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# Spectroscopic analysis of honey

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## Abstract

**Introduction**. The main objective of this investigation is to study the relationships of chemical composition, physical properties, botanical and geographical origin, and age of honey with its spectral parameters which can be used as taxonomic indices or indicators of honey state, quality and possible adulteration.

**Materials and methods.** Two non-destructive spectroscopic methods of honey authentication such as Near-Infrared Spectroscopy and Fluorescence Spectroscopy were used.

**Results and discussion**. The intensity of absorption spectra can be used as a criterion of geographical origin or age of honey.

The intensity of emission fluorescence spectra of honey depends on the geographical origin, its age, and on the type of honey.

The effect of temperature on the fluorescence intensity of honey demonstrated that the increasing of temperature provoked the decreasing of the fluorescence intensity. It was established the correlation between fluorescence properties of honey and the presence of water in it.

The reflectance spectra of honey samples in NIR part of the spectrum are characterized by a number of reflectance bands near 1,779 nm, 1,933 nm, and 2,290 nm; the relative intensity of the spectral bands depends on the type and age of the sample. It is very informative to use spectral parameters of honey in NIR part of spectrum for the non-destructive detection of honey adulteration.

**Conclusions**. Methods of near-infrared and fluorescence spectroscopy can be explored in honey-breeding. Both spectroscopic techniques can be used for non-destructive, fast and precise diagnostics of honey. The chemical composition, physical properties, botanical and geographical origin, and age of honey are closely related to spectral parameters of honey which can be used as taxonomic indices or indicators of honey state, quality, and aduteration.

## Introduction

### Definition of Honey

Honey, according to the accepted definition [Codex..., 1969], is the natural sweet substance produced by honey bees from the nectar of plants (*Blossom Honey* or *Nectar Honey*), or from secretions of living parts of plants, or excretions of plant sucking insects on the living parts of plants (*Honeydew Honey*), which the bees collect, transform by combining with specific secretions of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. Physical properties of honey are related to its state, age, presence of water and level of crystallization. All these factors affect the quality of honey.

### Composition of Honey

Honey is a product with complex chemical composition: it contains plant pigments (carotenes, xanthophylls, chlorophyll), mineral substances, sugars, and various impurities.

The main components of honey are sugars, which are presented by fructose (37.20%), glucose (31.28%), sucrose (1.31%), maltose (7.31%), etc. [Je'Anne, 1991]. Blossom honey differs from honey dew by the values of simple sugars, disaccharides, higher sugars, acids, mineral salts and nitrogen content. The concentration of sugars can be used as a criterion of honey adulteration, which is provoked with the artificial addition of syrup, sucrose that is hydrolyzed with acids, starch or beetroot treacle in honey [Chudakov, 1967]. That is why precise quality evaluation of honey has long been the goal of many investigators and specialists who are related to honey-breeding [Vorwohl, 1984, 1990; Gonnet, 1986; Vakhonina et al., 1987; Dustmann, 1993; Mautz, 1993; Campos, 1994]. The composition of honey also reflects the contaminants which are present in the area of bee activity.

### Honey Quality

High-quality honey can be distinguished by the aroma, taste and texture. Besides, good quality honey can not contain more than 18% water. High water level can cause the process of fermentation of honey and loss of its quality.

The quality of honey has been recognized by the European Directive (Council Directive, 1974) and the Food Standards Code (Codex Alimentarius standard, 1993). International Honey Commission, which was established in 1996, has revised the contents of these documents in recent years [Codex ..., 1993; Proposal ..., 1996; Bogdanov et al. 1999; Council Directive, 2001; Revised Codex ..., 2001] proposing specific quality parameters of honey and methods of its evaluation. The standard drafts include standards and methods for the determination of such quality factors as moisture, ash, acidity, HMF, apparent reducing sugars, apparent sucrose, diastase activity and water-insoluble matter. International honey standards for fructose/glucose content, the sucrose content and electrical conductivity were proposed. In addition, other quality factors, such as invertase activity, proline and specific rotation has been discussed.

The term non-destructive quality evaluation of honey means that the analysis of the honey sample and collection of its essential characteristics are carried out in such a way that physical and chemical properties of the sample are not changed.

### Spectroscopic Analysis of Honey

Traditional methods of honey analysis are time-consuming and costly. That’s why, there is a need for a new analytical technique that will enable non-destructive, fast and reproducible authentication of the botanical and geographic origins at low cost.

Development of non-destructive methods, which are based on the analysis of the sample without any alterations of product attributes, present the problem of great practical significance. Spectroscopic methods occupy an important place among the comprehensive tests [Posudin, 2005]. These methods include the measurement of difference between input and output light signals during interaction of light with the sample (absorption, transmission, reflection, scattering, re-emission) and analysis of the dependence of this difference on the wavelength. Besides, spectroscopic methods are rather fast and precise. Effects of honey type, age, temperature, water content and degree of sugar adulteration on the spectral properties of honey can also be studied.

Certain spectroscopic methods that have been applied to honey control are mentioned in the literature: spectrophotometry [Yao and Chen, 1985; Yao and Fan, 1985; Salinas et al., 1994,*a,b*], optical activity measurement [Juan et al., 1992], atomic spectroscopy [Petrovic et al., 1994; Salinas et al., 1994], nuclear magnetic resonance [Ohmenhaeuser et al., 2013]. However, the fact is that honey presents a non-transparent and opaque substance; the application of the abovementioned methods requires either the dilution, or special preparation of the samples. Method of NMR spectroscopy makes it possible to detect very fine structural components, but this technique is very expensive, time consuming, spectra take long time to interpret.

Near-infrared (NIR) spectroscopy is a spectroscopic method that uses the near-infrared (700 nm–2500 nm) region of the electromagnetic spectrum.

It was shown that methods of near infrared spectroscopy (NIR) and mid infrared spectroscopy (MIR) can be applied for honey detection, particularly for quality control analysis, determination of botanical and geographical origin and detection of adulteration of honey. The principle, technology path, accuracy, influence factors, and the development trend are discussed [Tu et al., 2010].

Method of Fourier transform infrared spectroscopy (FT-IR) was used for the determination of water, glucose, fructose, sucrose, melezitose and monosaccharide content in honey as well as fructose/glucose ratio, glucose/water ratio, electrical conductivity, pH-value and free acidity [Ruoff et al., 2006]. The results demonstrate that mid-infrared spectrometry is a valuable, rapid and non-destructive tool for the quantitative analysis of honey. More than 1600 samples of honey were analysed using FT-IR and reference methods to develop a partial least-square regression based calibration model for the major components of honey (sugars, proline, free acids, invertase, moisture, hydroxymethylfurfural, pH and electrical conductivity) [Lichtenberg-Kraag et al., 2002]. Fourier-transform infrared spectrometer was used to determine botanical origin of 144 samples of nine different unifloral honey types from different Croatian regions [Svečnjak et al., 2011].

The results of this study showed that FT-IR spectrometry provides reliable results, but also represents rapid, simple and cheap analytical tool in comparison to commonly used standard analytical methods.

Fourier transform infrared attenuated total reflectance (FT-IR ATR) spectroscopy was applied to 14 different samples of northeast Brazilian honey. The results showed that mid-infrared spectrometry can be used as a screening method for the routine analysis of Brazilian honey, with the advantages of being rapid, non-destructive, and accurate [Almeida-Muradian et al., 2014].

Honey is a classic object of adulteration through the addition to natural honey such substances as sucrose, sugar, glucose, partial invert cane and corn syrups, and beet sugar, dextrin, starch, unripe honey, molasses, honeydew, and artificial sweeteners. Some samples of honey can be contaminated with heavy metals, pesticides, and antibiotics. Various

spectroscopic methods of honey adulteration detection are used such as headspace-mass spectrometry, gas chromatography, combination of gas chromatography and mass spectrometry, terahertz time-domain spectroscopy, NIR spectroscopy, which can be used successfully to identify authentic honey from adulterated one [Posudin et al., 2015].

Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample.

It was shown that unifloral honeys with very characteristic fluorescence spectra, such as chestnut honey, can be easily recognised using only one of the single spectra recorded. Honey types having less characteristic spectra, such as alpine polyfloral or lowland polyfloral honeys, need a combination of several spectra for a reliable authentication [Ruoff et al., 2005].

Front-face fluorescence spectroscopy, directly applied on honey samples, was used for the authentication of 11 unifloral and polyfloral honey types (n = 371 samples) previously classified using traditional methods such as chemical, pollen, and sensory analysis. This study indicates that front-face fluorescence spectroscopy is a promising technique for the authentication of the botanical origin of honey and may also be useful for the determination of the geographical origin within the same unifloral honey type [Ruoff et al., 2006]. This technique combined with chemometrics was used to classify honey samples according to their botanical origin. Synchronous fluorescence spectra of three monofloral (linden, sunflower, and acacia), polyfloral (meadow mix), and fake (fake acacia and linden) honey types (109 samples) were studied. The results demonstrated that this method is a valuable and promising technique for honey authentication [Lenhardt et al., 2014].

Fluorescence spectroscopy coupled with parallel factor analysis (PARAFAC) and Partial least squares Discriminant Analysis (PLS DA) were used for characterization and classification of honey [Lenhardt et al., 2015].

Method of fluorescence spectroscopy showed that while the major contributor to the fluorescence of cane sugar syrup is the reduced form of nicotinamide adenine dinucleotide, the fluorescence of honey is dominated by flavins. The difference in the synchronous fluorescence spectra of honey and cane sugar syrup could be used to monitor adulteration of honey by cane sugar syrup [Ghosh et al., 2005].

Fluorescence spectroscopy was used for quality control of honey, particularly to describe the types of honey and to distinguish the honey samples with added artificial sweeteners from natural ones [Nikolova et al., 2014].

**The main objective of this investigation** is to evaluate two non-destructive spectroscopic methods of honey authentication: Near-Infrared Spectroscopy and Fluorescence Spectroscopy.

## Materials and methods

The samples of honeydew, which were used in these experiments, were taken from the collection of Bavarian Institute of Bee-Breeding (Erlangen, Germany)[1], and the samples of honey – from different regions of Ukraine: Kyiv, Dnepropetrovsk, and Crimea. Samples with different percentage of sucrose were prepared in National University of Life and

[1] The samples of honeydew were presented by the courtesy of Dr. D. Mautz.

Environmental Sciences of Ukraine, Kyiv, for obtaining useful information about adulteration of honey. Descriptions of all the samples are given in Table 1. The measurements of spectroscopic properties of Bavarian "Tanne" samples were performed in 1993, and of the Ukrainian honey samples in 1994.

**Table 1**

**Samples of honey which were used in the experiments**

| No | Type of Honeydew | Geographic Origin | Year of Collection |
|---|---|---|---|
| 1 | Abies alba, Schwarzwald (“Tanne") | Germany, Bavaria | 1989 |
| 2 | " | “ | 1992 |
| 3 | Abies alba, Bayerischerwald ("Tanne") | “ | 1990 |
| | **Type of Honey** | **Geographical Origin** | **Year of Collection** |
| 4 | Monofloral, Acacia | Ukraine, Crimea | 1993 |
| 5 | Monofloral, Sunflower | “ | “ |
| 6 | Polyfloral, Esparcet-Rape-Acacia | “ | “ |
| 7 | Polyfloral, Sage-Lavender | “ | “ |
| 8 | Polyfloral, Sonchus-Buckwheat | “ | “ |
| | Sunflower | “ | “ |
| 9 | Acacia | Ukraine, | |
| | " | Verkhniodniprovsk | 1993 |
| 10 | " | Ukraine, Pavlivka | “ |
| 11 | " | Ukraine, Motronivka | “ |
| 12 | " | Ukraine, Vodiane | “ |
| 13 | " | Ukraine, Didove | “ |
| 14 | " | Ukraine, Andriivka | “ |
| 15 | " | Ukraine, Malyi Bukryn | “ |
| 16 | " | Ukraine, Kyiv | “ |
| 17 | Lime-Tree | “ | 1996 |
| 18 | Multiherbal Collection | “ | “ |

**Instrumentation**

The spectra of absorption and reflection of honey in the ultraviolet and visible part of the spectrum were measured with spectroscopic complex KSVU-23 ("LOMO", Russia), which was equipped with a double monochromator, diffraction gratings, and computer.

The investigation of fluorescence spectra of honey was performed with the spectrofluorometer SDL-2 ("LOMO", Russia) in the regime of photon counting. The spectral range during these measurements was 200–700 nm; the errors of measurement were 2 nm for intensity of the bands and 5 nm for half-width of the bands.

The reflectance spectra in the near-infrared (NIR) part of spectrum (1,620–2,320 nm) were measured with analyzer Model 4250 ("Pacific Scientific", USA). This analyzer has three ranges: 1,620–1,800 nm, 1,890–2,115 nm, and 2,050–2,320 nm. Reproducibility of the results was better than 0.015 nm. The NIR spectrum was estimated as the dependence of optical density $D = \log(1/R)$ on the wavelength $\lambda$ (where $R$ is reflection coefficient). All measurements were performed at room temperature.

## Result and discussion

### Results of Spectroscopic Analysis of Honey

The absorption spectra of honeydew ("Tanne") in ultraviolet and visible parts of the spectrum are presented in Figure 1. A certain shoulder near 250–275 nm is standing out against the background which is decreasing monotonously from 200 to 700 nm. The presence of the shoulder in absorption (reflectance) spectra of honey testifies the participation of several honey components in formation of absorption (reflectance) spectra in the ultraviolet and visible parts of the spectrum. The intensity of absorption can be used as a criterion of geographic origin or age of honey.

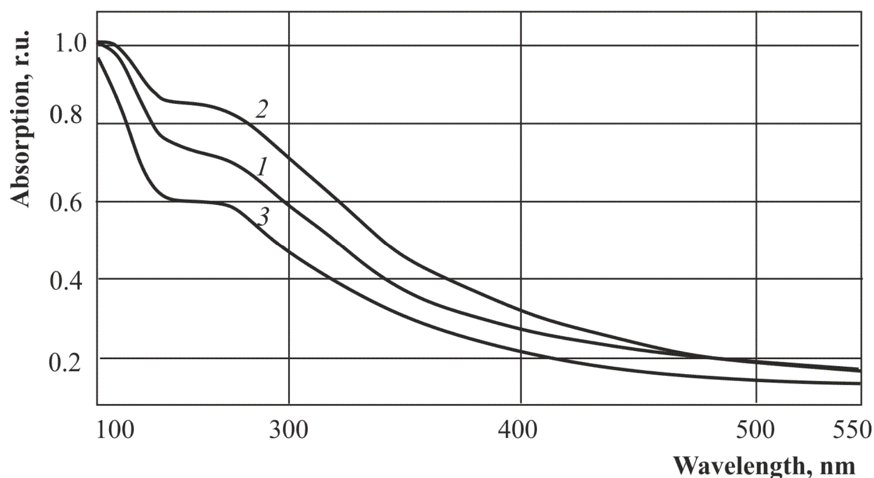


**Figure 1. Absorption spectra of honey in ultraviolet and visible parts of spectrum:**
1 – Schwarzwald "Tanne", 1989; 2 – Schwarzwald "Tanne", 1992;
3 – Bayerischer "Tanne", 1990
[Posudin et al., 1995]

The excitation fluorescence spectra of honeydew were investigated for different wavelengths of fluorescence emission (440 nm, 560 nm, and 575 nm).

The emission fluorescence spectra of honeydew are characterized by a broad band (about 100–150 nm); the spectral position of its maximum depends on the wavelength of excitation. These maxima are located near 420 nm ($\lambda_{exc}$ = 350 nm), 480 nm ($\lambda_{exc}$ = 400 nm), and 510 nm ($\lambda_{exc}$ = 450 nm). The maximal intensity of emission takes place during excitation in the ultraviolet part of the spectrum. The fluorescence intensity of honeydew depends on the geographical origin and its age. Typical fluorescence emission spectra of honeydew are presented in Figure 2.

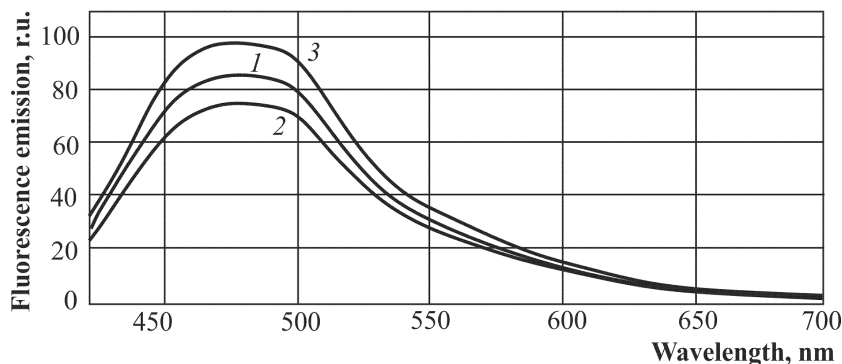


**Figure 2. Fluorescence emission spectra of honey (samples N1-N3).**
Wavelength of excitation 400 nm
[Posudin et al, 1995]

The fluorescence intensity of honey also depends on the type of honey (Table 2); the samples of the same type of honey (Acacia), which were collected from different parts of one region and of one age, demonstrated quasi equal intensity of fluorescence, but this intensity depends on the age of honey (Table 3).

**Table 2**

**Dependence of fluorescence intensity of honey on the geographic origin**

| **Number of sample** (From Table 1) | **Type of honey** | **Fluorescence Intensity** |
|---|---|---|
| 4 | Monofloral, Acacia | 0.29 ± 0.064 |
| 5 | Monofloral, Sunflower | 0.48 + 0.088 |
| 6 | Polyfloral, Esparcet-Rape-Acacia | 0.38 ± 0.031 |
| 7 | Polyfloral, Sage-Lavender | 0.69 ± 0.096 |
| 8 | Polyfloral, Sonchus-Buckwheat-Sunflower | 0.43 ± 0.045 |

**Table 3**

**Dependence of fluorescence intensity on the geographic origin and year of collection of honey**

| **Number of Sample (From Table 1)** | **Geographical Origin** | **Year** | **Year** |
|---|---|---|---|
| | | **1993** | **1994** |
| 9 | Verkhniodniprovsk | 0.30 ± 0.08 | 0.53 ± 0.07 |
| 10 | Pavlivka | 0.21 ± 0.05 | 0.68 ± 0.07 |
| 12 | Vodiane | 0.31 ± 0.09 | 0.82 ± 0.16 |
| 13 | Didove | 0.34 + 0.10 | 0.44 ± 0.03 |
| 14 | Andriivka | 0.28 + 0.09 | 0.50 ± 0.02 |
| 15 | Malyi Bukryn | 1.28 + 0.06 | 2.05 ± 0.09 |

The dependence of intensity, half-width, and spectral position of fluorescence spectra on the wavelength of excitation and emission means the participation of several fluorophores in formation of fluorescence spectra of honey.

The effect of temperature on the fluorescence intensity of honey is demonstrated in Figure 3.

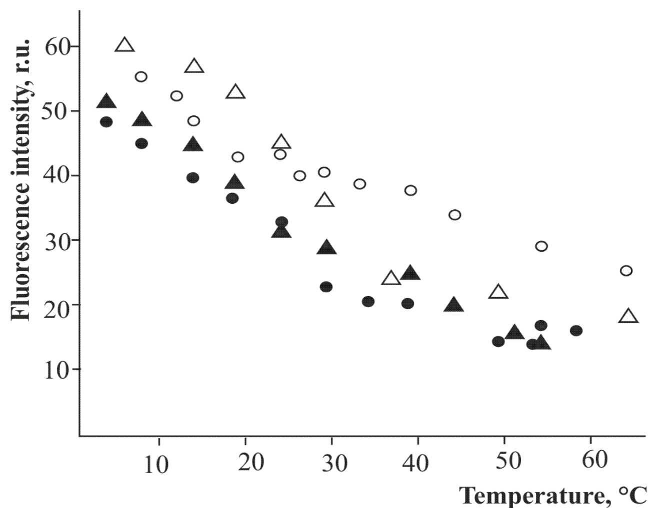


**Figure 3. Dependence of fluorescence intensity of honey on the temperature.**
– o – – monofloral, acacia, sample N4;
-▲- – monofloral, sunflower, sample N5;
-Δ- – polyfloral, sage-lavender, sample N7;
-●- – polyfloral, sonchus-buckwheat-sunflower, sample N8
[Posudin et al., 1995]

It is shown that the increasing of temperature provokes the decreasing of the fluorescence intensity.

The correlation between fluorescence properties of honey and the presence of water in it was also investigated. The quantity $W$ of water was estimated with the refractometric method according to the following relation [Aganin, 1989]:

$$W = 400[1.538 - n(20\ °C)] \quad (1)$$

where $n$ is the coefficient of refraction.

The results of these measurements are presented in Table 4.

The coefficients of correlation were calculated for polyfloral honey ($r$ = –0.96) and monofloral honey ($r$ = –0.87). This strong negative correlation between the presence of water in honey and fluorescence intensity can be used for quality evaluation of honey.
The reflectance spectra of honey samples in NIR part of the spectrum are characterized by a number of reflectance bands near 1,779 nm, 1,933 nm and 2,290 nm; the relative intensity of the spectral bands depends on the type and age of the sample (Figure 4). The samples taken from different parts of the same geographic region produced the same shape of the reflectance spectra, which were distinguished by the intensity of spectral bands only (Figs.5 and 6). However, the samples that were taken from different geographic zones demonstrated different shapes (Figs. 4 and 5).

Table 4

**Results of estimation of quantity *W* of water by fluorescence and refractometric methods**

| Index of Refraction (n) | Quantity of Water (W) | Fluorescence Intensity (I) | Index of Refraction (n) | Quantity of Water (W) | Fluorescence Intensity (I) |
|---|---|---|---|---|---|
| | Sage-Lavender, Sample N7 | | | Acacia, Sample N4 | |
| 1.4961 | 16.76 | 2.98 | 1.4976 | 16.16 | 1.45 |
| 1.4835 | 21.80 | 2.22 | 1.4785 | 23.80 | 1.28 |
| 1.4711 | 26.80 | 1.52 | 1.4651 | 29.20 | 0.96 |
| 1.4651 | 29.20 | 1.34 | 1.4565 | 32.60 | 0.88 |
| 1.4420 | 37.50 | 0.92 | 1.4282 | 44.00 | 0.70 |
| 1.4222 | 46.40 | 0.78 | 1.4082 | 51.90 | 0.64 |
| 1.4061 | 52.80 | 0.74 | 1.3871 | 60.40 | 0.62 |
| 1.3849 | 61.20 | 0.66 | 1.3600 | 71.20 | 0.50 |
| 1.3650 | 68.20 | 0 56 | 1.3500 | 75.20 | 0.32 |
| 1.3489 | 75.60 | 0.42 | 1.3380 | 80.00 | 0.24 |
| 1.3380 | 80.00 | 0.34 | - | - | - |

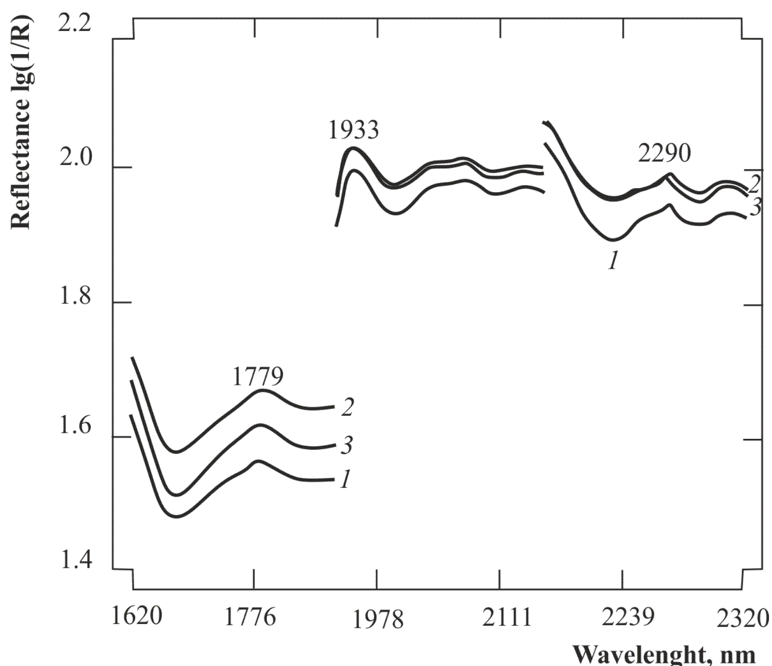


**Figure 4. Reflectance spectra of honey in near-infrared part of spectrum (samples N 1–3)**
[Posudin et al., 1995]

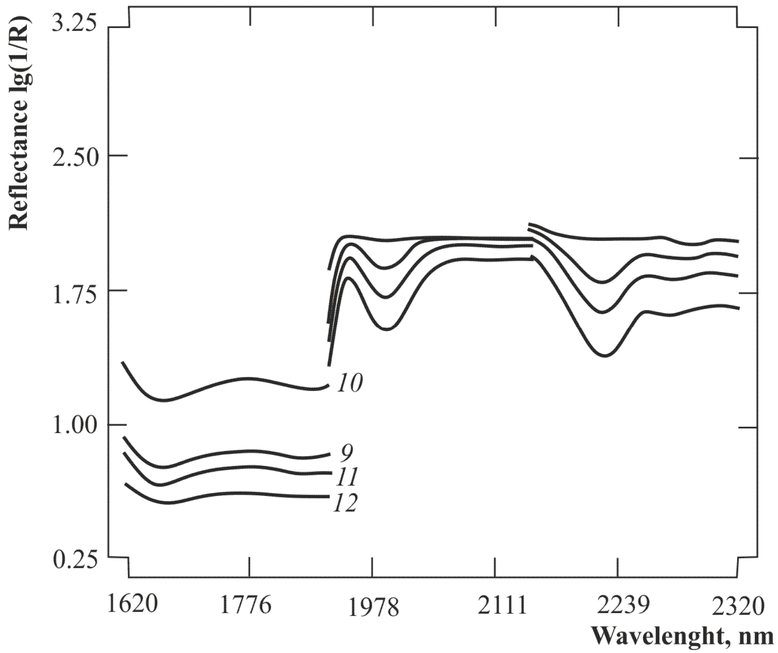


**Figure 5. Reflectance spectra of honey in near-infrared part of spectrum (samples N 9–12)**
[Posudin et al., 1995]

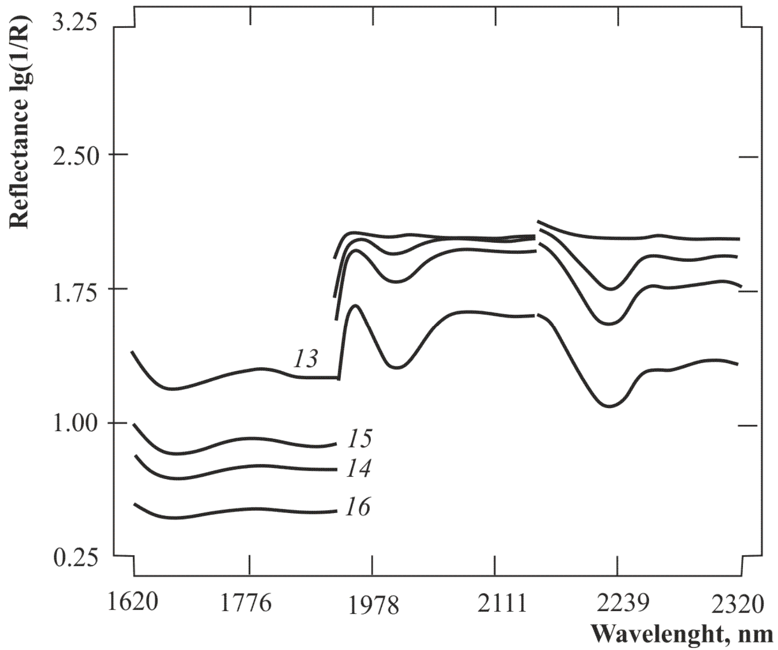


**Figure 6. Reflectance spectra of honey in near-infrared part of spectrum (samples N 13–16)**
[Posudin et al., 1995]

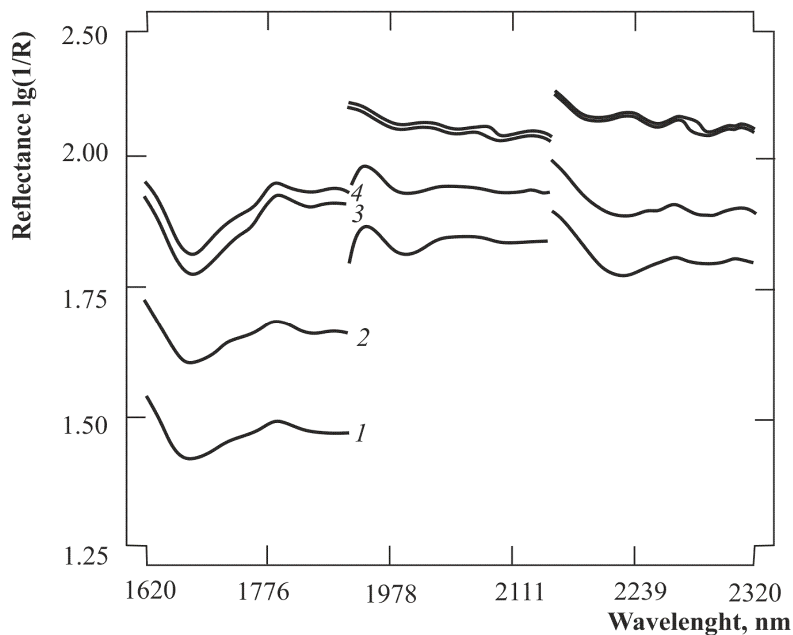


**Figure 7. Dependence of reflectance spectra of lime-tree honey (sample N 17) in near-infrared part of spectrum on the concentration of sucrose which was artificially added to honey**:
1 – natural honey: 2 – 20% sucrose; 3 – 40% sucrose; 4 – 60% sucrose [Posudin et al., 1995]

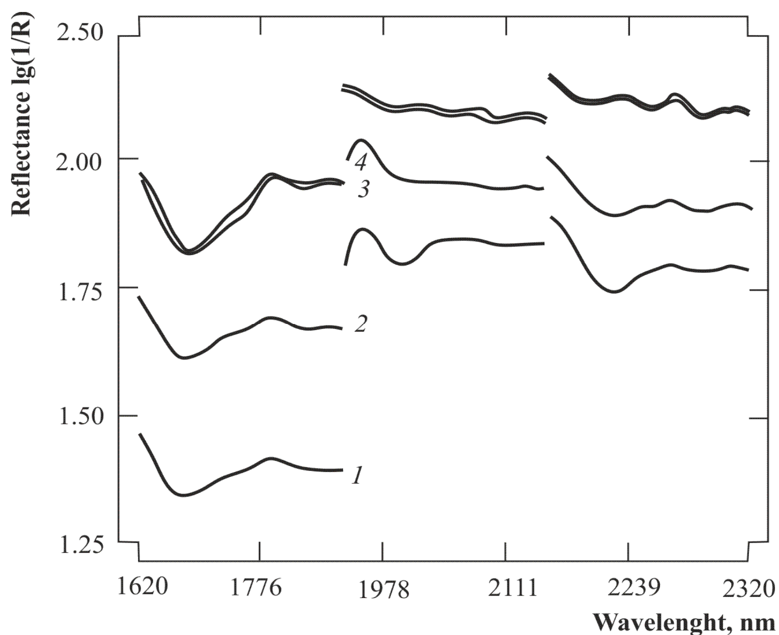


**Figure 8. Dependence of reflectance spectra of multiherbal honey (sample N 18) in near-infrared part of spectrum on the concentration of sucrose which was artificially added to honey**:
1 – natural honey: 2 – 20% sucrose; 3 – 40% sucrose; 4 – 60% sucrose [Posudin et al., 1995]

It is very informative to use spectral parameters of honey for the non-destructive detection of honey adulteration. Figures 7 and 8 demonstrate the evolution of the reflectance spectra for the samples with different concentration of sucrose, which was artificially added to honey from lime-tree and multiherbal collection respectively. The curves "reflectance versus sucrose concentration" which are presented in Figure 9, can be used for quantitative estimation of honey adulteration. The accuracy limits in these experiments did not exceed 5%.

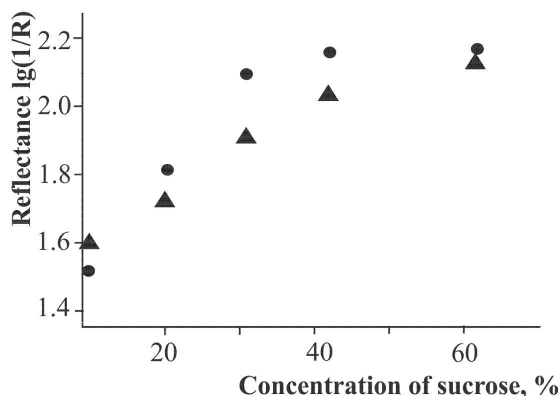


**Figure 9. Dependence of reflectance of honey on the concentration of sucrose**.
Wavelength is 1,779 nm.
-▲- – lime-tree honey;
–•– – multiherbal honey
[ Posudin et al., 1995]

Thus, honey is characterized by certain spectral properties in ultraviolet, visible, and near-infrared parts of the spectrum, which are related to its ability to absorb, transmit, reflect and re-emit optical radiation. The chemical composition and physical properties of honey are closely related to its spectral parameters, which can be used as taxonomic indices or indicators of honey state and quality.

## Conclusion

Methods of near-infrared and fluorescence spectroscopy can be explored in honey-breeding. Both spectroscopic techniques can be used in principle for non-destructive, fast and precise diagnostics of honey. The chemical composition, physical properties, botanical and geographic origin, and age of honey are closely related to spectral parameters of honey which can be used as taxonomic indices or indicators of honey state and quality.

The near-infrared reflectance spectroscopy and fluorescence spectroscopic methods are simple and inexpensive; they do not require particular sample preparation or special qualification of laboratory personnel. Both spectroscopic methods provide useful information about the adulteration of honey.

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# Composition and properties of partially hydrolyzed sunflower protein isolates

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## Abstract

**Introduction.** Partial enzyme hydrolysis of proteins is common method of modification of their functional properties. The characteristics of partially hydrolyzed proteins obtained from sunflower meal are presented in this work.

**Materials and methods.** Proteins of sunflower meal were extracted in the presence of two proteases viz. neutral protease and Alkalase during 40 min. Partially hydrolyzed protein samples were prepared by isoelectric precipitation and next drying. The polypeptide composition of protein isolates were studied by polyacrylamide gel electrophoresis, degree of protein hydrolysis, amino acid composition, surface activity and functional properties were estimated.

