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Methanol content in grape and fruit brandies: a major indicator for authenticity and safety

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Abstract

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Introduction. The methanol is constantly present compound in the brandies, regardless of fruit raw material from which they are produced. Its presence determines the authenticity of brandies, and its concentration levels are an indicator of the level of safety for consumption.

Materials and methods. The alcoholic content of ten different brandy samples – six grapes and four plums was determined by using of the automatic distillation unit Gibertiny BEE RV 10326. The content of methanol in brandies was evaluate by gas chromatograph Varian 3900 with a capillary column VF max MS (30 m, 0,25 mm ID, DF = 0,25 μ m), equipped with FID. The statistical analysis of the data was carried out by the standard deviation determining.

Results and discussion. The obtained data for the alcoholic content of grape brandies showed variation from 36.00 to 69.98 vol.% (Average 52.70 vol.%). In plum brandies this variation was in the range from 40.00 to 62.70 vol.% (Average 46.27 vol.%). Methanol was identified in all analyzed brandy samples. In the grape brandies it ranged from 0,20–0,56 g/dm³, while in the plum brandies this variation was in range 1,08–2.98 g/dm³.

The first three samples grape brandies showed lower levels of methanol, which is explained by the use of distillation unit with additional purification column and condenser. This leads to a better purification of methanol, in comparison to the other three samples grape brandies, which are distilled in ordinary still.

Established higher levels of methanol in plum brandies, compared with grape brandies, are normal trend. The reason for this is the higher content of pectin in the plum fruit. The pectin is a precursor of the methanol. The higher pectin levels normally lead to the formation of a higher amount methanol in the final product.

The identified concentrations of methanol in this study were within the normative values for the presence of this alcohol (maximum level to 10,00 g/dm³), documented in the Bulgarian and European legislation.

Conclusions. The identification of methanol in all tested brandies confirmed their authenticity. All established methanol concentrations in brandies meet the Bulgarian and European legislation. The studied brandies are safe for consumption.

Introduction

Brandies are alcoholic drinks produced by the method of distillation of fermented fruit pastes, fruit juices, or by-products of wine production [1]. Typical indicator of their authenticity is the presence of alcohol fermentation products.

The methanol is constantly present component of brandies, regardless of the raw material from which they are produced [2]. Its presence is due to the concentration level of its precursor (pectin) in fruits, the degree of the carboxyl groups methoxylation of the pectin, the degree of rotting of the fruit and the concentration and activity of fruit enzyme systems [1]. The presence of methanol in the brandies is indicator of their authenticity and safety for consumption. The methanol is formed on the base of several enzymatic transformations following order: initial decomposition of insoluble protopectin to soluble pectin under the action of the enzyme protopectinase; subsequent decomposition of the soluble pectin to polygalacturonic acid and methanol under the action of the pectinesterase enzyme [1, 3, 4, 5]. The toxic effect which possess methanol on the human body is associated with ingesting it at high concentration and due to its highly toxic end metabolic products – formaldehyde and formic acid [6, 7, 8, 9]. Poisoning with methanol can cause metabolic disorders, blindness, neurological dysfunction, surrogate toxicity and death [10, 11]. This requires constant and regulated control of its presence in alcoholic beverages and compliance to defined legally-regulated limits of its presence in alcoholic beverages.

Velkov [4] claimed that the concentration of methanol in grape brandies ranges from 0.40–2.00 g/dm³, for plum brandies (manufactured by qualitative, technological mature and healthy material) this range is within 2.00–5.00 g/dm³, while in the plum brandies obtained after the fermentation of partially rotten (unhealthy) plums, these levels rise and fall in the range of 4.00–10.00 g/dm³. According to Marinov [1], the methanol in grape brandies made from quality material is moving in the range 1.60–1.80 g/dm³ and must not exceed 2.00 g/dm³. In fruit brandies produced from healthy fruits, the methanol content varied in higher concentration range – 2.00–6.00 g/dm³, while when using of rotten fruit material, it rises and falls in the range of 4.00–12.00 g / dm³.

The requirements of the Bulgarian legislation on the composition of various types brandies produced in the territory of Bulgaria are defined in the Law on wine and alcoholic beverages (Law on Wine and Alcohol Beverages, Bulgaria, 2014). It confirms the following maximum levels of methanol in different groups brandies: wine brandy – a minimum alcohol content – 37.5 vol.%, the maximum allowed content of methanol – 200 g/hl a.a. (2 g/dm³); grape brandy – a minimum alcohol content – 40 vol.%, the maximum allowed content of methanol – 1000 g/hl a.a. (10 g/dm³); fruit brandy – the minimum alcohol content – 37.5 vol.%, the maximum permitted methanol content – 1000 g/hl a.a. (10 g/dm³).

The European legislation (Regulation № 110/2008 of the European Parliament and Council) [13] determines the maximum methanol content of fruit brandies to 1000 g/hl a.a. (10 g/dm³), but a slightly higher methanol levels for various fruit brandies are eligible. For brandies made from plums, apples, pears, raspberries, blueberries, apricots and peaches are allowed methanol concentrations to 1200 g/hl a.a. (12 g/dm³). For brandies made from currants, blackberries, elderberries, quince and juniper are allowed methanol content to 1350 g/hl a.a. (13.50 g/dm³).

The aim of this study is to examine the methanol content of various grape and fruit brandies and to evaluate their authenticity and safety.

Materials and methods

Origin of samples. Ten different brandy samples were provided for analysis: six grape brandies and four plum brandies. Samples were produced in the region of Pleven and Lovech, Bulgaria.

The first three grape brandy samples were obtained after distillation in still equipped with additional purification column, followed by condenser. The other three grape brandy samples were obtained after distillation in ordinary still. Plum brandies were obtained after distillation of fermented raw material in ordinary still too.

Determination of alcohol content. The alcohol content of the tested drinks was defined by specialized equipment with high precision – automatic distillation unit – Gibertiny BEE RV 10326 (Gibertiny Electronics Srl., Milano, Italy) and Gibertiny Densi Mat CE AM 148 (Gibertiny Electronics Srl., Milano, Italy).

Determination of methanol in brandies by GC-FID. The methanol content was determined by Method IS 3752:2005 (Indian Standard 3752:2005. Alcohol Drinks – Methods of Test) by preparing a standard solution. An amount of 1,0 g of methanol (purity 99.9%, Merck, Germany) was diluted to 100 ml with 40% ethanol solution. 10 ml of this solution was diluted to 100 ml with 40% ethanol solution. From this stock solution was prepared the standard methanol solution by adding 5 ml of the diluted solution in the 10 ml test tube, and adding 1 ml of the previously prepared solution of octanol (internal standard). The 2 µl of resulting standard solution of methanol and octanol was injected in gas chromatograph Varian 3900 with a capillary column VF max MS (30 m, 0,25 mm ID, DF = 0,25 µm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was generated and supplied to the chromatograph via a hydrogen generator (Parker Chroma Gas: Gas Generator 9200). The injection is manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220 °C; detector temperature – 250 °C, initial temperature of the oven – 35 °C/retention 1 min, rise to 55° C with step of 2 °C/min for 11 min, rise to 230 °C with step of 15 °C/min for 3 min. Total time of chromatography analysis – 25,67 min.

Identification of methanol and octanol in the standard solution is shown in the chromatogram – figure 1.

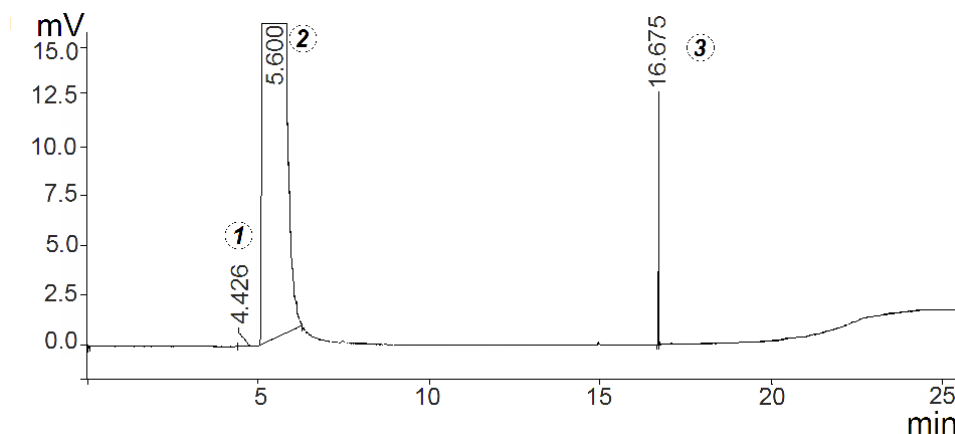


Fig. 1. Chromatogram of standard solution (methanol and octanol (IS)):
 1 – methanol (retention time = 4.426 min); 2 – ethanol (solvent);
 3 – octanol (internal standard; retention time = 16.675 min)

After determination of the retention times of methanol and octanol, we proceed to the identification and quantification of the methanol in the brandy samples. 5 ml of each brandy sample and 1 ml of internal standard solution (octanol) were placed in 10 ml test tubes with a stopper. Prepared samples were injected in an amount of 2 μ l in a gas chromatograph and was carried out an identification and quantification of the methanol content in each of them.

Statistical analysis. The statistical analysis of the data was carried out by determining the standard deviation (SD), with triple repetition of the analyses. It is performed with the Excel 2007 software application of the Microsoft Office 2007 suite (Microsoft Corporation, USA).

Results and discussion

The obtained data for the alcoholic content of the studied grape and fruit brandies were presented in Table 1.

The ethanol content (vol.%) in grape brandies varied in the range of 36.00 vol.% – 69.98 vol.% (Average 52.70 vol.%).

Variation of ethanol in plum brandies was in the range of 40.00 vol.% – 62.70 vol.% (Average 46.27 vol.%). These indicators were brought to standard alcoholic content for both group brandies – grape and fruit, regulated by the Bulgarian and European legislation (Law for Wine and alcoholic drinks, 2004; Regulation №110 / 2008 of the EU).

The chromatographic profiles of the analyzed grape brandies were presented in Figures 2-7.

Alcoholic content of the analyzed grape and fruit brandies

Table 1

Brandy sample	Content of ethanol, vol.%
Grape brandy 1	68.70
Grape brandy 2	69.98
Grape brandy 3	45.62
Grape brandy 4	40.20
Grape brandy 5	36.00
Grape brandy 6	55.70
Plum brandy 1	40.00
Plum brandy 2	62.70
Plum brandy 3	36.70
Plum brandy 4	45.68

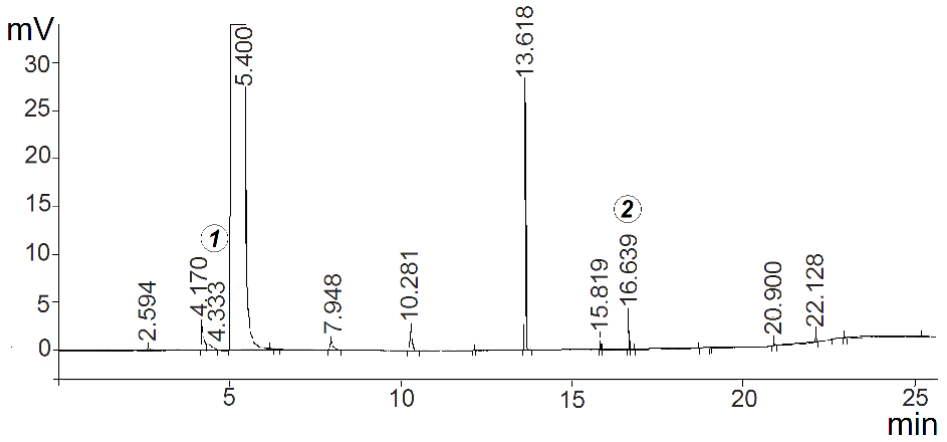


Fig. 2. Chromatographic profile of grape brandy 1:
1- methanol (retention time = 4.333 min);
2 – octanol (internal standard; retention time = 16.639 min)

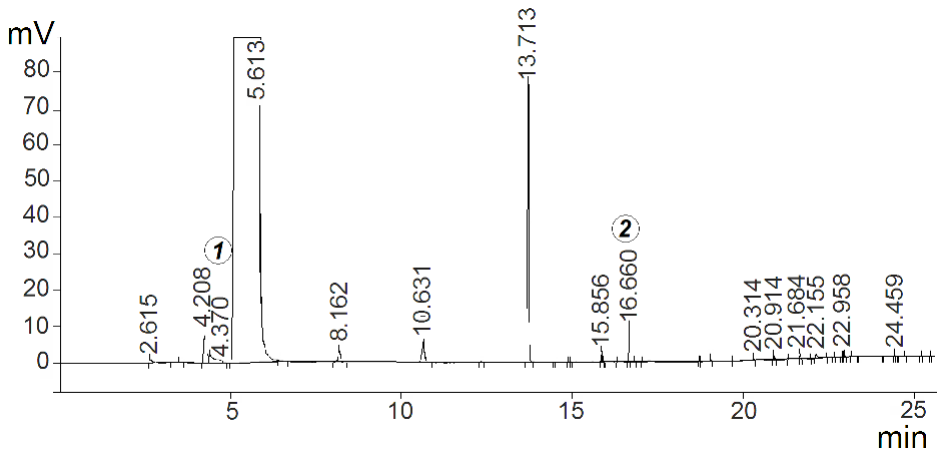


Fig. 3. Chromatographic profile of grape brandy 2:
1- methanol (retention time = 4.370 min);
2 – octanol (internal standard; retention time = 16.660 min)

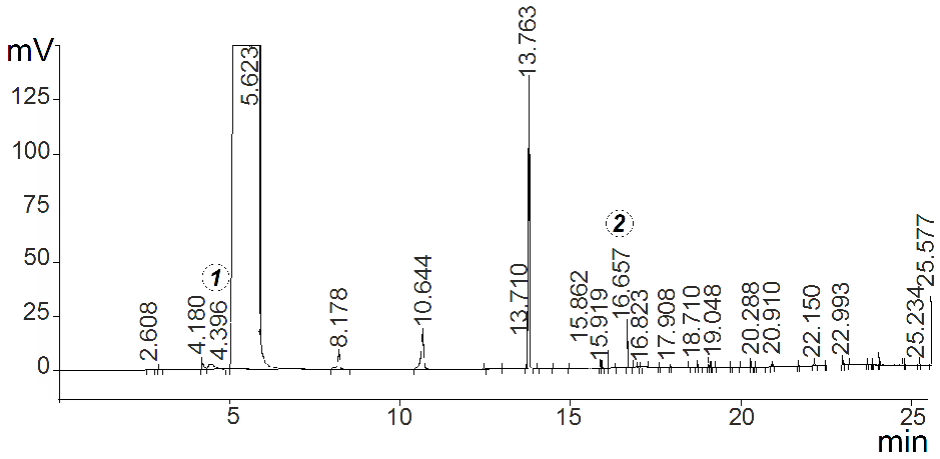


Fig. 4. Chromatographic profile of grape brandy 3:
1- methanol (retention time = 4.306 min);
2 – octanol (internal standard; retention time = 16.657 min)

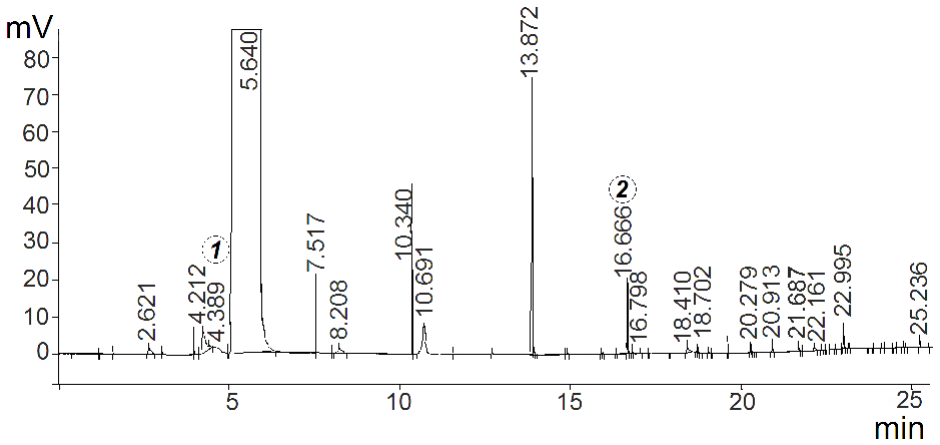


Fig. 5. Chromatographic profile of grape brandy 4:
1- methanol (retention time = 4.389 min);
2 – octanol (internal standard; retention time = 16.666 min)

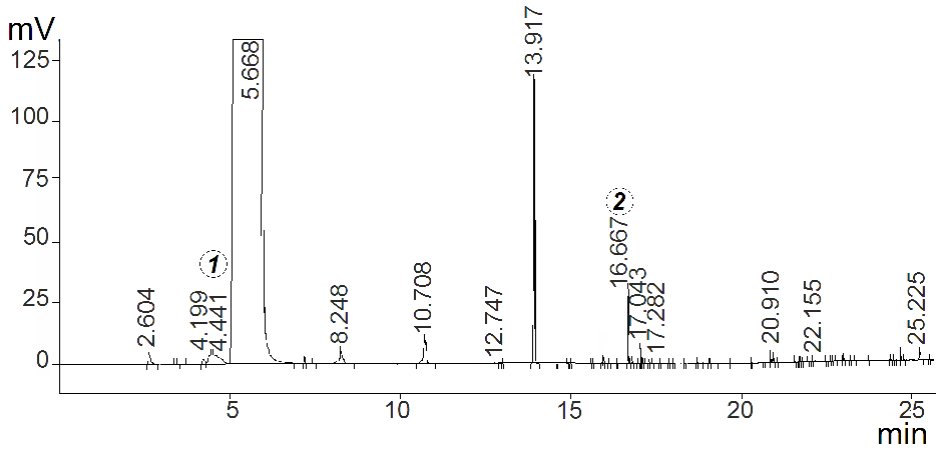


Fig. 6. Chromatographic profile of grape brandy 5:
1- methanol (retention time = 4.441 min);
2 – octanol (internal standard; retention time = 16.667 min)

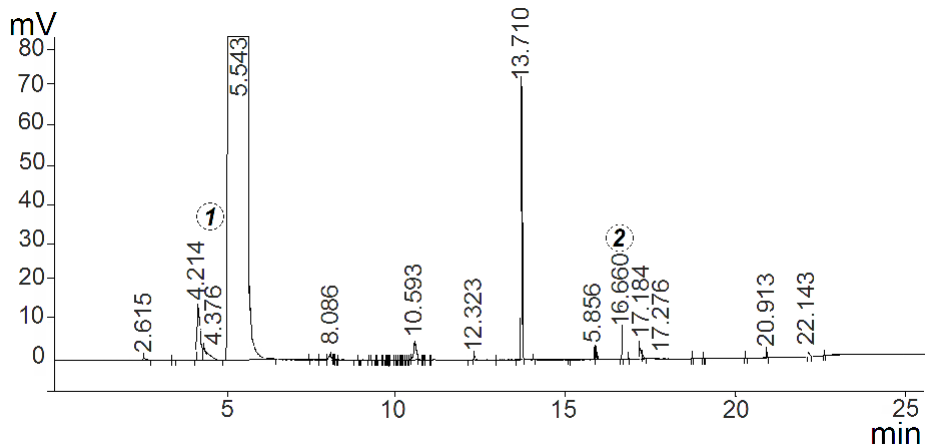


Fig. 7. Chromatographic profile of grape brandy 6:
1- methanol (retention time = 4.376 min);
2 – octanol (internal standard; retention time = 16.660 min)

The identification of methanol and octanol in brandy samples was performed according to established retention times of the two compounds in the standard solution. As is apparent from the chromatographic profiles, the methanol was identified in all six samples. This is an evidence that the samples were authentic grape brandies, made from grape raw material on the base of fermentation process. The presence of methanol in these samples exclude the possibility that they were falsified. The evidence supporting this proposition was the presence of other peaks were not identified, but indicated presence of other compounds that are likely to be products of the yeast metabolism.

The established concentration levels of methanol in grape brandies are presented in table 2.

Table 2

Methanol content of studied grape brandies

Brandy sample	Content of methanol, g/dm ³
Grape brandy 1	0.20 ± 0.03
Grape brandy 2	0.27 ± 0.01
Grape brandy 3	0.30 ± 0.02
Grape brandy 4	0.56 ± 0.01
Grape brandy 5	0.39 ± 0.01
Grape brandy 6	0.59 ± 0.02

It is noteworthy that the first three grape brandies showed lower methanol content than the other three. This is due to various distillation plants, on the basis of which brandies were obtained. The use of distillation in stills equipped with additional purification column and condenser for the first three samples resulted in better cleaning. This reflected in decreased methanol content in them.

The obtained results indicate the highest content of methanol in grape brandy 6—0.59 ± 0.02 g/dm³. The lowest found methanol level occurred in grape brandy 1—0.20 ± 0.03 g/dm³. The obtained data correlate with the values documented in the scientific works of Marinov [1] and Velkov [4]. The methanol content was in agreement with the requirements of the Bulgarian and European legislation. The obtained results for the content of methanol in analyzed grape brandies were clear evidence for their authenticity. The concentrations of methanol indicate that these brandies were harmless from a toxicological point of view, with acceptable levels of methanol.

The chromatographic profiles of the second studied group brandies – plum, were presented in figures 8-11.

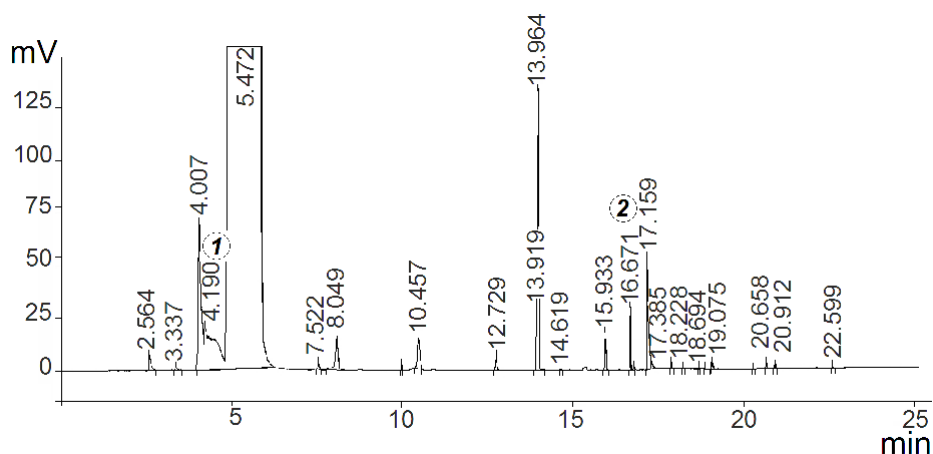


Fig. 8. Chromatographic profile of plum brandy 1:
 1- methanol (retention time = 4.190 min);
 2 – octanol (internal standard; retention time = 16.671 min)

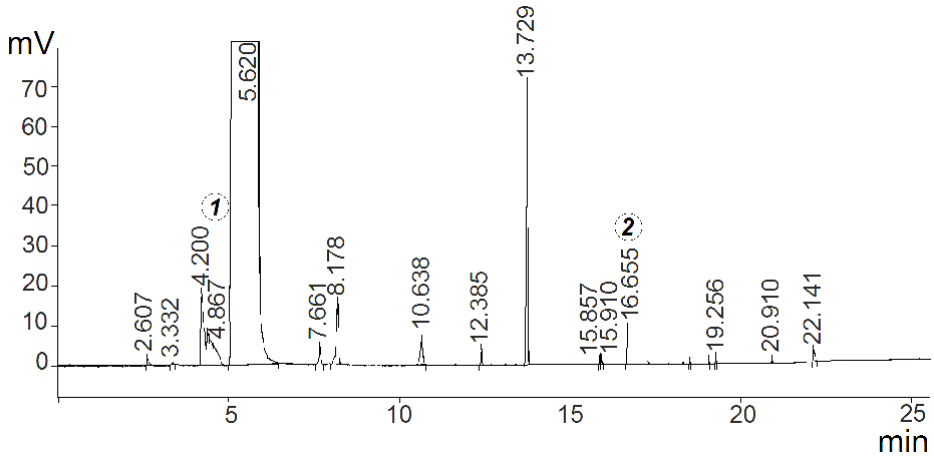


Fig. 9. Chromatographic profile of plum brandy 2:
1- methanol (retention time = 4.367 min);
2 – octanol (internal standard; retention time = 16.655 min)

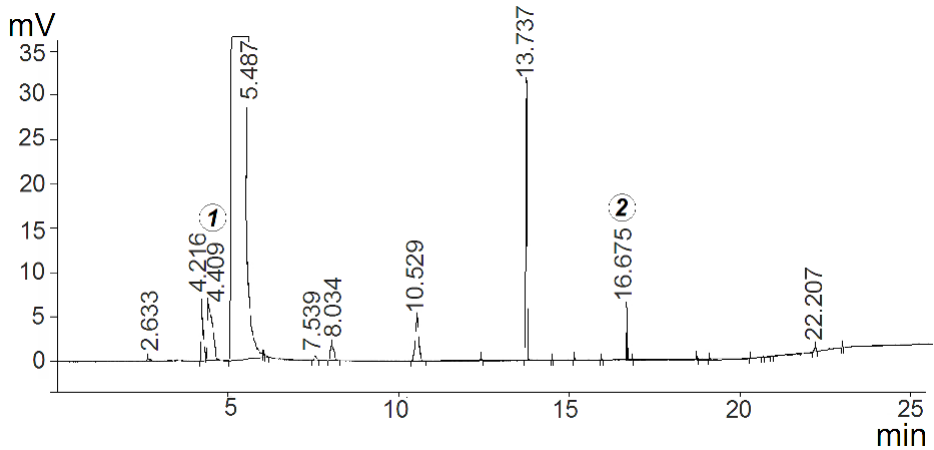


Fig. 10. Chromatographic profile of plum brandy 3:
1- methanol (retention time = 4.409 min);
2 – octanol (internal standard; retention time = 16.675 min)

The chromatographic profiles of brandies made from plum raw material, indicated the presence of methanol in all four tested brandies. This proves the authenticity of beverages. A comparison between the chromatographic profiles of the grape and plum brandies showed that the peaks of methanol in plum brandies occupy a larger area – an indicator of a higher amount of searched congener. The quantities of methanol in plum brandies, presented in table 3, confirm this clear trend.

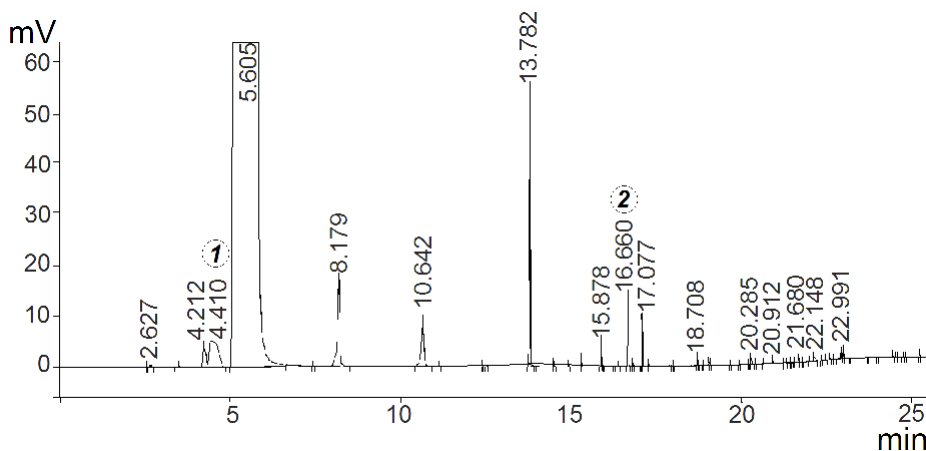


Fig. 11. Chromatographic profile of plum brandy 4:
1- methanol (retention time = 4.410 min);
2 – octanol (internal standard; retention time = 16.660 min)

Table 3

Content of methanol in plum (fruit) brandies

Plum brandy	Content of methanol, g/dm³
Plum brandy 1	2.98 ± 0.02
Plum brandy 2	1.64 ± 0.03
Plum brandy 3	2.36 ± 0.01
Plum brandy 4	1.08 ± 0.01

The presence of higher amounts methanol in plum brandies against the grape brandies, is a normal trend. This is for the reason that plum fruits has a higher content of pectin, which is a precursor for the formation of methanol. Higher pectin levels normal lead to higher amounts of methanol in the final product. The highest methanol content was found in plum brandy 1–2.98 ± 0.02 g/dm³. In all samples the methanol content was within the range of 1.00–5.00 g/dm³, which indicated that the used raw material was strong, quality and technologically mature and the distillation process was carried out in compliance with the technology of distillation. The identified concentrations of methanol in plum brandies categorize them as safe, because they cover the normative values for the presence of this alcohol (maximum acceptable levels to 10.00 g/dm³), documented in the Bulgarian Law of wine and alcohol beverages (2014) and Reg. №110/2008 of the EU.

Conclusions

The study, undertaken in order to evaluate the degree of authenticity and safety of six samples grape brandies and four samples plum brandies found:

1. Identification of methanol in all analyzed brandy samples, which confirms their authenticity and proves that they are made from raw material typical for them;
2. Established quantitative data for the presence of methanol confirm the identity of brandies corresponded to the raw material used for its production. The methanol content in brandies complied to the limits of this alcohol in both groups brandies – grape and fruit (plum);
3. The reported higher levels of methanol in plum brandies were indicator for their authenticity, because plum fruits are rich source of the methanol precursor – pectin;
4. All established methanol amounts in brandies meet the legal requirements, which is a direct confirmation for their safety consumption.

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Inactivation effect of microwave heating on pectin methylesterase in orange juice

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Abstract

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Introduction. Enzyme inactivation is a major objective in orange juice production. Conventional heating in elevated temperature causes adverse effects on the final products such as color alterations, flavor damages, ascorbic acid losses. For this, microwave heating (MW) was used in this study as thermal treatment on orange juice production for pectin methylesterase (PME) inactivation, and response surface methodology (RSM) was used for optimization of MW conditions.

Materials and methods. Oranges (*Citrus sinensis* Osb.) of Navel variety were used as raw material in this study. The effects of flow rate and power on PME activity were investigated. After optimization orange juice was produced with using optimized conditions and compared with untreated control juices and conventional thermally heated juices on the aspect of PME inactivation and some quality characteristics.

Results and discussion. The linear effects of flow rate (x_1) and power (x_2), as well as the quadratic effect of flow rate (x_1^2), power (x_2^2) and the interaction effect of flow rate-power (x_1x_2) were significant for PME inactivation by MW. Lack of fit of experimental data was not significant ($P>0.05$) for model. The coefficient of variation (C.V.) was 6.27%. The model showed an adequate precision of 6.788×10^{-3} . The determination coefficient (R^2) was 0.9793, while the adjusted determination coefficient (adjusted R^2) value was 0.9645. R^2 and adjusted R values for the models did not differ dramatically indicating non-significant terms have not been included in the model. Reduction of PME activities was found approximately 93–95% in MW groups. The PME inactivation rate was described satisfactorily as a function of microwave heating conditions. The PME can be inactivating in moderate temperatures by MW (40 mL/min-900W-83 °C) and MW (50 mL/min-900W-75 °C). D values were calculated for two optimum conditions of MW and CH treatments and found as 39.24 sec for MW (40 mL/min-900W), 38.76 sec for MW (50 mL/min-900W) and 70 sec for CH (95 °C–60 sec). Total pectin content was increased 17.2% after MW application. And the loss of ascorbic acid content for MW sample was found lower than other applications.

Conclusions. A synergistic effect of microwave energy and temperature on orange juice PME inactivation was found under microwave heating conditions. It was determined that MW (50 mL/min-900W) can be applied as a thermal treatment on orange juice production in moderate temperatures (75 °C) for PME inactivation and may improve functional properties of orange juice. And this result is extremely important in terms of cloud stability of orange juices.

Introduction

Orange juice is a widely consumed product owing to its high nutritional value and desirable sensory characteristics [1] and a common problem associated with orange juice (fresh squeezed, concentrated and preserved) is the loss of cloudiness [2], and it is a critical orange juice quality parameter imparting characteristic flavor, color and mouthfeel. The juice cloud, which is composed of finely divided particulates of pectin, cellulose, hemicellulose, proteins and lipids in suspension, is considered a desirable characteristic of orange juice [1, 3, 4]. Cloud stability is affected by the impact of pectic enzymes, particularly pectin methylesterase (PME) [5]. PME, a ubiquitous enzyme in plants, de-esterifies the methoxylated pectin in the plant cell wall. PME (EC 3.1.1.11) is also referred as pectin demethoxylase, pectin methoxylase, pectase, pectinoesterase and pectinesterase, released into juice during extraction [6, 7, 8]. The design for thermal pasteurization of orange juice is based on the thermal destruction characteristics of PME which is thermally stable than many vegetative microorganisms [9, 10]. Conventional thermal processing is the most common method to inactivate PME and to prevent juice cloud precipitation; additionally to control microbial growth in orange juice production. Pasteurization at 90-95 °C for 60-90 sec is used to inactivate the most tolerant PME isoenzyme. However it can be reduce freshness, affecting sensory and nutritional characteristics of orange juice. This conventional treatment in elevated temperature causes adverse effects on the final products such as color alterations, flavor damages, ascorbic acid losses [11-14].

As consumers are highly demanding minimally processed and fresh-like food products, the use of emerging technologies is gaining popularity. The food industry is interested in emerging technologies which inactivates enzymes and microorganisms without significant adverse effects on flavor and nutrients [15]. Microwave heating is an emerging technology in food processing, which bases its preservation properties in thermal reduction of the microbial counts in foods. Heating takes place due to the interaction of electromagnetic radiation at certain frequencies with dielectric materials; this is also called volumetric heating due to, in contrast with convection or conduction mechanisms, microwave radiation directly penetrates the material causing volumetric heat generation in the material, resulting in high-energy efficiency and lower heating times [16, 17]. This technology has found many applications in the food industry, as it has important advantages over conventional heating, such as reduced processing time, high-energy efficiency, and improved food quality [17]. Microwave heating as an alternative method for fruit juice pasteurization has now gained better acceptance as it offers several advantages over the conventional method. The advantages for the use of microwave energy in fruit juice processing are (i) heating the juice directly, (ii) no heat-transfer films, (iii) improved temperature control, (iv) rapid startup and cooldown, and (v) less heat lost to the environment.^[18] Microwave pasteurization of fruits and fruit juices, e.g. citrus juices, involving enzyme inactivation has not been commonly studied. There have been few reports on PME inactivation using microwave energy [7, 14, 16–18, 20–22]. However, no optimization data for microwave inactivation of PME has been reported.

Therefore the objective of this study was to investigate the effect of microwave heating on the inactivation of PME on orange juice and to optimize the moderate temperature conditions for electrical field application with response surface methodology (RSM) and to compare it with the conventional thermal treatment. Then effects of MW treatment at optimized conditions on the PME activity and some quality characteristics were determined by physical and chemical analyses.

Materials and methods

Material. Oranges (*Citrus sinensis* Osb.) of Navel variety were used as raw material in this study. The oranges were purchased from Zumdieck Frozen and Canned Food Company (Salihli-Manisa, TURKEY). They were stored in a refrigerator at 7 °C and 80–90% humidity for maximum of 48 hours before processing.

Processing methods.

Orange juice production: After washing and peeling applications oranges were processed to orange juice by using a juice extractor (Moulinex, JU5000-800W) and all groups (control, MW and CH) were sieved (mesh 3.5 mm). Then orange juices were divided into three groups; (i) control group (without any treatment) and (ii) MW application group, process conditions (flow rate and power) were optimized by using RSM; (iii) conventional thermal treatment group (CH) to determine PME inactivation and quality effects;

MW application: MW heating was used for the pasteurization of the orange juices. A modified MW oven (Model Arçelik MW 595, Istanbul, Turkey) with 2450 MHz operated at 540 to 900 W was used. The heating region of the MW oven contained a 3-m-long silicon hose (diameter of hose 8 mm-inside; 11 mm-outside) and a peristaltic pump for controlling flow (Watson Marlow [505U] Ltd., Falmouth, Cornwall, U.K.). Entrance and exit temperatures were measured by thermocouples. The maximum difference among the measured temperatures was approximately 1 °C. The experiments were replicated three times. The average temperature of the replicated heating experiments was accepted or used as the measured temperature values. Flow rates between 40 and 80 mL/min at 540, 720, and 900 W were studied. Flow rates and power were optimized for pasteurization process by RSM and the PME activity was taken as a response. By the ANOVA, flow rate and power were found to be significantly important on PME activity at 95% confidence interval. Model was tested for lack of fit.

CH application: Conventional thermal treatment was realized in a water bath (DKZ Series). Shelf stable orange juices, processing times for thermal pasteurization are equivalent to 90-95 °C for 60-90 sec [23, 24]. As given in literature; 200 mL bottled orange juice were heated until 95 °C and kept at the same temperature for 60 sec. The temperature was measured in the juice within the center vertical axis of the bottles without agitation. And the water bath temperature was 100 °C; all of the bottled orange juices were come up to 95 °C in 10 minutes and kept at the same temperature for 60 sec. Then orange juice was processed at optimized conditions to compare quality characteristics. Processed orange juices by MW were filled into 200 mL sterile bottles in aseptic conditions and closed leaving the minimum amount of headspace volume. After production of orange juices, all samples were cooled +4°C in an ice bath and analyses were conducted after production. After heating treatments decimal reduction times (D values) of PME were calculated for MW optimum points and conventional heating treatments.

Methods of analysis.

Response measurement techniques: RSM was used for optimization of MW conditions (Myers and Montgomery, 1995) [25]. The effects of flow rate and power (independent variables) were investigated on PME activity (response) of orange juice. A central composite rotatable design was used in designing the MW treatment of two variables at five levels (Design Expert 7.0.0 STAT-EASE, 2005). PME activities were determined after MW applications.

The model adequacies were checked by R^2 , adjusted- R^2 , predicted- R^2 and prediction error sum of squares (PRESS) [25]. A good model will have a large predicted R^2 , and a low PRESS. After model fitting was performed, residual analysis was conducted to validate the assumptions used in the ANOVA. This analysis included calculating case statistics to identify outliers and examining diagnostic plots such as normal probability plots and residual plots. Maximization and minimization of the polynomials thus fitted was performed by desirability function method and mapping of the fitted responses was achieved using Design Expert Version 7.0.0 software.

Chemical and physical analysis: PME activity and quality characteristics of orange juices were investigated and samples were analyzed to determine the following:

PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution using a pH meter (WTW InoLab, Weilheim, Germany) and 0.01 M NaOH. Routine assays were performed with a 0.5% pectin (Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.) solution (25 mL) containing 0.117 M NaCl (pH 7.0) at 30 °C. An activity unit (U) of PME is defined as the amount of enzyme required to release 1 μmol of carboxyl groups per minute [26, 27].

Pectin content was investigated according to AOAC (1968) [28]. The method is based on the extraction of pectin with ethanol after centrifugation (4000 rpm, 15 min, 20 °C) (CFC free Universal Hettich Zentrifugen, Tuttlingen, Germany); the precipitated part was treated with 5 mL NaOH and completed to 100 mL with deionized water. After filtration, samples were prepared with 0.5 mL carbazol (Merck, Darmstadt, Germany) and 0.5 mL ethanol. Sulfuric acid (Merck) (6 mL) was added to both samples, then they were placed in a water bath at 85 °C for 5 min. Absorbance values were taken at 525 nm with a Varian Cary 50 Scan (Sydney, Australia) spectrophotometer and the pectin content was calculated with the calibration curve that was made by using gallacturonic acid anhydrate standards (Sigma-Aldrich Corp.).

Ascorbic acid was determined according to Hışıl (2004) [29]. 10 mL of orange juice were homogenized in a Waring Blender (Waring Products Inc., Connecticut, USA) with 90 mL of 0.4% aqueous oxalic acid solution, filtrated through Whatman No 1 paper. 1 mL of filtrated sample was mixed with 9 mL 2,6 dichlorophenol-indophenol (Merck KGaA, Germany), and immediately the absorbance was measured with a spectrophotometer (Varian Cary 50 Scan, Australia) at 518 nm against the solution of sample mixed with distilled water (1:10). Standard curve was constructed by using L(+) ascorbic acid (Carlo Erba Reagenti SpA) solutions with known concentrations, and ascorbic acid content of the samples calculated against the standard curve.

Total soluble matter ($^{\circ}\text{Brix}$) of juices was measured with a refractometer at 20 °C (RFM 330; Bellingham+Stanley Limited, Tunbridge Wells, Kent, U.K.) [30]. The pH values of orange juices were measured with pH meter-WTW InoLab at 20 °C [30]. The color (L^* , a^* , b^*) values of orange juice were measured with a HunterLab Colorflex colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.) and total color differences (ΔE) and chroma (ΔC) values were calculated according to control group. Orange juices were placed on the light port using a 5 cm diameter glass dish with cover. Color parameters were recorded as L^* (lightness), a^* (redness) and b^* (yellowness) and total color differences (ΔE) values were calculated according to equations 1.

$$\Delta E = [(L - L_{\text{ref}})^2 + (a - a_{\text{ref}})^2 + (b - b_{\text{ref}})^2]^{0.5} \quad (1)$$

Statistical methods: Results were statistically analyzed by analysis of variance (ANOVA) using the software SPSS 13 (SPSS Inc., Chicago, IL, USA) with the Duncan test

to evaluate differences between treatments at levels of significance $P \leq 0.05$. Each experiment was repeated at least three times; means and standard deviations of results were calculated.

Results and discussion

Optimization ranges of MW application were determined by pretesting. By the aim of choosing the suitable flow rate interval some experiments were made between flow rate ranges of 20-100 mL/min at 900 W. Under 40 mL/min flow rate orange juice outlet temperatures were measured upper than conventional heating temperatures (95 °C). Above 80 mL/min application, PME was activated because of the low temperature (~55 °C). And the effects of power on PME inactivation were determined by three power levels as 540, 720 and 900 W. So independent variables (flow rate and power) interval were chosen as 40–80 mL/min; 540-900 W for RSM. By the ANOVA, flow rate and power were found significantly important on the PME inactivation of orange juice at 95% confidence interval. Model was tested for lack of fit. Table 1 shows the experimental design and responses.

Table 1
Central composite rotatable design with experimental values of response variables

Run #	Flow rate (mL/min) (x_1)	Power (W) (x_2)	PME activity* ($\mu\text{mol}/\text{min}/\text{mL}$)
1	60	720	0.243±0.02
2	60	540	0.281±0.01
3	88	720	0.373±0.03
4	40	540	0.173±0.01
5	60	720	0.276±0.01
6	32	720	0.130±0.03
7	60	900	0.179±0.02
8	60	720	0.276±0.02
9	80	540	0.366±0.04
10	60	720	0.276±0.02
11	80	900	0.260±0.02
12	40	900	0.098±0.01
13	60	720	0.281±0.01

* Results are presented as means \pm SD ($n = 3$).

The linear effects of flow rate (x_1) and power (x_2), as well as the quadratic effect of flow rate (x_1^2), power (x_2^2) and the interaction effect of flow rate-power (x_1x_2) were significant for PME inactivation by MW. Lack of fit of experimental data was not significant ($P > 0.05$) for model. The coefficient of variation (C.V.) was 6.27%. Adequate precision compares the model predicted values with its associated error, its signal to noise ratio. Ratios greater than 4 indicate adequate model discrimination. The model showed an adequate precision of 6.788×10^{-3} . The determination coefficient (R^2) was 0.9793, while the adjusted determination coefficient (adjusted R^2) value was 0.9645. R^2 and adjusted R values for the models did not differ dramatically indicating non-significant terms have not been included in the model. There was a high correlation between the experimental and predicted

values. These statistical parameters confirm the consistency of model, indicating that it is reliable to predict PME inactivation. Using the regression coefficients from the adjusted model (Table 2) the following model equation was generated (x_1 : flow rate, mL/min; x_2 : power, W):

$$\begin{aligned} \text{PME activity } (\mu\text{mol}/\text{min}/\text{mL}) = & 0.27 + 0.087 x_1 - 0.041 x_2 - 7.5x10^{-0.03} x_1 x_2 - \\ & - 0.014 x_1^2 - 0.025 x_2^2 \end{aligned} \quad (2)$$

$(x_1$: flow rate; x_2 : power)

Table 2
Anova table showing the variables as quadratic terms on response variable and coefficient for the prediction model

Source	DF**	Coefficient	Sum of squares	p-value*
Model	5	+0.27	0.080	< 0.0001
x_1	1	0.087	0.061	< 0.0001
x_2	1	-0.041	0.013	< 0.0001
$x_1 x_2$	1	$-7.5x10^{-0.03}$	$2.250x10^{-4}$	0.3659
x_1^2	1	-0.014	$1.307x10^{-3}$	0.0526
x_2^2	1	-0.025	$4.186x10^{-3}$	0.0042
Residual	7		$1.686x10^{-3}$	
Lack of fit	3		$7.487x10^{-4}$	0.4572
Pure error	4		$9.369x10^{-4}$	
Total	12		0.081	
R^2		0.9793		
Adj- R^2		0.9645		
Pred- R^2		0.9167		
PRESS		$6.788x10^{-3}$		
CV		6.27		

*p-value < 0.05 is significant at a = 0.05. Lack of fit is not significant at p-value > 0.05.

** DF: Degrass of freedom

Optimum condition for MW of orange juices was determined to obtain minimum PME activity. Second order polynomial models obtained in this study were utilized for response in order to determine the specified optimum conditions. These regression models are valid only in the selected experimental domain. In this study, flow rate and power were selected in the range of 32–88 mL/min, 540–900 W respectively. Figure 1 shows the effect of flow rate and power PME inactivation of the orange juice at MW application. By applying desirability function method, two solutions were obtained for the optimum covering the criteria. The first one is 40 mL/min for flow rate and 900 W for power. The second one is 50 mL/min for flow rate and 900 W for power. The results indicate that the high powers on lower flow rates can decrease PME activities and both solutions gave nearly same desirability values (0.9). So, the factor level combinations obtained both solutions were selected as the optimum. Production of orange juices was made at two optimum point for determining PME activities and quality properties. Predicted value by RSM was suitable with the observed value of PME activities as shown in Table 3.

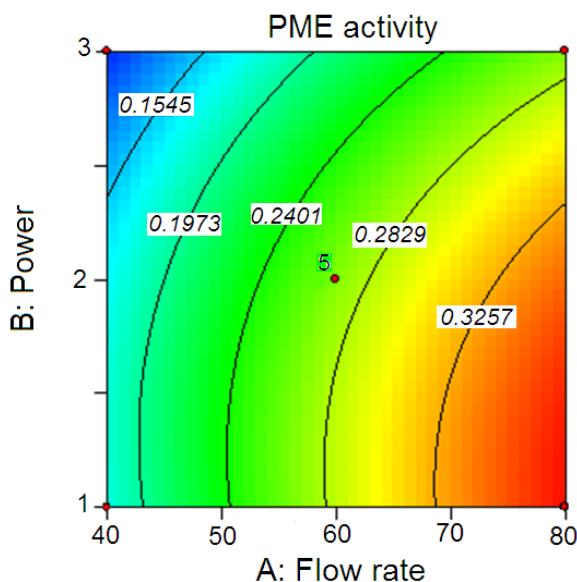


Figure 1. The effect of voltage gradient and temperature on pectin methylesterase inactivation of the orange juice at microwave heating applications

Table 3
Predicted and observed responses at optimum level of variables

Sample	Predicted value of response, PME activities ($\mu\text{mol}/\text{min}/\text{mL}$)	Observed response, PME activities** ($\mu\text{mol}/\text{min}/\text{mL}$)	Reduction of PME activities (%)*
MW (40 mL/min-900W)	0.0734	0.087 \pm 0.05 ^a	95.36
MW (50 mL/min-900W)	0.1221	0.130 \pm 0.04 ^b	93.04
CH (95 °C-60 sec)	-----	0.260 \pm 0.03 ^c	86.08
Control	-----	1.868 \pm 0.06 ^d	-----

* Reduction of PME activities (%) were calculated according to control PME activities

**Statistically significant difference shown levels a, b ($P \leq 0.05$); results are presented as means \pm SD ($n = 3$).

PME activities of orange juices were found as 0.087 $\mu\text{mol}/\text{min}/\text{mL}$ MW (40 mL/min-900W) and 0.130 $\mu\text{mol}/\text{min}/\text{mL}$ MW (50 mL/min-900W) whereas control group has 1.868 $\mu\text{mol}/\text{min}/\text{mL}$ PME activities. In CH (95 °C-60 sec) group PME activities of orange juices were found as 0.260 $\mu\text{mol}/\text{min}/\text{mL}$. It was found statistically significant ($P < 0.05$). Reduction of PME activities were found as 95.36%, 93.04%, 86.08% for MW (40 mL/min-900W), MW (50 mL/min-900W), CH (95 °C-60 sec); respectively (Table 3).

Results were in agreement with Nikdel et al., (1993) [18]. They found 98.5-99.5% reduction of PME activity during MW (>75 °C with 10-15 sec) compared to its activity in fresh orange juice. This compared to 99.0% inactivation by traditional pasteurization for 15 sec at 90.5 °C. Villamiel et al., (1998) reported that continuous microwave heating compares favourably with conventional heating at PME inactivation temperatures [14].

Tajchakavit and Ramaswamy (1995) found that the comparison of continuous-flow MW and thermal inactivation kinetics of PME at 60 °C indicated the MW inactivation rate to be 7.5 and 3.5 times faster than convectional thermal inactivation rate under batch type and continuous flow heating conditions indicating the possibility for some additive non-thermal effects [31]. Tajchakavit and Ramaswamy (1997) reported that the PME decimal reduction times for microwave heating were 38, 12, 4.0 and 1.3 sec at 55, 60, 65 and 70 °C, respectively [22]. And as compared with about 150 and 37 sec at 60 and 70 °C respectively, during conventional heating. In the literature, there are few reports on MW heating of orange juice for PME inactivation but there are some other studies on PME inactivation for orange juice by using high pressure, PEF etc. Yeom et al. (2002) found a 90% orange PME activity reduction with 125 pulses of 2- μ s-pulse-width at 25 kV/cm [32] and Elez-Martinez et al. (2003) reached an 80% activity reduction of orange PME after 375 pulses of 4- μ s-pulse-width at 35 kV/cm [33]. Moreover, Rodrigo et al. (2003) achieved an 81.3% PME reduction in orange-carrot juice after a 350- μ s HIPEF treatment of 35 kV/cm [34]. Giner et al. (2005) also observed an 86.8% PME inactivation in a commercial pectolytic enzyme preparation [35]. Different results achieved by employing techniques can be related to different temperature/time history of orange juice samples. In addition, this difference is the result of thermostable PME ratio of total-PME. The relative ratio of the thermostable PME to total-PME can vary between 0 and 33% depending on the citrus cultivars. In the case of oranges, the percentage of PME fractions depends on the variety of the oranges [36-38], geographic location, growth practice, post-harvest handling, seasonal differences [37], fruit tissues [8], and experimental changes in protocol [39]. A 5% thermostable PME fraction was observed for Valencia oranges [38]. In Navel oranges, Van den Broeck et al. (2000) [40] and Versteeg et al. (1980) [7] also found a 5% heat-stable PME fraction. In addition, according to Rombouts et al. (1982) [36], the thermostable PME represented 6% of the total activity in Navel oranges, 11% in Salustiana oranges and 7% in Shamouti oranges. And our results shows additional PME inactivation rates for Navel oranges by MW heating.

Thermal heating histories and D values of microwave heating (optimum points) and conventional thermally heated orange juices were shown in Table 4. D values were calculated for two optimum conditions of MW and CH treatments and found as 39.24 sec for MW (40 mL/min-900W), 38.76 sec for MW (50 mL/min-900W) and 70 sec for CH (95 °C – 60 sec). D value defined as the heating time required to result in 90% inactivation of initial activity. As seen from the results, to inactivate 90% of PME at MW conditions need shorter time than CH. And it shows the additional effects of electromagnetic energy. D values of PME inactivation in orange juice obtained following microwave heating were remarkably smaller than those obtained from conventional thermal inactivation. This indicates that microwave heating is more effective than conventional heating in inactivating PME in orange juice with some contributory nonthermal effects. Wicker and Temelli (1988) reported the D value of 0.225 sec at 90 °C for orange juice [41]. The calculated ranges of D values are 10-390 sec at 55 °C and 6-36 sec at 70 °C. In comparison with these values, the D values obtained under microwave heating conditions are smaller by an order of magnitude. This indicates that microwaves cause inactivation of PME in some way which cannot be solely explained by thermal effects. In other words, these results confirm the contributory nonthermal effects of microwaves resulting in enhanced inactivation of PME in orange juice as also observed in our study. Versteeg et al. (1980) showed three forms of PME in Navel oranges, one of which, the high molecular weight PME, was reported to be the most heat resistant fraction D value of 24 sec at 90 °C; of the other two forms of PME isozymes, one was more rapidly inactivated (D₆₀ of 47 sec) than the other (D₇₀ of 27 sec) [7]. These studies indicate that PME inactivation kinetics depend on several

factors: variety and composition (acidity, total solids, pulp content) of juice as well as heating method.

Table 4
Thermal heating histories of microwave and conventional heated orange juices

Heating treatments	F (mL/min)	P (W)	T _{in} (°C)	t _h (sec)	T _{out} (°C)	t _t (sec)	T _{c-out} (°C)	D* (sec)
Microwave	40	900	25±2	52	83±1	52	+4±1	39.24±0.2 ^a
Microwave	50	900	24±2	45	75±1	45	+4±1	38.76±0.5 ^a
Conventional heating	—	—	24±2	60	95±1	660	+4±1	70.00±0.2 ^b

F: flow rate; P: power T_{in}: inlet temperature; T_{out}: outlet temperature after heating treatments; T_{c-out}: outlet temperature after cooling;

t_h: total heating time; t_t: total treatment time; D: Decimal reduction times of PME;

*Statistically significant difference shown levels a, b (P ≤ 0.05); results are presented as means ±SD (n = 3).

Results of chemical and physical analyses of orange juice which produced at optimum conditions were shown in Table 5. Pectin content is important for cloudiness of orange juice. Total pectin content was increased 2.6% and 17.2% for MW (40 mL/min-900W) and MW (50 mL/min-900W) respectively. The difference between pectin content of samples was found statistically significant (P ≤ 0.05). Yıldız and Baysal (2006) [42]; investigated the effects of alternative current on pectin content in tomato samples and found 3.23% pectin content at 68 V/cm for 23 sec application (78°C) and 3.15% pectin content at 48 V/cm for 40 sec at (82°C). Rayman et al. (2011) investigated the effect of electroplasmolysis on carrot juice and the total pectin content was increased 14.78% after electroplasmolysis applications [43]. Demirdöven and Baysal (2009) found 18.4% increase in pectin content of orange juices after electroplasmolysis application at 27 V/cm [15].

Table 5
Results of chemical analyses of microwave and conventionally heated orange juices

Sample	Total pectin (GA-AH mg/L)*	Ascorbic acid (mg/100mL)*	°Brix*	pH*
MW (40 mL/min-900W)	418.67±1.5 ^a	50.4±0.02 ^b	12.4±0.1 ^a	3.8±0.1 ^a
MW (50 mL/min-900W)	478.2±2.3 ^b	51.3±0.10 ^c	12.3±0.1 ^a	3.8±0.1 ^a
CH (95 °C-60 sec)	411.1±1.0 ^c	40.5±0.00 ^a	12.5±0.1 ^a	3.7±0.2 ^a
Control	407.9±1.3 ^d	55.4±0.04 ^d	12.3 ±0.1 ^a	3.7±0.2 ^a

*Statistically significant difference shown levels a, b compared with same column (P ≤ 0.05); results are presented as means ±SD (n = 3)

Ascorbic acid was found in control sample as 55.4 mg/100 mL where, it was observed 40.5 mg/100 mL in CH group. Ascorbic acid contents of MW samples were found as 50.4 mg/100 mL and 51.3 mg/100 mL for MW (40 mL/min-900W) and MW (50 mL/min-900W) respectively. High ascorbic acid content of MW samples can be explained by moderate temperature applications. And also it can be explained by increasing in cell

permeability and components can be transferred to orange juice easily. The difference between ascorbic acid content of samples was also found statistically significant ($P \leq 0.05$). The content of vitamin C in fresh orange juice has been widely studied and the results obtained in the present work are in the range of those published in the literature, which varied from 25 mg/100 mL to 68 mg/100 mL [44–50]. Vikram et al. (2005) investigated the effects of different electro-heating method on vitamin C degradation [50]. They evaluated that the destruction of vitamin C was influenced by the method of heating and the temperature of processing as found in the present study. They found highest vitamin C degradation during microwave heating due to uncontrolled temperature generated during processing and ohmic heating gave the best result facilitating better vitamin retention at all temperatures. Lima et al. (1999), examined ascorbic acid degradation in pasteurized orange juice during conventional and ohmic heating [51]. They also found that the type of heating had no significant effect on vitamin C degradation. They measured a decrease of 21–23% in ascorbic acid during thermal treatments at 90 °C for 30 min.

There are no significant differences between the samples for water soluble matters and pH values ($P > 0.05$). Effects of PEF application on brix were investigated by some researchers. Rivas et al. (2006) applied thermal pasteurization and PEF to the blended orange–carrot juice and found brix values: 9.5 and 10.4 for control and pasteurized samples [52]. Torregrosa et al. (2006) who studied PEF determined comparable brix values for the pasteurized juice and juice treated by PEF [53]. Like the mentioned previous study Cserhalmi et al. (2006) reported that in citrus juices PEF treatment (50 pulses at 28 kV/cm) did not change the brix value significantly [54] as we found.

