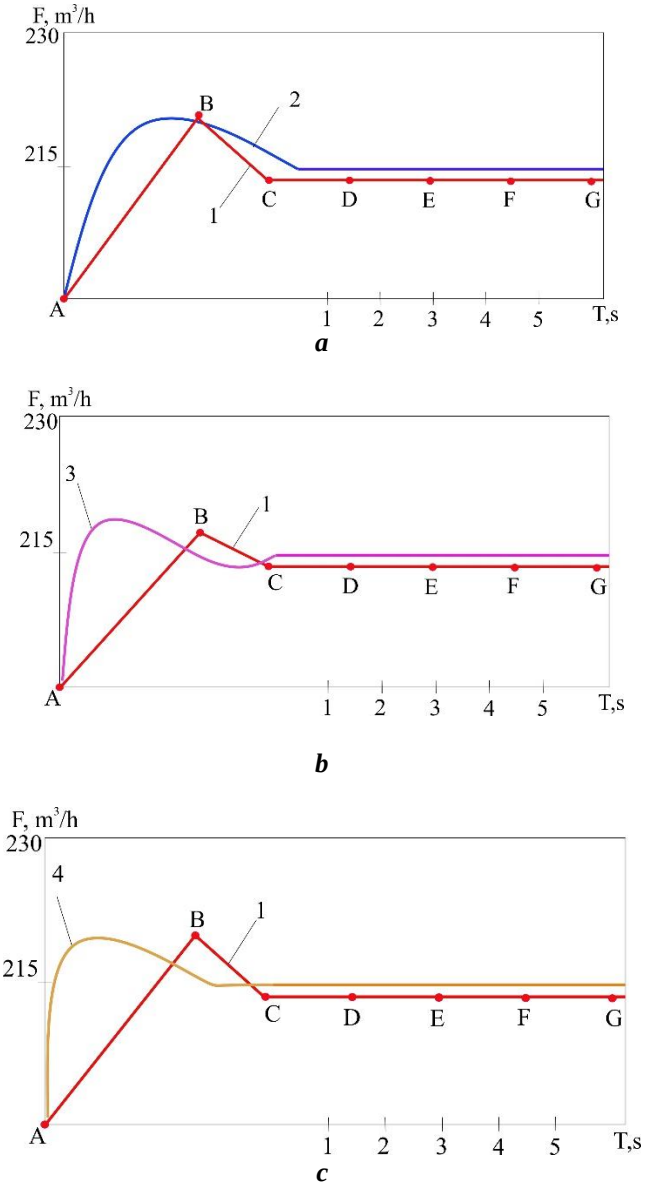

Figure 6. Comparison of the transients of the predicted and actual consumption of the syrup in the first building of the evaporation station using:

a – PID;

b – neurone-fuzzy;

c – neural network regulators;

1 (—) – the projected value of the syrup consumption;

2 (—) – the actual value of the syrup consumption (PID-regulator);

3 (—) – the actual value of the syrup consumption (neurone-defunt regulator);

4 (—) – actual value of syrup consumption (neural network regulator);

AB, BC, CD, ..., JK – time series intervals.

In this study, a forecasting method was used to compare methods for regulating the flow rate of syrup in the apparatus, which makes 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 and, thus, increase the efficiency of the evaporation station. The advantage of this method is its easy and quick implementation, which does not require large economic and energy costs. The disadvantage of this method is the need to break the transient process into separate time intervals of the number series manually and the direct dependence of the accuracy of the model on the number of elements of the time series.

In other studies, most of the problems of intelligent control in the process of evaporation remain unresolved. The use of neural regulators occurs only in certain specific cases. In addition, there is no comparison of the use of intelligent regulators with classic ones. There is also no coverage of the possibility of combining the work of several types of intelligent regulators if necessary. In addition, there are no clear means of forecasting the work of intelligent regulators and decision-making subsystems.