**Results and discussion.** The polypeptides of higher molecular weight (45–54 and about 32–35 kDa) were absent in polypeptide profile of partially hydrolyzed proteins either of proteases. At the same time they were abounded with 14–16 kDa polypeptides and polypeptides with molecular weight lower 14 kDa.

Partially hydrolyzed protein samples had higher protein content, lower content of ash and carbohydrates as compared with the control sample. The biological value of sunflower protein isolates were limited by three amino acids – sulfur containing amino acids (methionine and cysteine) and lysine. The content of methionine, cysteine and lysine has been increased in protein samples obtained with neutral protease, relative to protein isolate. Differential scanning calorimetric analyze of protein samples have demonstrated that partially hydrolyzed samples contained undenaturated proteins, but their denaturation degree was higher compared with control samples. Partial hydrolysis of sunflower seed proteins have improved their solubility in pH range from 2 to 8, water holding, oil binding, foaming, emulsifying capacities and surface activity.

**Conclusions.** Partially hydrolyzed protein samples had higher protein content, lighter color, lower degree of denaturation and better functional properties compared to the traditional protein isolates.

## Introduction

Partial enzyme hydrolysis of protein is common method of modification of their functional properties. The protein hydrolysates are usually prepared from milk or soy proteins under influence of different proteases. Another source of proteins can be used as substrate for enzyme hydrolysis too. Parrado et al. [1] and Villanueva et al. [2] have obtained sunflower protein hydrolysates from sunflower seed protein isolates, Vioque et al. [3] have produced rape seed protein hydrolysates.

The polypeptide composition of protein isolates have changed as result of protein hydrolysis under different proteases notably polypeptides of smaller size appeared [2, 4]. It was shown that soy and sunflower protein hydrolysates had higher solubility in wide pH range, in despite of native proteins which have destricted solubility at pH of isoelectric point [2, 4]. The pumpkin oil cake globulin hydrolysates obtained with different enzymes (hydrolysis degree 5.6–29.8%) had also increased solubility within the studied pH range [5]. In addition, hydrolysates of cucurbitin obtained with Alkalase and pepsin showed antioxidant activity.

In most cases limited protein hydrolysis can improve their emulsifying, foaming properties [3–7] and change sensory characteristics [8].

In our previous works we have shown that extraction of protein from industrial sunflower meal in the presence of trypsin [9] or microbial proteases [10, 11] resulted in increase of protein concentration in extracts and protein isolate yield. The protein concentration in extracts obtained in the presence of proteases was approximately twice higher than in control samples. The protein hydrolysis degree was sufficiently low and varied from 3.5% to 5.2% at moderate enzyme/substrate ratio and neutral protease [10]. When enzyme:substrate ratio increased the degree of hydrolysis was also rising and reached 9.0–9.5% during 40–60 min that is desirable for improvement of protein functional properties, higher hydrolysis level results in loss of protein functionality. The degree of protein hydrolysis varied from 8.5 to 14.5% when alkaline protease was used at every investigated enzyme:substrate ratio during 40–60 min. It was shown also that correlation (r=0.69) exists between concentration of proteins in extracts and degree of protein hydrolysis under influence of microbial proteases. The model was obtained for estimation of the affectivity of protein extraction as function of enzyme:substrate ratio and extraction time.

Changes in polypeptide composition of protein isolates under influence of proteases resulted in differences of amino acid composition between unhydrolyzed proteins and proteins partially hydrolyzed by various proteases. Amino acid composition of sunflower meal, protein isolates (PI) and partially hydrolyzed protein isolates (PHPI, hydrolysis degree 12.4%) were studied in our previous work [12]. Three amino acids were limiting in sunflower meal proteins almost in equal extent, notably, valine had amino acid score 71.2% to FAO/WHO reference protein, isoleucine – 72.5 and lysine – 73.5%, respectively. Amount of amino acids in PI and PHPI had changed comparing with sunflower meal that is content of sulfur containing amino acids and lysine in protein isolates decreased drastically. On the other hand, sunflower protein isolates, obtained by Villanueva et al. [2] and Ivanova et al. [13], were not limited by the content of methionine and cisteine.

In this work we present the next characteristics of partially hydrolyzed proteins (PHPI, hydrolysis degree 8–12%), obtained from sunflower meal with assistance of microbial proteases, notably their composition, solubility in the range of pH 2–8, their surface activity, emulsifying, foaming capacities, water and oil binding properties, denaturation temperature and enthalpy of denaturation.

## Materials and methods

**Materials.** Sunflower meal was collected from local oil extraction plant, Melitopol, Ukraine. The protein content of meal varied from 33 to 39%. Neutral protease from *Bacillus subtilis* (Protolad, 70 FIP-U/g, optimum pH 6.5–7.0, ENZYME, Ukraine) and alkaline protease from *Bacillus licheniformis* (Alkalase, 2.4 AU/g, optimum pH 8.5–9.0, ENZYME, Ukraine) were used for hydrolysis. All chemicals used for experiments were at least analytical grade.

**Preparation of protein isolate and partially hydrolyzed protein isolates.** Proteins were extracted from sunflower meal by sodium chloride solution (70 g/L, pH 7.0) under constant stirring, at 45 °C for 40–50 min, meal:solution ratio was 1:10 (w:v).

In order to obtain partly hydrolyzed samples, extraction of proteins from sunflower meal was carried out in the presence of Protolad or Alkalase, with meal:enzyme ratio 100:1. The protease activity was stopped by heating of reaction mixture at 80 °C for 15 min.

Afterwards, the insoluble residue was precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation at pH 4.0. After protein coagulation, pellet was separated by centrifugation (3,000 x g), washed with water solution (pH adjusted with HCl to 4.0), protein pellet was collected and dried to 6–8% humidity.

**Determination of the Protein Hydrolysis Degree.** The degree of protein hydrolysis (DH) was determined according to Popovic et al. [5] in some modification. To a 0.5-mL aliquot of the supernatant obtained after hydrolysis, an equal volume of 0.5 mol/dm$^3$ 3-chloro-acetic acid (TCA) was added. The mixture was incubated for 30 min at 4 °C. Thereafter, the mixture was centrifuged at 7 000 rpm for 10 min. The TCA-soluble fractions were analyzed to determine the protein content by the method of Lowry et al. [15]. The DH value was calculated as the increase of TCA-soluble protein concentration in the presence of protease ($C_{protease}$) to protein content in control samples ($C_{control}$), expressed as a percentage:

$$DH = \frac{C_{protease} - C_{control}}{C_{control}} \times 100$$

**Analysis of protein sample composition.** Moisture and ash content of protein samples was determined using the gravimetric method. Oil content was measured according to Soxhlet's method. For this purpose 5 g of sample were extracted for 24 hrs using hexane as a solvent. Crude protein (Nx6.25) was determined by the Kjeldahl method according to AOAC Method [16]. Crude protein content was calculated with a conversion factor of 6.25. Carbohydrate content was calculated as difference between dry substances mass and mass of protein, ash and oil.

**Determination of amino acid composition of protein samples.** The direct acid hydrolysis of protein isolates was used to obtain hydrolysates suitable for determination of all amino acids except cysteine and tryptophan. Hydrolysis was carried out in test tubes by adding of 1 mL of 6 M HCl to dry sample, corresponding to 2 mg of protein. The mixture was frozen in a bath at – 80 °C, evacuated, sealed and then samples were exposed at 106 °C for 24 h in a thermostat. After hydrolysis samples were cooled and HCl was removed from them by evacuating in dessicator containing NaOH pellet. After drying of samples 4 mL of deionized water was added and drying procedure was repeated. Dry samples were dissolved in citrate buffers (0.3 M/L, pH 2.2) and used for amino acid analyses.

Amino acid analyzer T 339 (Czech Republic) was used for amino acid content analysis. Standard amino acid mixture containing 0.5 μM of the 17 commonly occurring amino acid was used to calculate the amount of amino acids in the samples.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).** The polypeptide composition of the protein samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis by the method of Laemmli [17]. The gel system, containing 0.2% (wt/vol) SDS consisted of a 12% polyacrylamide-resolving gel (pH 8.8) and a 4% stacking gel (pH 6.8 ) in a minislabs system (Bio-Rad Mini-Protean II Model). The length of the resolving and stacking gel were 10 and 2 cm, respectively, with a gel thickness of 0.75 mm. Electrophoresis was perfomed at a constant current of 25 mA. Protein bands were stained by immersion of the gel in 0.05% Coomassie brilliant blue G–250 solution, in 45% methanol and 9% acetic solution.

Protein molecular weights were estimated using low MW markers (Pharmacia, Amersham, England) that included phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and a-lactalbumin (14,400).

**Differential scanning calorymetry (DSC).** Thermal denaturation of the protein suspensions was studied by DSC with a DSC Q 2000 calorimeter (V24.4 Build 116, TA Instruments, Germany). 8 mg of sample was placed in an aluminium pan and heated from 20 to 110 °C at a rate of 2 °C/min.

**Determination of protein solubility.** Determination of solubility was made in a pH region of 2.0 to 8.0 adjusted by 0.1 M HCl or NaOH. Protein samples were dissolved for 1 h at room temperature at concentration 1% wt/vol under constant stirring. Suspensions were centrifuged at 3,000 x *g* for 20 min. Protein concentration in supernatants were measured by method of Lowry et al. [15]. Solubility was expressed as grams of soluble protein/100 grams of sample.

**Determination of protein sample functional properties.** The water holding capacities (WHC) of proteins were measured as described by Ashraf et al. [18] taking 1 g of protein extract and resuspended in 10 mL of distilled water and mixed vigorously for 2 min, the supernatants obtained after centrifugation at 3,000 x g for 20 min were decanted and the weights of the sediments were determined, the WHC values expressed as gram of water absorbed per 100 g of protein.

The oil binding capacities (OBCs) of seed proteins were measured using the method of Ashraf et al. [18] taking 1g of protein, deposited and reweighed in 50 mL centrifuge tubes and thoroughly mixed for 3 min with 10 mL of vegetable oil. Samples were allowed to stand for 30 min and the mixtures were centrifuged at 3,000 x g for 20 min, the supernatants were carefully poured immediately after centrifugation and tubes with the sediments were weighted. The OBC values expressed as gram of oil absorbed per 100 g of protein isolates.

The emulsifying capacity (EC) of the protein samples were determined according to Karki et al. [19] taking 8.5 g of each sample and mixed with 50 mL of distilled water for 2 min using a blender and vegetable oil was adding slowly with continuous blending. The process was stopped after every 2 min to check for emulsion breakage. The maximum volumes of oil that was emulsified were measured and emulsifying capacity was determined as volume of oil relatively to 1 g of protein isolates.

The foaming capacity (FC) of the protein samples were determined according to Makri et al. [20] taking 1% of the samples and resuspended in deionized water, pH was adjusted

to 7.4 with 0.1 M NaOH and 0.1 M HCl. 100 mL of solution were blended for 3 minutes and poured into a 500 mL graduated cylinder. The volume of foam ($V_f$) and liquid ($V_l$) were immediately recorded and FC was calculated using the following equation:

$$FC = \frac{V_f}{V_l} \cdot 100$$

**Surface tension measurements.** Series of protein suspensions with concentrations from 0.03 to 0.5% (w/v) were prepared in water solution at pH 8.0 adjusted with 0.1 M NaOH. The surface tension measurements were made with a stalagmometer. Three readings were made on each sample to obtain an average value. All measurements were made at temperature (20 ± 1) °C. Distilled water was used as surface-tension standard. The surface tension of protein suspensions was calculated using the following equation:

$$\sigma = \sigma_0 \frac{\rho \times N_0}{\rho_0 \times N}$$

where $\sigma_0$, $\rho_0$ and $N_0$ are the surface tension, density and drop number of distilled water; $\rho$ and N are the surface tension, density and drop number of protein suspension respectively.

**Statistical analysis.** Data were expressed as means ± standard deviations for triplicate determination. Statistical analysis was performed using Microsoft Excel 2007. Differences were considered to be significant at validity of α=0.95.

## Results and discussion

**Chemical and polypeptide composition of sunflower seed isolated proteins.** The sunflower seed protein isolate used as a control sample had a protein content about 78% and high carbohydrate content (Table 1). Partially hydrolyzed protein sample obtained with neutral protease (hydrolysis degree 8.5%) contained about 90% proteins, lower amount of ash and more than two times less of carbohydrates than protein isolates. The protein sample obtained with Alkalase (hydrolysis degree 11.5%) also had higher protein content, lower content of ash and carbohydrates as compared with the control sample. Such influence of partial protein hydrolysis on the chemical composition of protein samples is obviously due to decrease of intermolecular aggregation of smaller polypeptides with other components of sunflower seed including carbohydrates. Low amount of concomitant substances are coprecipitated from the protein suspension together with small polypeptides at their isoelectric precipitation. And finally partial hydrolysis could result in readsorption of some contaminations from the protein surface.

Using of Protolad and Alkalase at extraction of proteins from sunflower meal resulted in changes of polypeptides composition of obtained partially hydrolyzed proteins (Figure 1).

Table 1

**Chemical composition of sunflower seed proteins calculated on a dry weight basis**

| Component | Protein isolate | Partially hydrolyzed proteins (Protolad) | Partially hydrolyzed proteins (Alkalase) |
|---|---|---|---|
| Protein | 78.4 ± 0.5 | 89.5 ± 0.4 | 87.2 ± 0.6 |
| Ash | 4.9 ± 0.2 | 2.8 ± 0.1 | 3.0 ± 0.2 |
| Carbohydrates | 16.0 ± 0.4 | 7.2 ± 0.3 | 9.2 ± 0.4 |
| Lipid | 0.7 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 |

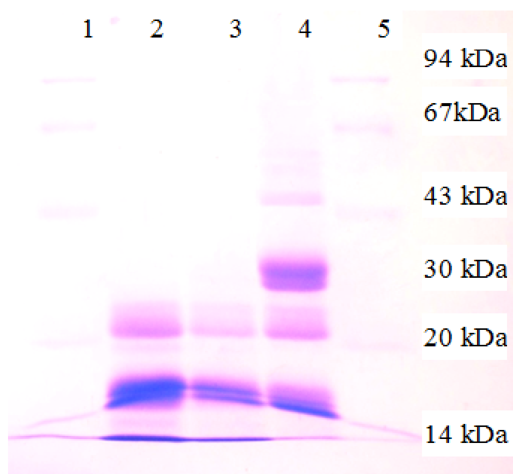


**Figure 1. Sodium dodecyl sulfate-polyacrilamide gel electrophores polypeptides profile of sunflower seed proteins isolate (lane 4), partially hydrolyzed by Protolad (lane 2) and Alkalase (lane 3), lane 1 and 5 – molecular weight markers (94, 67, 43, 30, 20 та 14 kDa).**

The polypeptides of higher molecular weight (45–54 and about 32–35 kDa) were absent in polypeptide profile of proteins partially hydrolyzed by either of proteases. They abounded with 14–16 kDa polypeptides and polypeptides with molecular weight lower 14 kDa. Some new polypeptides with molecular weight in rage of 10–20 kDa had appeared in their profiles, whereas control protein sample had relatively high content of 32–35 kDa polypeptides and low content of 16 kDa polypeptides.

The essential amino acid compositions of protein samples were sufficient different, indicating that limited hydrolysis of sunflower meal proteins under their extraction have changed the biological value of proteins (Figure 2). The biological value of sunflower protein isolate was limited by three amino acids – sulfur containing amino acids (methionine and cysteine), their content was only about 46% of FAO protein, and lysine which was 54% of references protein. Since content methionine and cysteine in sunflower meal proteins was 80% of FAO proteins [12] evidently, that proteins abounded by these hydrophobic amino acids are less soluble and are not extractable from the meal. Content of other essential amino acids exceeds their content in references protein. Limited hydrolysis of sunflower meal proteins by Protolad have increased amino acid score of every essential amino acid compared to the protein isolate, most significantly leucine, sum of aromatic amino acids and valine. This protein samples have increased content of methionine and cysteine by 26% and lysine – by 17% relative to protein isolate. On the contrary, essential amino acids content of protein samples limited hydrolyzed by Alkalase were lower compared to the protein isolate, these proteins were especially poor in sulfur containing amino acids (their content was only 23% relative to FAO pattern). This phenomenon could be due to loss of such amino acids with hydrolysis products which are not precipitated during isoelectric precipitation. Decrease of methionine and cysteine content were observed also in sunflower hydrolysate obtained with Alkalase [2].

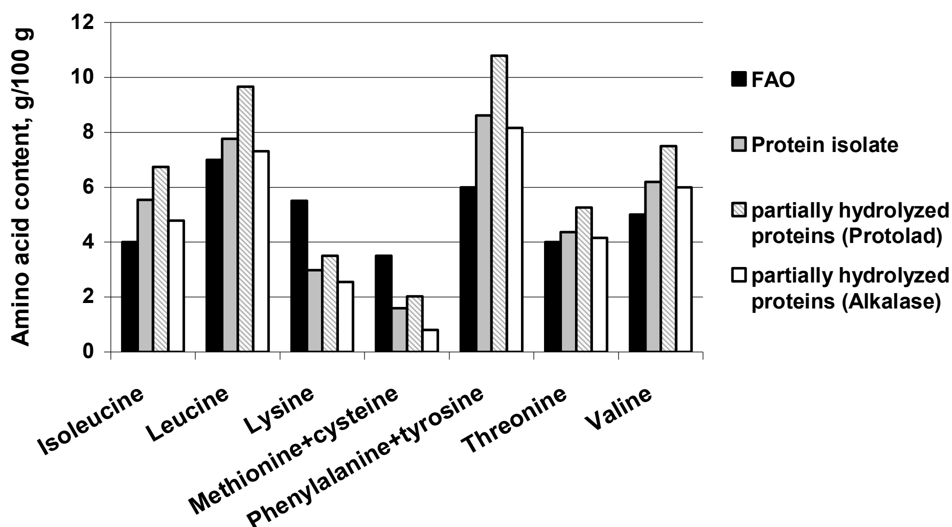


**Figure 2. Essential amino acid composition of sunflower seed protein samples**

**Protein denaturation degree of sunflower protein isolates.** Obtained protein samples had similar denaturation temperatures and enthalpies (Table 2, Figure 3). Dependences of thermal flow of protein suspensions from temperature were similar for partially hydrolyzed and unhydrolyzed protein samples (Figure 3). The denaturation temperatures of protein isolate was about 85 °C. Proteins of partially hydrolyzed samples had a little lower temperature stability, the denaturation temperature were 80.2 and 79.1 °C for partially hydrolyzed proteins by Protolad and Alkalasa respectively. Denaturation degree of partially hydrolyzed proteins were higher compared to the protein isolate, enthalpies of denaturation were 151.5 and 146.8 J/g proteins for Protolad and Alkalasa sample respectively. Thus, in spite of limited hydrolysis, protein samples obtained with proteases, contains macromolecules with preserved second and tertiary structure.

Table 2

**Denaturation temperature and enthalpies of sunflower protein products**

| **Sunflower protein products** | **Denaturation temperature, °C** | **Enthalpy, (ΔH, J/g protein)** |
|---|---|---|
| Protein isolate | 84.9 ± 1.7 | 184.7 ± 2.1 |
| Partially hydrolyzed proteins (Protolad) | 80.2 ± 1.5 | 151.5 ± 1.6 |
| Partially hydrolyzed proteins (Alkalase) | 79.1 ± 1.2 | 146.8 ± 1.4 |

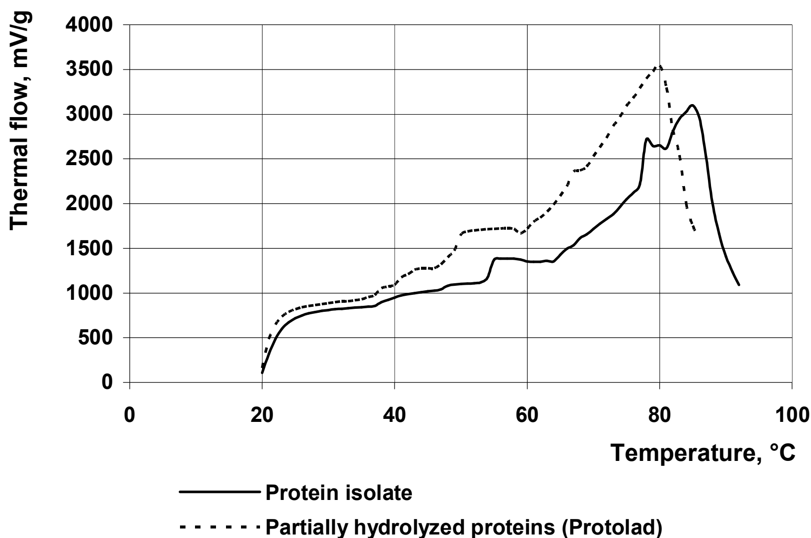


**Figure 3. Dependence of protein suspensions thermal flow from temperature.**

**Solubility of protein samples and their functional properties.** High solubility of isolated proteins is required in many foods because of soluble proteins provide a homogeneous dispersability of molecules in colloidal systems and many other functional properties of proteins are realized when they are in soluble state. The solubility of proteins usually increased over a wide range of pH as result of their hydrolysis [14]. Indeed, protein samples, containing limited hydrolyzed polypeptides, had higher solubility in pH range from 2 to 8 (Figure 4).

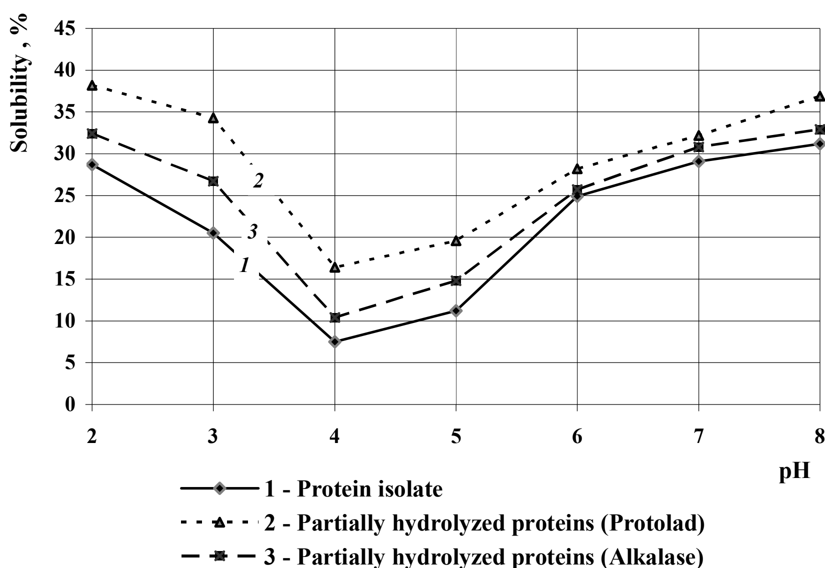


**Figure 4. Solubility of sunflower protein samples at different pH values**

The greatest increase of solubility was observed at acid medium (pH 2–5) for protein sample, partially hydrolyzed by Protolad, solubility differences between samples decreased at pH 6–8. Increase of solubility for protein sample, partially hydrolyzed by Alkalase, was insignificant at pH range 2–5 and very poor at higher pH, that could be determined not only specific effect of this protease and properties of hydrolysis products, but also composition of proteins which were extracted in alkaline solution.

Accordingly, the water holding capacity of partially hydrolyzed protein samples increased too (Figure5, a), for protein sample, partially hydrolyzed by Protolad, this increase was almost five times, for protein sample, partially hydrolyzed by Alkalase, this characteristic improved four times. The increase of oil binding capacities was statistically insignificant for both partially hydrolyzed protein samples.

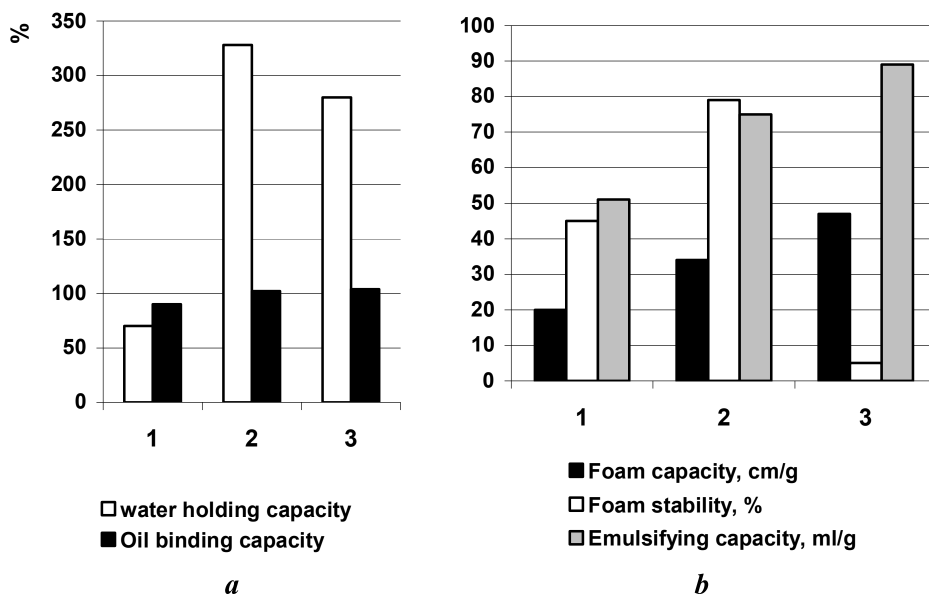


**Figure 5. Functional properties of sunflower protein samples:**
**1 – protein isolate, 2 – partially hydrolyzed proteins (Protolad),**
**3 – partially hydrolyzed proteins (Alkalase).**

Isolated proteins have surface activities and hence are active in creation of such disperse systems as foams and emulsions. Dependences of surface tension of protein suspensions from their concentration have demonstrated that interfacial properties partially hydrolyzed proteins have been improved under influence of limited hydrolysis (Figure 6). The surface activity of protein sample, partially hydrolyzed by Protolad, has increased most drastically, even at very low protein concentrations (<0.1%) surface tension coefficient decreased about 10% compared to the protein isolate.

Foaming and emulsifying capacities partially hydrolyzed proteins increased substantially (Figure 5, b). Foaming and emulsifying capacities were higher in sample, partially hydrolyzed by Alkalase, but foam stability of this sample was very poor. At the same time foam stability was highest in the sample, partially hydrolyzed by Protolad. Loss of foaming stabilizing capacity with increase of hydrolysis degree had been demonstrated for soy proteins too [4].

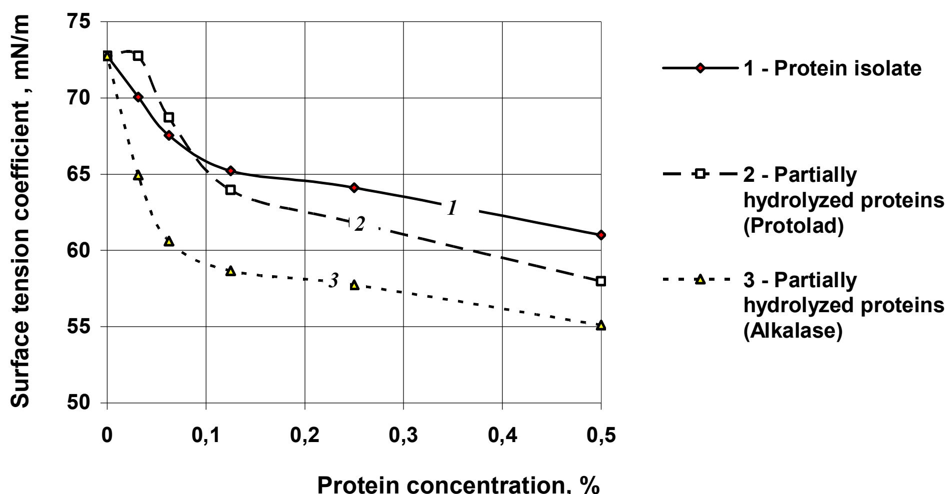


**Figure 6. Dependence of surface tension coefficient of protein suspensions from their concentration.**

## Conclusion

We have used two proteases during extraction of proteins from sunflower seed meal. We have obtained the partially hydrolyzed protein samples with different composition, color, denaturation degree and functional properties. These samples had higher protein content compared to the traditional protein isolates, protein sample partially hydrolyzed by neutral protease had higher biological value too. Protein solubility at pH 2–8, water holding, oil binding, foaming and emulsifying capacities of partially hydrolyzed protein samples were improved compared to the control.

*Acknowledgements*
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# Spoilage volatiles and sensory properties of a grilled stick meat product inoculated with *Pediococcus acidilactici* FLE07 as starter culture

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## Abstract

**Introduction**. Grilled meat products are normally eaten as snacks in many developed and developing countries usually during leisure. The use of biological agents such as lactic acid bacteria may help improve the associated volatiles and sensory appeal of such products.

**Materials and methods.** A grilled meat product (*Tsire*) was inoculated with 6 log CFU/g of *Pediococcus acidilactici* FLE07 as starter culture (IS), with the objective of improving the associated spoilage volatiles during a 4 day storage at 30 °C; while uninoculated sample of *Tsire* served as control (UC). Solid phase mass extraction gas chromatography-mass spectrometry (SPME-GCMS) and taste panel using hedonic scaling were used to evaluate the volatiles and sensory quality respectively during storage.

**Results and discussion.** In preliminary experiments, ten *Pediococcus* strains were evaluated for production of organic acids; among these, 34 $g_{acid}$ /$10^7$CFU was recorded as highest lactic acid production by *P. acidilactici* FLE07 and was chosen as starter culture for inoculation of *Tsire*. The strain was shown to produce acetic acid of concentration lower than 12 $g_{acid}$ /$10^7$ CFU. SPME-GCMS analysis of the grilled meat product showed that a total of fourty eight (48) volatiles, belonging to ketones (35.42%), acids (8.33%), alcohols (25%), aromatic/cyclic (14.58%) and nitrogenous compounds (16.67%), were detected during storage. Volatiles including acetone, 2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone, 2-hexanone, 2-heptanone and 1-hydroxy-2-propanone were among the prominent ketone compounds observed in the *Tsire* samples, and their total are units (TAUs) showed significant difference ($p<0.05$) between the IS and UC samples. There was reduction in spoilage volatile indicators; concentrations (µg/g) of 0.57, 1.98, 0.93 and 1.39 were recorded for heptanal, 1-octen-3-ol, 3-methyl-butanoic acid and nonanal respectively in inoculated samples (IS) compared to 2.43, 3.21, 2.94 and 2.94 obtained for uninoculated control (UC) on day 3. Sensory study with the use of 50 panelists to provide data showed that higher scores ($p<0.05$) were recorded in the aroma, appearance, tenderness, taste and general acceptability properties of IS than UC.

**Conclusion.** It was concluded that *Pediococcus acidilactici* FLE07 and other suitable starter culture(s) may be used in promoting sensory quality development and availability of the product beyond the day of production. This is the first report of this type on the grilled meat product.

## Introduction

Grilled meat products are normally prepared by grilling of shredded meat cuts hanged on sharpened edges of sticks, and *Tsire* is common example in Nigeria. The meat cuts are normally spiced with peanut cake, spices, vegetable oil, salt or other flavorings and then cooked by grilling. They are sprinkled with groundnut oil during the grilling process to simulate the traditional technique of avoiding burning or charring [1]; grilling is usually carried out around glowing charcoals. *Tsire* could be prepared from muscles of beef, goat, mutton or chicken, though preparation from beef is common [2]. It is a popular traditional meat product in Nigeria commonly eaten as delicacies, served or sold along streets or in club houses.

Cooking of meat involves a complex series of thermally induced reactions occurring between non-volatile components of lean and fatty tissues, resulting in a large number of reaction products (Lorenzo *et al.*, 2016); the volatile compounds formed in these reactions are largely responsible for the characteristic flavour of cooked meat [1]. Such products include aldehydes, carboxylic acids, alcohols, ketones, esters, sulfur compounds, nitrogenous compounds, terpenes, alkanes and alkenes, aromatic and cyclic hydrocarbons, which have been noted to contribute to flavour development in meat products [3].

*Pediococcus* is a genus under the group of lactic acid bacteria (LAB) which play positive role in fermentation and preservation of many foods, especially by their abilities to produce considerable amounts of volatile and organic compounds [2]. LAB have a generally regarded as safe (GRAS) status and have been widely used as starters in the industrial preservation of meats [4]. For instance, Olaoye *et al.* [5] reported the application of LAB cultures, *P. pentosaceus* GOAT 01 and *Lactobacillus plantarum* GOAT012, in the preservation of goat meat during storage at 30$^{o}$C. According to the authors, the LAB cultures were able to extend the shelf life of the meat for days beyond the uninoculated control samples. In another study, the use of *Pediococcus* cultures in the generation of antioxidant nitrogen compounds in Iberian dry-fermented sausages was reported by Fernández *et al.* [6]. Moreover, Lorenzo *et al.* [7] investigated the effect of commercial cultures of *Pediococcus* spp. on volatile compound profile and sensory characteristics of dry‐cured foal sausage during ripening. Olaoye [1] and Olaoye [3] reported studies on the effect of storage period on volatiles and consumers' acceptability of *Tsire* and pork *balangu* (a grilled meat product) respectively; the authors recommended that inoculation of the meat product with LAB cultures should be carried out in future studies for possible improvement in volatile characteristics.

In spite of the available reports on meat products, no study is currently available on the effect of LAB cultures on volatiles of *Tsire* during storage. Hence, the present study focused on evaluating the influence of *P. acidilactici* FLE07 culture on the volatile compounds, especially those that may be spoilage indicators, and sensory characteristics of the traditional meat product during storage.

## Materials and methods

**Source of meat and ingredients.** The beef used in this study was purchased from a butcher's shop in the city of Nottingham, UK, and conveyed to the laboratory over ice crystals for immediate use. The ingredients used included ground red pepper (*Capsicum* sp.), onions (*Alium cepa*), ginger (*Zingiber officinalis*), groundnut (*Arachis hypogaea*) and salt, obtained from a Nigerian shop in the same city.

**Sources of LAB used and growth conditions.** Ten presumptive LAB isolates, previously isolated and phenotypically identified from meat in a preliminary study were used in the present study [8]. They included *Pediococcus pentosaceus* LIV01, *P. acidilactici* FLE07, *P. acidilactici* FLE02, *P. pentosaceus* INT01, *P. pentosaceus* INT02, *Lactobacillus plantarum* FLE04, *P. acidilactici* INT04, *P. acidilactici* FLE07, *Leuconostoc mesenteroides* FLE03 and *P. pentosaceus* INT04. The optimum growth temperature of the isolates in MRS medium (Oxoid, UK) was 30 °C. The isolates were routinely maintained in MRS broth medium containing 20% glycerol at –20 °C as working cultures, and at –80 °C for long-term storage.