The color values (L^* , a^* and b^*), and the total color difference (ΔE) of samples in CIE Lab system are summarized in Table 6. The difference between L^* values of MW (50 mL/min-900W) samples was found as statistically significant ($P \leq 0.05$). The difference between a^* and b^* values of samples and the color differences (ΔE) of all samples were found as statistically important ($P \leq 0.05$). By MW and CH treatments a^* and b^* values decreased compared to control group, this can be explained by color of samples became lighter after thermal applications. Same effects were observed after electrical application in apple juices [55]. And the lower ΔE value measured on MW (50 mL/min-900W) heated samples.

Table 6
Color values of microwave and conventionally heated orange juices

Sample	L^*	a^*	b^*	ΔE
MW (40 mL/min-900W)	56.1±0.01 ^a	11.5±0.04 ^a	57.7±0.08 ^a	3.1±0.2 ^a
MW (50 mL/min-900W)	56.4±0.01 ^b	12.2±0.02 ^b	58.5±0.02 ^b	1.9±0.1 ^b
CH (95 °C-60 sec)	56.1±0.01 ^a	11.8±0.01 ^c	58.4±0.05 ^c	2.3±0.1 ^c
Control	56.1±0.01 ^a	13.2±0.06 ^d	60.2±0.01 ^d	-----

*Statistically significant difference shown levels a, b compared with same column ($P \leq 0.05$), results are presented as means ±SD ($n = 3$)

Demirdöven and Baysal (2009) found statistically significant decrease in L^* , a^* and b^* values of orange juices after electroplasmolysis application [15]. And Demirdöven and Baysal (2012) found statistically significant difference between a^* , b^* and ΔE values of ohmic and conventional heated orange juices [56]. In a previous study after treatment of PEF in citrus juices ΔE values were determined as 0.45 for grapefruit; 0.59 for lemon; 0.47 for orange and 2.44 for tangerine juices. L^* value of PEF treated (50 pulses at 28 kV/cm)

tangerine juice was found as 20.76 where control has 22.16 L* value [54]. Rivas et al.^[52] (2006) in blended orange–carrot juice investigated the effects of HTST (98°C, 21 sec) and PEF (25 kV/cm, 280 µs) treatments and found L* values for control, pasteurized and PEF treated; 62.80; 62.65; 63.08, respectively.

Conclusion

In this study, the effect of microwave heating on the inactivation of PME in orange juice and to optimize the microwave heating conditions for electrical field application with response surface methodology (RSM) in moderate temperatures (60–85 °C) were investigated. A synergistic effect of microwave energy and temperature on orange juice PME inactivation was found under microwave heating conditions. The PME inactivation rate was described satisfactorily as a function of microwave heating conditions. The PME can be inactivating in moderate temperatures by MW (40 mL/min-900W- 83 °C) and MW (50 mL/min-900W-75 °C). The flow rate and power combinations necessary to inactivate the labile fraction of PME can be estimated allowing selection of optimal process conditions that should also provide sensorial quality. Reduction of PME activities was found approximately 93–95% in MW groups where conventional thermally heated juice has 86% reduction value. Total pectin content was increased 17.2% after MW (50 mL/min-900W) applications. And the lost of ascorbic acid content of MW (50 mL/min-900W) sample was lower than other applications. Due to these results, there was an important improvement in functional properties particularly in pectin content of orange juice; and this result is extremely important in terms of cloud stability of orange juices. And it was determined that MW (50 mL/min-900W) heating can be applied as a thermal treatment on orange juice production in moderate temperatures (75 °C) for PME inactivation and improving functional properties of orange juice.

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Lactic acid bacteria compositions for application in the meat industry

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Abstract

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Introduction. Despite a large number of the bacterial preparations used in the meat industry, development of new starters and study of their influence on development of undesirable microbiota are still vital.

Materials and methods. We studied the halo- and thermo-resistance of 8 collection strains of the lactic acid bacteria and the compositions made of these lactic acid bacteria. The antagonistic activity toward the indicator strains, isolated from the raw meat, and the collection bacteria strains was determined by the well-diffusion method.

Results and discussion. At the maximum NaCl concentration (10.0%) in the growing medium, the strains of *L. plantarum* 12 and 1005 were characterized by high growth rate, *L. delbrueckii s/sp. lactis* 013 and *L. casei s/sp. tolerans* 290 – by the average one. Thermo-resistant (that is able to grow quickly at the temperature ranged from 5 to 25 °C) were the strains of *L. plantarum* 12, *L. delbrueckii s/sp.lactis* 013, *L. acidophilus* 147 and *L. casei s/sp. tolerans* 187 and 290. The lactic acid bacteria showed the antagonistic activity to both, the strains isolated from the raw meat, and the collection strains of bacteria. Growth of some indicator bacteria was slowed down only, while that of others – completely inhibited. The best antagonists were the strains of *L. plantarum* 12, *L. delbrueckii s/sp. lactis* 013 and *L. casei s/sp. tolerans* 290, which completely inhibited the growth of *Bacillus sp.* 3, *Kurthia sp.*, *Planacoccus sp.* 1, *sp.* 2, *Micrococcus sp.* 2, *Sarcina sp.* and *Staphylococcus sp.*, isolated from meat and the collection bacteria, such as: *Planacoccus citreus*, *Escherichia coli* and *Salmonella enteritidis*.

Based on the strains of *L. plantarum*, *L. delbrueckii s/ sp.lactis* 013 and *L. casei s/sp. tolerans* 290 nine variants of compositions were made and their biotechnological potential was studied. All the compositions were able to grow even at 0 °C. The most stable was the starter made of *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 at the ratio of 1:2, the growth rate of which at 5 °C was estimated as "exceptional". The lactic acid bacteria compositions significantly inhibited the growth of the indicator bacteria. The dimensions of the "no growth" areas for the bacteria isolated from meat ranged from 16 mm to 43 mm depending on the indicator strain and composition. The most sensitive bacteria from the collection ones were the gram-positive cocci *P. citreus* and *M. luteus*, for which the "no growth" area dimensions, depending on the composition, ranged from 34 mm to 42 mm and from 28 mm to 40 mm, respectively.

Conclusions. The results show an increase in the biotech activity of the lactic acid bacteria in the compositions. The most promising for testing in an industrial environment is a composition of *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 at the ratio of 1:2.

Introduction

Providing the population with the high-grade whole food, meat and meat products in particular, is one of the priorities of the contemporary food industry. High nutrition and biological value of meat, the possibility to get a thousand of finished products different in the form, taste, flavor, succulence, color and consistency are determined by the heterogeneity of its structure, composition and high variability of its properties [1, 2].

Since meat is a favorable breeding ground, there is a high risk of the development, reproduction and accumulation of microorganisms in it [5, 6]. The sources of the microbial contamination can be exogenous and endogenous.

To maintain quality, prevent development of deficiencies and extend the storage life of meat, various types of processing are offered. One of the current trends is the biotechnological methods aimed at modification and optimization of the functional and technological properties of the raw meat, and improvement of its quality characteristics during storage [7, 8, 9]. For this purpose, use of the lactic acid bacteria seems most promising, in particular, the *Lactobacillus* genus bacteria, producing organic acids, enzymes and other bioactive substances which promote the antagonistic properties in respect of the undesirable raw meat microbiota [10]. There are works of the domestic and foreign scientists devoted to search for the promising lactic acid bacteria and making compositions (consortia) based on them, the so called “bacterial starters” [11, 12, 13]. However, in general they relate to use of starters in manufacture of the meat products. There are few works related to use of the microorganism cultures to improve the raw meat quality and extend its storage period. The issue of choice of the bacterial composition and its influence on the contaminating microbiota development is still vita [14].

Therefore, the purpose of this work is selection of the lactic acid bacteria strains and making the compositions suitable for bioprotection of the raw meat (having antagonistic activity to the collection bacteria strains and those isolated from the raw meat).

Materials and methods

We studied 8 collection lactic acid bacteria strains (*Lactobacillus plantarum* 12, 1005, *L. delbrueckii s/sp. lactis* 013, *L. acidophilus* 147, *L. gasseri* 149, *L. casei s/sp. tolerans* 187 and 290, *L. casei s/sp. rhamnosus* 283); the lactic acid bacteria compositions made; the indicator bacteria isolated from the raw material (*Bacillus sp.* 1, 2, 3 *Kurthia sp.*, *Planacoccus sp.* 1, 2, *Micrococcus sp.* 1, 2, *Sarcina sp.*, *Staphylococcus sp.*) and the collection strains of saprophytic (*Planacoccus citreus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*), opportunistic pathogenic (*Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*) and pathogenic (*Salmonella enteritidis*) bacteria.

To activate the lactic acid bacteria strains, inoculation to the liquid MRS medium enriched with 5% glucose solution was carried out.

Resistance of the lactic acid bacteria and their compositions to salt was determined by growth rate in the culture medium (MRS-broth) with introduction of sodium chloride at the concentrations of 2.5; 5.0; 7.5 and 10.0%.

Growth rate at the low above-zero temperatures was measured at 0, 5, 10, 15, 20 and 25 °C for 14 days.

The antagonistic activity was determined by the well-diffusion method. As the indicator bacteria strains, we used *Bacillus sp.* 1, 2, 3, *Kurthia sp.*, *Planacoccus sp.* 1, 2, *Micrococcus sp.* 1, 2, *Sarcina sp.*, *Staphylococcus sp.*, isolated from the raw meat and *P.*

citreus, *M. luteus*, *B. subtilis*, *B. cereus*, *B. megaterium*, *S. aureus*, *E. coli*, *P. vulgaris* and *S. enteritidis* as the collection ones. In the experiment, we used the daily broth cultures: the lactic acid bacteria were grown in the MRS-broth, and the indicator bacteria – in a nutrient broth. The results were taken in 24 hours by measuring the delay and growth inhibition areas for the indicator microorganisms. When determining the antagonistic activity, only the cases of complete indicator organism growth inhibition were taken into account. If the size of a “no growth” area ranged from 10 mm to 20 mm, the degree of activity was considered average, if it was greater than 20 mm – high.

Results and discussion

The biological methods to treat the raw meat for extending its storage life are based on use of the bacterial starters consisting of the specially selected species and strains of microorganisms, in particular, the lactic acid bacteria of *Lactobacillus* genus. In selection of the lactic acid bacteria strains to make starters with a high biotechnological potential, their value measures included: halotolerance, thermo-resistance and symbiotic nature of the relationship between the cultures and antagonistic activity against the opportunistic pathogenic and pathogenic microbiota [3, 9, 11].

In this connection, at the first stage of work we studied the resistance of 8 collection lactic acid bacteria strains to salt and low above-zero temperatures. The results showed that adding NaCl to the culture medium at the concentrations of 2.5 to 7.5% did not influence the bacteria growth, and their growth rate was estimated as "exceptional". A 10.0% salt concentration in the culture medium inhibited the growth of the strains under study. With such NaCl content, only two strains (*L. plantarum* 12 and 1005) showed high growth rate and two strains (*L. delbrueckii s/sp. lactis* 013 and *L. casei s/sp. tolerans* 290) – the average one (Table 1).

Table 1

Resistance of the lactic acid bacteria to NaCl and temperature

Strain	Growth rate at	
	10.0% of NaCl	5 °C
<i>L. plantarum</i> 12	high	high
<i>L. delbrueckii s/sp. lactis</i> 013	average	high
<i>L. acidophilus</i> 147	average	high
<i>L. gasseri</i> 149	low	low
<i>L. casei s/sp. tolerans</i> 187	low	high
<i>L. casei s/sp. rhamnosus</i> 283	low	average
<i>L. casei s/sp. tolerans</i> 290	average	high
<i>L. plantarum</i> 1005	high	average

Similar studies with respect to the growth potency at the temperature ranged from 5 °C to 25 °C showed that most significantly the lactic acid bacteria growth slowed at 5°C. The obtained results are listed in Table 1 showing the growth rate of the selected strains at the lowest of the above temperatures. Such strains as *L. plantarum*, *L. delbrueckii s/sp. lactis* 013, *L. acidophilus* 147, *L. casei s/sp. tolerans* 187 and 290 appeared to be thermo-resistant.

Determination of the lactic acid bacteria antagonistic properties showed that they were active against both, the strains isolated from the raw meat and the collection ones. At that, growth of some indicator bacteria was slowed down only, while that of others – completely inhibited. Thus, all strains of the lactic acid bacteria studied only slowed down the growth of *Bacillus sp.* 1, isolated from the raw meat, and the collection *B. subtilis* and *B. cereus*; while the growth of *Planacoccus sp.* 2 (from meat), *Micrococcus sp.* 2 (from meat) and *S. enteritidis* (from collection) was inhibited completely. It should be noted that size of the “stasis” and “no growth” areas was different and depended both, from strain of the lactic acid bacteria, and an indicator microorganism. The bacteria isolated from the raw meat and the collection bacteria appeared to be most sensitive to the metabolites produced by the three lactic acid bacteria strains: *L. casei s/sp. tolerans* 290, *L. delbrueckii s/sp. lactis* 013 and *L. plantarum* 12. The results of determination of the antagonistic activity of the lactic acid bacteria are shown in Table 2.

Table 2

Antagonistic activity of the lactic acid bacteria strains

Strain	Size of delay and lack of growth areas of the indicator bacteria (mm)									
	isolated from raw meat									
	1	2	3	4	5	6	7	8	9	10
A	25 [^]	26 [^]	23*	26*	25*	35*	27 [^]	24*	23*	30*
B	25 [^]	28 [^]	29*	30*	24*	33*	28*	31*	30*	40*
C	25 [^]	20*	28*	32*	20*	28*	28 [^]	23*	45*	41*
Strain	collection									
	11	12	13	14	15	16	17	18	19	
A	27*	40*	25*	35*	20 [^]	29*	23 [^]	26 [^]	30 [^]	
B	29*	-	23*	27*	-	24*	22 [^]	24 [^]	40*	
B	44*	34*	24 [^]	26*	21 [^]	28*	24 [^]	34 [^]	25 [^]	

Note: “[^]” – stasis; “*” – no growth; “-” – no effect.

A – *L. plantarum* 12; B – *L. delbrueckii s/sp. lactis* 013; C – *L. casei s/sp. tolerans* 290.

1 – *Bacillus sp.* 1; 2 – *Bacillus sp.* 2; 3 – *Bacillus sp.* 3; 4 – *Kurthia sp.*; 5 – *Planacoccus sp.* 1; 6 – *Planacoccus sp.* 2; 7 – *Micrococcus sp.* 1; 8 – *Micrococcus sp.* 2; 9 – *Sarcina sp.*; 10 – *Staphylococcus sp.*; 11 – *Planacoccus citreus*; 12 – *Micrococcus luteus*;

13 – *Staphylococcus aureus*; 14 – *Escherichia coli*; 15 – *Proteus vulgaris*; 16 – *Salmonella enteritidis*; 17 – *Bacillus subtilis*; 18 – *Bacillus cereus*; 19 – *Bacillus megaterium*.

To estimate the antagonist activity of *L. casei s/sp. tolerans* 290, *L. delbrueckii s/sp. lactis* 013 and *L. plantarum* 12 we considered only the cases of complete indicator organism growth inhibition (lack of growth). As seen from the data in Table. 3, the lactic acid bacteria strains isolated from the raw meat and the collection bacteria showed high antagonistic activity, therewith, the size of the “no growth” areas for the indicator bacteria growth was different (Table 3).

Table 3

Antagonistic activity of the lactic acid bacteria strains

Strain	Activity in relation to the indicator bacteria																		
	isolated from raw meat										collection								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	D	D	H	H	H	H	D	H	H	H	H	H	H	D	H	D	D	D	
B	D	D	H	H	H	H	H	H	H	H	H	-	H	H	-	H	D	D	H
C	D	A	H	H	A	H	D	H	H	H	H	H	D	H	D	H	D	D	D

Note: "D" – stasis; "H" – high activity; "A" – average activity; "-" – no effect.

A – *L. plantarum* 12; B – *L. delbrueckii s/sp. lactis* 013; C – *L. casei s/sp. tolerans* 290.

1 – *Bacillus sp.* 1; 2 – *Bacillus sp.* 2; 3 – *Bacillus sp.* 3; 4 – *Kurthia sp.*; 5 – *Planacoccus sp.* 1; 6 – *Planacoccus sp.* 2; 7 – *Micrococcus sp.* 1; 8 – *Micrococcus sp.* 2; 9 – *Sarcina sp.*; 10 – *Staphylococcus sp.*; 11 – *Planacoccus citreus*; 12 – *Micrococcus luteus*; 13 – *Staphylococcus aureus*; 14 – *Escherichia coli*; 15 – *Proteus vulgaris*; 16 – *Salmonella enteritidis*; 17 – *Bacillus subtilis*; 18 – *Bacillus cereus*; 19 – *Bacillus megaterium*.

It is known that the starter multispecies are more active and resistant to the adverse environmental factors compared to the starters, made on the basis of certain cultures [5, 12, 13]. Therefore, considering the obtained results, three strains of the lactic acid bacteria – *L. casei s / sp. tolerans* 290, *L. delbrueckii s / sp. lactis* 013 and *L. plantarum* 12 – were chosen to make compositions (starters).

One of the important criteria for combining individual species into a multispecies starter is the compatibility of species and strains, i.e., first of all, they must not be reciprocal antagonists. The research of the intrageneric antagonism of the selected lactic acid bacteria showed no reciprocal growth inhibition.

So, to pursue further studies, we made 9 variants of the lactic acid bacteria compositions consisting of two lactic acid bacteria one-to-one: (1 – *L. plantarum* 12 + *L. casei s/sp. tolerans* 290; 2 – *L. plantarum* 12 + *L. delbrueckii s/sp. lactis* 013; 3 – *L. casei s/sp. tolerans* 290 + *L. delbrueckii s/sp. lactis* 013) и 1:2 (4 – *L. plantarum* 12 + *L. casei s/sp. tolerans* 290; 5 – *L. plantarum* 12 + *L. delbrueckii s/sp. lactis* 013; 6 – *L. casei s/sp. tolerans* 290 + *L. plantarum* 12; 7 – *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12; 8 – *L. casei s/sp. tolerans* 290 + *L. delbrueckii s/sp. lactis* 013; 9 – *L. delbrueckii s/sp. lactis* 013 + *L. casei s/sp. tolerans* 290) (Table 4).

Using starters in the meat industry, one shall take into account the temperature modes of production and storage of the raw meat.

Study of the growth rate of the lactic acid bacteria compositions at the temperature ranged from 0°C to 25°C showed that the effect of temperature on individual compositions was similar (Table 5). It was noted that the temperature of 10°C and 15°C had no effect on growth of the starters studied (growth rate was estimated as "very high"), at 5°C most starters (samples No. 2, 3, 4, 5 and 9) were characterized by "average" growth and development rate. Moreover, composition No. 7 consisting of *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 at the ratio of 1:2 appeared to be so resistant to the above temperature, that its growth was described as "exceptional".

Table 4

Combinations of the lactic acid bacteria compositions

Strain	Combinations					
	1:1			1:2		
	<i>L. plantarum</i> 12	<i>L. casei s/sp.</i> <i>tolerans</i> 290	<i>L. delbrueckii</i> <i>s/sp. lactis</i> 013	<i>L. plantarum</i> 12	<i>L. casei s/sp.</i> <i>tolerans</i> 290	<i>L. delbrueckii</i> <i>s/sp. lactis</i> 013
<i>L. plantarum</i> 12	-	+1:1	1:1	-	1:2	1:2
<i>L. casei s/sp. tolerans</i> 290	-	-	1:1	1:2	-	1:2
<i>L. delbrueckii s/sp. lactis</i> 013	-	-	-	1:2	1:2	-

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Table 5

Growth rate of the lactic acid bacteria compositions at different temperatures

Number of composition	0 °C	5 °C	10 °C	15 °C
1	+	+++	++++	++++
2	+	++	++++	++++
3	+	++	++++	++++
4	+	++	++++	++++
5	+	++	++++	++++
6	+	+++	++++	++++
7	+	++++	++++	++++
8	+	+++	++++	++++
9	+	++	++++	++++

Note: "++++" – exceptional growth rate, "+++” – high, "++” – average, "+" – low.

It should be noted that even at 0°C weak growth of all the lactic acid bacteria compositions was observed. Comparing the results obtained in the study of the growth rate at low above-zero temperatures of individual lactic acid bacteria strains (Table 1) and their compositions (Table 5) one can assume that combinations of cultures in the starters increases their thermo-resistance.

One of the most important criteria put forward both, to individual strains of the lactic acid bacteria, and the starters, made of them, is the antagonistic activity [11]. Considering

that the strains of *L. casei s/sp. tolerans* 290, *L. delbrueckii s/sp. lactis* 013 and *L. plantarum* 12 taken individually were active antagonists to the indicator bacteria (both, the collection and isolated from the raw meat) (Tables 2, 3), and in view of the existing requirements, it was appropriate to determine whether the starter's composition and the ratio of the lactic acid bacteria in it influence the antagonistic properties. The results are presented in Table 6.

Table 6

Antagonistic activity of the lactic acid bacteria compositions

Number of composition	Size of delay and lack of growth areas of the indicator bacteria (mm)									
	isolated from raw meat									
	1	2	3	4	5	6	7	8	9	10
1	26 [^]	22*	30*	31*	21*	30*	22*	40*	32*	34*
2	27 [^]	23*	27*	31*	28*	28*	27*	42*	31*	39*
3	25 [^]	21*	29*	32*	25*	28*	29*	42*	34*	36*
4	25 [^]	22*	32*	30*	25*	29*	25*	40*	32*	36*
5	22 [^]	16*	30*	27*	26*	38*	27*	39*	32*	43*
6	26 [^]	21*	26*	29*	25*	26*	26*	34*	34*	43*
7	29 [^]	21*	32*	30*	27*	25*	27*	39*	32*	43*
8	24 [^]	23*	28*	30*	25*	26*	27*	33*	35*	40*
9	26 [^]	21*	30*	31*	27*	27*	27*	34*	34*	31*

Number of composition	Size of delay and lack of growth areas of the indicator bacteria (mm)								
	collection								
	11	12	13	14	15	16	17	18	19
1	34*	34*	21*	27*	-	24*	25*	24*	26*
2	40*	40*	26*	38*	-	23*	26 [^]	21*	29*
3	42*	42*	21*	32*	-	21*	24 [^]	23*	25*
4	40*	40*	23*	30*	-	22*	28*	24*	23*
5	40*	40*	25*	28*	-	24*	27 [^]	28*	27*
6	37*	37*	23*	34*	24 [^]	25*	27 [^]	23*	24*
7	42*	42*	26*	37*	23*	29*	29 [^]	29*	27*
8	38*	38*	19*	26*	23*	23*	26 [^]	25*	22 [^]
9	40*	40*	20*	24*	19 [^]	23*	24 [^]	26 [^]	24 [^]

Note: “[^]” – stasis; “*” – no growth; “-” – no effect.

1 – *Bacillus sp.* 1; 2 – *Bacillus sp.* 2; 3 – *Bacillus sp.* 3; 4 – *Kurthia sp.*; 5 – *Planacoccus sp.* 1; 6 – *Planacoccus sp.* 2; 7 – *Micrococcus sp.* 1; 8 – *Micrococcus sp.* 2; 9 – *Sarcina sp.*; 10 – *Staphylococcus sp.*; 11 – *Planacoccus citreus*; 12 – *Micrococcus luteus*; 13 – *Staphylococcus aureus*; 14 – *Escherichia coli*; 15 – *Proteus vulgaris*; 16 – *Salmonella enteritidis*; 17 – *Bacillus subtilis*; 18 – *Bacillus cereus*; 19 – *Bacillus megaterium*

Growth of the bacteria isolated from meat (except for *Bacillus sp.* 1) was significantly inhibited by metabolites. The size of the “no growth” areas for the indicator bacteria ranged from 16 mm (determined for *Bacillus sp.* 2 under the effect of starter No. 5) to 43 mm (determined for *Staphylococcus sp.* in the presence of starters No. 5, 6 and 7). The most resistant strain was *Bacillus sp.* 1, the growth of which by all the starters was delayed only.

Table 7

Degree of antagonistic activity of the lactic acid bacteria compositions

Number of composition	Size of delay and lack of growth areas of the indicator bacteria (mm)									
	isolated from raw meat									
	1	2	3	4	5	6	7	8	9	10
1	D	H	H	H	H	H	H	H	H	H
2	D	H	H	H	H	H	H	H	H	H
3	D	H	H	H	H	H	H	H	H	H
4	D	H	H	H	H	H	H	H	H	H
5	D	A	H	H	H	H	H	H	H	H
6	D	H	H	H	H	H	H	H	H	H
7	D	H	H	H	H	H	H	H	H	H
8	D	H	H	H	H	H	H	H	H	H
9	D	H	H	H	H	H	H	H	H	H

Number of composition	Size of delay and lack of growth areas of the indicator bacteria (mm)									
	collection									
	11	12	13	14	15	16	17	18	19	
1	H	H	H	H	-	H	H	H	H	
2	H	H	H	H	-	H	D	H	H	
3	H	H	H	H	-	H	D	H	H	
4	H	H	H	H	-	H	H	H	H	
5	H	H	H	H	-	H	D	H	H	
6	H	H	H	H	D	H	D	H	H	
7	H	H	H	H	H	H	D	H	H	
8	H	H	A	H	H	H	D	H	D	
9	H	H	A	H	D	H	D	D	D	

Note: “D” – stasis; “H” – high activity; “A” – average activity; “-” – no effect
 1 – *Bacillus sp.* 1; 2 – *Bacillus sp.* 2; 3 – *Bacillus sp.* 3; 4 – *Kurthia sp.*; 5 – *Planococcus sp.* 1; 6 – *Planococcus sp.* 2; 7 – *Micrococcus sp.* 1; 8 – *Micrococcus sp.* 2; 9 – *Sarcina sp.*; 10 – *Staphylococcus sp.*; 11 – *Planococcus citreus*; 12 – *Micrococcus luteus*; 13 – *Staphylococcus aureus*; 14 – *Escherichia coli*; 15 – *Proteus vulgaris*; 16 – *Salmonella enteritidis*; 17 – *Bacillus subtilis*; 18 – *Bacillus cereus*; 19 – *Bacillus megaterium*.

From the collection bacteria, the most sensitive to metabolites of the compositions were the gram positive cocci *P. citreus* and *M. luteus*, the size of the “no growth” areas of which, depending on the composition, ranged from 34 mm to 42 mm and from 28 mm to 40 mm, respectively. The least exposed to the starter effect were olm (*P. vulgaris*), probably, due to a short lag-phase of this microorganism, and hay bacillus (*B. subtilis*), may be due to formation of resistance to the adverse conditions.

The most active was composition No. 7, in the presence of which the growth of almost every indicator bacteria was inhibited completely, and its activity was rated as "high".

As can be seen from the data presented, the lactic acid bacteria compositions inhibited the growth and development of the indicator bacteria. The degree of the antagonistic activity of the compositions under study to most indicator organisms was high (Table 7), while the sensitivity of individual, both the collection, and isolated from the raw meat microorganisms, was determined by a specific starter.

Comparing the data obtained in studying the antagonistic activity of individual lactic acid bacteria strains and their compositions, in most cases we observed an increased activity of the latter, presumably, due to the synergy phenomenon, when the effect of the metabolites produced by bacteria in the starters exceeds the effect of each taken individually.

Thus, the results give evidence of promising starter use in the industry to extend the storage life of the meat and meat products. With a view to industrial testing, composition No. 7 has been selected, consisting of *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 at the ratio of 1:2.

Conclusions

1. It is experimentally found that the strains of bacteria of *Lactobacillus* genus (*L. plantarum* 12 and 1005, *L. delbrueckii s/sp. lactis* 013, *L. acidophilus* 147, *L. gasseri* 149, *L. casei s/sp. tolerans* 187 and 290, and *L. casei s sp. rhamnosus* 283) are halo- and thermo-resistant, as well as antagonistically active against the bacteria isolated from the raw meat and the collection bacteria.
2. The choice of *L. plantarum* 12, *L. delbrueckii s/sp. lactis* 013 and *L. casei s/sp. tolerans* 290 to make the lactic acid bacteria compositions is proved.
3. It is shown that the biotech activity of the lactic acid bacteria in the compositions increased.
4. It is determined that the composition of *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 in the ratio of 1:2 is the most promising for testing in the industrial environment.

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Epiphytic and regulated microbial contaminants of food vegetable raw materials and products

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Abstract

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Introduction. Biological hazards as priorities at the evaluating degree of risk are caused by the presence of microorganisms in food products.

Materials and methods. As objects of study common types of fruits, vegetables and berries were used. Conventional microbiological techniques such as mesophilic aerobic and optional anaerobic bacteria were used, fungi and yeasts have been considered by inoculation under beef-extract agar (MPA) and word agar, respectively, coliforms was determined by inoculation in liquid culture media, *Bacillus cereus* and *Clostridium perfringens* was determined by ISO methods, the last with pre-treatment developed.

Results and discussion. Group composition of epiphytic microorganisms, which contaminated widespread types of fruits, vegetables, berries on indices of mesophilic aerobic and optionally-anaerobic microorganisms, mold fungi, yeasts, coliforms (BGEC), clostridia were studied. The considerable contamination of raw materials by mesophilic bacilli from $1,8 \cdot 10^2$ to $7,6 \cdot 10^8$ CFU/g was established. It was shown, that the main isolated morphotypes of bacilli can be ascribed to of *subtilis-licheniformis* group. The composition of vegetable raw materials microorganisms gives an indication of both the possibility of epidemiological risk and product high quality. Contrary to previous opinion about the dominance among epiphytic microbiota of fungi, our in some cases showed pre-printat content of rod-shaped microorganisms. Grown and picked in the same fruits of different varieties are distinguished in the predominant species of fungi. The concentration of patulin, depending on the degree of spoilage of fruits, was determined by priority method which we have developed. A large number of soil microorganisms, including *Bacillus* and *Clostridium*, are on the surface of the plant material, especially root vegetables. According to the studies, the probability of detecting dangerous to human *Clostridium perfringens* on the leaves of green plants is up to 61%, on vegetables – up to 39%. *Subtilis-licheniformis* microorganisms are the dominant raw materials contaminants and prevail in the composition of microbiota of product before sterilization. They were also detected in the residual microbiota of finished canning. The food poisoning agents were detected from plant raw materials among isolated bacteria – *Bacillus cereus* and others. *Bacillus cereus* was found in 6.2% of the investigated samples of fruits, 33% of samples of carrot, 21% of samples of parsley, and up to 9.5% of the samples of canned food.

Conclusions. High thermostability of spore-forming microorganisms of raw materials, including the test-cultures, was shown. This may account for their presence in canned products, cause deterioration of the organoleptic properties of foods and toxic effects on the human organism.

Introduction

The document CAC/GL 21 by the Codex Alimentarius, other directive documents of the EU (e.g. the report by the EU Commission «On the Strategy of the Microbiological Criteria Choice for Food Products in the Food Legislation of the EU», the leading document of the EU 2073 «On Microbiological Criteria for Foodstuffs»), and the leading documents of Food Drug Administration (the USA), provide with the general considerations concerning the principles of development and application of the microbiological criteria for the different types of food products. The Codex Alimentarius commission has developed the safety-control measures of food products, including biological safety according to the document CAC/GL 69. Evaluation of food safety in modern conditions is relevant throughout the world, including Ukraine. This is confirmed by the documents cited, as well as the Law of Ukraine "On safety and quality of food" and other government acts and regulations, modern conditions of food safety assessment and food quality are particularly relevant.

Ukraine, by its soil-climatic conditions, is similar to some foreign countries, thus the presented investigation for Ukrainian regions may be topical for other countries. The problem of safety as the most important characteristics of nutrition quality is becoming increasingly important due to the increasing pollution of the environment. And that leads to a permanent increase in the contamination of food raw materials and food products with biological agents and chemicals that affect human health [1-3].

In the food industry a number of microorganisms is used to provide necessary consumer properties of food or increase their shelf life. But the presence of certain other microorganisms has to be strictly controlled and in the raw materials processing to ensure restriction of their number, or the impossibility of their development. The latter include pathogens of such nutritional diseases as food poisoning or food infection. The former are caused by toxins of microorganisms developing in a food product, the later are infectious diseases, in which the food product serves only as an intermediary in the transmission of pathogens, claiming the lives of more than 1.8 million children each year. According to statistical data of annual economic losses, due to diseases, caused by multiple pathogenic microorganisms, constitutes up to 35 US billion dollars in the USA, in Australia to 2.6 billion Australian dollars, and social losses are irreplaceable [2, 4, 5].

Biological hazards as priorities at the evaluating degree of risk are caused by the presence of such microorganisms in food products as helminthes and protozoa, and insects (venomous or transmitting). The reason for rising of biological in nature dangers is a modern fashion for consumption of raw or minimally processed foods culinary, increasing the products proportion in diet, improperly cooked or stored for a long time, and the use of new types of food raw materials, made possible by the expansion of international trade [6 – 10].

Currently the bacteria become a source of food poisoning and infections are in the focus of attention of hygienists. These microorganisms are the kind *Salmonella*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Vibrio cholerae* O and non-O-1, *Vibrio parahaemolyticus*, *Vibrio vulnificus* genera and other representatives of the *Vibrio*, *Shigella*, *Streptococcus* genera, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, group of Gram-negative bacteria *Miscellaneous enterics*, and *Escherichia coli* [10–13]. Such viruses as the *Hepatitis* (A and E) *virus*, *Rotavirus*, *Norwalk virus* and others and parasites – helminthes and intestinal

pathogenic protozoa, the source of which may be water, shellfish, sick animals and people can also contribute their share of the risk of nutritional diseases.

Our own analytical review made it possible to investigate the morphological characteristics and specific cultural features of the growth of these microbial contaminants of food products on nutrient media. Given that the plant products, that are quantitatively dominant in the human diet, the experimental study on the qualitative and quantitative composition of the group of epiphytic microbiota group, the main most common types of juicy vegetable raw materials was conducted.

Aim of the researches was a study of qualitative and quantitative composition of microbial contaminants of vegetable raw materials and some products of its processing, their features and potential danger for a consumer.

Materials and methods

As objects of study common types of fruits, vegetables and berries were used. We studied the following species and varieties of vegetable raw materials: 4 apple varieties – *Renet Simirenko* var. apples (Odesa's and Poltava's regions), *Antonovka* var. apples, *Jonatan* var. apples and low-grade apples (Odesa's region), 2 varieties of pears – *Bergamot* var. pears and low-grade pears (Odesa's and Poltava's regions), 2 varieties of carrot – *Nantski* var. carrot and low-grade carrot (Odesa's and Poltava's regions), raspberries (Odesa's and Poltava's regions), sweet pepper, green pea, lettuce var. *Khutchiryavitz Odesskiy*, leaf parsley, cucumbers (Odesa's region); raspberry with and without peduncles, raspberries and strawberries of different maturity.

Conventional microbiological techniques were used: such as mesophylic aerobic and optional anaerobic bacteria (MAFAnM) have been considered by inoculation under beef-extract agar (BEA) ISO 4833-2:2013, fungi and yeasts was measured by wort agar ISO 13681:1995, ISO 21527-1:2008. Coliforms were determined by inoculation in liquid culture media by ISO 16649-2:2001, ISO 21528-2:2004, and *Bacillus cereus* was determined according to ISO 7932:2004. *Clostridium perfringens* have been considered by ISO 7937:2004 and in accordance with the method developed from the sample of microorganisms separated biomass primary cells by centrifugation at 1,500xg for 2 minutes and the supernatant filtered through nitrocellulose membrane filters of 0.22 micron diameter (Millipore) or 7,500xg centrifuged at for 10 minutes [14].

Results and discussion

Contaminants of fruits and vegetables were represented as typical and casual types of microorganisms that fall out from the soil, water, rainfall, wind-blown, birds, rodents, insects, and with the technological processing – also equipment, containers, vehicles, arms and clothing of workers and other objects.

The main source of contamination of vegetable raw materials is the soil, in 1 g which microbial quantity can range from 1 to 4 billion cells at a fairly high numbers of bacterial spores from a few thousands to several millions.

The information about microbial contamination and most widespread types of microorganisms in vegetable raw materials is given in Table 1.

Table 1
Microbiological description of fruits and vegetables raw materials going into processing and storage, (CFU/G)

Type of raw materials	Mold fungi	Yeasts	Bacteria	
			mesophilic bacteria	coliforms
The <i>Renet Simirenko</i> var. apples (Odesa's region)	69	$2,7 \cdot 10^2$	$1,8 \cdot 10^2$	7
The <i>Renet Simirenko</i> var. apples (Poltava's region)	$1,1 \cdot 10^2$	$2,0 \cdot 10^2$	$1,6 \cdot 10^2$	2
The <i>Antonovka</i> var. apples	81	$2,5 \cdot 10^2$	$3,9 \cdot 10^2$	5
The low-grade apples	101	$3,2 \cdot 10^2$	$4,3 \cdot 10^2$	15
The <i>Bergamot</i> var. pears	$1,2 \cdot 10^2$	$3,5 \cdot 10^2$	$7,4 \cdot 10^4$	3
The low-grade pears	$7,2 \cdot 10^2$	$5,0 \cdot 10^3$	$7,9 \cdot 10^4$	2
Sweet pepper	$2,4 \cdot 10^2$	$9,8 \cdot 10^5$	$6,5 \cdot 10^6$	4
Green pea	$2,8 \cdot 10^2$	$8,7 \cdot 10^5$	$6,1 \cdot 10^6$	6
The <i>Nantski</i> var. carrot	$9,4 \cdot 10^4$	$3,2 \cdot 10^5$	$9,2 \cdot 10^7$	$1,9 \cdot 10^3$
The low-grade carrot	$8,9 \cdot 10^4$	$4,1 \cdot 10^5$	$7,6 \cdot 10^8$	$2,9 \cdot 10^3$
Lettuce var. <i>Khutchiryavitz Odesskiy</i>	$3,1 \cdot 10^3$	$4,0 \cdot 10^2$	$6,7 \cdot 10^7$	51
Leaf parsley	$5,8 \cdot 10^3$	$7,9 \cdot 10^2$	$9,4 \cdot 10^6$	48
Cucumbers	$1,8 \cdot 10^2$	$9,2 \cdot 10^2$	$1,4 \cdot 10^5$	37

As it seen from the presented results, the dominating epiphytic microbiota group of vegetables and fruits studied are the bacteria. On vegetables are commonly occurred microorganisms of *Bacillus*, *Paenibacillus*, *Lactobacillus*, *Micrococcus* and *Alcaligenes* genera, and also then molds, among which the most typical representatives are *Penicillium*, *Fusarium*, *Alternaria*, *Botrytis*, *Sclerotinia*, *Risoctonia*. The surface of cucumbers, tomatoes and leaf vegetables is also the habitation zone of lactobacilli.

The special interest presents the berry's raw materials, which have comparatively delicate consistence, can cozily be traumatized, in consequence of which the epiphytic microbiota begins more actively develop (Table 2).

The qualitative composition of vegetable raw materials microorganisms gives an indication of both the possibility of epidemiological risk and product high quality. Contrary to previous opinion about the dominance among epiphytic microbiota of fungi, our results in some cases showed pre-printiat content of rod-shaped microorganisms.

Grown and picked in the same fruits of different varieties are distinguished in the predominant species of fungi. For example, on the surface Antonovka variety apples there are in average fungi of the such genera as *Alternaria* – 61%, *Mucor* – 10%, *Fusarium* – 9%, of other species – 20%. Whereas, the Renet Simirenko variety apples have fungi of the such genera as *Penicillium* (57%), *Aspergillus* (23%), other 20%, in average, the Jonatan variety apples have *Aspergillus* fungi (58%) (Figure 1).

Table 2
Microbiological description of berry raw materials going into processing and storage

Type of raw materials	Mold fungi	Yeasts	Bacteria	
			mesophylic bacteria	coliforms
The raspberry without peduncles, CFU/G	$2,7 \cdot 10^3$	$9,9 \cdot 10^4$	$6,4 \cdot 10^3$	102
The raspberry with peduncles, CFU/G	$3,3 \cdot 10^2$	$2,4 \cdot 10^4$	$7,9 \cdot 10^2$	12
The raspberry of picking maturity, %	10,6	86,2	3,2	0,09
The raspberries of technical maturity, %	2,5	92,1	5,4	0,005
The raspberry of overmatured, %	1,7	96,3	2,0	0,001
The strawberry of technical maturity, CFU/G	$1,8 \cdot 10^2$	$4,3 \cdot 10^3$	$3,9 \cdot 10^2$	35
The strawberry of technical maturity, %	3,4	87,9	8,0	0,7
The strawberry of overmatured, %	5,3	90,3	4,2	0,2

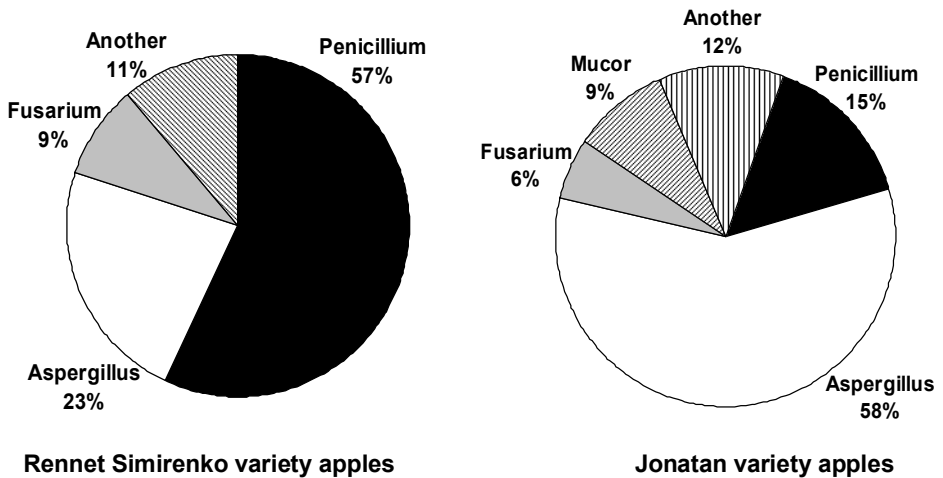


Figure 1. The group composition of fungi-epiphytes of apples

The comparative evaluation of contamination of raw materials from a variety of soil and climatic regions of microorganisms is shown in Figure 2.

The following fungi are capable of producing hazardous mycotoxins: *Penicillium patulum*, *Penicillium expansum*, *Penicillium urticae*, *Penicillium rugulosum*, *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium solani*, *Fusarium nivale*, *Fusarium circinatum*, etc. [5, 15, 16].

There are the methods for biological testing of toxic substances including the use of *Daphnia magna* S. in a variety of objects known [7, 17, 18]. The following Table 3 presents the results of patulin determination method which we have developed by using *Daphnia magna* S. [7] in tomato samples.

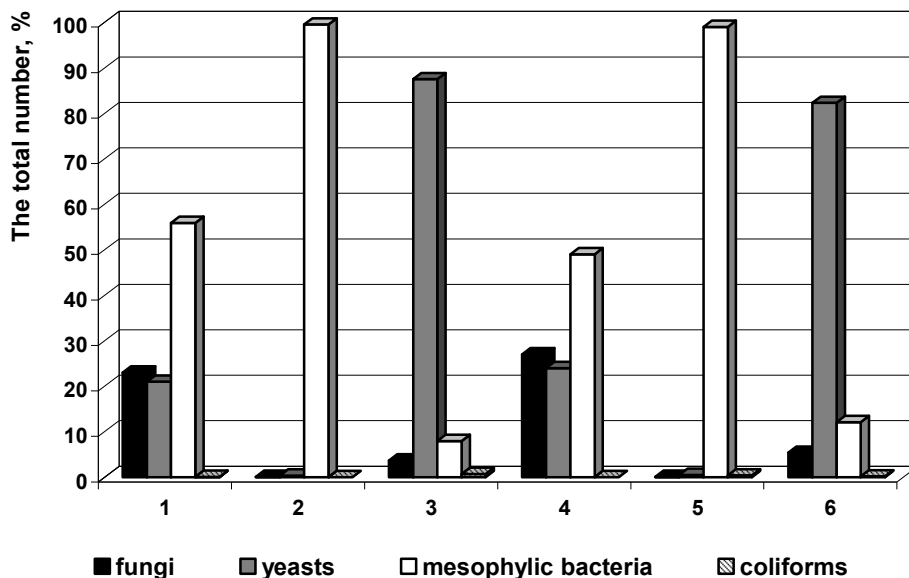


Figure 2. The microbial contaminants in raw materials:

1 – pears, Odesa`s region; 2 – carrot, Odesa`s region;
 3 – strawberry, Odesa`s region; 4 – pears, Poltava`s region;
 5 – carrot, Poltava`s region; 6 – strawberry, Poltava`s region

Table 3

The influence degree fruits spoilage on patulin accumulation

Samples	Approximate level of fruits destruction, % from total square	Patulin concentration, μkg
1	0 (without visible defects)	0,1
2	10	100
3	30	400
4	50	800
5	100	1800

As a result of investigation of juicy vegetable raw materials, the comparative numerical ratio of fungi, yeast, MAFAnM and coliforms as a part of epiphytic microflora of apples, grapes, peppers, carrots, and strawberry were found. Compared with other fruits the lowest number of microorganisms has been detected on the surface of apples. This can be explained by the presence of waxy coating on the surface of apples, which prevents microorganisms feeding. In some cases, the yeast on the surface of the berries dominates. A large number of microbial cells distinguish vegetables from fruits and berries.

A large number of soil microorganisms, including *Bacillus* and *Clostridium*, are on the surface of the plant material, especially root vegetables. According to the studies, the probability of detecting dangerous to human *Clostridium perfringens* on the leaves of green plants horse radish is up to 61%, on vegetables – up to 34%, based on the results of our study – up to 39%.

Subtilis-licheniformis microorganisms are the dominant raw materials contaminants and prevail in the composition of residual microbiota of product before sterilization. They were also detected in the residual microbiota of finished canning. The presence of aerobic bacillus does not always cause a significant change in the organoleptic properties of canned products. In some countries *Bacillus subtilis* are used as probiotics. However, analysis of the literature suggests the presence among them of microorganisms with toxigenic properties even in acceptable residual canned microbiota, which may lead to reduced immunity and cause various diseases in humans [19–21]. According to the information in Table 4, their high thermal stability contributes to their survival after technological processing of raw materials.

Table 4
Indices of the thermal stability of test organisms in phosphate buffer solution

Test organisms	pH	Temperature, °C	Thermal stability indices	
			$D_{T^{\circ}C}, \text{min}$	Z, °C
<i>Clostridium botulinum</i>	7,0	121,1	0,1...0,2	10,0
<i>Clostridium sporogenes</i>	7,0	121,1	0,6	10,0
<i>Bacillus (Geobacillus) stearothermophilus</i>	7,0	121,1	2,0...5,0	12,0
<i>Bacillus (Paenibacillus) polymyxa</i>	7,0	121,1	0,2	10,0
<i>Bacillus (Paenibacillus) macerans</i>	7,0	121,1	0,2	10,0

In experimental studies, aerobic spore-forming bacteria were isolated from 47 of the most popular canned products. Among these ones there were low acid canned vegetables (organic, and mixed, and puree). Pure cultures have been isolated from samples of sterile canned products. By their morphological, tinctorial, cultural and biochemical properties isolated microorganisms can be ascribed to *subtilis-licheniformis* group.

It should be noted that the food poisoning agents were detected from plant raw materials among isolated bacteria – *Bacillus cereus* and others. *Bacillus cereus* was found in 6.2% of the investigated samples of fruits, 33% of samples of carrot, 21% of samples of parsley, and up to 9.5% of the samples of canned food.

Conclusions

1. Group composition of epiphytic microbiota of different types of vegetable raw materials (vegetables, fruits, and berries) has been studied. Dominance of spore-forming rod-shaped organisms was detected in most samples.
2. Fungi have been found among the studied contaminants of vegetable raw materials samples. The concentration of the mycotoxin patulin, depending on the degree of spoilage fruits, was determined by the priority biological method developed by us.
3. High thermostability of spore-forming microorganisms of raw materials, including the test-cultures, was shown. This may account for their presence in canned products, cause deterioration of the organoleptic properties of foods and toxic effects on the human organism.
4. Development of modern methods of accelerated microbial detection is updated by abundance of microorganisms in the plant raw materials, and the duration and inaccurate identification of certain types of traditional methods of microbiological tests.

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Use of natural oils as bioactive ingredients of cosmetic products

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Abstract

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Introduction. Oils components embedded in the lipid structure of the horny layer of the epidermis, changing the properties of the epidermal barrier. The most important characteristic of fatty vegetable oils that determines the properties of cosmetic ingredient content is the esters of fatty acids.

Materials and methods. The mixes of oils (coconut, palm, almond, grape seed, olive, corn, sesame, wheat germ), which fatty acid composition imitated the structure of cell membranes were done by empirical method. It was made for creating cosmetic fat phase composition. Detection of fatty acids were carried out on the gas chromatograph production Hewlett-Packard NR6890 by conventional method.

Results and discussion. Empirical selection of possible mixtures of oils or mixtures of a particular calculation algorithm from an existing set of known oil fatty acid composition. The results of screening the fatty acid composition of traditional cosmetic oils show that fatty acids found in all known fats and oils, but their content varies widely. The most balanced composition is peanuts, wheat germ oil, olive, coconut, almond, palm and rapeseed oil. However, the compound of any of the individual oils do not meet the standards of cosmetology. Studied the characteristic ratio of linoleic and oleic acids for normal healthy skin is about 1:1,8, while for dry skin, it is about 1:4,7. The most optimal in terms of the content of mono- and poly unsaturated fatty acids is a composition comprising coconut, sesame oil and wheat. Value linoleic (C18:2) and oleic (C18:0) acids it is 1:8, which is adequate for normal healthy skin, and the ratio of polyunsaturated linoleic (C18:2) and alpha-linolenic (C18:3 ω -3) is close to the biologically effective level and is perfect 1:11 to 1:10.

Conclusions. Such cosmetic base consists entirely of natural plant oils and is designed for use in formulations and fat emulsion cosmetic care dry irritated skin, its supply and softening.

Introduction

After applying the oils on the skin as part of cosmetic nature their effects on the lipid barrier varies as the penetration into the deeper layers of the skin. First, spreading over the surface of the skin, the oil forms a protective hydrophobic film that reduces evaporation from the skin and prevents transepidermal water loss (TEWL). Oils consisting occlusive film interact with hydrolipid barrier and gradually absorbed by the skin. This range of physical and chemical interactions expanding their components embedded in the lipid structure of the horny layer of the epidermis, changing the properties of the epidermal barrier. Depending on the type and chemical composition of specific oils TEWL may decrease or increase, changing the penetration barrier for other ingredients. Some components of oils directly affect the properties of the lipid barrier, embedding in its structure, others involve in the synthesis of the structural elements of the skin.

The most important characteristic of fatty vegetable oils that determines the properties of cosmetic ingredient content is the esters of fatty acids (glycerol), called fatty acid composition. The most common of fatty acids in vegetable oils are composed of saturated palmitic (C16:0) and stearic (C18:0) acids, monounsaturated oleic acid (C18:1) and polyunsaturated linoleic (C18: 2) acid. Particular interest are the fats that contain essential fatty acids that are not synthesized in the body and must come from the outside: linoleic (C18:2 ω -6), alpha-linolenic (C18:3 ω -3) and gamma-linolenic (18:3 ω -6) acid. These acids are the starting material of metabolic reactions formation of prostaglandins, which can regulate inflammatory responses of the skin. That is why the failure of these fatty acids, the main of which is linoleic, the skin becomes irritated and prone to inflammation. We know now that the deficiency of alpha-linolenic (family ω -3) acid skin becomes irritated and prone to inflammation, while the lack of gamma-linolenic and arachidonic acid (Family ω - 6) lipid layers that form the barrier layer, lose strength and easily broken. [1–3] Fatty acid composition used in cosmetics as a separate ingredient or in natural glycerol lipids.

The aim of research was to create a fat phase composition of cosmetic products based on native vegetable oils, fatty acid composition is imitated to the composition of cell membranes, ensuring proper functioning and regeneration of the skin.

Materials and methods

The objects of study were chosen following oils: coconut, palm, almond, grape seed, olive, corn, sesame, wheat germ and other traditionally used technologies in cosmetic products. The subject of study were analysis of fatty acids called oils, their origin and quality. Fatty acid composition was determined according by conventional method. Detection of fatty acids were carried out on the gas chromatograph production Hewlett-Packard NR6890 with flame ionization detector, injector S/S of dividing flows Sp2380 column, length 100 m, inner diameter 0.25 mm, 0.2 micron coating thickness. Chromatography conditions: temperature 280 °C injector, dividing the flow of 100:1, the temperature detector 290 °C. The column operates in the constant flow speed of 1.2 ml/min helium carrier gas. Temperature gradient column thermostat is from 60 to 250 °C.

Results and discussion

Market offers mostly cosmetic additives preparations saturated fatty acids with a concentration of 65 to 99%. The source of essential polyunsaturated acids are the vegetable oils of natural origin. Cosmetic effect both synthetic and natural fatty acids appears the same way and does not depend on the origin of the preparation. Cosmetic effects common fatty acids are given in Table 1.

Table 1

Cosmetic properties of fatty acids

Fatty acids		Cosmetic properties
Symbolic designation	Nomenclature name INCI	
C12:0	Lauric Acid	Lauric acid enhances the antimicrobial properties of skin protective shell, has antimicrobial and antibacterial properties, negatively acts on a variety of pathogens, bacteria, yeast, fungi and viruses. Used in the manufacture of soaps, creams and other cosmetics. Lauric acid content in the product formulation of 99%.
C14:0	Myristic Acid	Myristic acid helps restore the protective properties of the skin, has high sliding and lubricating properties. It is used in cosmetics for thickening and stabilizing emulsions, as well as to enhance penetration into the skin of other components. Myristic acid content in the product formulation of 99%.
C16:0	Palmitic Acid	Palmitic acid and its derivatives are used as structure-, emulsifier, emollient. Palmitic acid content in the products of different brands 98%, 99%.
C18:0	Stearic Acid	Stearic acid helps restore the protective properties of the skin. Makes opaque mixture is used as a thickener in the manufacture of solid cosmetics. It is used in concentrations of 2–5% for creams and lotions, and 25% for solid of cosmetic products in the form flow. Stearic acid content in the product formulation 92–96%.
C18:1 9c	Oleic acid	Oleic acid activates lipid metabolism, restoring the barrier function epidermis and retains moisture in the skin. Slows lipid. Foods containing oleic acid, is well absorbed into the skin, it increases the penetration of other active components in the stratum corneum. Used in the production of creams and other cosmetic products. The content of oleic acid in the products of different brands of 65%, 70%, 75%.
C16:0, C18:0	Palmitic Acid, Stearic Acid	Used for the production of liquid cream. Blended products containing palmitic (55–60%) and stearic (39–45%) acid.

The composition and proportions of fatty acids (glycerol) that would ensure the most positive effect of a particular cosmetic composition seems reasonable approach, which uses glycerol those types that make up the lipid barrier of the skin in their natural composition, characteristic of normal healthy skin. In particular, in [5], citing [Houtsmuller U.M.T., 1981 and M. Mao-Quing, 1993] showed that a mixture of lipids that form the basis of the lipid barrier of the skin much more effectively supports the skin's ability volohoutrymuvalnu and restores lipid bar ' Pierre at external injuries than any of the components of the mixture separately. Moreover, the most effective influence mixes lipids observed in their natural proportions typical of normal healthy skin, as opposed to a mixture of the same components in sub-optimal proportions [6].

Biological activity of lipids can be based on two mechanisms: the biochemical reaction of eicosanoids and interact with specific receptors. Biological activity of essential fatty acids is the most thoroughly studied. In 1963 Morhauer N. and others first showed that linolenic acid deficiency caused growth inhibition in rats and its supplement decreased the signs of deficiency. In 1972, J. Polsrud and others found and A. Hansen and others in 1987 clarified that the lack of linolenic acid causes dermatitis in case of receipt of parenteral diet free of fat. Polyene fatty acids having two or more unsaturated (double) bonds, found in about 25 different fats, but the most important are three: linoleic, linolenic, arachidonic. These acids can occur in the form of isomers: cis, cis-cis, trans and trans-trans isomers; only cis-cis isomer has a physiological activity. Among the four groups of polyunsaturated fatty acids (PUFAs) can isolate family linoleic acid (omega-6 or ω -6) and linolenic acid family (ω -3) (Table 2).

Table 2

Representatives of essential fatty acids families

Omega-3	Omega-6
α -linolenic C 18:3	Linolenic C 18:2
Oktadekatetraenoic C 18:4	γ -linolenic C 18:3
Eykozatetraenoic C 20:4	Dihomo- γ -linolenic C 20:3
Eicosapentaenoic C 20:5	Arachidonic C 20:4
Docosahexaenoic C 22:6	Dokozapentaenoic C 22:6

The first signs of deficiency of polyunsaturated fatty acids in the skin occur in a variety of disorders in their intensity. According to the current level of knowledge, it appears that representatives of ω -6 family are more important for the normal functioning of human skin.

The source of essential polyunsaturated acids is the vegetable oils of natural origin. Cosmetic effects both synthetic and natural fatty acids appear the same way and do not depend on the origin of the drug.

For many years, these groups are summarized called «vitamin F». Now the name of the marketing considerations used in nutrition and cosmetics, but not in medicine. Linoleic and linolenic acids are the only truly exogenous essential fatty acids that are not synthesized in the body and must come from outside, mainly from food. Proved that the simultaneous presence of both essential fatty acids is not necessary because in the body there is a transition of some other acids. Since linoleic acid is formed as a result desaturative linolenic acid. By elongation (increase in chain length) and desaturation of gamma-linolenic acid, arachidonic acid can be obtained. This transition has been established by direct experiment with tracer [7].

In practice, empirical selection of possible mixtures of oils or mixtures of a particular calculation algorithm from an existing set of known oil fatty acid composition [8–9]. Table 3 presents a comparative analysis of fatty acids, 23 kinds of natural plant oils, traditionally used in the technology of fatty cosmetics.