Conclusions

1. After researching a large number of sources, it was concluded that in other studies, most of the problems of intelligent control in the evaporation process remain unresolved. The use of neural regulators occurs only in certain specific cases. Therefore, a model for predicting the operation of an evaporation station by the method of local tendency was built and a forecasting algorithm and an algorithm for the decision-making subsystem were developed.
2. An algorithm for assessing the priority of user requests according to the method of calculating and forecasting priorities was developed. The result of this algorithm was the determination of priority estimates, which showed that the most important in the output of information by the decision-making subsystem is the function of displaying information about the possible changes to the parameters of regulation of other regulatory circuits, since it has the highest priority rating.
3. A model of forecasting the operation of an evaporation station by the method of local tendency is constructed and a forecasting algorithm has been developed. The accuracy of the obtained forecasting model is also evaluated. The accuracy of the forecasting model was 98% for the PID controller, 95% for the neural non-fuzzy regulator and 96% for the neural network, which are high rates The advantage of this model is its high accuracy in general, but the disadvantage is that during the occurrence of oscillations in the transition process, there is an insignificant delay in predicting these fluctuations.
4. Statistical data of the behavior of the contours of the automation system in different modes of operation using intelligent and classical regulators were collected, a model for predicting the operation of an evaporation station by the method of local tendency was built and a forecasting algorithm was developed. The advantage of this method is its easy and quick implementation, which does not require large economic and energy costs. The disadvantage of this method is the need to break the transient process into separate time intervals of the number series manually and the direct dependence of the accuracy of the model on the number of elements of the time series.

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Анотації

Харчові технології

Вплив рисового борошна на конформаційні перетворення в тісті при виробництві пшеничного хліба

Анастасія Шевченко, Світлана Літвинчук
Національний університет технологій, Київ, Україна

Вступ. Метою роботи було визначення впливу рисового борошна на конформаційні перетворення в структурі тіста для виготовлення пшеничного хліба, до складу якого внесено лецитин.

Матеріали і методи. Було досліджено рисове борошно, його хімічний склад та фракційний склад білків. Конформаційні перетворення структурних елементів в тісті та хлібі були досліджені методом інфрачервоної спектроскопії у ближній інфрачервоній області.

Результати і обговорення. У рисовому борошні загальний вміст білків на 47% нижчий, ніж у пшеничному борошні, вміст харчових волокон у 8,5 разів нижчий. Фракційний склад білків показав вищий вміст альбуміну, проламіну та нерозчинних білків в пшеничному борошні, ніж в рисовому на 11, 90 та 75% відповідно. За вмістом глобулінів та глютелінів переважає борошно рисове на 7 та 183% відповідно, однак склад глютелінів у досліджуваних зразках різний. У пшеничному борошні це глютенін, який є клейковинним білком, утворює гетерогенну суміш полімерів через дисульфідні зв'язки поліпептидів. У рисовому борошні представником глютелінів є орізенін. Інфрачервоні спектри відбивання пшеничного та рисового борошна показали подібний характер спектрів: екстремуми спостерігаються на однакових довжинах хвиль, спектри між собою розташовані паралельно та відрізняються лише за інтенсивністю відбивання. Спектр лецитину соняшникового значно відрізняється через відмінний хімічний склад. Також на деяких довжинах хвиль на спектрі лецитину помітні зміщення екстремумів як в коротко-, так і довгохвильову область. Вторинна структура глютену зазнала змін в хлібі після впливу температури шляхом просування α-спіралей і β-поворотів і сприяла утворенню дисульфідних зв'язків.

Висновки. Проведені дослідження свідчать про доцільність застосування рисового борошна в технології хлібобулочних виробів на заміну пшеничного з метою мінімізувати вміст клітковини в хлібі.

Ключові слова: *хліб, рис, борошно, лецитин, ІЧ спектроскопія.*

Зменшення вмісту акриламіду в формованих картопляних чіпсах підвищеної харчової цінності

Аліна Ковтун, Володимир Ковбаса, Олег Бортнічук,
Олександр Шевченко, Олександр Дуборєзов
Національний університет харчових технологій, Київ, Україна

Вступ. Метою дослідження є визначення оптимальних температурних режимів випікання-висушування картопляного тіста для зменшення акриламіду в формованих картопляних чіпсах з підвищеною харчовою цінністю.

Матеріали і методи. В якості досліджуваної сировини обрано: картопляну крупку, висівки жита, ячменю, жмих гарбузового насіння, кріопорошки броколі та червоного буряка. Визначали кількість аспарагінової кислоти, редукувальних цукрів в основній та додатковій сировині, а також кількість утвореного акриламіду в процесі температурного оброблення тістової картопляної маси.