**Production of organic acids.** Prior to selection of one of the LAB isolates as starter culture, the isolates were evaluated for their abilities to produce organic acids (lactic and acetic acids), using the method of Olaoye *et al*. [8]. One of the isolates, *P. acidilactici* FLE07, showed considerable production of organic acids, and was hence chosen as starter culture for inoculation of the traditional meat product prior to storage. Concentrations of the organic acids were expressed in $g_{acid}$ /$10^7$ CFU (i.e grams of lactic/acetic acid per $10^7$ colony forming units)

**Confirmation of identity of presumptive *P. acidilactici* FLE07 used as starter culture.** The LAB isolate *P. acidilactici* FLE07 used as starter culture was presumptively identified in a previous study [8]. Full identity of the isolate was obtained in the present study using 16S rDNA nucleotide sequencing after successful amplification by PCR. Deoxyribonucleic acid (DNA) was extracted using a boiling method [9]. PCR amplification was performed using the following set of primers [10]: Forward, 5′-CCTACGGGAGGCAGCAG-3′ and Reverse, 5′-ATTACCGCGGCTGCTGG-3′, targeting approximately 200 bp of 16S rDNA gene (V3 region). PCR conditions used were as described by Olaoye *et al.* [5]. Electrophoresis of the 16S rDNA-PCR products, purification and sequencing were carried out as previously described [5]. The specific nucleotide sequences were subjected to BLAST programme of NCBI (website; http://www.ncbi.nlm.nih.gov/blast/) to determine the homology of the *Pediococcus* isolate with related genera and species [11].

**Preparation of *Tsire* and inoculation with *P. acidilactici* FLE07.** *Tsire* samples were prepared according to the method described by Olaoye [1]. Some of the *Tsire* samples were inoculated with 6 log CFU/g of *P. acidilactici* FLE07 culture according to the method of Olaoye and Dodd [2] while uninoculated samples served as control.

**Storage of *Tsire.*** The *Tsire* samples were wrapped in aluminium foils and stored for four days in a storage cabinet at 30°C to represent ambient temperature in Nigeria. Samples were taken daily, in three replicates, for analysis of thiobarbituric acids and volatiles compounds.

**Analysis of thiobarbituric acid in *Tsire.*** Thiobarbituric acid (TBA) values were determined for the *Tsire* samples as described by Olaoye and Onilude [12].

**Analysis of volatile compounds** in *Tsire* using solid phase mass extraction-gas chromatography mass spectrometry (SPME-GCMS)

The volatiles in the *Tsire* samples taken daily during storage were analyzed using SPME-GCMS. This was performed by placing 2 g of samples in 20 ml headspace vials (22.5 mm x 75.5 mm, Grace Alltech, UK). The vials were sealed with a magnetic cap (20mm diameter, 5mm centre, PTFE/Silicone Liner; Grace Alltech) using a Crimper (Part no 60045, Alltech Associates Inc., USA) and allowed to equilibrate at room temperature (22 °C) for 30 min before commencement of analysis.

A Stableflex fibre coated with 50/30 μm divinylbenzenecarboxen on polydimethylsiloxane bonded to a flexible fused silica core (Supelco, Bellefonte, PA, 16823–0048 USA) was used for the extraction of the flavour volatiles in the headspace of the vials. For volatile sampling, an extraction time of 15 min at room temperature was used, while desorption time was set to 4 min at 230 °C.

GCMS was carried out using a Trace GC Ultra gas chromatograph (Thermo Electron Corporation, UK) and a DSQ mass spectrometer (1.4.1 SP3 Thermo Electron Corporation, USA). Samples were injected in splitless mode into the GC with a PAL auto-sampler. Chromatography was carried out with a TRACE GC 2000 series gas chromatograph using a ZB-WAX capillary column (Serial no 162147, Order no 7HG-G007-22, L 30m x I.D. 0.25mm x df 1μm, USA). Helium was employed as the carrier gas, at a constant pressure of 15 psi and splitless time of 1 min. The oven temperature programme was as follows: an initial temperature of $40^{o}$C was maintained for 1 min, with ramps 8 °C/min to 200 °C and 10 °C/min to a final temperature of 230 °C. Mass spectrometry was performed with a DSQ mass spectrometer. The mass spectrometer was operated in positive ionisation electron impact mode (EI+) at electron energy of 70 eV. The scan time was 0.29 s. Samples for injections into the GC were prepared in three replicates. The detector was operated in scan mode, scanning from m/z 20 to 210. Source temperature was 200 °C. The data generated were processed with Xcalibur ™ 1.4 SR1 (Thermo Electron Corporation) software.

Volatile compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectral library and/or by calculation of the retention indices relative to a series of n-alkanes (C5–C19; Sigma-Aldrich, UK) and matching them with standard compounds and data reported in the literature [13,14]. The results were reported as relative abundance expressed as total area counts, TAUs ($\times 10^4$).

**Sensory study.** Sensory study was conducted on the *Tsire* samples that depended on inoculation with or without *P. acidilactici* FLE07 and storage time. Samples were evaluated for the sensory properties of aroma, appearance, tenderness, taste and general acceptability using a 50 member panel ($n$=50), composing of Nigerians who were already familiar with the product. Freshly prepared *Tsire* samples were used as reference for comparison of sensory properties of other samples during storage. Panelists were asked to allocate scores to three coded replicates of samples, using a 9-point hedonic scale, from 1-dislike extremely to 9-like extremely. Data obtained were subjected to statistical analysis to determine significant differences among samples.

**Statistical data analysis.** Results which depended on starter culture and storage time were analyzed according to a completely randomized design with three replicates. The data obtained were subjected to one way analysis of variance (ANOVA) to evaluate the effect of starter culture on the samples. Differences between means were evaluated by Duncan's multiple range test and significant difference was expressed at $p < 0.05$. The SPSS statistic programme (version 10.01) was used.

The relationship between the inoculated and uninoculated control samples, storage time and their volatile compounds was evaluated by principal component analysis (PCA) using Xlstat software (ver. 17.3.01.19703; Addinsoft, NY).

## Results and discussion

In this study, ten LAB isolates previously isolated and presumptively identified from meat in a preliminary study [8], were screened for production of organic acids (lactic and acetic acids), with the objective of selecting suitable candidate(s) as starter culture for possible improvement of volatiles and sensory properties of a Nigerian traditional meat product (*Tsire*) during storage. Among the ten isolates, *Pediococcus pentosaceus* LIV01, *P. acidilactici* FLE01, *P. acidilactici* FLE02 and *P. acidilactici* FLE07 produced lactic acid higher than 25 $g_{acid}$ /$10^7$ CFU (Figure 1); however, *P. acidilactici* FLE07 had the highest production of the acid (34 $g_{acid}$ /$10^7$ CFU) and was chosen as starter culture for inoculation of *Tsire*. Acetic acid production by the LAB isolates was generally lower than 12 $g_{acid}$ /$10^7$CFU.

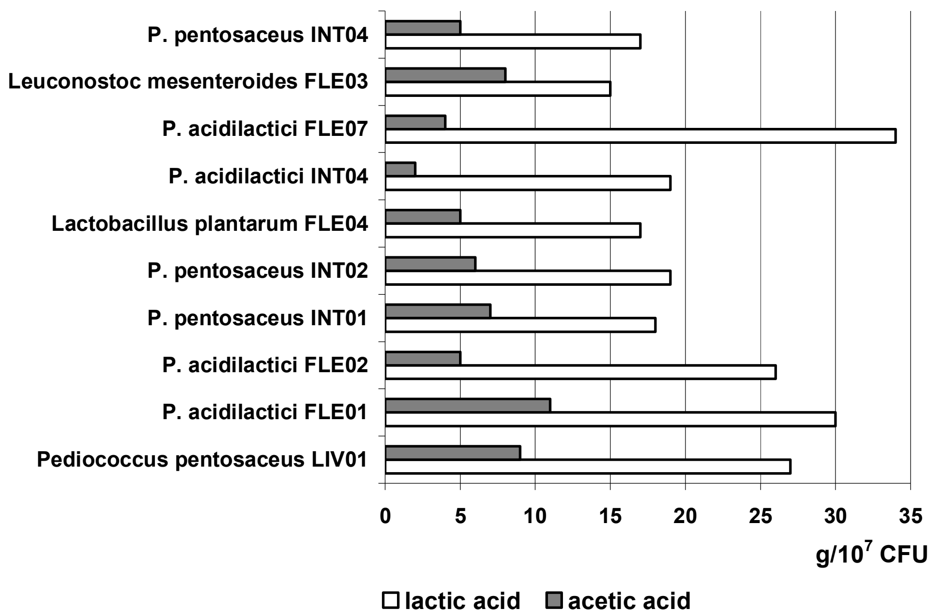


**Figure 1. Concentrations of lactic and acetic acids ($g_{acid}$/$10^7$ CFU/g) produced by the LAB isolates**

Sixty four (48) volatile compounds were detected in the meat product during storage, and they were categorized into different classes including ketones (class 1; 35.42%), acids (class 2; 8.33%), aromatic/cyclic hydrocarbons (class 3; 14.58%), nitrogenous compounds (class 4; 16.67%) and alcohols (class 5; 25.0%); these are presented in Tables 1 and 2.

Similar reports of detection of these classes of volatiles in meat products have been made by other research investigators [3,15].

*(In the Table 1: Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; P-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library)*

**Table 1. Total area units (x10$^4$) of ketone and acid compounds in *Tsire***

| Compounds | RI | *Tsire* inoculated with *P. acidilactici* FLE07 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD1 | StdD | SD2 | StdD | SD3 | StdD | SD4 | StdD |
| **Class 1 – Ketones** | | | | | | | | | |
| Acetone | <600 | 5862 | 134 | 4102 | 129 | 2981 | 172 | 2410 | 74 |
| 3-methyl-2-butanone | <600 | 25 | 4 | 17 | 5 | 25 | 3 | 53 | 12 |
| Methyl-isobutyl-ketone | 612 | 0.79 | 0 | 2 | 1 | 6 | 2 | 46 | 13 |
| 2-butanone | 618 | 540 | 20 | 520 | 112 | 605 | 47 | 725 | 129 |
| 2,3-butanedione | 621 | 541 | 64 | 323 | 32 | 275 | 12 | 172 | 17 |
| 3-methyl-2-pentanone | 634 | 0.11 | 0.01 | 6 | 2 | 13 | 3 | 68 | 6 |
| 5-methyl–2-hexanone | 687 | 81 | 19 | 76 | 7 | 69 | 14 | 58 | 10 |
| 2-hexanone | 699 | 24 | 4 | 20 | 8 | 16 | 2 | 13 | 3 |
| 2,5-hexanedione | 712 | 92 | 12 | 178 | 36 | 471 | 86 | 1290 | 128 |
| 2-heptanone | 719 | 2 | 0.8 | 11 | 1 | 19 | 4 | 50 | 3 |
| 2-octanone | 720 | 5 | 3 | 11 | 2 | 24 | 17 | 18 | 5 |
| 6-methyl-2-heptanone | 723 | 1 | - | 1.2 | 0.3 | 4 | 1 | 20 | 3 |
| 5-methyl-2-heptanone | 725 | 1.3 | 0.5 | 2 | 1 | 9 | 3 | 52 | 4 |
| 3-hydroxy-2-butanone | 727 | 251 | 89 | 264 | 101 | 272 | 86 | 281 | 27 |
| 1-hydroxy-2-propanone | 733 | 21 | 12 | 45 | 15 | 85 | 12 | 31 | 5 |
| 6-methyl-5-hepten-2-one | 742 | 21 | 2 | 25 | 7 | 29 | 12 | 35 | 8 |
| cyclopentanone | 748 | 32 | 2 | 39 | 13 | 47 | 9 | 64 | 21 |
| **Class 2 – acids** | | | | | | | | | |
| 2-methyl-propanoic acid | 752 | 2 | 0 | 20 | 4 | 18 | 2 | 13 | 3 |
| 2-methyl-hexanoic acid | 768 | 6 | 2 | 42 | 7 | 46 | 7 | 71 | 16 |
| 3-methyl-butanoic acid | 756 | 156 | 25 | 87 | 8 | 34 | 8 | 50 | 13 |
| Hexanoic acid | 772 | 12 | 3 | 20 | 6 | 21 | 12 | 10 | 2 |

| Compounds | Uninoculated *Tsire* | | | | | | | | *P*-value | MI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SD1 | StdD | SD2 | StdD | SD3 | StdD | SD4 | StdD | | |
| **Class 1 – Ketones** | | | | | | | | | | |
| Acetone | 5852 | 43 | 5574 | 116 | 5574 | 321 | 5404 | 373 | 0.002 | 1 |
| 3-methyl-2-butanone | 9 | 3 | 35 | 13 | 50 | 10 | 53 | 9 | 0.0004 | 2 |
| Methyl-isobutyl-ketone | 0.93 | 0 | 10 | 3 | 29 | 3 | 47 | 9 | 0.0004 | 2 |
| 2-butanone | 539 | 12 | 732 | 103 | 842 | 101 | 1431 | 108 | 0.035 | 2 |
| 2,3-butanedione | 514 | 98 | 451 | 75 | 351 | 45 | 367 | 43 | 0.003 | 1 |
| 3-methyl-2-pentanone | 9.37 | 0.3 | 20 | 9 | 66 | 17 | 77 | 9 | 0.0016 | 2 |
| 5-methyl-2-hexanone | 80 | 13 | 89 | 23 | 93 | 18 | 126 | 16 | 0.019 | 2 |
| 2-hexanone | 24 | 7 | 22 | 5 | 21 | 3 | 21 | 2 | 0.012 | 1,2 |
| 2,5-Hexanedione | 91 | 27 | 167 | 31 | 251 | 65 | 452 | 98 | 0.0021 | 2 |
| 2-heptanone | 3 | 1 | 38 | 18 | 108 | 33 | 120 | 12 | 0.0013 | 2 |
| 2-octanone | 6 | 1 | 12 | 5 | 24 | 9 | 37 | 4 | 0.002 | 1 |
| 6-methyl-2-heptanone | 0.38 | 0 | 5 | 1 | 24 | 14 | 34 | 6 | 0.001 | 2 |
| 5-methyl-2-heptanone | 2 | 1 | 15 | 4 | 50 | 21 | 76 | 10 | 0.0005 | 2 |
| 3-hydroxy-2-butanone | 262 | 92 | 321 | 103 | 461 | 122 | 519 | 97 | 0.007 | 2 |
| 1-hydroxy-2-propanone | 7 | 2 | 16 | 4 | 55 | 23 | 31 | 9 | 0.031 | 2 |
| 6-methyl-5-hepten-2-one | 22 | 5 | 21 | 7 | 40 | 13 | 62 | 11 | 0.0014 | 2 |
| Cyclopentanone | 33 | 10 | 41 | 5 | 54 | 11 | 71 | 14 | 0.073 | 2 |
| **Class 2 – Acids** | | | | | | | | | | |
| 2-methyl-propanoic acid | 0.8 | 0 | 16 | 2 | 18 | 3 | 12 | 2 | 0.01 | 2 |
| 2-methyl-hexanoic acid | 4 | 3 | 52 | 5 | 112 | 28 | 62 | 12 | 0.003 | 2 |
| 3-methyl-butanoic acid | 156 | 12 | 136 | 18 | 112 | 28 | 63 | 13 | 0.036 | 2 |
| Hexanoic acid | 6 | 1 | 7 | 2 | 9 | 1 | 12 | 5 | 0.281 | 1,2 |

Table 2

**Total area units (x10$^4$) of aromatic/cyclic hydrocarbon, nitrogenous and alcohol compounds in *Tsire***

| Compounds | RI | *Tsire* inoculated with *P. acidilactici* FLE07 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD1 | StdD | SD2 | StdD | SD3 | StdD | SD4 | StdD |
| ***Class 3 – Aromatic/cyclic*** | | | | | | | | | |
| Cyclopentene | <600 | 15 | 2 | 24 | 3 | 27 | 13 | 125 | 20 |
| 1-pentyl-propyl-Cyclopropane | 742 | 7 | 1 | 4 | 2 | 3 | 0 | 4 | 1 |
| Ethylbenzene | 747 | 13 | 3 | 8 | 2 | 3 | 0.64 | 3 | 0.33 |
| 2-methoxy-2-propenyl-Benzene | 754 | 41 | 5 | 35 | 7 | 32 | 9 | 24 | 5 |
| O-xylene | 756 | 8 | 1 | 5 | 1 | 3 | 0.79 | 3 | 0.57 |
| Tetramethyltricyclo-Undec-2-ene | >1000 | 10 | 2 | 10 | 2 | 8 | 1 | 9 | 0 |
| Hexamethyl-cyclotrisiloxane | >1000 | 7 | 2 | 8 | 2 | 6 | 3 | 7 | 0 |
| ***Class 4 – nitrogenous*** | | | | | | | | | |
| 1-methyl-1h-pyrrole | 761 | 99 | 23 | 86 | 7 | 75 | 8 | 58 | 15 |
| 3-methyl-butanenitrile | 873 | 14 | 4 | 12 | 6 | 11 | 3 | 9 | 1 |
| 2-methyl-pyrazine | 881 | 7 | 2 | 11 | 1 | 14 | 2 | 37 | 2 |
| Trimethyl-pyrazine | 903 | 1017 | 19 | 879 | 89 | 724 | 92 | 584 | 198 |
| Tetramethyl-pyrazine | 910 | 175 | 23 | 138 | 37 | 52 | 8 | 31 | 8 |
| 3-ethyl-2,5-dimethyl-Pyrazine | 921 | 4 | 1 | 5 | 0 | 5 | 2 | 12 | 2 |
| 2,5-dimethyl-pyrazine | 954 | 48 | 9 | 149 | 62 | 225 | 34 | 1760 | 143 |
| 2,3-dimethyl-pyrazine | 962 | 34 | 5 | 27 | 4 | 16 | 2 | 16 | 5 |
| ***Class 5 – alcohols*** | | | | | | | | | |
| 1-propanol | 641 | 2 | 0 | 3 | 0 | 4 | 0 | 8 | 4 |
| 2,6-dimethyl-4-heptanol | 645 | 3 | 1 | 6 | 1 | 10 | 2 | 13 | 4 |
| 1-pentanol | 676 | 27 | 4 | 16 | 2 | 6 | 2 | 5 | 2 |
| 3-methyl-1-butanol | 701 | 14 | 2 | 21 | 4 | 41 | 10 | 325 | 28 |
| 4-methyl-2-hexanol | 729 | 15 | 5 | 29 | 8 | 27 | 3 | 46 | 13 |
| 3-methyl-3-buten-1-ol | 732 | 1 | 0 | 4 | 1 | 13 | 3 | 13 | 1 |
| 1-octen-3-ol | 741 | 53 | 7 | 57 | 18 | 48 | 9 | 39 | 11 |
| 3-methyl-2-butanol | 748 | 3 | 1 | 4 | 1 | 9 | 4 | 33 | 12 |
| P-menth–1-en–8-ol | 749 | 10 | 1 | 8 | 1 | 7 | 1 | 7 | 1 |
| Tricyclo(1,5)dec-5-en-8-ol | 759 | 25 | 6 | 15 | 2 | 10 | 2 | 6 | 1 |
| Phenylethyl-alcohol | 792 | 2 | 0 | 3 | 1 | 3 | 0 | 33 | 9 |
| Ursane-3,16-diol | 812 | 17 | 7 | 11 | 4 | 10 | 6 | 23 | 5 |

**Table 2**
***(continuation)***

| **Compounds** | **Uninoculated *Tsire*** | | | | | | | | ***P*-value** | **MI** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **SD1** | **StdD** | **SD2** | **StdD** | **SD3** | **StdD** | **SD4** | **StdD** | | |
| ***Class 3 – Aromatic/cyclic*** | | | | | | | | | | |
| Cyclopentene | 77 | 12 | 79 | 9 | 76 | 22 | 174 | 17 | 0.005 | 2 |
| 1-pentyl-propyl-Cyclopropane | 7 | 2 | 3 | 1 | 7 | 2 | 9 | 2 | 0.0061 | 2 |
| Ethylbenzene | 12 | 2 | 9 | 3 | 6 | 0.63 | 6 | 1 | <0.001 | 2 |
| 2-methoxy–2-propenyl-Benzene | 40 | 12 | 48 | 9 | 50 | 11 | 71 | 5 | 0.004 | 2 |
| O-xylene | 8 | 3 | 6 | 2 | 4 | 0.87 | 4 | 0.63 | 0.005 | 2 |
| Tetramethyltricyclo-Undec–2-ene | 8 | 2 | 8 | 3 | 9 | 2 | 9 | 3 | 0.2719 | 2 |
| Hexamethyl-cyclotrisiloxane | 3 | 1 | 5 | 2 | 6 | 2 | 12 | 3 | 0.0331 | 2 |
| ***Class 4 – nitrogenous*** | | | | | | | | | | |
| 1-methyl-1h-pyrrole | 98 | 21 | 103 | 31 | 113 | 14 | 144 | 28 | 0.004 | 2 |
| 3-methyl-butanenitrile | 14 | 2 | 13 | 5 | 16 | 8 | 21 | 3 | 0.0017 | 1 |
| 2-methyl-pyrazine | 11 | 2 | 17 | 5 | 51 | 11 | 73 | 3 | 0.0004 | 2 |
| Trimethyl-pyrazine | 1020 | 37 | 987 | 29 | 975 | 184 | 760 | 35 | 0.03 | 2 |
| Tetramethyl-pyrazine | 176 | 18 | 156 | 39 | 129 | 10 | 46 | 9 | 0.024 | 2 |
| 3-ethyl-2,5-dimethyl-pyrazine | 4 | 0 | 9 | 4 | 39 | 9 | 33 | 3 | 0.0728 | 2 |
| 2,5-dimethyl-pyrazine | 68 | 12 | 820 | 123 | 2462 | 128 | 3783 | 210 | 0.0003 | 2 |
| 2,3-dimethyl-pyrazine | 31 | 13 | 29 | 5 | 22 | 2 | 21 | 0 | 0.013 | 2 |
| ***Class 5 – alcohols*** | | | | | | | | | | |
| 1-propanol | 2 | 0 | 5 | 2 | 13 | 6 | 18 | 10 | 0.0009 | 1 |
| 2,6-dimethyl-4-heptanol | 4 | 1 | 10 | 2 | 19 | 4 | 27 | 7 | 0.004 | 2 |
| 1-pentanol | 27 | 6 | 19 | 2 | 10 | 2 | 8 | 2 | 0.005 | 1 |
| 3-methyl-1-butanol | 19 | 4 | 33 | 11 | 910 | 40 | 1455 | 300 | 0.0035 | 2 |
| 4-methyl-2-hexanol | 14 | 2 | 22 | 12 | 42 | 4 | 59 | 6 | <0.0001 | 2 |
| 3-methyl-3-buten-1-ol | 1 | 0 | 6 | 1 | 13 | 1 | 20 | 2 | 0.0001 | 2 |
| 1-octen-3-ol | 53 | 8 | 59 | 11 | 64 | 10 | 97 | 19 | 0.019 | 2 |
| 3-methyl-2-butanol | 2 | 0 | 16 | 7 | 27 | 6 | 35 | 10 | 0.0005 | 2 |
| P-menth–1-en-8-ol | 9 | 1 | 7 | 1 | 9 | 3 | 11 | 3 | 0.0189 | 2 |
| Tricyclo(1,5)dec-5-en-8-ol | 25 | 3 | 21 | 6 | 14 | 2 | 9 | 1 | 0.017 | 2 |
| Phenylethyl-alcohol | 2 | 1 | 3 | 1 | 19 | 4 | 36 | 5 | 0.0004 | 2 |
| Ursane-3,16-diol | 6 | 1 | 17 | 3 | 13 | 4 | 20 | 3 | 0.009 | 2 |

Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; *P*-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library

The total area units (TAUs) of the class of ketone compounds identified in the meat product are shown in Table 1. The volatiles acetone, 2-butanone, 2,3-butanedione, 3-hydroxy–2-butanone, 2-hexanone, 2-heptanone and 1-hydroxy–2-propanone were among the prominent ketone compounds which recorded significant differences ($p<0.05$) in their TAUs between the IS and UC samples. Some of the ketone volatiles have been noted to play important roles in sensory characteristics of meat products [7]. One of the important ketone compound was 3-hydroxy–2-butanone (acetoin), a product of degradation due to maillard reaction; its identification from meat products has been reported [3,16]. Presence of this compound especially in relatively high concentration may cause spoilage of food [17]. Lower values of TAUs of acetoin ($p=0.0004$) were recorded in IS than UC samples, indicating that inoculation of the meat product with starter culture had significant effect on the compound; this observation may help enhance shelf life of the product. The reduced TAU of acetoin in the IS sample may be attributed to possible antioxidative property of *P. acidilactici* FLE07 used as starter culture [18,19,20], which may bring about reduction of undesirable volatile compounds in meat products. Acetoin has been reported as a spoilage molecule associated with in meat products during storage [21].

The compounds, 2-hexanone, 2-heptanone and 2-butanone were among the ketone compounds identified in the present study, and they have been noted as contributors to off flavour development in meat products [22]; however their TAUs were lower ($p<0.05$) in the IS samples than UC. This may therefore translate that they are present in reduced concentration in IS than UC samples, indicating that there may be reduction of off flavour development in the meat product inoculated with the starter culture. Another ketone compound identified in this study was 2,3-butanedione (diacetyl), which has been reported as a product of lactose and citrate metabolism by the action of bacteria, especially LAB [23]. The occurrence of the compound in *Tsire* is in support of a similar report by Huan *et al*. [24] in a research investigation during storage of a Chinese meat product – *Jinhua ham*. Diacetyl may be of technological importance as it possesses anti-microbial properties against many unwanted microorganisms, especially the spoilage types, in foods [25].

The volatile compounds in class 2 comprised of 2-methyl-propanoic acid, hexanoic acid, 3-methyl-butanoic acid and 2-methyl-hexanoic acid, all of which had higher TAUs in IS than UC (Table 1). Significant difference ($p<0.05$) was recorded in the acids of IS and UC, with the exception of hexanoic acid. Lower values of TAUs were recorded for the acids ($p<0.05$) in IS than UC, and this may be of significance as a result of possible association of certain acids, especially 3-methyl-butanoic acid, butanoic acid (and some of its derivatives) with meat spoilage [17]

The seven volatile compounds of aromatic/cyclic hydrocarbons belonging to class 3 compounds in IS were significantly different ($p<0.05$) from those of UC (Table 2), with the only exception of tetramethyltricyclo-undec-2-ene. They have reduced TAUs in the IS samples compared to UC, indicating possible influence of the starter culture. One of the compounds belonging to class 3 was ethylbenzene, which presence in the meat product may be very significant as it may be associated with spoilage of meat and fish [17,26]. The reduced values of TAUs of the compound in the IS samples is therefore desirable towards possible reduction of spoilage in the meat product. The nitrogenous compounds (class 4) consisted mostly of pyrazines, which are regarded as products of maillard reactions; their formation in meat products could be attributed to application of heat and salting during processing [26]. Contribution of pyrazines to development of desirable sensory characteristics of grilled and roasted meat has been reported [16]. The identification of nitrogenous compounds, 2,3-dimethyl-pyrazine and tetramethyl-pyrazine in meat products was observed by Gianelli *et al.* [16], thus supporting their occurrence in *Tsire* in the present study. The class 5 volatile compounds consisted of twelve alcohol compounds (Table 2), most of which recorded lower values of TAUs ($p<0.02$) in IS samples than UC. Among

these compounds, 1-octen-3-ol and 3-methyl-1-butanol have been noted as spoilage molecules in meat products [21]. The lower values of TAUs of these compounds in IS samples than UC may thus indicate possible extension in shelf life of the former over the latter.

The chart of PCA carried out on the volatile compounds during storage of *Tsire* samples inoculated with starter culture is presented in Figure 2. At the upper portion in the left hand side of the chart are represented the principal components associated with the meat samples on storage day 1 (Sd1). The principal components on Sd1 were composed of 2,3-dimethyl-pyrazine, o-xylene, p-menth-1-en-8-ol, ethylbenzene, hexamethyl-cyclotrisiloxane, 3-methyl-butanoic acid, 1,2-diepoxyhexadecane, 1-pentyl-2-propyl-cyclopropane, tetramethyltricyclo-undec-2-ene, and 3-methyl-butanenitrile, which represent 15.6% of the total volatile compounds (TVCs) in the meat product. On Sd2 (lower portion of the left hand side), the principal components included octane, 2-hexanone, 1-methyl-1h-pyrrole, 2,3-butanedione, acetone, 1-pentanol, (e)-3-dodecene, heptane, trimethyl-pyrazine, 2-pentyl-furan, tricycle(1,5)dec-5-en-8-ol, tetramethyl-pyrazine, 2-methoxy-2-propenyl-benzene and 5-methyl-2-hexanone, representing 21.9% of TVCs. A major cluster was formed on Sd2 consisting mainly of ketones, alcohols and pyrazines, suggesting that these compounds were the major ones associated with the product on second day of storage. On Sd3 (lower portion in the right hand side of chart), the principal components identified were 3-methyl-3-buten-1-ol, 2-octanone, 2-methyl-propanoic acid, 1-hydroxy-2-propanone, nonanal and hexanoic acid, forming 9.4% of TVCs. Twenty two principal components (representing 34.4% of TVCs) occurred on Sd4 (upper portion in the right hand side of chart), belonging mainly to alcohols, ketones, nitrogenous compounds, acids and pyrazines.

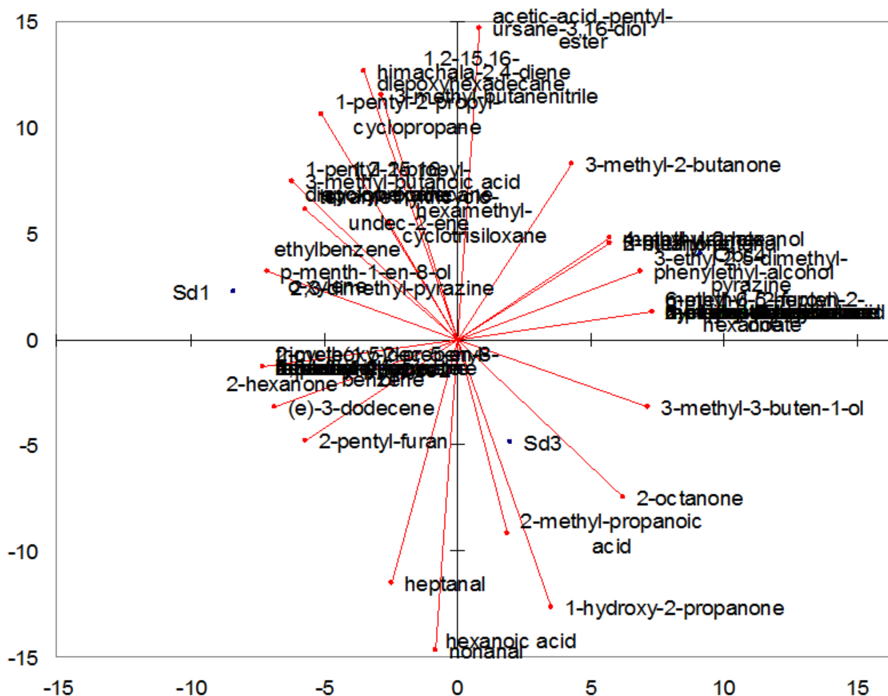


**Figure 2. Principal component analysis of volatiles and SPME-GCMS peak area data from the headspace of inoculated *Tsire***

Sd1, Storage day 1; Sd2, Storage day 2; Sd3, Storage day 3; Sd4, Storage day 4.

The mean quantities (µg/g) of some volatile molecules identified in the meat product are shown in Table 3. Result indicated that compounds which have been regarded as spoilage indicators of meat had higher TAUs in UC than IS samples ($p<0.05$), suggesting the influence of the starter culture used. The compounds included 3-hydroxy-2-butanone, heptanal, nonanal, 2-butanone, 1-octen-3-ol and 3-methyl-butanoic acid, some of which had been noted earlier.

**Table 3**

**Mean quantities (µg/g) of some head space compounds in *Tsire***

| Compounds | RI | *Tsire* inoculated with *P. acidilactici* FLE07 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD1 | StdD | SD2 | StdD | SD3 | StdD | SD4 | StdD |
| 2-butanone | 618 | 38.52 | 2.78 | 38.47 | 4.84 | 42.17 | 9.26 | 47.39 | 11.93 |
| 2,3-butanedione | 621 | 37.23 | 11.02 | 29.83 | 5.4 | 30.03 | 5.82 | 16.73 | 3.29 |
| 2-hexanone | 699 | 1.9 | 0.51 | 1.76 | 0.25 | 1.7 | 0.42 | 1.21 | 3 |
| 3-methy-1-butanol | 701 | 1.26 | 0.54 | 1.82 | 0.37 | 3.37 | 0.09 | 29.93 | 7.26 |
| 2-heptanone | 719 | 0.23 | 0.01 | 1.16 | 0.09 | 1.65 | 0.39 | 2.17 | 0.82 |
| Heptanal | 725 | 1.62 | 0.08 | 0.58 | 0.03 | 0.57 | 0.07 | 0.49 | 0.11 |
| 3-hydroxy-2-butanone (acetoin) | 727 | 23.26 | 9.18 | 25.01 | 4.19 | 25.92 | 7.02 | 27.28 | 5.4 |
| 1-octen-3-ol | 741 | 2.36 | 0.19 | 2.41 | 0.24 | 1.98 | 0.08 | 2.13 | 0.17 |
| Ethylbenzene | 747 | 1.22 | 0.18 | 0.69 | 0.04 | 0.31 | 0.07 | 0.32 | 0.01 |
| 3-methyl-butanoic acid | 756 | 2.31 | 0.77 | 1.29 | 0.14 | 0.93 | 0.04 | 0.77 | 0.12 |
| Nonanal | 982 | 1.59 | 0.09 | 1.62 | 0.42 | 1.39 | 0.41 | 0.89 | 0.02 |

| Compounds | Uninoculated *Tsire* | | | | | | | | *P*-value | MI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SD1 | StdD | SD2 | StdD | SD3 | StdD | SD4 | StdD | | |
| 2-butanone | 38.51 | 9.28 | 47.83 | 7.31 | 49.15 | 8.97 | 59.53 | 9.28 | 0.035 | 2 |
| 2,3-butanedione | 37.19 | 5.47 | 33.89 | 10.29 | 30.95 | 12.4 | 31.28 | 8.35 | 0.013 | 1 |
| 2-hexanone | 1.87 | 0.24 | 1.85 | 0.71 | 1.83 | 0.61 | 1.84 | 0.12 | 0.007 | 1,2 |
| 3-methyl-1-butanol | 1.28 | 0.08 | 1.97 | 0.28 | 50.17 | 10.3 | 61.29 | 14.29 | 0.005 | 2 |
| 2-heptanone | 0.22 | 0.03 | 2.27 | 0.93 | 10.41 | 2.19 | 12.86 | 0.84 | 0.009 | 2 |
| Heptanal | 1.6 | 0.18 | 1.89 | 0.55 | 2.43 | 0.82 | 5.86 | 0.93 | 0.008 | 1 |
| 3-hydroxy-2-butanone (acetoin) | 23.75 | 4.5 | 29.72 | 10.2 | 34.12 | 2.93 | 37.84 | 4.72 | 0.005 | 2 |
| 1-octen-3-ol | 2.42 | 0.26 | 2.97 | 0.36 | 3.21 | 1.02 | 4.32 | 0.63 | 0.008 | 2 |
| Ethylbenzene | 0.93 | 0.03 | 2.92 | 0.07 | 3.1 | 0.74 | 3.96 | 1.01 | 0.01 | 2 |
| 3-methyl-butanoic acid | 2.32 | 0.09 | 2.83 | 1.29 | 2.94 | 0.91 | 2.89 | 0.72 | 0.027 | 2 |
| Nonanal | 1.6 | 0.16 | 2.43 | 0.34 | 2.94 | 1.04 | 3.72 | 0.65 | 0.005 | 1 |

Each value is mean of three replicates of samples; SD1, storage day 1; SD2, storage day 2; SD3, storage day 3; SD4, storage day 4; StdD, standard deviation; *P*-value, probability value; RI, retention index; MI, method of identification; 1, Identification using authentic standards; 2, Identification using retention indices from the literature and their mass spectra with the NIST mass spectral library

Result of sensory study carried out on the meat samples during storage is presented in Table 4. It was observed that IS samples recorded higher mean scores by the panelists than UC ($p<0.05$) from 24 h of storage in the sensory properties of aroma, appearance, tenderness and taste. The IS samples also recorded higher preference than UC in term of general acceptability ($p<0.05$). The result of sensory study corroborates the report of Calo-Mata *et al.* [27] who noted that LAB cultures may be used to develop desirable sensory characteristic properties in food products. The number of panelist who allocated scores higher than 5 to the meat samples decreased gradually as storage period progressed, the decrease was however more pronounced in UC than IS samples. Result further indicated that IS samples recorded higher acceptability by consumers than UC ($p<0.05$).