Table 3

Fatty acids compounds of traditional cosmetic oils

Name of oil	Content of the main fatty acids, %			Ratio characterizing the biological effectiveness		
	MNSFA	PNSFA	NSFA	MNSFA: PNSFA: NSFA	C18:2: C18:1	C18:3: C18:2
Ideal lipid	33,3	33,3	33,3	1:1:1	1:1,8	1:10
Apricot	73,43	20,64	5,93	12:3,5:1	1:3,5	0:20
Amaranth	26,08	55,48	18,44	1,4:3:1	1:0,5	1:41
Peanut	48,50	33,30	18,20	2,7:1,8:1	1:1,4	0:33
Grape seed	19,88	68,60	11,52	1,7:6:1	1:0,3	1:115
Pumpkin	21,66	58,54	19,80	1,1:3:1	1:0	1:417
Mustard	69,63	25,54	4,83	14,4:5,3:1	1:5,4	1:1
Walnut	16,84	74,96	8,20	2,9:1:1	1:0	1:5
Wheat germ	16,32	64,08	19,60	1:3,9:1,2	1:0,3	1:9
Coffee	9,65	45,00	45,35	1:4,7:4,7	1:0,2	1:33
Cedar	27,64	64,81	7,55	3,7:8,6:1	1:0,6	1:2
Coconut oil	2,65	0,54	96,81	4,9:1:179	1:5,0	0:0,5
Hemp	14,90	74,34	10,76	1,4:6,9:1	1:0,2	1:3
Corn	27,87	61,07	11,06	2,5:5,5:1	1:0,5	1:500
Sesame	39,27	45,40	15,33	2,6:3:1	1:0,9	1:124
Flax	15,02	73,66	11,32	1,3:6,5:1	1:0,9	1:0,5
Almond	71,02	21,71	7,27	9,8:3:1	1:3,2	0:22
Sea buckthorn	48,81	22,00	29,19	2,2:1:1,3	1:0,4	1:3
Olive oil	73,39	11,04	15,57	6,6:1:1,4	1:10,1	1:12
Palm oil	26,08	5,18	68,74	5:1:13,3	1:5,1	0:5
Ccamelina	31,17	58,89	9,94	3,1:5,9:1	1:0,8	1:1
Rapeseed	65,32	27,82	6,86	9,5:4:1	1:3,2	1:2
Soybean	21,44	63,01	15,55	1,4:4:1	1:0,4	1:10
Sunflower	25,93	62,70	11,37	2,3:5,5:1	1:0,4	1:626

The results of screening the fatty acid composition of traditional cosmetic oils show that fatty acids found in all known fats and oils, but their content varies widely. In particular, palmitic C16:0 and stearic C18:0 acids found in all samples analyzed. Vegetable oil, which at room temperature are in liquid form (i.e., all the oils studied, except coconut

and palm) contain more palmitic acid than stearic. The most widely distributed in nature monounsaturated fatty acid with one double bond. In rare unsaturated fat acids found in much larger quantities than saturated constitute 80-90% of the total fatty acid compound. Vegetable oils often contain unsaturated oleic C18:1 and 9c elaidic acid C18:1 9t fatty acids with 18 carbon atoms and a much smaller quantities linoleic acid. About 70% oleic acid containing oil from apricot pits and Almond, 58-59% of a mustard seed and rapeseed. Linoleic C18:2 acid oils absent in stone – apricot, grape and almond. Among the vegetable oils most linolenic acid C18:3 ω -3 contains linseed oil, its content is 55,53%; γ -linolenic acid found in pine 18,81% and 2,57% hemp oils [10]. The most balanced composition is peanuts, wheat germ oil, olive, coconut, almond, palm and rapeseed oil. However, the compound of any of the individual oils do not meet the standards of cosmetology.

Behavior of cosmetic oils on the skin is similar to sebum, primarily because of its spreading and lubrication, resulting in the alignment of the upper layer of horny skin flakes. In this case they act as emollients. However, due to the natural oils inherent biological activity should take into account the nature of their interaction with lipid layers and effects on lipid metabolism in the epidermis.

With a lack of polyunsaturated fatty acids or violating their optimal ratio of liquid crystal membrane structures are replaced missing polyunsaturated acids to saturated or monounsaturated, changing the viscosity of the cell membrane, and consequently disrupted the normal functioning lipid barrier [11, 12, 17]. External manifestation of this change is to increase transepidermal water loss, increased exfoliation of cells of the stratum corneum and visible change in skin appearance.

Therefore, when compiling the optimum mix of triglycerides is crucial not only to the presence of essential fatty acids, but is very significant and their relationships. In [4, 14, 15] studied the characteristic ratio of linoleic and oleic acids for normal healthy skin is about 1:1,8, while for dry skin, it is about 1:4,7. The authors Patent RU 2218324 recommend the optimal ratio of linoleic and linolenic acids as 10:1, which is typical for normal healthy skin. Group correlation of saturated, mono- and poly unsaturated fatty acid as a 1:1:1 submitted by the recommendations of the Institute of Nutrition [16, 17].

Note that in the most cases enough to carry out the selection of the most physiologically active families glycerol, although some tasks may be necessary to consider other groups glycerol.

With an arbitrary set of oils accounted linear combination coefficients determined by the least squares method, given the above criteria. By calculation determined optimal composition of mixtures of oils that meet the requirements of balanced fatty acid composition (Table 4).

Table 4 presents a list of the oils compounds, the estimated composition of which is close to the recommended standards. The most optimal in terms of the content of mono- and poly unsaturated fatty acids is a composition comprising coconut, sesame oil and wheat. Value linoleic (C18:2) and oleic (C18:0) acids it is 1:8, which is adequate for normal healthy skin, and the ratio of polyunsaturated linoleic (C18:2) and alpha-linolenic (C18:3 ω -3) is close to the biologically effective level and is perfect 1:11 to 1:10. Other developed blends yield the composition, including the content of acid groups MNSFA:PNSFA:NSFA.

After applying the oils on the skin as part of cosmetic nature of their effects on the lipid barrier varies as the penetration into the deeper layers of the skin.

Table 4

Main ratio of fatty acids in the invented fat compounds

Name of sample	Content of the main fatty acids, %			Ratio characterizing the biological effectiveness		
	MNSFA	PNSFA	NSFA	MNSFA: PNSFA: NSFA	C18:2: C18:1	C18:3: C18:2
Optimum lipid	33,3	33,3	33,3	1:1:1	1:1,8	1:10
Compound of oils (1:1:1)						
Coconut – Sesame – Wheat germ	31,50	33,00	35,50	1:1:1,1	1:1,8	1:11
Coconut – Grape seed oil – Rapeseed	28,99	32,00	39,01	1:1,1:1,3	1:0,9	1:9
Coconut – Pumpkin – Rapeseed	29,58	28,68	41,74	1:1:1,5	1:0,8	1:8,3
Coconut – Peanut – Walnut	22,44	35,90	41,66	1:1,6:1,8	1:0,8	1:6
Coconut – Sunflower – Hemp	14,32	45,40	40,28	1:3,1:2,8	1:0,3	1:6,6
Coconut – Almond – Amaranth	32,92	25,65	41,43	1,3:1:1,6	1:1,3	1:57
Coconut – Grapeseed – Sea buckthorn	23,54	30,08	46,38	1:1,3:2	1:3	1:15,8
Coconut – Cedar – Walnut	15,55	46,30	38,15	1:3:2,5	1:1	1:3,2
Palm – Grape seed – Wheat germ	20,55	45,49	33,96	1:2,2:1,7	1:0,4	1:18
Grape seed – Sea buckthorn – Peanut	38,67	40,89	20,44	1,9:2:1	1:0,8	1:98
Grape seed – Sea buckthorn – Sunflower	34,52	40,59	24,89	1,4:1,6:1	1:0,4	1:27

Some components of oils directly affect the properties of the lipid barrier, embedding in its structure, others involved in the synthesis of the structural elements of the skin.

Since the 80s of XX century cosmetic affects PUFA described by two mechanisms. Physico-chemical mechanism PUFAs as lipids with high saturation are not able to directly influence the structure of intercellular substance of the horny layer. Additionally, PUFAs have biological activity by metabolites – eicosanoids. In the works of Katarzyna Pytkowska there is the third mechanism of PUFA: the ability to interact with receptors that are activated peroxisomal prolyferatorom PPAR, hormonal levels. Explanation PPAR involvement in the physiology of the skin converts natural lipids and their derivatives in a number of biologically active components of cosmetics.

Table 5

Cosmetic properties of oils elaborated compositions

Type of Oil		Cosmetic Properties
Trivial Name	Nomenclature name INCI	
Coconut Oil	Coconut Oil Refined	Coconut oil is a hardened vegetable fat with a specific smell; contains 50% lauric and 23% myristic acid which act as emollients. It forms a protective film on the skin and hair, perfectly softens the skin, as introduced in the formulation of cosmetics for dry skin, protective equipment for the skin and hair.
Sesame Oil	Sesame Oil Refined	Sesame oil is exclusive agent in cosmetics to care for dry skin damage, skin eyelids and child care, because it contains 48% linoleic acid, which activates lipid metabolism and restores the barrier function of the epidermis. It is used mainly in nourishing creams and masks for dry and damaged sensitive skin, massage products and balms hair.
Wheat-Germ Oil	Wheat-Germ Oil Refined	Wheat germ oil contains unsaponifiable fats, vitamins E, A and phyosterols in high concentrations, and therefore has unique regenerating and antioxidant properties. Widely used in cosmetics for the care of dry sensitive skin, maintains moisture balance of the epidermis. One of the best oils for the skin of the eyelids and lip balms because restores hydrolipid mantle, smoothes wrinkles, relieves peeling and irritation. For use in pediatric formulations of cosmetics.

Conclusions

Behavior cosmetic oils on the skin similar to sebum, is primarily spreading and lubrication, resulting in the alignment of the upper layer of horny skin flakes. In this case they act as emollients. However, due to the natural oils inherent biological activity should take into account the nature of their interaction with lipid layers and effects on lipid metabolism in the epidermis. Advantage calculated formula is not only optimal ratio of polyunsaturated acids both among themselves and with oleic acid, but the optimal balance between saturated, mono and poly unsaturated acids. Developed compounds are consist of natural vegetable oils for further using in fat and emulsion formulations of cosmetics to care for dry irritated skin, its nutrition and suppleness, softness.

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Rheological properties of gelatine solutions for production of gluten-free pasta

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Abstract

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Introduction. For formation of gluten-free pasta products of corn meal that does not form gluten, the selection of a structure forming substance, determining the way of its introduction and dosage on the basis of the research of the rheological properties of its solutions and impact on the quality of the goods are important.

Materials and methods. The rheological properties of colloid gelatine solutions with the concentration of 0,50–1,25%, which are prepared at the water temperature of 20 °C and 40 °C and the swelling duration of 40 min and at 60 °C without the swelling are examined. The viscosity of these solutions is determined by the Reotest-2 viscometer at the temperature of 20 °C. According to the data obtained, the rheological viscosity and fluidity curves are built and the rheological properties of the solutions are calculated. The impact of the forming substance solutions on the quality indicators of pasta products is determined.

Results and discussion. At the gelatine swelling temperature of 20 °C, the dynamic viscosity of the undistorted structure of the colloid solution decreases from 59,10 Pa·s to 21,89 Pa·s as its concentration increases from 0,50% to 1,25%, except for the solution with the concentration of 1,00%, for which the viscosity abnormality is noticed, and its viscosity is equal to 531,90 Pa·s. The similar research carried out at swelling at the water temperature of 40 °C showed that all colloid gelatine solutions are pseudoplastic liquids ($P_{K1} = 0$) at the concentration of 0,50–1,25% and have much lower dynamic viscosity for both the distorted and undistorted structure and lower strength of the structural frame than the same at the swelling temperature of 20 °C. For the sample with the concentration of 0,75%, the viscosity abnormality is noticed: at such concentration, the solution has the greatest dynamic viscosity of the undistorted structure, the greatest dynamic viscosity of distorted structure, 94,56 and 1,35 Pa·s respectively, the greatest value of $\eta_0 - \eta_m$, 93,21 Pa·s, and at the same time, the greatest strength of 425,52 Pa of the formed structural frame. The pasta products manufactured with the use of such solutions are of the best quality. At the temperature of 60 °C, the solutions have low viscosity and strength, thus forming weak gels that do not provide the formation of a solid structural frame and good quality of pasta products.

Conclusion. The gelatine dosage of 0,75–1,0% of the meal mass and the parameters of its preparation for production such as swelling in course of 40 min at the temperature of 40–20 °C respectively, which provide the highest viscosity of gelatine solutions of 94,6–531,9 Pa·s and facilitate the derivation of good-quality products, have been established.

Introduction

Over the last twenty years, the assortment of products in the pasta industry has narrowed dramatically. In particular, the products with enhanced nutritive value and those intended for health improvement and dietary purpose virtually are not produced.

Meanwhile, the number of metabolic diseases involving protein substances such as phenylketonuria and celiac disease among the population is growing. Prevention and treatment of these diseases consist in a special diet. For patients with celiac disease, products containing gluten are excluded from the food ration [13]. The food ration for the patients is very limited, so the promising line of diversification of the dietary pasta products assortment is production of gluten-free products [14, 15]. On the market of Ukraine, there are an insignificant number of non-domestic products for celiac disease patients [12]. The development of gluten-free pasta technology will facilitate providing the Ukraine's population with high-quality domestic products.

The raw substance for production of gluten-free pasta products is the by-products derived from corn, rice, buckwheat. The use of corn meal also provides an opportunity to create new products with enhanced chemical composition by means of their enrichment with the substances that are essential for the organism such as polyunsaturated fatty acids, fibre, β -carotene, iron and so forth.

Owing to the fact that corn meal does not form gluten that is of technological importance and is a principal structure forming substance in dough, the essential condition for creation of the gluten-free pasta products is the use of structure forming substances that may function in lieu of gluten.

Structure forming substances of various origins have different structure and therefore manifest themselves in different ways in the process of production of food products. Protein additives of both the plant and animal origins are used traditionally for enrichment of pasta products. Protein products of animal origin have the most valuable and balanced amino acid composition. References [1] include the information on the use of dry egg albumen and gelatine for enhancement of the quality and increasing the biological value of pasta products of wheat meal. It is expedient to examine the opportunity to use gelatine as a structure forming substance for production of gluten-free products of corn meal. This structure forming substance is a food product that is relatively cheap and widely presented on the market.

Gelatine is a protein product obtained by acid and alkaline hydrolysis of the connective tissues of the animal raw substance with the following extraction with hot water [2, 3, 6]. The key property of gelatine is the ability to form jelly in water solutions. This ability is caused by the asymmetry of high-polymer chains that are formed by the gelatine solution [5, 11]. The more the asymmetry, the easier is the formation of a reticulate spatial frame of jelly, within the frame grid of which the water is contained.

There is a strict relation of solution viscosity, thickening and melting temperature with the molecular mass of gelatine and size of high-polymer chains [3, 7]. The lesser the molecular mass of gelatine, the lower the physical and chemical characteristics of its solutions.

The research of rheological properties of gelatine solutions and their impact on the quality of pasta products of corn meal will make it available to substantiate the parameters for the technological process of pasta products manufacturing and explain the mechanism of action of gelatine as a structure forming substance in dough.

Materials and methods

The rheological characteristics of colloid gelatine solutions and their impact on the quality of gluten-free pasta products have been examined. The corn meal of fine grinding and fast-soluble food gelatine are used as basic raw substances for pasta products manufacturing.

Colloid gelatine solutions are prepared with the concentration of 0,50–1,25% of the portion of water intended for preparation of dough at the water temperature of 20 °C, 40 °C and swelling duration of 40 min, and 60 °C without swelling. The remainder of the water required for mixing is introduced directly into the dough at the temperature of 60 °C. The gelatine dosage in the amount of 0,50–1,25% of the wheat mass is selected subject to the manufacturers' guidelines with regard to production of jelly products. The examination of rheological characteristics of these solutions is performed with the Reotest-2 viscometer at the temperature of 20 °C. The curves of system viscosity and fluidity are built according to the results obtained. At the treatment of the curves, the following viscosity and strength characteristics are calculated: dynamic viscosity of the undistorted structure (η_0 , Pa·s), dynamic viscosity of distorted structure (η_m , Pa·s), viscosity abnormality value ($\eta_0 - \eta_m$, Pa·s), static limit of the system's flowing ability, (P_{K1} , Pa), dynamic limit of the system's flowing ability (P_{K2} , Pa), strength of the structural frame formed (P_m , Pa), strength of structural relations (P_{K1}/P_{K2}), range of stress (P_m/P_{K1}). The nature of the structure formed (pseudoplastic fluid, structured solid-like body, Newtonian fluid, thixotropic solid-like body, etc.) is determined according to the fluidity curves.

For examination of the impact of colloid gelatine solutions on the quality of pasta products, the dough is mixed in the laboratory press MAKMA-M with the mass fraction of moisture of 36%, the mixing duration is equal to 10 min. The pasta products are formed as noodles, dried to the moisture of 13,0–13,5% at the room temperature.

The quality indicators of pasta products such as surface condition, colour, microcracks availability, fracture condition, taste, smell, strength, acidity and cooking properties (mass (K_m) and volume gain (K_v) coefficients, transition of dry substances into cooking water) are determined. The strength of the pasta is measured in Newtons (N).

Results and discussion

The experimental data analysis (Table 1) shows that, at the gelatine swelling temperature of 20 °C, the dynamic viscosity of the undistorted structure of the colloid solution decreases from 59,10 Pa·s to 21,89 Pa·s as its concentration increases from 0,50% to 1,25%, except for the solution with the concentration of 1,00%, for which the viscosity abnormality is noticed, and its viscosity is equal to 531,90 Pa·s. The dynamic viscosity of the distorted structure of these colloid solutions is much lesser, 0,72–6,23 Pa·s, and it grows with the increase of gelatine concentration within the examined range.

Value P_{K1} shows that the colloid gelatine solution, at the concentration of 0,50%, is a pseudoplastic fluid ($P_{K1} = 0$), and a structured solid-like body at greater concentration ($P_{K1} > 0$). As the gelatine concentration within the examined range increases, the dynamic limit of the system's flowing ability significantly grows (from 166,97 Pa to 1899,04 Pa) and so does the strength of the formed structural frame (from 236,40 Pa to 2364,00 Pa). Indicator P_{K1}/P_{K2} characterising the strength of structural relations within the system is the highest for the solution with the gelatine concentration of 1,00%, for which the viscosity abnormality is noticed.

Table 1
Rheological characteristics of the gelatine solutions with the concentration of 0,50–1,25% in relation to the swelling (dissolution) temperature

Gelatine concentration, %	Rheological characteristics of colloid solutions							
	$\eta_0, \text{Pa}\cdot\text{s}$	$\eta_m, \text{Pa}\cdot\text{s}$	$\eta_0-\eta_m, \text{Pa}\cdot\text{s}$	P_{k1}, Pa	$P_{k2}, \text{Pa}\cdot\text{s}$	P_m, Pa	P_{k1}/P_{k2}	P_m/P_{k1}
at swelling at the water temperature of 20 °C								
0,50	59,10	0,72	58,38	0,00	166,97	236,40	0,00	-
0,75	55,16	1,30	53,86	23,64	406,55	502,35	0,06	21,2
1,00	531,90	4,86	527,04	531,90	1023,33	1595,70	0,52	3,0
1,25	21,89	6,23	15,66	23,64	1899,04	2364,0	0,01	100,0
at swelling at the water temperature of 40 °C								
0,50	7,88	0,68	7,20	0	81,21	165,48	0	-
0,75	94,56	1,36	93,20	0	299,04	425,52	0	-
1,00	7,88	0,68	7,20	0	51,94	141,84	0	-
1,25	1,09	0,24	0,85	0	6,510	65,01	0	-
at dissolution at the water temperature of 60 °C								
0,50	0,16	0,09	0,07	0	10,0	24,3	0	-
0,75	0,26	0,14	0,12	0	12,5	35,2	0	-
1,00	0,31	0,19	0,12	0	30,0	55,5	0	-
1,25	0,54	0,49	0,05	0	25,0	75,7	0	-

Consequently, at the swelling temperature of 20 °C, the viscosity abnormality for the gelatine solution takes place at its concentration of 1,00%, and the highest viscosity value and quite high value of the system structure strength are achieved, which are determined by the high strength of structural relations. It is likely that such rheological properties of the solution of the structure forming substance may provide optimal structural and mechanical characteristics of dough and high quality of products. It is known [5] that the best quality of pasta products is achieved at the highest plasticity and strength of dough.

The similar research carried out at swelling at the water temperature of 40 °C showed that all colloid gelatine solutions are pseudoplastic liquids ($P_{K1} = 0$) at the concentration of 0,50–1,25% and have much lower dynamic viscosity for both the distorted and undistorted structure and lower strength of the structural frame than the same at the swelling temperature of 20 °C. At that, as the gelatine concentration increases, the dynamic viscosity of the solution and the strength of the formed structural frame decreases for both undistorted and distorted systems. It is evident that, at the temperature of 40 °C, the partial dissolution of gelatine and decrease of the strength of structural relations take place, which is evidenced by value P_{k1}/P_{k2} . However, for the sample with the concentration of 0,75%, the viscosity abnormality is noticed: at such concentration, the solution has the greatest dynamic viscosity of the undistorted structure, the greatest dynamic viscosity of distorted structure, 94,56 and 1,35 Pa·s respectively, the greatest value of $\eta_0-\eta_m$, 93,21 Pa·s, and, at the same time, the greatest strength of 425,52 Pa of the formed structural frame.

Supposedly, the viscosity abnormality at the concentration of the solution of 0,75% may be explained by the fact that, at the water temperature of 35–40 °C, gelatines

act as unordered spirals that may take the infinite number of unsteady configurations, as the aggregation of the solution takes place at cooling. At the concentration of 1,00% and more, depending on the gelatine quality and its pH, a colloid solution is formed, which is gel as for its structure. Authors [4, 8, 9] consider the areas of gelatine chains which are rich of pyrrolidine to be a centre of formation of possible connective zones, where the collagen-like triple spiral is formed at the aggregation of these chains, which functions as the points or zones of gel formation. These zones are stabilized with the hydrogen links within the chain, which disintegrate at the temperature of 35–40 °C due to the thermolability at the concentration of gelatine of 1,00% and more, which causes the melting of gel. It is evident that, at the concentration of the colloid gelatine solution of 0,75%, gel does not melt yet and to a greatest extent manifests the properties of a structure forming substance.

For the fast-soluble gelatine, it is recommended by the manufacturer that the dissolution temperature be 60 °C, therefore the solution is not held at such temperature for swelling. The relation of the dynamic viscosity of the solution with the shear stress is given in Figure 1, and Table 1 data shows almost complete dissolution of gelatine: the dynamic viscosity of undistorted and distorted structure is very low and slightly changes as the concentration of the solution increases, and it is equal to $\eta_0 - 0,16-0,54$, $\eta_m - 0,05-0,12$ Pa·s respectively. The solutions are pseudoplastic liquids, which are characterised by the low strength of the structural frame and structural relations.

As it is known [4, 10], in the gelatine gel held at a high temperature, a few collagen-like chains are formed and the remainder of each polypeptide chain interrupts the order, which entails the formation of weak gels. It is evident that the weak gels formed cannot fully prove to be structure-forming substances for pasta products.

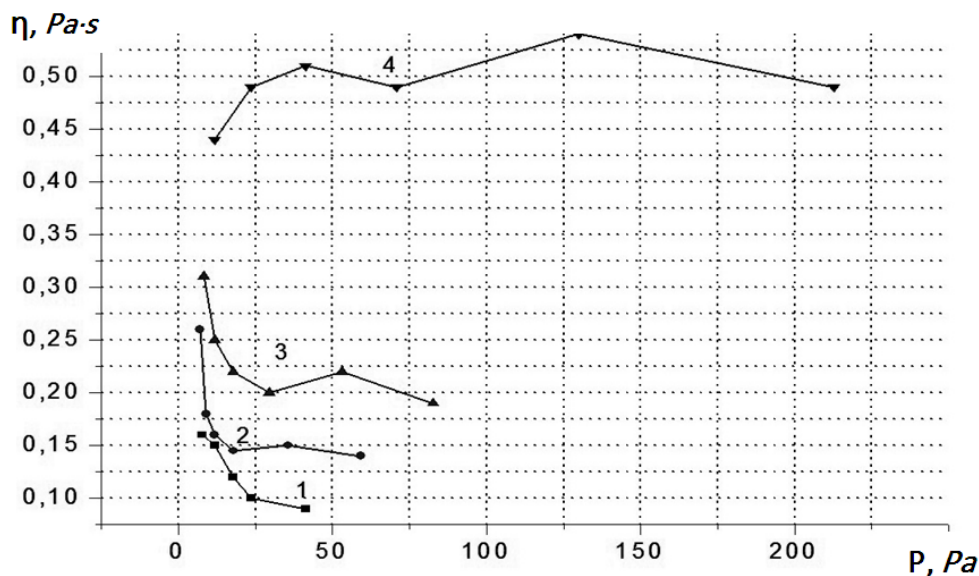


Fig. 1. Curves of the dynamic viscosity relation of the colloid gelatine solution at the water temperature of 60 °C (η) with the shear stress (P):
1 – c=0,5%; 2 – c=0,75%; 3 – c=1,00%; 4 – c=1,25%

The results of the analysis of the quality indicators of the products manufactured with the use of the gelatine solution obtained at the swelling temperature of 20 °C are presented in Table 2.

Table 2

Quality indicators of gluten-free pasta products of corn meal with the gelatine solution prepared at the water temperature of 20 °C

Indicators	Products with gelatine dosage%			
	0,50	0,75	1,00	1,25
<i>Organoleptic indicators</i>				
Surface	rough	slightly rough	smooth	slightly rough
Colour	creamy		light yellow	
Fracture	mealy	slightly mealy	glassy	
Microcracks availability	solitary		absent	
Smell	peculiar to corn meal			
Taste	peculiar to corn meal			
<i>Physical and chemical indicators</i>				
Acidity, degrees	3,4	3,2	3,4	3,4
Strength, N	5,8	5,9	6,9	6,3
<i>Cooking properties</i>				
Form preservation	lost	retained, products do not stick together		
Mass gain coefficient, Km	1,7	1,7	1,8	1,6
Volume gain coefficient, Kv	1,6	1,8	1,8	1,7
Transition of dry substances into cooking water, % of DS	28,5	23,2	21,1	22,8

It is established that the quality of products as for organoleptic indicators, strength and cooking properties improves as the dosage of gelatine increases. All samples, except for the products with the use of 0,50% of gelatine, have acceptable quality, however, the sample of pasta products of corn meal with the gelatine dosage of 1.00% is of the best quality. In terms of its organoleptic quality properties, this sample is distinguished from others by its light yellow colour, smooth surface, glassy fracture, high strength of 6,9 N and the lowest transition of dry substances into cooking water, 21,1% of dry substances. This particular dosage provides the highest rheological characteristics of the solution. Gaining the dosage over 1,00% also provides relatively good product quality, but it is still lower.

The quality indicators of gluten-free corn meal pasta products manufactured with the use of the colloid gelatine solution at the swelling temperature of 40 °C are presented in Table 3.

The data obtained shows that, at such temperature of gelatine preparation for production, the product quality is slightly lower compared to the use of the solution obtained at the swelling temperature of 20 °C, except for the sample with the gelatine dosage of 0,50%. The product strength, transition of dry substances into cooking water and the coefficients of mass and volume gain at the time of boiling are reduced. However, the best product quality is achieved at the dosage of 0,75%. This sample has the light yellow colour, slightly rough surface, glassy fracture, a bit higher strength, 4,8 N, and the lowest

percentage of transition of dry substances into cooking water, 22,2% of dry substances. At this particular dosage of the solution, the viscosity abnormality of the colloid solution and the highest values of viscosity and strength of its structural frame are noticed.

Table 3
Quality indicators of gluten-free corn meal pasta products with the colloid gelatine solution at the water temperature of 40 °C

Indicators	Products with the dosage of gelatine, %			
	0,50	0,75	1,00	1,25
<i>Organoleptic indicators</i>				
Surface	rough	smooth		slightly rough
Colour	creamy	light yellow		
Fracture	mealy	glassy	slightly mealy	glassy
Microcracks availability	available	absent	solitary	
Taste	peculiar to corn meal			
<i>Physical and chemical characteristics</i>				
Acidity, degrees	2,8	2,6	2,8	3
Strength, N	4,3	4,8	4,6	4,5
<i>Cooking properties</i>				
Form preservation	retained, products do not stick together			
Mass gain coefficient, Km	1,5	1,6	1,7	1,6
Volume gain coefficient, Kv	1,6	1,7	1,7	1,7
Transition of dry substances into cooking water, % of DS	24,3	22,2	23,6	23,6

The quality indicators of the gluten-free corn meal pasta products manufactured with the use of the gelatine solution obtained at the water temperature of 60 °C are presented in Table 4.

It has been established that, in this case, the quality of pasta products as for the indicators of glassiness and strength is almost on par with such of the products manufactured with the use of the gelatine solution obtained at the swelling temperature of 40 °C. However, in terms of the cooking properties such as transition of dry substances into cooking water, product mass and volume gain coefficients, they are much worse and have unacceptable quality as for the indicator of transition of dry substances into cooking water, nearly 25% of DR and higher.

Consequently, basing on the results of the examination of the rheological characteristics of colloid gelatine solutions with the concentration of 0,50–1,25% at the water temperature of 20 °C, 40 °C and 60 °C, it has been proven that the best quality of pasta products of corn meal is achieved at the gelatine dosage of 0,75–1,00% of the mass of meal and the parameters of its preparation for production such as swelling in course of 40 min at the temperature of 40–20 °C respectively, which provide the highest viscosity of gelatine solutions, 94,6–531,9 Pa·s. The pasta product quality deteriorates as the solution viscosity decreases.

Table 4

Quality indicators of gluten-free corn meal pasta products with the colloid gelatine solution at the water temperature of 60 °C

Indicators	Products with the dosage of gelatine, %			
	0,50	0,75	1,00	1,25
<i>Organoleptic indicators</i>				
Surface	rough	slightly rough	smooth	slightly rough
Colour	creamy	light yellow		
Fracture	glassy			
Microcracks availability	solitary	absent		
Taste	peculiar to corn meal			
Colour	peculiar to corn meal			
<i>Physical and chemical characteristics</i>				
Acidity, degrees	3,2	3	3,2	3,4
Strength, N	4,2	4,4	4,5	4,6
<i>Cooking properties</i>				
Form preservation	retained, products do not stick together			
Mass gain coefficient, Km	1,6	1,5	1,5	1,5
Volume gain coefficient, Kv	1,7	1,7	1,6	1,7
Transition of dry substances into cooking water, % of DS	28,8	25,9	24,5	24,8

Conclusions

Basing on the conducted research, the structure forming ability of gelatine and feasibility of its use for the formation of gluten-free pasta products of corn meal that does not contain gluten has been proven.

It has been established that the viscosity of gelatine solutions is reduced with an increase in the swelling temperature up to 40 °C by virtue of partial dissolution of gelatine, and that their viscosity is significantly reduced at the water temperature of 60 °C.

It has been established that, at the gelatine swelling temperature of 20 °C, the viscosity abnormality of the solutions with the concentration of 1,00% takes place, and that is the case at the temperature of 40 °C with the concentration of 0,75%. The viscosity of these solutions is equal to 531,9 Pa·s and 94,56 Pa·s respectively.

It has been established that the best quality of the pasta products of corn meal is achieved with adding 1,00% of gelatine to the meal mass in a form of a colloid solution at the water temperature of 20 °C. At the temperature of 40 °C, the pasta products with the dosage of gelatine of 0,75% are of the best quality. At the temperature of 60 °C, the dissolution of gelatine takes place, and there are weak gels formed, which cannot prove to be structure forming substances.

Upon the rheological research results, the optimal viscosity range of the colloid gelatine solutions has been initially established for production of gluten-free corn meal pasta products of good quality, and it is 94,6 up to 531,9 Pa·s.

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Microbiological investigation of wild, cultivated mussels (*Mytilus galloprovincialis* L. 1819) and stuffed mussels in Sinop–Turkey

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Abstract

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Introduction. To purpose of study – to investigate the microbiological properties of wild and cultivated mussels and stuffed mussels sold by restaurants and street vendor in August and September.

Materials and methods. In total 68 mussel (*Mytilus galloprovincialis* L. 1819) and stuffed mussel samples were investigated by using total aerobic mesophilic bacteria, total Coliform bacteria, *Escherichia coli* and *Vibrio* spp. were performed by standard procedures. The sampling was carried out aseptically for the microbiological analysis. All of the microbiological analyses were conducted in triplicate.

Results and discussion. The initial total aerobic mesophilic bacteria, total Coliform bacteria, *E. coli* counts of wild and cultivated mussels in August were 4.04 Log CFU/g and 3.55 Log CFU/g, 3.69 Log CFU/g and 3.09 Log CFU/g, 0.59 Log CFU/g and 0.39 Log CFU/g respectively. Total bacteria, total coliform and *Vibrio* spp. numbers of wild mussels were higher than cultivated mussels ($p < 0.05$). *Vibrio* spp. were not found associated with cultivated mussels.

The number of total aerobic mesophilic bacteria, total Coliform bacteria and *E. coli* in stuffed mussels sold by street vendors were determined higher than that found associated with stuffed mussels sold in restaurant ($p < 0.05$) in August. In September, *E. coli* were not detected in stuffed mussels sold by restaurants, and street vendor. No stuffed mussel samples exceeded an acceptable limit value (6 Log CFU/g) for aerobic plate count in the months of August and September. In stuffed mussels *Vibrio* spp. were found except for stuffed mussels sold by street vendor in September.

The stuffed mussels were made from wild mussels and the aerobic mesophilic bacteria, total Coliform bacteria and *E.coli* numbers of them were more than wild mussel.

Conclusion. *E. coli* were not found in stuffed mussels sold in restaurant in both two months, whereas *Vibrio* spp. were detected in twenty seven of total forty eight stuffed mussel samples collected from street vendors and restaurant.

Introduction

The Mediterranean mussel, *Mytilus galloprovincialis* (Lamarck), is a bivalve and it occurs naturally in Black Sea coast and the other coast of Turkey. In 2012, 2093.4 tons of *M. galloprovincialis* were caught in Turkey and around 92% of it was in Western Black Sea region [1]. Especially in Sinop region, collecting and consumption of wild mussel are developed. Mussel's edible portion contains approximately 80% water, 9–13% protein, 0–2% fat and 1–7% glycogen depending on the season, feeding, maturity and water temperature [2]. Moreover, Mediterranean mussel (*M. galloprovincialis*) harvested from the Black Sea coast contains about 82.99–86.06% moisture, 10.24–10.30% protein, 1.49–1.14% fat and 1.14–0.95% ash [3, 4].

M. galloprovincialis present along the national coast and consumer appreciate it because of its organoleptic properties; it is retained also after processing, and for the competitive price if compared with other bivalves [5]. In our country, the mussels are generally exported. In addition, they are widely consumed as fried in some restaurants. In addition, some of them are canned or frozen [6].

Street food is defined as “ready-to-eat foods and beverages prepared and/or sold by street vendors and hawkers, especially in the street or other similar public places” by FAO [7]. The most popular local street foods in Turkey are meat and chicken doner, bagel, stuffed mussel, fried mussel, ice cream, fish bread, raw meatballs. Stuffed mussel is a delicious traditional food like an appetizer especially in coastal areas in Turkey. Generally, street vendors sell stuffed mussel so that it is called as a street food. Stuffed mussels along the Sinop coast are generally sold in August and September by street vendors and restaurants.

Kışla and Üzgün [8] defined stuffed mussel produced in Turkey as “mussel shells are cleaned with water by scraping with a knife, and any beards are removed; mixture is prepared with rice, vegetable oil, salt, and spices; mixture is stuffed into each shell included mussel flesh, and shells are closed tightly before cooking by steaming; vegetable oil is sprayed on the shells of stuffed mussels because of bright surface”.

The microbial load of seafood after collecting is closely related to environmental conditions such as; water temperature, salt content, distance between localization of collected and polluted areas (human and animal feces), natural occurrence of bacteria in water, methods of harvest, handling, storage practices and chilling factors [9, 10].

Most food vendors ignore of good food handling practices, exposing foods to dangerous conditions such as unsuitable conditions, unsafe storage and poor time-temperature conditions, so that it can cause food poisoning. In Selangor-Malaysia in 1993, two cholera outbreaks were linked to street food [11, 12]. Ready-to-eat foods such as stuffed mussels sold in the open areas without any precautions could be a major cause of food-poisoning and foodborne diseases [13].

The purpose of this study was to examine; a) the microbiological quality differences of wild and culture Mediterranean mussel (*M. galloprovincialis*) collected from Sinop region, and b) the microbiological qualities of stuffed mussel sold by street vendors and restaurants in Sinop.

Materials and methods

Materials. Mediterranean mussels (n= 20; 10 wild, 10 cultivated) (Figure 1) were collected from Black Sea and transported within half an hour to the laboratory on August.

Cultured mussels were collected in submerged long line mussel culture system in offshore in Black Sea.



Fig 1. Wild (left) and cultivated (right) mussels (Photos: D. Kocatepe)

Stuffed mussels (n=48) were sold in the afternoon (roughly 5:00 p.m.) from street vendors and restaurants in August and September. The stuffed mussels investigated in this study were produced from wild mussels. Products were prepared in the morning and they were put up for sale throughout the day on the bench by street vendors but they are kept at cold and offered for sale at bench gradually at the restaurant.

Methods. In the sampling days, the mean of weather temperature in August and September were 28°C and 24°C, respectively.

The sampling was carried out aseptically for the microbiological analysis. Each mussel meat and stuffing mixture was removed from the shells, mixed, and a 10-g portion transferred into 90 ml of sterile 0.1% peptone water containing 3% NaCl except from *Vibrio* spp. analysis. The sample was homogenized for 2 min and serially diluted as needed for plating. The following media and incubation condition were used. Total mesophilic aerobic bacteria (TMAB) were determined using Plate count agar (PCA, Merck code: 105463.0500) after incubation for 2 day at 30°C. Total coliform bacteria (TCB) were enumerated on violet red bile agar (Merck 1.15525, Lancashire, UK) by the double-layer pour plate method and incubated at 35°C for 24h [14]. For *E. coli* bacteria used violet red bile+Mug agar (Merck 1.4030, Lancashire, UK) and incubated at 37°C for 18h. Colonies with blue fluorescence under UV light were counted [15].

To determine *Vibrio* spp.; 25 grams of homogenized sample mixture were transferred into sterile bottles. Then 225 ml of alkali-peptone water (Merck 1.01800, Lancashire, UK) was added and incubated at 35–37 °C for 8 h. Then a loop full enrichment broth was streak plated onto thiosulfate–citrate–bile salt sucrose agar (Merck 1.10263, Lancashire, UK) and plates incubated at the same temperature for 24 h [15, 16]. After incubation, gram-stained and tested for oxidase activity and ability to ferment glucose (using modified Hugh Leifson's medium including 26g NaCl g/L [8]. The results were given as the number of positive samples.

All of the microbiological analyses were conducted in triplicate. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU g⁻¹).

Microsoft Excel (Microsoft Corp., Redmond, WA) and Minitab Release 13.20 (Minitab, Inc., State College, PA) evaluated statistical analysis. One-way variance analysis used for statistical evaluation of data [18].

Results and discussion

The results of microbial analysis of wild and cultivated mussels at Black Sea are shown in Table 1. Mussels pump large quantities of water through their bodies, so they accumulate the toxic substances and microorganisms present in ambient water [19].

Table 1
The microbial flora of wild mussels and cultivated mussels in Sinop, south of Black sea

Month Temperature	Product	TMAB (Log CFUg ⁻¹)	TCB (Log CFUg ⁻¹)	<i>E. coli</i> (Log CFUg ⁻¹)	<i>Vibrio</i> spp.*
August 28°C	Wild mussels	4.04 ± 0.03 ^a	3.69±0.02 ^a	0.59±0.24 ^a	10(10) ^a
	Cultivated mussels	3.55 ± 0.02 ^b	3.09 ±0.02 ^b	0.39 ± 0.21 ^a	- (10) ^b

Note:

TMAB: Total aerobic mesophilic bacteria. TCB: Total Coliform bacteria.

* Values in parentheses are number of samples; values outside of parentheses are number of positive samples.

a, b, c (↓): Means in the same column with the same letter do not differ at the level of 0.05 significance.

“-“ Not detected.

The initial TMAB counts of wild and cultivated mussels in August were 4.04 Log CFU/g and 3.55 Log CFU/g, respectively (Table 1). TMAB and TCB numbers of cultivated mussels were lower than wild mussel ($p < 0.05$). While the *Vibrio* spp. was detected in 100% of wild mussels, *Vibrio* spp. were not found in none of cultivated mussel samples. The differences between wild and cultivated mussels were statistically significant ($p < 0.05$). Çağlak et al. [18] and Ulusoy [20] reported that the number of total viable bacteria of mussels (*M. galloprovincialis*) on day harvesting were 3.25 Log CFU/g, 2.34 Log CFU/g, respectively. The total bacteria, *Coliform* and *V. parahaemolyticus* counts of mussels collected from three different stations on the coast of Trabzon in Black Sea during a year were maximum 5.62, 4.77, 3.47 Log CFU/g, respectively [21]. According to the results, cultivated mussels contained less microorganisms than wild mussels. The microbiological properties of water may affect the microbial flora of mussels. Microbiological load of wild mussels caught in coast of Sinop is higher than mussels harvested from the cultivating system in the open sea area, because of sewage, shipyards and garbage.

The microbiological results of stuffed mussels were sold by street vendors and restaurant in Sinop coast of Turkey are shown in Table 2.

Table 2

The microbial flora of stuffed mussels sold by street vendors and restaurant

Month Temperature	Product	TMAB (log CFUg ⁻¹)	TCB (log CFUg ⁻¹)	<i>E. coli</i> (log CFUg ⁻¹)	<i>Vibrio</i> spp.*
August 28°C	Stuffed mussels sold by street vendor	5.35±0.07 ^a	4.14±0.01 ^a	1.17±0.39 ^a	13 (16) ^a
	Stuffed mussels sold by restaurant	5.02±0.02 ^{bA}	3.31±0.02 ^{bA}	- ^{bA}	8 (16) ^{aA}
September 24°C	Stuffed mussels sold by street vendor	/	/	/	/
	Stuffed mussels sold by restaurant	4.96 ± 0.01 ^A	3.08±0.01 ^B	- ^A	6 (16) ^A

TMAB: Total aerobic mesophilic bacteria. TCB: Total Coliform bacteria.

“/” : Sample was not found.

*Values in parentheses are number of samples; values outside of parentheses are number of positive samples.

a, b, c (↓): For the two groups, means in the same column with the same letter do not differ significantly at the level of 0.05 significance on August

A, B (↓): For the stuffed mussels sold by restaurant, means in the same column with the same letter do not differ significantly at the level of 0.05 significance on August and September.

“-“ Not detected

Stuffed mussels are prepared from wild mussels collected from the coastal areas and then sold from different sale points like restaurants, street vendors, and cafes. No stuffed mussel samples exceeded an acceptable limit value (6 Log CFU/g) for aerobic plate count indicated by TGK [22]. In this study, the numbers of TMAB, TCB and *E. coli* in stuffed mussels sold in street vendors were higher than stuffed mussels sold by restaurants (p<0.05) in August. Kök et al. [19] reported that the microbiological analysis of stuffed mussel samples showed that the TMAB were ranging between <2 and 6.44 log CFU/g in Aydın and İzmir, Turkey.

Maximum acceptable *E. coli* number for prepared foods (like snacks) and meat products (cooked) is <10 CFU/g [23]. Stuffed mussels were sold by street vendor in August exceeded this limit value. Whereas, *E. coli* were not detected in stuffed mussels sold by restaurant during August and September. Ateş et al. [13] emphasized in their study that 76.6% samples of stuffed mussel were unacceptable.

Comparing the samples collected from restaurant (it was kept at cold and offered for sale at bench gradually) and street vendors; the stuffed mussels sold in restaurant had lower bacteria than the other had. Hampikyan et al. [24] reported that the total viable bacteria,

Coliform and *E. coli* counts of stuffed mussels sold in Istanbul were maximum 2.3×10^7 , 5.8×10^6 , 4.0×10^1 CFU/g, respectively. According to the studies, the number of bacteria detected from stuffed mussels in Sinop was lower than that sold in Istanbul. There was no differences ($p > 0.05$) in point of positive sample numbers of *Vibrio* spp. between the stuffed mussels sold in street and restaurant in August. In September, there could not be found sample from street vendors. TMAB, *E. coli*, *Vibrio* spp. in the stuffed mussel sold in restaurant in August and September were similar ($p > 0.05$), meanwhile; number of TCB in September significantly decreased ($p < 0.05$). This may be because of weather temperature.

Total number of bacteria in mussels can be consumed, it should not have been out of 5 billion/g [25]. The total number of mesophyll bacteria of the wild mussels, cultivated mussels and stuffed mussels in Sinop did not exceed consumable limit. Whereas *Vibrio* spp. were determined in stuffed mussels, therefore the importance of hygiene during processing and initial microbial load of raw mussels must be emphasized. The similarly as our results, Kök et al. [19] indicated that stuffed mussels might constitute a potential health hazard, especially when kept at high ambient temperatures, depending on contamination level and lack of sanitary practices, and therefore, handling practices should require more attention and improvement.

Conclusion

Mussels filter the water for feeding, therefore; they also take the unwanted substance (industrial waste, petrol compounds, heavy metals, agricultural waste, sewage, pathogenic microorganisms) from the area. Microbial loads of wild mussels show an alteration in cultivated mussels. In our study; microbiological loads of wild mussels obtained from the coast of Sinop and cultivated mussels were compared. Microbiological loads of cultivated mussels were found lower than wild mussels. Consuming of raw mussels collected from sea and stuffed mussels prepared from wild mussels may be dangerous for public health because of especially *Vibrio* spp. number. It can be said that using of cultivated mussels for stuffing is more appropriate than wild mussel. However, we can safely consume or use the mussels collected from the clean sea/water in terms of microbiological.

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Enhancement of microbial transglutaminase production from *Streptomyces* sp.

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Abstract

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Introduction. Enhancement of microbial transglutaminase (MTGase) production was investigated in terms of creating a model for future studies about the usage of microbial transglutaminase which might become popular in the next years.

Materials and methods. In order to determine the highest enzyme activity, the effects of temperature, pH, medium and type of strains have been investigated. Five different strains were selected to produce MTGase: *Streptomyces mobaraensis* (NRRL B-3729), *S. ladakanum* (NRRL ISP-5587), *S. lividans* (NRRL B-12275), *S. sioyaensis* (NRRL B-5408) and *S. platensis* (NRRL B-5486). The fermentation process carried out using two fermentation media based on glucose-starch and soy with different pH and temperatures (6.0, 7.0, 8.0 pH and 20, 30, 40 °C). MTGase activity had been determined for 28 days by hydroxamate-based colorimetric method.

Results and discussions. *S. mobaraensis*, *S. ladakanum* and *S. lividans* showed greater growth rates than *S. sioyaensis* and *S. platensis*. In that case, *S. mobaraensis*, *S. ladakanum* and *S. lividans* were selected for the enzyme production. At pH 6.0, the highest enzyme activity (0.036 U/ml) was achieved by *S. mobaraensis* in glucose-starch medium at 30 °C for 14 days and the enzyme activity decreased dramatically after 14th day of fermentation for all strains. At pH 7.0, the highest enzyme activity was seen on 28th day of incubation for *S. mobaraensis* at 30 °C. At pH 8.0, MTGase could not be produced in any of the culture media, or at any temperatures and pH value. For initial pH 6.0 value, the increasing rate of MTGase activity in glucose-starch based medium was higher than in soy based medium at all different temperatures (20, 30 and 40 °C). *S. ladakanum* and *S. lividans* could not produce MTGase for none of the conditions.

Conclusions. *S. mobaraensis* yielded the highest enzyme activity when compared with other strains. Glucose-starch based medium was the most suitable medium for MTGase production. pH and temperature changes affected the enzyme activity and pH 6.0 and 30 °C were the best conditions for the production of MTGase.

Introduction

Transglutaminase (TGase; protein-glutamine-glutamyl-transferase, EC 2.3.2.13) is an enzyme capable of catalyzing acyl transfer reactions by introducing covalent cross-links between proteins as well as peptides and various primary amines [1]. When the amine substrates are absent in the medium, TGase can catalyze the hydrolysis of the γ -carboxyamide group of the glutaminyl residue, resulting in deamination. When the ε -amino group of a peptide-bound lysyl residue is the substrate, peptide chains are covalently connected through by TGase ε -(γ -glutamyl) lysine (G-L) bonds [2]. Transglutaminase has been found in animals, plants, and microorganisms [3]. Recently, TGase has captured peoples' interest due to its potential application for various food products [4, 5].

There are three ways to produce TGase. The first approach is extraction and purification of the enzyme from body fluids and tissues of animals. Factor XIII, extracted from the blood of cattle, was the first commercially produced TGase in Europe [6]. However, the blood enzyme needed thrombin to be active and when used for food products, red pigmentation affected the appearance. The second approach is producing the enzyme by using genetic manipulation. In this purpose, many researchers experimented to produce TGase within the host microorganisms as *E. coli*, *Bacillus* and *Aspergillus* [7–9]. Although being a low-costed method, because of consumers disclaim, second approach could not be an applicable method to obtain commercial TGase [2]. The third approach is finding an appropriate microorganism and produce TGase by using fermentation technology. Several reviews on the application of microbial transglutaminase (MTGase) in food and other areas are already available in the literature [2, 3, 5, 10].

The production of MTGase by *Streptovorticillium mobaraense* was first reported by Ando et al. [11]. It was reported that MTGase can catalyze acyl transfer reactions by introducing covalent cross-links between proteins like blood and tissue TGase. Furthermore, MTGase do not require Ca^{+2} or thrombin for activation. For commercial production, MTGase can be easily isolated from the culture broth. MTGase from *Streptovorticillium* sp. used for several food applications such as producing polymers of casein and soybean proteins and gelatinizing sodium caseinate and skim milk gels [12, 13].

Streptomyces species are able to produce MTGase in different structures and these species are commonly used for the industrial production of MTGase. Accordingly, researchers are focused on enhancement of the production of MTGase by using *Streptomyces* species in terms of factors affecting enzyme activity such as substrate optimization, metabolic optimization, and extrinsic factors (pH, dissolved oxygen and temperature etc.). Altering the parameters about fermentation may cause to increase the enzyme activity and hereby increasing the enzyme activity may lower the costs of the production [14]. Because of consideration in the usage of MTGase will become popular, in this study enhancement of MTGase production was investigated in terms of creating a model for future studies. For determination of the highest enzyme activity, the effects of temperature, pH, medium and type of strains have been investigated.

Materials and methods

Materials. All the chemicals used were of analytical grade and mainly purchased either from Sigma Chemical Co., Ltd. or Merck Millipore Corporation. Z-Gln-Gly (γ -glutamyl donor substrate), was purchased from Sigma Chemical Co., Ltd. *Streptomyces mobaraensis* NRRL B-3729, *S. ladakanum* NRRL ISP-5587, *S. lividans* NRRL B-12275, *S. sioyaensis* NRRL B-5408 and *S. platensis* NRRL B-5486 were obtained from USDA Agricultural

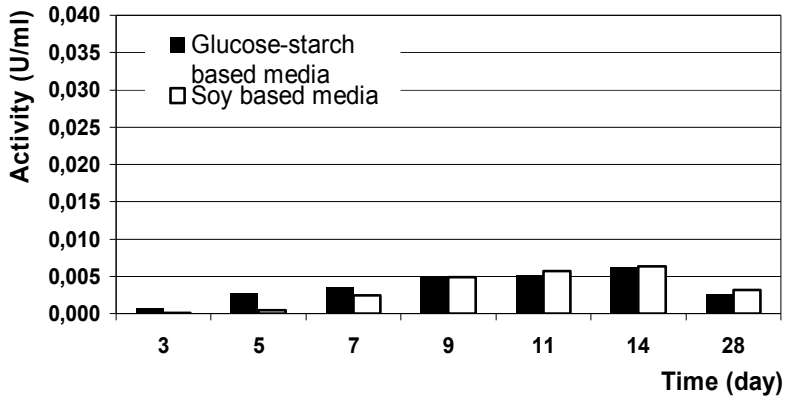
Research Service. Perkin-Elmer Lambda EZ 201 spectrophotometer was used for the enzyme assays.

Culture conditions and MTGase Production. All strains were pre-cultured on a medium composed of: glucose (4.0 g/L), yeast extract (4.0 g/L) and malt extract (10.0 g/L) at pH 7.2, 30 °C. After 6 days of incubation, *S. mobaraensis*, *S. ladakanum* and *S. lividans* showed greater growth rates than *S. sioyaensis* and *S. platensis*. In that case, *S. mobaraensis*, *S. ladakanum* and *S. lividans* were selected for the enzyme production. Two different media were prepared for MTGase production. The first medium based on glucose-starch composed of: glucose (15 g/L), starch (15 g/L), peptone (15 g/L), yeast extract (4 g/L), MgSO₄ (2 g/L), KHPO₄ (2 g/L) and KH₂PO₄ (2 g/L) [15]. The second medium based on soy composed of: soy (20 g/L), glycerol (10 g/L), peptone (15 g/L), starch (5 g/L), glucose (5 g/L), yeast extract (5 g/L), CaCO₃ (5 g/L), MgSO₄ (2 g/L), KHPO₄ (2 g/L) and KH₂PO₄ (2 g/L) [1]. To determine the effect of different temperatures and pH values on MTGase production, *S. mobaraensis*, *S. ladakanum* and *S. lividans* were inoculated in both glucose-starch based medium and soy based medium, initial pH values were adjusted to 6.0, 7.0 and 8.0. Samples had been incubated for 28 days at 20, 30 and 40 °C were analyzed to determine MTGase activity.

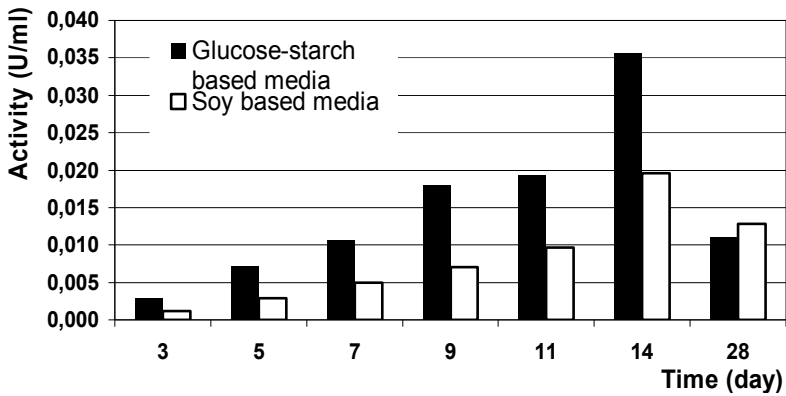
Determination of MTGase activity. MTGase enzyme activity was determined by Hydroxamate formation with the specific substrate of Z-Gln-Gly. The reaction mixture contained 100 µl enzyme solution and 325 µl substrate solution (200 µl of 200 mM Tris-HCl, 25 µl of 12.5 mM reduced glutathione, 25 µl of 125 mM hydroxylamine and 75 µl of 37.5 mM Z-Gln-Gly). The solution was incubated at 37 °C for 24 hours and then it was ended by adding 425 µl of stop reagent (consisting of 15% TCA and 5% FeCl₃). After centrifugation at 11000 rpm for 5 minutes, the absorbance of the supernatant was measured at 525 nm. Assay was also carried out using the enzyme blanks. One unit of MTGase activity was described as the amount of enzyme which caused the formation of 1.0 µmole of hydroxamate per minute by catalyzing the reaction between Z-Gln-Gly and hydroxylamine at pH 6.0 and 37 °C by using L-glutamic acid γ-monohydroxamate as a standard [16, 17].

Results and discussion

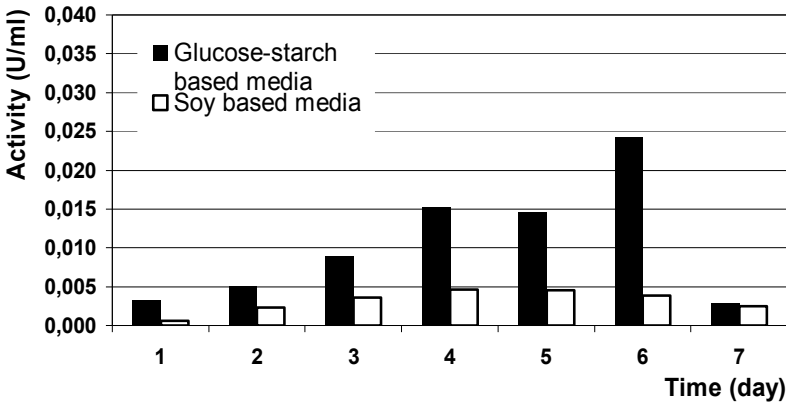
All five lyophilized strains obtained from USDA were pre-cultured on a medium composed of glucose, yeast extract and malt extract. *S. mobaraensis*, *S. lividans* and *S. ladakanum* were selected for the MTGase production of enzyme. These strains were reproduced once in two days for the first week, and this procedure was repeated every couple of weeks. For preparation of the stock culture, cultured media were centrifuged and bacterial pellet was obtained. Following the centrifugation, sterilized pre-culturing medium containing 30% glycerol (v/v) was added on the bacterial pellet and stock cultures were stored inside cryotubes at -65 °C. For the enzyme production, fresh cultures which obtained from pre-culturing medium were inoculated in (1%) glucose-starch and soy based media. After 28 days of incubation, *S. mobaraensis* yielded the highest enzyme activity. However, *S. ladakanum* and *S. lividans* could not produce MTGase for none of the culture mediums, temperatures and pH value. Some researchers attempted to produce MTGase by genetic modification methods from *S. ladakanum* and *S. lividans* strains which produce MTGase very slightly [18, 19]. On the other hand, according to the Tellez-Luis et al. [20], *S. ladakanum* is able to produce MTGase (0.725 U/ml) in the presence of casein and glycerol in the broth medium. In the same way, Ho et al. [21] produced MTGase (2.40 U/ml) by using agitating fermenter from *S. ladakanum*.



a



b



c

Figure 1. Effect of the different incubation temperatures on the enzyme activity of *S. mobaraensis* for glucose-starch based medium (dark grey) and soy based medium (light grey) at initial pH of 6.0:

a – 20 °C, *b* – 30 °C, *c* – 40 °C.