Результати і обговорення. Для підвищення харчової цінності формованих картопляних чіпсів розширено традиційну сировинну базу за рахунок застосування різних висівок зернових, жмиху, харчових волокон, порошків овочів, тощо. Рекомендовані оптимальні параметри оброблення картопляного тіста при температурі 125°С та тривалості 4,5 хв без застосування рослинних олій на відміну від традиційних способів виробництва формованих картопляних чіпсів. Визначено кількість аспарагінової кислоти – 190,5 мг/г білка і редукувальних цукрів – 0,6 % в картопляній крупці. У висівках жита та ячменю кількість аспарагінової кислоти 77,5 та 72,6 мг/г білка відповідно, у жмиху гарбузового насіння – 80,5 мг/г білка, у кріопорошках броколі та червоного буряка – 72,5 та72,9 мг/г білка відповідно. Масова частка редукувальних цукрів у висівках жита та ячменю складає 0,74 та 0,8 %, у жмиху гарбузового насіння – 0,5 %, у кріопорошках броколі та червоного буряка – 0,3 та0,5 %відповідно. Отримані кінцеві продукти, в яких не виявлено акриламіду завдяки зміни класичної технології і параметрів виробництва формованих картопляних чіпсів. Досліджено, що при застосуванні класичної технології формованих картопляних чіпсів кількість акриламіду у готових виробах становила 61 мкг в 100 г продукту. В формованих картопляних чіпсах без додавання висівок, жмиху та кріопорошків, які випікалися-висушувалися,кількість акриламіду була 9,35 мкг в 100 г продукту.

Висновки. Утворення акриламіду залежить від хімічного складу сировини, тривалості та температури випікання-висушування та технології виробництва формованих картопляних чіпсів.

Ключові слова: *картопляна крупка, чіпси, висівки, кріопорошок, акриламід.*

Вплив знакозмінних імпульсів тиску на сенсорні характеристики в бродильній технології

Ірина Дубовкіна
Інститут технічної теплофізики Національної академії наук України, Київ, Україна

Вступ. Метою наукової роботи є дослідження впливу знакозмінних імпульсів тиску в харчових виробництвах під час одержання дослідних зразків вина, кріпленого вина та асоційованих водних систем і розчинів на сенсорні характеристики та дегустаційне оцінювання.

Матеріали та методи. Було виконано аналіз зміни фізико-хімічних параметрів зразків вина, кріпленого вина та асоційованих водних систем і розчинів під час оброблення із застосуванням знакозмінних імпульсів тиску з використанням різних технологічних режимів. В роботі використані загальнонаукові та спеціальні методи досліджень, а саме електрохімічні методи. Окрім цього в роботі використаний метод сенсорного аналізу зразків вина, кріпленого вина та асоційованих водних систем і розчинів.

Результати і обговорення. В результаті застосування знакозмінних імпульсів тиску під час одержання кріпленого вина, загальний дегустаційний бал підвищився на 7,3%, у порівнянні з контрольними зразками, що є досить вагомим показником якості готового продукту. Під час проведення оброблення знакозмінними імпульсами тиску варіювалось число кавітації від 0.1 до 0.5, що дозволило одержати під час дегустаційного оцінювання найвищий загальний бал 8.8. Обгрунтовано технологію одержання кріпленого вина, що полягає у дробленні винограду, гребеневідділенні, настоюванні сусла на м'яззі, пресування, зброджування сусла, купажування, спиртування. Спиртування вина проводять із застосуванням знакозмінних імпульсів тиску в умовах гідродинамічної кавітації з числом кавітації 0,3, швидкістю зсуву потоку $2{,}6 \cdot 105 c^{-1}$ та напруженням зсуву потоку 260 Па.

Висновки. Загальний бал зразків вина та кріпленого вина, які були одержані в умовах знакозмінних імпульсів тиску, мав підвищені показники якості у порівнянні з контрольними зразками. Це позитивним чином впливає на якість готового продукту.

Ключові слова: *випробування, аналіз, рідкий, оброблення, тиск.*

Біотехнологія, мікробіологія

Вплив наночастинок подвійного дво- та тривалентного оксиду заліза на бактеріостатичні властивості насіння льону

Микола Рябчиков[1], Ірина Цихановська[2],
Олександр Александров[2], Дєніс Ковильов[2]
1 – Луцький національний технічний університет, Луцьк, Україна,
2 – Українська інженерно-педагогічна академія, Харків, Україна

Вступ. Вивчено вплив наночастинок подвійного дво- та тривалентного оксиду заліза (НЧ $FeO \times Fe_2O_3$ – наномагнетиту) на бактеріостатичні (захисні) властивості насіння льону проти грибкових інфекцій та встановлено залежність бактеріостатичних властивостей від кількості наномагнетиту (НЧ $FeO \times Fe_2O_3$).

Матеріали і методи. Мікросопічне **в**изначення морфологічних та культуральних особливостей мікроміцетів (культури дріжджів *Saccharomyces cerevisiae* та міцеліальних грибів *Mucor racemosus*) на агаризованому живильному середовищі. Дослідні зразки мікроміцетів одержували шляхом посіву стандартного мікробного препарату у вигляді суспензії (вихідне *розведення мікробної суспензії* 1:100) в чашки Петрі (чашковий метод).