**Table 4**

**Result of sensory study on the *Tsire* samples during storage**

| SP (h) | Samples | Aroma | Appearance | Tenderness | Taste | G.acceptability | % Acceptability (% n who scored > 5) |
|---|---|---|---|---|---|---|---|
| 0 | F | 7.5 ± 1.29 | 6.9 ± 0.18 | 7.8 ± 0.76 | 7.6 ± 2.01 | 8.1 ± 1.29 | 100 |
| 24 | IS | 7.3 ± 1.20 | 7.0 ± 0.92 | 7.4 ± 0.87 | 7.1 ± 2.30 | 7.5 ± 2.17 | 90 |
| | US | 6.9 ± 0.98 | 6.1 ± 1.28 | 6.2 ± 1.07 | 6.7 ± 0.29 | 6.9 ± 1.55 | 75 |
| 48 | IS | 7.0 ± 2.08 | 6.5 ± 1.33 | 6.9 ± 0.94 | 6.8 ± 0.67 | 6.4 ± 1.36 | 87 |
| | US | 5.8 ± 0.93 | 5.3 ± 0.77 | 5.1 ± 1.25 | 5.3 ± 1.33 | 5.2 ± 0.55 | 59 |
| 72 | IS | 6.2 ± 1.20 | 6.0 ± 0.88 | 6.3 ± 1.29 | 6.1 ± 0.82 | 5.9 ± 0.72 | 74 |
| | US | 4.7 ± 0.73 | 4.2 ± 0.15 | 4.3 ± 1.08 | 3.9 ± 0.73 | 3.8 ± 0.88 | 36 |

Each value is mean of three replicates of samples; SP, Storage period; *F*, freshly prepared *Tsire*; *IS*, *Tsire* samples inoculated with starter culture; *US*, uninoculated *Tsire* samples; G.acceptability, General acceptability; *n*, number of panelists

From the results of this study, it was concluded that inoculation of *Tsire* with *P. acidilactici* FLE07 as starter culture had significant and desirable influence on the associated volatile compounds and sensory properties. This observation is in support of previous studies which reported impact of LAB cultures on volatile compounds of meat products. It was further concluded that the use of *P. acidilactici* FLE07 may contribute to extended shelf life of *Tsire* during storage as a result of reduction recorded in some of the known spoilage molecules. Suitable LAB starter cultures may therefore be applied towards promoting sensory quality development and availability of the traditional meat product beyond the day of production.

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# Improving the nutritional value of grains by biological activation

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## Abstract

**Introduction.** Cereal products are a source of carbohydrates, protein, macro and micronutrients, vitamins, enzymes, dietary fiber, phospholipids. Sprouting grains is one of the methods for biological activation.

**Materials and methods.** Grains of wheat, triticale and naked oats have been studied. Protein was determined by Bradford method, the starch content – by poalrimetric method. Fat was determined by exhaustive extraction with chemically pure hexane. Vitamins $B_1$, $B_2$, $B_3$, $B_6$ were determined by the method of fluorometry. Vitamins PP and E were determined colorimetrically. Determination of vitamin C was performed by a titration method.

**Results and discussion.** An important task in the process of preparing raw materials for health products is increasing their food and biological value. We proposed a regime of hydrothermal processing of grain at temperatures 12–16 °C. Under these conditions, activation of the enzyme complex, reduction of grain density and increase of its per unit volume; activation of the synthesis of vitamins and vitamin-like substance take place. In the process of biological activation of grain the bioavailability of proteins, carbohydrates, fat increases due to their partial hydrolysis. It has been studied, that the content of fibres,which is natural food sorbent, in biologically activated grains of wheat, naked oats and triticale is respectively 2,68, 2,34, 2,62%. It has been found that in the proposed processing of wheat, triticale and naked oats the quantity of vitamin C increased more than twice. The content of tocopherols increases tenfold, routine – 2,5–3 times. It was established that after hydrothermal treatment the total number of colony-forming units of mesophilic aerobic and facultative anaerobic microorganisms in native and dried corn samples is within the norms established by standards.

**Conclusions.** The results are of practical importance, as they allow to recommend the use of biologically activated wheat, triticale, naked oats for the production of food of health, functional and health-care purposes.

## Introduction

Production of functional foods is a priority for all developed countries. The inclusion of functional foods to the daily diet will allow to improve physical and cognitive functions of organism, reduce the risk of many diseases, improve the genetic potential of humanity.

Grain raw materials is one of the main food basics for functional foods creation.

The aim of work is to research the increase of nutritional value of grain cereal crops through biological activation under the proposed regimes for its use in the creation of health food, functional and preventive nutrition.

Objectives of research:

- To propose mode of treatment of grain of cereals to increase its nutritional value;
- To explore the change in content of basic substances of grain in the proposed mode of treatment;
- To explore the change in content of vitamins in wheat, triticale and naked oats during processing;
- To determine the microbiological quality of grain, prepared by the proposed regime.

## Analysis of the literature

Foods from whole grain cereals, which include shell of the grain aleurone layer and the germ contain powerful antioxidants – vitamin E, C, carotenoids, choline, folate; cofactors of antioxidant enzymes – microelements Se, Cu, Mg, food sorbents – cellulose, lignin, lignin [1].

Scientists studied the total number of phenolic compounds, flavonoid content and antioxidant activity of extracts of different fractions of grain cereals grinding. It has been established that the flour of various sizes has lower antioxidant potential than bran fractions (fine and coarse bran). The extract of barley had the highest values of antioxidant activity and polyphenol content [2].

Improving the quality of raw materials is an important task of the food industry.

Recently, new technologies of unconventional processing of grain cereals that provide germination process have been developed.

Sprouting as a method of biological activation, is used to increase the nutritional value of grain and other raw materials.

During germination of wheat, the bioavailability of dietary compounds is increased by partial hydrolysis of starch, protein, hemicellulose and cellulose; increase in content of vitamins, bioelements and other biologically active substances. In addition, the activity of some antialimentary active substances (inhibitors of enzymes, hemagglutinin) decreases during germination, which promotes full absorption of grain nutrients [3].

Due to utility of germinated grains, they were used to produce malt extracts from oats, barley, wheat, corn and peas. These products show a strong recreational effect on the human body, stimulate metabolism, improve efficiency, increase the activity of the body and its resistance to harmful factors [4].

Sprouted grain is a holistic living organism that possesses natural biological properties and is in the stage of maximum vitality. Sprouted grain is a natural biogenic product that has high energy potential, the total antioxidant capacity of which is 3 –10 times higher (depending on culture) compared to the native grain.

Regular consumption of sprouted grain stimulates metabolism, hematopoiesis , improves immunity, compensates for vitamin and mineral deficiency, normalizes the acid-

alkaline balance, cleans the body of toxins, contributes to good digestion, increases potency and slows the aging process [5].

Sprouted grain wheat, which contains plant protein, is recommended to be included in the diet intended as general strengthening of the body and enhancing or restoring sexual activity in particular [6], reducing the risk of the emergence and development of cancer [7], enrichment of child's body in biologically active substances [8]. The consumption of sprouted grains improves reproductive function [9].

During properly organized process of germination of grain its basic nutrients – proteins, fats, carbohydrates – split under the influence of enzymes to form more simple components that are easily absorbed by the human body, with a significant increase in amounts of vitamins and vitamin-like substances. In addition, the germinated seeds contain some kind of natural food sorbents – cellulose, hemicellulose, lignin, which are indispensable components of food. That is why sprouted grain is used in many developed countries as a valuable supplement to the diet, which promotes recovery and longevity.

There are data on the significant increase in the content of carotenoids and vitamin E during germination of wheat, and the authors note that the maximum content of tocopherols is increased on the second day, and the amount of carotenoids on the seventh day of germination [10].

To improve the nutritional value of cereal grains and legumes, his soaking and sprouting in water, solutions of sorbic acid and sea salt is recommended to combine with the deep freeze, which contributes to the increase in number of reduced sugars [11].

Scientists say that the flour obtained from grain sprouted in sea salt solution contains more amino acids and fatty acids as a result of activity of enzymes. In addition, mineral composition is improved: the content of magnesium, calcium, potassium, iron, zinc, copper increases 14.8; 7.5; 3.0; 4.7; 4.2; 6.3 times respectively compared with ordinary wheat flour [12].

Sprouting for 48 hours improves the chemical composition of the fruit of the African yam, a combination of such raw materials with sprouted grain of corn allows to get a biologically valuable supplement to the diet [13].

Application of sodium selenate ($Na_2SeO_4$) during germination of rye allows to get grain enriched with this important microelement, besides, increases extractivity of received malt [14].

Analyzing the effect of duration of germination on the nutritional value of wheat, scientists found that after biological activation of wheat for 24 h the amount of vitamin E increases by 6.5 times, the amount of niacin (PP) increases by 1.3 times, the content of vitamins $B_2$ and C 6 increases respectively by 26 and 65%. The total number of amino acids decreases by 17.4% [15].

It has been investigated that B-group vitamins, vitamins C and E, β-carotene are accumulated in the process of grain sprouting gradually. The maximum increase in the content of folic acid by 3.6 times in germinated seeds of wheat and by 1.7–3.8 times in rye was fixed on the fifth day of germination [16].

It has been established that the rate of creating of vitamins E, C, B is maximum during the development of germ from burgeoning to the size of 2–3 mm, which indicates the feasibility of using sprouted grains for producing health foods in the initial stage of germination.

The use of biologically activated wheat in the bakery makes it possible to increase the biological value of bakery products [17].

The biologically activated wheat contains significant amounts of vitamins and fiber, so its use is appropriate for the enrichment of bread. During the germination of seeds redox

systems that have B vitamins in their structure are formed. It has been studied that the content of B vitamins, including $B_1$, $B_2$, $B_5$, $B_6$ 5–10 times higher in sprouted wheat grain than in mature one. Also, vitamin C is synthesized during the process of grain sprouting. The inclusion of biologically activated wheat to bread recipe in amount of 15% allows to get a product with good organoleptic and technological characteristics [18].

A new kind of corn bread from sprouted wheat "Colossus", which has high quality, including increased specific volume of the bread pan, well-developed uniform crumb porosity, improved microbiological indicators, extended shelf life has been developed [19].

The influence of biological activation of grain cereals, including wheat, triticale, naked oats on the change of the content of its basic nutrients is a key issue; in literature no relevant data are available.

## Materials and methods

The object of research is the grain of wheat, triticale and naked oats of such breeds as accordingly Myronivska 137, Molfar, Solomon of harvest of 2015 were used for studies.

Protein was determined by Bradford method [20], the starch content – by physical method. The physical method based on starch dissolving and determination of the solution poalrimetric deviation. Fat was determined by exhaustive extraction with chemically pure hexane. Vitamins $B_1$, $B_2$, $B_3$, $B_6$ were determined by the method of fluorometry. Vitamins PP and E were determined colorimetrically.

Determination of vitamin C was performed by a titration method. The method is based on extraction of vitamin C from the sample with solution of acid (hydrochloric, metaphosphoric acid or a mixture of acetic and metaphosphoric), followed by further titration potentiometrically or visually with solution of 2,6-dyhlorfenolindyfenolyat sodium**.**

Microbiological samples of grain of wheat, triticale and naked oats native and biologically activated was determined after drying to a moisture content of 11–12%.

With this aim the studied samples were plated on agar surface of nourishing source: meatpepton agar (detection of mesophilic aerobic and facultative anaerobic microorganisms) wort-agar (yeast and fungi).

Cups of crops were incubated for 2–3 days at 37 °C for determining the total number of microorganisms. Crops in the cup of the wort-agar medium for detection of fungi and yeast were incubated at a temperature of 28 °C for 5–7 days.

## Results and discussion

Hydrothermal processing of grain is used in flour and cereal industries, the production of animal feed. It is known that hydrothermal processing of grain influences the anatomical, physical, chemical, structural, mechanical and biochemical properties of grain, changing its technological properties. Traditional modes of cold air-conditioning in the production of flour and grain provide moistening and long moistening – for 4–8 or 16–24 hr., depending on the type of wheat, to a moisture content of 15,5–17% at 20 to 40 °C. Under these conditions the scarifiering of endosperm, the formation of cracks in it; changing the thickness of the shells and aleurone layer, increasing of their flexibility and strength take place, the moisture of corn increases by 1,5–2%. These factors increase the degree of extraction of endosperm, flour yield, reduce energy consumption for grinding grain. In

cereal production the aim of hydrothermal treatment is to increase core strength, providing a higher yield of whole grains. So, hydrothermal treatment regimes include steaming under pressure and tempering of grain. The production of wheat cereals of cold conditioning is carried out at a temperature of 30–40 °C.

We proposed the regime of hydrothermal processing – cold air conditioning at temperatures 12–16 °C for 25–30 hours. Under these conditions, grain moisture increases by 30–35%, which leads to activation of the enzyme complex, decrease in grain density and increase in its per unit volume. As a result of the intensification of enzymatic processes a partial hydrolysis of carbohydrates and proteins, change of conformation of proteins, activation of the synthesis of vitamins and vitamin – like substances take place.

The relative change in weight of wheat, triticale and naked oats in the process of hydrothermal treatment has been investigated. Setting the intensity of mass change that characterizes the percentage of moisture absorbed by grain during each hour in the process of humidification showed that the highest intensity of grain swelling occurs within four hours of active humidification. This pattern of moisture absorption occurs during the first cycle of hydrothermal processing of grain. After reaching significance humidity of 30 – 35% of the swelling process slows down dramatically.

The change in content of essential nutrients of grain cereals in the proposed regime of hydrothermal processing has been investigated, the data are presented in table 1.

**Table 1**

**The content of organic substances in major grain cereals**

| Cereals | Proteins,g | Fats, g | Starch, g | Cellulose, g |
|---|---|---|---|---|
| Native grains | | | | |
| Wheat | 12,5 | 1,72 | 68,04 | 2,45 |
| Oat | 16,8 | 5,3 | 40,52 | 2,26 |
| Triticale | 13,0 | 1,97 | 65,41 | 2,59 |
| Biologically activated grain | | | | |
| Wheat | 10,8 | 2,52 | 54,36 | 2,68 |
| Oat | 14,2 | 6,52 | 34,61 | 2,34 |
| Triticale | 11,8 | 2,7 | 52,3 | 2,62 |

The decrease in the total number of proteins was marked iIn the process of biological activation of grain that is consistent with the literature and is explained by the removal of protein molecules from amino acids that take a direct part in metabolic processes occurring in plant tissues and cells. Fat content in the grain increases during processing, which is associated with the mutual conversion of carbohydrates proteins and fats. A significant decrease in the number of carbohydrates due to hydrolysis to sugars has been marked.

Dietary fiber is a complex of natural polysaccharides of plant origin, which have water-retaining, fat-connecting, gelling and sorption properties. Dietary fiber can adsorb toxic substances, heavy metals, radionuclides, bile acids, cholesterol. Fiber as one of the main components of dietary fiber is an activator of digestive enzymes, their stabilization occurs on its surface and enhances the activity of enzyme systems, improving detoxification processes.

It has been studied that fiber content in the biologically activated grain cereals of wheat, naked oats and triticale is respectively 2,68, 2,34, 2,62%.

Due to the high water-retaining capacity, fibers have positive effect on digestion, they occupy a significant volume of the intestine and increase its motility.

We investigated the water-retaining capacity of fiber of biologically activated wheat, oats and triticale. When the temperature rises the water-retaining capacity increases significantly. The research was conducted at room temperature and at 36 °C, to bring to the terms of stay of dietary fiber in the gastrointestinal tract of the human body. Figure 1 shows indicators of water-capacity of studied fiber grain cereals.

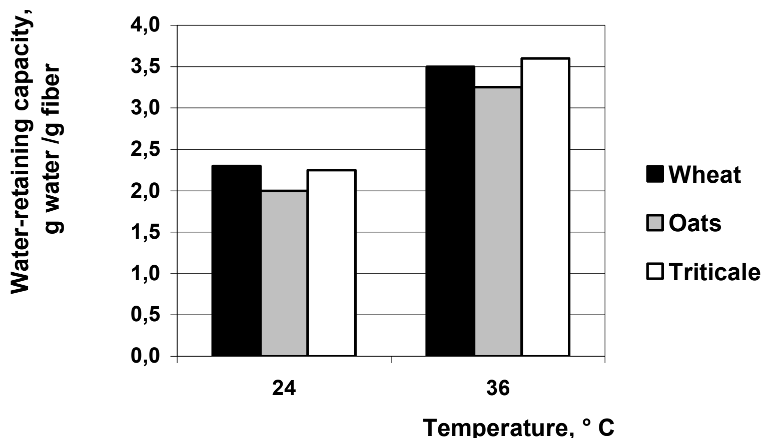


**Figure 1. The water-retaining capacity of fiber of wheat, oats and triticale**

So, from the received data we can conclude that for their waterhold ability dietary fibers of wheat, oats and triticale relate to water-retaining fibers that connect from 2 to 8 g water/g of fiber.

During the hydrothermal treatment under the proposed regime the change of content of vitamins that exhibit antioxidant properties of tocopherol, ascorbic acid, rutin has been researched (Tab. 2).

**Table 2**

**The content of vitamins C, E, and P in grain cereals**

| Cereals | Vitamin P, mg% | Vitamin E, mg% | Vitamin C, mg% |
|---|---|---|---|
| Native grain | | | |
| Wheat | 3,9 | 0,34 | 2,6 |
| Oat | 3,4 | 0,21 | 1,2 |
| Triticale | 4,5 | 0,47 | 2,3 |
| Biologically activated grain | | | |
| Wheat | 9,3 | 10,73 | 5,7 |
| Oat | 7,9 | 9,26 | 3,8 |
| Triticale | 9,2 | 10,82 | 4,1 |

Vitamin E (tocopherol) is found in a germ of grain. Its main role is to protect unsaturated fatty acids, which are important for the integrity of cell membranes, against free radicals. In human body, vitamin E improves blood circulation, prevents blood clots and is a synergist of vitamin A.

Vitamin C is a component of the oxidation-reduction systems, it prevents the formation of excess of oxidative free radicals, promotes the oxidation of cholesterol, involved in the synthesis of connective tissue structures and the formation of a number of hormones that positively affect most parts of the immune system.

Substances with P-vitamin activity strengthen capillary walls and thereby reduce their permeability. They are involved in tissue respiration, increase the effect of ascorbic acid.

Experimental studies have established that during the proposed preparation of wheat, triticale and naked oats vitamin C increases more than twice. The content of tocopherols increases tenfold, routine – by 2,5–3 times.

It has been investigated that during hydrothermal treatment under the proposed regime the content of water-soluble vitamins in grains of wheat, triticale and naked oats also significantly increases: the number of thiamine and riboflavin increases by 2–2,5 times; the content of pantothenic acid, pyridoxine, nicotinic acid and inositol increases by 1,5–2 times.

The total number of colony forming units of mesophilic aerobic and facultative anaerobic microorganisms (CFU MANFAnM) was determined in native and dried corn samples after hydrothermal treatment. The results of the research of microbiological indicators of grain are given in Table 3.

**Table 3**

**Microbiological indicators of grain**

| **A sample of grain** | **Microbiological parameters** | | |
|---|---|---|---|
| | **Quantity MAFAnM, CFU/g, not more** | **Molds, CFU/g, not more** | **Pathogenic microorganisms, including Salmonella in 25 g** |
| Cereal grains, normative values | $5 \cdot 10^3$ | 50 | Not allowed |
| Grain of wheat | $2 \cdot 10^2$ | 30 | Not found |
| Grain of oat | $3 \cdot 10^2$ | 25 | Not found |
| Grain of triticale | $2 \cdot 10^2$ | 21 | Not found |
| Grain of wheat, biologically activated | $3 \cdot 10^2$ | 25 | Not found |
| Grain of oat, biologically activated | $2 \cdot 10^2$ | 20 | Not found |
| Grain of triticale, biologically activated | $3 \cdot 10^2$ | 20 | Not found |

As a result of studies it has been found that microbiological fertilizing of native wheat, triticale and naked oats and biologically activated is not higher than permissible values of microbiological fertilizing, therefore the grain after the proposed treatment is secure raw materials for food production in terms of microbiological purity.

## Conclusions

It has been established that during hydrothermal processing of grain of wheat, triticale, naked oats by the proposed regime its food and biological value increases through the partial conversion of the main components – proteins, fats and carbohydrates in a more accessible for assimilation form; a significant increase in content of vitamins, especially those that exhibit antioxidant properties, B vitamins. Biologically activated grain cereals with intact structure, without separation of membranes are a source of natural food sorbents. The received results are of practical importance, as they allow to recommend the use of biologically activated wheat, triticale, oats for the production of health, functional and health-care purposes foods.

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# Raspberry and blackberry pomaces as potential sources of bioactive compounds

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## Abstract

**Introduction.** The concern for obtaining some secondary metabolites with health-beneficial effects has initiated studies on valorization of by-products. In this sense, fruit processing by-products have attracted great interest as sources of bioactive compounds.

**Materials and methods.** Pomaces of raspberry and blackberry were obtained after juice separation. These byproducts were characterized in terms of total phenolic content (TPC) by Folin-Ciocalteu method and total monomeric anthocyanins content (TAC) by pH differential method. Also, antioxidant activity of pomaces against stable 1,1-diphenyl–2-picrylhydrazyl (DPPH) radicals was evaluated.

**Results and discussion.** Pomace of blackberry was characterized by significantly higher TPC (10.1 mgGAE/g) and TAC (6 mg C3GE/g) compared to raspberry (8.2 and 3.6 mg/g, respectively). DPPH radical scavenging activity was similar, with slightly higher values in pomace of raspberry (11.7 μmolTrolox/g) than in blackberry (10.9 μmolTrolox/g). The total soluble solids was 9.3 $^{o}$Bx in RP sample, significantly lower than in BP sample with value 14.5 $^{o}$Bx.

This study indicates that both, raspberry and blackberry pomaces, as wastes of juice production, could be used as a cheap source of bioactive compounds with strong antioxidant activity. Therefore, these pomaces should be considered as a raw material for production of valuable dietary supplements and natural colorants for development of new products with high value added. By-products of fruit processing could be used due to convenience, accessibility and low cost, as suitable way to increase health properties of food. The potential utilization of fruit pomaces could be the enrichment of different products such as bakery products, biscuits, cookies, paste, ice cream, fruit yogurts etc.

Additionally, the potential applications of the ethanol used for bioactive compounds extraction in this study must be considered on the basis of the solvent percent choice, liquid–solid ratio, temperature, and time applied.

**Conclusion.** Raspberry and blackberry pomaces, discarded during juice production, represent significant sources of bioactive compounds, such as phenolic antioxidants.

## Introduction

In recent years, food industry has been facing to high expectations that food products should meet consumers' demands for a healthy life style. Therefore, the role of food is not only to satisfy hunger and provide necessary nutrients, but also to prevent nutrition-related diseases and improve consumers' physical and mental well-being [1]. The growing interest in functional food leads to examination for new sources of bioactive compounds [2]. In this regard, the importance of plant secondary metabolites and their potential effects on human health have been intensively studied. Researches indicate that by-products of food processing represent a disposal problem, but also they are promising sources of compounds which could be used because of their convenient technological or high nutritive properties [3].

Berries are rich sources of antioxidants and other bioactive compounds. Many studies claim that the dietary intake of these fruits has positive effects on human health, performance and disease [4, 5]. Besides high contents of fibers, vitamins and essentials minerals, raspberries and blackberries are known for their high contents of phenolic compounds, such as phenolic acids, tannins, flavonoids and anthocyanins [6, 7]. Raspberries (*Rubus idaeus* L.) and blackberries (*Rubus fruticosus* L.) are produced in more than 30 countries worldwide. Despite those berry fruits are often sold and consumed fresh, it represents less than 10% of produced berries, due to their perishability contributes to nutritional and microbiological deterioration and diminishes quality and health benefits. Thus, large fractions are processed into juices, jams, jellies, syrups, fruit wines, confectionery products, yogurts, and as ingredients of various foods [5, 6, 8, 9].

The high amounts of residues rich in phenolic antioxidants and fibers are produced during industry manufacturing [10]. Nowadays, there is great interest in the recovery of these by-products as raw materials for development of natural food additives and functional food [5]. Additionally, from the economic point of view, fruit processing by-products are the best sources of bioactive compounds and their utilization ensures more effective by-product management [7, 11]. The palatable taste and functional properties make berries suitable for obtaining functional food enriched by the valuable compounds [10]. For example, for this purpose, strawberry, sour cherry, raspberry and blackcurrant pomaces were used in order to increase the nutritional values of muffins [11], while raspberry pomace was used as replacement of flour in cookies [2].

The aim of this study was to investigate the potentials of raspberry and blackberry pomaces as sources of bioactive compounds. In this context, the contents of total phenolic compounds and monomeric anthocyanins, as well as 2,2-dyphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity were determined.

## Materials and methods

Meeker variety raspberry and Čačanska bestrna variety blackberry were cultivated in Aleksandrovac, Serbia. Folin-Ciocalteu reagent and gallic acid were purchased from Merck (Darmstadt, Germany). Sodium carbonate and sodium acetate were supplied by Centrohem (Belgrade, Serbia). DPPH (2,2-dyphenyl–1-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were provided by Aldrich (Sigma-Aldrich Chemie Steinheim, Germany). Ethanol was procured from Vrenje Spiritana (Belgrade, Serbia). All other chemicals used for the experimental procedures were of analytical grade and used as such without further purification.

Samples of the berries were stored at –20 °C prior to analysis. Pomaces of raspberry (RP) and blackberry (BP) were obtained after juice separation. The extraction of the pomaces was performed with 70% ethanol solution at room temperature. The weight ratio of pomaces and ethanol was 1:2 in favor of ethanol.

The concentration of soluble solids in samples was measured using Abbe refractometer (Bellinghan & Stanley Ltd., UK).

Total phenolic content of raspberry and blackberry pomace extracts was determined spectrophotometrically according to the Folin-Ciocalteu method [12]. It is accepted that Folin-Ciocalteu reagent contains complex of phosphotungstate and phosphomolybdate. This method is based on the transfer of electrons from phenolic compounds to molybdenum in alkaline conditions. The result of the reaction is blue complex whose absorbance is measured at 765 nm.

Briefly, 0.5 mL of diluted extracts were mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and allowed to react for 5 minutes. 2 mL of sodium carbonate solution (75 g/L) was added to the mixture and shaken. After 2 h of reaction at room temperature, the absorbance of the developed blue coloration was measured at 765 nm against a blank sample using UV-Vis double beam spectrophotometer (HALO-DB/2S, Dynamica, Switzerland). The calibration curve was prepared using solutions of gallic acid as the standard and the results were expressed as milligrams of gallic acid equivalents per gram of pomace (mg GAE/g).

Total monomeric anthocyanins content of raspberry and blackberry pomace extracts was estimated spectrophotometrically using UV-Vis double beam spectrophotometer (HALO-DB/2S, Dynamica, Switzerland) by pH differential method [13]. The base of this method is the structural transformation of anthocyanins in change in pH. The monomeric anthocyanins are subject to reversible structural transformation as a function of pH wherein at pH = 1 are in the form of intensively stained flavylium cation and at pH = 4.5 taking the form of a colorless hemiketal. The content of anthocyanins is determined by measuring the change in absorbance at two different pH values (pH = 1 and pH = 4.5). The difference in absorbance of a pigment at wavelength of 520 nm is proportional to the concentration of the pigment. Anthocyanins were quantified as cyanidin-3-glucoside equivalents and resulting values were expressed in terms of milligrams of cyanidin-3-glucoside equivalents per gram of pomace according to the following formula:

$$Anthocyanin\ content\ (cyanidin-3-glucoside\ equivalents, \frac{mg}{L}) = \frac{A \cdot MW \cdot DF \cdot 10^3}{\varepsilon \cdot l}$$

where:

$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH\,4.5}$;

MW = molecular weight of cyanidin–3-glucoside;

DF = dilution factor;

l = path lenght (1 cm);

ε = molar extinction coefficient (26900 L/mol × cm);

$10^3$ = conversion factor.

DPPH radical-scavenging activity of pomace extracts was investigated spectrophotometrically according to the slightly modified procedure described by Kaneda et al. [14] using UV-Vis double beam spectrophotometer (HALO-DB/2S, Dynamica, Switzerland). 2,2-dyphenyl-1-picrylhydrazyl (DPPH) is purple stable free radical with a

delocalized unpaired electron and with maximum absorbance at 515 nm. Dark purple radical becomes pale yellow hydrazine as the result of reaction between DPPH and antioxidants.

Briefly, diluted extract (0.2 mL) was added to the DPPH working solution (2.8 mL, mixture of $1.86 \times 10^{-4}$ mol/L DPPH in ethanol and 0.1 M acetate buffer (pH = 4.3) in ratio 2:1 (v/v)). The mixture was shaken and left at room temperature for 6 min. The absorbance was measured at 515 nm. The Trolox calibration curve was plotted as a function of the inhibition percentage of DPPH radical. The results were expressed as micromoles of Trolox equivalents per gram of pomase (umol TE/g).

All results were obtained in three independent measurements and expressed as mean ± standard deviation. The experimental data were subjected to One-way analysis of variance (ANOVA) and Tukey's test was used to detect difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program STATISTICA 12 (Data Analysis Software System, Stat-Soft, Inc., USA).

## Results and discussion

The extracts of raspberry and blackberry pomace were comparatively evaluated in terms of their antioxidant phytochemicals through assessment of total phenolic and anthocyanin contents, as well as DPPH radical scavenging activity (Figure 1).

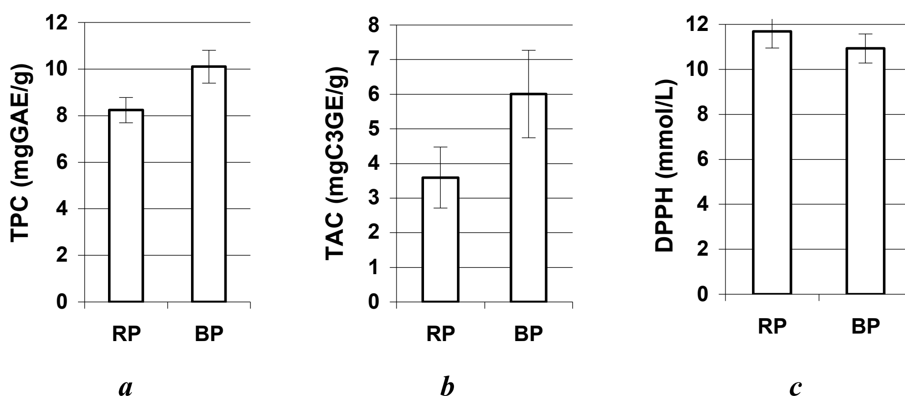


**Fig 1. Total phenolic content (a), total anthocyanin content (b) and antioxidant activity (c) of raspberry (RP) and blackberry pomace (BP)**

*The results are expressed as the mean ± standard deviation (n=3)

The results of total phenolic content determined by Folin-Ciocalteu method proved that raspberry and blackberry pomaces contain a high amount of these compounds. As can be seen on Figure 1a pomace of blackberry was characterized by higher TPC (10.1 mg GAE/g) than raspberry (8.2 mg GAE/g). The observed difference was statistically significant at the level of 5%. The combination of pure water with ethanol, as an organic solvent, creates a moderately polar medium favoring the extraction of polyphenols from berries which have moderately polar nature [7, 15, 16]. The obtained results are in accordance with results published by other authors that have also investigated extracts of berry by-products as sources of antioxidant polyphenols [5, 7, 16]. The major concern with polyphenol

extraction is the effect of increased temperature due to thermal reaction leads to degradation of the polyphenols and formation of Maillard reaction products [16]. All above-mentioned data indicate that the extraction procedure applied in this study represents a good method for the production of phenolic extracts from fruit processing by-products.

The level of total anthocyanins content varied depending on the types of fruit used for juice production. Namely, significantly higher difference has been detected for TAC, where the BP sample (6 mg C3GE/g) is remarkably dominant compared to RP (3.6 mg C3GE/g) (Figure 1b). It could be explained to some extent with berry structure and composition, such as higher content of total solids and thus potential bindings with anthocyanins and/or similar molecules with sugar or fibers. Specifically in this study, the total soluble solids was 9.3 $^{o}$Bx in RP sample, significantly lower than in BP sample with value 14.5 $^{o}$Bx. It could be concluded that these extracts have a potential as replacements for synthetic food colorants thanks to content of anthocyanins.