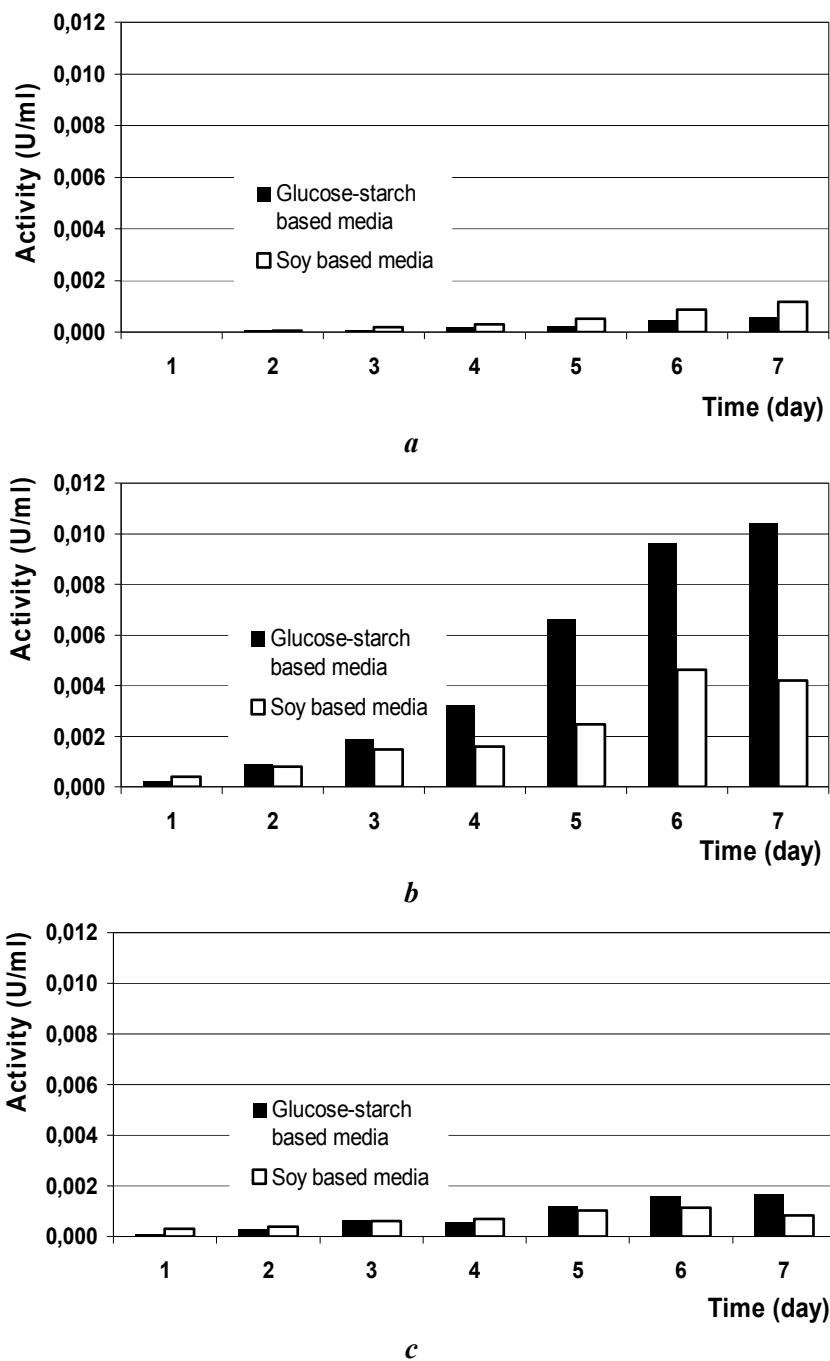


Figure 2. Effect of the different incubation temperatures on the enzyme activity of *S. mobaraensis* for glucose-starch based medium (dark grey) and soy based medium (light grey) at initial pH of 7.0:
a – 20 °C, *b* – 30 °C, *c* – 40 °C.

Figure 1 shows that the highest activity for all experimental conditions was found at 30 °C, 14th day of incubation. The results for glucose-starch based medium and soy based medium were 0.036 U/ml and 0.020 U/ml, respectively. In the same way, Zheng et al. [22] carried out the effects of the temperature to the fermentation of *Streptovericillium mobaraense* and reached the highest enzyme activity at 30 °C (2.90 U/ml at pH 6.5). The glucose-starch based medium had the lowest enzyme activity at 20 °C. Likewise, soy based medium showed the lowest enzyme activity at 20 and 40 °C. The highest activity at 20 °C was reached on the 14th day of incubation for both media (~0.006 U/ml). Temperature might considerably affect the specific cell growth rate of microorganisms. Therefore, the amount of MTGase activity can be related with specific cell growth rate of *S. mobaraensis* [22]. Glucose-starch based medium had an acceptable enzyme activity (0.024 U/ml) at 40 °C as well. However, the enzyme activity decreased dramatically after 14th day of incubation for all samples at 20, 30 and 40 °C.

For initial pH 6.0, the increasing rate of MTGase activity in glucose-starch based medium was higher than in soy based medium at all different temperatures (20, 30 and 40 °C). Tellez-Luis et al. [20] reported that using of glycerol and casein in the medium had positive impact on producing MTGase from *S. ladakanum*. On the other hand, in the medium, cross-linking of peptides may occur by MTGase. For our soy based medium, free amino acids may be probably limited the MTGase production.

As shown in Figure 2, at initial pH 7.0, the highest enzyme activities for both glucose-starch based medium and soy based medium at 30 °C were 0.010 U/ml, 0.004 U/ml, respectively similar to initial pH 6.0. We concluded that glucose-starch based medium had high amount of enzyme activity when compared with soy based medium likewise at pH 6.0. For the temperatures 20 and 40 °C, there were slight changes on the MTGase activity. At pH 7.0, the highest enzyme activity was seen on 28th day of incubation unlike pH 6.0. In our study, because of having low enzyme activity at pH 8.0 for both media, the data was not shown in this paper. Meiyong et al. [23] stated that *S. mobaraense* cannot grow normally in acidic or slightly alkaline medium. Hydrogen bonds may not be formed; consequently, protein molecule bond formation cannot be established. They suggested that to maximize the specific cell growth rate the pH value should be controlled at 7.0. However, we reached the highest enzyme activity value at initial pH 6.0.

Conclusions

S. mobaraensis showed the highest MTGase activity when compared with *S. lividans* and *S. ladakanum*. MTGase activity of *S. mobaraensis* was crucially affected by medium type, different temperatures and pH values. The effect of temperature (20, 30 and 40 °C), pH (6.0, 7.0 and 8.0) and two different media (glucose-starch based and soy based medium) on the enzyme activity was investigated and it was reached that the highest enzyme activity (0.036 U/ml) at 30 °C, pH 6.0 and in glucose-starch based medium. To maintain maximum MTGase production, in addition to the temperature and pH, other parameters such as dissolved oxygen and agitation may also be changed. Instead of ready to use medium, molasses or waste products can be used as carbon source for the production of MTGase.

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The influence of technological parameters of creams fermentation on formation of functional peculiarities of cultured butter

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Abstract

Keywords:

Fermentation
Cream
Flora Danica
Lactobacillus
Acidophilus La-5
Butter
Fatty
Acid

Introduction. The determining factors of cultured butter production is fermentation processes (selection starting cultures, their relations and determinations of optimal technological parameters of fermentation) and physical cream maturation.

Materials and methods. The activity of acid formation during cream fermentation was determined by volumetric change and active acidity. Number of viable cells *Flora Danica* and *Lactobacillus acidophilus* La-5 was calculated by sowing while using nutrient environment M17 Agar CM-0785 and Lactobacillus MRS Agar M 641-500G (Himedia). Fat acid composition of oil samples were investigated by gas-liquid chromatography using gas chromatograph Hewlett Packard HP-6890.

Results and discussion. The use of dairy products compositions in the manufacture along with some lactobacilli, containing monocultures of probiotic strains can become irreplaceable in terms of modern nutrition food with probiotic, health and given special properties.

Subject to the technological instructions recommended temperature fermentation and compromise temperature for microbial cultures selected drugs were chosen two temperatures – 20 and 30 °C for cream fermentation. It was established that the highest growth rate recorded in titrated acidity cream sample, which was used for fermentation *Flora Danica* + *Lactobacillus acidophilus* La-5 and temperature 30 °C.

As the results demonstrated, like the joint cultivation *Flora Danica* + *Lactobacillus acidophilus* La-5 for fermentation temperature 30 °C shows the best dynamics of biomass growth for fermentation and physical maturation cream, because the concentration of viable cells in this version was the largest.

As for the content of fat acids that exhibit a strong biological effects, their contents showed a clear tendency for the sample of cultured butter rise, which used a combination of mixed cultures of mesophilic and thermophilic acidophilic bacillus fermentation and cream at temperature 30 °C.

Conclusions. The use of technology of cultured butter composition, composed of mixed cultures of mesophilic was proposed to use for the first time.

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Introduction

The role of probiotic products grows in the modern world daily. The demand of consumers for new nutrition products is very large; today the consumer ability of the world functional foods market is estimated about 1.4–1.7 million US dollars, from them 65% – constitute functional milk products [1, 2, 3]. Milk and functional milk products, occupying a substantial place in the daily ration of Ukrainians, are on the one of the first positions among functional foods today, that prevents an origin and progress of dysbacteriosis [4].

The correct choice of cultures for the fermented dairy product provides the obtaining of the product of certain type with the characteristic and rationed indexes of quality and forecast probiotic properties. The market of functional milk products with probiotics, mainly, is presented by soul-milk drinks of the functional setting. Cultured butter with the probiotic bacteria at the market of Ukraine, countries of the CIS and European Union is not presented. The usage of ferment compositions in the dairies production, that are next to certain lactic acid bacterias, contain monoculture of probiotic strains, that allows to get irreplaceable, from the point of view of modern dietetics, food product with probiotic, health and set of special properties [5]. While common cultivation of two cultures, the origin of both synergism and antagonism is possible, that is why the necessary stage of experimental researches at the production of cultured butter is the feature establishment of cross-coupling the cultures of *Flora Danica* and probiotic monoculture *Lactobacillus acidophilus* of probiotic strain La-5 at a cultivation in creams [6].

Cultured butter is an enough popular product in the European countries, unlike our state. The reason of such low demand of cultured butter in Ukraine are not only differences in tastes of consumers, but also contradictions in relation to the features of production technology, disadaptation of technological modes to differences in the composition and properties of domestic raw materials [7]. This, in its turn, caused the interest to the revival of cultured butter technology. The commercial success of probiotics at the market of soul-milk foods made developers appeal to other types of dairies, including cultured butter.

The strain of *Lactobacillus acidophilus* La-5 is a strain analogical to the one, that is in the gastrointestinal tract of people. La-5 is characterized by high firmness to muriatic and suckling acids during the long contact with them, that can be considered as a guarantor of the maintenance of their viability at transit through the sour environment of stomach and at storage of soul-milk foods [8].

The maintenance of high level of viable amount of cells of probiotic in the fermented food products is not a simple task. The viability of cultures lactic acid bacterias is influenced by: acidity of product, co-operation of ferment cultures inter se and condition of their storage [9]. However, there are a few reports, that present sale milk products contain the insufficient amount of viable cages of probiotic (to $<10^6$ cfu/g on the dead-line of storage), the same way diminishing the positive influence on a health of man [10]. Thus, a survival of probiotics and development of methods for support of their vital functions during all expiration date are the important subject of researches.

In our time in the different countries around the world of *Lactobacillus acidophilus* is entered in a monoculture, or in a complex with the various types of lactobacillus in composition of soul-milk foods [11, 12, 13]. The possibility and expediency of general cultivation of lactic acid bacterias and acidophilic bacillus are proven [14, 15, 16], that allows to get the high concentration of viable cages of both groups of microorganisms in a product.

The once developed fermented dairy product is based on the use of cultures of *Lactobacillus acidophilus* La-5, *Lactobacillus casei* 431, BB-12 and *Flora Danica*. The

best viable property, during the storage for temperature 4°C, was characteristic feature of monoculture of *Lactobacillus acidophilus* La-5 in combination with the mixed cultures of *Flora Danica* [17, 18].

The rational correlation of lactic acid bacterias and acidophilic bacillus in a composition with ferment compositions may allow to produce acidophilic foods with the maximally high concentration of probiotic cultures, well organoleptics, rationed microbiological and physical and chemical indexes, in particular, with the not high level of acidity, and also prolonged expiration date [8].

Creams are the special environment for cultivation of lactate bacterias, and have marked features and temperature parameters of their fermentation for the production of butter, in addition, the special requirements are pulled out to the aroma and taste of cultured butter, that is why an important problem is forming of microbial composition.

The possibility of involving cultures of probiotic to fermentation of creams is an unstudied question. It needs the special attention from the point of view of temperature conditions and combination of processes of the biological and physical ripening of creams, as cultures for fermentation of creams are mesophilic, thus one should pick up strains with maximal activity at mionectic temperatures, and cultures of probiotic are thermophilic. For soul-milk foods with probiotic properties qualificatory is the viability of probiotic cultures and conservation of them in an amount that is necessary to give to the product the functional properties. Therefore, the determinations of conditions, at which the cultures of probiotic will save viability, simultaneously with forming the excellent organoleptic properties and rationed physical and chemical parameters are actual task [19].

The latest reports certify the positive connection between the consumption of full-milk and milk products, including, with high content of fat (butter) and health of people [20, 21].

The special attention is focused on sublimity of content of conjugated linoleic acids (CLA) in milk and milk products. CLA in milk preliminary appears in the deck-house of cattle as a mediator of microbial hydrogenation of polyunsaturated of fat acids. Except the microorganisms of scar, a few lactobacillus synthesize CLA [22, 23]. It is also reported that the synthesis of CLA by lactobacillus while enriching milk with oil rich for linolic acid as substrate [24]. It is determined that the synthesis of CLA by preparation that contained *Lac. lactis* (CI4b). The content of CLA increased from 0.41 to 1.21 g/100 g of suckling fat in the fermented milk without addition of any substrates [25].

Without regard to considerable efforts of scientists, the content of CLA in the diets of people remains very low in the comparison with a level, necessary to provide the benefit for a health. Huth and other (2006) proved that 0.42 g of CLA in a day can result in anticarcinogenic effects for people. Strengthening of commercially accessible CLA-isomers that would become the effective method of increase of content of CLA in a dairy product, however, it is related with many defects, in particular, with the presence of other position of trans-isomers that show other effect, and also with the grant of taste defects [26]. The other authors in literature reports about the synthesis of CLA report a lactate microflora [27, 28, 29].

The search for ways of natural increase of content of CLA in dairy products is actual. One of them is the use of lactobacillus. This possibility to enrich CLA with dairy butter is unexplored.

A research aim was establishment of feature of cross-coupling of the cultures DVS *Flora Danica* and monoculture of probiotic *L. acidophilus* La-5 at a cultivation in creams while the production of cultured butter.

To reach a set aim, such tasks were solved:

- to set possibility of combination of *Flora Danica* from *L. acidophilus* La-5 of fermentation of creams;
- to investigate influence of ferment cultures and temperature of fermentation of creams on activity of formation of lactic acid;
- to define influence of temperature of fermentation of creams on the amount of viable cages during fermentation and physical ripening of creams;
- to investigate influence of ferment cultures and temperature of fermentation of creams on composition of fatty acids of cultured butter;
- to give recommendations in relation to the scientific ground of technology of cultured butter with a probiotic properties.

Materials and methods

The first research was conducted in the laboratory of the department of Milk and milk products technology at Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyi and in the laboratory of CSK FOOD Enrichment-Ukraine. Milk raw material for the production of cultured butter was obtained in a spring-summer period of year. For this purpose used milk with mass part of fat 3,4% was separated at a temperature 40-45 °C, received creams with mass part of fat 33% were pasteurized at a temperature 95 °C without a withstand. Creams after pasteurization were cooled to the temperature of fermentation.

It were applied two ferment cultures DVS for the fermentation of creams: cultures of *Flora Danica* – FD (*Lactococcus lactis* subsp. *lactis*; *Lactococcus lactis* subsp. *cremoris*; *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*; *Leuconostoc mesenteroides* subsp. *cremoris*), and also monoculture of probiotic of *Lactobacillus acidophilus* La-5 – La-5 (Chr. Hansen, Denmark) in correlation 1:1, the initial concentration of cultures at a ferment in creams was $0,5 \cdot 10^5$ and $0,5 \cdot 10^5$ CFU/ml namely (for the standards of K2 and K3). For research four standards were made:

– sample 1 – CB1 accordingly – FD; the fermentation of creams at a temperature 20 C and physical ripening at a temperature 5 C is an initial concentration of cultures in creams $0,5 \cdot 10^5$ CFU/ml;

– sample 2 – CB2 accordingly – FD in combination with La-5; fermentation of creams at a temperature 20 °C and ripening at a temperature 5 C;

– sample 3 – CB3 accordingly – FD in combination with La-5; fermentation of creams at a temperature 30 °C and ripening at a temperature 5 °C;

– sample 4 – SB – sweet butter.

The activity of formation of lactic acid was selected the initial factors during fermentation of creams, that was determined after the change of titrated and active acidity, by the amount of viable cages in creams in 1 ml, organoleptic properties and composition of fatty acids of lipids of butter. Butter was made using the method of rafting of creams with a triple reiteration that was packed in polystyrene glasses of the capacity of 200 ml and kept for temperatures 0...5 C.

The common amount of the mixed cultures of FD was determined the by the parallel sowing of breeding of standards of butter in double-dish on the environment of M17 Agar CM of a 0785 firm Himedia with next incubation in a thermostat at a temperature 30 C during 3 days in anaerobic terms. Common amount of viable cells of La-5 was determined with the parallel sowing of breeding of standards of butter in double-dish on the

environment of Lactobacillus MRS Agar M 641-500G (Himedia) with next incubation in a thermostat at a temperature 37 °C during 3 days in anaerobic conditions.

The composition of fat acids was investigated by the method of gas-liquid chromatography on gaschromatography of Hewlett Packard HP-6890 with application of capillary column of HP-88 (88 lyanopropyl aryl-polysiloxane, Agilent Technologies) length a 100 m, with an internal diameter a 0,25 mm and in thick immobile phase of 0,2 mkm at next terms: flow rate of gas-transmitter-1,2 ml/min, coefficient of division of stream – 1:100, temperature of vaporizer – 280 °C, temperature of detector (UNDER) – 290 °C, a temperature condition of column is the gradual heating from 60 °C to 230 °C.

It was used a mixture of methyl ethers of fat acids 37 Component FAME Mix firm of Supelco (executioner. № 47885-U) and mixture of methyl ethers of CLA firm of Sigma (executioner. № 05632) for authentication of chromatography peaks and account of chromatogram.

Registration and reatment of chromatogram was carried out by means of the personal computer equipped by HP ChemStation software.

Results and discussion

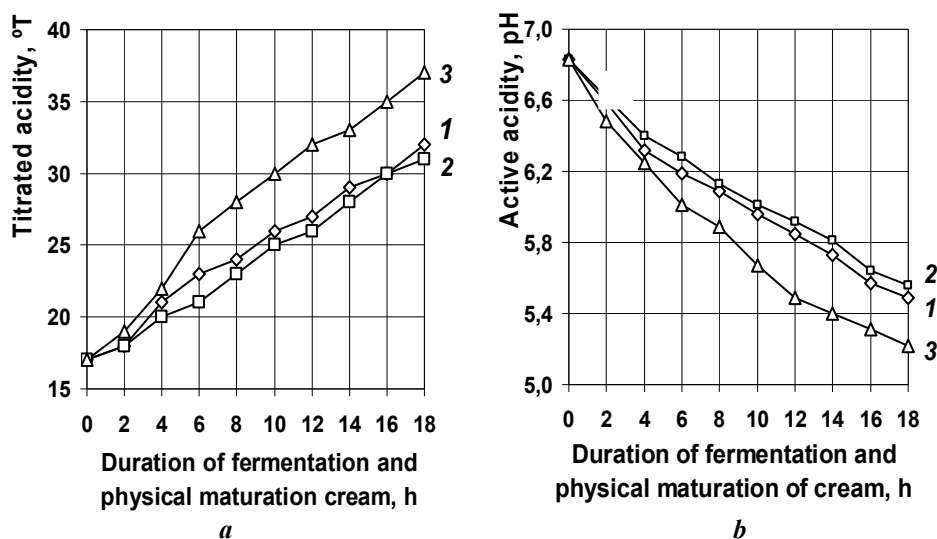
To establish the possibility of combination of FD with the acidophilic bacillus of strain of La-5 at making of cultured butter were conducted fermentation of creams at different temperatures, in fact a temperature has substantial influence on the dynamics of fermentation of creams, and in further on a organoleptic estimation and microbiological indexes of product. According to the review of literary data and technological instructions [31, 32] it is known that the optimal temperature of fermentation of creams at the production of cultured butter is a temperature of 16...20 °C. Taking into account the recommended for the cultures fermentation instructions and a compromise temperature for microbial cultures of selected preparations, were chosen two temperatures conditions 20 and 30 °C for fermentation of creams.

The duration of hold of the leavened creams at every temperature depended on activity of ferment culture, namely speeds of growth of titrated acidity of plasma to the necessary value – 55°T (the value of titrated acidity directly for creams presents 37°T), in obedience to a calculation driven to [32]. Cooling of the fermented creams began, when titrated acidity was on 8–10 °C [31] less than necessary, for avoidance of superfluous growth of acidity.

Titrated acidity of creams during fermentation for the standard of CB3 (8 h) grows from 17°T to 28°T, while for CB1 and CB2 for 10 h to 25–26°T. It was established that the greatest rate of increase of titrated acidity of creams is registered for the standard of CB3, for fermentation of that is used FD+La-5 and temperature of fermentation 30 °C. During a 18 h of fermentation and physical ripening of creams titration for CB3 grew from 17°T to 37°T, while for CB2 grew from 17°T to 31°T titration of creams 37°T answers 55°T acidity plasma. In the samples of CB1 and CB2, for fermentation of creams of that applied FD independently and combination with La-5 and temperature 20 °C, the general duration of fermentation and physical ripening presented also 18 h; during this time titration grew on 15°T, while for CB3 on 20°T. It is explained by subzero activity of FD and La-5 for the temperatures of fermentation of creams (20±1) °C.

The maximum accumulation of diacetyl occurs at the active environment acidity pH 4.7–5.2 [33]. After the fermentation and physical maturation, the active cream acidity was within pH 5.56–5.22. Analogically to the volumetric change of acidity in the experimental

samples of cream, the highest rate of decrease acidity active registered sample CB3, which is used for fermentation FD+La-5 and temperature (30±1) °C. During the cream fermentation in the sample cream CB2 active acidity for 10 h decreased by 0.87 whereas the sample CB3 for 8 h – by pH 0.94.



**Fig. 1. Change of titrated (a), active (b) of acidity in the fermentation and physical maturation cream cultures FD and La-5:
1 – SB1; 2 – SB2; 3 – SB3.**

At the beginning of the fermentation the number of viable cells FD and La-5 was $0.5 \cdot 10^5$ CFU/cm³ for all samples. While the fermentation of creams with the FD culture the at a temperature 20 and 30 °C, the number of viable cells in samples CB1-CB3 8...10 h increased from 4.5 to 6.4–6,6 lg CFU/cm³, and the number of cells La-5 (CB2-CB3) – up to 6.6–6.7 lg CFU/cm³. The usage of fermented cultures CB1-CB3 composed from FD and La-5, made it possible to get clusters of cream with the number of viable cells at the end of physical cream maturation FD 6.8–7.2 lg CFU/cm³, and La-5 – 7.0–7.4 lg CFU/cm³. The most intensive increase in the number of viable cells occurred during the fermentation of cream; in the future, the number of viable cells has changed slightly, due to the low activity of cultures at low temperature of physical maturation (5±1) °C. However, as it is certified by the results, sample with the joint cultivation of FD and La-5 at a fermentation temperature (30±1) °C shows the best dynamics, because the concentration of viable cells in this version was the largest.

However, in all tested items such number of viable cells is insufficient to ensure the probiotic properties of the finished product – cultured butter, since the further technological operations connected with the removal of plasma, thus, significant decrease in the number of viable cells. Therefore, further studies are required to search the dose of inoculation of fermented cultures and immediate introduction of FD and La-5 and determination of their value to increase the concentration of viable cells.

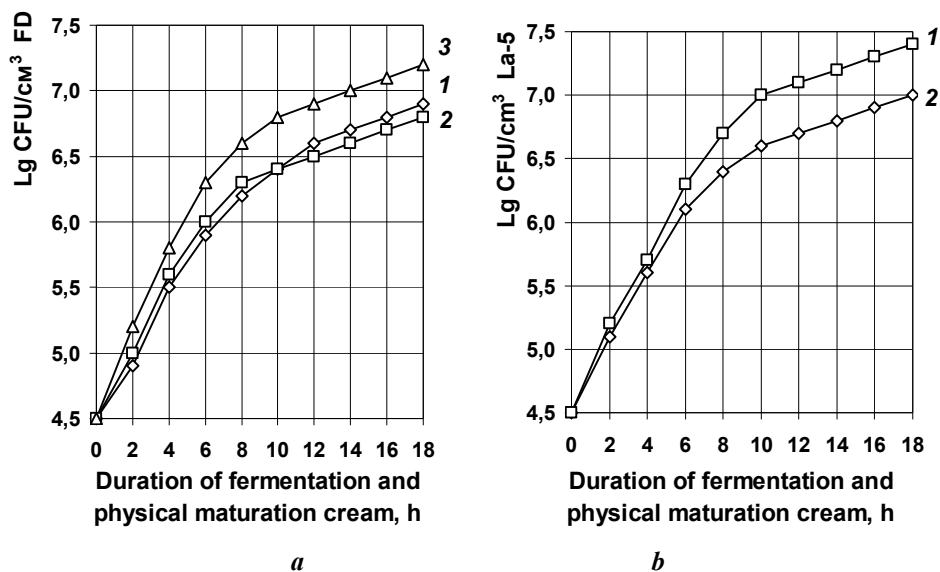


Fig. 2. Change of the number of viable cells FD (a) and La-5 (b) 1 cm³ cream during fermentation and physical maturation cream cultures DVS

**Table 1
Organoleptic butter samples**

Samples of butter	Characterization
SB	Clean, with characteristic pleasant taste and aroma from pasteurized cream flavor. Homogeneous, plastic, solid surface butter on the cut shiny and dry in appearance to the presence of single the smallest droplets of moisture. Color yellow, uniform throughout the mass of color.
CB1	Insufficiently pronounced sour taste and smell. Homogeneous, not enough plastic, solid surface butter on the cut slightly shiny and dry in appearance to the presence of single the smallest droplets of moisture. Color light yellow, homogeneous throughout the mass.
CB2	Clean, without the tastes and smells of weakly expressed yogurt taste and smell. Homogeneous, plastic, solid surface butter on the cut slightly shiny and dry in appearance to the presence of single the smallest droplets of moisture. Color light yellow, homogeneous throughout the mass.
CB3	Clean, without the tastes and smells of the expressed pleasant yogurt flavor and aroma. Homogeneous, plastic, solid surface butter on the cut shiny and dry in appearance to the presence of single the smallest droplets of moisture. Color yellow, uniform throughout the mass of color.

Table 2
Fat acid composition of lipids in samples CB1-CB3, compared with sweet butter, % of total fat acids

Fat acids	Samples of butter			
	SB	CB1	CB2	CB3
C4:0	4.07	4.42*	4.35*	4.42*
C6:0	1.59	1.67	1.67	1.67
C8:0	0.93	0.96	0.96	0.96
C10:0	1.99	2.02	2.04	2.03
C12:0	2.40	2.42	2.45	2.44
C14:0	9.56	9.59	9.63	9.58
iso-C14:0	0.45	0.45	0.45	0.45
anteiso-C14:0	0.81	-	-	-
C14:1	0.97	1.74**	1.74**	1.74**
C15:0	1.57	1.57	1.57	1.56
C16:0	25.04	24.96	24.96	24.82
iso-C17:0	0.78	0.77	0.77	0.77
C16:1 c9	1.40	1.39	1.40	1.41
anteiso-C17:0	0.58	0.58	0.58	0.58
C17:0	0.91	0.90	0.90	0.90
C18:0	11.29	11.22	11.19	11.10
C18:1 t6	0.33	0.28	0.30	0.33
C18:1 t9	0.26	0.29	0.28	0.24
C18:1 t11	4.03	4.01	3.99	4.00
C18:1 c6	0.26	0.24	0.26	0.24
C18:1 c9	22.82	22.66*	22.67*	22.71
C18:1 c11	0.63	0.62	0.62	0.62
C18:1 c12	0.15	0.15	0.15	0.15
C19:0	0.14	-	-	0.14
C18:2 t9, c12	0.12	0.12	0.12	0.12
C18:2 c9, t12	0.62	0.61	0.60	0.61
C18:2 c9, c12	1.33	1.32	1.33	1.33
C20:0	0.24	0.23	0.23	0.23
C18:3 c9, c12, c15	1.22	1.21	1.21	1.21
C 20:1 c11	0.21	0.21	0.20	0.21
C18:2 CLA c9, t11	1.84	1.92	1.87	1.93*
C18:2 c10, c12	0.02	0.01	0.01	0.01
C18:2 t10, c12	0.01	0.01	0.02	0.01
C18:2CLA c11, t13	0.14	0.14	0.14	0.13
C21:0	-	0.08	0.08	0.05
C18:2 CLA c9, c11	0.03	0.02	0.02	0.03
C18:2 CLA t11, t13	0.02	0.02	0.02	0.02
C20:2	0.03	0.03	0.02	0.02
C18:2 CLA t9, c11	0.09	0.11	0.09	0.11
C22:0	0.15	0.12	0.12	0.12
C20:3 c8, c11, c14	0.05	0.08	0.07	0.07
C20:3 c11, c14, c17	0.02	0.03	0.03	0.02
C20:4 c5 c8, c11, c14, c17	0.11	0.12	0.13	0.13
C23:0	0.08	0.08	0.07	0.07
C20:4	0.10	0.10	0.10	0.09
C20:5 c5, c8, c11, c14, c17	0.14	0.14	0.15	0.22*
C24:0	0.09	0.09	0.09	0.09
C22:5 c7, c10, c13, c16, c19	0.30	0.31	0.32	0.29

Note: * – the difference is likely to control * – P<0.05; ** – P<0.01; *** – P<0.001

According to the organoleptic evaluation, sample CB3 with a combination of cultures FD and La-5 and fermentation at a temperature 30 °C was characterized with clean, without the other tastes and smells of the expressed pleasant yogurt flavor and aroma. Other samples were characterized by insufficient or poorly marked yogurt flavor and aroma. Sweet butter is characterized by a pleasant taste and aroma from pasteurized cream flavor. Color samples of oil from light yellow to yellow, homogeneous throughout the mass.

Therefore, to development of promising technologies of cultured butter with probiotic properties are the composition of FD and La-5 and fermentation temperature of fermentation cream – (30±1) °C.

The fermentation of creams cultures of immediate introduction of DVS FD and La-5 while cultured butter production affected the fat acid composition of milk fat, as evidenced by the data presented in table 2.

The results show that sweet butter and cultured butter are characterized by a large range of fat acids, which include acids iso-forms and anteiso, as well as acid chain of length of more than 20 carbons (C21:0, C22:0, C23:0, C24:0). The main changes include increasing the proportion of butyric acid (C4:0) 8.6% for CB1 and CB3 ($p < 0.05$) and 6.9% for CB2 ($p < 0.05$), respectively, compared with sweet butter. This is a unique milk fat acid which shows anticarcinogenic effect, synthesized *de novo* in the secretory cells of the breast and is the dominant short-fatty acids of milk lipids ruminants.

Typical components of milk fat are fatty acids with branched carbon chain (iso-C14:0, anteiso-C14:0, iso-C17:0, anteiso-C17:0), which are components of lipid microbial cells. The largest number of this group of acids in the butter sample SB was acid anteiso-C14:0. In samples of cultured butter this acid was not identified.

The most undesirable fat acids of milk fat is saturated C12:0, C14:0 and C16:0, because they contribute to raising the level of cholesterol and low-density lipoprotein in the blood and thus show atherogenic and thrombogenic properties. The content of acid C14:1 in samples of cultured butter increased by 2 times that may be a sign of activity of desaturated microflora under the influence of $\Delta 9$ -desaturase. Among the family of acids n-6 in samples of butter dominated linoleic (C18:2 c9, c12), the content of which was 1.32–1.33%.

The presence in dairy products trans-isomers of unsaturated fat acids is associated with the risk to human health. However, the main trans-acids of milk fat is C18:1 trans-11 and dienes trans-11 conjugated of linoleic acid, exhibiting the diverse positive biological effects on the human body. The most studied linoleic acid isomer is cis-9, trans-11 diene conjugates that has anticarcinogenic, antiatherogenic, antidiabetic, anti-inflammatory and immunomodulatory effects [36, 37]. The results showed that the content of CLA cis-9, trans-11 consisting in milk fat was 1.84% in SB and 1.92, 1.87, 1.93% respectively in CB1, CB2, CB3. The sum of all isomers CLA in the experimental samples of butter ranged from 2.08 to 2.13%, it should be emphasized that the content of trans-9 isomers in a CB3 was 0.24 versus 0.26 in SB. The content of cis-9, trans-11 CLA showed a tendency to increase in samples CB1 and CB3. These results of the content of trans-11 isomer in the sample CB2 may presuppose that the joint cultivation of lactic acid bacteria FD and acidophilic bacillus probiotic strains La-5 at a fermentation temperature (30±1) °C these isomers are synthesized by lactic acid bacterias.

The increase of unsaturated fat acids in the samples of cultured butter reflected in the tendency to increase the ratio of unsaturated/saturated fat acids – 0.61 in CB1–CB3 versus 0.59 in SB. In the butter samples the total content of branched fat acids ranged within 1.8–2.6% of the sum of fat acids. The sum of odd fat acids was similar in all groups as well.

The ratio of fatty acids n-3/n-6 were roughly the same in all tested items, but the amount of families acids n-3 and n-6 showed a tendency to increase in samples of cultured butter.

As for the content of fatty acids that exhibit a strong biological effects, their contents showed a clear tendency to increase the sample of cultured butter CB3, in which were used a combination of mixed cultures of mesophilic and thermophilic acidophilic bacillus fermentation and cream at temperature (30±1) °C.

Conclusions

The possibility of a combination of mixed cultures of *Flora Danica* and *L. acidophilus* La-5 strain during fermentation of cream in the technology of cultured butter was determined.

Fat acid composition of samples butter was researched. As for the content of fatty acids that exhibit a strong biological effects, their contents showed a tendency to increase in the sample of cultured butter, in which was used a combination of mixed cultures of mesophilic lactic acid bacterias and thermophilic acidophilic bacillus and cream fermentation at temperature (30±1) °C.

It was proposed to use in the technology of a cultured butter, ferment composition, composed from mixed mesophilic cultures FD and thermophile La-5 and a temperature of cream fermentation of (30±1) °C.

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Cu(II) complexes of 4- and 5- nitro-substituted heteroaryl cinnamoyl derivatives and determining their anticoagulant activity

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Abstract

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Introduction. This article presents the synthesis of Cu(II) complexes of 4- and 5- nitro-substituted heteroaryl cinnamoyl derivatives and the research on their anticoagulant activity.

Materials and methods. All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined with a digital melting point apparatus SMP 10. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F₂₅₄, 0.2 mm Merck plates, eluent system (vol. ratio): CH₂Cl₂ : CH₃COCH₃ = 1 : 1. IR spectra were taken on spectrometer Perkin-Elmer FTIR-1600 in KBr discs.

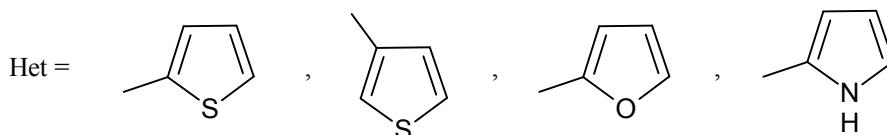
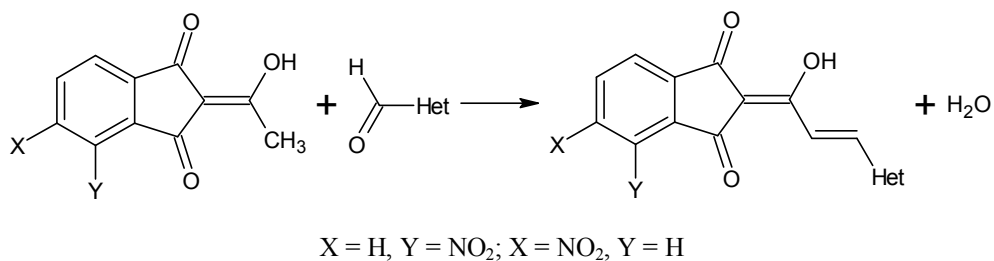
Results and discussion. Ligands, needed for the obtaining of the corresponding complexes, are obtained from 4- and 5-nitro-substituted 2-acyl-1,3-indandiones by two different methods: Mosher and Meier and Rotberg and Oshkaya, and the new thing in our method is the use of pyrrolidine as catalyst. The resulting ligand is dissolved in dioxane and Cu(CH₃COO)₂ · H₂O is dissolved in methanol and this solution is added to the solution of the ligand, wherein the complex crystallizes upon cooling. The physicochemical parameters and the corresponding spectral studies of the newly identified compounds have been defined. Based on the results obtained it is suggested that the composition of the complexes is [M(L)₂]. Based on the experimental data, the most probable structure for the Cu(II) complexes has been suggested with two deprotonated OH groups of 4- and 5-nitro- cinnamoyl derivatives. The spectroscopic data on the amorphous samples indicate a square planar or a tetrahedral structure geometry of the Cu(II) complexes. Anticoagulant activity of the synthesized complexes has been determined by their prothrombin time and has been compared with that of the starting 4- and 5-nitro-cinnamoyl derivatives. It has been shown that the 5-nitroderivatives have a higher anticoagulant activity than the corresponding 4-nitroderivatives.

Conclusion. New Cu(II) complexes of 4- and 5- nitro-substituted heteroaryl cinnamoyl derivatives were successfully obtained and their anticoagulant activity was determined. These complexes may be used in future tests of food products of plant and animal origin.

Introduction

In our previous work [1] we described the synthesis of 4- and 5-nitro-substituted heteroaryl cinnamoyl derivatives from the corresponding 4- and 5-nitro-2-acetyl-1,3-indandiones with heteroaldehydes in the presence of pyrrolidine as catalyst. Their structure has been proved by elemental analysis and spectral analysis methods.

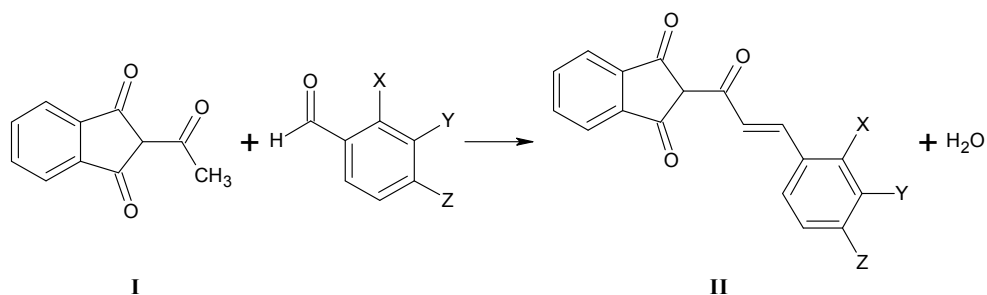
The compounds were obtained from 4- and 5-nitro-substituted 2-acetyl-1,3-indandiones by two different methods: Mosher and Meier [2] and Rotberg and Oshkaya [3], and the new thing in our method was the use of pyrrolidine as catalyst.



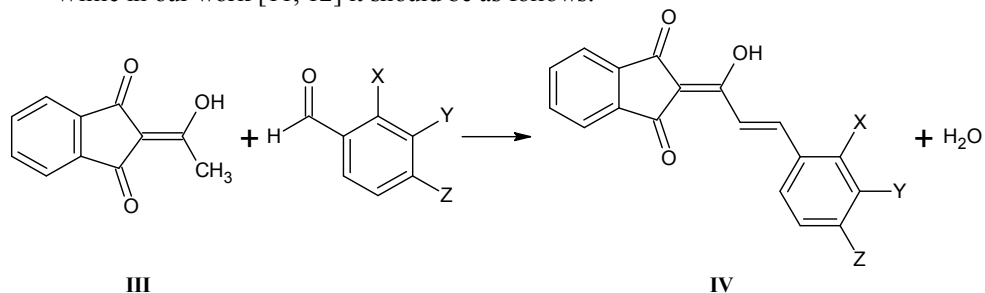
In the literature, there are quite a few examples showing that the replacement of saturated with unsaturated radical very often enhances the physiological effect or have particularly specific effect [4, 5].

But the introduction of an unsaturated radical becomes very difficult through the methods described [2, 3, 6-9]. For this purpose a new methodology [10] has been developed.

In the absence of catalysts, for example, 2-acetyl-1,3-indandione with benzaldehyde does not react, but in particularly hard conditions in the presence of alkaline or acidic catalysts, the reaction proceeds to the obtaining of the corresponding cinnamoyl derivatives. In all of these publications the reaction is indicated to proceed as follows:



While in our work [11, 12] it should be as follows:



The tautomeric form III has been proved to be energetically the most favourable form of the existing five tautomeric forms [13]. While up to now, 4- and 5-nitro cinnamoyl derivatives have not been received, pyrrolidine proved to be particularly successful catalyst.

The structure of the product of the condensation reaction between 2-acetylindan-1,3-dione and aniline was investigated in gas phase, solution and solid state using a combination of quantum-chemical calculations, IR- and ^1H -, ^{13}C -NMR spectroscopy, and by using a combination of 2D NMR experiments, which included ^1H - ^1H COSY, HMQC and HMBC sequences, and X-ray crystallography [14]. A series of six 2-acyl-1,3-indandione derivatives were determined in crystalline state and in gas phase using X-ray diffraction and theoretical methods (HF, DFT and TD-DFT) [12].

Synthesis, structure, spectral and coordination properties of a crown ether derivative of 1,3-indandione were presented by Ahmedova et al. [15]. The crystal structure of the newly synthesized crown ether derivative of 2-benzylidene-1,3-indandione was determined by means of single-crystal X-ray diffraction. The compound crystallizes in the monoclinic P21/n space group. There are four molecules in the unit cell. The structure of complexes with alkaline and alkaline-earth metal ions (K^+ , Na^+ , Mg^{2+} , Sr^{2+} and Ba^{2+}) were verified by elemental analyses, IR-, UV-Vis and also studied by means of quantum chemical methods. A racemic mixture of an octahedral Fe(III) complex was studied by means of single-crystal X-ray diffraction, Mössbauer and EPR spectroscopy [16]. Six organophosphine/phosphite silver(I) complexes of 2-acetyl-1,3-indandione (2-AID) have been obtained and characterized by elemental analysis, ^1H -, $^{13}\text{C}\{\text{H}\}$ NMR, IR spectroscopy, and thermal analysis (TG and DSC), respectively. The molecular structure of one of them has been determined by X-ray single crystal analysis in which the silver atom is in a distorted trigonal geometry [17]. Eu^{+3} complexes with 2-AID, 2-isovaleryl-1,3-indandione and 2-benzyl-1,3-indandione have been synthesized and characterized by Teotonio et al. [18]. A series of new Cu(II), Zn(II), Pb(II) Cd(II) complexes of 2AID were synthesized and based on NMR (in solution and solid state) and EPR spectroscopy for paramagnetic complexes were characterized [19]. Some of the studies concern excited state twisting, as for N,N-dimethylaminobenzylidene-1,3-indandione and derivatives [20-22]. Recently, selective electrochemical fluorination (SEF) of 1-indanone, 2-indanone and 1,3-indandione were carried out in $\text{Et}_3\text{N}\cdot 4\text{HF}$ ionic liquid by Ilayaraja and Noel [23]. Extensive publications and review, mainly from Alfimov et al., focus on the study of the molecular receptors based on photochromic crown ethers and complex formation [24]. No complexes with 4- and 5-nitro-substituted heteroaryl cinnamoyl derivatives have been obtained, so their anticoagulant activity has not been examined.

That is why, the research described here is focused on the synthesis of Cu(II) complexes of 4- and 5-nitro-substituted heteroaryl cinnamoyl derivatives and their characterization by elemental analysis, UV-Vis and IR spectroscopy.

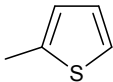
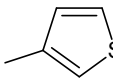
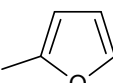
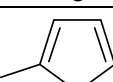
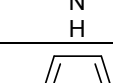
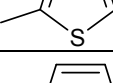
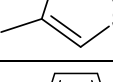
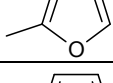
Materials and methods

All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined with a digital melting point apparatus SMP 10. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F₂₅₄, 0.2 mm Merck plates, eluent system (vol. ratio): CH₂Cl₂ : CH₃COCH₃ = 1 : 1. IR spectra were taken on spectrometer Perkin-Elmer FTIR-1600 in KBr discs. UV/Vis spectra was measured on a Lambda 9 Perkin-Elmer UV/Vis/NIR Spectrophotometer from 200 nm to 1000 nm.

The obtaining of the Cu (II) complexes of the 4- and 5-nitro-cinnamoyl derivatives was conducted according to the following common methodology.

In a flask of joint of 100 ml, 0.001 mol of the corresponding nitro-cinnamoyl derivative are dissolved by refluxing. 0.0005 mol of the copper salt (Cu(CH₃COO)₂).H₂O are dissolved with gentle heating. The ligand is dissolved in a medium of dioxan (7 ml), and the copper salt – in an medium of methanol (10 ml). After addition of the solution of the copper salt to that of the nitro-cinnamoyl product, the solution is cooled to room temperature. The resulting complex is filtered and dried at room temperature and then in a desiccator over silica gel. The resulting complexes are light brown to dark brown crystalline substances with melting points above 370 °C. The yields, melting points and R_f are given in Table 1.

Table 1

X	Y	Het	Yeld, %	M.p., °C	R _f
H	NO ₂		57.17	> 370	0.47
H	NO ₂		18.00	> 370	0.49
H	NO ₂		43.77	> 370	0.40
H	NO ₂		38.33	> 370	0.45
NO ₂	H		48.71	> 370	0.48
NO ₂	H		50.62	> 370	0.42
NO ₂	H		53.51	> 370	0.51
NO ₂	H		39.13	> 370	0.40

Results and discussion

Through spectroscopic methods for structural analysis the structure of 2-acetyl-1,3-indandione and its derivatives have been defined in detail. Based on this, the possibility for receiving of several tautomeric forms, as well as a tautomeric equilibrium, have been established. V. Enchev et. al. [13] in 1999 demonstrated the realization of intramolecular proton transfer. The proof of proton transfer and the failure to register a high energy form led to the assumption of reversible and relatively fast transfer of passing of enol forms in one another.

A number of researchers have been directed to synthesis, determining the structure and properties of derivatives of 2-acetyl-1,3-indandione. Of particular interest is the nitro group introduced in the phthaloyl ring, due to the strong electron acceptor character due to a significant degree to the change of the distributed electron density, as a result of which change in acidity and other physicochemical properties of 2-acetyl-1,3-indandione can be expected. It has been found that the introduction of a nitro group in the phthaloyl core of 2-acetyl-1,3-indandiones greatly increases the acidity of the triketones. The increase in the acid properties is associated with an increase in the acidity of the anionic form, which in its turn is explained by the involvement of the nitro group in the negatively charged delocalized anion of the 5-nitro-2-acetyl-1,3-indandione. The nitro group in the fourth position due to the spatial problems, does not detract from the plain position and thus does not have such a strong negative conjugation effect to the triketone group as the nitro group located in the fifth place.

Despite the well-known chelato-creating ability of the 2-acetyl-1,3-indandiones and their derivatives, significantly small is the number of the studies in this area. That is why we turned to the study of the 4- and 5- nitro-cinnamoyl derivatives with five-cycle heteroaldehyde and the ability to form complexes with transition metals, in this case Cu^{2+} .

The relative stability of the potential enol tautomers in the cinnamoyl derivatives, unsubstituted in the phthaloyl core, has been established [25] through optimization by HF and DTF methods [25]. The resulting relative energies indicate that the exocyclic enol form (a) is the most favourable. In accordance with the Hartree-Fock calculations the endocyclic enol form (b) and the rotamer acis are very poor in energy. DFT results show energy differences between these two isomers as the rotamer acis is more stable than the tautomer (c) (Fig. 1).

Due to the existence of geometrical isomerism, the number of isomers has been increased to eight, as energetically most advantageous is structure (a), and this is namely the E-form (a) (Fig. 2).

The names of the respective forms, depending on the substituents, are given in the Table. 2.

UV/Vis spectra were measured on a Lambda 9 Perkin-Elmer UV/Vis/NIR Spectrophotometer from 200 nm to 1000 nm.

All UV-Vis spectra were registered in DMSO. Maxima in the electronic spectra of Cu(II) complexes with 4-nitro-substituted heteroaryl cinnamoyl derivatives were observed at 255, 305, 376 for Va (Cu(II) complexes with 4-nitro-cinnamoyl derivatives); 256, 323, 382 for Vb; 256, 320, 411 for Vc nm, respectively. Maxima in the UV-Vis spectra of the Cu(II) complexes with 5-nitro-substituted heteroaryl cinnamoyl derivatives were observed at 256, 395 for Va₁ (Cu(II) complexes with 5-nitro-cinnamoyl derivatives); 256, 284 for Vb₁; 256, 317, 386 for Vc₁ nm, respectively.

For the course of the reaction, we judge not only by the colour changes, but also by the spectral data of the resulting complexes, compared with those of the starting cinnamoyl

derivatives published by us earlier [1]. The full reaction is traced with TLC. First, there has been a reduction of the frequency of oscillation of the hydroxyl group with $40-85\text{ cm}^{-1}$ for the various complex compounds. The oscillations of one of the carbonyl groups have been retained, unengaged with the chelato-creation, as well as the double $\text{C}=\text{C}$ bond, while for the other change it is significant, the difference is $20-45\text{ cm}^{-1}$. Coordination of the 2-cinnamoyl-1,3-indandiones after deprotonation of the enolic OH group is supposed after comparing the IR spectra of the metal complexes with that of the free ligands. The physicochemical parameters, as well as the IR-spectral data for Cu(II) complexes with 4- and 5-nitro-substituted heteroaryl cinnamoyl derivatives, are listed in Table 1, Table 3 and Table 4.