Результати і обговорення. Відзначено здатність наночастинок подвійного дво- та тривалентного оксиду заліза (НЧ $FeO \times Fe_2O_3$ – наномагнетиту) сприяти покращенню бактеріостатичних (захисних) властивості насіння льону: додавання 0,1%; 0,15%; 0,2% наномагнетиту в (8–20 разів) пригнічує розвиток мікрофлори (мікроміцетів) у зразках насіння льону.

Встановлено зменшення (порівняно з контролем): кількості в (8–10) разів та розміру в (10–20) разів колоній мікроміцетів (дріжджів *Saccharomyces cerevisiae* та міцеліальних грибів *Mucor racemosus*). Визначено раціональний вміст наночастинок подвійного дво- та тривалентного оксиду заліза (НЧ $FeO \times Fe_2O_3$ – наномагнетиту) – 0,15% від маси рецептурної суміші.

Запропонована математична модель дозволяє прогнозувати ефективність використання НЧ $FeO \times Fe_2O_3$ – наномагнетиту в пригніченні росту міцеліальних грибів (мікроміцетів) для забезпечення бактеріостатичних властивостей сировинних інгредієнтів, зокрема насіння льону.

Висновок. Вперше досліджено вплив наночастинок подвійного дво- та тривалентного оксиду заліза (НЧ $FeO \times Fe_2O_3$ – наномагнетиту) на бактеріостатичні (захисні) властивості насіння льону.

Ключові слова: *насіння, льон, наночастинка, $FeO \times Fe_2O_3$, наномагнетит, бактеріостатичний.*

Процеси, обладнання і системи контролю

Автоматизовані методи керування витрати сиропу у випарному апараті з підсистемами підтримки прийняття рішень та прогнозування

Михайло Грама, Віктор Сідлецький
Національний університет харчових технологій, Київ, Україна

Вступ. Мета представленого дослідження – обґрунтування методів регулювання витрати сиропу у випарному апараті з підсистемою прогнозування, що дозволить спрогнозувати поведінку системи та підсистемою прийняття рішень, яка дозволить знизити вплив людського фактору на перебіг процесу випарювання.

Матеріали і методи. Досліджується робота випарної установки з підсистемою прогнозування та підтримки прийняття рішень при регулюванні витрати сиропу. В схемі автоматизації регулювання витрати сиропу в якості датчика використовуються індукційні витратоміри. В якості виконавчих механізмів використано пневматичні сідельні клапани, з вбудованим дроселем та електро-пневмоперетворювачем.

Результати і обговорення. Використання нейронечітких регуляторів відбувається лише в окремих специфічних випадках інтелектуального керування процесу випарювання, відсутні дані порівняння застосування інтелектуальних регуляторів з класичними, можливості комбінування роботи кількох типів інтелектуальних регуляторів, а також чітких засобів прогнозування їх роботи та підтримки прийняття рішень. Тому у даній роботі обгрунтовано підсистему прийняття рішень, яка дозволила оцінити пріоритети запитів користувачів при використанні людино-машинного інтерфейсу. Найвищий пріоритет отримав запит на виведення інформації про можливе внесення змін до параметрів регулювання інших контурів регулювання. Також було використано метод прогнозування для порівняння методів регулювання витрати сиропу в апараті, що дозволило спрогнозувати поведінку системи при формуванні управляючого діяння та вивести готовий прогноз на екран оператора та, таким чином, підвищити ефективність роботи випарної станції. Було зібрано статистичні дані поведінки контурів системи автоматизації у різних режимах роботи з використанням інтелектуальних та класичних регуляторів і побудовано модель прогнозування роботи випарної станції методом локальної тенденції та розроблено алгоритм прогнозування. Також оцінено точність отриманої моделі прогнозування. Точність моделі прогнозування склала 98% для ПІД-регулятора, 95% для нейронечіткого регулятора та 96% для нейромережевого.

Висновки. Модель для прогнозування роботи випарної станції характеризується високою точністю в цілому, але під час виникнення коливань у перехідному процесі виникає несуттєве запізнення прогнозування цих коливань. Найбільш важливою при виведенні інформації підсистемою прийняття рішень є функція виведення інформації про можливе внесення змін до параметрів регулювання інших контурів регулювання.

Ключові слова: *випарювання, витрата, сироп, система, прогнозування.*

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харчових технологій
Вул. Володимирська, 68
Київ
01601
Україна

E-mail:

Ukrfoodscience@meta.ua

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