Contrary to the results of TPC and TAC, DPPH radical scavenging activity of RP (11.7 μmolTrolox/g) and BP (10.9 μmolTrolox/g) have been statistically insignificantly different, with slightly higher values in RP sample (Figure 1c). It could be explained by the extraction of ellagic acid which expresses strong antioxidant activity and presents the most abundant phenolic compound of raspberry. Additionally, extraction of other compounds, such as vitamin C could contribute to higher antioxidant activity [17]. Additionally, the antioxidant activity of phenolic compounds depends on their structural features and represents ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations [6]. In that regard, it is necessary to determine the chemical profile of the obtained extracts. Since high portion of these pomaces represents seeds it should be kept in mind that seed oils are rich in not only phenols, but essential omega fatty acids tocopherols, sterols, and carotenoids that have antioxidant properties [18].

As can be seen from the Table 1, coefficient of determination has been higher in case of BP and statistically significant among TPC and DPPH for same sample.

**Table 1**

**Coefficient of determination between methods used in the study**

| $R^2$ | TPC | | TAC | | DPPH | |
|---|---|---|---|---|---|---|
| | RP | BP | RP | BP | RP | BP |
| **TPC** | --- | | 0,73 | 1,00 | 0,89 | **1,00** |
| **TAC** | 0,73 | 1,00 | --- | | 0,60 | 1,00 |
| **DPPH** | 0,89 | **1,00** | 0,60 | 1,00 | --- | |

*Bolded numbers are statistically significant

Generally, very important factors represent (pre)treatments applied during processing of berries (e.g. juices production). Application of different temperatures, filtration, enzymes and other biocatalysts have strong influence on the content of bioactive compounds that remains in pomace, beside cultivars as raw material [8, 19, 20, 21]. Also, these compounds are enclosed in complex insoluble structures, such as vacuoles of plant cells, thus their extraction is complicated and the potential of new technologies should be studied [22].

## Conclusions

This study points out that raspberry and blackberry pomaces, discarded during juice production, represent significant sources of bioactive compounds, such as phenolic antioxidants. Resulting residues could be used as cheap sources of potential dietary additives, even natural colorants for development of new products with high value added.

The potential applications of the ethanol for bioactive compounds extraction must be studied on the basis of the solvent percent choice, liquid–solid ratio, temperature, and time applied. Since an economical issue is crucial one for the industrial implementation, the combination of effective extraction and low-cost raw materials is desirable. It represent and environmental and economical solution at the same time, contrary to conventional extraction methods where large amounts of organic solvents and long extraction times are required.

The use of the novel processing technologies will reduce food processing wastes and facilitate the production of natural valuable products which will guarantee food sustainability and also, meet consumer demands for product with healthy constituents.

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# Chemical composition of essential oil from *Rosa Damascena* mill., growing in new region of Bulgaria

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## Abstract

**Introduction**. The objectives of this study are to present the chemical composition of essential oils from *Rosa damascena* Mill., growing in a new region of Bulgaria.

**Materials and methods**. The rose flowers were harvested in 2016 in the vicinity of the town of Vidin (North-West Bulgaria) in the stage of flowering in two periods – 10 May (sample 1) and 26 May (sample 2).

The chemical composition of the oil is determined chromatographically.

**Result and discussion.** The moisture of the plants is 82.70% (for sample 1) and 79.04% (for sample 2). The yield of essential oil is 0.08% and 0.03%, respectively.

Thirty-nine components were identified in the oil in sample 1 and sample 2. Two groups of compounds were found in hydro-distillated rose oils, – odor carriers and odor fixators. Terpene alcohols are the main components responsible for the characteristic odor of rose oil and represent about 56% of total identified substances. High geraniol content with combination of citronellol, farnesol and nerol results in a strong, sweet, floral fresh rosaceous character of the produced rose oils.

The chromatographic profile of the rose oil revealed a significant presence of aliphatic hydrocarbons (31%) which are the main components responsible for the odor stability.

The main compounds of essential oils were as follows: β-citronellol (30.24–31.15%); trans-geraniol (20.62–21.24%), n-heneicosane (8.79–9.05%), n-nonadecane (8.51–8.77%), nonadecene (4.42–4.55%) and phenylethyl alcohol (4.04–4.16%).

**Conclusion.** For the first time in new region of Bulgaria essential oil from *Rosa damasena* Mill. was obtained.

## Introduction

Bulgaria is relatively small country in South-East Europe and located in the centre of the Balkan Peninsula. The climate ranges from moderate continental in the northern part to the Mediterranean (subtropical in southwest and southeast regions). As a result of the climatic conditions, soils and other natural factors, Bulgaria has been a producer of rose oil from *Rosa damascena* Mill for more than 400 years. The rose plants were cultivated in Central Bulgaria in the "Valley of the Roses" which is between the towns Karlovo and Kazanlik (350 m elev.) [1].

*Rosa damascena* Mill. is the most common in Rosaceae family and the aromatic products are used many different industries such as perfume, cosmetic, pharmaceutical and food. The plant is cultivated and used in Bulgaria, Turkey, Iran, India, China, Libya and other countries [1].

The industrial processing of rose sets its beginning in the middle of 17th century century probably from the Damascus area of Syria. The rose area decreased almost by 50% – from about 2500 ha in 1990 to about 30 000 dka in 2011. Currently, the Bulgarian essential oil industry employs about 12000 people full-time. During the months May to September, the total number involved in cultivation and processing operations increases to 40000.

The essential oil is deposited mostly in the petal leaves (about 93% of the total content of the flowers) – in the cells of the epidermis and the parenchyma. The average oil content in the flowers is 0.035%, but varies among varieties from 0.009% to 0.062% [1]. Roses are known to pass through six phases of flower development from bud-forming to fading, and the transition between each of the stages brings about changes in the quantity and the composition of the essential oil.

In full bloom (Phase IV) the oil content is maximum (0.071%), and the content of terpene alcohols (totaling 43%) is highest, while the amount of stearoptene is minimum (16%). With the lowest content of terpene alcohols and the highest of stearoptene are characterized the oils from just-opening buds (Phases I and II). The excess presence of buds and overblown blossom in the processed raw material delivers essential oil with modified composition, i.e. non-standard oil.

Rose oil is an oily, clear liquid or a heterogeneous mass, with yellow to yellow-green color and specific odor. Approximately 400 oil components have been identified. About 62–77% terpene compounds are found in oil's composition: hydrocarbons (monoterpenes up to 2% and sesquiterpenes 3–5%), oxygen-containing derivatives (monoterpenes 64–71% and sesquiterpenes 0.5–2%), fatty hydrocarbons and their oxygen derivates (18–25%), phenylpropanoids (3–5%), and others (0.5–2%) [1].

The distillation itself is the simple process of 'water-distillation'. The flowers are soaked in water and are distilled in containers of 1, 2, 5 to 10 $m^3$. The distillate separates continuously in a Florentine flask into water and oil (the so-called 'direct oil'). The water phase, containing emulsified oil and dissolved polar components, yields by a second distillation, involving cohobation, the so-called 'water-oil' and rose water. The two types of oil are mixed together to form the final product, known as rose oil.

The annual production of rose oil in Bulgaria over the last 18 years has varied between 750 to 1900 kg, except for the poor weather year of 2002 t hat yielded only 620 kg. The peak rose oil output of 1650 kg over the last 30 years occurred in 1975–76. Along with the maturation of the new rose plantations, our aim is to achieve an annual production of 1400–1500 kg of rose oil in the next 3–4 years.

Rose oil composition is varied over the different conditions, for example harvesting period [2], ecological factors [3, 4, 5, 6, 7] *etc*. [8, 9, 10]. It is known that the rose essential oil has an antimicrobial activity [5, 9].

The essential oil is extensively applied in perfumery, cosmetics and medicine. Rose essential oil is distinguished by a multifaceted pharmacological activity – it is used in the treatment of gall stones, in cases of impaired lipid metabolism; it performs antisclerotic, antispasmodic, and hepatoprotective actions [1].

The objectives of this study are the following: to present the chemical composition of essential oils from Rosa damascena Mill., growing in a new region of Bulgaria; comparing of the obtained results with composition of rose oil standardized in ISO 9842:2006 in order to validate their quality.

## Materials and methods

**Materials.** The rose flowers were harvested in 2016 in the vicinity of the town of Vidin (North-West Bulgaria, 150–200 m elev.) in the stage of flowering in two periods – 10 May (sample 1) and 26 May (sample 2).

**Sample preparation.** The oils were prepared by hydrodistillation for 2 h 30 min in laboratory glass apparatus of British Pharmacopoeia, modified by Balinova and Diakov [12]. The oils were dried over anhydrous sulfate and stored in tightly closed dark vials at 4°C until analysis.

**Moisture content measurement.** The row materials moisture content was determined by drying up to constant weight, at 105 ºC. The water content is expressed as the percentage, by weight, of the dry sample [11].

**Chemical compositon determination.** GC analysis was performed using gas chromatograph Agilent 7890A; column HP–5 ms (30 m x 250 µm x 0.25 µm); temperature: 35°C/3 min, 5°C/min to 250°C for 3 min, total 49 min; carrier gas helium 1ml/min constant speed; split ratio 30:1. GC/MS analysis was carried out on a mass spectrometer Agilent 5975C, carrier gas helium, column and temperature as the same as the GC analysis. The components of the oils were identified by their retention indices and by comparison of their mass spectra whit those of authentic samples or with data already available in the literature.

**Statistical analysis.** Variance analysis of the results was carried out by least square method with application of Microsoft Office Excel program. Differences were considered statistically significant if probability was greater than 95% ($q < 5\%$). Experimental results are represented according to standard rules.

## Results and discussion

The moisture of the plants is 82.70% (for sample 1) and 79.04% (for sample 2). The yield of essential oil is 0.08% and 0.03%, respectively.

Chemical compositions of the oils are listed in Table 1.

Thirty-nine components representing 96.61% of the total content were identified in the oil in sample 1. Ten of them were in concentrations over 1% and the rest 29 constituents were in concentrations under 1%. As seen the major constituents (up 3%) of the oil are as

follows: β-citronellol (30.24%); trans-geraniol (20.62%), n-heneicosane (8.79%), n-nonadecane (8.51%), nonadecene (4.42%) and phenylethyl alcohol (4.04%).

A total of thirty-nine components representing 99.54% of the total content were identified in the oil in sample 2. Ten of them were in concentrations over 1% and the rest 29 constituents were in concentrations under 1%. As seen the major constituents (up 3%) of the oil are as follows: β-citronellol (31.15%); trans-geraniol (21.24%), n-heneicosane (9.05%), n-nonadecane (8.77%), nonadecene (4.55%) and phenylethyl alcohol (4.16%).

**Table 1**

**Chemical composition of rose oils,%**

| Compounds | RI[a] | Sample 1 (10 May) | Sample 2 (26 May) | Compounds | RI[a] | Sample 1 (10 May) | Sample 2 (26 May) |
|---|---|---|---|---|---|---|---|
| α-Pinene | 939 | 0.17 | 0.18 | α-Humulene | 1454 | 0.84 | 0.87 |
| Camphene | 954 | 0.13 | 0.13 | δ-Cadinene | 1525 | 0.16 | 0.16 |
| Sabinene | 971 | 0.05 | 0.05 | n-Hexadecane | 1600 | 0.23 | 0.24 |
| β-Pinene | 979 | 0.43 | 0.44 | 8-Heptadecene | 1683 | 0.18 | 0.19 |
| β-myrcene | 991 | 0.08 | 0.08 | n-Heptadecane | 1700 | 0.35 | 0.36 |
| Phenylethyl alcohol | 1110 | 4.04 | 4.16 | Farnesyl alcohol | 1725 | 0.21 | 0.22 |
| β-Linalool | 1097 | 0.85 | 0.87 | n-Octadecane | 1800 | 0.19 | 0.20 |
| cis-Rose oxide | 1106 | 0.16 | 0.18 | Nonadecene | 1880 | 4.42 | 4.55 |
| trans-Rose oxide | 1124 | 0.09 | 0.09 | n-Nonadecane | 1901 | 8.51 | 8.77 |
| Terpinene-4-ol | 1179 | 0.18 | 0.19 | n-Eicosane | 2000 | 2.03 | 2.09 |
| α-Terpineol | 1187 | 0.86 | 0.89 | 10-Heneicosene | 2093 | 0.92 | 0.95 |
| β-Citronellol | 1225 | 30.24 | 31.15 | n-Heneicosane | 2100 | 8.79 | 9.05 |
| cis-Geraniol | 1229 | 3.28 | 3.38 | n-Docosane | 2200 | 0.31 | 0.32 |
| trans-Geraniol | 1248 | 20.62 | 21.24 | (Z)-9-Tricosene | 2294 | 0.55 | 0.57 |
| Citronellyl acetate | 1351 | 0.57 | 0.59 | n-Tricosane | 2300 | 2.22 | 2.29 |
| Geranyl acetate | 1382 | 1.82 | 1.87 | n-Tetracosane | 2400 | 0.15 | 0.15 |
| Eugenol | 1386 | 0.75 | 0.77 | n-Tricosanol-1 | 2495 | 0.26 | 0.27 |
| β-Elemene | 1390 | 0.42 | 0.43 | n-Pentacosane | 2500 | 0.11 | 0.11 |
| Methyleugenol | 1405 | 0.05 | 0.05 | n-Hexacosane | 2600 | 0.88 | 0.91 |
| β-Caryophyllene | 1419 | 0.51 | 0.53 | | | | |

%, relative percentage of ionic current, [a] Retention indices (RI)

Two groups of compounds were found in hydro-distillated rose oils, – odor carriers and odor fixators. Terpene alcohols are the main components responsible for the characteristic odor of rose oil and represent about 56% of total identified substances.

The chromatographic profile of the rose oil revealed a significant presence of aliphatic hydrocarbons (31%) which are the main components responsible for the odor stability (Table 2). Distribution of aroma substances in the two samples shown that the dominant group is oxygen monoterpenes, followed by aliphatic hydrocarbons and phenyl propanoids.

**Table 2**

**Chromatographic profile of the rose oil, %**

| Groups of compounds | Sample 1 (10 May) | Sample 2 (26 May) |
|---|---|---|
| Aliphatic hydrocarbons | 31.16 | 31.16 |
| Monoterpene hydrocarbons | 0.89 | 0.88 |
| Oxygen monoterpenes | 60.73 | 60.73 |
| Sesquiterpene hydrocarbons | 2.00 | 2.00 |
| Oxigen sesquiterpenes | 0.22 | 0.22 |
| Phenyl propanoids,% | 5.00 | 5.01 |

According to qualitative and quantitative content of the major constituents the produced oils are equal to the rose oil (see Table 3).

Citronellol dominates, followed by geraniol and nerol and their concentrations are similar to those regulated by ISO 9842:2006 (Table 3). Citronellol/geraniol ratio was used to evaluating the odor quality of rose oil. A value between 1.25–1.30 is considered as a reference for the best odor [14].

The data in Table 3 shows that the ratio is 1.47 for the both samples of rose oils which guarantees their excellent properties. High geraniol content with combination of citronellol, farnesol and nerol results in a strong, sweet, floral fresh rosaceous character of the produced rose oils [15].

**Table 3**

**Comparative chemical composition of rose aromatic products**

| No | Compounds, % | Essential oils (samples 1 and 2) | Essential oil [13] |
|---|---|---|---|
| 1. | Phenylethyl alcohol | 4.04–4.16 | max 3.5 |
| 2. | Citronellol | 30.24–31.15 | 31.15 |
| 3. | Nerol | 3.28–3.38 | 5.0–12.0 |
| 4. | Geraniol | 20.62–21.24 | 15.0–22.0 |
| 5. | Eugenol | 0.75–0.77 | - |
| 6. | Methyleugenol | 0.05 | - |
| 7. | n-Heptadecane | 0.23–0.24 | 1.0–2.5 |
| 8. | n-Nonadecane | 8.51–8.77 | 8.0–15.0 |
| 9. | n-Heneicosane | 8.79–9.05 | 3.0–5.5 |

The difference in chemical composition of the our investigations (higher content of n-heneicosane and lower of n-heptadecane) and the reported data may be due to environmental conditions under which the plant has grown.

## Conclusion

For the first time in new region of Bulgaria essential oil from *Rosa damasena* Mill. was obtained. The GC-MS chromatograph profile revealed a significant presence of β-citronellol (30.24–31.15%); trans-geraniol (20.62–21.24%), n-heneicosane (8.79–9.05%), n-nonadecane (8.51–8.77%), nonadecene (4.42–4.55%) and phenylethyl alcohol (4.04–4.16%).

Bulgaria has the necessary favourable natural conditions, a long production tradition, highly educated professionals and the goodwill to preserve and further develop its essential oils industry. This sector is a one of the priority areas for development in the Bulgarian government economic program.

The industry is export oriented and the Bulgarian companies are making continuous efforts to introduce the best European practice in essential oils production by adoption of EU Directives on manufacturing, quality control, safety and market performance in the branch. Bulgaria today imports large quantities of perfumery and cosmetic products from the well-known international brand name companies.

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# Milk fat in structure formation of dairy products: a review

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## Abstract

**Introduction.** This article primarily focuses on published information addressing how milk fat affects the structure of dairy products, published within the last 15 years. Texture is an important parameter in determining the consumer's acceptance of dairy foods as well as the sensory manifestation of product's structure. Therefore, establishment and maintenance of optimal structure are critical to high quality of product.

**Material and methods.** The structure formation of dairy products is an investigation object of this review article. Scientific articles as well as theses and monographs of dairy science have been analysed. Subjects of this research are properties of milk fat and wide variety of processes applied to the milk; the influence of milk fat on the structure of different dairy products.

**Results and discussion.** Milk fat occurs naturally in milk and cream, forming an oil-in-water emulsion. In the emulsion, milk fat is contained within milk fat globules, surrounded by the milk fat globule membrane. From a practical viewpoint, milk lipids are very important as they confer distinctive physical, chemical, textural and organoleptic properties on dairy products, such as cream, butter, ice cream, whipping cream and cheese.

Milk fat-based products, such as butter, are water-in-oil emulsions consisting of crystallized fat in a continuous phase, in which water droplets, milk fat globules, and partially damaged fat globules are dispersed. A microstructure dominated by numerous small crystals is shown to increase the hardness by up to 20% compared to a microstructure with few, but large crystals, a difference that from consumer perspective, would be noticed by different mouthfeel, brittleness, and spreadability.

Milk fat is essential for the formation of stable whipped cream and ice cream, which depend on the interaction between fat globules, air bubbles and plasma components (esp. proteins). During whipping and cooling fat globules partially coalesce in chains and clusters, and adsorb to and spread around the air bubbles. As the result, air bubbles are stabilized. Furthermore, fat is special importance for a solid ice cream structure to be formed during freezing and therefore for consistency, appearance, and melting resistance.

The presence of milk fat in cheese is necessary to develop the characteristic flavour and mouthfeel. Moreover, fat globules have an impact on texture by partially disrupting the casein fibrous matrix to soften the texture.

**Conclusion.** Milk fat is valued for its pleasant flavour but its properties often need to be modified to make it more suitable for food applications, which in turn can have a marked effect on the structure formation of dairy products, such as butter, whipping cream, ice cream and cheese.

## Introduction

The presence of fat globules in milk was first reported by Van Leeuwenhoek in 1674, after microscopic analysis of milk placed in a fine capillary tube. Since then, the physical and colloidal properties of milk fat globules and their size distribution have been the subject of considerable study.

Bovine milk typically contains $>10^{10}$ fat globules per mL. Milk fat is present predominantly in spherical droplets ranging from about 0.2 to 15.0 µm in diameter, with the bulk of the fat being in globules 1.0 to 8.0 µm diameter [1]. The fat globules in milk are stabilized by an adsorbed layer of protein and phospholipid called the ‘milk fat globule membrane’ (MFGM), which is distinct from the aqueous phase protein. The MFGM, acting as an emulsifier, stabilizes the fat globules in the emulsion by lowering the surface tension [2, 3, 4]. In addition, it protects the fat from chemical reactions such as hydrolysis and oxidation [5]. The average composition of the MFGM has been estimated to be about 48% protein, 33% phospholipid, and 11% water, with the remainder made up of other minor lipid components [6, 7]. The phospholipid fraction of the membrane is composed of lecithin, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositide, plasmalogens and sphingomyelin. Phospholipids are important food emulsifiers in their own right [8, 9]. Milk fat, on the other hand, contains a wide range of triglycerides, with a broad thermal range of melting points ranging from –50 to 80 °C.

## Material and methods

The structure formation of dairy products is an investigation object of this review article. Scientific articles as well as theses and monographs of dairy science have been analysed. Subjects of this research are properties of milk fat and wide variety of processes applied to the milk; the influence of milk fat on the structure of different dairy products.

Methodology of the investigation is based upon the use of the methods of analysis, comparison and synthesis.

## Results and discussion

Whole milk or cream can be regarded as an emulsion of milk fat globules in milk plasma. The physico-chemical properties of the milk fat globules affect many properties of dairy products. The physico-chemical properties of the milk fat globules can be influenced through a wide variety of processes. These processes can be efficiently used to give products desired characteristics (e.g., in terms of storage stability or rheological properties) [10, 11, 12].

The shelf-life of many dairy emulsions depends on rheological characteristics of the emulsion’s phases. For example, the rate of creaming of milk depends on the viscosity of milk plasma [13]. The content of lactose or the whey proteins in milk influence the viscosity of milk only little; fat content has a major influence, although by far the greatest influence is that of the casein content [8, 14]. If fat globules are present as separate particles, the fat content is less 40% and the milk fat completely molten, milk and cream behave as Newtonian fluids at intermediate and high shear rates. At a temperature below 40 °C, milk does not behave as a Newtonion fluid. The deviation from Newtonian flow becomes larger as the temperature decreases. Viscosity of milk decreases with increasing shear rate at a temperature below 40 °C, which may be due to disruption of clusters of milk fat globules, which were formed as a result of cold agglutination.

**Heating.** When fat globules are heated in the absence of whey proteins, high molecular weight complexes form between butyrophilin and Xanthine oxidoreductase in less than 10 min at a temperature as low as 60 °C [7]. In the presence of whey proteins, large amounts of β-lactoglobulin and α-lactalbumin associate with the MFGM [15]. Direct evidence of heat-induced covalent disulfide interactions between whey proteins and MFGM proteins can be obtained by electrophoresis and isoelectric focusing of the heated MFGM [7, 16, 17]. The heat-induced formation of protein complexes on the surface of the MFGM may include denaturation of the individual proteins with the formation of aggregates containing MFGM proteins alone or MFGM proteins with whey proteins.

**Mechanical treatments** such as agitation, pumping and high shear can cause changes in the composition of the MFGM, as well as changes in the size of the fat globules. Homogenization is often employed to reduce the size of the fat globules, improve stability and delay creaming [18, 19]. During homogenization, the interfacial area increases significantly. Rearrangement of the original MFGM material occurs and considerably more protein is necessary to cover the newly formed interface. For this reason, casein micelles are adsorbed on the milk fat globules. This effect explains the observation that the fat globules in homogenized milk have a much higher protein load than untreated fat globules. Homogenization and heating are unit operations that are usually combined during milk processing [8, 10, 20, 21].

The formation of complexes between skim milk-derived proteins and MFGM proteins is of significance in milk processing [22]. When raw cream is homogenized without being subjected immediately to high-temperature pasteurization, indigenous milk lipoprotein lipase penetrates the secondary membrane of fat globules (which has higher interfacial tension than native membrane) and hydrolyzes triglycerides to free fatty acids within a few minutes, resulting in intense rancidity [19, 20].

**Fat Destabilization.** While homogenization is the principal method for achieving stabilization of the fat emulsion in milk, fat destabilization is necessary for structure formation in butter, whipping cream and ice cream [10, 13]. Fat destabilization refers to the process of clustering and clumping (partial coalescence) of the fat globules, which leads to the development of a continuous internal fat network or matrix structure in the product. Fat destabilization (sometimes "fat agglomeration") is a general term that describes the summation of several different phenomena [8, 13, 23]. These include [9]:

*Coalescence:* an irreversible increase in the size of fat globules and a loss of identity of the coalescing globules;

*Flocculation:* a reversible (with minor energy input) agglomeration/clustering of fat globules with no loss of identity of the globules. The fat globules that flocculate, they can be easily redispersed if they are held together by weak forces, or they might be harder to redisperse to they share part of their interfacial layers;

*Partial coalescence:* an irreversible agglomeration/clustering of fat globules, held together by a combination of fat crystals and liquid fat, and a retention of identity of individual globules as long as the crystal structure is maintained (i.e., temperature dependent, once the crystals melt, the cluster coalesces). They usually come together in a shear field, as in whipping, and it is envisioned that the crystals at the surface of the droplets are responsible for causing colliding globules to stick together, while the liquid fat partially flows between they and acts as the "cement". Partial coalescence dominates structure formation in whipped, aerated dairy emulsions, crystals within the emulsion droplets are responsible for its occurrence.

The **crystallization behavior** of milk fat is extremely important to the processing and texture of dairy foods. For example, the crystal network structure of butter depends on its composition and the crystallization behavior of the milk fat present. In turn, these properties

determine the end use applications, spreadability, mouthfeel, appearance and even the taste of butter [10].

Milk fat is composed of literally hundreds of unique and varied triacylglycerol (TAG) species. This results in milk fat having complicated crystallization, melting, and rheological behaviour [24, 25, 26].

Crystallization refers to the change from a liquid to a solid state and is an exothermic process. It involves nucleation, crystal growth and crystal rearrangements. When a fat is cooled to a temperature below its melting point, the molecules are "supercooled". Supercooling is equivalent to supersaturation and is the thermodynamic driving force for crystallization to occur. In this non-equilibrium state, molecules begin to aggregate into tiny clusters (i.e., embryos), which continuously form and dissolve until some critical size is reached. At this point, the cluster is referred to as a nucleus [9, 27].

The fat crystal network is held together by crystal–crystal interactions, characterized by strong irreversible (primary) bonds and weak reversible van der Waals (secondary) bonds [8, 23]. The irreversible are formed upon crystal growth, as the crystals get mechanically interlinked. Fat crystal networks develop from initial nucleation sites, which grow into crystals as more TAGs crystallize (there may be further nucleation during growth) [25]. Growing crystals become primary particles, or microstructural elements (collection of primary crystallites or single crystals), of approximately uniform size (< 5 μm). These microstructural elements then aggregate into clusters, or microstructures (>100μm). They constitute the largest structural building block of the fat crystal network. The liquid phase (oil) of the network is interspersed between the microstructural elements and microstructure [10, 28].

The properties of the fat crystal network depend on the interactions between the crystals. Crystal size, shape, and polymorphic structure, as determined by processing conditions and chemical composition, affect the crystal–crystal interactions. [24, 25, 29, 30].

So, milk fat can be affected greatly by various processes (e.g., heat treatment, cooling or homogenization), which has significant influence on its application in a manufacturing of different dairy products.

**Butter** is water-in-oil emulsions consisting of crystallized fat in a continuous phase, in which water droplets (2.3–10.6 μm) [31, 32], milk fat globules (2.5 μm) [33], and partially damaged fat globules are dispersed. It contains 80% of fat, which is partly crystallized. Figure 1 shows a schematic representation of the physical changes involved.

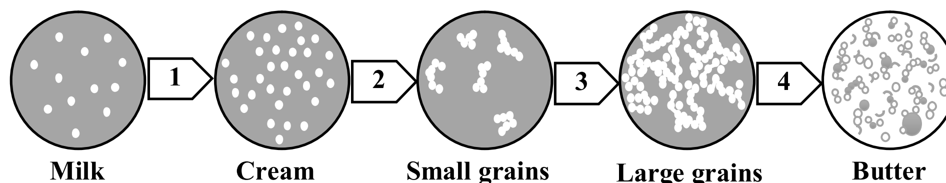


**Figure 1. Stages in the formation of butter: grey represents the aqueous phase; white represents fat (1 – separation, 2 – churning, 3 – churning and draining, 4 – working) [34]**

In most cases, the churning is achieved by beating in of air. Rapid beating in of air in milk or cream causes a new airwater interface to be continually formed, and fat spread over the interface [8,10]. If the fat is fully liquid, the subsequent breaking up of air bubbles

covered with fat causes disruption of the fat. If the globules also contain solid fat, they become attached to the air bubbles. As the air surface area diminishes (because air bubbles coalesce), the attached fat globules are driven nearer to each other. The liquid fat spread over the air bubble surface, readily causes the globules to form granules [34] (Figure 2).

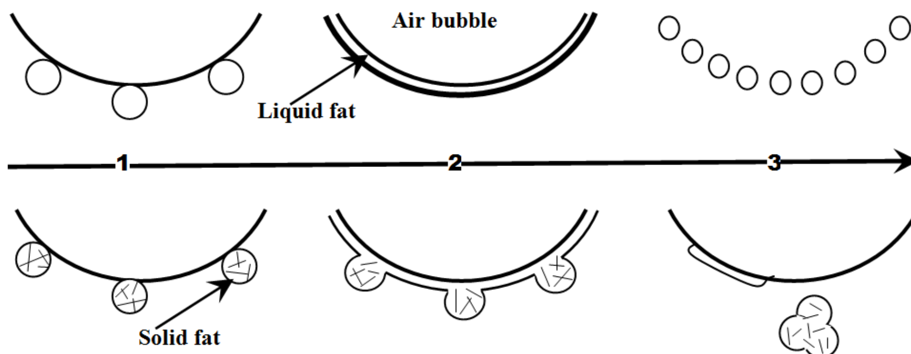


**Figure 2. Schematic representation of the interactions between fat globules and air bubbles during churning**

Further aggregation of granules yields butter grains, in which a phase inversion has apparently taken place. However, the grains still contain fat globules and moisture droplets. Concentrating and then working the grains removes excessive moisture and reduces the moisture droplets in size. In this way, butter is obtained. Figure 3 illustrate the microstructure of butter.

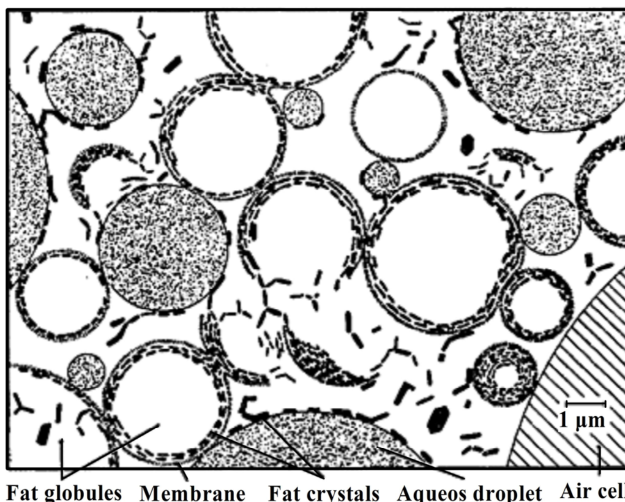


**Figure 3. Schematic presentation of butter microstructure at room temperature: liquid fat is white; membrane thickness is greatly (about ten times) exaggerated [34]**

Several factors affect both the rate and the efficiency of the churning process. The type and filling level of the churn, as well as the turning speed of the churn, naturally influence the churning process [10, 20].

The proportion of solid fat is crucial. If the fat is fully liquid, a kind of homogenizing rather than churning occurs. Also, if the globules contain very little solid fat, then the cream does not churn. The clumps formed are soon pulled to pieces. But for the rest, the higher the proportion of solid fat, the slower the churning and the lower the fat content in the buttermilk. If the fat globules contain relatively little liquid fat, they can still be attached to the air bubbles, and the first stages of the churning, in which flotation predominates, do occur. The temperature therefore has a considerable effect on the churning. If the precooling is not sufficiently deep, undercooled (that is, liquid) fat globules will still be present, and the fat content of the buttermilk will substantially increase.

A partial phase inversion does occur during the churning; a continuous fat phase has developed in the butter granules (Figure 1). Nevertheless, in the whole mass of butter grains, the aqueous phase is still continuous. The working accomplishes a further phase inversion. In this stage, excessive moisture is squeezed out and the remaining moisture droplets are disrupted into smaller ones. This does not concern the very small moisture droplets that are left between individual clumped fat globules; these are too small (on average about 2 μm) to be disrupted by the working.

Increasing the working speed causes the droplets to become smaller, the butter becomes ‘dry’. Likewise, moisture can be incorporated into the butter. During working at a very slow speed, larger droplets emerge again, especially at low temperature; the butter becomes ‘wet’ i.e., visible droplets appear. In that way, excessive moisture can be worked out of the butter. The butter can also readily become ‘wet’ during repackaging in retail packages.

A striking and important difference with, for example, margarine is the presence of several intact fat globules. Their number depends on the method of manufacture, and it decreases strongly with intensive working. Note that most crystals in the fat globules are tangentially arranged. The continuous phase is liquid fat. Sometimes a continuous aqueous phase persists, especially in insufficiently worked butter. This aqueous phase partly passes through the surface layers of the fat globules. The fact that displacement of water through butter can occur generally has another cause: approximately 0.2% (v/v) water can dissolve in liquid fat, which implies that water can diffuse through the continuous oil phase. The ratio between liquid and solid fat is of utmost importance for the rheological properties of butter and spreads. Without solid fat, a milk fat-based product is fully liquid. Without liquid fat, it would appear hard and brittle [35, 36, 37].

The rheological properties of fat-based products are influenced by the fat crystal structure, solidification, and transformation behaviour [38]. The fat crystals consist of triglyceride molecules, forming nanoscale structured elements (150- to 350-nm long and 10- to 60-nm thick) assembled into network of fat crystals (20 to 100 μm) [39]. A microstructure dominated by numerous small crystals is shown to increase the hardness by up to 20% compared to a microstructure with few, but large crystals, a difference that from consumer perspective, would be noticed by different mouthfeel, brittleness, and spreadability [40]. For milk fat, the broad range of triglycerides results in different polymorphic forms due to varying chain length and degree of saturation. The term polymorphism describes the type of lateral packing of the aliphatic triglyceride chains [38]. The lateral packing is influenced by factors such as cooling rate, agitation temperature, and the mechanical treatment [11, 14, 18,41].

A significant part of the crystalline fat may be inside the fat globules because during churning liquid fat is extruded from the globules, mainly by spreading over the air bubbles. However, there are also crystals outside the globules, and these aggregate to a continuous

network and may grow together to form a solid structure, which is mainly responsible for the firmness of butter. The crystals inside the globules do not participate in this network and, therefore, they hardly make the butter firmer.

The crystals outside the fat globules thus make up a continuous network, in which part of the water droplets and damaged fat globules may participate. This network retains the liquid fat as a sponge. When the temperature increases, many crystals melt and the network becomes less dense and coarser.

The structural stability and rheological behavior of butter and milk fat-based products is primarily determined by stabilization by the fat crystal network [42–44]. The fat crystal network is strengthened by formation of more and stronger crystal–crystal interactions due to mechanically interlinked fat crystals as occurring during crystal growth. Initially, the crystals are kept together by van der Waals forces, but they soon become much more strongly bonded to each other due to sintering. Part of the fat is in fat globules, and crystals in these globules do not participate in the fat crystal network. If this is a substantial portion of the solid fat, the firmness of the butter will be decreased. Very intensive or prolonged working can reduce the quantity of globular fat.