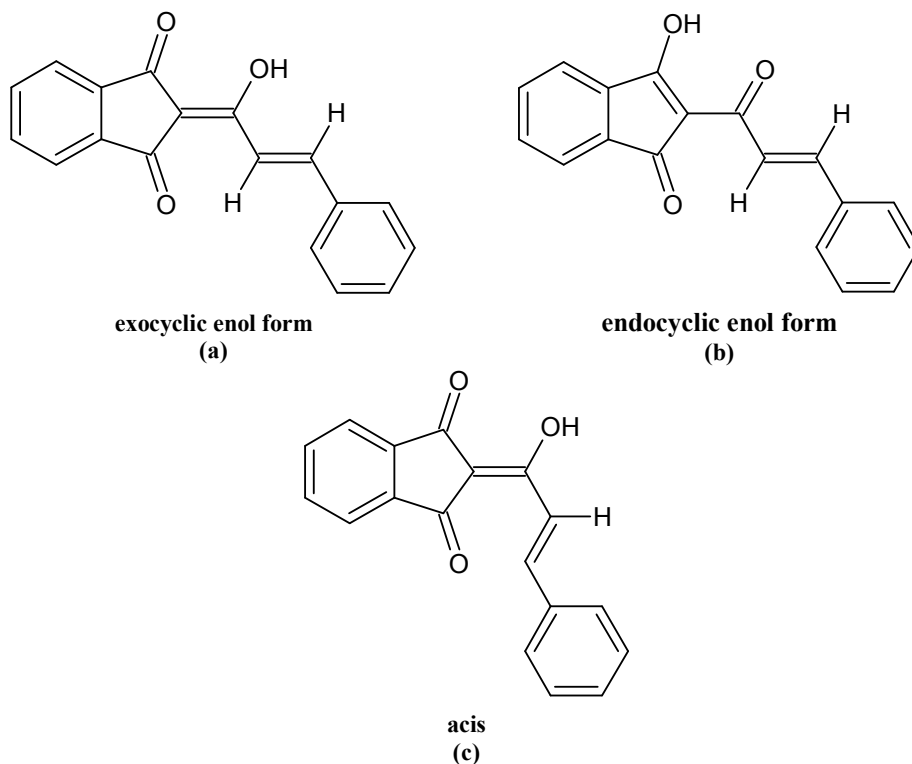


Fig. 1

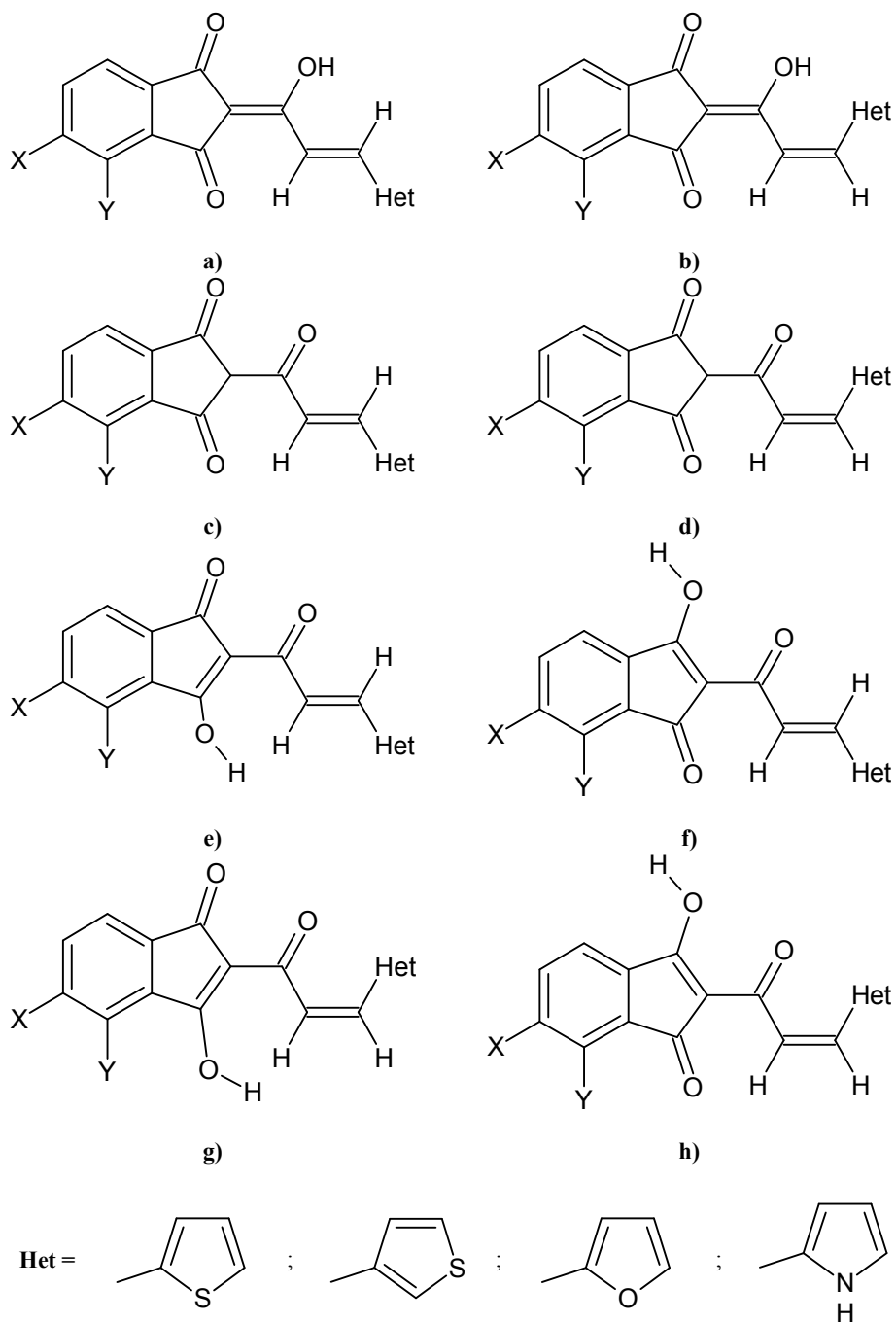
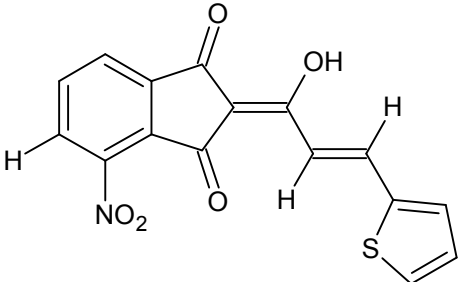
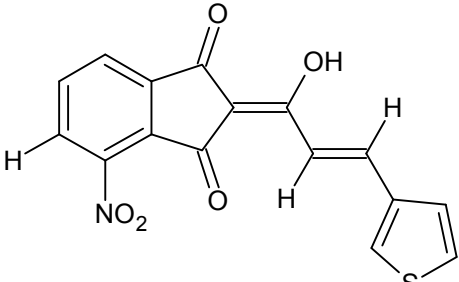
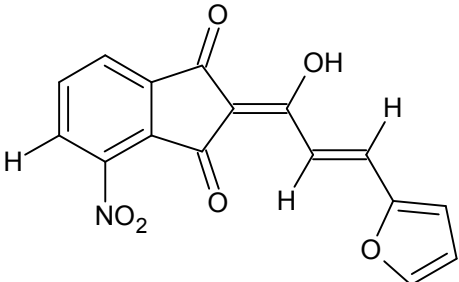
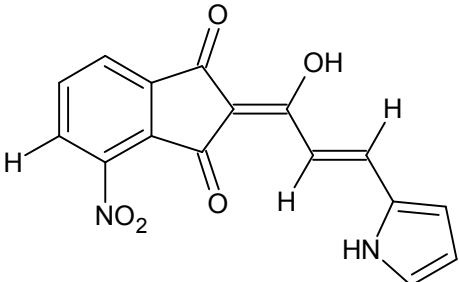
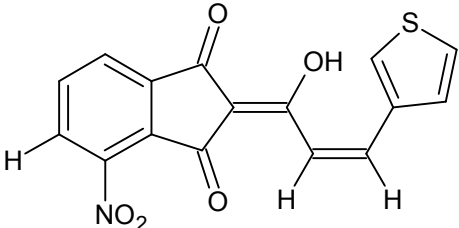
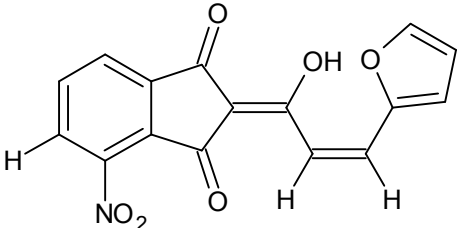
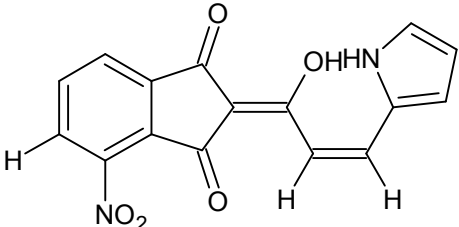
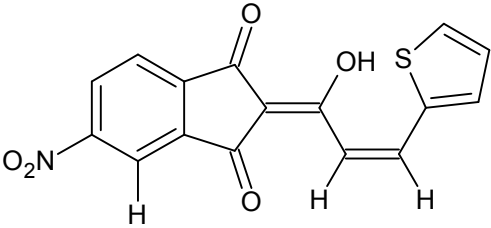
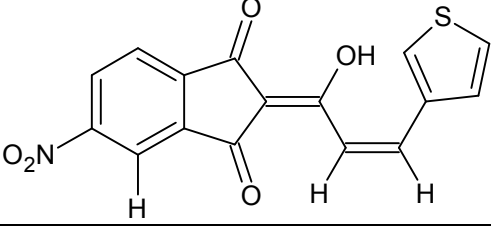
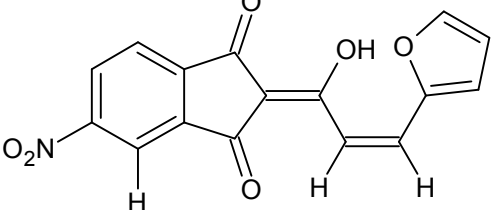


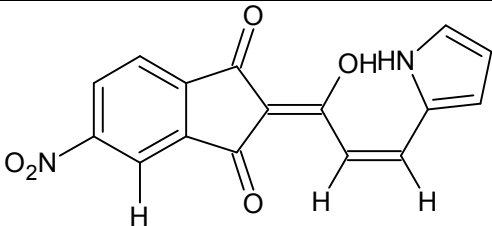
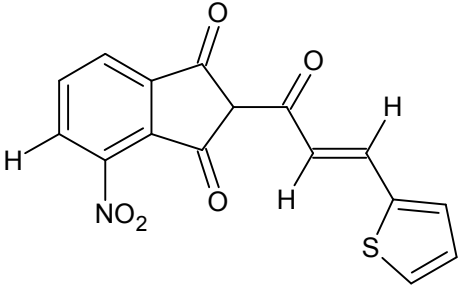
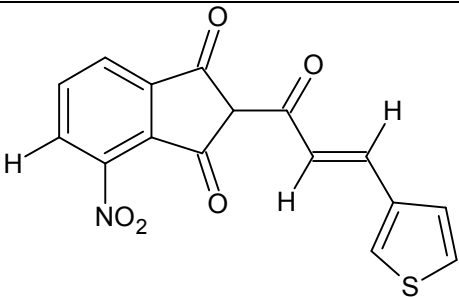
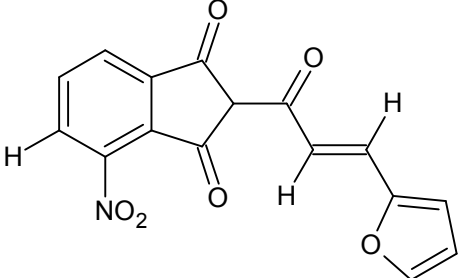
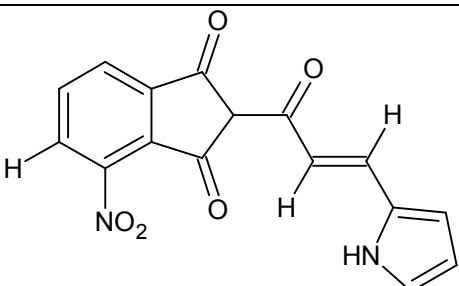
Fig. 2

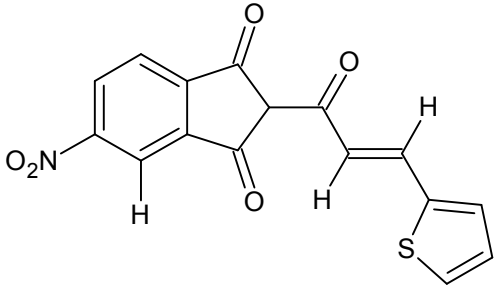
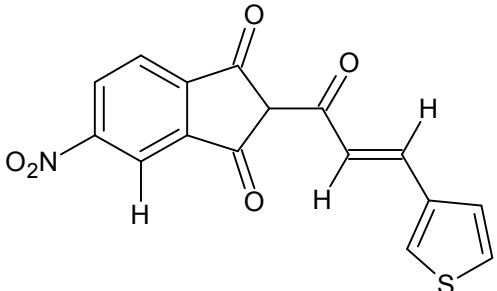
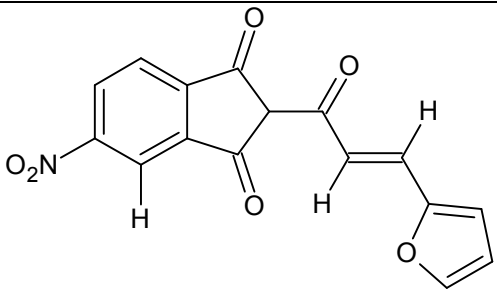
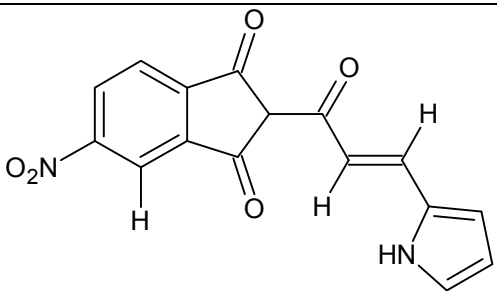
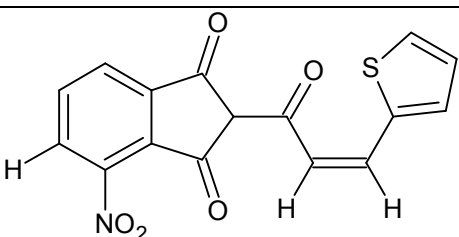
Table 2

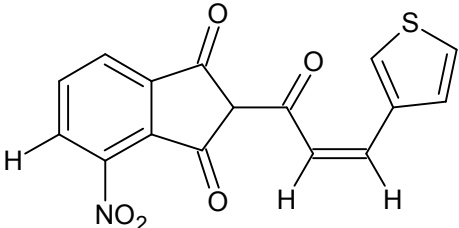
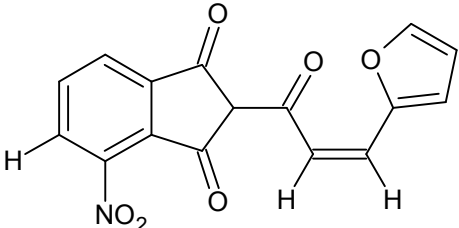
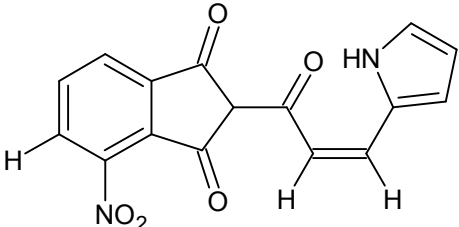
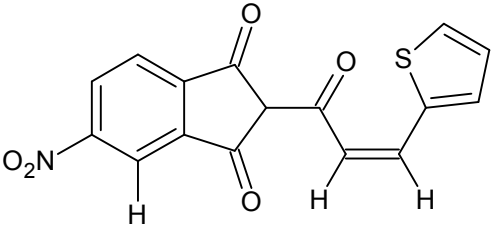
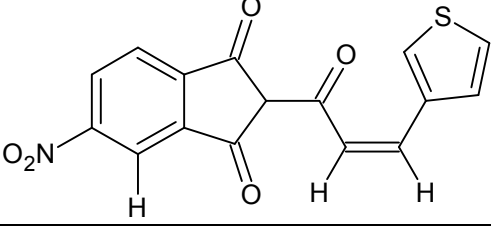
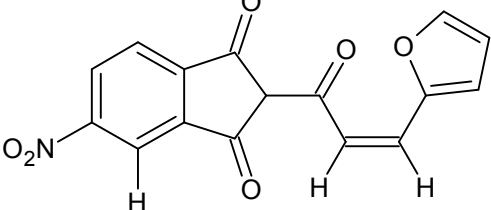
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		<p>(2E)-2-[(2E)-1-hydroxy-3-(thiophen-3-yl)prop-2-en-1-ylidene]-4-nitro-1H-indene-1,3(2H)-dione</p>
		<p>(2E)-2-[(2E)-3-(furan-2-yl)-1-hydroxyprop-2-en-1-ylidene]-4-nitro-1H-indene-1,3(2H)-dione</p>
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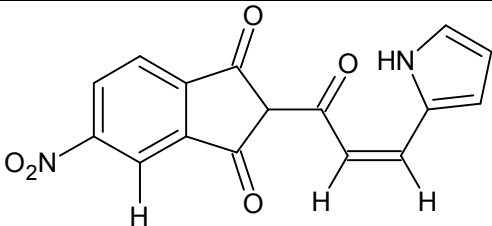
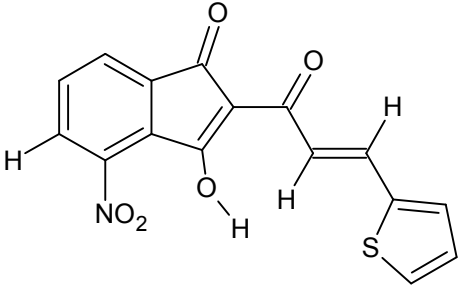
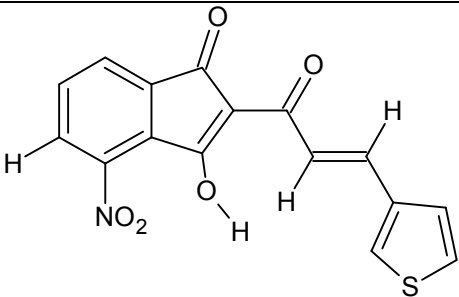
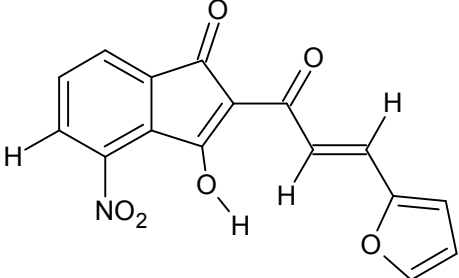
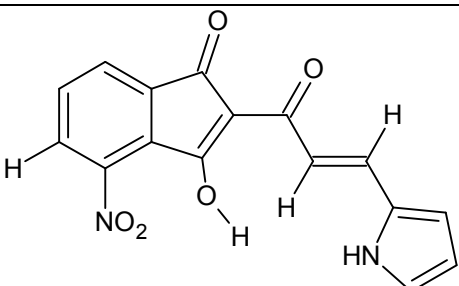
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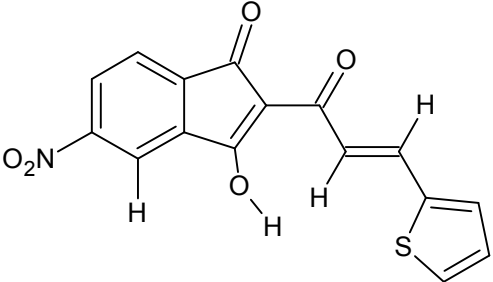
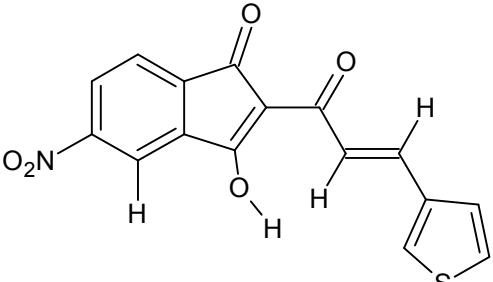
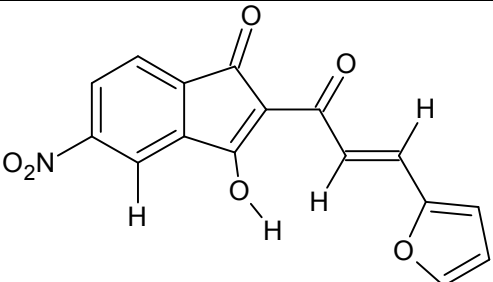
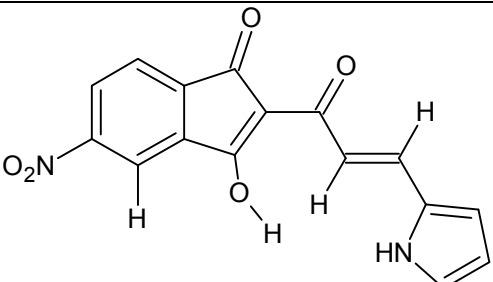
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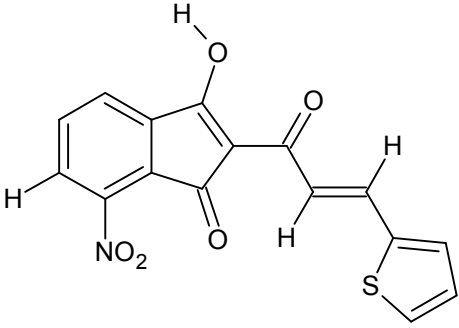
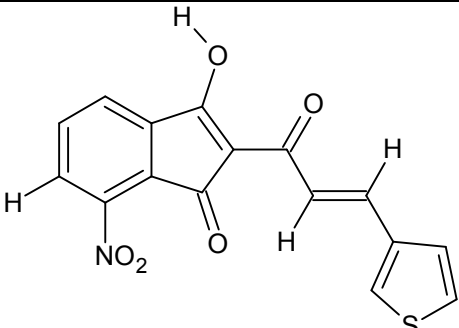
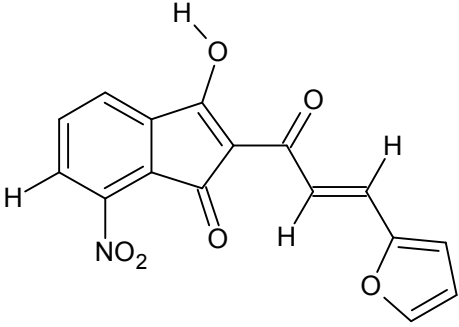
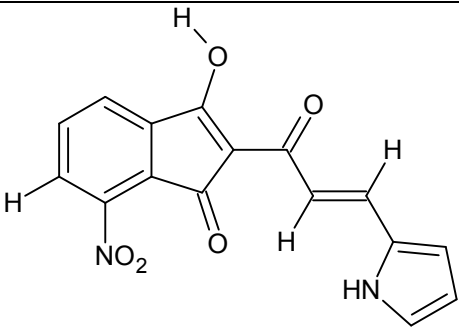
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c		<p>4-nitro-2-[(2E)-3-(thiophen-2-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
		<p>4-nitro-2-[(2E)-3-(thiophen-3-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
		<p>2-[(2E)-3-(furan-2-yl)prop-2-enoyl]-4-nitro-1H-indene-1,3(2H)-dione</p>
		<p>4-nitro-2-[(2E)-3-(1H-pyrrol-2-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>

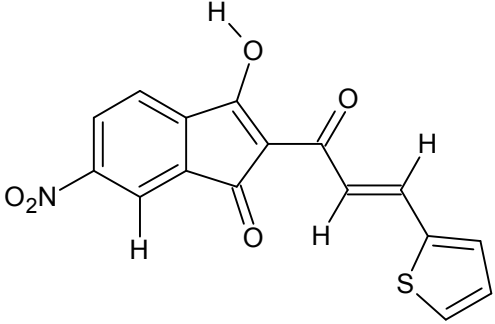
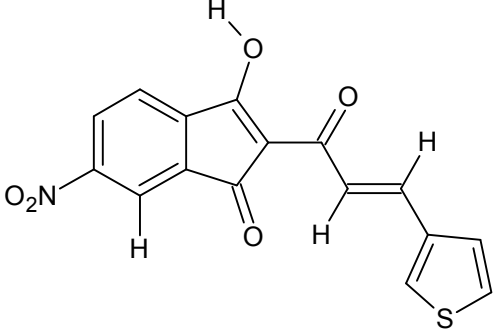
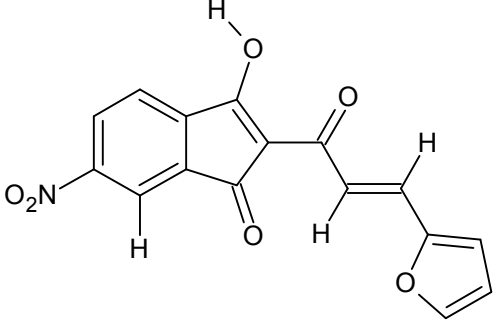
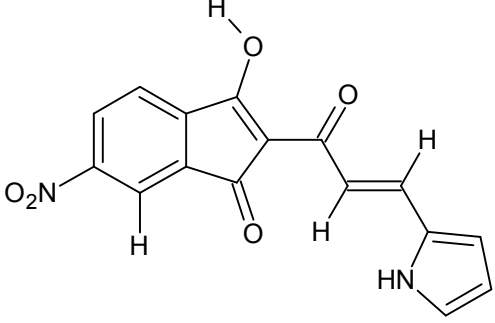
		<p>5-nitro-2-[(2<i>E</i>)-3-(thiophen-2-yl)prop-2-enoyl]-1<i>H</i>-indene-1,3(2<i>H</i>)-dione</p>
		<p>5-nitro-2-[(2<i>E</i>)-3-(thiophen-3-yl)prop-2-enoyl]-1<i>H</i>-indene-1,3(2<i>H</i>)-dione</p>
		<p>2-[(2<i>E</i>)-3-(furan-2-yl)prop-2-enoyl]-5-nitro-1<i>H</i>-indene-1,3(2<i>H</i>)-dione</p>
		<p>5-nitro-2-[(2<i>E</i>)-3-(1<i>H</i>-pyrrol-2-yl)prop-2-enoyl]-1<i>H</i>-indene-1,3(2<i>H</i>)-dione</p>
d		<p>4-nitro-2-[(2<i>Z</i>)-3-(thiophen-2-yl)prop-2-enoyl]-1<i>H</i>-indene-1,3(2<i>H</i>)-dione</p>

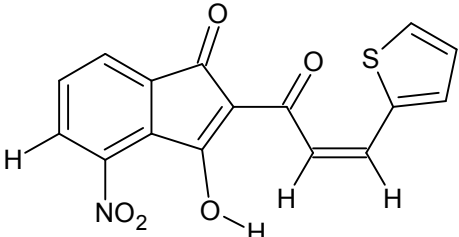
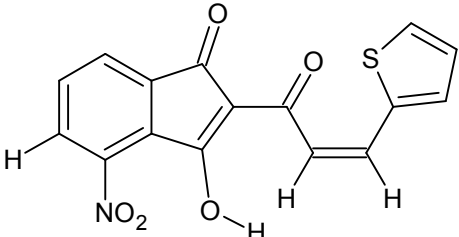
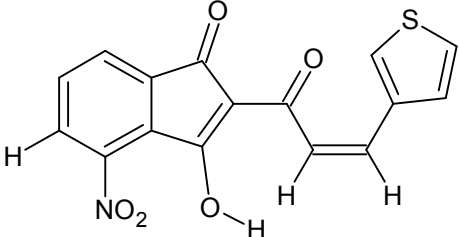
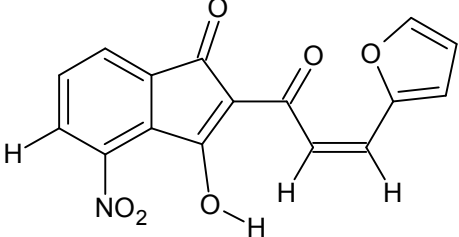
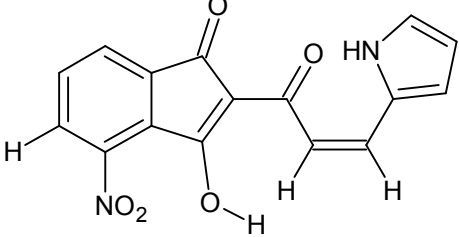
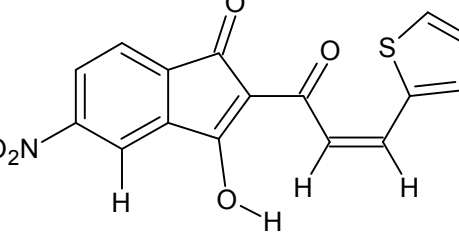
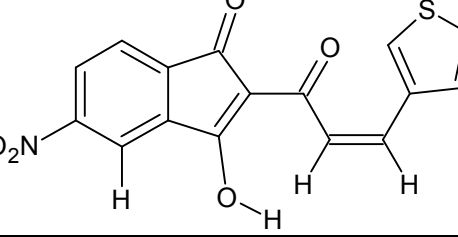
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 4-position and a (2Z)-3-(thiophen-3-yl)prop-2-enoyl group at the 2-position. The thiophene ring is attached at the 3-position.</p>	<p>4-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 4-position and a (2Z)-3-(furan-2-yl)prop-2-enoyl group at the 2-position. The furan ring is attached at the 2-position.</p>	<p>2-[(2Z)-3-(furan-2-yl)prop-2-enoyl]-4-nitro-1H-indene-1,3(2H)-dione</p>
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 4-position and a (2Z)-3-(1H-pyrrol-2-yl)prop-2-enoyl group at the 2-position. The pyrrole ring is attached at the 2-position.</p>	<p>4-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 5-position and a (2Z)-3-(thiophen-2-yl)prop-2-enoyl group at the 2-position. The thiophene ring is attached at the 2-position.</p>	<p>5-nitro-2-[(2Z)-3-(thiophen-2-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 5-position and a (2Z)-3-(thiophen-3-yl)prop-2-enoyl group at the 2-position. The thiophene ring is attached at the 3-position.</p>	<p>5-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enoyl]-1H-indene-1,3(2H)-dione</p>
 <p>The structure shows a 1H-indene-1,3-dione core with a nitro group at the 5-position and a (2Z)-3-(furan-2-yl)prop-2-enoyl group at the 2-position. The furan ring is attached at the 2-position.</p>	<p>2-[(2Z)-3-(furan-2-yl)prop-2-enoyl]-5-nitro-1H-indene-1,3(2H)-dione</p>

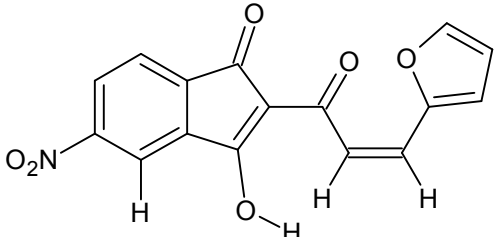
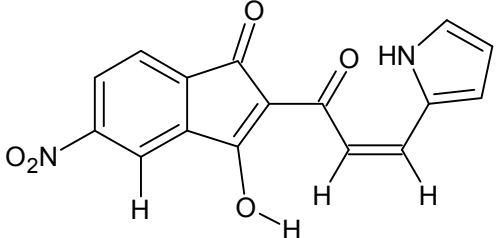
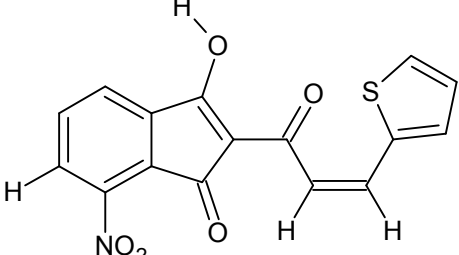
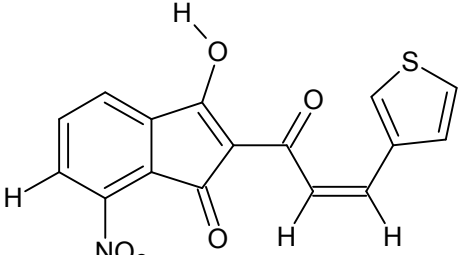
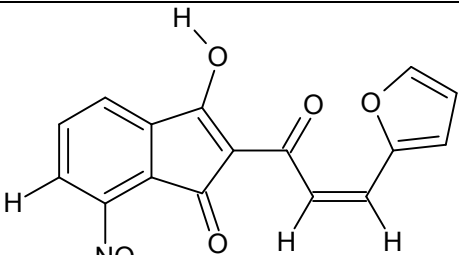
		<p>5-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-indene-1,3(2H)-dione</p>
e		<p>3-hydroxy-4-nitro-2-[(2E)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one</p>
		<p>3-hydroxy-4-nitro-2-[(2E)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one</p>
		<p>2-[(2E)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-4-nitro-1H-inden-1-one</p>
		<p>3-hydroxy-4-nitro-2-[(2E)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one</p>

	<p>3-hydroxy-5-nitro-2-[(2E)-3-(thiophen-2-yl)prop-2-enoyl]-1H-inden-1-one</p>
	<p>3-hydroxy-5-nitro-2-[(2E)-3-(thiophen-3-yl)prop-2-enoyl]-1H-inden-1-one</p>
	<p>2-[(2E)-3-(furan-2-yl)prop-2-enoyl]-3-hydroxy-5-nitro-1H-inden-1-one</p>
	<p>3-hydroxy-5-nitro-2-[(2E)-3-(1H-pyrrol-2-yl)prop-2-enoyl]-1H-inden-1-one</p>

<p>f</p>		<p>3-hydroxy-7-nitro-2-[(2<i>E</i>)-3-(thiophen-2-yl)prop-2-enyl]-1<i>H</i>-inden-1-one</p>
		<p>3-hydroxy-7-nitro-2-[(2<i>E</i>)-3-(thiophen-3-yl)prop-2-enyl]-1<i>H</i>-inden-1-one</p>
		<p>2-[(2<i>E</i>)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-7-nitro-1<i>H</i>-inden-1-one</p>
		<p>3-hydroxy-7-nitro-2-[(2<i>E</i>)-3-(1<i>H</i>-pyrrol-2-yl)prop-2-enyl]-1<i>H</i>-inden-1-one</p>

	<p>3-hydroxy-6-nitro-2-[(2E)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one</p>
	<p>3-hydroxy-6-nitro-2-[(2E)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one</p>
	<p>2-[(2E)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-6-nitro-1H-inden-1-one</p>
	<p>3-hydroxy-6-nitro-2-[(2E)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one</p>

		3-hydroxy-4-nitro-2-[(2Z)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one
		3-hydroxy-4-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one
		2-[(2Z)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-4-nitro-1H-inden-1-one
		3-hydroxy-4-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one
		3-hydroxy-5-nitro-2-[(2Z)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one
		3-hydroxy-5-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one

		2-[(2Z)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-5-nitro-1H-inden-1-one
		3-hydroxy-5-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one
h		3-hydroxy-7-nitro-2-[(2Z)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one
		3-hydroxy-7-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one
		2-[(2Z)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-7-nitro-1H-inden-1-one

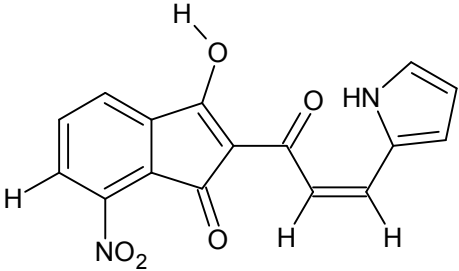
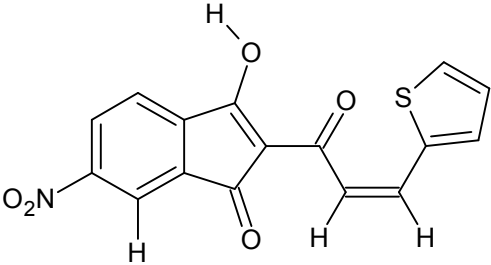
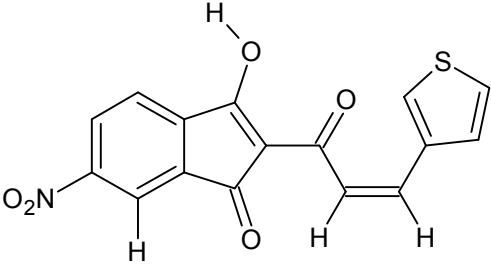
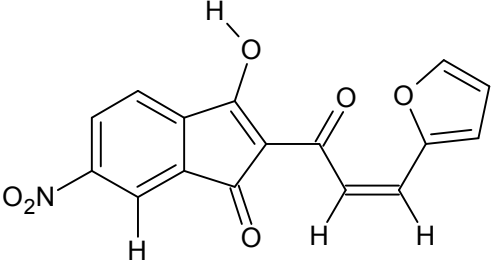
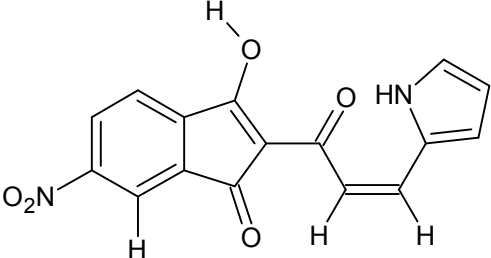
	<p>3-hydroxy-7-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one</p>
	<p>3-hydroxy-6-nitro-2-[(2Z)-3-(thiophen-2-yl)prop-2-enyl]-1H-inden-1-one</p>
	<p>3-hydroxy-6-nitro-2-[(2Z)-3-(thiophen-3-yl)prop-2-enyl]-1H-inden-1-one</p>
	<p>2-[(2Z)-3-(furan-2-yl)prop-2-enyl]-3-hydroxy-6-nitro-1H-inden-1-one</p>
	<p>3-hydroxy-6-nitro-2-[(2Z)-3-(1H-pyrrol-2-yl)prop-2-enyl]-1H-inden-1-one</p>

Table 3









№	Het	ν_{OH}	ν_{NH}	ν_{ar}	$\nu_{C=O}$	$\nu_{C=O'}$	$\nu_{C=C}$	$\nu_{C=C(Het)}$	ν_{asNO_2}	ν_{sNO_2}	ν_{OH}	$\nu_{=CH}$	$\nu_{2-tioph}$	$\nu_{3-tioph}$
Va		3444		3062	1701	1632	1614	1571	1537	1351	1254	962	825	
Vb		3443		3088	1697	1632	1610	1573	1537	1351	1276	967		768
Vc		3483		3071	1698	1636	1612	1572	1548	1351	1271	968		
Vd		3471	3364	3055	1697	1633	1610	1572	1538	1351	1271	967		

Table 4

№	Het	ν_{OH}	ν_{NH}	ν_{ar}	$\nu_{C=O}$	$\nu_{C=O'}$	$\nu_{C=C}$	$\nu_{C=C(Het)}$	ν_{asNO_2}	ν_{sNO_2}	ν_{OH}	$\nu_{=CH}$	$\nu_{2-tioph}$	$\nu_{3-tioph}$
Va ₁		3495		3096	1698	1625	1607	1563	1533	1342	1254	951	838	
Vb ₁		3427		3096	1697	1626	1608	1586	1533	1342	1253	955		798
Vc ₁		3495		3097	1698	1631	1612	1571	1549	1342	1254	950		
Vd ₁		3475	3381	3096	1697	1626	1610	1573	1540	1342	1253	951		

Data from the elemental analysis of the ligands and the complexes are shown in Table 5 and Table 6. The elemental analyses data show composition of the complexes with 1 : 2 metal-to-ligand ratio ($[M(L)_2]$). The ligands coordinate in a bidentate way as monoanions after deprotonation of the enolic OH group. This is confirmed by the IR data of the metal complexes, which are compared with those of the free ligands and presented in Table 3 and 4. The IR data of the 4- and 5-nitro-substituted heteroaryl cinnamoyl derivatives are presented in reference 1.

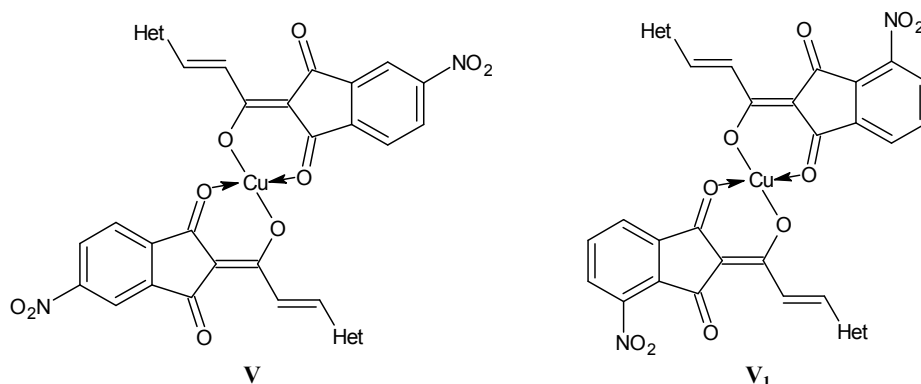
Table 5

Compound	C, %		H, %		N, %		S, %		Cu, %	
	calc.	found	calc.	found	calc.	found	calc.	found	calc.	found
L-Va	58.71	58.65	2.77	2.71	4.28	4.25	9.80	9.59		
CuL	53.67	53.55	2.25	2.17	3.91	3.83	8.95	8.77	8.87	8.75
L-Vb	58.71	58.58	2.77	2.64	4.28	4.18	9.80	9.64		
CuL	53.67	53.48	2.25	2.13	3.91	3.86	8.95	8.79	8.87	8.69
L-Vc	61.74	61.65	2.91	2.83	4.50	4.44				
CuL	56.19	56.00	2.36	2.27	4.10	3.95			9.29	9.18
L-Vd	61.94	61.89	3.25	3.15	9.03	8.94				
CuL	56.35	56.29	2.66	2.51	8.21	8.06			9.32	9.22

Table 6

Compound	C, %		H, %		N, %		S, %		Cu, %	
	calc.	found	calc.	found	calc.	found	calc.	found	calc.	found
L-Va ₁	58.71	58.57	2.77	2.61	4.28	4.24	9.80	9.67		
CuL	53.67	53.48	2.25	2.21	3.91	3.79	8.95	8.78	8.87	8.71
L-Vb ₁	58.71	58.57	2.77	2.63	4.28	4.08	9.80	9.59		
CuL	53.67	53.61	2.25	2.15	3.91	3.82	8.95	8.83	8.87	8.79
L-Vc ₁	61.74	61.55	2.91	2.81	4.50	4.35				
CuL	56.19	56.03	2.36	2.27	4.10	3.95			9.29	9.11
L-Vd ₁	61.94	61.66	3.25	3.15	9.03	8.97				
CuL	56.35	56.17	2.66	2.55	8.21	8.06			9.32	9.26

The most probable structure of the resulting complexes, in our opinion, can be presented as follows:



Based on the results obtained it is suggested that the composition of the complexes is $[M(L)_2]$. Based on the experimental data, the most probable structure for the Cu(II) complexes has been suggested with two deprotonated OH groups of 4- and 5-nitrocinnamoyl derivatives. The spectroscopic data on the amorphous samples indicate a square planar or a tetrahedral structure geometry of the Cu(II) complexes.

Anticoagulant activity of the synthesized complexes has been determined by their prothrombin time and has been compared with that of the starting 4- and 5-nitrocinnamoyl derivatives (Table 7 and Table 8).

Table 7

N ^o	PT, %	INR	PT, s
Va	76.0	1.32	16.4
Va+Cu ²⁺	95.6	1.57	25.4
Vb	129.5	0.44	6.6
Vb+Cu ²⁺	135.6	0.55	9.3
Vc	106.4	0.58	8.5
Vc+Cu ²⁺	114.7	0.76	12.2

PT – Prothrombin Time

INR – International Normal Ratio

Table 8

N ₂	PT, %	INR	PT, s
Va ₁	82.0	1.41	19.6
Va ₁ +Cu ²⁺	101.5	1.63	31.3
Vb ₁	132.3	0.64	10.5
Vb ₁ +Cu ²⁺	140.1	0.66	13.8
Vc ₁	108.4	0.68	9.5
Vc ₁ +Cu ²⁺	111.1	0.84	16.6

PT – Prothrombin Time

INR – International Normal Ratio

It is evident that the 5-nitroderivatives and their complexes manifest a higher anticoagulant activity.

Conclusion

Synthesized and characterized, still not described in the literature, are new 4- and 5-nitro-cinnamoyl derivatives of five-cycle hetero-aldehyde with Cu²⁺. A series of non-charged complexes with Cu(II) has been isolated and analyzed by elemental analyses, UV-Vis and IR spectroscopy. For the paramagnetic Cu(II) complexes a tetrahedral or square planar structure has been proposed.

The anticoagulant activity of the resulting complexes has been determined by defining their prothrombin time, it has been shown that the 5-nitroderivatives have a higher anticoagulant activity than the corresponding 4-nitroderivatives.

References

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Non-stationary sucrose diffusion mass flow calculation for sucrose solution cells from the «larger sugar crystal-larger sugar crystal sucrose solution-less sugar crystal sucrose solution-smaller sugar crystal-masseccuite» system cells depending on the boiling sugar masseccuite time

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Abstract

Keywords:

Sucrose
Diffusion
Solution
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Masseccuite

Introduction. In this paper is proposed one of the following steps to create the sucrose crystallization process mathematical model.

Materials and methods. To obtain the non-stationary diffusion mass sucrose solutions flow quantities for sucrose cells solved simultaneously system with 7 unsteady heat conduction problems in each separate area with constant and with variable thermophysical coefficients, and three separate unsteady diffusion mass transfer problems for four sucrose solution areas with constant and variable diffusion mass transfer coefficients applied numerical methods (method of controlling volume).

Results and discussion. The unsteady mass diffusion sucrose solutions flow distribution found for sucrose areas considered entire system cells for ten cases relative time boiling sugar masseccuite τ/τ_c ($\tau/\tau_c = 0.15; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9, 1.0$) based on four simultaneous systems solution of the non-stationary parabolic type differential equations in partial derivatives (first system – for unsteady heat conduction problem; and three systems – for non-stationary diffusion mass transfer problems). For the first time based on the calculations found that the process dissolved sucrose flow from the one crystal sucrose solution cell to other crystal sucrose solution cell really is and in which direction it is going. Also for the first time were evaluated quantitative value sucrose diffusion mass flow between sucrose solutions cells areas of different sugar crystals. At the time relative boiling sugar masseccuite $\tau/\tau_c = 0.15$ is the substances (sucrose) transfer from the area 4 (left sucrose solution of crystal 2 cell) in the region 3 (right sucrose solution of crystal 1 cell). Approximately at $\tau_c = 2$ s is reached their minimum. Since at time $\tau_c = 2.58$ s for calculating options with constant thermophysical coefficients situation is reversed, ie the sucrose transfer is already from the area 3 in the area 4. With all variable thermal characteristics per stay system cells in the heating tube the sucrose transfer is still going on field 4 in area 3 and at the exit system cells from heating pipes approaches to zero, that is virtually absent. So in this case were clearly defined minimum diffusion mass flow. At the time relative boiling sugar masseccuite $\tau/\tau_c = 1.0$ were clearly defined minimum and maximum for both constant and variable for all thermal characteristics.

Conclusions. For each sucrose solution area received unsteady diffusion mass sucrose flow value depending on the contact time system cell with a heating tube. The first time the diffusion mass flow value and direction between the two regions sucrose solutions first and second sugar crystals.

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Introduction

The crystalline sucrose in sugar process production is the most energy intensive part.

On the basis of literary analysis revealed that single issue crystallization process of sugar crystal, mass sucrose crystallization and related processes that directly affect these complex processes up to date engaged several authors: Tetiana Vasylenko and Sergii Vasylenko [1], Myronchuk and Dmitrenko [2], Hugot E. [3, 7], Jenkins G.H. [4], Jiahui Chen [5], Baikow V.E. [6], Lauret P. [8], Alewijn W.F. [9], Senglali Aouragh Hassani [10] and Thomas R. Gillett [11].

From the literature review we can conclude that describe the crystallization of sucrose, taking into account all factors that influence this process is extremely difficult. In addition can be said that today there is no single universally accepted approach on this issue.

Therefore, in this paper were implemented one of the next steps in the development and creation as the most complete mathematical model of mass crystallization of sucrose.

It is necessary that mathematical model is developed, more fully described process of simultaneous heat and mass transfer, which takes place between the components of the multiphase system, which is a sugar util.

Just note that all the above described process with all the technological, thermal and hydrodynamic characteristics that affect the sucrose mass crystallization process, practically very difficult or even impossible.

On this basis the number of simplifications was adopted. Therefore, developed a mass crystallization mathematical model is attributed to the idealized model.

So, to continue [12, 13, 14] sugar massecuite also represented as a cellular model [15, 16, 17].

Considered that each sugar crystal cell [16] surrounded by a corresponding sucrose solution cell [17] for the whole sugar massecuite boiling time.

Also assume that hydrodynamic interactions between cells occur only between crystalline sucrose solutions.

At the same time, heat exchange and mass transfer processes occurring inside the system cells and between them.

Modeling simultaneously unsteady heat and mass transfer processes for the entire system cells is very complex, so held in several stages.

In the earlier stages of constructing a mathematical model was found unsteady temperature distribution in all cells of the system.

In the first, a simple case [12] considered unsteady temperature distribution for the system cells that consisted of only one crystal sugar. In the second, more complex cases [13] system cells are made up of two sugar crystals.

In the future creation of a mathematical model of crystallization process considered system cells for two sugar crystals.

So the next step had to find the sucrose transferred value between the cells and the sugar crystal amount will crystallize (or dissolve) in each cell of crystal sugar.

The results of simultaneous unsteady temperature distribution calculation in the «larger sugar crystal–larger sugar crystal sucrose solution–less sugar crystal sucrose solution–smaller sugar crystal–massecuite» system cells and the sucrose concentration in the solutions cells of the same system have been received and are described in detail in [14] for the two sugar crystals case.

The problem in finding the value transferred between cells sucrose sucrose solution and sugar crystal amount will crystallize (or dissolve) in each the sugar crystal cell because of sufficient complexity had to be considered in several stages.

The first step is to find the diffusion mass flow value on the boundaries of each sucrose solution cell entire system cells.

That this issue and the subject of this work.

Based on these calculations finally have the opportunity to find sucrose value transferred between sucrose solution cells and the sugar crystal amount will crystallize (or dissolve) in each sugar crystal cell. What should also be implemented at a later stage to create a mathematical model of sucrose mass crystallization.

So, in this paper we find the diffusion mass flow value on the boundaries of each sucrose solution cell entire system cells which consists of two sugar crystals.

It is understood that solution to this problem is completely based on the simultaneous solution of the unsteady diffusion mass transfer problems between sucrose solution cells and unsteady heat transfer problem for the whole of the system cells.

In this work, to continue [12, 13, 14] the results of mathematical modeling at once unsteady heat process and mass transfer unsteady diffusion processes for two sugar crystals, which are surrounded by respective sucrose solution cells and which simultaneously interact with masseccuite is presented.

Simulation of unsteady diffusion mass transfer process for a system with two sugar crystals was carried out based on the simultaneous solution of three separate non-stationary diffusion mass transfer problems. As a result, the distribution concentration was determined in each cell sucrose solution discussed above system.

Non-stationary heat exchange and mass transfer processes between system components to cells are considered case of system cell contact with the heating (boiling) tube surface of the heating chamber vacuum machine.

Accepted the initial time $\tau_{c,0} = 0$, when the whole cells system adjudged (included) to the bottom of a vertically oriented heating tube.

Final $\tau_{c,end}$ is the one time when the whole system comes out simultaneously with heating tubes in its upper part.

Research regarding the residence time $\tau/\tau_{c,end}$ system cells at heating tube depending on the relative boiling sugar masseccuite time τ/τ_c in detail was considered in [18].

Note also that the crystal's cells, sucrose solution's cells and masseccuite's cell thermal characteristics and diffusion mass transfer coefficient of sucrose solutions cells will depend on the relative boiling sugar masseccuite time τ/τ_c .

Materials and methods

In this paper, the search unsteady sucrose diffusion mass flow distribution on the sucrose solutions cells boundaries entire system cells completely based on obtained mutual unsteady temperature distribution in all system cells components and simultaneously received on the between the solution cells concentrations distribution of the whole system cells.

Methods of obtaining temperature distribution in the whole system cells simultaneously interference the distribution concentration counting only in the sucrose solution the same system cells was examined in detail in [14].

Similarly in [13], first consider the volumetric case system of cells: «larger sugar crystal–larger sugar crystal sucrose solution–less sugar crystal sucrose solution–smaller sugar crystal–masseccuite». By a similar method [13] made the transition from the volume cell model to equivalent one-dimensional model.

Similarly, the work [14], the non-stationary heat and mass transfer problems considered next 7 dimensional regions (Fig. 1), simultaneously pairs in contact with each other:

1 – left area larger crystal sucrose solution;

- 2 – larger crystal sugar;
- 3 – rights area larger crystal sucrose solution;
- 4 – sucrose solution left area smaller crystal;
- 5 – smaller crystal sugar;
- 6 – rights area smaller crystal sucrose solution
- 7 – massecuite.

The problem of unsteady heat transfer process simultaneously for the entire system cells has been considered by a non-stationary heat problem solution. This heat conduction problem consists of system non-stationary heat conduction problems with appropriate initial and boundary conditions [13]. Thus, the system unsteady heat transfer problems considered simultaneously for all 7 one-dimensional regions (Fig. 1), in pairs in contact with each other.

The non-stationary diffusion mass transfer process problem only for sucrose solutions has been considered by three separate unsteady mass transfer problems solution.

For three individual unsteady mass transfer problems considered one-dimensional four areas (Fig. 1): 1, 3, 4, and 6:

1 – the first unsteady diffusion mass transfer problem involved the first area, representing the left larger crystal sucrose solution region (Fig. 1);

2 – the second unsteady diffusion mass transfer problem involved the simultaneous contact areas 3 and 4 of the ideal mass transfer between them (Fig. 1);

3 – the third unsteady diffusion mass transfer problem was about the sixth area, representing the right smaller crystal sucrose solution region (Fig. 1).

Finding the unsteady heat conduction problem solution for the entire system cells and three unsteady diffusion mass transfer problems solutions for sucrose solution areas were interrelated and considered as one large system of equations.

To solve such a complex system of non-stationary differential equations with constant and with variable thermophysical characteristics of analytical methods [19] is difficult and almost impossible.

Therefore, in this case, similar to the cases examined [12, 13, 14] were applied numerical methods using well-known methods of controlling volume [20, 21].

Thus, when writing programs algorithm to obtain the unsteady heat conduction problem solution for the entire system cells and at the same time three unsteady diffusion mass transfer problems solutions for sucrose solution areas at each step conducting of the time and to coordinate calculations it was considered that they are interconnected.

The calculation of non-stationary sucrose solutions diffusion mass flow for the entire system cells also performed using numerical methods on the basis of the non-stationary concentration distribution problems solutions in their respective fields sucrose solution.

Assume the following notation. Diffusion mass flow on the boundary of two areas simultaneously in contact with each other, denoted by the value j_{mn} , ($(mn) = \{01; 21; 23; 34; 54; 56; 67\}$). Where the value of m is the number area from which comes (flowing) sucrose mass flow, and the value n is the number field which includes (enters) sucrose mass flow (Fig. 1). Region "zero" formally put in writing the algorithm calculation program for recording left boundary condition for the first sucrose solution area (Fig. 1).

Assume the following signs agreement concerning the value of sucrose mass flow j_{mn} , ($(mn) = \{21; 23; 34; 54; 56\}$):

- the substance (sucrose) comes out of the region m in region n , then the sign of the mass flow value j_{mn} , ($(mn) = \{21; 23; 34; 54; 56\}$) will be positive $j_{mn} > 0$ (Fig. 1)

- if the same substance (sucrose) comes out of the field n in the region m , is a sign of the mass flow value j_{mn} , ($(mn) = \{21; 23; 34; 54; 56\}$) will be negative $j_{mn} < 0$ (Fig. 1).

In Fig. 1 the footnotes show all considered in this paper cases diffusion mass flow of sucrose at appropriate boundary. Direction arrows on each of these points footnotes received positive direction of the sucrose mass flow at appropriate boundaries neighboring areas.

Setting and solution unsteady heat and mass transfer problems similar to [14] considered for future constant and variable cases selection thermal characteristics and diffusion mass transfer coefficient:

I) all the thermal characteristics in the non-stationary heat transfer problem calculation and the diffusion coefficient in the non-stationary diffusion mass transfer problem calculations is constant in all sucrose solution areas;

I) all the thermal characteristics in the non-stationary heat transfer problem calculation and the diffusion coefficient in the non-stationary diffusion mass transfer problem calculations is constant in all sucrose solution areas:

II, a) at every calculating time step all variable thermal characteristics (density, the thermal conductivity and the heat capacity) in all regions depend only on the current (variable) temperature of the corresponding cell.

In calculating non-stationary mass transfer problem the diffusion mass transfer coefficient in sucrose solution areas depended only on the current (variable) temperature corresponding cell.

A concentration of sucrose content in each area was fixed and taken an equal (steel) content of sucrose concentration in massecuite at a given relative time τ/τ_c .

II, b) as in the previous paragraph in the calculation of non-stationary heat problem at every step of calculating time-variable thermal properties (density, thermal conductivity and heat capacity) in all regions dependent only on the current (variable) temperature corresponding cell.

In calculating non-stationary mass transfer problem the diffusion mass transfer coefficient in sucrose solution areas are dependent on current (variable) temperature and the current (variable) sucrose content in each respective region at this time relative τ/τ_c .

II, c) the non-stationary heat problem calculation at every step of calculating time thermal characteristics (only density and heat capacity) in all regions dependent only on the current (variable) temperature corresponding cell. All other parameters change (factors) are dependent on the density and heat capacity were fixed for each respective region at this time relative τ/τ_c .

At every step of calculating time a variable thermal conductivity in the areas of both sugar crystals and massecuite depend only on the current (variable) temperature. Current solids content in the cell massecuite was fixed (constant) in this relative time τ/τ_c .

At every time calculating step a variable thermal conductivity in areas sucrose solution depends on the current (variable) temperature and current of dry matter content in each respective field of cell sucrose solution.

At every time calculating step a variable thermal conductivity in areas sucrose solution depends on the current (variable) temperature and current of dry matter content in each corresponding sucrose solution area cell.

In calculating non-stationary mass transfer problem at every calculating time step variable mass transfer diffusion coefficient in sucrose solution areas depends on the current (variable) temperature and the current sucrose content in each corresponding area cell sucrose solution.

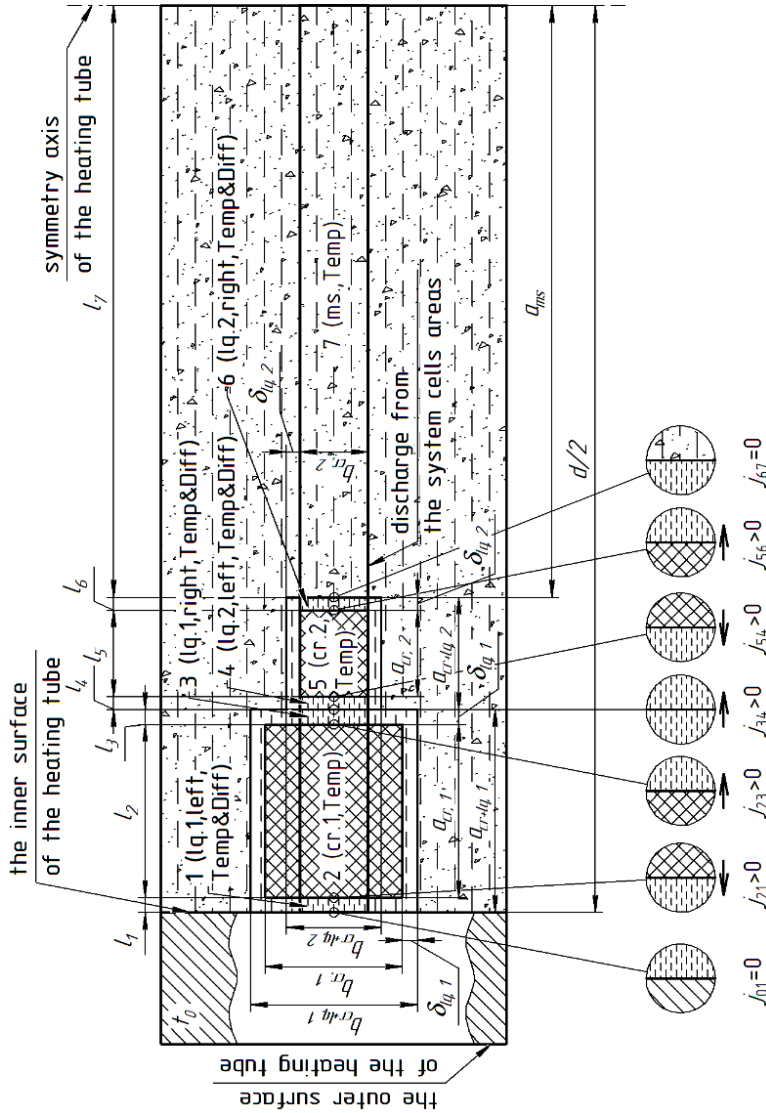


Fig. 1. One-dimensional case system cells, "the left side larger crystal sucrose solution cell—larger sugar crystal cell—right side larger crystal sucrose solution cell—left side smaller crystal sucrose solution cell—smaller sugar crystal cell—right side smaller crystal sucrose solution cell—massecuite" simultaneously take participate in non-stationary heat exchange and mass transfer diffusion processes for the calculation of unsteady sucrose diffusion mass flow.

Designation:

- *) For areas: Temp—considered only unsteady heat conduction problems;
- Temp&Diff—considered simultaneously unsteady heat problem and nonstationary mass diffusion transfer problem.
- *) For sucrose diffusion mass flow J_{ms} : (mn) = {21; 23; 34; 54; 56}:

Formulation of non-stationary heat conduction problems for the steady case (I) and variable cases (II, a and II, b) thermal characteristics and methods of solving such problems by numerical methods was discussed in detail in [13].

Production of non-stationary heat conduction problems for the variable case (II, c) thermal characteristics and methods of solving such problems by numerical methods was similar to the method considered in [13].

Production of non-stationary diffusion mass transfer problems in the steady case (I) and variable cases (II, a and II, b) thermal characteristics and methods of solving such problems by numerical methods similar to that discussed in detail in [14].

Formulation of unsteady three separate tasks diffusion mass transfer in the variable case (II, c) thermal characteristics and methods of solving such problems by numerical methods was discussed in detail in [14].

Remind once again, that [14] considered three separate simultaneous solution of of unsteady diffusion mass transfer problems with a solution of non-stationary heat problem.

On the basis of these three unsteady diffusion mass transfer problems [14] write the following equation to determine the diffusion mass flow in the sucrose solution boundary.

Note that previously put the diffusion mass flow value j_{mn} , (mn) = {01; 21; 23; 34; 54; 56; 67} calculated per unit area and its dimension in this paper is $\text{kg}/(\text{m}^2 \cdot \text{sec})$.

Consider the issue of boundary conditions formation for each area that reflects the appropriate cell sucrose solution.

First, we note the following. We accept that the concentration value at each border sucrose solution region in contact with the appropriate crystal sugar equals the saturation concentration sucrose solution. The value is calculated saturated concentrations at the current temperature of the crystal surface, which is in contact this sucrose solution area.

This applies to all sucrose solutions areas for all three specific unsteady diffusion mass transfer problems.

Thus, the first unsteady diffusion mass transfer problem, which concerned one area on the left border of the diffusion mass flow is absent. This fact get from a physical point of view, because there is one area in contact with the heating tubes surface, which is not leading mass.

As a result, we get the following equation:

$$j_{01} = -\rho_1(t_1, Pr_1, DS_1) \cdot D_1(t_1, Cx_1) \frac{\partial C_1}{\partial x} \Big|_{x=0} = 0. \quad (1)$$

As in the previous case, it was assumed that the diffusion mass transfer area between sucrose solution region 6 and massecuite region not happens (or is so small that it can be ignored):

$$j_{67} = -\rho_6(t_6, Pr_6, DS_6) \cdot D_6(t_6, Cx_6) \frac{\partial C_6}{\partial x} \Big|_{x=\sum_{k=1}^6 l_k} = 0. \quad (2)$$

All other sucrose diffusion flows at the diffusion mass transfer processes were calculated based on the following equation:

$$j_{21} = -\rho_1(t_1, Pr_1, DS_1) \cdot D_1(t_1, Cx_1) \frac{\partial C_1}{\partial x} \Big|_{x=l_1} \quad (3)$$

$$j_{23} = -\rho_3(t_3, Pr_3, DS_3) \cdot D_3(t_3, CX_3) \frac{\partial C_3}{\partial x} \Big|_{x=l_1+l_2} \quad (4)$$

$$\begin{aligned} j_{34} &= -\rho_3(t_3, Pr_3, DS_3) \cdot D_3(t_3, CX_3) \frac{\partial C_3}{\partial x} \Big|_{x=l_1+l_2+l_3} = \\ &= -\rho_4(t_4, Pr_4, DS_4) \cdot D_4(t_4, CX_4) \frac{\partial C_4}{\partial x} \Big|_{x=l_1+l_2+l_3} \end{aligned} \quad (5)$$

$$j_{54} = -\rho_4(t_4, Pr_4, DS_4) \cdot D_4(t_4, CX_4) \frac{\partial C_4}{\partial x} \Big|_{x=l_1+l_2+l_3+l_4} \quad (6)$$

$$j_{56} = -\rho_6(t_6, Pr_6, DS_6) \cdot D_6(t_6, CX_6) \frac{\partial C_6}{\partial x} \Big|_{x=l_1+l_2+l_3+l_4+l_5} \quad (7)$$

To calculate the diffusion mass flow values j_{mn} , ($m=2, 3, 5, n=1, 3, 4, 6, m \neq n$), the equations (3)–(7) also applied numerical simulation. In applying of numerical methods the equations (1)–(7) conducted approximation for the first and second order accuracy.

Note that in this study the results of diffusion flows calculations between sucrose solution areas using a second-order approximation.

As in [12, 13, 14], the initial temperature of the system cells (Fig. 1) assumed equally to all areas simultaneously and equal 75°C.

As in [12, 13, 14], the initial concentration for each area between the crystal sucrose solution calculated with a coefficient supersaturation $S=1$. Thus, it shall be taken as in the saturation concentration state under already accepted the initial temperature and equal to 77,594%.

The temperature of the heating tube's inner wall assumed constant over the tube entire height and equal 100 °C.

Also, [14], it is assumed that between areas 3 and 4 sucrose solutions entire system cells is a perfect law of mass transfer.

Thus, in this case, as cases [12, 13, 14] were applied numerical methods using well-known methods of controlling volume [20, 21].

Discretization in time was $\Delta\tau_c = 0,01$ s.

The coordinate discretization for each area 1–6 was uniform, and for the area 7 (Fig. 1) masseccuite was uneven.

Each region separately (Fig. 1) smashed on the corresponding control volumes number: $n_1 = n_3 = n_4 = n_6 = 10, n_2 = n_5 = 20, n_7 = 100$.

The cells values are accepted the following sizes: $a_{cr,1} = 5,0 \cdot 10^{-4}$ m, $\delta_{lq,1} = 4,29 \cdot 10^{-5}$ m, $a_{cr,2} = 2,5 \cdot 10^{-4}$ m, $\delta_{lq,2} = 3,73 \cdot 10^{-5}$ m, $a_{ms} = 4,83896 \cdot 10^{-2}$ m.

Based on the calculations [18], the end contact time of the cell system with the heating tubes wall for boiling relative time $\tau/\tau_c = 0,15$ is $\tau_{c,end} = 3,95$ sec, and with $\tau/\tau_c = 1,0$ is $\tau_{c,end} = 67,93$ sec.

Results and discussion

The calculations for the above-mentioned non-stationary heat conduction problems and three non-stationary diffusion mass transfer problems were conducted for all areas of system cells the following values relative sugar massecuite boiling time $\tau/\tau_u = 0,15; 0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8; 0,9; 1,0$.

Because of limited volume in this paper are given only two cases relative boiling sugar massecuite time τ/τ_c : at the winding crystals time ($\tau/\tau_c = 0,15$) and complete the boiling sugar massecuite time ($\tau/\tau_c = 1,0$).

The unsteady sucrose diffusion mass flows distribution calculations results in an appropriate areas border 1, 3, 4, and 6 (Fig. 1) sucrose solution at $\tau/\tau_c = 0,15$ are given:

- diffusion mass flow j_{21} substances (sucrose) from region 2 (first crystal) to the sucrose solution region 1 – in Fig. 2;
- diffusion mass flow j_{23} substances (sucrose) from region 2 (first crystal) to the sucrose solution region 3 – in Fig. 3;
- diffusion mass flow j_{34} substances (sucrose) from the sucrose solution region 3 to the sucrose solution region 4 – in Fig. 4;
- diffusion mass flow j_{54} substances (sucrose) from the region 5 (second crystal) to sucrose solution region 4 – in Fig. 5;
- diffusion mass flow j_{56} substances (sucrose) with region 5 (second crystal) to sucrose solution 6 – in Fig. 6.

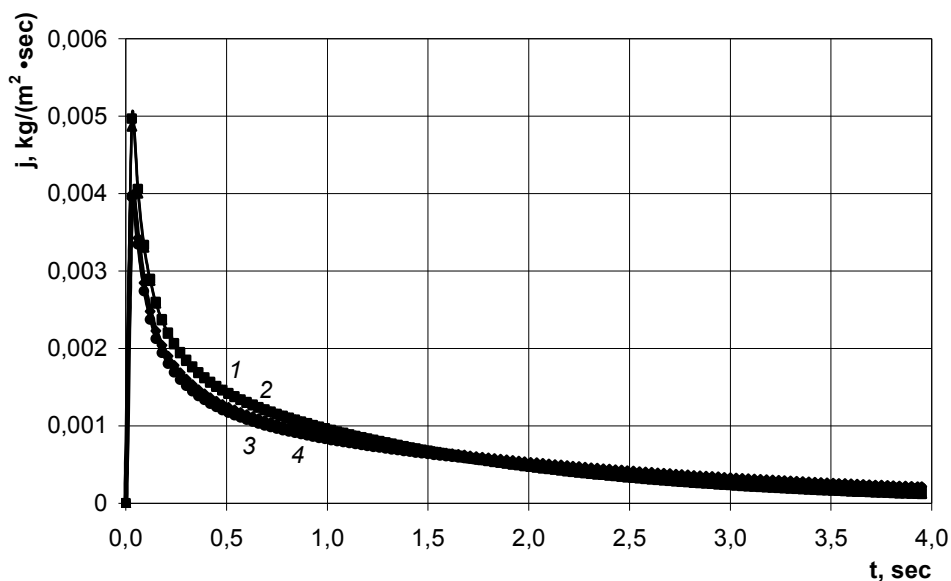


Fig. 2. Sucrose diffusion mass flow j_{21} on the boundary (Fig. 1) region 2 (the first sugar crystal) and region 1 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time massecuite boiling $\tau/\tau_c = 0,15$; [value $j_{21} > 0$ if the substance (sucrose) is transferred from the region 2 (the first sugar crystal) in the region 1 (sucrose solution)].

*Designations:

- 1 – all thermal characteristics and diffusion mass transfer coefficient are a constant (option I);
- 2 – thermal characteristics and diffusion mass transfer coefficient are variables (variant II, a);
- 3 – thermal characteristics and diffusion mass transfer coefficient are variables (variant II, b);
- 4 – thermal characteristics and diffusion mass transfer coefficient are variables (variant II, c);

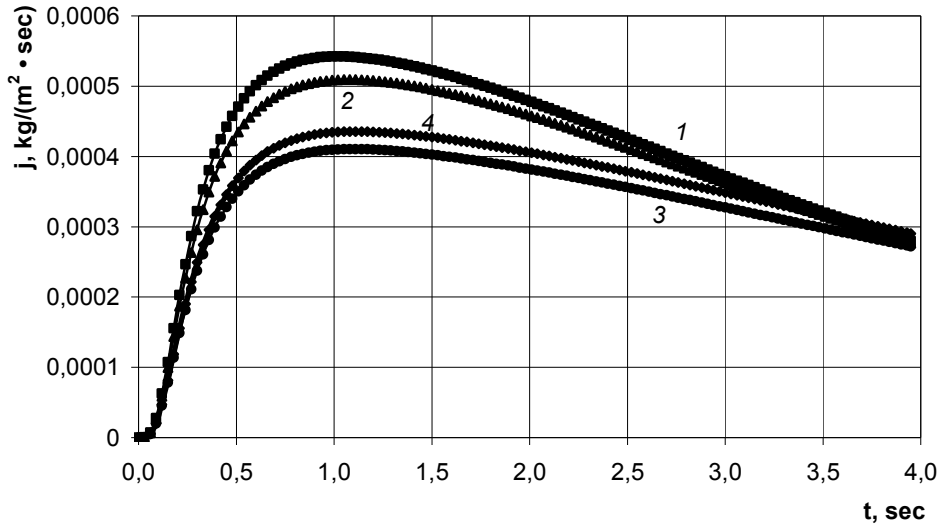


Fig. 3. Sucrose diffusion mass flow j_{23} on the boundary (Fig. 1) region 2 (the first sugar crystal) and region 3 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time masseccuite boiling $\tau/\tau_c=0,15$; [value $j_{23}>0$ if the substance (sucrose) is transferred from the region 2 (the first sugar crystal) in the region 3 (sucrose solution)]. *Designations the same as in Fig. 2.

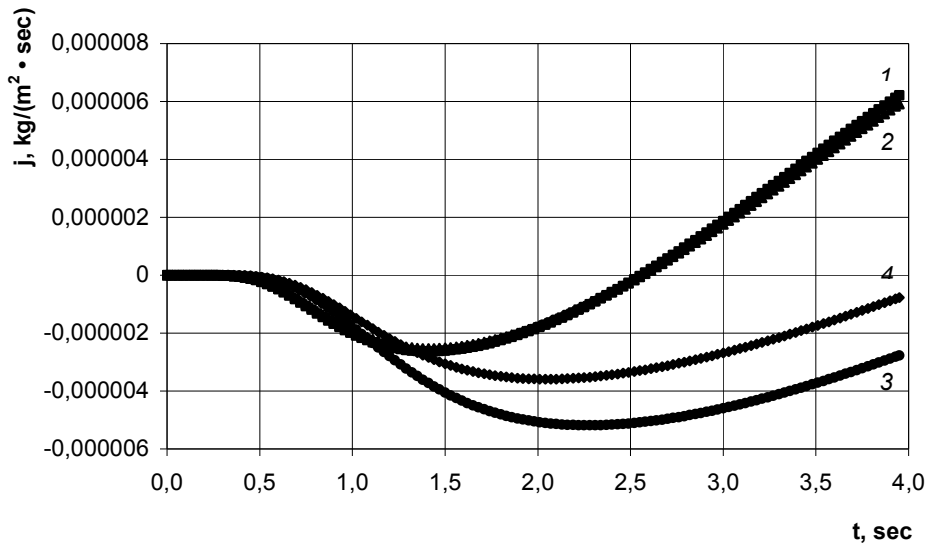


Fig. 4. Sucrose diffusion mass flow j_{34} on the boundary (Fig. 1) region 3 and region 4 (sucrose solutions both) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time masseccuite boiling $\tau/\tau_c=0,15$; [value $j_{34}>0$ if the substance (sucrose) is transferred from the region 3 (sucrose solution) in the region 4 (sucrose solution)]. *Designations the same as in Fig. 2.

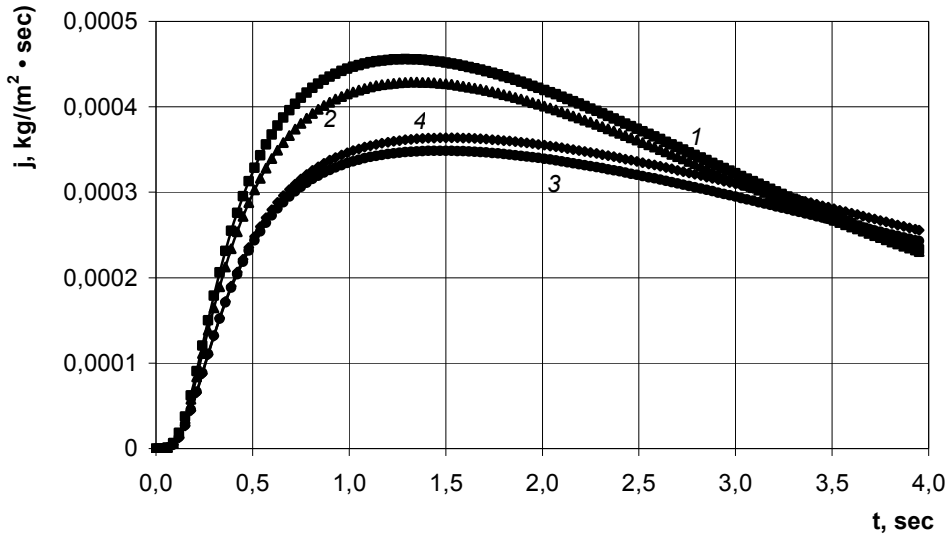


Fig. 5. Sucrose diffusion mass flow j_{54} on the boundary (Fig. 1) region 5 (the second sugar crystal) and region 4 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time massecuite boiling $\tau/\tau_c=0,15$; [value $j_{54}>0$ if the substance (sucrose) is transferred from the region 5 (the second sugar crystal) in the region 4 (sucrose solution)]. *Designations the same as in Fig. 2.

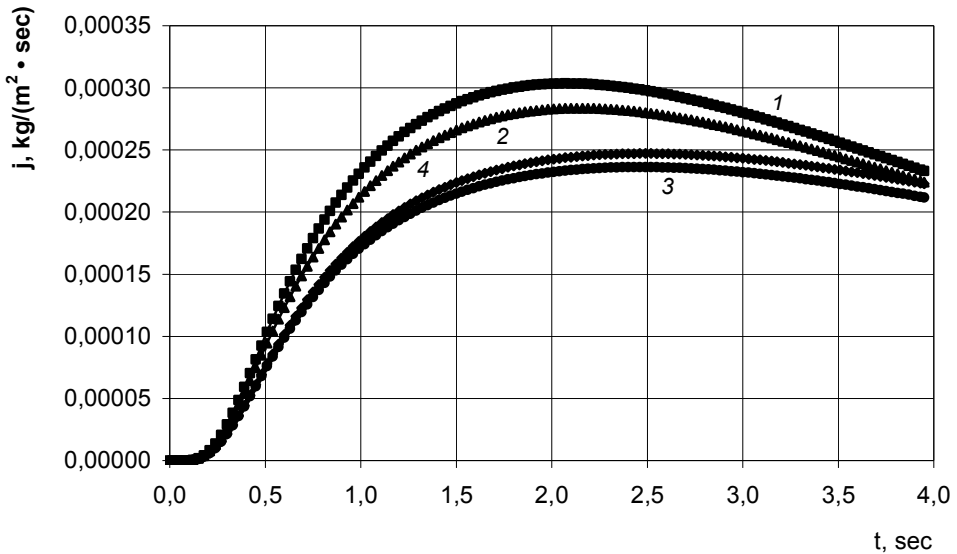


Fig. 6. Sucrose diffusion mass flow j_{56} on the boundary (Fig. 1) region 5 (the second sugar crystal) and region 6 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time massecuite boiling $\tau/\tau_c=0,15$; [value $j_{56}>0$ if the substance (sucrose) is transferred from the region 5 (the second sugar crystal) in the region 6 (sucrose solution)]. *Designations the same as in Fig. 2.

Finally, we present the calculations results of unsteady sucrose diffusion mass flows distribution in sucrose solutions in an appropriate areas border 1, 3, 4, and 6 (Fig. 1) sucrose solution at $\tau/\tau_c=1.0$:

- diffusion mass flow j_{21} substances (sucrose) from region 2 (first crystal) to the sucrose solution region 1 – in Fig. 7;
- diffusion mass flow j_{23} substances (sucrose) from region 2 (first crystal) to the sucrose solution region 3 – in Fig. 8;
- diffusion mass flow j_{34} substances (sucrose) from the sucrose solution region 3 to the sucrose solution region 4 – in Fig. 9;
- diffusion mass flow j_{54} substances (sucrose) from the region 5 (second crystal) to sucrose solution region 4 – in Fig. 10;
- diffusion mass flow j_{56} substances (sucrose) with region 5 (second crystal) to sucrose solution 6 — in Fig. 11.

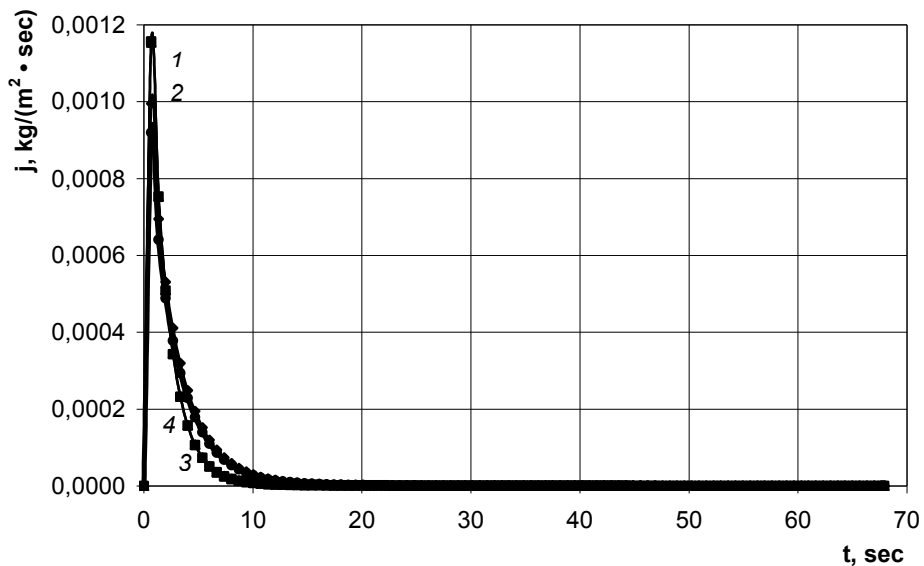


Fig. 7. Sucrose diffusion mass flow j_{21} on the boundary (Fig. 1) region 2 (the first sugar crystal) and region 1 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time masseuite boiling $\tau/\tau_c=1,0$; [value $j_{21}>0$ if the substance (sucrose) is transferred from the region 2 (the first sugar crystal) in the region 1 (sucrose solution)].

*Designations the same as in Fig. 2.

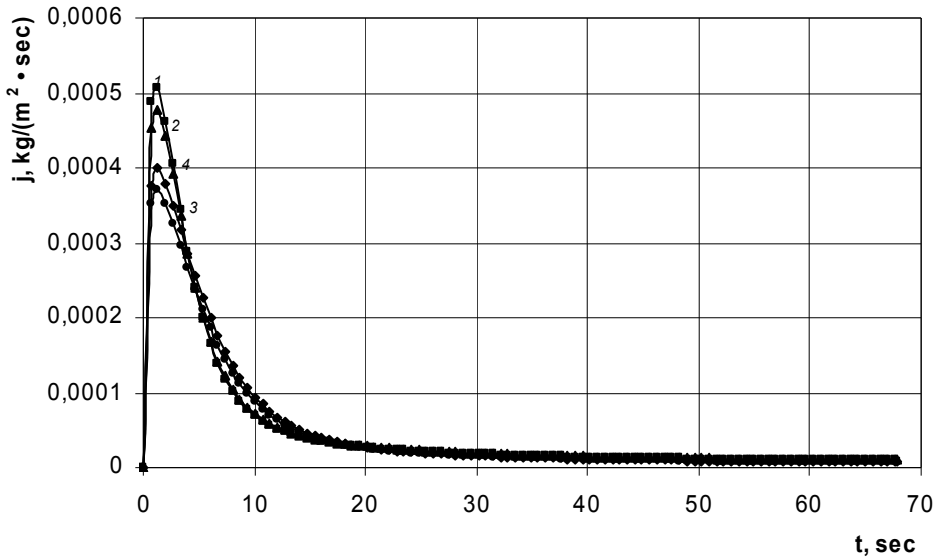


Fig. 8. Sucrose diffusion mass flow j_{23} on the boundary (Fig. 1) region 2 (the first sugar crystal) and region 3 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time massecuite boiling $\tau/\tau_c=1,0$; [value $j_{23}>0$ if the substance (sucrose) is transferred from the region 2 (the first sugar crystal) in the region 3 (sucrose solution)]. *Designations the same as in Fig. 2.

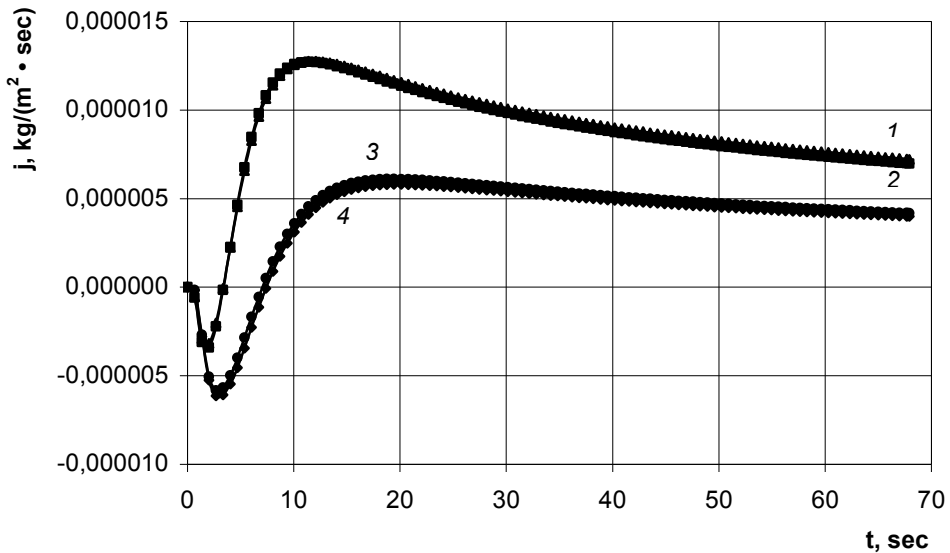


Fig. 9. Sucrose diffusion mass flow j_{34} on the boundary (Fig. 1) region 3 and region 4 (sucrose solutions both) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time massecuite boiling $\tau/\tau_c=1,0$; [value $j_{34}>0$ if the substance (sucrose) is transferred from the region 3 (sucrose solution) in the region 4 (sucrose solution)]. Designations the same as in Fig. 2.

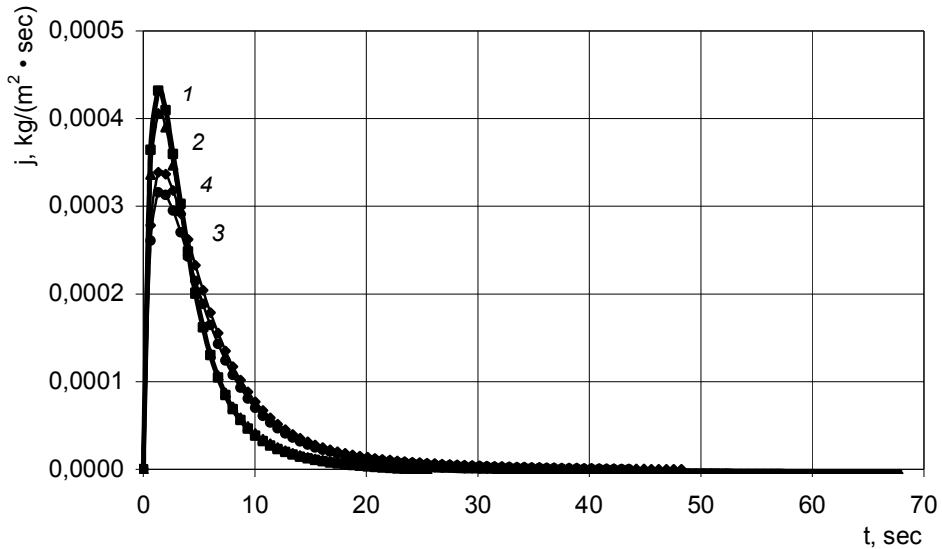


Fig. 10. Sucrose diffusion mass flow j_{54} on the boundary (Fig. 1) region 5 (the second sugar crystal) and region 4 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time masseccuite boiling $\tau/\tau_c=1,0$; [value $j_{54}>0$ if the substance (sucrose) is transferred from the region 5 (the second sugar crystal) in the region 4 (sucrose solution)]. *Designations the same as in Fig. 2.

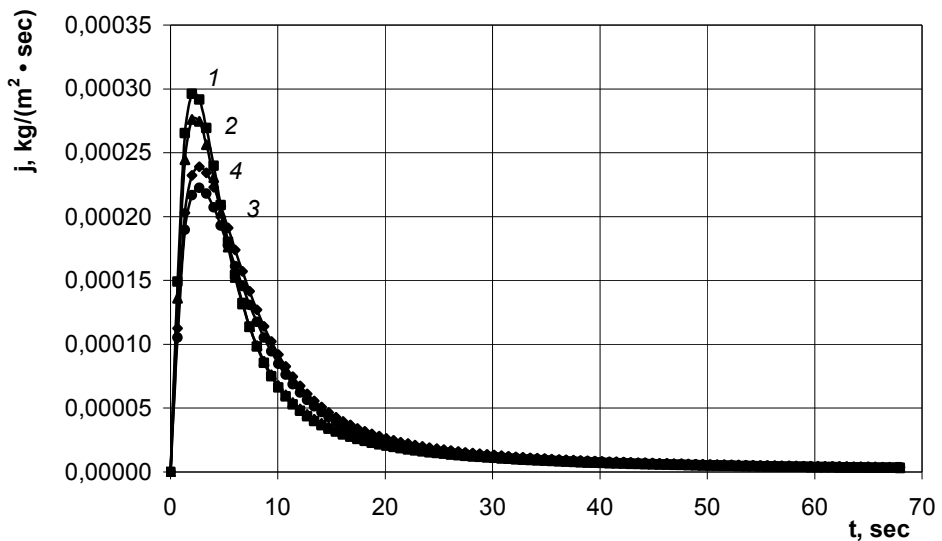


Fig. 11. Sucrose diffusion mass flow j_{56} on the boundary (Fig. 1) region 5 (the second sugar crystal) and region 6 (sucrose solution) depending on the contact time τ system cells from the inner surface of the heating tubes with a relative time masseccuite boiling $\tau/\tau_c=1,0$; [value $j_{56}>0$ if the substance (sucrose) is transferred from the region 5 (the second sugar crystal) in the region 6 (sucrose solution)]. *Designations the same as in Fig. 2.

As can be seen from the graphs diffusion mass flows of sucrose:

- Fig. 2 and Fig. 7 the value $j_{21} > 0$ for all the calculations cases (ie, constant thermal coefficient case (I) and variable thermal coefficient (II, a), (II, b) and (II, c)). That is the solute (sucrose) transfer from region 2 (the first sugar crystal) in region 1 (sucrose solution). Thus, under these conditions the first crystal dissolves. This result could be expected, because, recall, supersaturation coefficient in this study was taken to 1. Further, as shown in (Fig. 2, Fig. 7), diffusion mass flow for all versions calculations differ little between them. Also shows that in both cases $\tau/\tau_c = 0,15$ and $\tau/\tau_c = 1,0$ at the outlet of the heating tube diffusion mass flow is almost close to zero, ie, disappears.

- Fig. 3 and Fig. 8 the value $j_{23} > 0$ for all the calculations cases ((I), (II, a), (II, b) and (II, c)). That is the solute (sucrose) transfer from region 2 (the first sugar crystal) in region 3 (sucrose solution). Thus, under these conditions the first crystal dissolves also. Further, as shown in (Fig. 3, Fig. 8), diffusion mass flow in variant (I) and (II, a), calculations differ little between them. As well had differing versions of calculations (II, b) and (II, c). In $\tau/\tau_c = 0,15$, as shown in Fig. 3, the output of the heating tube diffusion mass flow is 51–67% of the its maximum value. Also shows in the case $\tau/\tau_c = 1,0$ at the outlet of the heating tube diffusion mass flow is almost close to zero, ie, disappears.

- Fig. 4 and Fig. 9 diffusion mass flow j_{34} calculations in different variants ((I), (II, a), (II, b) and (II, c)) has the largest difference from each other. Note that the calculations results for options (I) and (II a) almost coincide with each other (Fig. 4, Fig. 9). Calculations for variants (II b) and (II a) and almost identical to each other.

Note that in Fig. 4 clearly shows that the first $j_{34} < 0$. That is, transfer agents (sucrose) from the field 4 (the first crystal sucrose solution) in region 3 (the second crystal sucrose solution). Around the time $\tau_c = 2$ s is reached their minimum.

For calculation options (II, b) and (II, c) the sucrose diffusion mass flow value is close to zero, and the substance transfer direction remains the same.

Since at time $\tau_c = 2,58$ s for calculation options (I) and (II a) carrying direction changes to the opposite sucrose as $j_{34} > 0$. So sucrose begins transferred from region 3 (the first crystal sucrose solution) in 4 (the second crystal solution sucrose).

Further, in Fig. 9 clearly shows that at first substance (sucrose) is transferred also from the region 4 (the first crystal sucrose solution) in region 3 (the second crystal sucrose solution). Approximately at time $\tau_c = 2$ s is reached their minimum.

Then the situation is reversed. In other words, sucrose begins transferred from the region 3 (the first crystal sucrose solution) in 4 (the second crystal sucrose solution). For calculation options (I) and (II a) value j_{34} reaches its maximum respectively at time $\tau_c = 11,39$ sec and $\tau_c = 12,06$ sec. For calculation options (I, b), and (II, c) the value of j_{34} reaches its maximum respectively at time $\tau_c = 19,43$ sec and $\tau_c = 20,10$ sec. Further, in all the calculations cases after reaching its maximum sucrose diffusion mass flow start to decrease to the leaving heating tube time.

The research at $\tau/\tau_c = 0,15$ showed that the ratio of the minimum diffusion mass flow value $j_{34, \min}$ (between regions 3 and 4 both sucrose solutions) compared with the maximum diffusion mass flow value $j_{23, \max}$ (from region 2 (the first crystal) in region 3 (sucrose solution)) and value $j_{54, \max}$ (from region 5 (the second crystal) in region 4 (sucrose solution)) for different calculation variants is:

- within 0,49–0,58% for the calculation option (I);
- within 0,50–0,59% for the calculation option (II, a);
- within 1,26–1,49% for the calculation option (II, b);
- within 0,82–0,98% for the calculation option (II, c).

The research at $\tau/\tau_c=0,15$ showed that the ratio of the maximum diffusion mass flow value $j_{34,\max}$ (between regions 3 and 4 both sucrose solutions) compared with the maximum diffusion mass flow value $j_{23,\max}$ (from region 2 (the first crystal) in region 3 (sucrose solution)) and value $j_{54,\max}$ (from region 5 (the second crystal) in region 4 (sucrose solution)) for different calculation variants is:

- within 2,52–2,95% for the calculation option (I);
- within 2,68–3,14% for the calculation option (II, a);
- within 1,64–1,93% for the calculation option (II, b);
- within 1,45–1,71% for the calculation option (II, c).

Most importantly, in this paper for the first time found that the process dissolved sucrose flow from one solution cell to another really happening (even supersaturation factor equal to 1). Also for the first time obtained a quantitative diffusion mass flow value j_{34} between sucrose solutions cells (Fig. 4, Fig. 9).

- Fig. 5 and Fig. 10 the value $j_{54}>0$ for all the calculations cases ((I), (II, a), (II, b) and (II, c)). That is the solute (sucrose) transfer from region 5 (the second sugar crystal) in region 4 (sucrose solution). Thus, under these conditions the second crystal dissolves also. This result could be expected, because, recall, supersaturation coefficient in this study was taken to 1. As shown in (Fig. 5, Fig. 10), diffusion mass flow in variant (II, b) and (II, c) calculations differ little between them.

- Fig. 6 and Fig. 11 the value $j_{56}>0$ for all the calculations cases ((I), (II, a), (II, b) and (II, c)). That is the solute (sucrose) transfer from region 5 (the second sugar crystal) in region 6 (sucrose solution). Thus, under these conditions the second crystal dissolves also. As shown in (Fig. 6, Fig. 11), diffusion mass flow in variant (II, b) and (II, c) calculations differ little between them.

Thus, as in [14] can also be concluded that variants calculations (II, b) and (II, c) coincide with each other. Thus, based on the unsteady diffusion mass flow calculations between the system cells components also may be advisable in future to carry out calculations is the variable thermal characteristics case, variant (II, b).

Conclusions

In this work was the non-stationary sucrose diffusion mass flow calculation was conducted for sucrose solution cells from the «larger sugar crystal–larger sugar crystal sucrose solution–less sugar crystal sucrose solution–smaller sugar crystal–massecuite» system cells depending on the boiling sugar massecuite time.

The results is obtained from the simultaneous non-stationary heat conduction problems and three of unsteady diffusion mass transfer problems system solution by numerical methods.

Calculations were made for relative boiling sugar massecuite time $\tau/\tau_c = 0.15; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 1.0$.

This paper presents the results only for two cases relative boiling time: $\tau/\tau_c = 0,15$ and $\tau/\tau_c = 1,0$.

For each options of τ/τ_c in this paper considered four different computing variants: with constant (variant I) and three variants (II, a, II, b and II, c) with variable thermophysical characteristics and the mass transfer diffusion coefficient.

With further research to create a mathematical model of crystallization process according to the author should choose the formulation and solution of non-stationary heat

conduction problems and mass transfer in the case of variable thermal characteristics of the option (II, b). This is the match the real physical process of mass sucrose crystallization of these variants (I), (II, a), (II, b), although it will concede variant calculations (II, c).

Most importantly, in this paper for the first time found that the dissolved sucrose flow process from one cell to another solution really is. Also for the first time were evaluated quantitative unsteady diffusion mass flow magnitude between sucrose solutions cells one and other sugar crystals (Fig. 4, Fig. 9).

The unsteady diffusion mass flow calculations results needed in the future directly for:

- the number between cells sucrose transferred values between sucrose solution for one and second sugar crystal;
- the number of sugar crystal that will crystallize (or dissolve) in each sugar crystal cell of the considered system cells.

The system consists of two sugar crystals, each of which is surrounded by a corresponding sucrose solution amount.

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Intensification of mass transfer processes in gas-liquid media by discrete – pulse energy input method

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Abstract

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Introduction. The aim of this study was the intensification of the aeration of culture media in a fermenter by the method of discrete – pulse input of energy, which is being implemented in a rotor – pulsating apparatus.

Materials and methods. The process of aeration of culture media in the technology of yeast *Saccharomyces cerevisiae* growing by discrete – pulse energy input. The mass transfer rate of oxygen was determined by the number of yeast biomass grown for cultivation period.

Results and discussion. During the experiments on cultivation of yeast on molasses solutions the mass transfer rate of oxygen dependence on the angular rate of the rotor unit in the culture medium with a solids content of 3 – 10% was determined. With the reduction of the solids content from 10 to 5% by treatment with an angular rate of rotor of 48 rps, the mass transfer rate is increased by 1.9 times. As the frequency of the flow pulsations increases from 2 to 3.85 kHz, mass transfer increases from 4 to 6.3 g / l per h at solids content – 3% and from 2,2 to 4 g / l per h at solids content – 10%. A further increase of the frequency of pulsations leads to inactivation of the yeast cells. It was also found that the optimal value of the flow shear rate is $90 - 100 \cdot 10^3 \text{ s}^{-1}$.

Conclusions. The results of this study suggest that the use of the DPIE method in absorption technologies can significantly intensify the processes of mass transfer.

Introduction

The dissolution of sparingly soluble gases in technological liquids is an essential process in a number of food industry plants. Thus, the process of oxidation of technological water with oxygen contained in the air during water treatment of alcohol, brewing and other industries allows to remove from the water undesirable impurities such as iron, manganese, hydrogen sulfide. Wastewater treatment alcohol and brewing industries, causing biological pollution, also occurred by chemical and biological oxidation of waste. Aeration of the culture media used in biological productions during culturing aerobic microorganisms. Oxygen breathing of organisms, being an order of magnitude more energetically favorable than the alcoholic fermentation, is at the heart of the process of growing biomass of yeast for bakery, alcohol industry, brewing, etc [1]. The main problem to be solved in the process of yeast growing is to provide a sufficient number of cells of dissolved oxygen during the entire culture period. The low equilibrium concentration of dissolved oxygen in water and nutrient media at a temperature of the process (30 °C) with a high rate of oxygen consumption by yeast cells is a constant need for supplying oxygen in the air into the culture medium.

Literary analysis

By the process of aeration of culture media continued interest displayed by the scientific community. It focuses on two aspects – the study of the impact of aeration on the growth or production of target metabolites by microorganisms, as well as ways to change the characteristics of mass transfer operation of the aeration devices [2–4].

Air flow rate passing through the culture medium in the bubble machines, mainly used for process of yeast growing an achieve significant volumes [5]. This is related to the fact that occurring during the bubbling air bubbles coalesce quickly, substantially reducing the surface contact between the phases. In addition, the terms of flow aeration process such that the mass of yeast unevenly distributed over the volume of the apparatus, which has a negative impact on results of process. The process of dissolving oxygen in the culture media is generally dependent upon the difference of oxygen concentration in the gas and liquid phases, the contact area of the phases, as well as hydrodynamic conditions at the interface. It should be noted that the main resistance to the mass transfer of oxygen has liquid. The mass transfer resistance inside the gas phase, as well as resistance at the liquid – cell interface is usually neglected.

Thus, the intensification of the mass transfer process is directed action on each of these parameters.

One method of providing directional effect on the treated liquid medium in the dispersion process, dissolving, emulsifying, mixing, catalysis, etc., is a method of discretely – pulsed power input [6]. The principle of the method consists in that previously entered stationary and randomly distributed in the working volume energy to accumulate (concentrate) in the local discrete points of system to achieve the desired effects. The aim of the method is the intensification of heat and mass transfer and hydrodynamic processes in technological media, as well as the creation of methods of optimization and control methods. Realization of the method involves the creation of a large number of uniformly distributed in a dispersion medium working elements or working elements which transform the fixed thermal, mechanical or other types of energy in the energy-power pulses, discrete in time and space. Accompanying these phenomena shock waves, interfacial turbulence, cavitation, penetrating cumulative microjet, vortices caused at interfaces such as Rayleigh –

Taylor and Kelvin-Helmholtz instability, which leads a significant increase in the total surface of phase contact and improve mass and heat transfer processes [7]. The discrete pulse energy input method is realized in a variety of devices. The most common are the impulse pulsator, rotary – disk and rotary – pulsation apparatus. The use of each of these types of devices, as well as the design features of the working parts and processing modes defined treatment goals, physical – chemical properties of the media, energy consumption. The use of certain apparatus allows you to boost the role of cavitation, or the role of shear stress, and so on. This allows to effectively solve technological problems in each particular case. The discrete pulse energy input method as a method of intensifying mass transfer processes at the gas – liquid has been applied in biotechnology in aquaculture ponds aeration processes, the activation of yeast in alcohol production [8–9].

The aim of this work is the intensification of the process of mass transfer of oxygen by discrete – pulse input energy in a rotary – pulsating apparatus in the fermentation unit at the aeration of culture media, as well as the effect of this treatment on the process of yeast growing .

Materials and methods

During the work the process of aeration of culture medium during the process of growing *Saccharomyces cerevisiae* yeast growing on molasses solutions in the fermentation unit with discrete – pulse energy input was studied.

The fermentation unit consists of the following units: the tank – storage, rotor – pulsation apparatus, loop recycling, the refrigerant circuit, the control and monitoring unit.

Capacity storage tank is a useful volume of 50 liters and serves for the culture medium processing.

In order to maintain a constant temperature process, the tank is provided cooling (heating) jacket, containing the inlet and outlet port for connection to the water mains. To determine the filling of the tank was a level gauge. Inside the tank storage removable inner glass can be provided, which serves for receiving the treated culture fluid of the recirculation system.

In the upper part of the tank – storage is welded pipe for the connection of the recirculation pipe. The cover of a tank – storage provides technologies connections for input into the workspace seed yeast, nutrients and defoamer, the pressure valve. The cover is provided with a viewing window. At the bottom of the tank valve drive is provided for the change of the volume flow.

Rotary – pulsating apparatus is intended for transforming electric energy input by method DPIE to the physical, hydrodynamic, acoustic impact on the culture medium.

Fully assembled apparatus consist of a disc mounted on a shaft with blades – the centrifugal pump impeller, a rotary – pulsating unit representing a settling down in the housing two fixed stator and rotor mounted on the motor shaft. The stator and rotor are in the form of shells with rectangular slits. Work RPA volume was $1.5 \cdot 10^{-3} \text{ m}^3$. The air in the aeration is supplied through a filter self-priming. The air supply is due to local discharge in the discharge line. Multiple processing is carried out at the expense of recycling of the culture medium along the contour of tank-storage – rotary – pulsating apparatus – tank – storage.

Control and monitoring unit is designed for controlling, monitoring and control of electrical equipment. The unit consists of a magnetic starter, frequency inverter, ammeter, electricity meter.

Oxygen mass transfer rate was determined by equating the rate of oxygen dissolution rate to its consumption by yeast cells. Taking the position that all the oxygen is consumed by the cell, it is spent on the process of division of cells the consumption rate is calculated based on the amount of growth of yeast mass.

This condition allowed to determine the oxygen absorption rate by determining the amount of biomass of yeast grown during cultivation period.

Methods of obtaining initial data for determining the mass flow rate was as follows.

The tank-storage supplied pre-prepared culture medium. Clarification of molasses made of acid – cold method. The resulting solution (1: 1, solids content – 37%) diluted with artesian water to a content of solids content provided program of experiment and was adjusted to 30 °C temperature.

The solids content was determined by the saccharometer. Preparation nutrient medium acidified with phosphoric acid to pH 5.0. The solutions of nutrient salts, growth substances prepared separately.

The tank – storage was filled with mixture of molasses solution and nutrient. Capacity was determined by the height of the filling level gauge.

Frequency converter sets the frequency of the output shaft speed. After switching on the device temperature of the medium was adjusted to 30°C. The air valve opened. Prepared seed yeast was diluted with water to the yeast concentration, provided research program, and then fed into a tank – storage. After 5 minutes after seeding a sample the culture medium was taken, which was the control. A determination of yeast biomass, determination of the number of dead cells was made. Samples were collected during the period of cultivation for each hour. Temperature of cultivation was maintained by the start of cold water into the jacket.

Foam height was determined visually through the viewing window. When exceeding the critical level the oleic acid emulsion into the tank – storage supplied. The concentrated culture medium (1:1), nutrient growth substances and salt solutions were calculated by the method based on the intended specific growth rate. Supply of nutrients and growth substances carried out every half hour throughout the entire period of cultivation.

Obtained mass of yeast was determined by weighing on an analytical balance according to standard procedure.

The number of dead cells was determined by direct counting in Goryaev chamber.

The main parameters that have changed in the course of processing the culture media, was the frequency of the flow pulsations and flow shear rate. The first parameter is determined by the number of slots in the shell and characterizes the frequency of exposure to the culture medium pulses of pressure generated when slots of the rotor and stator are at overlapping. The second parameter takes into account the impact of gap between rotor and stator on processing the culture medium.

Results and discussion

The initial concentration of yeast cells in the culture fluid volume of 30 liters was 20 g / l. Yeast was cultured for 8 hours. The magnitude of biomass growth per hour was determined depending on the concentration of yeast biomass at different processing modes. In Figure 1–3 shows the dependence of the concentration of yeast and quantities of hourly biomass growth in the processing method DPIE with a variety of flow shear rates.

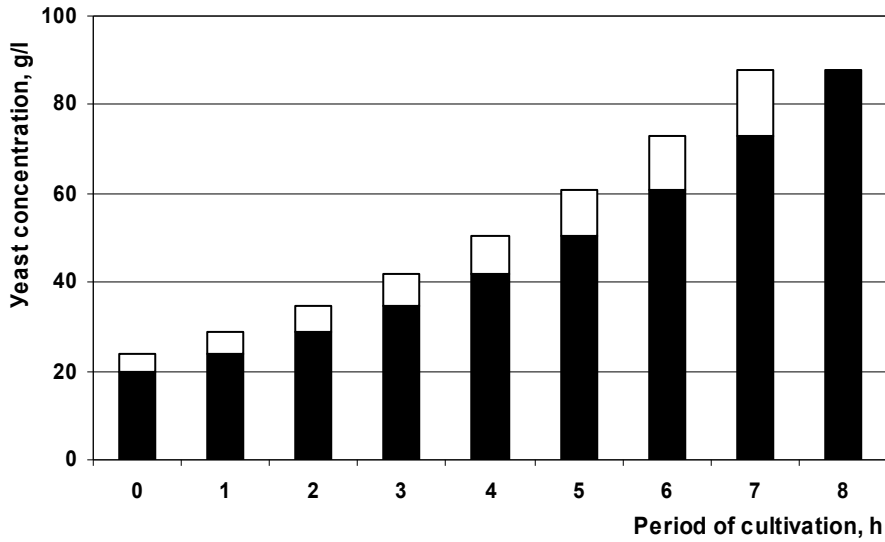


Fig.1. Yeast concentration dependence of the duration of culturing at a shear rate of flow of $85,46 \cdot 10^3 \text{ s}^{-1}$

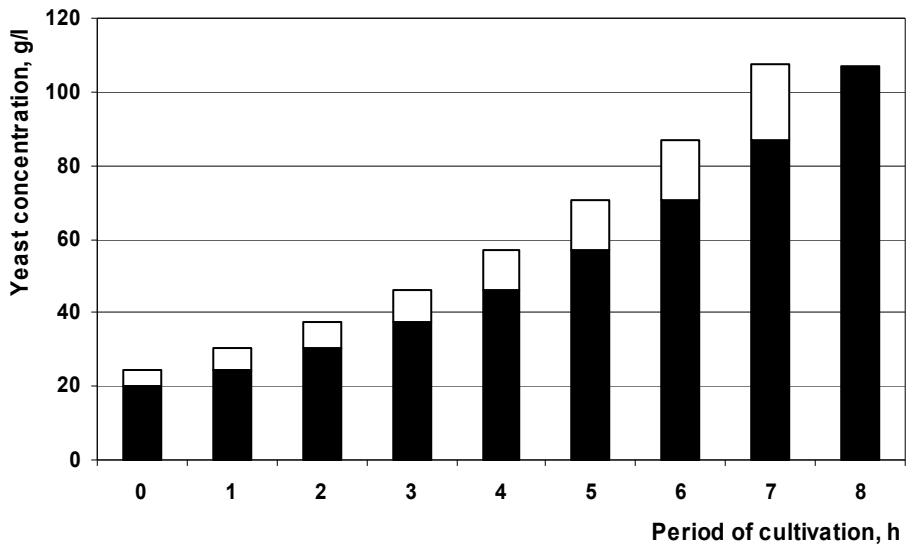


Fig.2. Yeast concentration dependence of the duration of culturing at a shear rate of flow of $114 \cdot 10^3 \text{ s}^{-1}$

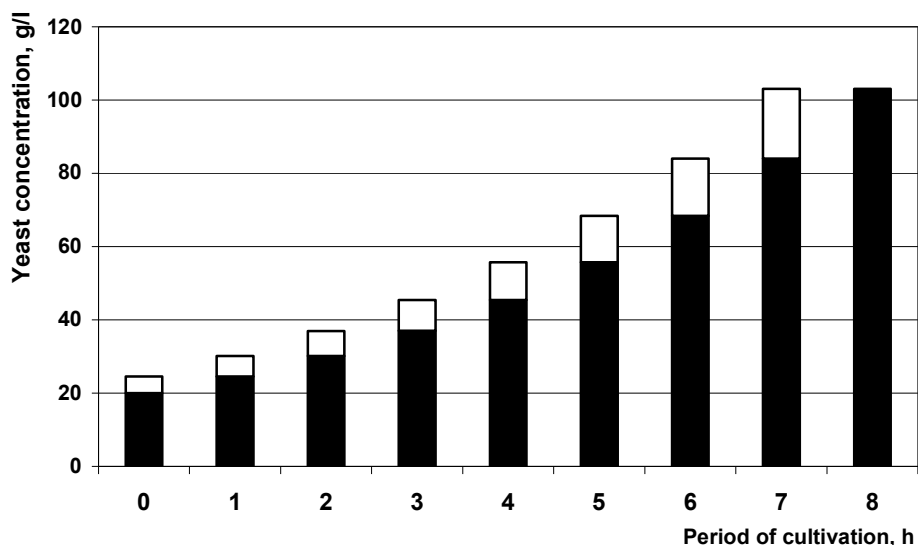


Fig. 3. Yeast concentration dependence of the duration of culturing at a shear rate of flow of $125,3 \cdot 10^3 \text{ s}^{-1}$

Based on the obtained data of biomass growth for the last hour the mass transfer rate of oxygen into the culture media was determined.

Table 1 shows the dependence of the mass flow rate of the angular speed of the rotor at different solids content in culture medium

Table 1

Dependence of the rate of mass transfer on an angular rate of the rotor at different solids content

Solids content	Angular rate of the rotor, rps			
	38,2	43	47,75	52,52
10%	3,4	3,8	4,065	3,82
5%	4,15	4,7	4,88	4,59
3%	5,4	6	6,33	5,9

It should be noted that with increasing angular rate of the rotor from about 38.2 to 47.75 rps rate of mass transfer is increased. It is found that the mass transfer rate depends on the concentration of solids content in the medium. With decreasing solids content from 10 to 5% by treatment with an angular rate of 47.75 rps the mass transfer rate increases 1.9 times.

In conducting research of interest is the effect on the rate of mass transfer rate the frequency of flow pulsations at the different solids content (Table 2).

The table shows that the increase frequency flow pulsations from 2 to 3.85 kHz mass transfer leads to an increase from 4 to 6.3 g / l per h at 3% of solids content, from 3,0 to 4,88 g / l per h at 5% of solids content and of 2,2 to 4 g / l per h at 10% of solids content. A marked increase in the frequency of pulsation leads to a "tightening" mode of operation, which negatively affects the dynamics of the growth of yeast.

Table 2
Dependence of the rate of mass transfer on frequency flow pulsations at different solids content

Solids content	Frequency of the flow pulsations, kHz			
	2	2,483	2,865	3,15
10%	2,2	3,6	4,065	3,82
5%	3,0	4,5	4,88	4,59
3%	4	5,8	6,33	5,9

When determining the effect of DPIE mechanisms on the rate of mass transfer is an important factor a gap between rotor and stator, which takes into account the influence of an indicator such as the flow rate of the shift, which is defined as the ratio of flow rate radially to the thickness gap between rotor and stator.

Experimental data showing the dependence of the flow shear rate on the rate of mass transfer at different solids contents in medium shown in Table 3.

Table 3
Dependence of the rate of mass transfer on flow shear rate at different solids content

Solids content	Flow shear rate, $\cdot 10^3 \text{ s}^{-1}$			
	57	85,46	114	125,3
10%	1,6	3,4	4,065	3,82
5%	2,5	4,35	4,88	4,59
3%	3,4	5,4	6,33	5,9

As in previous studies have shown that an increase in the flow shear rate of mass transfer rate increases, but the increase in value of this magnitude leads to a need for small (less than 100 micron) gap, which is technically difficult to implement. The optimum range is the shear rate of flow is within $90 - 114 \cdot 10^3 \text{ s}^{-1}$. The table also shows that an increase in solids content of the medium the mass transfer rate decreases.

High values of the growth rate on the dilute media suggests that the oxygen in such media dissolves better, however, the rapid uptake of yeast weight carbonaceous nutrient forced feeding fresh medium practically continuously, which creates a certain difficulty in maintaining a stable growth dynamics, thus the initial concentration of solids in the culture medium should not be below 10%.

Reduced oxygen consumption rate by increasing the frequency of flow pulsations and flow shear rate associated with increasing numbers of dead cells, which is confirmed by data obtained by microscopy.

Conclusions

DPIE mechanisms controlling and changing the design features of the rotary – pulsating apparatus, it is possible to influence the rate of mass transfer of oxygen into the culture medium. This can be explained with decrease by the mean diameter of the air bubbles in the space between the working parts of apparatus that greatly increases the contact area of the interface.

Increasing the mass flow rate of 4.1 g / 1·h allows to intensify the process of cultivation of yeasts, namely to reduce the process time duration from 12 to 8 hours and to

increase the final concentration in the yeast in 2 times compared to the conventional technology.

Increasing the degree of exposure on culture medium a flow shear rate higher than $114 \cdot 10^3 \text{ s}^{-1}$ leads to decrease in the yield of finished product. This may be due to the fact that increased physical and hydrodynamic effects in the apparatus leads to the inactivation of some cells.

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Heat transfer enhancement in a corrugated tube heat exchanger

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Abstract

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Introduction. The use of corrugated tubes is an effective method of intensification of heat transfer in tubular heat exchangers, but there is currently no universal method of calculation and design of such heat exchangers.

Materials and methods. The heat transfer characteristics of flexible corrugated stainless steel tubes with different corrugation profile were studied. The laboratory stand is a "tube in tube" type heat exchanger with outer smooth tube and inner corrugated tube, equipped with sensors for measuring temperature and hydraulic flow parameters.

Results and discussion. The research of heat transfer and hydrodynamics in the "tube in tube" type heat exchanger with a corrugated inner tube found considerable intensification of heat transfer compared with traditional smooth tube heat exchanger in the range of Reynolds numbers from 4000 to 40000. The increasing of heat transfer coefficient was from 2.0 to 2.6 times during the increase of the hydraulic resistance in 1.9...2.0 times. It was found that the tubes with the small corrugation height and the big corrugating pitch (height/pitch ratio – 1.9/4.0 mm) have 15...20% higher convective component of the heat transfer coefficient in comparison with tubes with the higher corrugation height and the small corrugation pitch (height/pitch ratio – 2.4/3.2 mm) under identical flow conditions.

Two-dimensional axially symmetric computer model of the unit cell of the heat exchanger was developed to estimate the effect of the tube geometry on the intensity of the heat transfer process. Numerical simulation of hydrodynamics and heat transfer in the unit cell channel showed that mathematical calculations are quite close to the experimental studies.

The heat exchanger with capacity of 350 kW for the administrative building heating is designed and engineered using criterial dependences. The experimental operation of the heat exchanger confirmed the effectiveness of the proposed technical solutions.

Conclusions. The using of corrugating tubes allowed to increase the heat transfer coefficient. The received criterial dependences allow to calculate and optimize the process of heat transfer in a tubular heat exchanger with flexible corrugated pipes.

Introduction

Heat exchangers play an important role in industrial power system. It is a great group of heat-power equipment with considerable industrial areas and often 20% or more of the total cost of equipment used in thermal power, chemical industry, refining industry, food processing industry, utilities and other industries [1].

Creation of modern heat-exchange equipment is the complicated and multiplane purpose. The expansion of activities focused on the intensification of heat exchange, reducing the weight and dimensions of heat exchangers, increasing their productivity is needed to decrease energy and material consumption as well as saving the cost of heat exchange equipment. The real rationalization of heat exchanger design is considered nearly exhausted today [2].

Heat treatment of liquid products in tubular heat exchangers is often used in the food industry [3]. The high heat transfer performance and low flow resistance losses, compact size and lightness are the main requirement for heat exchangers. The achievement of these requirements is only possible using the intensification of heat exchange.

The intensification of heat exchange is one of the most important directions for development of modern heat-exchange equipment. The intensification of heat exchange is usually of interest providing gain in cost in comparison with standard samples. The method of intensification depends on the availability of materials, safety and reliability. The correlation properties of heat exchangers are presented in Figure 1.

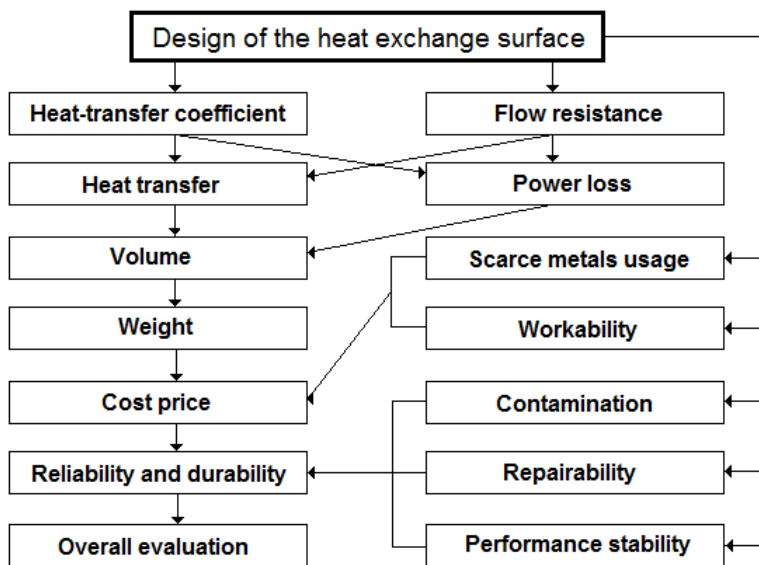


Fig.1. Correlation properties of heat exchangers

The scientific works of foreign and native scientists are constantly studied for search of methods of heat exchange intensification [4–11]. The methods of passive heat exchange intensification and the use of developed heat exchange surfaces are widely applied today. No universal methods and no criteria are developed for efficiency assessment of the heat exchange surfaces. Further in-depth study of the thermal and hydrodynamic processes

occurring in the developed heat exchange surfaces is required in order to obtain general dependences for calculating the thermal and hydraulic characteristics and to evaluate their efficiency.

The efficiency of the surface itself, its manufacture adaptability, technological efficiency of the heat exchanger, strength requirements, and surface contamination is necessary to consider choosing the method of heat exchange intensification for the practical application.

Materials and methods

Thin-walled corrugated flexible stainless steel tubes of various modifications are produced by a number of domestic companies today (Figure 2). These tubes are relatively cheap and used extensively for hot and cold water supply systems, heating systems and gas consuming equipment [12].