Microstructural changes of fat crystals during storage are, however, the biggest challenge to maintain the initially induced rheological differences in milk fat-based products. During subsequent storage, the continuously super-cooled fat will eventually crystallize and thereby strengthen the fat crystal network independently of processing conditions [45].

**Whipping cream** ranks among premium food products and is consumed for its pure flavor. The whipping of pasteurized cream containing more than 30% fat is possible only after adequate cooling since the transformation of the original o/w emulsion into a stable foam requires that part of the fat is solid. The initial stage of whipping involves stabilization of the trapped air bubbles by a temporary interfacial film of soluble whey proteins and β-casein. On mechanical treatment, fat globules increasingly loose at least segments of their natural membrane, thereby exposing strongly hydrophobic surface areas of pure fat. Subsequently, these partly destabilized fat globules adsorb at the air/serum interface of the air bubbles (figure 4). The leakage of liquid fat from mechanically stressed and deformed fat globules supports globule agglomeration and partial coalescence. These agglomerates also interact with the air bubbles and may form bridges between them [9, 13, 20].

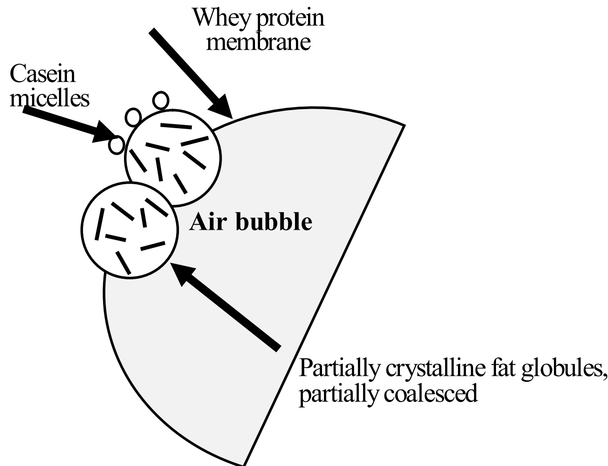


**Figure 4. Schematic representation of the air bubble stabilization in whipping cream**

As the fat partially coalesces, it causes one fat-stabilized air bubble to be linked to the next, and so on. The whipped cream soon starts to become stiff and dry appearing and takes on a smooth texture. This results from the formation of this partially coalesced fat structure stabilizing the air bubbles. The water, lactose and proteins are trapped in the spaces around the fat-stabilized air bubbles. The crystalline fat content is essential (hence, whipping of cream is very temperature dependent) so that the fat globules partially coalesce into a 3-dimensional structure rather than fully coalesce into larger and larger globules that are not capable of structure-building. This is caused by the crystals within the globules that cause them to stick together into chains and clusters, but still retain the individual identity of the globules [10, 34].

In homogenized cream, partial coalescence is too slow, because the fat globules are too small and their proteinaceous surface layers provide good stability. However, homogenization at low pressure (1 to 4 MPa), gives rise to small homogenization clusters and such a cream can be whipped [8, 13]. Another measure to enhance whippability is the addition of a suitable small-molecule surfactant (usually called emulsifier) that displaces (part of) the protein from the globule surface. This enhances the susceptibility of the globules to partial coalescence and markedly affects whipping properties.

The structure of whipped cream is very similar to the fat and air structure that exists in ice cream.

The fat component of frozen dairy dessert mixes increases the richness of flavor, is a good carrier and synergist for added flavor compounds, produces a characteristic smooth texture by lubricating the palate, helps to give structure through the process of partial coalescence and foam stabilization and aids in producing desirable melting properties [46]. The fat content is an indicator of the perceived quality and/or value of ice cream**.** A high fat content leads to a dry, almost grainy texture, a low fat content to a smooth, homogeneous, somewhat slimy texture [47, 48].

To describe the role of fat in the structure thoroughly, it is necessary to begin with the formation of the emulsion at the time of homogenization and the role of the ingredients present at the time of homogenization, with particular reference to the fat, proteins and emulsifiers [49]. The milk fat exists in tiny globules that have been formed by the homogenization. The creation of a large population of small, discrete droplets is a

prerequisite for the development of structure during dynamic freezing, utilizing these droplets. Thus, homogenization conditions can have a large impact on ice cream structure [50–53]. There are many proteins that act as emulsifiers and give the fat emulsion its needed stability. The emulsifiers are added to ice cream to actually reduce the stability of this fat emulsion by replacing proteins on the fat surface [54, 55] (figure 5), leading to a thinner membrane more prone to coalescence during whipping [13, 56].

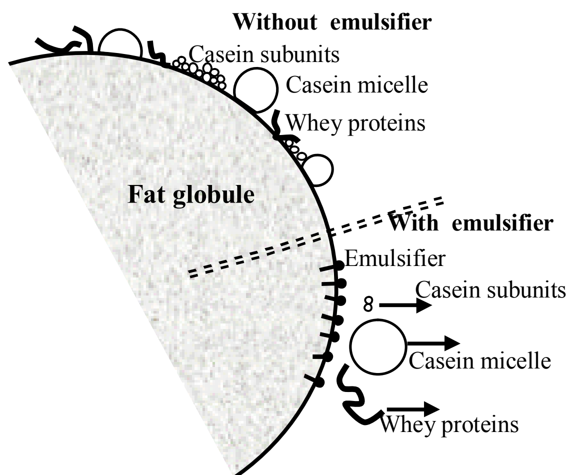


**Figure5. Schematic representation of the interactions between fat globules and emulsifier in ice cream**

Fat crystallization occurs during ageing, creating a highly intricate structure of needle-like crystals within the globule. A partially crystalline fat droplet is necessary for optimal fat structure formation to occur during freezing [57, 58]. When the mix is subjected to the whipping action of the freezer, the fat emulsion begins to partially break down and the fat globules begin to flocculate or destabilize. The air bubbles which are being incorporated into the mix are stabilized by this partially coalesced fat [59]. If emulsifiers were not added, the fat globules would have so much ability to resist this coalescing, due to the proteins being adsorbed to the fat globule, that the air bubbles would not be properly stabilized and the ice cream would not have the same smooth texture (due to this fat structure) that it has.

The clumped fat globules, together with the air cells to which they are attached, form a continuous network throughout the liquid (Figure 6). The clusters of fat globules formed during the process of partial coalescence are responsible for adsorbing to, and stabilizing, the air cells [56, 60] and creating a semi-continuous network or matrix of fat throughout the product that crosses the lamellae between the air cells [50, 61]. Hence, a liner distribution of air bubbles, resulting in thinner lamellae, also helps to produce optimal shape retention during extrusion and melting [62]. Optimal formation of fat structure and air bubble size may also help to slow down ice recrystallization [60].

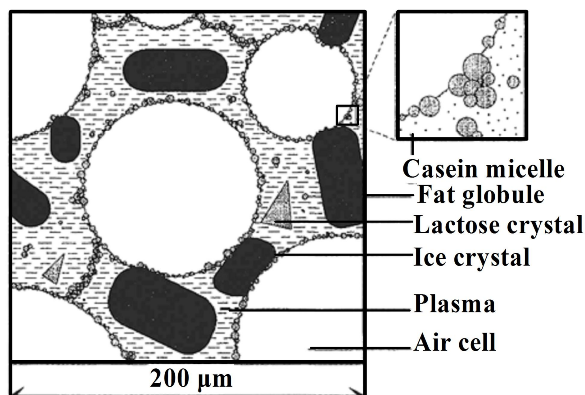


**Figure 6. Schematic presentation of the microstructure of ice cream [34]**

The network of clumped fat globules is formed during freezing. Although the air bubbles become almost completely covered with fat globules, flotation churning presumably does not occur, because too little liquid fat is available to spread over the air bubbles. In all likelihood, clumping is predominantly caused by mechanical forces, i.e., the fat globules are pushed together during beating because of the presence of ice crystals and are damaged by them. The lower temperature occurs more ice and faster clumping.

If unhomogenized cream would be taken, all the fat globules together would just suffice to cover air cells. But natural milk fat globules clump rapidly, and the aggregates formed are not nearly sufficient to fully 'encapsulate' such air cells. This implies that the cells would be unstable during and after freezing and large air bubbles develop, causing a coarse texture. The fat globules become much smaller in size by homogenization of the cream, and then they can cover a much larger air cell surface even after clumping [51,52]. The homogenized fat globules will, however, hardly clump, so the desired network of clumped fat globules does not form. However, these globules increasingly tend to clump if a suitable, small-molecule surfactant (emulsifier) is added. This is due to the emulsifier displacing part of the protein from the surface layers, which decreases the stability of the fat globules to partial coalescence; the globules also become more readily attached to the air bubbles [60].

Fat is a major component in most cheese types. The presence of fat in cheese is necessary, in most cases, to develop the characteristic flavour profile and mouth-feel. Fat globules also have an impact on texture by partially disrupting the casein fibrous matrix to soften the texture [63]. The level of fat influences several aspects of cheese, including composition, biochemistry, microstructure, yield, rheological and textural properties [64, 65]. Moreover, for a given fat content, the type of fat (melting point) and the state of the fat (non-globular, free fat, homogenized, globule size distribution, solid-to-liquid ratio) has a major impact on the rheology, flavor and cooking properties of cheese [66, 67].

There is some debate about whether fat globules participate directly in cheese microstructure by binding to the casein matrix, or act as inert filler material by partially disrupting the casein matrix. Undoubtedly, both mechanisms occur to some extent, as large fat globules are more likely to disrupt the matrix compared to the smaller globules occluded within the protein void spaces. Large fat globules are more likely to be distorted, and

perhaps exist as free fat trapped within the protein void spaces compared to small fat globules [68, 69].

Cheese is essentially a concentrated protein gel, which occludes fat, moisture, and other materials. The gelation of milk is characterized by aggregation of the rennet-altered casein micelles into interconnected clusters and forming a network in which fat globules are interspersed as loose inclusions [70]. As the protein matrix contracts and adjoining curd particles mat through their fat-depleted surface layers, these fat-depleted areas become part of the internal structure of the cheese. The enmeshed fat globules occupy the spaces between the protein strands and may be considered to impede physically the aggregation of the para-casein matrix, to a degree dependent on their volume fraction and size distribution [63]. Consequently, a higher fat level leads to slower syneresis during manufacture [71], and an increase in the level of MNFS in the cheese. The increase in MNFS has a major impact on cheese yield and quality.

Some clumping and/or coalescence of fat globules generally occur in most cheese varieties [10]. In contrast to Cheddar and Mozzarella, relatively little clumping and coalescence of fat globules is evident in other cheese types such as Cheshire, Gouda or Meshanger cheese. The relatively high degree of fat globule coalescence in Cheddar and Mozzarella is probably due to the relatively large displacement of neighboring layers of protein matrix, between which fat globules and fat globule clusters are sandwiched during the cheddaring and/or kneading/ stretching stages of manufacture. Such displacement can be expected to "stretch" the fat globules and, consequently, shear and disrupt their membranes [20, 71].

At the temperatures used in the manufacture of cheese (~30–55 °C) much, or all, of milk fat is liquid and can therefore flow and aggregate, leading to coalescence on the application of stress. A significant portion of the fat (~20–30% total) may be liquid at the ripening temperatures (~4–7 °C), and will aggregate, leading to coalescence [8, 72].

Compared to natural cheese, there is less clumping or coalescence of fat globules in Pasteurized Processed Cheese Products and Analogue Cheese Products. Consequently, the mean fat globule size tends to be generally smaller, although it varies depending on the type and level of emulsifying salt, types and levels of milk protein added, processing time. Generally, for most emulsifying salts, the fat globule size decreases as the processing time at a high temperature increases [72–75].

Increasing the fat content results in a reduction in the volume fraction and continuity of the casein matrix, which becomes more interrupted by fat globules [20]. Concomitantly, the fat globules become more numerous and varied in size and shape, and the degree of clumping and coalescence of the fat globules increase. The increased degree of fat globule aggregation is expected because the number of encounters of the fat globules within a given volume of the casein matrix increases as the enveloping protein matrix contracts during manufacture and as the curd particles undergo deformation during the various stages of cheese making [10, 62, 71]. Conversely, as the fat content of cheese is reduced there are longer stretches of uninterrupted casein matrix and the fat globules become more uniformly dispersed and less clumping is evident.

In Camembert cheese fat globules can both interact with the surrounding protein matrix or serve as protein matrix breakers. Larger fat globules in Camembert cheese tend to be more aggregated and are surrounded by thick protein strands, whereas smaller globules are enveloped by thinner protein strands [76]. The higher total surface area of smaller fat globules may allow greater water binding and moisture retention in the cheese. In addition, small globules are associated with small voids in the protein matrix that more effectively trap water, resulting in a higher-moisture cheese.

## Conclusions

Milk fat can be influenced through a wide variety of processes applied to the milk, particularly homogenization, cooling, heating, which has significant implications for the properties of milk fat globules. These processes are efficiently used to give products desired characteristics and milk fat can have different effect on the structure formation of dairy products. The structural stability and rheological behaviour of butter and milk fat-based products is primarily determined by stabilization by the fat crystal network. Stronger crystal–crystal interactions are very important for good quality of butter. The formation of stable structure of whipped products, such as ice cream and whipping cream, depends on the interaction between fat globules, air bubbles and proteins. Partly destabilized fat globules adsorb at the air/serum interface of the air bubbles. As the fat partially coalesces, it causes one fat-stabilized air bubble to be linked to the next, and foam structure are formed. In cheese's structure formation milk fat is an accessory structure element.

A more detailed understanding is needed of how the presence of milk fat globules is related to the rheological behaviour and structure formation of dairy products. For further studies, it would be relevant to relate the rheological differences observed as a function of thermal and mechanical treatments, and the milk composition of different mammals to the sensory quality of milk-based products. A detailed study addressing this statement would provide valuable knowledge at the industrial level. However, several steps are needed to link the existing knowledge to industrial applications of new products with a high functional value and nutritional quality.

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# Using of bioinformatics and computer morphometry in study of *Fusarium* spp. causing potato dry rot

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## Abstract

**Introduction**. The purpose of research – to evaluate the possibility of using the methods of computer morphometry and bioinformatic analysis in studying of reactive changes in *Fusarium* spp. causing potato dry rot.

**Materials and methods**. Tubers of "Nevski" potato with *Fusarium* contamination signs were selected. Three areas of the damaged tuber potatoes were studied: the epicenter of contaminated plant tissue; peripheral area of contamination; plant tissue outside of contamination epicenter. Morphological research and computer morphometry of fixed preparations of microorganisms stained by 1,0% methylene blue solution were made under magnification x 400, x 1000.

**Results and discussion.** Macroconidium morphometric characteristics of *Fusarium* spp. (square, perimeter, length, number) were obtained by automatic image analyzer "Micros". The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. macroconidium cells causing potato dry rot was created to study bioinformatic cell characteristics.

The following information characteristics of fungal cells have been calculated: H – information entropy characterizing the real structural diversity; $H_{max}$ – the storage capacity characterizing the maximum structural time-diversity of biological system; R (%) – information redundancy coefficient characterizing the relative organization of biosystem.

The *Fusarium* macroconidium cells have demonstrated low values of H (0,002) compared to $H_{max}$ (0,5). The values of R in *Fusarium* cells were 99,40% – 99,69% thus demonstrated a high degree of orderliness and relative organization in microbial cellss. Our research proves the maximum adaptation level of *Fusarium* cells as parasites in biocenosis with plant cells. It should be expected that in the case of the reaction of *Fusarium* cells on chemical or biological preparations the value of H should increase up to values of $H_{max}$, and the value of R will decrease. It's may be occured as a result of disruption structural and functional integrity of *Fusarium* cell system.

**Conclusions**. The method of bioinformatic analysis of *Fusarium* spp. cells causing potato dry rot can be recommended as a testing system in development of new plant protecting preparations.

## Introduction

Safety of food and agricultural products during production and turnover is a keystone in development of food market in many countries [1].

The potato is one of the leading agricultural crops in many countries. The total volume of its production in EU countries in 2013 amounted to about 60 million tons [2]. Despite the fact that the potato is a high yielding culture, total number of potato tubers has significantly reduced in some unfavourable years. The main reason of the potato harvest decrease is the damage of the potato tubers by bacterial and fungal infections such as *Rhizoctonia*, phoma rot, late blight, *Fusarium* and others [3].

Fusarium dry rot is a second most common plant disease after late blight. *Fusarium* spp. belongs to the division Ascomycota and causes many important diseases. These fungi may contaminate food products with mycotoxins, threatening to animal and human health, can cause a wide spectrum of human infections, especially in immune compromised patients (cancer) or HIV positive patients [4, 5].

The fungal genus of *Fusarium* spp. are wide spread in all regions of Northern Hemisphere and other regions [6, 7, 8]. *Fusarium sambucinum*, *Fusarium coeruleum*, *Fusarium solani App.*et. Wr., *Fusarium avenaceum* are the main potato pathogens (Figure1).

**Figure1. Tuber contaminated by *Fusarium* spp. causing potato dry rot.**

A new molecular method of PCR is widely used in science experiments together with traditional morphological and physiological research methods of *Fusarium* spp. [9, 10].

The following measures are used to prevent *Fusarium* plant contamination: mechanical cleaning of tubers, disinfection of storage containers, treatment storage period, keeping the optimum temperature, early diagnostics of tuber infection, chemical treatment of seed material [11, 12].

Biological protection of agricultural plants is one of the alternative methods to reduce soil damage against mineral fertilizers. Biologicals suppress the tissue pathogenic flora by their own microorganisms or plant biologically active extracts [13].

The chemical compound of plant tissue is a good growth medium for microorganisms. That´s why the fungi of the genus *Fusarium* should be considered as one of the components of biological ecosystem.

New informative methods to characterize morphological and functional state of *Fusarium* cells are required in searching of chemical and biological plant protective means [14].

There is no data on using the bioinformatic modelling in study of morphofunctional state of *Fusarium* spp.

There are several articles on the bioinformatic status of human tissues [15]. It was demonstrated that the bioentropy reflects morphofunctional adaptive capacity of organism and is related to changes in bioinformatic status and development of pathological processes in tissues and organs [16, 17].

Our work was to explore the possibility of using bioinformatics and computer morphometry in studying the reactive changes of *Fusarium* spp. causing potato dry rot.

## Materials and methods

Tubers of "Nevski" potato grown in the Leningrad region of Russia were selected as an object to study. The potato "Nevsky" belongs to intensive type of varieties and well responds to fertilizer application. Investigated tubers were in storage for 3 months after the harvest in 2015, average storage temperature was + 18 º C, relative humidity was 75%.

Sampling from potato lot was carried out in accordance with ISO 7002:1986. Potatoes tubers with *Fusarium* contamination signs were selected. The changes of tuber with dry rot were: drying up, shrivelling and falling off the epithelial tissue.

The tuber was cut with a knife along the longitudinal axis through the stolon and examined the tuber flesh. The flesh looked like a dry rotten black mass with voids, filled with fluffy mycelium of the fungus – the epicenter of contaminated plant tissue.

Three areas of the damaged potato tuber were studied:

- the epicenter of contaminated potato tuber tissue;
- peripheral area of potato tuber tissue contamination;
- potato tuber tissue outside the epicenter of contamination.

The qualitative method has been used to detect the presence or absence of *Fusarium* sp. in potato tissues (ISO 7218–2011). Microbiological study of tuber tissues was carried out using "imprint method" ("contact method") by analogy of horizontal sampling techniques from surfaces using contact plates (ISO 18593:2006). A glass slide was applied for 5–10 seconds to the each areas of contaminated potato tissues. Samples of potatoe tissues were stained by 1% methylene blue solution [18]. Morphological research of microorganism fixed preparations was made on microscope "Micros" (Austria), model MS 100 (XP), under magnification x 400 or x 1000.

Morphometric macroconidium cell characteristics of *Fusarium* spp. (square, perimeter, length, number) were obtained by automatic image analyzer "Micros". The quantification of microorganisms was made in 10 view fields for each sample [20].

Morphologic identification of fungi of the genus *Fusarium* was determined by curvature of macroconidium, the shape of the apical cells. The species identification of *Fusarium* according to the morphological characteristics of their macro – and microconidia wasn´t studied.

The following information characteristics of fungal cells have been calculated:

- H – information entropy characterizing the real structural diversity.
- $H_{max}$ – the storage capacity characterizing the maximum structural time-diversity of biological systems.
- R (%) – information redundancy coefficient that characterizing the true biosystem organization.

Statistical data processing was provided with program "STATGRAPHICS".

## Results and discussion

The results of recent study of morphofunctional state of yeast and bacterial producers of biotech products by bioinformatics and morphometric analysis show the usefulness of these methods in research of the adaptive reactions to some microorganisms [15]. The research purpose was to determine the possibility of using bioinformatics and computer morphometry to the fungal pathogens of valuable agricultural crops, including *Fusarium* causing potato dry rot.

The results of microscopic study of potato tuber tissues contaminated by *Fusarium* spp. are shown on Figure2.

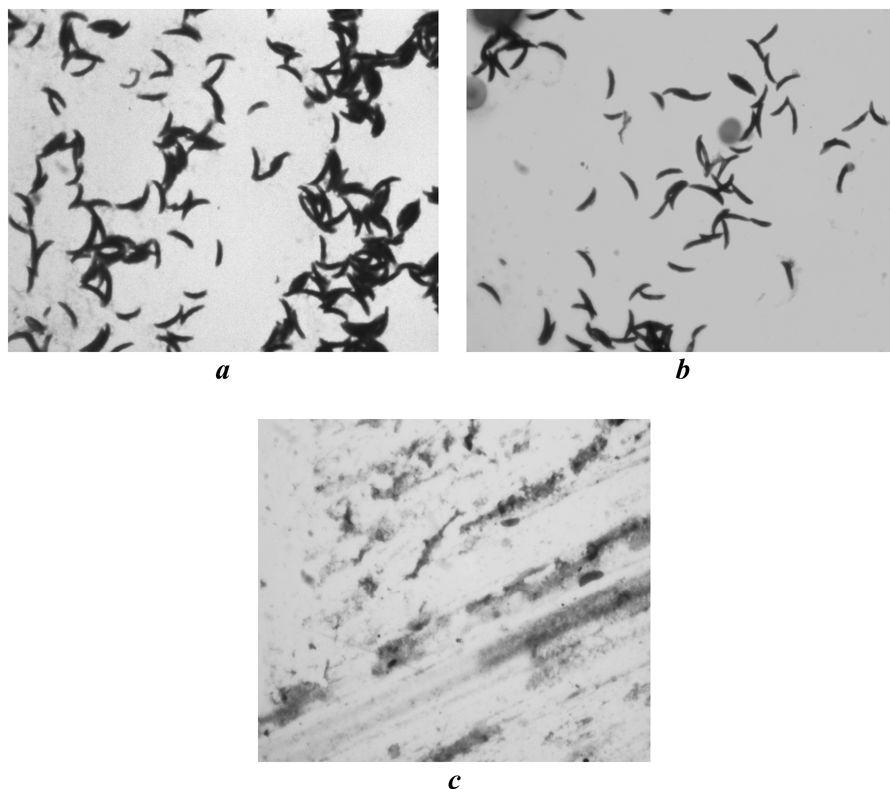


**Figure 2. Conidia of the *Fusarium* spp. in potato tuber tissues:**
a – the epicenter of contaminated potato tuber tissue;
b – peripheral area of potato tuber tissue contamination;
c – potato tuber tissue outside the epicenter of contamination.
Methylene blue. Magnification x 400.

The maximum concentration of the *Fusarium* macroconidium cells was determined in the epicenter of contaminated potato tuber tissue (Figure 2-a). The amount of the *Fusarium* macroconidium cells decreased in the peripheral area of contamination (Figure 2-b). The *Fusarium* cells (only microconidium) were found in the plant tissue outside the epicenter of contamination in a single amount (Figure2-c).

The macroconidium of *Fusarium* spp. were from fusiform to lanceolate, mostly with a visible three partitions. Cells in the central part are typically square. The apical cell is slightly elongated, gradually and evenly tapering, and somewhat curved (Figure 3).

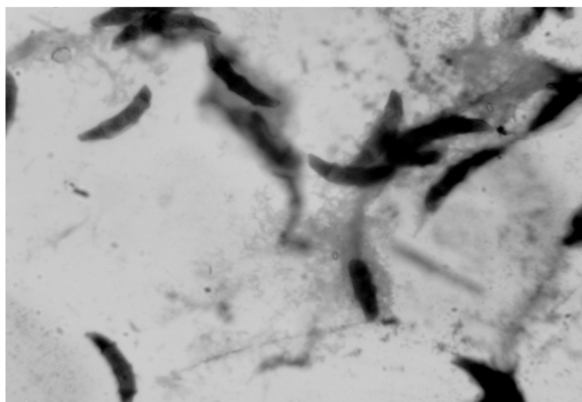

**Figure 3. Macroconidium of *Fusarium* spp. causing potato dry rot. Methylene blue. Magnification x 1000.**

The results of morphometric measurements of the *Fusarium* macroconidium cells in the epicenter of contaminated potato tuber tissue were: the area size was 30084,64 $\mu m^2$ ± 0,02, the cell perimeter – 996,15 µm ± 0,01, the cell length – µm 416,31 µm ± 0,02.

The results of morphometric measurements of the *Fusarium* macroconidium cells in the peripheral area of potato tuber tissue contamination were: the area size was 25670,66 $\mu m^2$ ± 0,03, the cell perimeter – 1010,54 µm ± 0,01, the cell length – 452,67 µm ± 0,04.

The morphometric measurments of *Fusarium* microconidium cells in the plant tissue outside the epicenter of contamination weren't to carry.

The results obtained show that *Fusarium* cells have their individual morphometric characteristics in two zones of contamination. The area size of *Fusarium* cells in the epicenter of contaminated potato tuber tissue was 1,7 times larger then in the peripheral area of potato tuber tissue contamination.

Integral criteria of the morphofunctional state of *Fusarium* cells causing potato dry rot were obtained by using the basic concepts of thermodynamics and informatics: entropy H as a function of system state, the information redundancy [15].

The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. were created to calculate cell characteristics such as information entropy (H) and storage capacity ($H_{max}$). Due to values of H and $H_{max}$, we've calculated the values of the information redundancy coefficient (R,%) that characterizes a measure of the biological system reliability, i.e. its ability to resist external and internal effect factors.

The data of H, $H_{max}$ and R for *Fusarium* cells causing potato dry rot are presented in Table 1.

**Table 1**

**Informational characteristics of *Fusarium* cells causing potato dry rot**

| Num | Area of potato tuber tissue | Value of information entropy (H) | Value of storage capacity ($H_{max}$). | Value of information redundancy coefficient (R,%) |
|---|---|---|---|---|
| 1 | The epicenter of contaminated potato tuber tissue | 0,002 | 0,5 | 99,69 |
| 2 | The peripheral area of potato tuber tissue contamination | 0,002 | 0,5 | 99,4 |
| 3 | The potato tuber tissue outside the epicenter of contamination | - | - | - |

These data demonstrate that microscopic fungi cells have some differences in information characteristics of two areas of contaminated plant tissues.

The destruction of potato tuber tissue was maximally expressed in the epicenter of contamination. The redundancy coefficient R of *Fusarium* cells in this zone was 99,69%. The redundancy coefficient R of *Fusarium* cells in peripheral area of potato tuber tissue contamination was slightly reduced to 99,4%.

The values of information entropy (H) of the microorganisms cells were 0,002, the storage capacity ($H_{max}$) 0,5 in both areas of plant tissue contamination.

Previously we've obtained informative characteristics for yeast and bacteria cells – producers of biotechnological foodstuff [19]. The information redundancy coefficient R was calculated for each type of microorganism.

For different yeast strains the values of redundancy coefficient R (%) were:

*Saccharomyces cerevisiae* – 82,02; *Mycoderma vini* – 68,84; *Rhodotorula gracilis* – 81,12. For different bacterial species the values of redundancy coefficient R (%) were: *Escherichia coli* – 88,46; *Baccilus subtilis* – 65,94; *Micrococcus sulfaticus* – 49,29; *Pseudomonas fluorescens* – 38,83.

Comparative analysis of information characteristics of the researched microorganisms (*Fusarium*, yeast, bacteria) showed that the values of the redundancy

coefficient R in the *Fusarium* cells causing potato dry rot were higher in 1.3 times than that of yeast cells. The values of the redundancy coefficient R in the *Fusarium* cells causing potato dry rot were higher in 1.6 times than that of bacterial cells.

These data should be explained by some facts. The yeast strains and bacterial species were grown in a nutrient culture media prepared to ISO 11133–1–2011. The microorganisms were in resting state in culture media. The *Fusarium* cells have demonstrated low values of information entropy H (0,002) compared to information capacity $H_{max}$ (0,5). The redundancy coefficient R in *Fusarium* cells had high values (99,40% to 99,69%) thus demonstrated a high degree of orderliness and relative organization in microbial cells contaminating potato tubers.

It is known that phytopathogenic fungi of the genus *Fusarium* contaminating potato tubers are parasites. The fungi cells penetrate plant tissue and multiply in it by activating their metabolism [21]. Our research proves the maximum adaptation level of *Fusarium* cells in biocenosis with plant cells during microbial contamination of potato tubers.

It is important to study the *Fusarium* cell information characteristics in case of development and introduction new chemical and biological plant protecting preparations. It should be expected that in the case of the reaction of *Fusarium* cells on chemical or biological preparations the value of information entropy H should increase up to values of information capacity $H_{max}$, and the value of the redundancy coefficient R will decrease. This may be accured as a result of disruption structural and functional integrity of *Fusarium* cell system and will indicate the efficiency of new preparations .

## Conclusions

The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. macroconidium cells causing potato dry rot was created to study bioinformatic cell characteristics.

The values of bioinformatic cell characteristics such as the information entropy (H), the storage capacity ($H_{max}$) and the information redundancy coefficient (R) were obtained to *Fusarium* spp. macroconidium cells causing potato dry rot.

The method of the bioinformatic modelling is demonstrated to help in study of adaptive reactions of *Fusarium* spp. causing potato dry rot and can be recommended as a testing system in development of new plant protecting preparations.

The method of the bioinformatic modelling can be recommended for the diagnostics of Fusarium infections in medicine.

The authors disclose that they have no conflict of interests.

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# Ethanol prodution from mucilage and pulp of processed coffee

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**Abstract**

**Introduction**: The present investigation is to evaluate ethanol production from coffee pulp and mucilage as coffee by-products, which was used as substrate.

**Material and methods**: Wort extracted from pulp and mucilage was fermented; molasses sugar was used to optimize in terms of concentration and increase reducing sugars for fermentation process; acid hydrolysis was performed to breakdown complex polysaccharides from pulp and mucilage into simple monomers. Dry yeasts used as Saccharomyces cerevisiae were rehydrated and supplemented by nutrients rich in nitrogen, magnesium and carbon before being pitching in the wort; appropriate materials for hermetic process of fermentation were used and regular controlled.

**Results and discussion**: The variation of reducing sugars in a substance shows the evolution of fermentation process. During this fermentation process, it has been realized that the sugar content has decreased, by comparing with the initial level and final, before and after fermentation, is directly related to the fermentation efficiency. The end of process was realised when the density has decreased below 6%. The obtain results after fermentation, was also continued by conducting distillation; the 6.5l as amount of wort produced 2.3l of ethanol with 45% absolutely volume, equivalent to 35.5% of wort volume. Since pH of wort was optimised to 4–5 for fermentation, the results show that ethanol production can be produced and viable using coffee pulp and mucilage as by-products and reduce the contamination of them to the environment. For this case study, by optimizing alcohol concentration, the pH, and temperature have been kept constant for all samples.

**Conclusion:** Processing of the coffee mucilage and pulp as by-products to ethanol can increase raw material resources suitable for ethanol production for various purposes in Rwanda and this confirm that ethanol production from mucilage and pulp of coffee is possible as results show the potential to use them instead of considering them as waste.

## Introduction

Rwandan coffee has a balanced flavour, aroma and fruity taste which make it among the top rated coffee in USA, European and the Asian coffee markets. The main by-products from coffee processing are waste water, mucilage and pulp.

The by-products are rich in carbohydrates thus useful for various purposes including the potential for ethanol production. Types of coffee processing produce slightly different by-products.

Wet processing produces different by-products compared to dry method. In wet processing the coffee fruit is pressed in water through a screen which leaves a part of the pulp, the mucilage and the parchment still attached to the seeds (Belitz, et al. 2009).

The composition of Coffee pulp is ether extract: (0.48%); crude fibre: (21.4%); crude protein: (7.8%); Pectic substances: (6.5%); non-reducing sugar: (2.0%); reducing sugar: (12.4%); chlogenic acid: (2.6%); caffeine: (2.3%) and total caffeic acid: (1.6%) (Gathuon, et al., 1991).

The coffee mucilage fraction remains adhered to the coffee bean in the wet processing which allows separation and concentration of the fraction. The composition of the mucilage is: water: 84.2%; protein: 8.9%; glucose (reducing): 2.5%; sucrose (nonproducing): 1.6%; pectin: 1.0% and ash: 0.7% (Clifford, Wilson, 1985).

This research studied ethanol production from a mixture of coffee by-products particularly pulp and mucilage of coffee. The Fermentation and distillation processes of the raw materials and evaluation of the main process variables of coffee processing as by-products was conducted. Ethanol production as one of the applications of these wastes, as they contain carbohydrates which can be used for fermentation. The objective of the research:

- Minimize negative effects of coffee pulp and mucilage to the environment;
- Value addition to the coffee pulp and mucilage;
- Increase raw material resources suitable for ethanol production.

## Materials and methods

### Source of samples

The coffee by-products used in the study were of coffee Arabica variety from coffee station in the eastern province of Rwanda specifically 35 kgof pulp and mucilage from one of the coffee washing stations in Rwamagana were used.

### Samples preparation

Samples were prepared from pulp and mucilage based on different sugar contents. After the extraction of pulp and mucilage, samples of mixtures of all by-products were prepared, for each by-product different weight and Bx were used: 11 kg (sample A) with 10 degree Bx; 8 kg(sample B) with 20 degree Bx and 2 samples of 6.5 kg with 15 degree Bx (C and D). The other raw materials used were coffee water, water and molasses.

### Wort preparation

The pulp with mucilage adhering on it, was passed through a mesh then through a manual extractor for juice extraction, care was taken not to cause physical damage to the structure. The juice obtained was collected in a well cleaned bucket in the laboratory. The juice was mixed with 10 kg of coffee waste water for wort preparation.

All samples were sterilized by heating at 95 °C for 900 seconds. Acid hydrolysis and regulation of fermentable sugars were simultaneously done to each sample. Yeasts were also prepared for each sample pulp by considering sample size obtained in extraction. It was necessary also to increase fermentable sugars by acid hydrolysis, where the hydrolysis of pectic substances in mucilage such as protopectin, increase the total sugar content (Fengel, 1979; Diana et al., 2011).

**Fermentation**

All samples were fermented separately, wort well aerated and cooled to 20–22 °C were subjected to fermentation. The pH of wort was optimised to 4–5 for fermentation. Yeast solutions were also prepared for each sample and were mixed with the corresponding samples to facilitate fermentation process. Air bubbles in the containers as well as the level of sugar content were monitored in the process and ethanol was progressively produced.

Ethanol (alcohol) measurement after fermentation this involves the measurement of the density of the wort before fermentation (FG). The ethanol concentration obtained was calculated by using Cyril JJ Berry formula:

$$\% ABV=(OG - TG)/0.736)\cdot 100$$

where OG – original specific gravity;
FG – final specific gravity;
7.36 – ethanol density.

**Distillation and identification**

Each sample was distilled separately and ethanol concentration was determined using an alcoholmeter, chemical reaction and combustions.

## Results and discussions

**Handling of raw materials**

After reception of the raw materials, it was observed that their decomposition occurred immediately after processing. The decomposition was related to changes in soluble solids, pH, color and odor over time, is associated with factors such as the quantity and quality of the water used in the processing of coffee, which was not of drinking quality.

**Fermentation process**

The process of fermentation was conducted and the obtained results are shown in the following Figure 1.

When the fermentation is going on, sugars are converted by yeasts to ethanol. The variation of reducing sugars in a substance shows the evolution of fermentation process. The fermentation is finished when the density has decreased by below 6%.

Many times the fermentation stops before reaching that value, or can continue and goes below that value. It may be due to many factors related to pH, sugar content, yeasts preparations etc.

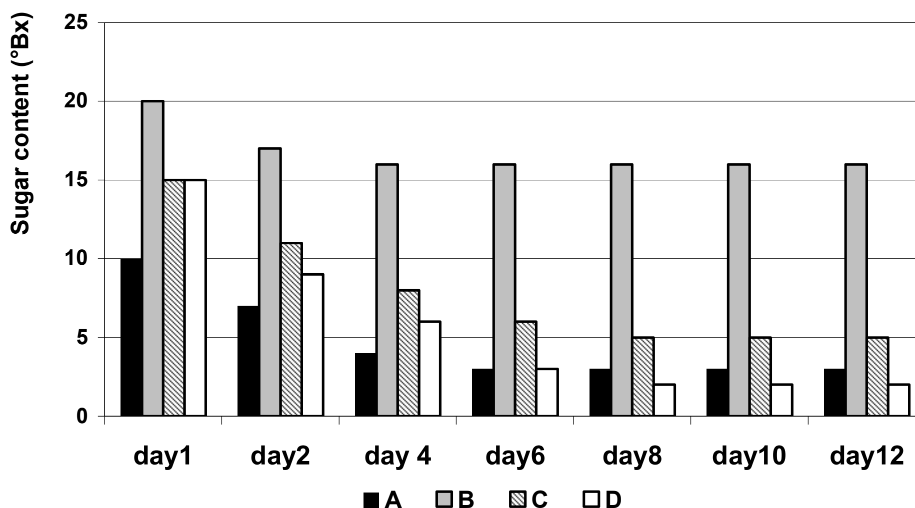


**Figure 1. Variation in sugar content (°Bx) over time, during fermentation in the sample A, B, C, D.**

This results in very sweet or dry wines when they are for drinking, but for this case study, the non-well fermented wort will result in low yield of alcohol (i.e. ethanol concentration).

From Figure 1, it easy to see that sugar content varied differently over time, in the 4 samples:

- For Sample A, the Bx level decreased considerably from 10 to 2 °Bx;
- For Sample B, the Bx level decreased by only 3 °Bx, it means from 20 to 17 °Bx;
- For Sample C, the Bx level decreased moderately from 15 to 5.5 °Bx;
- For Sample D, the Bx level decreased considerably from 15 to 2 °Bx.

The way sugar content have decreased during fermentation, by comparing the initial level before fermentation and the final level after fermentation is directly related to the fermentation efficiency.

For this case, for Sample D, where sugar content varied considerably from 15 to 2 °Bx, it means that fermentation have been powerful, Although for Sample B, where sugar content decreased by only 3 °Bx, from 20 to 17 °Bx, it means that the fermentation have been inefficient.

When the fermentation has been inefficient, it means that sugars have not been converted into ethanol adequately, which means that the alcohol concentration in the fermented product should be low.

**Optimization of alcohol concentration**

From the following Figure 2, it is shown that the alcohol concentration varies for the 4 samples. It may due to the parameters which condition the fermentation process such as: yeasts preparation, temperature, pH adjustments, and sugar content.

For this case study, pH, and the temperature have been kept constant for all samples, which means that the difference between the alcohol concentrations resides in 2 factors:

- Sugar concentration
- Yeasts preparation

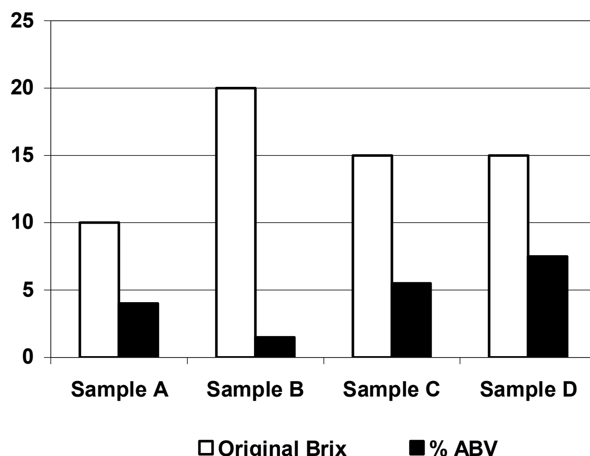


**Figure 2. Comparison between the increase in Original Brix and%ABV for all samples**

## Optimization of alcohol quantity yield

During fermentation, sugars are converted into alcohol, but all the sugars are not converted completely. After the fermentation; residues of sugar not fermented, dead yeasts, and other materials contained in wort which are not fermentable, sink in the bottom of fermenting vessel. Minimizing the available quantity of alcohol mixture in order to use it in the distillation process.

**Table 1**

**Alcohol quantity produced**

| | Sample A | Sample B | Sample C | Sample D |
|---|---|---|---|---|
| Initial weight of wort ( kg) | **11** | **8** | **6.5** | **6.5** |
| Weight of alcohol mixture ( kg) | **8** | - | **4.5** | **5.5** |
| Percentage yield | **72%** | - | **69%** | **85%** |
| Apparent attenuation (%) | **80%** | **15.7%** | **63%** | **93.7%** |

When sugars are not fermented sufficiently many sugar residues sink at the bottom, which results in a loss in the alcohol recuperation. By calculating the apparent attenuation, it was immediately anticipate the loss in alcohol recuperation as an increase in apparent attenuation corresponds to the increase in quantity available for distillation.

Thus, in order to increase the quantity of fermented substance which is available or usable in distillation, (which influences also the final ethanol concentration obtained after distillation), is to make sure that is done in the way of controlling all factors (Yeasts preparation, Optimum pH, temperature, Not high level of sugars etc… as cited above).

As it is shown on Figure3, the Sample D has produced a high quantity of alcohol, both in the quantity and in concentration, as shown by Figure 2. On the other hand, the Sample B, has not produced any valuable quantity for use, because its fermentation got stuck as shown, in figure 1.

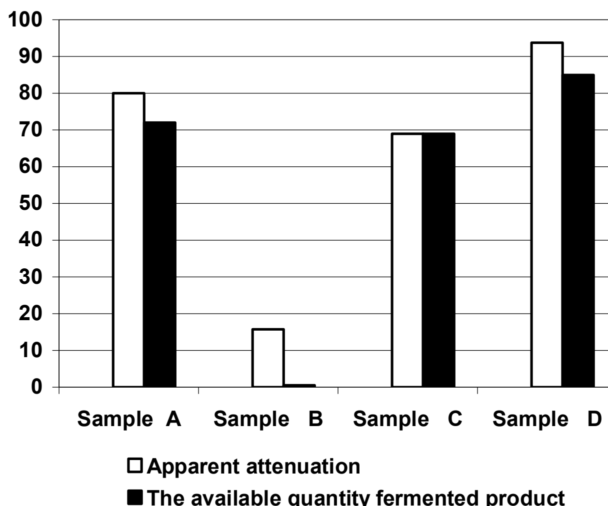


**Figure 3. Relation between apparent attenuation increase and the increase of available quantity fermented product**

### Quantity analysis of ethanol yield production

During the distillation process all wine, beer, or fermented product content are not evaporated, because they are not ethanol or water. Thus, there will be some liquid which remains in distilling flask. These substances have been weighed and compared to the weight of the ethanol produced.

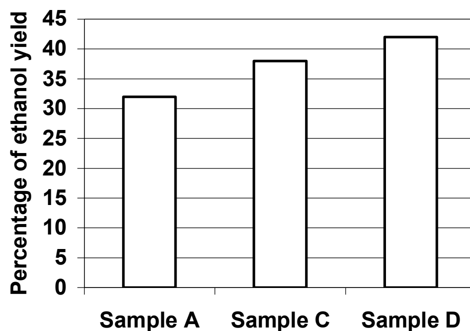


**Figure 4. Ethanol yield percentage**

The figure 4 shows that the production of ethanol in samples A, C and D was good and the best production of ethanol was high in the sample D compare to others.

## Conclusions

- Further studies on this topic can be conducted for moderation since it is a complex process and it necessitates more time and powerful equipment to explore each opportunity by analyzing deeply all details;
- The application of this project can help various institutions in Rwanda, dealing with environment, to reduce the environment threat caused by coffee processing by-products, and produce a valuable substance such as ethanol, which can be even used to produce energy.

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# Technology features of using gums in a creation of gel bases

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## Abstract

**Introduction.** The aim of this research is the studying of technological, physical-chemical and rheological properties of the gel bases, based on hydrocolloids, for further development of gel food products.

**Materials and methods.** As objects of the research we have selected xanthan gum, guar gum and locust bean gum, also gel bases with different concentration of these gums. Samples were prepared, using 3 methods, to study the technological, physical-chemical and rheological properties of gel bases. The study of structural and mechanical properties of the samples were performed on the rotational viscometer.

**Results and the discussion.** It was investigated the influence of a gelling agent concentration, water temperature and the duration of swelling on the viscosity of colloidal solutions and on the limiting shift stress. Gel bases, prepared using 3 methods with chosen hydrocolloids, belong to structured systems. The analysis of the experimental data showed that the greatest value of the maximum viscosity that meets virtually indestructible system, for samples of gel bases with xanthan gum, prepared by the method 3, is 106,3 Pa·s, with locust bean gum it is 1,97 Pa·s, with guar gum it is 17,7 Pa·s, at a concentration of 1%.

Based on the experimental data it was also calculated the structural and mechanical characteristics. For the xanthan and guar gum the value tension of change is more than zero, this indicates that the investigated samples are structured bodies like solid. For the samples with locust bean gum the value tension of change is zero, this indicates that the investigated samples appear to be pseudo-plastic liquid. The most dynamic limit of ability to flow was observed in samples with xanthan gum, prepared by the second and the third methods, with locust bean gum and guar gum, prepared by the first and the third methods at a concentration of 1%.

A comparison of hydrocolloids solutions, obtained at different ways of preparation, showed that the most appropriate preparation of gels is the third, because under these conditions the viscosity of solutions (at the same concentration of a gelling agent) was higher and the structure of the gel was more homogeneous.

**Conclusions.** All the experimental samples of gels are characterized by non-Newtonian pseudo-plastic type of stream. The most appropriate is the preparation of gels by the third method, which consisted in swelling of hydrocolloid for 40 minutes in water of room temperature, heating to 60 °C and cooling. The optimum concentration of gelling agents – 0.5%.

## Introduction

Structured products have become widespread, they are easily digested and by allowing the inclusion various ingredients to them, they can extend the range of food products with high biological value. The creation of gel products is based on the concept of the formation of the suspension of vital substances that are ready for absorption by the organism, through being in a microenvironment of a gel. They are ready for consumption and quick assimilation always and anywhere [1].

It is known that in recent years it is a tendency to create products, based on natural substances, so studying the properties of xanthan gum, locust bean gum, guar gum is an urgent task [2]. The determining of the structural viscosity, thixotropic degree can objectively estimate the quality of a developed gel product.

The aim of this work is the studying of technological, physical-chemical and rheological properties of the gel bases, based on hydrocolloids, for further development of gel food products.

## Literature review

The design and the implementation of new generation food occur mainly in two ways: the development of analogs of products, which quality criteria are the characteristics of known by population products, and the manufacturing of products with new properties and composition. The development of new forms of products, studying their consumer characteristics, quality and efficiency become more and more important[1–3].

As a result of years of research, scientists have concluded that the most effective way to deliver the necessary substances to the organism is a product in a gel form, which facilitates the biological digestion of products.

Functional products in the form of gels deliver equally well as hydrophilic and hydrophobic substances into the body. The number of active components is unlimited and typically gel products are multi-component composition [2]. Thickeners play a role not only of supporting components, but they are biologically active, having a beneficial effect on the human body.

Food stabilizers, thickeners and gelling agents are derived from many natural sources, including bacteria, terrestrial and marine plants and connecting tissues of animals. The possibility and feasibility of using these ingredients are determined by the totality of their properties, price and availability [3, 4].

The ingredients, which are used in the food industry, are received by the extraction from various natural materials and are brought in food to get a certain structure and the necessary stability, fluidity and consumer characteristics.

These additives include traditional materials such as starch and gelatin. The most spread structural polymer of terrestrial plants is the cellulose and gums and products from algae. Also in the food industry it is allowed the use of microbial polysaccharides, which are xanthan, gellan and polluan [3–5].

Hydrocolloids have a significant effect on the properties of food and cosmetic products with content from several mg / kg to high concentrations. Constant attention of scientists to these substances is caused by the importance of hydrocolloids for food technologies. With the use of modern methods of research in recent years it has been established the structure of these polymers and the formation of mesh structures by them and mechanisms of their interaction with other polymers. These structures exhibit many properties of ready products, including the stability of emulsions, the lasting stability of suspensions [2, 6].

"They are present in nearly all manufactured products of food and cosmetic industry, but at the same time they are not well investigated" [2]. This statement clearly shows the role of stabilizers, thickeners and gelling agents. It is impossible to enumerate all the functions of these additives, that have an influence on the appearance, "sensation in the mouth" and the taste of products not only because a lot of them, but also because of constant changes, because all the time there are new data of the use of vehicles and useful properties of these compounds.

All stabilizers, thickeners and gelling agents are united by common name hydrocolloids, have vegetable matter, some of them are received using biotechnology, and gelatin belongs to the products of animal origin .

One of the most important properties of hydrocolloids, that affects the texture, is their property to gelling. For example, the addition of even small amounts of carrageenan to jelly dairy desserts forms stable gels. Other classic gelling agents include pectin, gelatin and agar [3, 7].

One of the perspective gelling agents is gum. Gums are the natural polysaccharides; used for a manufacturing of food products as a stabilizer, thickener, gelling agent.

Xanthan gum (E415) is a natural polysaccharide, natural food additive that is used for food production as a stabilizer, thickener, gelling agent and means for encapsulation. Xanthan gum is obtained by fermentation of sugar syrup by cultures of *Xanthomonas campestris*. The substance is easily soluble in water, almost independent of mechanical influences, doesn't lose its properties in a wide range of pH and temperatures [8].

Locust bean gum (E410) is a plant polysaccharide, which is obtained by grinding the endosperm of plants Ceratonia siligua. This polysaccharide is spread in the food industry where it is widely used for thickening aqueous phase and prevent syneresis [9].

Guar gum (E412) is a powder white or yellowish color with a characteristic odor. It is extracted from grinded endosperm of guar beans. In food guar gum is used as a thickener, which has the following properties: regulates viscosity, stabilizes emulsions and provides creamy texture of products [10].

## Materials and methods

As objects of the research we have selected xanthan gum, guar gum and locust bean gum, also gel bases with different concentration of these gums.

It was investigated three ways to study the technological conditions of preparation of gel bases.

By the way 1: to distilled water of room temperature with constant stirring we added hydrocolloid by parts and left for a day until the complete dissolution of a gelling agent. By the way 2: to distilled water, heated to 60 ºC, with constant stirring we added a gelling agent by parts to the formation of a gel. By the way 3: to purified water of room temperature we added the structure-made substance, heated to 60 °C and stirred to the formation of a gel. As the result we obtained transparent gel bases pale-white color, homogenous, various viscosities.

The study of structural and mechanical properties of the samples were performed on viscometer [11, 13].

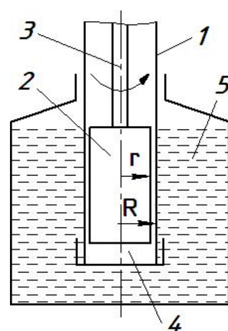


**Figure 1. Device "Rheotest – 2"**

Viscosity measurements were carried out using the device "Rheotest–2", which operates on a constant shear rate.

1. Prepare a sample of 100 cubic centimeters of the set system, mix her and maintain 20–30 minutes.
2. In the immobile external cylinder of device 1 (Figure 1) inundate 30–40 $cm^3$ of the investigated system.
3. Put an internal cylinder 2 on an axis 3 that is connected with an electric engine.
4. An external cylinder 1 with the structured system is put on the fixed internal cylinder 2 and lift to support.
5. Fix position of external cylinder by means of nut. The investigated system is evenly distributed in a gap 4 between coaxial cylinders (external 1 and internal 2). If necessary the system is maintained in a thermostat 5 at a certain temperature (Figure 1)
6. On condition of permanent tension of change P=const to the internal cylinder deformations (12 or 24) give certain permanent speed, here an external cylinder stays still.
7. Register velocity of circulation of movable cylinder 2 by means of potentiometer. Velocity of circulation of cylinder is proportional to speed of deformation of the investigated system.
8. Tensions of change of P expect, that arises up in the system, after equalization:

$$P = z \times a$$

Z – became internal cylinder (driven to the passport of device, for example, for the cylinder of S2 – Z=5,39 Pa); *a* – it is a value of scale on an indicatory device (potentiometer).

9. The values of gradients of deformation $\acute{\varepsilon}$ for every velocity of circulation (12 values) take from passport data.
10. After the values of tension of change of *P* and gradient of deformation $\acute{\varepsilon}$ expect dynamic viscosity η:

$$\eta = P / \acute{\varepsilon}$$

η – dynamic viscosity, Pa·s; P – is tension of change, Pa; $\acute{\varepsilon}$ – it is speed of change, $s^{-1.}$
The experimental data build complete rheological curves of viscosity $\eta = f(P)$ and fluidity $\acute{\varepsilon} = f(P)$ [11–13].

## Results and discussion

It was investigated the influence of a gelling agent concentration, water temperature and the duration of swelling on the viscosity of colloidal solutions and on the limiting shift stress.

It was calculated structural and mechanical properties of obtained colloidal systems.

For systems with xanthan gum, locust bean gum and guar gum with little stress we see an avalanche destruction of the structure, characterized by a sharp decrease of system viscosity. With further increase of the stress structure is destroyed more slowly, the viscosity decreases.

So, gel bases prepared by 3 ways with selected hydrocolloids belong to structured systems. Samples, prepared by the first method (Figure 1,4) for xanthan and locust bean gum didn't meet the required structural parameters.

The analysis of the experimental data showed that the greatest value of the maximum viscosity $\eta_0$ that meets virtually indestructible system [13–15], for samples of gel bases with xanthan gum, prepared by the method 3, is 106,3 Pa·s at a concentration of 1% (Figure3). The lowest value of the minimum viscosity $\eta_m$, corresponding to the almost destroyed system [13–15], is characteristic to the sample with xanthan gum, prepared by method 2, and it is 0,16 Pa·s at a concentration of 0.1% (Figure 2,3). The largest anomaly of the viscosity $\eta_0$-$\eta_m$ is characteristic for the systems with xanthan gum, prepared by 3 method, and it is 105,3 Pa·s at a concentration of 1%.

Based on the experimental data it was also calculated the structural and mechanical characteristics. For the xanthan gum the value $P_{k1}$> 0, this indicates that the investigated samples are structured bodies like solid [15–17]. The most dynamic limit of ability to flow was observed in samples with xanthan gum, prepared by the second and the third methods at a concentration of 1%. An indicator $P_{k1}$/ $P_{k2}$, which characterizes the strength of structural links in the system[15–17], indicates that samples prepared by the third method have the strongest structural links.

The analysis of the experimental data showed that the greatest value of the maximum viscosity $\eta_0$ that meets virtually indestructible system [13–15], for samples of gel bases with locust bean gum, prepared by the method 3, is 1,97 Pa·s at a concentration of 1% (Figure6). The lowest value of the minimum viscosity $\eta_m$, corresponding to the almost destroyed system[13–15], is characteristic to the sample with the locust bean gum, prepared by method 2, and it is 0,18 Pa·s at a concentration of 0.1% (Figure5). The largest anomaly of the viscosity $\eta_0$-$\eta_m$ is characteristic for the systems with locust bean gum, prepared by the method 3, and it is 1,589 Pa·s at a concentration of 1%.

Based on the experimental data it was also calculated the structural and mechanical characteristics. For the locust bean gum the value $P_{k1}$=0, this indicates that the investigated samples appear to be pseudo-plastic liquid [15–17]. The most dynamic limit of ability to flow was observed in samples with locust bean gum, prepared by the first and the third methods at a concentration of 1%. An indicator $P_{k1}$/ $P_{k2}$, which characterizes the strength of structural links in the system [15–17], indicates that samples prepared by the third method have the strongest structural links.

The analysis of the experimental data showed that the greatest value of the maximum viscosity $\eta_0$ that meets virtually indestructible system [13–15], for samples of gel bases with guar gum, prepared by the method 3, is 17,7 Pa·s at a concentration of 1% (Figure9). The lowest value of the minimum viscosity $\eta_m$, corresponding to the almost destroyed system [13–15], is characteristic to the sample with the guar gum, prepared by method 2, and it is 0,43 Pa·s at a concentration of 0,1% (Figure8). The largest anomaly of the viscosity $\eta_0$-$\eta_m$ is characteristic for the systems with guar gum, prepared by the method 3, and it is 16,96 Pa·s at a concentration of 1%.

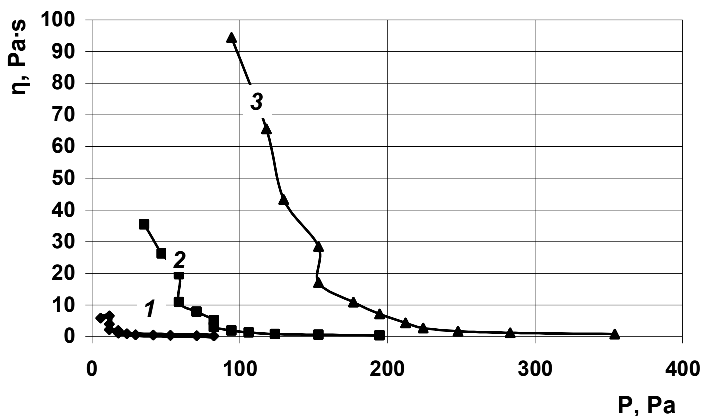


**Figure 1. The viscosity curve of samples with xanthan gum, prepared by method 1**

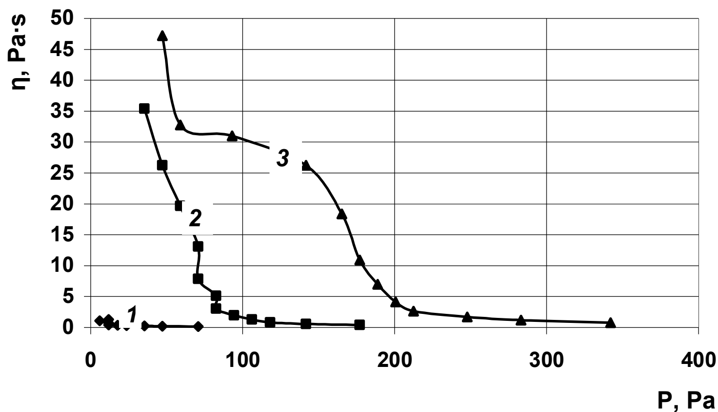


**Figure 2. The viscosity curve of samples with xanthan gum, prepared by method 2**

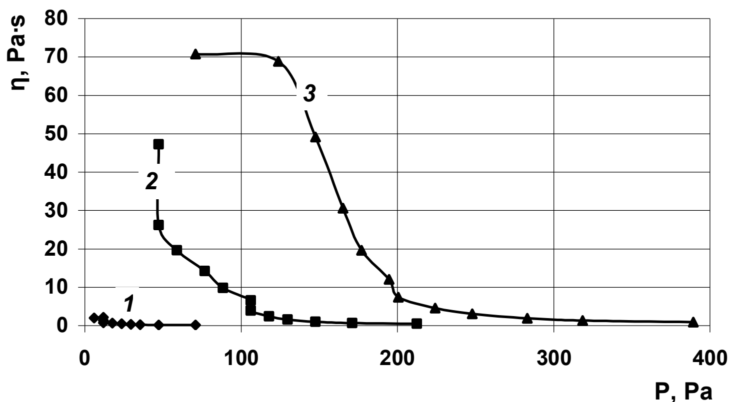


**Figure 3. The viscosity curve of samples with xanthan gum, prepared by method 3**

**The concentration: 1 – 0,1%; 2 – 0,5%; 3 – 1,0%**

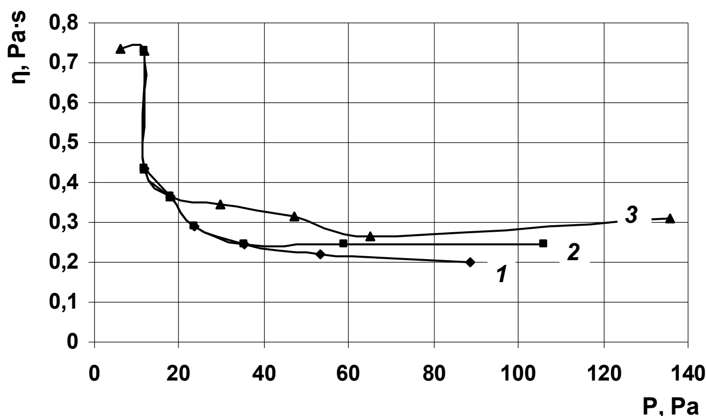


**Figure 4. The viscosity curve of samples with loc. bean gum, prepared by method 1**

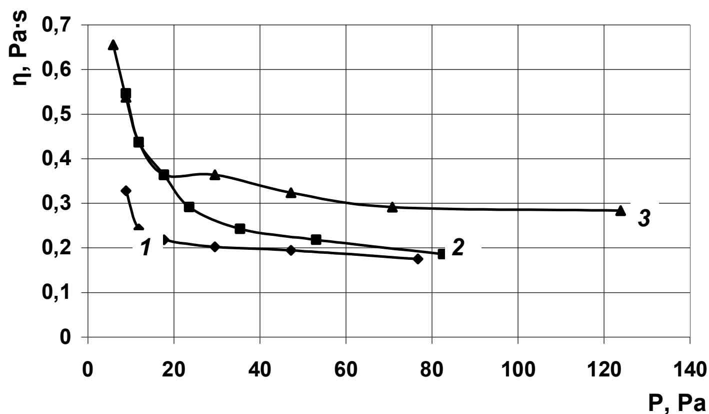


**Figure 5. The viscosity curve of samples with loc. bean gum, prepared by method 2**

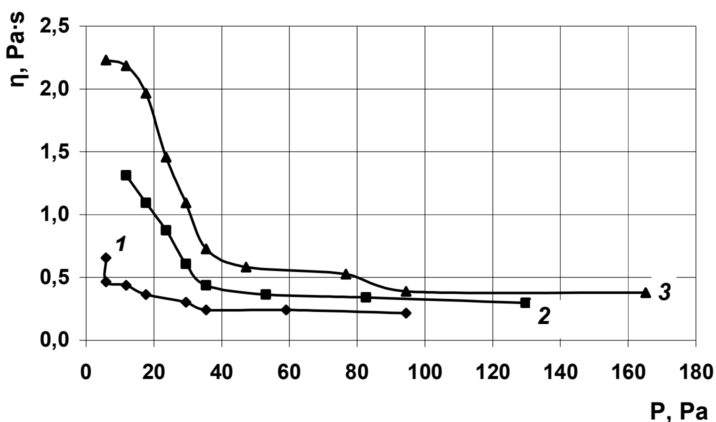


**Figure 6. The viscosity curve of samples with loc. bean gum, prepared by method 3**

**The concentration: 1 – 0,1%; 2 – 0,5%; 3 – 1,0%**

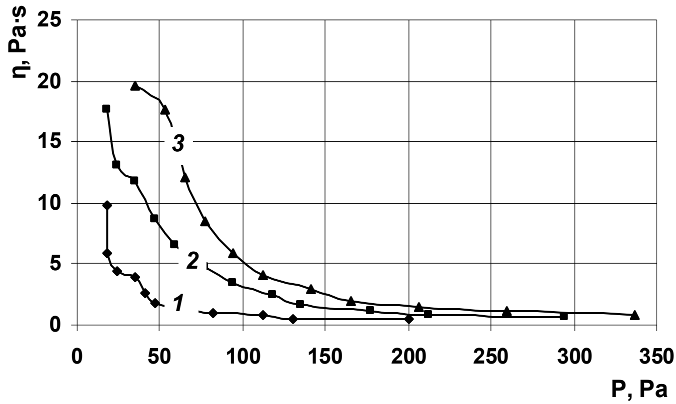


**Figure 7. The viscosity curve of samples with guar gum, prepared by method 1**

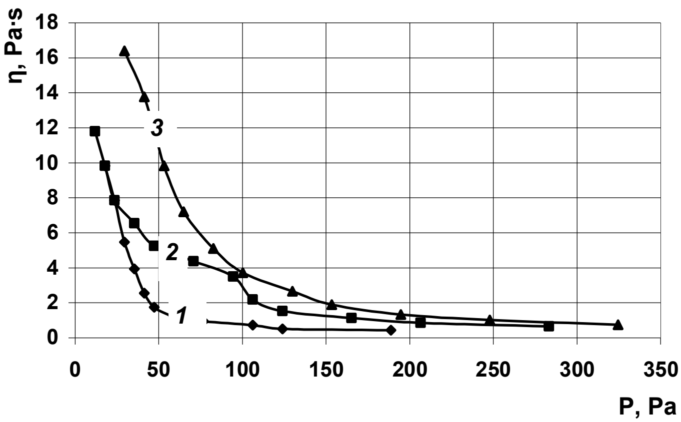


**Figure 8. The viscosity curve of samples with guar gum, prepared by method 2**

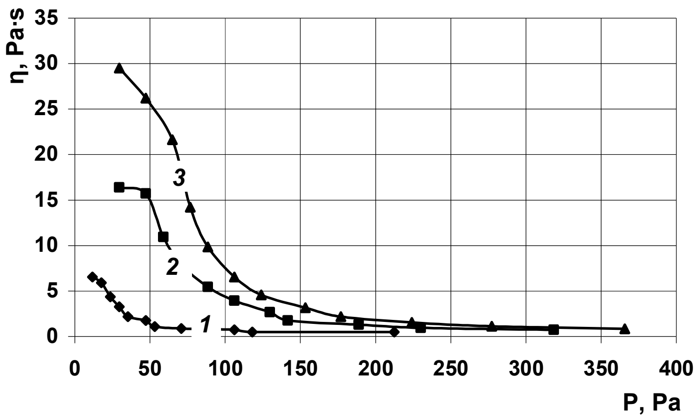


**Figure 9. The viscosity curve of samples with guar gum, prepared by method 3**

**The concentration: 1 – 0,1%; 2 – 0,5%; 3 – 1,0%**

Based on the experimental data it was also calculated the structural and mechanical characteristics. For the guar gum the value $P_{k1}>0$, this indicates that the investigated samples are structured bodies like solid [15–17]. The most dynamic limit of ability to flow was observed in samples with guar gum, prepared by the first and the third methods at a concentration of 1%. An indicator $P_{k1}/P_{k2}$, which characterizes the strength of structural links in the system [15–17], indicates that samples prepared by the third method have the strongest structural links.

A comparison of hydrocolloids solutions, obtained at different ways of preparation, showed that the most appropriate preparation of gels is the third, because under these conditions the viscosity of solutions (at the same concentration of a gelling agent) was higher and the structure of the gel was more homogeneous. A process for preparing the hydrocolloid gels don't affect the fluidity of the systems.

## Conclusions

All the experimental samples of gels are characterized by non-Newtonian pseudo-plastic type of stream. In the study of dependence of the structural viscosity on the shift stress of samples it is found that the viscosity of xanthan gels, prepared by 3 ways, decreased with the increasing of shift stress. This dependence characterizes xanthan gels as structured dispersed systems.

The most appropriate is the preparation of gels by the third method, which consisted in swelling of hydrocolloid for 40 minutes in water of room temperature, heating to 60 °C and cooling. The viscosity of the samples, prepared by this way (with the same concentration of a gelling agent), was higher and the structure of the gel was homogeneous.

The optimum concentration of gelling agents, which ensures the effective structure of mudflow base, is 0,5%. These gels have greater strength of structural links and greater range of stresses of structure destruction; also they meet the necessary organoleptic properties: have a more uniform structure, without lumps and do not stratify over time.

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# Mechanism of antibacterial effect of plant based antimicrobials

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## Abstract

**Introduction.** In recent years, natural antimicrobials have gained popularity and food producers are trying to meet the new trends. For this purpose, plant based antimicrobials have been used for inhibiting or controlling the growth of microorganisms which cause food borne diseases or food spoilage.

**Materials and methods.** In this study, it was aimed to review the mechanisms of antimicrobial effects of plant based antimicrobials which were used in foods. The referenced literatures were obtained from bibliographic databases by searching through AGRICOLA, CAB Abstracts, EBSCO, FSTA, Global Health, Google Scholar, Index Copernicus, PubMed, Scopus, TUBITAK ULAKBIM Life Sciences Database, and Web of Science.

**Results and discussion.** Several fruit and vegetable juices, herbs and spices have been used as acidifying and flavoring agent for traditional meals, salads and appetizers. All these products themselves and also their extracts have been used extensively in food industry as plant based natural antimicrobials. These plant based additives have antimicrobial effects on microorganisms, mainly based on their organic acid composition and/or phenolic content. Organic acids and phenolics which are fundamental compounds in plants were mostly associated with the antimicrobial activity. However, the effect of antimicrobial component varies depending on the genotype or cell wall structure or initial population of the target microorganism, as well as the activity mechanism of the antimicrobial agent varies depending on the defecting way or binding zone in the cell. On the other hand, the inhibitory effect of plant based antimicrobials on different microorganisms were studied for several times. However, there is still a lack of understanding of their exact antimicrobial mechanism which cause the cidal or static effect. It is important to determine the mechanisms of antimicrobial action exactly to make it available, comprehensible and applicable for industrial applications.