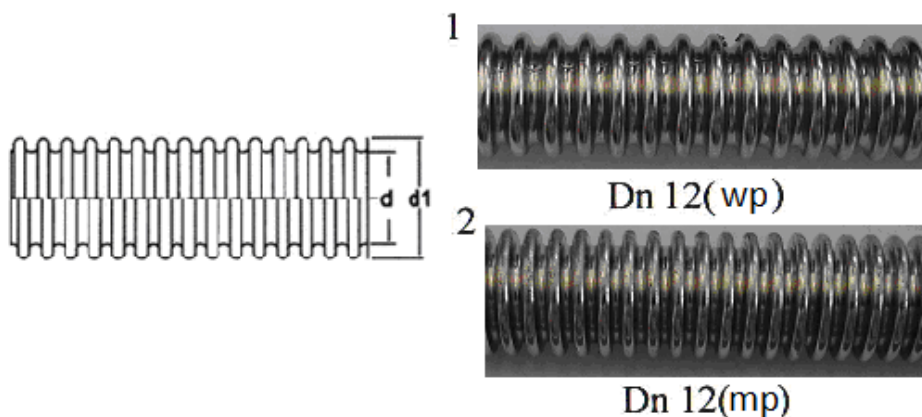
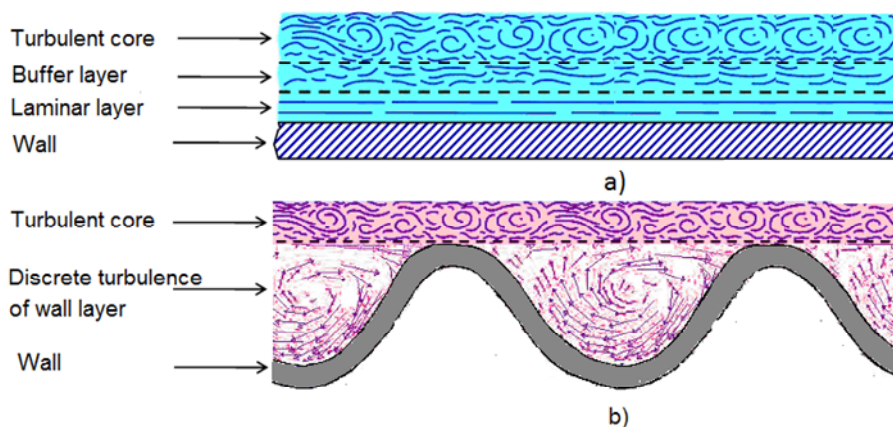


Fig. 2. Thin-walled corrugated tubes of various modifications

- 1 – Tube Dn12 with wide pitch distance of discrete vortex generators;
- 2 – tube Dn12 with medium pitch distance of discrete vortex generators

The corrugated tubes are not afraid of defrosting and water stress, compensate linear expansion and can operate at temperatures up to 150 °C and pressure of 25 kg/cm². These tubes are long service life due to the corrosion resistance and high tube strength and characterized by reliability and resistance to external and internal mechanical and hydraulic loads due to the material properties, high flexibility and elasticity.

The use of corrugated tubes of various modifications is the effective way to improve the thermal and hydraulic characteristics of heat exchangers. The corrugated tubes provide turbulence near the surface of the tube wall reducing the thickness of the thermal boundary layer. There is improved mixing of the liquid near the tube wall, due to detached disturbance from the wall to the main stream, and as a result the overall heat transfer coefficient of heat exchange systems is increased (see Fig. 3).



**Fig. 3. Scheme of flow formation in tubes of various geometries:
a – smooth tube; b – tube with discrete vortex generators.**

The test laboratory stand was created to research experimental models of heat exchangers and to determine their performance. (Fig. 4) The stand was equipped with a tubular heat exchanger type "tube in tube" with a corrugated inner tube (with replacement tubes with other geometry) 2. The water meters 5, 11 (vane) to measure the flow of hot and cold coolant were installed at the stand. Thermocouple chromel-kopel (HC) was used for determining the temperature of the coolant inlet and outlet (in the tube, between and on the walls of the tube) of the heat exchanger 14. The pressure sensors 12, 13 (DANFFOS company) for fixing the flow pressure at the inlet and outlet of the heat exchanger tubes were installed. The analog-to-digital converter (ADC) of the type I-7018 was included to stand hardware system to ensure the conversion of input signals from the thermocouples and pressure transducers to digital form and transmitting the data to the computer 15. The sensors to measure the thermal hydraulic flow conditions were used at the stand. The comparative analysis of operation modes of the heat exchanger with a corrugated inner tube (various modifications) and the heat exchanger with a smooth inner tube was carried out according to the results of experimental studies.

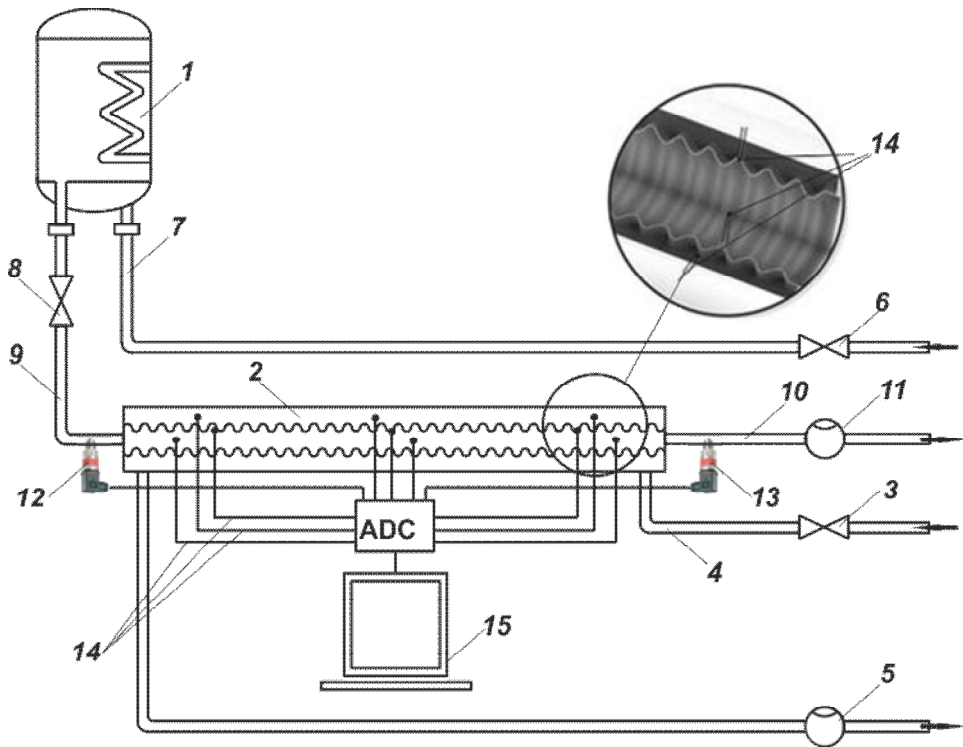


Fig. 4. Schematic diagram of the laboratory stand for the experimental study of heat transfer in corrugated tube heat exchangers based on tubes with discrete vortex generators:

- 1 – insulated boiler; 2 – tube in tube heat exchanger; 3 – cold coolant supply valve;
- 4 – cold coolant feeding; 5 – cold coolant flow meter; 6 – cold water supply valve;
- 7 – cold water feeding; 8 – hot coolant supply valve; 9 – hot coolant feeding;
- 10 – hot coolant drain; 11 – hot coolant flow meter; 12, 13 – sensors (DANFFOS company) for measuring the pressure at the inlet and outlet of the heat exchanger;
- 14 – thermocouples Chromel-Kopel (CK); 15 – personal computer.

Results and discussion

The results and heat transfer coefficient according to the coolant flow rate in corrugated tubes comparing with a smooth tube are demonstrated in Fig. 5 – 6. The convective heat transfer coefficient of corrugated tubes comparing with a smooth tube is considerably increased under the same flow conditions. The efficiency rise of the heat transfer coefficient is in the range of 2,0 to 2.6, and goes up with increasing Reynolds number. The increase of heat transfer is more than growth of hydraulic resistance at the same time.

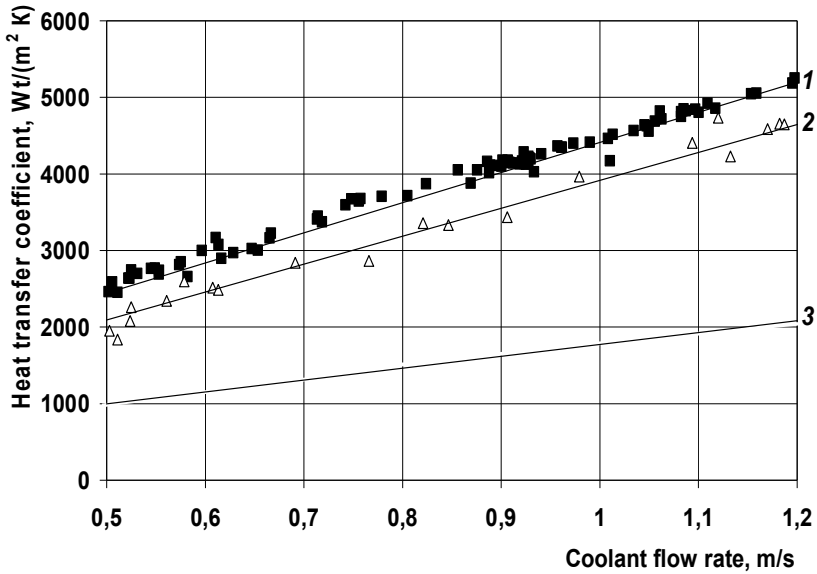


Fig. 5. Dependence of the heat transfer coefficient of the corrugated and smooth tubes on coolant flow rate:
1 – tube Dn12 with wide pitch distance of discrete vortex generators;
2 – tube Dn12 with medium pitch distance of discrete vortex generators;
3 – smooth tube.

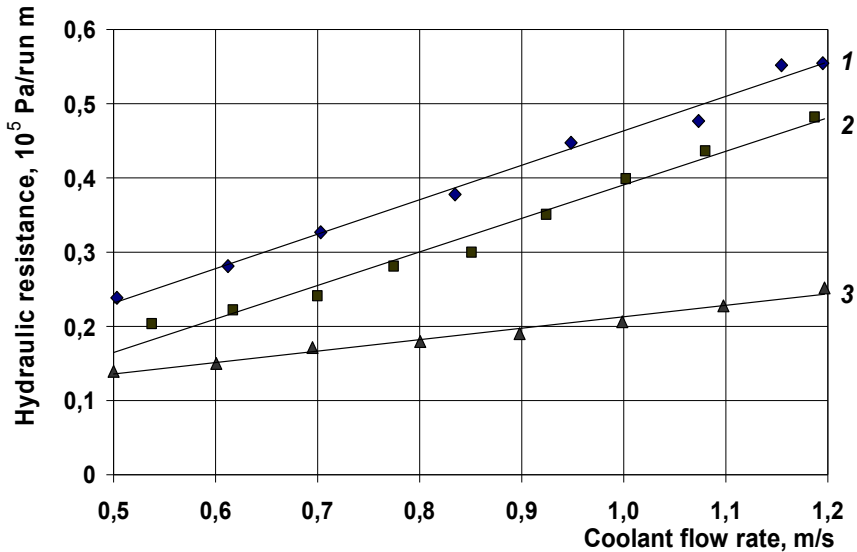


Fig. 6. Dependence of hydraulic resistance of the corrugated and smooth tubes on coolant flow rate:
1 – tube Dn12 with wide pitch distance of discrete vortex generators;
2 – tube Dn12 with medium pitch distance of discrete vortex generators;
3 – smooth tube.

The criterial dependences of the flow inside fold and in the annular fold of the corrugated tube were obtained in integrating experimental data. As the basic the equation for the tube flow was used to generalize the experimental data for inner fold of the tubes with discrete vortex generators:

$$\overline{Nu}_d = C \cdot Re_d^m \cdot Pr_{lg}^{0,43} \cdot \left(\frac{Pr_{lg}}{Pr_w} \right)^{0,25} \quad (1)$$

the value of the constant C and the exponent m were determined by the statistical processing of the experimental data. For this purpose the experimental data was presented in a logarithmic coordinate system:

$$F^* = \lg \left(\frac{\overline{Nu}_{lg}}{Pr_f^{0,43} \cdot (Pr_f / Pr_w)^{0,25}} \right) = \lg C + m \lg(Re_d) \quad (2)$$

where the experimental data are located near the straight line (Fig. 7) with a constant $C = 0.79$ and the exponent $m = 0,66$ in Equation (2).

The value of the exponent $m = 0.66$ generally corresponds to the transitional regime. Since the experiments were carried out at Reynolds numbers of more than 9000, it can be considered that the flow in the corrugated tube was turbulent. Additional processing of the experimental data was performed in the equation (1) with an exponent $m = 0.8$ at Reynolds number.

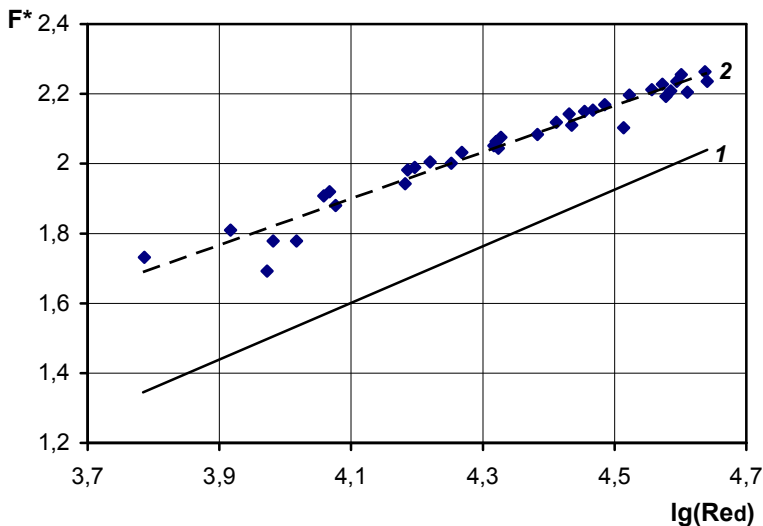


Fig. 7. Generalized results of experimental data of heat transfer inside corrugated tube:

- 1 – equation for the average heat exchange in tubes at the stabilized flow $\overline{Nu}_d = 0,021 \cdot Re_d^{0,8} \cdot Pr_{lg}^{0,43}$;
- 2 – approximation of experimental data.

The constant C is not constant, as it follows from the represented coordinate system Fig. 7, and depends on the Reynolds number and is determined by the equations:

$$\lg(C) = -0.02 \cdot \lg(\text{Re}_d) + 0.12 \quad (3)$$

$$C = 10^{-0.02 \cdot \lg(\text{Re}_d) + 0.12} \quad (4)$$

In this case, the equation for the average heat exchange inside fold of tube takes the following form:

$$\overline{Nu}_d = 10^{-0.02 \cdot \lg(\text{Re}_d) + 0.12} \cdot \text{Re}_d^{0.8} \cdot \text{Pr}_{lg}^{0.43} \cdot \left(\frac{\text{Pr}_{lg}}{\text{Pr}_w} \right)^{0.25} \quad (5)$$

It is used in Figure 8:

$$F^{**} = \lg \left(\frac{\overline{Nu}_d}{\text{Pr}_f^{0.43} \cdot (\text{Pr}_f / \text{Pr}_w)^{0.25} \cdot \text{Re}_d^{0.8}} \right)$$

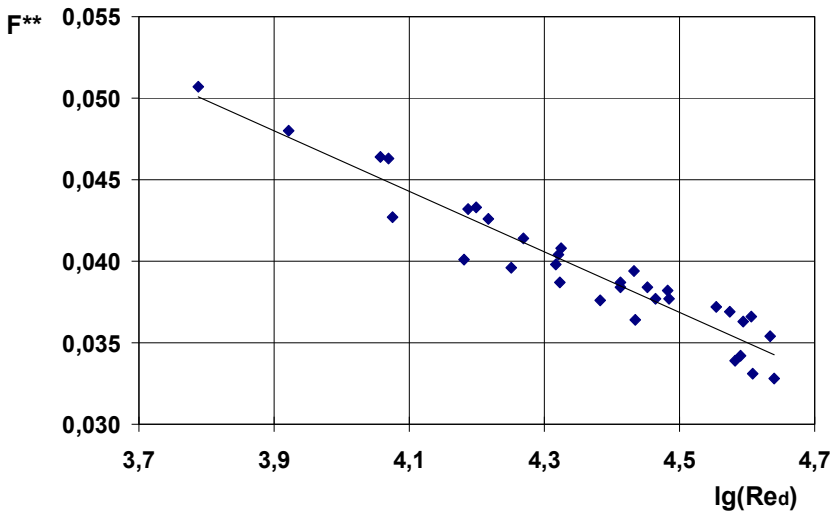


Fig. 8. Generalized results of experimental data of heat transfer inside fold of tube with exponent $m = 0.8$ at Reynolds number

The analogical generalization of experimental data was performed for the average heat exchange in the annular fold. The results in semi-log plot with the definition of the constant C and exponent m are shown in Fig. 9. In this case, the constant C is equal to 1.77, and the exponent $m = 1.0$.

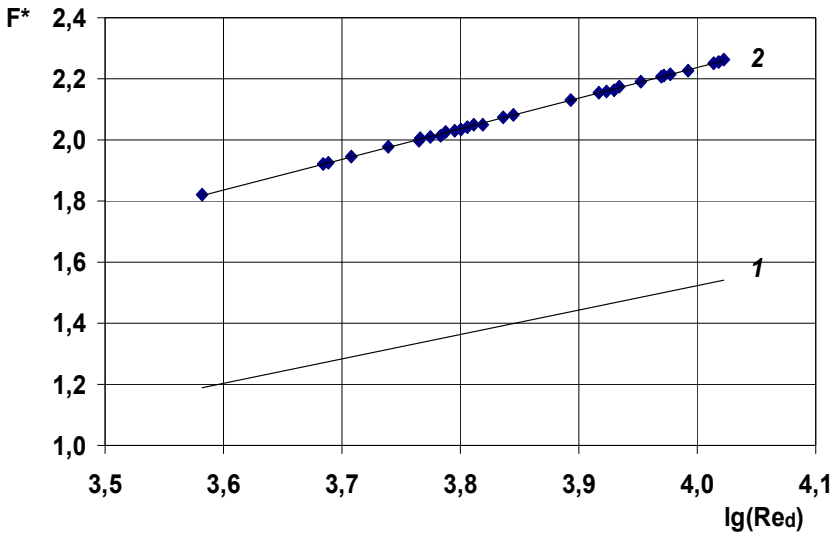


Fig. 9. Generalized results of experimental data of heat transfer inside annular fold:

1 – equation for the average heat exchange in tubes at the stabilized flow $\overline{Nu}_d = 0,021 \cdot Re_d^{0,8} \cdot Pr_{iq}^{0,43}$;
 2 – generalizing straight line.

The value of the exponent m does not correspond to the physical heat exchange in the annular fold because the data was obtained at Reynolds number more of 4000. There is assumed a turbulent regime in the annular fold despite the low Reynolds number corresponding to the transition mode during axial flow. Figure 10 shows generalization of experimental data performed with the exponent $m=0.8$ at Reynolds number.

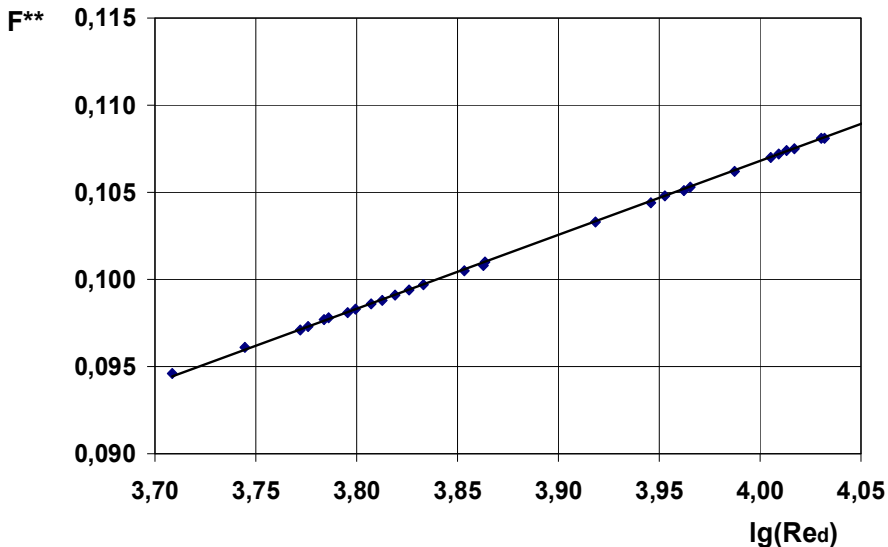


Fig. 10. Generalized results of experimental data of heat transfer inside annular fold of corrugated tube with exponent $m = 0.8$ at Reynolds number

The equation for the constant C is defined by the relationship in the present coordinate system:

$$\lg(C) = 0.05 \cdot \lg(\text{Re}_d) - 0,08 \quad (6)$$

$$C = 10^{0.05 \cdot \lg(\text{Re}_d) - 0.08} \quad (7)$$

and the equation for the average heat transfer in the annular fold takes the following form:

$$\overline{Nu}_d = 10^{0.05 \cdot \lg(\text{Re}_d) - 0.08} \cdot \text{Re}_d^{0.8} \cdot \text{Pr}_{fg}^{0.43} \cdot \left(\frac{\text{Pr}_{fg}}{\text{Pr}_w} \right)^{0.25} \quad (8)$$

The results show that the mathematical calculations are quite close to the experimental research. The obtained criterial dependences allow developing a methodology for engineering calculations for the design of new effective heat exchangers.

Theoretical and experimental studies show the prospects of this direction and allow creating effective heat exchangers with thin-walled stainless steel flexible corrugated tubes. Comparative heat engineering tests of the new heat exchanger 350 kW were successfully carried out in the administrative building of the IET of NASU.

Conclusions

1. The heat transfer coefficient in new corrugated tube heat exchangers with discrete vortex generators tubes is 1.1 – 1.2 times more the average value of k for plate heat exchangers.
2. The metal consumption of new corrugated tube heat exchangers with discrete vortex generator tubes is almost 1.5 times less compared to plate heat exchanger of the same capacity.
3. The unit cost of heat exchange surface of corrugated tube heat exchangers is much less compared to that of plate heat exchangers. It is a significant reserve to reduce the cost of heat exchange equipment and to create competitive heat exchange systems.

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Methodical bases of rating of investment appeal of Ukraine rural areas

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Abstract

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Introduction. The article deals with the problem of methodological and practical principles of rating evaluation of rural regions investment attractiveness.

Material and methods. It was studying the investment attractiveness of rural areas on the example of Ukraine regions. It was used synergistic method, the methods of integrated comparison and evaluation. It was used ranking approach, which is the most common for the evaluation of investment attractiveness using index-indicator system.

Results and discussion. The evaluation algorithm of investment attractiveness of the region was offered. It involves a sequence of actions by which the first three stages are preparative; the following four stages are the direct assessment rating of the region at different levels.

The assessment methodology investment and innovation attractiveness of regions was offered, which is based on the using of standardization and rating methods and the using of the comparison method too. The three-level index-indicator system of assessing the investment attractiveness of rural areas was offered. The first stage of which contains the performance component of investment potential, the second stage contains indicators of investment risk and the last stage contains the generalized indicators of investment attractiveness of regions.

On the basis of the rating, we have constructed the typology of rural areas. Rural areas are represented practically in all rating groups approximately in the same proportion in relation to the number of groups and thus represent investment environment indicators for all regions of Ukraine.

It is defined that the rural investment attractiveness in the majority depends on the social and economic development. The type of investment climate 2B (average potential, moderate risk) includes 6 rural regions; the type 3B1 (reduced potential, moderate risk) includes 4 rural regions; the type 3B2 (great potential, moderate risk), 2C (average potential, high risk), 3C1 (reduced potential, high risk) and 3C2 (great potential, high risk) include 3 rural regions. The regions with the minimal risk (types 1A, 2A, 3A, 1B) according to the calculations are not available.

Conclusion. Based on the analysis of the investment climate, the distribution of rural regions is represented according to the investment types. The typology of rural regions is based on the basis rating.

Introduction

In the modern conditions of managing the competitive struggle for the attraction of additional capital sources for stabilization and economic development is constantly increasing. It is in the process at all economic levels, and especially at the mesolevel or the level of the regional economy. In the world practice of government regulation of the regional economy, funds are invested into the most powerful territorial and sectoral groupings which, due to its rapid and profitable growth, help to support necessary market transformation in the areas with lower or slower level of development. It actualizes the theme of rating evaluation of investment attractiveness of regions. Moreover private investors' important issue is the choice of the most attractive areas of strategic management, investments into which will allow to recover and increase the initial capital, that to attract reinvested funds.

Realization of innovative projects, including the national project "State Target Program of Ukrainian Village Development for the Period up to 2015" provides more active investment policy. At the same time, the investment processes in agriculture continues to lag behind other industries. For the years of independence the investment policy of the government was not aimed at changing the current situation. Normative Acts aimed at intensifying of investment activities were not implemented. For example, the mentioned "State Target Program of Ukrainian Village Development for the Period up to 2015" was not financed and did not fully play the role designated to it. The analysis of the existing system of AIC state support shows that it is aimed primarily at solving current problems, and the strategic issues of agricultural development do not find the proper solution.

This policy has led to a decline in the share of fixed capital investments into agricultural production in Ukraine from 14.3% of the value of gross output in 1991 to 4.5% in 2010. The "the investment corridor" providing the optimal level of savings for the economy in general on a number of assessments should be 21–23% of GDP. In rural areas the share of investment in 2013 ranged from 0.5 to 16.4% of gross added value produced in agriculture (GVA in agriculture). More than 10% is in Lviv region (16.4%), Chernihiv region (12.5%), Cherkasy region (15.1%), Kherson region (12.2%), Mykolaiv region (11.3%), Zhytomyr region (11, 5%), Odessa and Zaporizhya regions (10.2%). In Lugansk region it is 9.9%. Less than 5% is Sumy region (4.8%), Chernivtsi region (4.6%), Transcarpathia region (4.3%), Rivne region (4.1%), the Crimean Republic (3.9%). The remaining 12 rural areas have from 5 to 10%. Thus, the existing level of investment in agriculture does not ensure the implementation of innovative projects and is unacceptably low even in the most rural regions referred to the group with the highest socio-economic development.

It is known that investment attractiveness is the integrating indicator of the investment climate in a region. In the implementation of the national rural development projects, a significant role is given to administrations, and therefore the investment attractiveness of the region is crucial in attracting investments.

Analysis of recent research

There is a great number of approaches to evaluate the investment attractiveness of regions, industries and individual businesses currently. Indicators of investment attractiveness are quite diverse and vary depending on the place of the subject in the relevant territorial, sector, management hierarchy. The rating approach is one of the most common in the evaluation practice of investment attractiveness of regions. This approach

(in the variety of its modifications) is put into the basis of techniques, particularly those offered by such scientists as I. Blank [1], S. Sonko [2], A. Datsishin [3] etc., expert groups of the Institute of Reforms, "SOCIS Gallup International", RA "Expert Rating" and others. [4–8]. Authorities of Ukraine have developed the techniques in the direction of evaluation: methods of assessing the level of business activity in the region, which is developed by the Cabinet of Ministers of Ukraine [9]; methods of evaluation of investment attractiveness, approved by the Ministry of Economy and European Integration of Ukraine; the method of calculating the integrated regional indices of economic development adopted by the State Statistics Committee of Ukraine and others.

In regard to administrative entities and industries the investment attractiveness is determined by the investment climate, which is economic, political, financial conditions affecting the supply of domestic and external investments into the economy. According to the classification of A. Ageenko [10] all indicators of investment attraction and economic entity can be divided into two groups. The first group includes social indicators that characterize the political climate of the region, the environmental situation, demographics, ethnic relations, and others. These factors are not amenable to precise quantitative evaluation, linkage to a particular industry or organization. They characterize the region as a whole. Different researchers apply subjective expert methods to them when determining their impact on the overall potential economic entity. The second group is formed by economic factors contained in the official statistical and accounting records. They help to assess the production potential, financial results, investment activity, and labor potential of economic entity. The association of outlined set of multi-level indicators makes the information base for an integrated assessment of investment attractiveness of the rural region and its major subsystems.

Material and methods

It was studying the investment attractiveness of rural areas on the example of Ukraine regions.

It was used ranking approach, which is the most common for the evaluation of investment attractiveness using index-indicator system.

The evaluation algorithm of investment region attractiveness is proposed, which provides a certain sequence of actions, according to which the first three stages are preparatory, and the next four are the direct rating assessment of a region at different levels (at the level of individual indicators, their groups, associations of these groups and integrated region assessment). The suggested technique of an estimation of investment and innovation attractiveness of regions is based on the usage of the methods of standardization and regulation, as well as on the method of comparison. Synergistic and systematic approaches are used in the study as well.

Result and discussion

According to existing methods the investment climate of regional agents is evaluated on the basis of indicators of investment potential and investment risk. Investment potential takes into account basic macroeconomic characteristics, area saturation factors of production, consumer demand and other indicators. The value of the investment risk describes the probability of investments loss and income from them. When calculating the integrated rankings of a region by the investment potential and risk, expert assessments of potential components and risk are used as well as the rejection of risk components of a

region on average. Final ratings define sequence numbers of regions so that the smaller the number, the higher potential and thus the lower risk (the first number corresponds to the highest potential and the lowest risk). In cases where the actual values of statistical parameters of several regions are the same, the entire group is assigned an average rating value.

The summary typology of regions based on the investment climate is offered in the following stratification:

Type 1 A. Maximum potential – minimum risk.

Type 2A. Average capacity – minimum risk.

Type 3A. Low potential – minimal risk.

Type 1B. High potential – moderate risk.

Type 2B. Medium potential – moderate risk.

Type 3B1. Decreased potential – moderate risk.

Type 3B2. Minor potential – moderate risk.

Type 2C. Average capacity – high risk.

Type 3C1. Decreased potential – high risk.

Type 3C2. Minor potential – high risk.

Type 3D. Low potential – extreme risk.

For the current situation when it is necessary to develop investment policies at both the national and regional levels, it is important to evaluate how the introduced indicators and rankings affect the real actions of investors. This research was conducted by G. Untura [11]. The investment activity in the part of the actually made investments was measured by the size of domestic and, separately, foreign investments per capita of regions. These values served as dependent variables in the equation of regression where the independent variables were either investment potential or investment risk. Calculations, carried out on the materials of 1990–2013, for each year individually, revealed statistical dependency significance, increased reaction of investors on ratings given and adequate perception of them (with some lag). The expediency of regular adjustment of methods of ranking calculating with the change of socio-economic and political situation in the country was also noted. Among the shortcomings of the methodology the incomplete set of indicators which are calculated on the basis of ratings of regions' investment attractiveness and possible subjectivity of expert assessments in the indicator calculation have been marked.

In regard to the abovementioned, we proposed the algorithm for the evaluating of the region investment attractiveness, which provides a sequence of actions, according to which the first three stages are preparatory, and the next four are direct rating assessment of the region at different levels (at the level of individual indicators, their groups, associations of these groups and complex estimation of the region). Thus, the main stages of the proposed algorithm for evaluation of investment attractiveness of the region are:

1 – establishing of eight evaluation criteria of investment attractiveness of a region (its general economic development, financial support of the economy, innovation and investment and social development as well as the development of regional infrastructure, efficient use of production resources and business activity), forming of eight groups of indicators that correspond to the mentioned criteria (six groups of absolute indicators to characterize the level of socio-economic development of the region and two groups of relative indicators for the detection of the efficient usage of fixed types of resources in the region;

2 – identifying and averaging the actual level (or standardized values) of each of the output indicators used to evaluate the investment attractiveness of the region;

3 – predicting the level of each of the normalized indices (indicators of investment attractiveness of the region), taking into account the dynamics of the actual values of these indices for the selected period;

4 – regions' ratings plotting of the studied population according to their investment attractiveness for each of the average actual and for each of the forecast values of the standardized indices;

5 – group rating plotting of areas for each of the selected criteria of the investment attractiveness based on the results calculated for each region of complex indicators which average actual and projected levels of investment attractiveness according to a particular group of indicators accordingly;

6 – plotting of general (or integrated) ratings of regions (individually for the characterization of socio-economic level supply of the region development according the first five groups of indicators and for the establishment of the efficiency level of fixed kinds of resources in the following two groups of indicators) on the basis of calculation of integrated indicators according to the totals of group value (actual and forecast) indicators of investment attractiveness, defined in the previous (the fifth) assessment stage;

7 – plotting of complex (or final) rating of areas through integrated indicators identified by combining the corresponding integral indicators of investment attractiveness.

The proposed method of estimating the investment and innovation attractiveness of regions is based on the use of methods of standardization and regulation, as well as on the method of comparison. In addition, in the study the synergistic and systematic approaches are used.

The only complexity in the implementation of the algorithm of the investment attractiveness of rural areas is to build a wider system of indicators characterizing the investment potential of the region, and the calculation of partial coefficients of risk investment activity within the rural area. Taking into account the research process and assessment of the attractiveness a three-tier system of indicators is formed (Table 1).

Based on the analysis of the investment climate, table 2 shows the distribution of rural areas according to investment types.

On the basis of the rating of 2012–2013, we have constructed the typology of rural areas. Rural areas are represented practically in all rating groups approximately in the same proportion in relation to the number of groups and thus represent investment environment indicators for all regions of Ukraine. Regions with low-risk (groups 1A, 2A, 3A, 1B) according to calculations are missing.

Analysis of Table 2 suggests that for rural areas investment attractiveness mostly depends on the level of socio-economic development. The highest investment attractiveness (type 3B1: Medium potential – moderate risk) is marked for the regions with the highest complex rating assessment of socio-economic development. The decrease in the level of socio-economic development is accompanied by the decrease in investment potential and growth of the investment risk. The decrease in the level of socio-economic development is accompanied by the decrease in investment potential and growth of the investment risk. In Table 3 it is shown by simultaneous movement of regions to the right and down. In particular, if the regions with the type of investment environment 3B1 (reduced potential – moderate risk) are approximately evenly distributed between groups with differently rated socio-economic development, for the following types of investment environment the movement of regions into the lower groups of socio-economic development is observed, and all rural areas with the lowest investment climate (type 3C2: little potential – high risk) are concentrated in the third worst group in the ranking of socio-economic development.

Table 1

The system of index-indicators for assessing the investment attractiveness of a region

Indicators and levels of difficulty	The order of calculating
Level 1. Performance components of investment potential	
Indicators of general economic level of urban area development	$e = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{ije} - a_{ie}}{S_{ie}} \right)^2}{\max \frac{a_{ije} - a_{ie}}{S_{ie}}}}$
Indicators of financial support of rural regions economy	$f = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{if} - a_{if}}{S_{if}} \right)^2}{\max \frac{a_{if} - a_{if}}{S_{if}}}}$
Indicators of innovative development of rural regions	$inv = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{iinv} - a_{iinv}}{S_{iinv}} \right)^2}{\max \frac{a_{iinv} - a_{iinv}}{S_{iinv}}}}$
Indicators of investment development level of rural regions	$in = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{iin} - a_{iin}}{S_{iin}} \right)^2}{\max \frac{a_{iin} - a_{iin}}{S_{iin}}}}$
Indicators of social development level of rural regions	$s = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{is} - a_{is}}{S_{is}} \right)^2}{\max \frac{a_{is} - a_{is}}{S_{is}}}}$
Indicators of infrastructure development of rural regions	$inf = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{inif} - a_{inif}}{S_{inif}} \right)^2}{\max \frac{a_{inif} - a_{inif}}{S_{inif}}}}$
Indicators of efficiency of basic types of resources for the rural region	$v = \sqrt{\sum_{i=1}^m \frac{\left(\frac{a_{iv} - a_{iv}}{S_{iv}} \right)^2}{\max \frac{a_{iv} - a_{iv}}{S_{iv}}}}$

Indicators of business activity of the rural region	$ba = \sqrt{\sum_{i=1}^m \left(\frac{a_{ijba} - \overline{a_{iba}}}{s_{iba}} \right)^2 \frac{a_{ijba} - a_{iba}}{\max s_{iba}}}$
Level 2. Indicators of investment risk	
Economic risk coefficient	$k_1 = 0,3 \left(\frac{(100 - IPP_i)}{\max DP} + \frac{L_i^{GRP}}{\max L^{GRP}} \right) + 0,2 \left(\frac{\max IIP - IIP_i}{\max IIP} \right) + 0,1 \left(\frac{(\max ID^{ag} - ID_i^{ag})}{\max ID^{ag}} + \frac{E_i^u}{\max E^u} \right)$
Financial risk coefficient	$k_2 = 0,1 \left(\frac{P_i}{\max P} \right) + 0,3 \left(\frac{F_i^d}{\max F^d} \right) + 0,2 \left(\frac{L_i}{\max L} \right) + 0,4 \left(\frac{FR_i^e}{\max FR^e} \right)$
Social risk coefficient	$k_2 = 0,4 \left(\frac{W_i}{\max W} \right) + 0,2 \left(\frac{U_i}{\max U} + \frac{E_i}{\max E} + \frac{\max G - G_i}{\max G} \right)$
Legislative risk coefficient	Share of normative acts of the <i>i</i> -th region the total number of acts regulating investment activities
Political risk coefficient	Percentage of individuals who supported the ruling party, the number of people voting
External economic risk coefficient	$k_6 = 0,2 \left(\frac{IG_i^U}{\max IG^U} \right) + 0,8 \left(\frac{IG_i^{GRP}}{\max IG^{GRP}} \right)$
Environmental risk coefficient	$k_7 = 0,4 \left(\frac{EHS_i}{\max EHS} \right) + 0,3 \left(\frac{HS_i^{km2}}{\max HS^{km2}} + \frac{HS_i^p}{\max HS^p} \right)$
Criminal risk coefficient	$k_8 = 0,5 \left(\frac{R_i}{\max R} + \frac{O_i}{\max O} \right)$
Level 3. Generalized indicators of investment attractiveness of a region	
Generalized indicator of investment potential	$P = \frac{e + f + inv + in + s + inf + r + ba}{8}$
Generalized indicator of investment risk	$R = \frac{\sum_{i=1}^n k_i}{n}$
Generalized indicator of investment attractiveness	$IP = P \cdot (1 - R)$

Legend: a_{ij} – value of *i*-th index of the *j*-th region of corresponding components of investment and innovation capacity; s_i – standard deviations of a_{ij} for corresponding

component of investment and innovation capacity; \bar{a}_i – average value of the i -th indicator of a corresponding component of investment potential; IIP_i – index of industrial production of the i -th region, %; DP – the largest decline in production in the region; L_i^{GRP} – share of loss in GRP of the i -th region; ID_i^{ag} – index of agricultural production development of the i -th region; E_i^u – share of unprofitable enterprises of the i -th region; P_i – level of enterprises profitability of the i -th region; F_i^d – financial dependence of enterprises of the i -th region; L_i – current enterprises liquidity of the i -th region; FR_i^e – financial risk of enterprises of the i -th region; W_i – the share of workers of the i -th region who took part in strikes, %; U_i – unemployment rate, in% to the economically active population of the i -th region; E_i – population number with an average income below the subsistence minimum in% to the total population of the i -th region; G_i – dynamics of the real money incomes of population, in% in comparison to the previous year of the i -th region; IG_i^U – import of the i -th region in the total amount of imported goods to Ukraine, %; IG_i^{GRP} – share of import in the economy of the i -th region; EHS_i – emissions of harmful substances which into get into the atmosphere, in% of total of the i -th region; $HS_i^{km^2}$ – amount of harmful substances per area of 1 km² of the i -th region; HS_i^p – amount of harmful substances per 1 person of resident population of the i -th region; R_i – share of persons prosecuted to the administrative responsibility, in an aggregate number of resident population of the i -th region, %; O_i – index of number of offenses registered in the area of the i -th region, %.

Table 2

Distribution of rural areas by the type of investment environment

Type of investment climatey	Number of rural areas	Rural areas
2B	6	Regions: Donetsk, Dnipropetrovsk, Kyiv, Lviv, Cherkasy, Mykolayiv
3B1	4	Regions: Zhytomyr, Sumy, Luhansk, Poltava
3B2	3	Regions: Chernihiv, Rivne, Kherson
2C	3	Regions: Odessa, Zaporizhzhya; Crimea
3C1	3	Regions: Vinnytsya, Transcarpathian region, Kharkiv
3C2	3	Regions: Volyn, Ivano-Frankivsk, Chernivtsi

Table 3

Distribution of rural areas by the type of investment climate and ranking of socio-economic development

Type of investment climate	Group according to the rating of socioeconomic development		
	1	2	3
3B1	Regions: Zhytomyr, Poltava, Lugansk, Poltava, Kyiv	Regions: Rivne, Kherson	Regions: Odesa, Vinnytsya
3B2	Crimea, Chernihiv region	Regions: Zaporizhzhia, Kharkiv, Chernivtsi	Regions: Transcarpathian, Lviv
2C	Donetsk region		
3C1	Dnipropetrovsk region		
3C2			Volyn, Ivano-Frankivsk region

1 – the highest level of economic development of its own food supply and purchasing power of population;

2 – close to the Ukrainian national average of these indicators;

3 – the lowest indicator values.

It is also necessary to mention that Poltava, Cherkasy region are included in ten regions with the least integrated investment risks since 2006 (annually); in certain years there were the autonomous republic of Crimea, Kyiv, Luhansk, Zhytomyr regions; for 1–2 years – Odesa, Vinnytsya regions. In other words, we have introduced factors and built classifications which are quite productive for the assessment of investment attractiveness of rural areas as well corresponded to the integral estimates, based on more complete system of indicators, including indicators that are rated expertly.

Reduction of investment risk is the main task of administrations of the regions represented in the three lower lanes in Table 3.

In recent years, it is the local levels of management which have become the most important factor in changes of the investment climate in the region. Revitalization of the regional strategies and programs development is accompanied by the creation of new tools and the improvement of methods of interaction with investors. According to survey results, investors traditionally believe that local "law" defines mostly the investment climate. Regional administrations define different forms of support for investors who realize their projects in the region.

Conclusions

Rating evaluation of investment attractiveness of a region is extremely important if taking into consideration a wide range of users and possibilities to use its results. These users are primarily private investors, for whom the set ratings will serve as indicators of current and future benefit indicators and weaknesses, opportunities and threats during the substantiation of investment capital directions into regional entities. Bodies of state and local government which manage the allocation of expenditure of respective budgets for investment purposes in different objects on the regional level will also be interested in an objective rating assessment of the region, because this approach will help them to justify

the optimal structure of expenditures and increase economic efficiency of the budget use. In addition, the detection of complex problems and strong sides of certain regions in different areas for regional socio-economic development support and according to the efficiency level of the use of basic types of region resources can act as a reliable basis for the formulation and implementation of national programs and strategies for country development.

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Анотації

Харчові технології

Вміст метанолу у виноградних і плодових бренді: основний показник оригінальності та безпеки

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Вступ. Метанол є неодмінним складником бренді незалежно від того, з якої саме фруктової сировини був зроблений напій. Вміст метанолу підтверджує оригінальність бренді, а його концентрація є показником рівня безпеки бренді для споживача.

Матеріали і методи. Вміст алкоголю в десяти різних зразках бренді - шести на основі винограду і чотирьох на основі слив - визначали за допомогою автоматичного блока дистиляції Gibertiny BEE RV 10326. Зміст метанолу в бренді визначали за допомогою газового хроматографа Varian 3900 з капілярною колонкою VF max MS (30 м, 0,25 мм ID, DF = 0,25 μm), оснащеної FID.

Результати і обговорення. Дослідження показало, що вміст алкоголю у виноградному бренді становив від 36,00 до 69,98 об.% (у середньому 52,70 об.%). Для сливових бренді значення цього показника становило від 40,00 до 62,70 об.% (у середньому 46,27 об.%). Метанол був виявлений у всіх досліджених зразках бренді. Для виноградних бренді вміст метанолу склав 0,20-0,56 г/м³, а для сливових - 1,08-2,98 г/дм³.

Перші три зразки виноградного бренді містять менше метанолу, що пояснюється використанням перегонної установки з додатковою колоною очищення і конденсатором. Завдяки цьому досягається більш вищий ступінь очищення метанолу порівняно з іншими трьома зразками виноградного бренді, дистиляція яких відбувалася в звичайних умовах.

Більш високий вміст метанолу в сливовому бренді порівняно з виноградним пояснюється більш високим вмістом пектину в плодах сливи. Пектин є речовиною-попередником метанолу. Високий вміст пектину зазвичай призводить до утворення більшої кількості метанолу в кінцевому продукті.

Вміст метанолу в досліджених зразках бренді не перевищував значень, встановлених бельгійським і європейським законодавством (максимально допустимий показник становить 10,00 г/дм³).

Висновки. Визначений у цьому дослідженні рівень метанолу в усіх зразках бренді підтвердив оригінальність напою. Зміст метанолу у всіх зразках відповідає вимогам бельгійського та європейського законодавства. Досліджені зразки бренді безпечні для споживання.

Ключові слова: метанол, виноград, слива, бренді, дистиляція, етанол.

Інактивувальний ефект мікрохвильового нагрівання на пектинметилестеразу в апельсиновому соці

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Вступ. Інактивація ферментів є основною складністю під час виробництва апельсинового соку. Звичайне нагрівання до високих температур негативно впливає на готовий продукт, викликаючи зміну кольору, погіршення аромату і втрату аскорбінової кислоти. В даному дослідженні для інактивації пектинметилестерази (ПМЕ) під час виробництва апельсинового соку було використано метод мікрохвильового нагріву (МН), а для оптимізації умов МН використовували методологію поверхні відгуку (МПВ).

Матеріали і методи. Як сировину використовували апельсини (*Citrus sinensis* Osb.) сорту Навел. Було досліджено вплив швидкості потоку і потужності на активність ПМЕ. Зразки апельсинового соку виробляли в оптимізованих умовах, після чого їх якісні характеристики й параметри інактивації ПМЕ порівнювали з параметрами необроблених контрольних зразків соку і зі зразками соку, що пройшли звичайну термічну обробку.

Результати і обговорення. Лінійні ефекти швидкості потоку (x_1) і потужності (x_2), а також квадратичний ефект швидкості потоку (x_1^2), потужності (x_2^2), ефект взаємодії швидкості потоку і потужності (x_1, x_2) суттєво вплинули на інактивацію ПМЕ за допомогою МН. Невідповідність експериментальних даних не мала статистичної значимості ($P > 0,05$) для моделі. Коефіцієнт варіації (К.В.) склав 6,27%. Точність моделі склала $6,788 \times 10^{-3}$, що є задовільним результатом. Коефіцієнт детермінації (R^2) склав 0,9793, в той час як скоригований коефіцієнт детермінації (скоригований R^2) склав 0,9645. Значення R^2 і скоригованого коефіцієнта R^2 практично не відрізнялися між собою, що показує відсутність кардинальних відмінностей в моделі. Зниження активності ПМЕ було виявлено в 93-95% груп МН. Інактивація ПМЕ проходить досить швидко залежно від умов СВЧ-нагрівання. Інактивувати ПМЕ можна за помірних температур за допомогою МН (40 мл/хв - 900 Вт - 83 °C) і МН (50 мл/хв - 900 Вт - 75 °C). Значення D були обчислені для двох оптимальних умов МН і СН, які склали 39,24 сек для МН (40 мл/хв - 900 Вт), 38,76 сек для МН (50 мл/хв - 900 Вт) і 70 сек для СН (95 °C - 60 сек). Загальний вміст пектину збільшився на 17,2% після застосування МН. Втрата аскорбінової кислоти в зразку, підданому МН, була нижчою, ніж в інших зразках соку.

Висновки. Під час мікрохвильового нагрівання був виявлений синергетичний ефект впливу мікрохвильової енергії й температури на інактивацію ПМЕ в апельсиновому соку. МН (50 мл/хв - 900 Вт) можна застосовувати для термічної обробки апельсинового соку за помірних температур (75 °C) для інактивації ПМЕ, а також для поліпшення функціональних характеристик апельсинового соку. Отриманий результат має велике значення для правильного зберігання апельсинового соку.

Ключові слова: апельсин, сік, мікрохвильовий нагрів, пектин, метилестераза.

Композиції лактобактерій для застосування в м'ясопереробній промисловості

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Вступ. Незважаючи на велику кількість бактеріальних препаратів, що застосовуються в м'ясопереробній промисловості, актуальною залишається розробка нових заквасок і вивчення їх впливу на розвиток небажаної мікробіоти.

Матеріали і методи. Досліджували гало- і терморезистентність 8 колекційних штамів лактобактерій і створених на їх основі лактобактеріальних композицій. Антагоністичну активність у відношенні індикаторних, виділених з м'ясної сировини і колекційних штамів бактерій визначали ямково-дифузійним методом.

Результати та обговорення. За максимальної (10,0%) концентрації NaCl в середовищі культивування штами *L. plantarum* 12 і 1005 характеризувалися високою інтенсивністю росту, *L. delbrueckii s/sp. lactis* 013 і *L. casei s/sp. tolerans* 290 – середньою. Терморезистентність (здатними з високою інтенсивністю рости в діапазоні від 5 до 25 °С) виявилися штами *L. Plantarum* 12, , *L. delbrueckii s/sp. lactis* 013, *L. acidophilus* 147, *L. casei s/sp. tolerans* 187 и 290. Лактобактерії проявили антагоністичну активність і щодо виділених з м'ясної сировини, і колекційних штамів бактерій. Зростання деяких індикаторних бактерій вони тільки затримували, інших – повністю придушували. Найкращими антагоністами виявилися штами *L. Plantarum* 12, , *L. delbrueckii s/sp. lactis* 013 и *L. casei s/sp. tolerans* 290, повністю пригнічують ріст *Bacillus sp.* 3, *Kurthia sp.*, *Planacoccus sp.* 1, *sp.* 2, *Micrococcus sp.* 2, *Sarcina sp.* і *Staphylococcus sp.*, виділених з м'яса, і колекційних – *Planacoccus citreus*, *Escherichia coli*, *Salmonella enteritidis*.

На основі штамів *L. Plantarum* 12, *L. delbrueckii s/sp. lactis* 013 і *L. casei s/sp. tolerans* 290 були створені 9 варіантів композицій та вивчено їх біотехнологічний потенціал. Всі композиції були здатні рости навіть при 0 °С. Найбільш стійкою виявилася закваска *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 в співвідношенні 1:2, зростання якої при 5 °С було оцінено як «дуже інтенсивне». Композиції лактобактерій істотно пригнічували ріст індикаторних бактерій. Розміри зон відсутності росту бактерій, виділених з м'яса, коливалися від 16 мм до 43 мм в залежності й від індикаторного штаму, і від композиції. З колекційних бактерій найбільш чутливими виявилися коки *P. citreus* і *M. luteus*, розміри зон відсутності росту яких в залежності від композиції коливалися від 34 мм до 42 мм і від 28 мм до 40 мм, відповідно.

Висновки. Отримані результати свідчать про підвищення біотехнологічної активності лактобактерій в композиціях. Найбільш перспективною для апробації в промислових умовах є композиція *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 в співвідношенні 1:2.

Ключові слова: м'ясо, лактобактерія, галорезистентність, терморезистентність, антагоністична активність.

Епіфітні і регламентовані мікробні контамінанти харчової рослинної сировини і продуктів

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Вступ. Біологічні небезпеки як пріоритетні під час оцінки ступеня ризику пов'язані з присутністю в харчових продуктах мікроорганізмів.

Матеріали та методи. Досліджено поширені види фруктів, овочів і ягід. Використовували загальноприйняті мікробіологічні методи: мезофільні аеробні і факультативно-анаеробні бактерії (МАФАНМ), гриби і дріжджі враховували посівом під м'ясо-пептонний агар (МПА) і агаризоване сусло відповідно, коліформи (БГКП)

визначали посівом в рідкі поживні середовища, *Bacillus cereus* та *Clostridium perfringens* – методами ISO, останній з розробленою попередньою обробкою.

Результати та обговорення. Вивчено груповий склад епіфітних мікробних контамінантів поширених видів фруктів, овочів, ягід за показниками: МАФАНМ, гриби, дріжджі, БГКП. Встановлено значну забрудненість сировини мезофільними бацилами від $1,8 \cdot 10^2$ до $7,6 \cdot 10^8$ КУО/г. Показано, що основні ізольовані морфотипи бацил можна віднести до групи *subtilis-licheniformis*. Склад мікроорганізмів рослинної сировини дозволяє судити як про можливість епідеміологічної небезпеки, так і про доброякісність продукції. Всупереч наявної раніше думки про домінування серед епіфітної мікробіоти грибів, наші результати показали для більшості зразків переважний вміст паличковидних мікроорганізмів. Плоди різних сортів, вирощені в однакових умовах і одночасно зібрані, розрізняються переважаючими видами грибів. Пріоритетним розробленим нами методом визначена концентрація папуліну в залежності від ступеня псування плодів. Велика кількість ґрунтових мікроорганізмів, включаючи дуже стійкі до нагрівання спори та бактерії родів *Bacillus* і *Clostridium*, знаходяться на поверхні рослинної сировини, особливо коренеплодів. Як показали проведені дослідження, ймовірність виявлення небезпечного для здоров'я людей *Clostridium perfringens* на листках зелених рослин становить до 61%, на овочах – до 39%. Мікроорганізми групи *subtilis-licheniformis* є домінуючими контамінантами сировини, превалюють в складі мікробіоти продукту перед стерилізацією і виявлені в залишковій мікробіоті готових консервів. Серед виділених з рослинної сировини бактерій були виявлені збудники харчових отруєнь – *Bacillus cereus* та ін. *Bacillus cereus* виявлений в 6,2% досліджених зразків фруктів, 33% проб моркви, 21% – петрушки, до 9,5% проб консервованих продуктів.

Висновки. Висока термостійкість спороносних мікроорганізмів сировини, в тому числі використовуваних як тест-культури, може обумовлювати їх наявність в консервованих продуктах, бути причиною погіршення органолептичних властивостей продуктів і викликати токсичний вплив на організм.

Ключові слова: епіфіт, мікроорганізм, рослина, консервування, папулін.

Застосування природних олій в якості біологічно активних інгредієнтів косметичних засобів

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Вступ. Компоненти олій вбудовуються в ліпідні структури рогового шару епідермісу, змінюючи властивості епідермального бар'єру. Найважливішою характеристикою жирних рослинних олій, що визначає їх властивості косметичного інгредієнта, є вміст складних ефірів жирних кислот.

Матеріали та методи. Для створення композиції жирової фази косметичних засобів емпіричним методом складали суміші рослинних олій (кокосова, пальмова, мигдальна, виноградних кісточок, оливкова, кукурудзяна, кунжутна, зародків пшениці та інші), жирнокислотний склад яких імітує склад клітинних мембран. Детекція жирних кислот здійснювались на газовому хроматографі виробництва Hewlett-Packard HP6890 за загальноприйнятою методикою.

Результати і обговорення. Можливий емпіричний підбір суміші олій, або розрахунок суміші за певним алгоритмом з наявного набору олій з відомим

жирнокислотним складом. Результати скринінгу жирнокислотного складу традиційних косметичних олій показують, що жирні кислоти містяться в усіх відомих жирах і оліях, однак їх вміст коливається у широких межах. Найбільш збалансованими за складом є арахісова, олія зародків пшениці, оливкова, кокосова, мигдальна, пальмова та ріпакова олії. Однак склад жодної з наведених індивідуальних олій не відповідає нормам косметології. Досліджено характерне співвідношення лінолевої і олеїнової кислот, що для нормальної здорової шкіри становить порядку 1:1,8, у той час як для сухої шкіри воно становить приблизно 1:4,7. Найбільш оптимальною з точки зору вмісту моно- та поліненасичених жирних кислот є композиція, що містить кокосову, кунжутну та пшеничну олії. Співвідношення лінолевої (C18:2) та олеїнової (C18:0) кислот у ній становить 1:8, що є адекватним для нормальної здорової шкіри, а співвідношення поліненасичених лінолевої (C18:2) та альфа-лінолевої (C18:3 ω-3) наближається до біологічно ефективного рівня і становить 1:11 проти ідеального 1:10.

Висновки. Така косметична база повністю складається з натуральних рослинних олій і призначена до застосування в рецептурах жирових та емульсійних косметичних засобів для догляду за сухою подразненою шкірою, її живлення і пом'якшення.

Ключові слова: олія, косметика, шкіра, склад.

Дослідження реологічних властивостей розчинів желатину для виробництва безглютенових макаронних виробів

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Вступ. Для формування безглютенових макаронних виробів з кукурудзяного борошна, яке не утворює клейковину, важливим є вибір структуроутворювача, визначення способу його внесення та дозування на підставі вивчення реологічних властивостей його розчинів та впливу на якість виробів.

Матеріали і методи. Досліджено реологічні властивості колоїдних розчинів желатину концентрацією 0,50–1,25%, приготовлених за температури води 20 °C та 40 °C і тривалості набухання 40 хв. та за 60 °C без набухання. Визначали в'язкість цих розчинів на віскозиметрі Реотест-2 за температури 20 °C. За отриманими даними будували реологічні криві в'язкості та плинності, розраховували реологічні характеристики цих розчинів. Визначено вплив розчинів структуроутворювачів на показники якості макаронних виробів.

Результати. За температури набухання желатину 20 °C динамічна в'язкість незруйнованої структури колоїдного розчину зі збільшенням його концентрації з 0,50% до 1,25% знижується від 59,10 Па·с до 21,89 Па·с, за винятком розчину з концентрацією 1,00%, для якого спостерігається аномалія в'язкості, а в'язкість дорівнює 531,90 Па·с. Аналогічні дослідження, проведені під час набухання за температури води 40 °C, показали, що всі колоїдні розчини желатину за концентрації 0,50–1,25% є псевдопластичними рідинами ($R_{K1} = 0$), мають значно нижчу динамічну в'язкість як зруйнованої, так і незруйнованої структури та нижчу міцність структурного каркасу, ніж за температури набухання 20 °C. Для зразка з концентрацією 0,75% спостерігається аномалія в'язкості: за цієї концентрації розчин має найбільшу динамічну в'язкість незруйнованої структури й найбільшу динамічну

в'язкість зруйнованої структури, відповідно 94,56 та 1,35 Па·с, та найбільше значення η_0 - η_m – 93,21 Па·с і одночасно найбільшу міцність утвореного структурного каркасу 425,52 Па. Макаронні вироби, виготовлені з використанням таких розчинів, мають найкращу якість. За температури 60 °С розчини мають низьку в'язкість і міцність, тобто утворюють слабкі гелі, які не забезпечують утворення міцного структурного каркасу і хорошої якості макаронних виробів.

Висновок. Встановлено оптимальну кількість желатину 0,75–1,0% до маси борошна та параметри підготовки його до виробництва – набухання протягом 40 хв. за температури 40–20 °С відповідно, які забезпечують найвищу в'язкість розчинів желатину 94,6–531,9 Па·с і сприяють отриманню виробів високої якості.

Ключові слова: желатин, дозування, в'язкість, розчин, якість.

Біотехнологія, мікробіологія

Мікробіологічне дослідження диких, культивованих (*Mytilus galloprovincialis* L. 1819) і фаршированих мідій

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Вступ. У статті досліджуються мікробіологічні властивості диких і культивованих мідій, а також фаршированих мідій, які продаються в ресторанах і вуличних торговельних точках в серпні і вересні у м. Синоп.

Матеріали і методи. За допомогою стандартних процедур були досліджені 68 зразків мідій (*Mytilus galloprovincialis* L. 1819), а також фаршировані мідії, в яких визначали загальний вміст аеробних мезофільних бактерій, бактерій *Coliform*, *Escherichia coli* і *Vibrio* spp. Відбір проб для мікробіологічного аналізу проводили в асептичних умовах. Усі мікробіологічні аналізи проводилися триразово.

Результати і обговорення. Початкові показники сумарної кількості аеробних мезофільних бактерій, колиформних бактерій, *E. coli* в диких і культивованих мідіях в серпні склали, відповідно, 4,04 ln Кое/г і 3,55 ln Кое/г; 3,69 ln Кое/г і 3,09 ln Кое/г; 0,59 логарифма Кое/г і 0,39 логарифма Кое/г. Загальна кількість бактерій, колиформних бактерій і бактерій *Vibrio* spp. в диких мідіях була вищою, ніж у культивованих ($p < 0,05$). У культивованих мідіях бактерій *Vibrio* spp. виявлено не було.

Загальна кількість аеробних мезофільних бактерій, колиформних бактерій і бактерій *E. coli* у фаршированих мідіях, які продавалися у вуличних торговельних точках у серпні, перевищувала цей показник для фаршированих мідій, що продавалися в ресторанах у цей же час ($p < 0,05$). У вересні бактерії *E. coli* не були виявлені в зразках фаршированих мідій, які продавалися як у ресторанах, так і у вуличних торговельних точках. У серпні та вересні вміст аеробних бактерій в зразках фаршированих мідій не перевищував допустимих граничних значень (6 ln Кое/г). Бактерії *Vibrio* spp. були виявлені у всіх зразках фаршированих мідій, крім тих, які продавалися у вуличних торговельних точках у вересні. Фаршировані мідії були приготовлені з диких мідій і загальний вміст аеробних мезофільних бактерій,

коліформних бактерій і бактерій *E.coli* у них перевищував вміст цих же бактерій у диких мідіях.

Висновки. Бактерії *E. coli* не були виявлені в фаршированих мідіях, які продавалися в ресторанах в серпні й вересні, а бактерії *Vibrio* spp. були виявлені в двадцяти семи з сорока восьми зразків фаршированих мідій, які продавалися в ресторанах і вуличних торговельних точках.

Ключові слова: мідія, фарширована мідія, *e.coli*, *coliform*, здоров'я.

Вплив технологічних параметрів ферментації вершків на формування функціональних властивостей кисловершкового масла

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Вступ. Визначальними чинниками виготовлення кисловершкового масла є процеси ферментації (підбір заквашувальних культур, їх співвідношення та визначення оптимальних технологічних параметрів ферментації) та фізичного визрівання вершків.

Матеріали та методи. Активність кислотоутворення при ферментації вершків визначали за зміною титрованої та активної кислотності. Кількість життєздатних клітин *Flora Danica* та *Lactobacillus acidophilus La-5* була підрахована шляхом посіву при використанні середовища M17 Agar CM 0785 та *Lactobacillus* MRS Agar M 641-500G (HiMedia). Жирнокислотний склад зразків масла досліджували методом газорідинної хроматографії на газовому хроматографі Hewlett Packard HP-6890.

Результати і обговорення. Використання у виробництві кисломолочних продуктів заквашувальних композицій, які поряд з певними лактобактеріями, містять монокультури пробіотичних штамів, дозволяє отримати незамінний з точки зору сучасної дієтології продукт харчування з пробіотичними, оздоровчими та заданими спеціальними властивостями.

Із урахуванням рекомендованих технологічними інструкціями температур ферментації та компромісної температури для мікробіальних культур вибраних препаратів обрали два температурні режими – 20 і 30 °C для ферментації вершків. Встановлено, що найвищий темп зростання титрованої кислотності вершків зареєстровано для зразка, для ферментації якого використовували *FD+La-5* і температуру 30 °C.

Як засвідчують результати, зразок під час спільного культивування *FD* і *La-5* за температури ферментації 30 °C демонструє найкращу динаміку наростання біомаси протягом ферментації і фізичного визрівання вершків, оскільки концентрація життєздатних клітин у цьому варіанті була найбільшою.

Щодо вмісту жирних кислот, які проявляють виражену біологічну дію, то їх вміст проявляв чітку тенденцію до збільшення у зразку кисловершкового масла, де застосовували поєднання змішаних мезофільних культур і термофільної ацидофільної палички та ферментацію вершків за температури 30 °C.

Висновки. Рекомендується використовувати у технології кисловершкового масла заквашувальну композицію, складену із змішаних мезофільних культур *Flora*

Danica та термофільної монокультури *Lactobacillus acidophilus* La-5 та температуру ферментації вершків 30 °С.

Ключові слова: ферментація, вершки, *Flora Danica*, *Lactobacillus acidophilus* La-5, масло.

Оптимізація виробництва мікробної трансглютамінази із *Streptomyces* sp.

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Вступ. З метою подальшого створення моделі для майбутніх досліджень на тему використання мікробної трансглютамінази були вивчені методи оптимізації виробництва мікробної трансглютамінази (МТГ).

Матеріали і методи. Вплив температури, рН, середовища і типу штаму було досліджено з метою визначення максимальної активності ферментів. Для виробництва МТГ були відібрані п'ять різних штамів: *Streptomyces mobaraensis* (NRRL B-3729), *S. ladakanum* (NRRL ISP-5587), *S. lividans* (NRRL B-12275), *S. sioyaensis* (NRRL B-5408) і *S. platensis* (NRRL B-5486). Процес ферментації проходив у двох ферментаційних середовищах – глюкозно-крохмальному і соєвому, які характеризувалися різними показниками рН і температури (6,0; 7,0; 8,0 рН і 20; 30; 40 °С). Активність МТГ визначалася протягом 28 днів колориметричним методом.

Результати і обговорення. Бактерії *S. mobaraensis*, *S. ladakanum* і *S. lividans* продемонстрували більш високі темпи зростання, ніж *S. sioyaensis* і *S. platensis*. Отже, для виробництва ферментів були обрані бактерії *S. mobaraensis*, *S. ladakanum* і *S. lividans*. За значень рН 6,0 найвища активність ферментів (0,036 од/мл) була досягнута за 14 днів для бактерій *S. mobaraensis* в глюкозно-крохмальному середовищі за 30 °С, а на 14-й день ферментації активність ферментів різко знизилася для всіх штамів бактерій. За значення рН 7,0 найвища активність ферментів була досягнута на 28-й день для бактерій *S. mobaraensis* за температури 30 °С. За значення рН 8,0 МТГ не вдалося отримати в жодному живильному середовищі ні за якої температури. Для початкового значення рН 6,0 зростаючий рівень активності МТГ в глюкозно-крохмальному середовищі за різних температур (20, 30 і 40 °С) був вищим, ніж у соєвому середовищі. Бактерії *S. ladakanum* and *S. lividans* не можуть виробляти МТГ за жодних умов.

Висновки. Бактерії *S. mobaraensis* показали найвищу ферментативну активність порівняно з іншими штамми. Глюкозно-крохмальне середовище є найбільш оптимальним для отримання МТГ. Зміна значень рН і температури впливає на активність ферментів. Найкращими умовами для отримання МТГ є рівень рН 6,0 і температура 30 °С.

Ключові слова: мікробна трансглютаміназа, *S. Mobaraensis*, фермент, активність, ферментація.

Харчова хімія

Комплекси 4- і 5-нітрозаміщених гетероарильних цинамоїлпохідних з Cu(II) і визначення їхньої антикоагулянтної активності

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Вступ. Описано синтез комплексів Cu(II) з 4- і 5-нітрозаміщеними гетероарильними цинамоїлохідними і досліджено їхню антикоагулянтну активність.

Матеріали і методи. Всі використані хімічні реактиви були придбані за каталогами компаній Merck і Sigma-Aldrich. Температури плавлення речовин визначені на цифровому пристрої SMP 10. Дані елементного аналізу одержані на аналізаторі Carlo Erba 1106. Чистота речовин перевірена методом тонкошарової хроматографії з використанням 0,2 мм платівок Kieselgel 60 F₂₅₄ (Merck, Німеччина), елюент: суміш CH₂Cl₂ : CH₃COCH₃ = 1 : 1 (об'ємні співвідношення). Спектри ІЧ-спектроскопії записані на спектрометрі Perkin-Elmer FTIR-1600 (зразки у вигляді таблеток з KBr).

Результати і обговорення. Ліганди, необхідні для утворення відповідних комплексів, були одержані з 4- і 5-нітрозаміщених 2-ацил-1,3-індантіонів двома способами: за методом Mosher і Meier, Rotberg і Oshkaya, а також авторським методом, новизною якого є використання піролідину як каталізатора. Для одержання комплексних сполук до розчинів кінцевих лігандів у діоксані додавали метанольний розчин Cu(CH₃COO)₂ · H₂O. Утворені комплексні сполуки кристалізувалися після охолодження суміші. Для новоодержаних сполук визначені фізико-хімічні параметри, а також проведено спектральні дослідження. На підставі експериментальних даних запропоновано склад комплексів [M(L)₂] і зроблено висновок, що найбільш імовірна структура комплексів Cu(II) передбачає наявність двох депротонованих OH груп 4- і 5-нітроцинамоїлпохідних. Спектроскопічні дослідження аморфних зразків показали, що комплекси Cu(II) характеризуються чотиригранною геометричною структурою. Антикоагулянтна активність синтезованих комплексів охарактеризована їхнім протромбіновим часом і порівняна з часом вихідних 4- і 5-нітрозаміщених похідних. Показано, що 5-нітропохідні мають вищу антикоагулянтну активність, ніж відповідні 4-нітропохідні.

Висновок. Одержані нові комплекси Cu(II) з 4- і 5-нітрозаміщеними гетероарильними цинамоїлпохідними і визначена їхня антикоагулянтна активність. Комплекси можуть бути використані у майбутніх тестуваннях харчових продуктів рослинного і тваринного походження.

Ключові слова: 1,3-індантіони, Cu(II)-комплекс, IR, PT, INR.

Процеси і обладнання харчових виробництв

Розрахунок нестационарних дифузійних масових потоків сахарози для комірок міжкристалічних розчинів сахарози системи: «більший кристал цукру–розчин сахарози більшого кристалу–менший кристал цукру–розчин сахарози меншого кристалу–утфель» в залежності від часу уварювання цукрового утфелю

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Вступ. В даній роботі запропоновано один із наступних етапів створення математичної моделі процесу кристалізації сахарози.

Матеріали та методи. Для отримання величин нестационарних дифузійних масових потоків сахарози для комірок міжкристалічних розчинів сахарози розв'язано одночасно систему із 7 нестационарних задач теплопровідності для кожної окремої області зі сталими та зі змінними теплофізичними коефіцієнтами, а також три окремих нестационарних задач дифузійного масообміну для чотирьох областей міжкристалічного розчину сахарози зі сталими та змінними коефіцієнтами дифузійного масообміну чисельними методами (метод контрольного об'єму).

Результати і обговорення. Для десяти випадків відносного часу уварювання цукрового утфелю $\tau/\tau_{\text{ц}}$ ($\tau/\tau_{\text{ц}} = 0,15; 0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8; 0,9; 1,0$) на основі одночасного розв'язку чотирьох систем нестационарних диференціальних рівнянь в частинних похідних параболічного типу (перша система – для нестационарної задачі теплопровідності; та три системи – для нестационарних задач дифузійного масообміну) знайдено розподіл нестационарних дифузійних масових потоків сахарози для областей міжкристалічних розчинів сахарози всієї розглянутої системи комірок. Вперше на основі проведених розрахунків встановлено, що процес перетікання розчиненої сахарози з комірки міжкристалічного розчину одного кристалу в комірку міжкристалічного розчину сахарози іншого кристалу дійсно відбувається і в якому напрямку він відбувається. Також вперше отриману кількісну величину дифузійного масового потоку сахарози між областями, що представляють комірками міжкристалічних розчинів різних кристалів цукру. За відносного часу уварювання цукрового утфелю $\tau/\tau_{\text{ц}} = 0,15$ відбувається перенесення речовини (сахарози) з області 4 лівої комірки міжкристалічного розчину кристалу 2 в область 3 правої комірки міжкристалічного розчину кристалу 1. Приблизно в за $\tau_{\text{к}} = 2$ с досягається їхній мінімум. Починаючи з моменту часу $\tau_{\text{к}} = 2,58$ с для варіанту розрахунку зі сталими теплофізичними коефіцієнтами ситуація змінюється на протилежну, тобто перенесення сахарози відбувається вже з області 3 в область 4. За всіх же змінних теплофізичних характеристиках перенесення сахарози за час перебування системи комірок в нагрівальній трубці все ще відбувається з області 4 в область 3, а під час виходу системи комірок з нагрівальної трубки прагне до нуля, тобто, практично відсутня. Отже, в цьому випадку отримали чітко виражений мінімум дифузійного масового потоку. При відносному часі уварювання цукрового утфелю $\tau/\tau_{\text{ц}} = 1,0$ отримали чітко виражений мінімум та максимум як для сталих, так і для всіх змінних теплофізичних характеристик.

Висновки. Для кожної області, що представляє собою міжкристалічний розчин сахарози, отримано величину нестационарного дифузійного масового потоку сахарози в залежності від часу контакту системи комірок з нагрівальною трубкою. Вперше встановлено величину та напрямок дифузійного масового потоку між двома областями міжкристалічних розчинів сахарози першого та другого кристалів цукру.

Ключові слова: сахароза, дифузія, розчин, кристал, утфель.

Інтенсифікація процесів масоперенесення в газово-рідких середовищах дискретним – імпульсним методом введення енергії.

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Вступ. Метою даного дослідження була інтенсифікація процесу аерації культуральних середовищ методом дискретно – імпульсного введення енергії, який реалізується в роторно – пульсаційному апараті.

Матеріали та методи. Досліджено процес аерації культуральних середовищ в технології вирощування дріжджів *Saccharomyces cerevisiae* методом дискретно – імпульсного введення енергії. Швидкість масоперенесення кисню визначалася за кількістю біомаси дріжджів, вирощених за період культивування.

Результати та обговорення. В ході експериментів з культивування дріжджів на м'ясних розчинах була визначена залежність швидкості масоперенесення кисню від кутової швидкості обертання роторного вузла в культуральних середовищах з вмістом сухих речовин 3 – 10%. Зі зменшенням вмісту сухих речовин від 10 до 5% при обробці з кутовою швидкістю 48 об/с, швидкість масопереносу збільшується в 1,9 рази. Зі збільшенням частоти пульсацій від 2 до 3,85 кГц, швидкість масоперенесення зростає від 4 до 6,3 г/л · год при вмісті сухих речовин – 3% і від 2,2 до 4 г/л · год при вмісті сухих речовин – 10%. Подальше підвищення частоти пульсацій призводить до інактивації частини дріжджових клітин. Встановлено також, що оптимальне значення швидкості зсуву потоку становить $90 - 100 \cdot 10^3 \text{ c}^{-1}$.

Висновки. Результати цього дослідження свідчать про те, що застосування методу ДІВЕ в абсорбційних технологіях дозволяє значно інтенсифікувати масообмінні процеси.

Ключові слова: масоперенесення, абсорбція, мікроорганізм, інтенсифікація.

Підвищення ефективності теплопередачі в трубчастому теплообміннику з гофрованими трубами

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Вступ. Дієвим методом інтенсифікації теплопередачі в трубчастих теплообмінниках є застосування гофрованих труб, але в даний час немає універсальної методики розрахунку і проектування таких теплообмінників.

Матеріали та методи. Вивчено теплопередаючі характеристики гнучких гофрованих труб з нержавіючої сталі з різним профілем гофр. Випробувальний стенд являє собою теплообмінник типу "труба в трубі" з гладкою зовнішньою і профільованою внутрішньою трубою, обладнаний датчиками для вимірювання температурних і гідравлічних параметрів потоку.

Результати і обговорення. Дослідження теплопереносу та гідродинаміки в теплообміннику «труба в трубі» з гофрованою внутрішньою трубою показало, що в діапазоні чисел Рейнольдса від 4000 до 40000 досягається значна інтенсифікація

теплообміну в порівнянні з традиційним гладкотрубним теплообмінником Збільшення коефіцієнта теплопередачі склало від 2,0 до 2,6 разів при зростанні гідравлічного опору в 1,9..2,0 рази. Встановлено, що при однакових умовах потоку труби з малою висотою гофри і великим кроком гофрування (співвідношення висота/крок – 1,9/4,0 мм) мають на 15..20% більшу конвективну складову коефіцієнта теплопередачі в порівнянні з трубами з високими гофрама і дрібним кроком гофрування (співвідношення висота/крок – 2,4/3,2 мм).

Для оцінки впливу геометрії труб на інтенсивність процесу теплообміну була розроблена двовимірний осесиметричний комп'ютерний модель одиничного елемента теплообмінного апарату. Чисельне моделювання гідродинаміки і теплообміну в каналі одиничного елемента показало, що математичні розрахунки досить близькі до експериментальних досліджень.

З використанням даних критеріальних залежностей розрахований і спроектований теплообмінник потужністю 350 кВт для системи опалення адміністративного корпусу, дослідна експлуатація якого підтвердила ефективність запропонованих технічних рішень.

Висновки. Використання гофрованих труб дозволило збільшити коефіцієнт теплопередачі. Отримані критеріальні залежності дозволяють розрахувати та оптимізувати процес теплопередачі в трубчастому теплообміннику з гнучкими гофрованими трубами.

Ключові слова: теплообмінник, гофротруба, турбулізатор, теплопередача.

Економіка і управління

Методичні засади рейтингової оцінки інвестиційної привабливості сільських регіонів України

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Вступ. Стаття присвячена дослідженню методичних та практичних засад рейтингового оцінювання інвестиційної привабливості сільських регіонів.

Матеріали та методи. Досліджується інвестиційна привабливість сільських регіонів на прикладі областей України. Для оцінки інвестиційної привабливості застосовано синергетичний метод, метод порівняння та інтегральної оцінки. Використано рейтинговий підхід, який є найпоширенішим у практиці оцінювання інвестиційної привабливості з використанням системи показників-індикаторів.

Результати та обговорення. Запропонований алгоритм оцінювання інвестиційної привабливості регіону, що передбачає певну послідовність дій, згідно з якою перші три етапи є підготовчими, а наступні чотири є безпосередньою рейтинговою оцінкою регіону на різних рівнях.

Запропонована методика оцінки інвестиційно-інноваційної привабливості областей, яка ґрунтується на використанні методів стандартизації та нормування, а також на застосуванні методу порівняння. Наведена трьохрівнева система показників-індикаторів оцінки інвестиційної привабливості сільських регіонів, до першого рівня якої включені показники складових інвестиційного потенціалу, до

другого рівня – показники інвестиційного ризику і до третього рівня – узагальнені показники інвестиційної привабливості регіонів.

На основі результатів рейтингу здійснений розподіл сільських регіонів за типами інвестиційного клімату. Сільські регіони представлені практично у всіх рейтингових групах приблизно в однаковій пропорції по відношенню до чисельності груп і, таким чином, репрезентують за показниками інвестиційного клімату всі регіони України.

Встановлено, що для сільських регіонів інвестиційна привабливість у більшості залежить від рівня соціально-економічного розвитку. До типу інвестиційного клімату 2В (середній потенціал, помірний ризик) відносяться 6 сільських регіонів, до типу 3В1 (знижений потенціал, помірний ризик) – 4 сільських регіонів, до типів 3В2 (незначний потенціал, помірний ризик), 2С (середній потенціал, високий ризик), 3С1 (знижений потенціал, високий ризик) та 3С2 (незначний потенціал, високий ризик) відносяться по три сільських регіонів. Регіони з мінімальним ризиком (типи 1А, 2А, 3А, 1В) за розрахунками відсутні.

Висновки. На основі аналізу інвестиційного клімату наведений розподіл сільських регіонів за інвестиційними типами. На основі результатів рейтингу побудована типологія сільських регіонів.

Ключові слова: *інвестиція, привабливість, розвиток, регіон, рейтинг.*

Аннотации

Пищевые технологии

Содержание метанола в виноградных и плодовых бренди: основной показатель подлинности и безопасности

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Введение. Метанол всегда входит в состав бренди независимо от того, из какого именно фруктового сырья был произведен напиток. Содержание метанола подтверждает подлинность бренди, а его концентрация является показателем уровня безопасности бренди для потребителя.

Материалы и методы. Содержание алкоголя в десяти различных образцах бренди - шести на основе винограда и четырех на основе слив - определяли с помощью автоматического блока дистилляции Gibertiny BEE RV 10326. Содержание метанола в бренди определяли с помощью газового хроматографа Varian 3900 с капиллярной колонкой VF max MS (30 м, 0,25 мм ID, DF = 0,25 μm), оснащенной FID. Статистический анализ данных проводили с помощью стандартного метода определения погрешности.

Результаты и обсуждение. Исследование показало, что содержание алкоголя в виноградном бренди составляло от 36,00 до 69,98 об.% (в среднем 52,70 об.%). Для сливовых бренди значение этого показателя составляло от 40,00 до 62,70 об.% (в среднем 46,27 об.%). Метанол был обнаружен во всех исследуемых образцах бренди. Для виноградных бренди содержание метанола составило 0,20-0,56 г/м³, а для сливовых - 1,08-2,98 г/дм³.

Первые три образца виноградного бренди содержат меньше метанола, что объясняется использованием перегонной установки с дополнительной колонной очистки и конденсатором. Благодаря этому достигается лучшая степень очистки метанола по сравнению с другими тремя образцами виноградного бренди, дистилляция которых происходила в обычных условиях.

Более высокое содержание метанола в сливовом бренди по сравнению с виноградным является нормальной тенденцией, которая объясняется более высоким содержанием пектина в плодах сливы. Пектин является веществом-предшественником метанола. Высокое содержание пектина обычно приводит к образованию большего количества метанола в конечном продукте.

Содержание метанола в исследуемых образцах бренди не превышало значений, установленных бельгийским и европейским законодательством (максимально допустимый показатель составляет 10,00 г/дм³).

Выводы. Определенный в этом исследовании уровень метанола во всех образцах бренди подтвердил подлинность напитка. Содержание метанола во всех образцах отвечает требованиям бельгийского и европейского законодательства. Исследованные образцы бренди безопасны для потребления.

Ключевые слова: метанол, виноград, слива, бренди, дистилляция, этанол.

Инактивирующий эффект микроволнового нагрева на пектинметилэстеразу в апельсиновом соке

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Введение. Инактивация ферментов представляет основную сложность при производстве апельсинового сока. Обычный нагрев до высоких температур плохо влияет на готовый продукт, вызывая изменение цвета, ухудшение аромата и потерю аскорбиновой кислоты. В данном исследовании для инактивации пектинметилэстеразы (ПМЭ) при производстве апельсинового сока был использован метод микроволнового нагрева (МН), а для оптимизации условий МН использовали методологию поверхности отклика (МПО).

Материалы и методы. В качестве сырья использовали апельсины (*Citrus sinensis* Osb.) сорта Навел. Было исследовано влияние скорости потока и мощности на активность ПМЭ. Образцы апельсинового сока производили в оптимизированных условиях, после чего их качественные характеристики и параметры инактивации ПМЭ сравнивали с параметрами необработанных контрольных образцов сока и с образцами сока, прошедшими обычную термическую обработку.

Результаты и обсуждение. Линейные эффекты скорости потока (x_1) и мощности (x_2), а также квадратичный эффект скорости потока (x_1^2), мощность (x_2^2) и эффект взаимодействия скорости потока и мощности (x_1x_2) оказывали значительное влияние на инактивацию ПМЭ с помощью МН. Несоответствие экспериментальных данных не было статистически значимым ($P > 0,05$) для модели. Коэффициент вариации (К.В.) составил 6,27%. Точность модели составила $6,788 \times 10^{-3}$, что является удовлетворительным результатом. Коэффициент детерминации (R^2) составил 0,9793, в то время как скорректированный коэффициент детерминации (скорректированный R^2) составил 0,9645. Значения R^2 и скорректированного коэффициента R^2 практически не отличались между собой, что показывает отсутствие кардинальных различий в модели. Снижение активности ПМЭ было обнаружено в 93-95% групп МН. Инактивация ПМЭ проходит достаточно быстро в зависимости от условий СВЧ-нагрева. Инактивировать ПМЭ можно при умеренных температурах с помощью МН (40 мл/мин - 900 Вт - 83°C) и СН (50 мл/мин - 900 Вт - 75°C). Значения D, вычисленные для двух оптимальных условий МН и СН, составили 39,24 сек для МН (40 мл/мин - 900 Вт), 38,76 сек для МН (50 мл/мин - 900 Вт) и 70 сек для СН (95 °C - 60 сек). Общее содержание пектина увеличилось на 17,2% после применения МН. Потеря аскорбиновой кислоты в образце, подвергнутом МН, была ниже, чем в других образцах сока.

Выводы. При микроволновом нагреве был обнаружен синергетический эффект воздействия микроволновой энергии и температуры на инактивацию ПМЭ в апельсиновом соке. МН (50 мл/мин - 900 Вт) можно применять для термической обработки апельсинового сока при умеренных температурах (75 °C) для инактивации ПМЭ, а также для улучшения функциональных характеристик апельсинового сока. Полученный результат имеет большое значение для правильного хранения апельсинового сока.

Ключевые слова: *апельсин, сок, микроволновой нагрев, пектин, метилэстераза.*

Композиции лактобактерий для применения в мясоперерабатывающей промышленности

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Введение. Несмотря на большое количество бактериальных препаратов, применяемых в мясоперерабатывающей промышленности, актуальной остается разработка новых заквасок и изучение влияния их на развитие нежелательной микробиоты.

Материалы и методы. Исследовали гало- и терморезистентность 8 коллекционных штаммов лактобактерий и созданных на их основе лактобактериальных композиций. Антагонистическую активность в отношении индикаторных выделенных из мясного сырья и коллекционных штаммов бактерий определяли луночно-диффузионным методом.

Результаты и обсуждение. При максимальной (10,0%) концентрации NaCl в среде культивирования штаммы *L. plantarum* 12 и 1005 характеризовались высокой интенсивностью роста, штаммы *L. delbrueckii s/sp. lactis* 013 и *L. casei s/sp. tolerans* 290 – средней. Терморезистентными (способными с высокой интенсивностью расти в диапазоне от 5 до 25 °C) выявились штаммы *L. plantarum* 12, *L. delbrueckii s/sp. lactis* 013, *L. acidophilus* 147, *L. casei s/sp. tolerans* 187 и 290. Лактобактерии проявили антагонистическую активность и в отношении выделенных из мясного сырья, и коллекционных штаммов бактерий. Рост некоторых индикаторных бактерий они только задерживали, других – полностью подавляли. Наилучшими антагонистами оказались штаммы *L. plantarum* 12, *L. delbrueckii s/sp. lactis* 013 и *L. casei s/sp. tolerans* 290, полностью подавляющие рост *Bacillus sp.* 3, *Kurthia sp.*, *Planacoccus sp.* 1, *sp.* 2, *Micrococcus sp.* 2, *Sarcina sp.* и *Staphylococcus sp.*, выделенных из мяса, и коллекционных – *Planacoccus citreus*, *Escherichia coli*, *Salmonella enteritidis*.

На основе штаммов *L. plantarum* 12, *L. delbrueckii s/sp. lactis* 013 и *L. casei s/sp. tolerans* 290 были созданы 9 вариантов композиций и изучен их биотехнологический потенциал. Все композиции были способны расти даже при 0 °C. Наиболее устойчивой оказалась закваска *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 в соотношении 1:2, рост которой при 5 °C был оценен как «очень интенсивный». Композиции лактобактерий существенно угнетали рост индикаторных бактерий. Размеры зон отсутствия роста бактерий, выделенных из мяса колебались от 16 мм до 43 мм в зависимости и от индикаторного штамма, и от композиции. Из коллекционных бактерий наиболее чувствительными выявились грамположительные кокки *P. citreus* и *M. luteus*, размеры зон отсутствия роста которых в зависимости от композиции колебались от 34 мм до 42 мм и от 28 мм до 40 мм, соответственно.

Выводы. Полученные результаты свидетельствуют о повышении биотехнологической активности лактобактерий в композициях. Наиболее перспективной для апробации в промышленных условиях является композиция *L. delbrueckii s/sp. lactis* 013 + *L. plantarum* 12 в соотношении 1:2.

Ключевые слова: мясо, лактобактерия, галорезистентность, терморезистентность, антагонистическая активность.

Эпифитные и регламентируемые микробные контаминанты пищевого растительного сырья и продуктов

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Введение. Биологические опасности как приоритетные при оценке степени риска вызываются присутствием в пищевых продуктах микроорганизмов.

Материалы и методы. В качестве объектов исследования использовали распространенные виды фруктов, овощей и ягод. Использовали общепринятые микробиологические методы: мезофильные аэробные и факультативно-анаэробные бактерии, грибы и дрожжи учитывали посевом под мясо-пептонный агар (МПА) и агаризованное сусло соответственно, БГКП определяли посевом в жидкие питательные среды, *Bacillus cereus* и *Clostridium perfringens* определяли методами ISO, последний с разработанной предварительной обработкой.

Результаты и обсуждение. Изучен групповой состав эпифитных микроорганизмов, контаминирующих распространенные виды фруктов, овощей, ягод по показателям: мезофильные аэробные и факультативно-анаэробные микроорганизмы (МАФАНМ), грибы, дрожжи, колиформы (БГКП). Установлена значительная обсемененность сырья мезофильными бациллами от $1,8 \cdot 10^2$ до $7,6 \cdot 10^8$ КОЕ/г. Показано, что основные изолированные морфотипы бацилл можно отнести к группе *subtilis-licheniformis*. Состав микроорганизмов растительного сырья позволяет судить как о возможности эпидемиологической опасности, так и о доброкачественности продукции. Вопреки существовавшему ранее мнению о доминировании среди эпифитной микробиоты грибов, наши результаты показали в ряде случаев преимущественное содержание палочковидных микроорганизмов. Плоды разных сортов, выращенные в одинаковых условиях и одновременно собранные, различаются по преобладающим видам грибов. Приоритетным разработанным нами методом определена концентрация патулина в зависимости от степени порчи плодов. Большое количество почвенных микроорганизмов, включая очень стойкие к нагреванию споры и бактерии родов *Bacillus* и *Clostridium* находятся на поверхности растительного сырья, особенно корнеплодов. Как показали проведенные исследования, вероятность обнаружения опасного для здоровья людей *Clostridium perfringens* на листьях зелёных растений составляет до 61%, на овощах – до 39%. Микроорганизмы группы *subtilis-licheniformis* являются доминирующими контаминантами сырья, преобладают в составе микробиоты продукта перед стерилизацией и обнаружены в остаточной микробиоте готовых консервов. Среди выделенных из растительного сырья бактерий были обнаружены агенты пищевых отравлений – *Bacillus cereus* и др. *Bacillus cereus* обнаружен в 6,2% исследованных образцов фруктов, 33% проб моркови, 21% проб петрушки, до 9,5% проб консервированных продуктов.

Выводы. Высокая термоустойчивость спорозоных микроорганизмов сырья, в том числе используемых в качестве тест-культур, может обуславливать их наличие в консервированных продуктах, быть причиной ухудшения органолептических свойств продуктов и вызывать токсические воздействия на организм.

Ключевые слова: эпифит, микроорганизм, растение, консервирование, патулин.

Применение природных масел в качестве биологически активных ингредиентов косметических средств

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Введение. Компоненты масел встраиваются в липидные структуры рогового слоя эпидермиса, изменяя свойства эпидермального барьера. Важнейшей характеристикой жирных растительных масел, определяющей их свойства косметических ингредиентов, является содержание сложных эфиров жирных кислот.

Материалы и методы. Для создания композиции жирной фазы косметических средств эмпирическим методом составляли смеси растительных масел (кокосовое, пальмовое, миндальное, виноградных косточек, оливковое, кукурузное, кунжутное, зародышей пшеницы и другие), жирнокислотный состав которых имитирует состав клеточных мембран. Детекция жирных кислот осуществлялись на газовом хроматографе производства Hewlett-Packard HP6890 по общепринятой методике.

Результаты и обсуждение. Возможен эмпирический подбор смеси масел, или расчет смеси по определенному алгоритму из имеющегося набора масел с известным жирнокислотным составом. Результаты скрининга жирнокислотного состава традиционных косметических масел показывают, что жирные кислоты содержатся во всех известных жирах и маслах, однако их содержание колеблется в широких пределах. Наиболее сбалансированными по составу являются арахисовое, масло зародышей пшеницы, оливковое, кокосовое, миндальное, пальмовое и рапсовое масла. Однако состав ни одного из приведенных индивидуальных масел не соответствует нормам косметологии. Исследовано характерное соотношение линолевой и олеиновой кислот, которое для нормальной здоровой кожи составляет порядка 1:1,8, в то время как для сухой кожи оно составляет примерно 1:4,7. Наиболее оптимальной с точки зрения содержания моно- и полиненасыщенных жирных кислот является композиция, содержащая кокосовое, кунжутное и пшеничное масла. Соотношение линолевой (C18:2) и олеиновой (C18:0) кислот в ней составляет 1:8 и является адекватным для нормальной здоровой кожи, а соотношение полиненасыщенных линолевой (C18:2) и альфа-линоленовой (C18:3 ω -3) приближается к биологически эффективного уровня и составляет 1:11 против идеального 1:10.

Выводы. Такая косметическая база полностью состоит из натуральных растительных масел и предназначена для применения в рецептурах жировых и эмульсионных косметических средств для ухода за сухой раздраженной кожей, ее питания и смягчения.

Ключевые слова: *масло, косметика, кожа, состав.*

Исследование реологических свойств растворов желатина для производства безглютеновых макаронных изделий

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Введение. Для формирования безглютеновых макаронных изделий из кукурузной муки, важным является выбор структурообразователя, определение

способа его внесения и дозировки на основании изучения реологических свойств его растворов и влияния на качество изделий.

Материалы и методы. Исследованы реологические свойства коллоидных растворов желатина концентрацией 0,50–1,25%, приготовленных при температуре воды 20 °С и 40 °С и продолжительности набухания 40 мин. и 60 °С без набухания. Определяли вязкость этих растворов на вискозиметре Реотест-2, при температуре 20 °С. Определено влияние растворов структурообразователя на показатели качества макаронных изделий.

Результаты. При температуре набухания желатина 20 °С динамическая вязкость разрушенной структуры коллоидного раствора с увеличением его концентрации с 0,50% до 1,25% снижается от 59,10 Па·с до 21,89 Па·с, за исключением раствора с концентрацией 1,00%, для которого наблюдается аномалия вязкости, а вязкость равна 531,90 Па·с. Аналогичные исследования, проведенные при набухании при температуре воды 40 °С, показали, что все коллоидные растворы желатина при концентрации 0,50–1,25% являются псевдопластическими жидкостями ($P_{k1} = 0$), имеют значительно более низкую динамическую вязкость как разрушенной, так и неразрушенной структуры и низкую прочность структурного каркаса, чем при температуре набухания 20 °С. Для образца с концентрацией 0,75% наблюдается аномалия вязкости: при этой концентрации раствор имеет наибольшую динамическую вязкость неразрушенной структуры и динамическую вязкость разрушенной структуры, соответственно 94,56 и 1,35 Па·с, наибольшее значение $\eta_0 - \eta_m - 93,21$ Па·с и одновременно наибольшую прочность образованного структурного каркаса 425,52 Па. Макароны, изготовленные с использованием таких растворов, имеют лучшее качество. При температуре 60 °С растворы имеют низкую вязкость и прочность, то есть образуют слабые гели, которые не обеспечивают и хорошего качества макаронных изделий.

Вывод. Установлено дозирование желатина 0,75–1,0% к массе муки и параметры подготовки его к производству – набухание в течение 40 мин. при температуре 40–20 °С соответственно, которые обеспечивают самую высокую вязкость растворов желатина 94,6–531,9 Па·с и способствуют получению изделий хорошего качества.

Ключевые слова: *желатин, дозировка, вязкость, раствор, качество.*

Микробиология, биотехнология

Микробиологическое исследование диких, культивированных (*Mytilus galloprovincialis* L. 1819) и фаршированных мидий

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Введение. В работе исследуются микробиологические свойства диких и культивированных мидий, а также фаршированных мидий, которые продаются в ресторанах и уличных торговых точках в августе и сентябре в г. Синоп.

Материалы и методы. С помощью стандартных процедур были исследованы 68 образцов мидий (*Mytilus galloprovincialis* L. 1819), а также фаршированные мидии, в которых определяли общее содержание аэробных мезофильных бактерий, бактерий *Coliform*, *Escherichia coli* и *Vibrio* spp. Отбор проб для микробиологического анализа проводили в асептических условиях. Все микробиологические анализы проводились троекратно.

Результаты и обсуждение. Начальные показатели суммарного количества аэробных мезофильных бактерий, колиформных бактерий *E. coli* в диких и культивированных мидиях в августе составили, соответственно, 4,04 ln КОЭ/г и 3,55 ln КОЭ/г; 3,69 ln КОЭ/г и 3,09 ln КОЭ/г; 0,59 ln КОЭ/г и 0,39 ln КОЭ/г. Общее количество бактерий, колиформных бактерий и бактерий *Vibrio* spp. в диких мидиях было выше, чем в культивированных ($p < 0,05$). В культивированных мидиях бактерий *Vibrio* spp. обнаружено не было.

Общее количество аэробных мезофильных бактерий, колиформных бактерий и бактерий *E. coli* в фаршированных мидиях, которые продавались в уличных торговых точках в августе, превышало этот показатель для фаршированных мидий, которые продавались в ресторанах в это же время ($p < 0,05$). В сентябре бактерии *E. coli* не были обнаружены в образцах фаршированных мидий, которые продавались как в ресторанах, так и в уличных торговых точках. В августе и сентябре содержание аэробных бактерий в образцах фаршированных мидий не превышало допустимых предельных значений (6 ln КОЭ/г). Бактерии *Vibrio* spp. были обнаружены во всех образцах фаршированных мидий, кроме тех фаршированных мидий, которые продавались в уличных торговых точках в сентябре.

Фаршированные мидии были приготовлены из диких мидий и общее содержание аэробных мезофильных бактерий, колиформных бактерий и бактерий *E. coli* в них превышало содержание этих же бактерий в диких мидиях.

Выводы. Бактерии *E. coli* не были обнаружены в фаршированных мидиях, которые продавались в ресторанах в августе и сентябре, а бактерии *Vibrio* spp. обнаружены в двадцати семи из сорока восьми образцов фаршированных мидий, которые продавались в ресторанах и уличных торговых точках.

Ключевые слова: мидия, фаршированная мидия, *e.coli*, *coliform*, здравоохранение.

Оптимизация производства микробной трансглутаминазы из *Streptomyces* sp.

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Введение. С целью последующего создания модели для будущих исследований на тему использования микробной трансглутаминазы в работе были изучены методы оптимизации производства микробной трансглутаминазы (МТГ).

Материалы и методы. Влияние температуры, pH, среды и типа штамма было исследовано с целью определения максимальной активности ферментов. Для

производства МТГ были отобраны пять различных штаммов: *Streptomyces mobaraensis* (NRRL B-3729), *S. ladakanum* (NRRL ISP-5587), *S. lividans* (NRRL B-12275), *S. sioyaensis* (NRRL B-5408) и *S. platensis* (NRRL B-5486). Процесс ферментации проходил в двух ферментационных средах, глюкозно-крахмальной и соевой, которые характеризовались различными показателями рН и температуры (6,0; 7,0; 8,0 рН и 20; 30; 40 °С). Активность МТГ определялась в течение 28 дней колориметрическим методом.

Результаты и обсуждение. Бактерии *S. mobaraensis*, *S. ladakanum* и *S. lividans* продемонстрировали более высокие темпы роста, чем *S. sioyaensis* и *S. platensis*. Следовательно, для производства ферментов были выбраны бактерии *S. mobaraensis*, *S. ladakanum* и *S. lividans*. При значении рН 6,0 наивысшая активность ферментов (0,036 ед/мл) была достигнута за 14 дней для бактерий *S. Mobaraensis* в глюкозно-крахмальной среде при 30 °С, а на 14-й день ферментации активность ферментов резко снизилась для всех штаммов бактерий. При значении рН 7,0 наивысшая активность ферментов была достигнута на 28-й день для бактерий *S. mobaraensis* при температуре 30 °С. При значении рН 8,0 МТГ не удалось получить ни в одной питательной среде ни при какой температуре. Для начального значения рН 6,0 растущий уровень активности МТГ в глюкозно-крахмальной среде при различных температурах (20, 30 и 40 °С) был выше, чем в соевой среде. Бактерии *S. ladakanum* and *S. lividans* не могут вырабатывать МТГ ни при каких условиях.

Выводы. Бактерии *S. mobaraensis* показали самую высокую ферментативную активность по сравнению с другими штаммами. Глюкозно-крахмальная среда больше всего подходит для получения МТГ. Изменение значений рН и температуры влияет на активность ферментов. Наилучшими условиями для получения МТГ являются уровень рН 6,0 и температура 30 °С.

Ключевые слова: микробная трансглутаминаза, *s. Mobaraensis*, фермент, активность, ферментация.

Влияние технологических параметров ферментации сливок на формирование функциональных свойств кисломолочного масла

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Введение. Определяющими факторами производства кисломолочного масла являются процессы ферментации (подбор заквасочных культур, их соотношение и определения оптимальных технологических параметров ферментации) и физического созревания сливок.

Материалы и методы. Активность кислотообразования при ферментации сливок определяли по изменению титруемой и активной кислотности. Количество жизнеспособных клеток *Flora Danica* и *Lactobacillus acidophilus* La-5 была подсчитана путем посева при использовании среды M17 Agar CM 0785 и *Lactobacillus* MRS Agar M 641-500G (Himedia). Жирнокислотный состав образцов масла исследовали методом газожидкостной хроматографии на газовом хроматографе Hewlett Packard HP-6890.

Результаты и обсуждение. Использование в производстве кисломолочных продуктов композиций, которые наряду с определенными лактобактериями,

содержащие монокультуры пробиотических штаммов, позволяет получить незаменимый с точки зрения современной диетологии продукт питания с пробиотическими, оздоровительными и заданными специальными свойствами.

С учетом рекомендованных технологическими инструкциями температур ферментации и компромиссной температуры для микробиальных культур выбранных препаратов выбрали два температурных режима – 20 и 30 °С для ферментации сливок. Установлено, что высокий темп роста титруемой кислотности сливок зарегистрировано для образца, для ферментации которого использовали *Flora Danica* + *Lactobacillus acidophilus* La-5 и температуру 30 °С.

Как свидетельствуют результаты, образец при совместном культивировании *Flora Danica* и *Lactobacillus acidophilus* La-5 при температуре ферментации 30 °С демонстрирует лучшую динамику нарастания биомассы в течение ферментации и физического созревания сливок, поскольку концентрация жизнеспособных клеток в этом варианте была наибольшей.

По содержанию жирных кислот, которые проявляют выраженное биологическое действие, то их содержание проявлял четкую тенденцию к увеличению в образце кисломолочного масла, где применяли сочетание смешанных мезофильных культур и термофильной ацидофильной палочки и ферментацию сливок при температуре 30 °С.

Выводы. Рекомендуется использовать в технологии кисломолочного масла композицию, составленную из смешанных мезофильных культур *Flora Danica* и термофильной монокультуры *Lactobacillus acidophilus* La-5 и температуру ферментации сливок 30 °С.

Ключевые слова: ферментация, сливки, *Flora Danica*, *Lactobacillus acidophilus* La-5, масло.

Пищевая химия

Комплексы 4- и 5-нитрозамещённых гетероарильных цинамоилпроизводных с Cu (II) и определение их антикоагулянтной активности

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Введение. Описан синтез комплексов Cu (II) с 4- и 5-нитрозамещёнными гетероарильными цинамоилпроизводных и исследована их антикоагулянтная активность.

Материалы и методы. Все использованные химические реактивы были приобретены по каталогам компаний Merck и Sigma-Aldrich. Температуры плавления веществ определены на цифровом устройстве SMP 10. Данные элементного анализа получены на анализаторе Carlo Erba 1106. Чистота веществ проверена методом тонкослойной хроматографии с использованием 0,2 мм пластинок Kieselgel 60 F254 (Merck, Германия), элюент: смесь CH₂Cl₂ : CH₃COCH₃ = 1 : 1 (объемные соотношения). Спектры ИК-спектроскопии записаны на спектрометре Perkin-Elmer FTIR-1600 (образцы в виде таблеток с KBr).

Результаты и обсуждение. Лиганды, необходимые для образования соответствующих комплексов, были получены с 4- и 5-нитрозамещённых 2-ацил-1,3-индандионов двумя способами: методом Mosher и Meier, Rotberg и Oshkaya, а также авторским методом, новизной которого является использование пирролидина в качестве катализатора. Для получения комплексных соединений к растворам конечных лигандов в диоксане добавляли метанольный раствор $\text{Cu}(\text{CH}^3\text{COO})_2 \cdot \text{H}_2\text{O}$. Образующиеся комплексные соединения кристаллизовались после охлаждения смеси. Для новополученных соединений определены физико-химические параметры, а также проведены спектральные исследования. На основании экспериментальных данных предложен состав комплексов $[\text{M}(\text{L})_2]$ и сделан вывод, что наиболее вероятно структура комплексов $\text{Cu}(\text{II})$ предполагает наличие двух депротонированных ОН групп 4- и 5-нитроцинамоилпроизводных. Спектроскопические исследования аморфных образцов показали, что комплексы $\text{Cu}(\text{II})$ характеризуются четырехгранной геометрической структурой. Антикоагулянтная активность синтезированных комплексов охарактеризована их протромбиновым временем и сравнима со временем выходных 4- и 5-нитрозамещённых производных. Показано, что 5-нитропроизводные имеют высшую антикоагулянтную активность, чем соответствующие 4-нитропроизводные.

Вывод. Получены новые комплексы $\text{Cu}(\text{II})$ с 4- и 5-нитрозамещёнными гетероарильными цинамоилпроизводными и определена их антикоагулянтная активность. Комплексы могут быть использованы в будущих тестированиях пищевых продуктов растительного и животного происхождения.

Ключевые слова: 1,3-индандионы, $\text{Cu}(\text{II})$ -комплексы, IR, PT, INR.

Процессы и оборудование пищевых производств

Расчет нестационарных диффузных массовых потоков сахарозы для ячеек межкристалльных растворов сахарозы системы «большой кристалл сахара–раствор сахарозы большего кристалла–меньший кристалл сахара–раствор сахарозы меньшего кристалла–уфеле» в зависимости от времени уваривания сахарного уфеля

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Введение. В данной работе реализован один из следующих этапов создания математической модели процесса кристаллизации сахарозы.

Материалы и методы. Для получения нестационарных диффузионных массовых потоков сахарозы для ячеек межкристалльных растворов сахарозы решено одновременно систему из 7 нестационарных задач теплопроводности по каждой отдельной области с постоянными и с переменными теплофизическими коэффициентами, а также три отдельных нестационарных задачи диффузионного массообмена для четырех областей межкристалльных растворов сахарозы с постоянными и с переменными коэффициентами диффузионного массообмена с применением численных методов (метод контрольного объема).

Результаты и обсуждение. Для десяти случаев относительного времени уваривания сахарного уфеля $\tau/\tau_{\text{ц}}$ ($\tau/\tau_{\text{ц}} = 0,15; 0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8; 0,9; 1,0$) на основании решения одновременного решения четырех систем нестационарных дифференциальных уравнений в частных производных параболического типа (первая

система — для нестационарной задачи теплопроводности; и три системы — для нестационарных задач диффузионного массообмена) найдено распределение нестационарных диффузионных массовых потоков сахарозы в каждой области межкристалльного раствора сахарозы всей рассматриваемой системы ячеек. Впервые на основании проведенных расчетов установлено, что процесс перетекания растворенной сахарозы из ячейки межкристалльного раствора одного кристалла в ячейку межкристалльного раствора сахарозы другого кристалла действительно происходит и в каком направлении. Также впервые получено количественную величину диффузионного массового потока сахарозы между областями, представляющие ячейки межкристалльного растворов различных кристаллов сахара. При относительном времени уваривания сахарного утфеля $\tau/\tau_{ц} = 0,15$ происходит перенос вещества (сахарозы) из области 4 левой ячейки между кристалльного раствора кристалла 2 в область 3 правой ячейки межкристалльного раствора кристалла 1. Примерно в при $\tau_{к}=2$ с достигается их минимум. Начиная с момента времени $\tau_{к}=2,58$ с для варианта расчета с постоянными теплофизическими коэффициентами ситуация меняется на противоположную, то есть перенос сахарозы происходит уже из области 3 в область 4. При всех же переменных теплофизических характеристиках процесс переноса сахарозы за все время пребывания системы ячеек на нагревательной трубке все еще происходит из области 4 в область 3, а при выходе системы ячеек с нагревательной трубки стремится к нулю, то есть, практически отсутствует. Итак, в этом случае получили четко выраженный минимум диффузионного массового потока. При относительном времени уваривания сахарного утфеля $\tau/\tau_{ц}=1,0$ получили четко выраженный минимум и максимум как для постоянных, так и для всех переменных теплофизических характеристик.

Вывод. Для каждой области, которая представляет собой межкристалльный раствор сахарозы получено величину нестационарного диффузионного массового потока сахарозы в зависимости от времени контакта системы ячеек с нагревательной трубкой. Впервые установлено величину и направление диффузионного массового потока между двумя областями межкристалльного растворов сахарозы первого и второго кристаллов сахара.

Ключевые слова: сахароза, диффузия, раствор, кристалл, утфель.

Интенсификация процессов массопереноса в газо-жидких средах дискретным – импульсным методом ввода энергии

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Введение. Целью данного исследования была интенсификация аэрации культуральных сред в ферментёре методом дискретно – импульсного ввода энергии, который реализуется в роторно – пульсационном аппарате.

Материалы и методы. Исследован процесс аэрации культуральных сред в технологии получения дрожжей *Saccharomyces cerevisiae* методом дискретно – импульсного ввода энергии. Скорость массопереноса кислорода определялась по количеству биомассы дрожжей, выращенных за период культивирования.

Результаты и обсуждение. В ходе экспериментов по культивированию дрожжей на мелассных растворах была определена зависимость скорости массопереноса кислорода от угловой скорости вращения роторного узла в культуральных средах с

содержанием сухих веществ 3 – 10%. С уменьшением содержания сухих веществ от 10 до 5% при обработке с угловой скоростью 48 об / с, скорость массопереноса увеличивается в 1,9 раза. С увеличением частоты пульсаций от 2 до 3,85 кГц, массопереноса возрастает от 4 до 6,3 г / л·ч при содержании сухих веществ – 3% и от 2,2 до 4 г / л·ч при содержании сухих веществ – 10%. Дальнейшее повышение частоты пульсаций приводит к инактивации части дрожжевых клеток. Установлено также, что оптимальное значение скорости сдвига потока составляет $90 - 100 \cdot 10^3 \text{ с}^{-1}$.

Выводы. Результаты этого исследования свидетельствуют о том, что применение метода ДИВЭ в абсорбционных технологиях позволяет значительно интенсифицировать массообменные процессы.

Ключевые слова: *массоперенос, абсорбция, микроорганизм, интенсификация.*

Повышение эффективности теплопередачи в трубчатом теплообменнике с гофрированными трубами

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Введение. Действенным методом интенсификации теплопередачи в трубчатых теплообменниках является применение гофрированных труб, но в настоящее время нет универсальной методики расчета и проектирования таких теплообменников.

Материалы и методы. Изучены теплопередающие характеристики гибких гофрированных труб из нержавеющей стали с различным профилем гофр. Испытательный стенд представляет собой теплообменник типа "труба в трубе" с гладкой наружной и профилированной внутренней трубой, оборудованный датчиками для измерения температурных и гидравлических параметров потока.

Результаты и обсуждение. Исследование теплопереноса и гидродинамики в теплообменнике «труба в трубе» с гофрированных внутренней трубой показало, что в диапазоне чисел Рейнольдса от 4000 до 40000 достигается значительная интенсификация теплообмена по сравнению с традиционным гладкотрубным теплообменником. Увеличение коэффициента теплопередачи составило от 2,0 до 2,6 раз при росте гидравлического сопротивления в 1,9..2,0 раза. Установлено, что при одинаковых условиях потока трубы с малой высотой гофр и большим шагом гофрирования (соотношение высота/шаг – 1,9/4,0 мм) имеют на 15–20% большую конвективную составляющую коэффициента теплопередачи по сравнению с трубами с высокими гофрами и мелким шагом гофрирования (соотношение высота/шаг – 2,4/3,2 мм).

Для оценки влияния геометрии труб на интенсивность процесса теплообмена была разработана двумерная осесимметричная компьютерная модель единичного элемента теплообменного аппарата. Численное моделирование гидродинамики и теплообмена в канале единичного элемента показало, что математические расчеты достаточно близки к экспериментальным исследованиям.

С использованием данных критериальных зависимостей рассчитан и спроектирован теплообменник мощностью 350 кВт для системы отопления административного корпуса, опытная эксплуатация которого подтвердила эффективность предложенных технических решений.

Выводы. Использование гофрированных труб позволило увеличить коэффициенты теплопередачи. Полученные критериальные зависимости позволяют рассчитать и оптимизировать процесс теплопередачи в трубчатом теплообменнике с гибкими гофрированными трубами.

Ключевые слова: теплообменник, гофротруба, турбулизатор, теплопередача.

Экономика и управление

Методические основы рейтинговой оценки инвестиционной привлекательности сельских регионов Украины

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Введение. Статья посвящена исследованию методических и практических основ рейтинговой оценки инвестиционной привлекательности сельских регионов.

Материалы и методы. Исследуется инвестиционная привлекательность сельских регионов на примере областей Украины. Для оценки инвестиционной привлекательности применен синергетический метод, метод сравнения и интегральной оценки. Использован рейтинговый подход, который является наиболее распространенным в практике оценки инвестиционной привлекательности с использованием системы показателей-индикаторов.

Результаты и обсуждения. Предложенная методика оценки инвестиционно-инновационной привлекательности областей, основанная на использовании методов стандартизации и нормирования, а также на применении метода сравнения. Приведенная трехуровневая система показателей-индикаторов оценки инвестиционной привлекательности сельских регионов, в первый уровень которой включены показатели составляющих инвестиционного потенциала, во второй уровень – показатели инвестиционного риска и в третий уровень – обобщенные показатели инвестиционной привлекательности регионов.

На основе результатов рейтинга осуществлено распределение сельских регионов по типам инвестиционного климата. Сельские регионы представлены практически во всех рейтинговых группах примерно в равной пропорции по отношению к численности групп и, таким образом, представляют по показателям инвестиционного климата все регионы Украины.

Установлено, что для сельских регионов инвестиционная привлекательность в большинстве зависит от уровня социально-экономического развития. К типу инвестиционного климата 2В (средний потенциал, умеренный риск) относятся 6 сельских регионов, к типу 3В1 (пониженный потенциал, умеренный риск) – 4 сельских регионов, типам 3В2 (незначительный потенциал, умеренный риск), 2С (средний потенциал, высокий риск), 3С1 (пониженный потенциал, высокий риск) и 3С2 (незначительный потенциал, высокий риск) относятся по три сельских регионов. Регионы с минимальным риском (типа 1А, 2А, 3А, 1В) по расчетам отсутствуют.

Выводы. На основе анализа инвестиционного климата приведено распределение сельских регионов по инвестиционным типами. На основе результатов рейтинга построена типология сельских регионов.

Ключевые слова: инвестиция, привлекательность, развитие, регион, рейтинг.

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Requirements for article:

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Size of the article – 8–15 pages in Microsoft Word 2003 and earlier versions with filename extension *.doc (!)

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5. Key words.

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Мови статей – англійська, українська, російська
Рекомендований обсяг статті – **8–15 сторінок** формату А4.
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1. Посилання на статтю:

Автори А.А. (рік видання), Назва статті, Назва журналу (курсивом), Том (номер), сторінки.

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Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies*, 2(2), pp. 104–108.

2. Посилання на книгу:

Автори (рік), Назва книги (курсивом), Видавництво, Місто.

Ініціали пишуться після прізвища.

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Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

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1. (2013), *Svitovi naukovometrychni bazy*, available at:

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2. Cheung T. (2011), *World's 50 most delicious drinks [Text]*, available at:

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