**Conclusion.** Determining the exact mechanism of antimicrobial activity is important to make these antimicrobials available, comprehensible and applicable for the food industry.

## Introduction

The development Foodborne diseases are increasingly important public health issues all over the world, even in developed countries. On the other hand, spoilage of foods is still a critical point for the economy. In spite of modern techniques in the food industry, “food preservation” and “food safety” are still major problems [1–3]. While “food preservation” is a continuous fight against saprophyte microorganisms to extend the shelf life of foods [4], the “food safety” ensures to inhibit or to control the growth of pathogenic microorganisms [5]. Several conventional methods, such as: chilling, freezing, drying and heat treatment, as well as novel techniques such as: gamma irradiation, modified atmosphere packaging, high pressure and preservatives and/or additives have been used for food safety and food preservation [6,7]. However, some of these methods damage the organoleptic properties of foods. Synthetic/chemical preservatives especially have adverse effects on human health [2,7]. Therefore, the demands of the consumers focus on foods which are minimally processed and also fresh, preservative free, natural, safe and beneficial for health [4,6,8]. The regulatory agencies have restricted or permitted the usage levels of some currently accepted preservatives in foods and modified the legislations [4,8]. However, these regulations and modifications caused problems for food preservation and food safety in the food industry, and it made researchers look for natural and available alternatives which could replace the declined preservatives [2,9,10]. As a result, plant based antimicrobials have gained popularity as natural antimicrobial agents and the researchers focused on them. Application of natural antimicrobials has been outlined by regulatory agencies in the USA and the EU countries. Essential oils of cinnamon, clove, lemon grass and their active compounds (cinnamaldehyde, eugenol, citral) are generally recognized as safe (GRAS) due to Code of Federal Regulation 21 CFR part 182.20. Carvacrol, carvone, cinnamaldehyde, citral, p-ctmene, eugenol, limonene, menthol and thymol have been registered and recognized as safe-to-use in the EU countries [2,11–13].

## Food antimicrobials

Antimicrobials are known as the substances that delay the growth of microorganisms or cause inhibition of them. When they show these functions in a food matrix, they are called “food antimicrobials” [7]. There are two main reasons for antimicrobial application in foods: the first one is ensuring food preservation and extending shelf-life by controlling the growth of saprophytic microorganisms whose metabolic end products or enzymes cause off-odors, off-flavors, textural problems and/or discoloration; and the second one is ensuring food safety by preventing the survival of pathogenic microorganisms which produce toxins [7,13,14].

Food antimicrobials could be categorized as “natural antimicrobials” or “synthetic antimicrobials” based on their origins. Natural food antimicrobials could be obtained from various origins such as animals, microorganisms and plants [15]. The effects of animal-origin antimicrobials (lysozyme, lactoferrin, etc.), microbial-origin antimicrobials (nisin, reuterin, etc.) and plant-origin antimicrobials (thyme, lemon juice, etc.) against pathogenic and/or saprophytic microorganisms have been demonstrated in several studies [15,16]. The plant based antimicrobials are the most popular and useful group among the others because of low cost, availability and sounding familiar.

## Plant based antimicrobials

The plants themselves (leaves, stems, buds, flowers, fruits, seeds, bulbs and rhizomes) and some compounds held from plants (extracts, essential oils) have been used as plant based antimicrobials to ensure the food safety in the food industry [13,7]. In the studies on the antimicrobial effects of plants and their extracts, it was declared that plant based antimicrobials led to inhibition of many types of microorganisms such as *Bacillus cereus, Bacillus coagulans, Bacillus megaterium*, *Bacillus subtilis, Bacteroides* spp., *Citrobacter* spp., *Clostridium* spp., *Corynebacteriumxerosis*, *Enterococcus feacalis, Escherichia coli, Escherichia coli* O157:H7, *Klebsiella pneumonia, Listeria monocytogenes, Micrococcus luteus, Porphyromonas*spp., *Pseudomonas aeruginosa, Staphlococcus aureus, Salmonella* Typhimurium, *Salmonella* Paratyphi, *Serratia* spp., *Shigella flexnerri* and *Yersinia enterocolitica* [18–27].

Fresh juice, sauce and concentrate of fruits and vegetables (lemon, pomegranate, grape, unripe grape, mulberry, orange, sour orange, black carrot, garlic, onion, leek, red pepper, garden radish and horseradish), herbs and spices (allspice, bay leaf, cumin, coriander, fenugreek, oregano, rosemary, cloves, parsley, lemongrass, sage, ginger, vanilla) were studied to determine their antimicrobial effects on foodborne pathogens by several researchers [28–31]. Additionally, some essential oils were obtained from plants (cardamom, cloves, thyme, cumin, etc.) containing active components (carvacrol, terpene, eugenol, etc.), and some extracts (from sumac, cinnamon, cranberry, red cabbage, sour cherry pomace, etc.) extracted by water or ethanol were also investigated as plant based antimicrobials [2,4,13,16,32–35]. *Laurus nobilis* L. and *Myrtus communis* L. essential oils were tested against five Gram positive and four Gram negative bacteria including *L. monocytogenes* and *E. coli.* It was detected that the essential oils were effective on the testing pathogens, and the diameters of inhibition zones ranged between 7.2–37.7 mm [36]. Antimicrobial activity of *Curcuma longa* extracts against *Klebsiella pneumoniae, Shigella flexneri*, *Candida albicans* were determined and the diameters of the inhibition zone were measured as 6 mm, 12 mm and 13 mm, respectively [37]. Antibacterial effect of pomegranate peel flour on six pathogens was studied and the minimum inhibitory concentration (MIC) values were between 20–50 mg/mL [38]. *Allium sativum* has antimicrobial effect against *E. coli, B. subtilis, S. aureus, A. niger* and *P. chrysogenum* and it caused inhibition zones ranged from 9 mm to 17 mm [39]. Antimicrobial effect of pomegranate juice was treated on seven bacteria and seven moulds. While, the maximum inhibitory effect was determined against *S. aureus* and *A. niger*, the minimum effect was determined against to *B. subtilis* and *T. Reesei*. The inhibition zones for all of the tested bacteria were measured between 15–26 mm, and they were between 10–19 mm for the tested moulds [26].

## Mechanism of antimicrobial effect

Plant based antimicrobial agents have been used as natural preservatives in foods for a long while. Although, the antimicrobial effect against pathogenic and saprophytic microorganisms was studied several times, the exact mechanism of inhibition could not be defined clearly. The cell inhibition is expressed by perturbation of the membrane with phenolic compounds, interacting genetic material with coumarins, chelating metals with flavonoids and flavonols. For all that, inhibition may occur simultaneously as a result of different consecutive reactions. It was also observed that compounds which damage the cell

membrane were affected by leakage of cell contents, disruption of metabolic enzymes and active transport, and loss of ATP [1,7,40].

There are lots of remarks according to the results of studies on metabolic activity and effective component of antimicrobial agents that control or inhibit the target (pathogenic or saprophytic) microorganism. In these studies, it was summarized that the lethal effect was related to degradation of the cell wall, inhibition of the cytoplasmic membrane and proteins, coagulation of cytoplasm and depletion of proton motive force (PMF) [7,41].

The antimicrobial effect of a certain antimicrobial agent depends on three main factors: the psycho-chemical properties of the antimicrobial agent (dissociation constant of the acids, solubility, organoleptic properties and hydrophobicity/lipophilicity ratio), the environmental factors (pH, water activity, temperature and structure of food), and the microbiological factors (initial and/or competitive microflora and type, genus, species and strain of the target microorganism) [2,5–7]. In addition, the antimicrobial effect of a plant based antimicrobial agent is mostly related with characteristic properties of the plant material such as genetic and environmental factors, part of the plant, methods of isolation, harvesting time and geographical location [4].

The microbial cells have complex metabolisms like prokaryote (bacteria) or eukaryote (yeasts and molds). So, an antimicrobial does not have a single site of action, it affects several systems in target microorganism [16]. Even if the plant based antimicrobials are obtained from different sources by different methods, the antimicrobial agent has three ways to inhibit a bacterial cell. These targets are destruction of the cell wall, inactivation of essential enzymes and damage of genetic material [16]. Plant based antimicrobials that are effective on microbial cells with different mechanisms are generally more effective on Gram-positive than Gram-negative bacteria. Gram-negative bacteria possess an outer membrane that surrounds the cell wall and protects diffusion of hydrophobic substances through its lipopolysaccharide covering [4,13,42].

The specific characteristics of the food matrix are very important. The physical structure of the food may influence the antimicrobial activity due to the limitation of diffusion [2]. Effectiveness of some antimicrobials could be limited by the high amount of lipids, particularly with hydrophobic properties. In other words, hydrophobic antimicrobials cannot be solved in water and so they cannot be used for dipping/rinsing solutions [16]. Furthermore, the high amounts of lipid and/or protein in foods protect the bacteria against to damage of antimicrobials [2].

The chemical reaction among antimicrobials in foods with other food components such as lipids, proteins, carbohydrates and food additives can affect the antimicrobial activity. These reactions could decrease the antimicrobial activity as well as occurring the formation of off-flavors, -odor, and –colors. Besides this, many antimicrobials should be used at high concentrations to completely inhibit the spoilage causing and/or pathogenic microorganism. However, these amounts are not available for food processing because of unacceptable flavor, odor, or texture they caused in foods [43].

Organic acids and phenolics which are fundamental compounds in plants were mostly associated with the antimicrobial activity, and their mechanisms of antimicrobial activity were explained and clarified in most of studies [13,44].

## Organic acids

Organic acids have been used in the food industry as preservative agents for a long time due to their activity on survival and growth of microorganisms in foods. Organic acids change the concentration of hydrogen ions (pH) in foods so they can control the microbial

growth. Although, the optimum pH values for growing of bacteria are close to neutrality, they could tolerate the pH values between 4 and 9 [16]. Antimicrobial efficiency of the organic acids depends on type (acetic, benzoic, etc.) and concentration of the acid, environmental conditions (pH, temperature), and structure of the target microorganism [16]. However, the pH value is the most important factor among the others.

The strong or weak acid terms are used to describe the acids that donate a proton or dissociate in aqueous solutions [45]. Strong acids such hydrochloric or sulfuric acids which are fully dissociated, do not penetrate to the cell membrane and they exert their effectiveness by the denaturing the enzymes which on the surface of the cell. On the other side, the weak acids which are lipophilic, penetrate thorough into the cell membrane. Thus, the primary effect of weak acids is decreasing the pH value of cytoplasm [46]. Most of the acids in foods are weak acids such as lactic, acetic and citric acid, and they exist in a pH-dependent equilibrium between the two states as fully dissociated or un-dissociated [6,8]. Organic acids have an optimal antimicrobial effect at a low pH values because they are in an un-dissociated state which can penetrate the cell membrane and reach to cytoplasm easily. The interior cell pH is neutral, so the acid is forced to dissociate into anions and protons [47,48]. The bacteria pump the accumulation of $H^+$ ions outside to the cell to protect the intracellular pH. Hence the transportation membrane proteins and transport enzymes are denaturized in turn, the membrane permeability is increased [16]. Antimicrobial activity of organic acids mainly depend on disruption of membrane and cell signaling, accumulation of toxic anions, inhibition of glycolysis, active transport and essential metabolic reactions [1,8,16]. The antibacterial activity is pH dependent. It was assosiated with pH reduction of the substrate, depression of the intracellular pH or disruption of substrate transport by alteration of cell membrane permeability [50,51].

In conclusion, inactivation of microorganisms by using organic acids was attributed to disruption of membrane and cell signaling, accumulation of toxic anions, inhibition of glycolysis, inhibition of active transport and inhibition of essential metabolic reactions [1,8,16].

**Phenolic compounds**

Phenolics are important compounds for the antimicrobial activity of plants and antimicrobial activity of plant phenolics has been intensively studied [34]. Phenolics may influence the growth and metabolism of bacteria. The inhibition of bacteria are included several mechanisms such as adsorption to cell membrane, destabilization of cytoplasmic membrane, permeabilization of plasma membrane, interaction with enzymes, substrate and metal ion deprivation, and inhibition of extracellular microbial enzymes[52,53].

The presence of the hydroxyl group in phenolic compounds plays an important role in antimicrobial activity [2]. The hydroxyl group reacts with the cell membrane and disrupts the structure of it, and the cell components are lost from the interior. This group promotes the delocalization of electrons which act as proton exchangers. Thus, it decreases in a gradient across the cytoplasmic membrane. When the gradient is reduced, PMF collapses and the ATP pool depletes. Eventually, it results in cell death. At the same time, -OH groups alter the cell metabolism by binding to the active site of enzymes [17]. The effects of phenolic compounds could vary depending on their concentration in foods. At low concentrations, the phenols impact the enzymes responsible for energy production, while at high concentrations, they raise the denaturation of proteins [15,49].

As a result, inhibition mechanism of phenolic compounds can be explained by damaging the cytoplasmic membrane, collapsing the PMF, disruption of electron flow and

depletion of active transport, thereby cell components become coagulated [2,4]. Phenolic compounds cause the leakage of macromolecules such as ribose and Na glutamate by altering the microbial cell membrane permeability. They could also interfere with membrane functions such as electron transport, nutrition, enzyme activity, protein and nucleic acid synthesis, and they interact with membrane proteins, causing disruption of the structures and functionality [15].

## Conclusion

There has been an increasing demand of safe and natural food products. The food legislations have promoted the producers to use natural additives instead of synthetics. This drives the search of the researchers and food industry for alternative techniques to ensure food safety and preservation. In this context, natural food additives with a broad spectrum of antimicrobial activity are gaining popularity. Many studies in the literature have stated that several plants or plant derivatives are effective on inhibiting microorganisms which cause food spoilage or foodborne diseases. The antimicrobial effect of plant based antimicrobials is well documented; however, there is still a lack of understanding of their antimicrobial mechanisms of action. On the other hand, the explanation of the mechanisms of antimicrobial action should be available, comprehensible and applicable for industrial applications. While plant based antimicrobials can attain the desirable effects on foods, the inhibitory concentrations could be limited by undesirable organoleptic characteristics they cause. In the context of the "hurdle effect," antimicrobial effects of the plant based antimicrobials could be increased by the synergistic effects of other techniques, such as non-thermal processing, and the inhibitory concentrations could be decreased by this way. Moreover, specific regulations in food legislations are necessary for using plant based antimicrobials and labeling them.

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# Three-dimensional model of non-Newtonian fluid flow in the rectangular channel

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**Abstract**

**Introduction.** The three-dimensional mathematical model of non-Newtonian fluid flow was introduced, with viscosity that depends of shear velocity in rectangular channels of food equipment.

**Materials and methods.** Applying the method of superposition allows to build a field of longitudinal flow of non-Newtonian fluid in rectangular channel of technological equipment with moving borders and thus to define values of velocity and pressure at any point inside the channel.

**Results and discussion.** We developed a model of longitudinal flow of non-Newtonian fluid in rectangular channels on borders of which different longitudinal velocities are set. The model is based on the solution of one-dimensional problem of the Couette flow in the channel. The composition of flows in the slit channels with mutually perpendicular pair of borders allows to receive a flow rate formula which satisfies the principle of limit correspondence between the currents in a rectangular and slit channels. We suggest a method of construction of the velocity field which is a subdivision of the final section of the channel into sections with a different dependence on the coordinates so that in some areas the velocity depends only on a single coordinate, while in others – only on the other coordinate. We obtain the equations of lines delimiting these areas, and how to determine the shape of boundaries.

**Conclusion.** The analytical formulas allow defining macrokinetic characteristics of each channel at the boundary with arbitrary distribution of velocities of the flow of non-Newtonian fluid the viscosity of which depends on the shear speed.

## Introduction

The movement of non-linear fluids is a part if hydrodynamics for laminar or Stokes flows. In technical literature there are a lot of ways to solve problems related to flow of viscoplastic fluids. The objective of any solution of the problem of fluid flow is to find the pressure and the velocity vector at each point within the channel [1, 2, 3].

## Materials and methods

Three-dimensional flows are usually studied using the numerical methods. Results obtained this way are more accurate. To generalize it in order to isolate the impact of individual parameters the analysis of large amount of numerical information is required. The form of representing results of such impacts is mostly descriptive and may contain many errors. There is another approach to the problem of constructing a picture of the three-dimensional flow, the purpose of which is the analytical solution of a problem, which is simpler yet retains all the important parameters of the flow. Usually this problem is one-dimensional. Then, a three-dimensional solution to the problem is constructed using heuristics compositions based on the results of one-dimensional problem solving. Then, a three-dimensional solution to the problem is constructed using heuristics compositions based on the results of one-dimensional problem solving. This solution has a lower accuracy than the numerical one, but allow the researcher to obtain physically reasonable parameter combinations as opposed to random combinations of a descriptive nature, which are obtained by numerical solution of the problem. Solutions based on the analytical methods have more methodical value, and can be used by other researchers for a variety of other tasks. As a means of studying the movement of nonlinear fluids authors adhere to the analytical approach followed by composition [4]. The basic problem in this case is the problem of the Couette flow in a slit channel. Rectangular channel and boundary conditions that create a three-dimensional flow in it are shown on Figure 1.

Knowledge of pressure and velocity at each interior point of the channel makes it possible to calculate such quantities: flow rate, the dissipative heat, shear rate and shear stress [5, 6].

Speed pressure fields are totally dependent on the boundary conditions on the channel walls. These conditions are determined by the construction of the working chamber through the helix lead angle of the worm and its pitch and speed of the machine shaft [1, 7, 8].

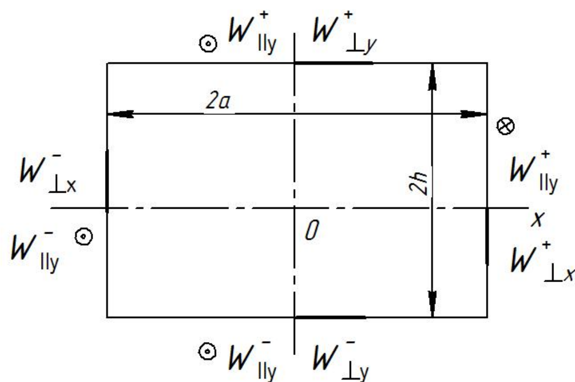


**Figure 1. Rectangular channel and the boundary conditions of three-dimensional flow in channel:**

$W^{\pm}_{IIY}$ – value of longitudinal velocity on the channel borders which are perpendicular to OY axis;

$W^{\pm}_{IIX}$ – value of longitudinal velocity on the channel borders which are perpendicular to OX axis;

$W^{\pm}_{\perp X}, W^{\pm}_{\perp Y}$ – value of transverse velocities on the channel borders

The boundary conditions for the basic problem of Couette flow are shown on Figure 2.

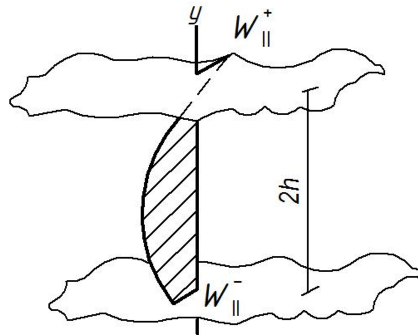


**Figure 2. Fragment of the slit channel with boundary conditions**

## Results and discussion

We studied the longitudinal flow of non-Newtonian fluid in rectangular channel, which has longitudinal velocities sent on its borders. The distribution of these boundary velocities is displayed on Figure 3.

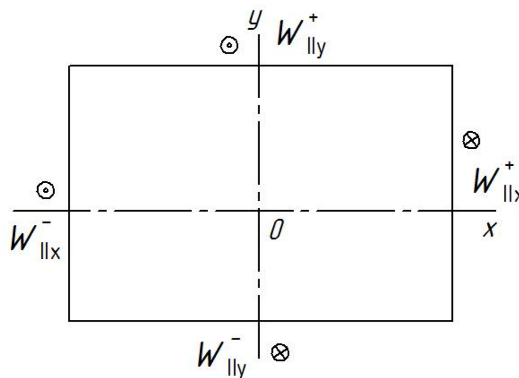


**Figure 3. Longitudinal flow in rectangular channel and boundary conditions on its borders**

The flow on this figure is a special case of the general three-dimensional flow, shown on Figure 1. Longitudinal flow has one velocity component $v_2$, which depends on two coordinates $x$ and $y$ in rectangular cross-section of the channel. The equation of liquid state is defined by viscosity $\mu$, which has the following form:

$$\dot{I}_2 = \left(\frac{\partial \upsilon_z}{\partial x}\right)^2 + \left(\frac{\partial \upsilon_z}{\partial y}\right)^2 \dot{I}_2 = \left(\frac{\partial \upsilon_z}{\partial x}\right)^2 + \left(\frac{\partial \upsilon_z}{\partial y}\right)^2 \tag{1}$$

in which $\alpha$ and $\beta$ – parameters that depend on molecular characteristics of liquid and that are determined by an experiment. The equation of the flow is represented in the following form:

$$\frac{dP}{dz} = \frac{\partial}{\partial x}\left\{\left(\alpha + \beta\sqrt{\dot{I}_2}\right)\frac{\partial \upsilon_z}{\partial x}\right\} + \frac{\partial}{\partial y}\left\{\left(\alpha + \beta\sqrt{\dot{I}_2}\right)\frac{\partial \upsilon_z}{\partial y}\right\}. \tag{2}$$

In accordance with the above, this problem should be reduced to a form which corresponds to the problem of longitudinal slit flow. This should be done twice: at first problem (1) should be transformed into problem of slit flow with borders, which are parallel to *ox* axis; then it should be transformed into problem of slit flow with borders, which are parallel to *oy* axis. Afterwards, the general flow in rectangular channel is constructed using composition method. Following this plan, it is necessary to express the derivatives $\partial\upsilon_z/\partial x$ и $\partial\upsilon_z/\partial y$ through each other. Relationship between the derivatives has the form: $\partial\upsilon_z/\partial x \sim x \;\; \partial\upsilon_z/\partial y$, where $x=h/a$, while $h$ and $a$ are the sides of the rectangle on the Figure 3. This relationship is defined up to a factor, the form of which depends on the degree of convexity or concavity of the velocity profile. Using derivative $\partial\upsilon_z/\partial y$ as a primary one, and then derivative $\partial\upsilon_z/\partial x$ we can come to the following equations of the slit flow:

$$\frac{dP}{dz}=\frac{\partial}{\partial y}\left\{\left[\alpha\left(1+x\right)+\frac{\beta\left(1+x\right)^{\frac{3}{2}}}{2}\left|\frac{\partial\upsilon_z}{\partial y}\right|\right]\frac{\partial\upsilon_z}{\partial y}\right\}=0\,.$$

$$\frac{dP}{dz}=\frac{\partial}{\partial x}\left\{\left[\alpha\left(1+\frac{1}{x}\right)+\frac{\beta\left(1+\frac{1}{x2}\right)}{2}\left|\frac{\partial\upsilon_z}{\partial y}\right|\right]\frac{\partial\upsilon_z}{\partial y}\right\}=0\,. \quad (3)$$

Below, both problems are solved simultaneously, since they have the same structure. In order to solve them variables $x$ and $y$ are denoted as $x_i$, where $i=x,y$; parameters of both problems, multiplied by factors, which depend on parameter $x$, are denoted as $d_i$, $\beta_i$, where $i=x, y$; rectangle sizes $h$ and $a$ are denotes as $l_i$, thus $l_x=a$, $l_x=h$. By virtue of inclusion of absolute value of velocity derivative, the solution to problem (3) consists of two branches which intersect at point $x^*_i$

$$v_{z^i}^{+1}=-\frac{\alpha_i}{2\beta_i}+\sqrt{\frac{\alpha_i^2}{4\beta_i^2}+\frac{x_i}{\beta_i}\frac{dP}{dz}+c_i}\,,$$

$$v_{z^i}^{-1}=-\frac{\alpha_i}{2\beta_i}+\sqrt{\frac{\alpha_i^2}{4\beta_i^2}-\frac{x_i}{\beta_i}\frac{dP}{dz}+c_i}\,. \quad (4)$$

The choice of characters is dictated by the fact that one branch should have a positive derivative, while the other – a negative one. At point $x^*_i$ where branches intersect, the derivative turns to zero. This condition along with boundary condition (see Figure 3), lead to the following expressions for velocity $v^{\pm}_z$:

$$v_{z^i}^{+}(x_i)=\frac{\alpha_i}{2\beta_i}\left(l_i-x_i\right)+\left(\frac{\alpha_i^2}{4\beta_i^2}+\frac{x_i-x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\cdot\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}-\left(\frac{\alpha_i^2}{4\beta_i^2}+\frac{l_i-x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\cdot\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}+w_i^{+}\,,$$

$$v_{z^i}^{-}(x_i)=\frac{\alpha_i}{2\beta_i}\left(l_i+x_i\right)+\left(\frac{\alpha_i^2}{4\beta_i^2}+\frac{x_i+x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\cdot\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}-\left(\frac{\alpha_i^2}{4\beta_i^2}+\frac{l_i+x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\cdot\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}+w_i^{-}\,. \quad (5)$$

Values $x^*_i$, which are calculated from the condition of continuity of velocity $v_i$ at points $x^*_i$, have the following representation [5, 6]:

$$x_i^* = -\frac{w_i^+ - w_i^-}{\frac{l_i}{\beta_i} - 2\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i}{\beta_i}\frac{dP}{dz}\right)^{1/2}}. \quad (6)$$

By integrating expression (5) on interval ($-l_i$, $x^*_i$) for branch $v_i$ and on interval ($x^*_i$, $l_i$) for branch $v^+_i$ we can come to the following expression for the $\dot{v}_i$ flow rate:

$$\dot{v}_i = w_i^+\left(l_i - x_i^*\right) + w_i^-\left(l_i + x_i^*\right) + \frac{\alpha_i}{2\beta_i}\left(l_i^2 + x_i^{*2}\right) + \frac{4}{15}\left(\frac{\beta_i}{\frac{dP}{dz}}\right)^2 \times$$
$$\times\left\{\left[\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i - x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{5}{2}} - \left(\frac{\alpha_i^2}{4\beta_i^2}\right)^{\frac{5}{2}}\right] + \left[\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i + x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{5}{2}} - \left(\frac{\alpha_i^2}{4\beta_i^2}\right)^{\frac{5}{2}}\right]\right\} -$$
$$-\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}\left\{\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i - x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\left(l_i - x_i^*\right) + \left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i + x_i^*}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}}\left(l_i + x_i^*\right)\right\}. \quad (7)$$

The expression (7) can be somewhat simplified by compromising accuracy. To do so, the terms in powers 5/2 and 3/2 should be expanded in Taylor series up to and including the new term by the degree of smallness. The value ($x^*_i\, dP/dz$)/ $\beta_i$ should be used as an expansion parameter. This expansion gives an error of not more than 16% for the case of inequality $\left|x_i^*\right| < l_i$. If such expansion is performed, then expression (7) for the flow rate turns into a square polynomial with respect to the value of $x^*_i$ The result is as follows:

$$\dot{v}_i = w_i^+\left(l_i - x_i^*\right) + w_i^-\left(l_i + x_i^*\right) + \frac{\alpha_i}{2\beta_i}\left(l_i^2 + x_i^{*2}\right) + \frac{8}{15}\left(\frac{\beta_i}{\frac{dP}{dz}}\right)^2\left[\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i}{\beta_i}\frac{dP}{dz}\right)^{\frac{5}{2}} - \left(\frac{\alpha_i^2}{4\beta_i^2}\right)^{\frac{5}{2}}\right] - .$$
$$-\frac{2}{3}\frac{\beta_i}{\frac{dP}{dz}}\left[2l_i\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i}{\beta_i} + \frac{l_i}{\beta_i}\frac{dP}{dz}\right)^{\frac{3}{2}} + 3\frac{1}{\beta_i}\frac{dP}{dz}\left(\frac{\alpha_i^2}{4\beta_i^2} + \frac{l_i}{\beta_i}\frac{dP}{dz}\right)^{\frac{1}{2}} x_i^{*2}\right]. \quad (8)$$

With the help of the formulas (7) or (8) it is possible construct a formula for the longitudinal flow rate, but in the rectangular channel, not a slit one. In order to perform such construction, we should use the considerations of limit compliance, which implies that if parameter $x$, which is present in definition of values $\alpha_i$, $\beta_i$, tends to zero, we get a slit channel in channel with borders along the *ox* axis. If, however, this parameter tends to infinity, we get the slit flow in channel with borders along the *oy* axis.

Based on these two extreme cases, the rate of the general flow can be represented as the composition of slit flows rate with weighting factors that depend on parameter $x$ and satisfy the compliance with the limit. Such composition is ambiguous. Its final concrete form is determined empirically. However, the simplest kind of composite factors is used. Based on what said above, the form of such composition can be the following:

$$\dot{v} = \dot{v}_y \left( \frac{1}{1+x^m} \right)^n + \dot{v}_x \left( \frac{x^m}{1+x^m} \right)^n , \tag{9}$$

where $m$ and $n$ – composition parameters. The simplest choice is to set $n$=1. The following considerations can be made with regards to the value of the parameter $m$. A common feature of slow (Stokes) viscous fluids is that the influence of bounds on the velocity profile shaping distributes over the distance into the flow region of the order of the corresponding border length. From this, it follows that for rectangular channels, that are highly elongated along the $x$ axis, the flow with velocity $v_y(y)$ distributes over an area of exponent $ah\text{-}h^2$, while the flow with velocity $v_x(y)$ distributes over the area of exponent $h^2$. For channels, that are highly elongated along the $oy$ axis, the situation is exactly the opposite: flow with velocity $v_x(y)$ distributes over the area of exponent $ah\text{-}a^2$, while flow with velocity $v_y(y)$ distributes over the area of exponent $a^2$. For these the expression for the rates can be represented in the following form:

$$\dot{v}(x=1) = \dot{v}_y (x=0) \frac{a^2}{ah} + \dot{v}_x (x=\infty) \frac{ah-a^2}{ah} \tag{10}$$

Comparing formulas (9) and (10) we can conclude that (10) leads to the fact that in (9) parameter $n$ should be set to 1.

Now we must construct the velocity field from the expressions (5). In order to do this we must refer to the Newtonian fluid flow in rectangular channel. Such a flow due to the linearity of the problem can be divided into two: the current, which is caused by the movement of the walls; and the flow caused by pressure drop. For the first flow, the rate is equal to the product of the average speed on the cross-sectional area and the velocity by a factor equal to the quantity ratio of the length of the moving part of the perimeter length of the cross section to the entire perimeter. This fact can also be interpreted in a way that the influence of the moving borders distributes not throughout the entire cross-sectional area but through its part. From physical considerations, it is clear that this part should be adjacent to the moving borders. The remaining part of the cross-section is influence by immovable walls and must adjust to non-moving borders. The influence areas of the borders are qualitatively presented on Figure 4.

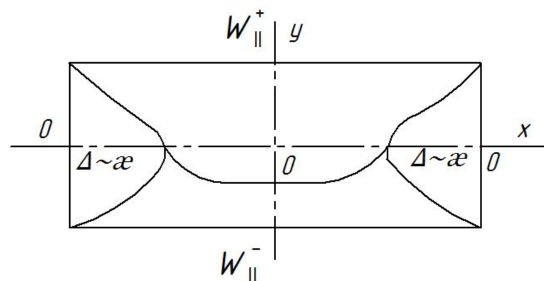


**Figure 4. Areas of influences of the boundary conditions on rectangular channel borders:**
$\Delta$ – the depth of penetration of the zero boundary conditions.

The figure shows that the size of the area adjacent to immovable borders has the exponent of $x/(1+x)$. From this follows the fact that is all the rectangle area is taken as 1 then the size of the area influenced by borders movement is $1/(1+x)$. Then for the parts elongated along the cross-sections the remaining area will actually be $x/(1+x)$. From this and from Figure 4 follows the fact that this value is the same as the depth of penetration of influence of zero boundaries. For the second flow slightly different considerations should be used. If the flow rate is taken as 1 and remove a pair of borders which are perpendicular to $ox$ axis, then adding this pair of borders decreases the flow rate to $1/(1+x^2)$ times. This fact can be interpreted as decreasing the maximum velocity in the same amount of times. The method of composing solutions (5) has the simple nature and can be used in engineering. In lies in using the corresponding solution from (5) for each pair of borders. Areas, in which different solutions work, have the shared borders. The shape of these borders can be defined from conditions of continuity of velocity fields on these borders. If we write continuity conditions for different solutions then there will be four conditions: $v^+_z(y)=v^+_z(x)$; $v^-_z(y)=v^+_z(x)$; $v^+_z(y)=v^-_z(x)$; $v^-_z(y)=v^-_z(x)$.

Each of continuity conditions leads to one line. The above statements are presented on Figure 5.

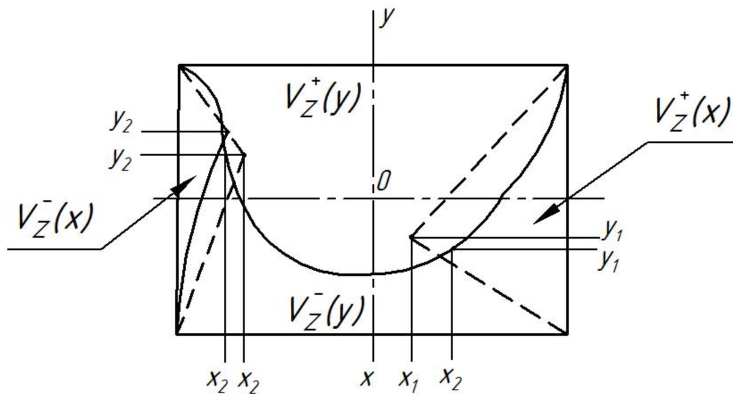


**Figure 5. Partitioning of channel cross-section into areas with velocity profiles $v_z^{\pm}(y), v_z^{\pm}(x)$:**
solid line – actual partitioning; dashed line – approximate partitioning

The conditions can be represented in the following expanded form:

$$a^- + b^- y + (c^- + d^- y)^{\frac{3}{2}} = m^+ + n^+ x + (l^+ + p^+ x)^{\frac{3}{2}},$$

$$a^+ + b^+ y + (c^+ + d^+ y)^{\frac{3}{2}} = m^- + n^- x + (l^- + p^- x)^{\frac{3}{2}},$$

$$a^- + b^- y + (c^- + d^- y)^{\frac{3}{2}} = m^- + n^- x + (l^- + p^- x)^{\frac{3}{2}} \qquad (11)$$

in which values $a^{\pm}, b^{\pm}, c^{\pm}, d^{\pm}, m^{\pm}, l^{\pm}, p^{\pm}$ are defined in the obvious way from (5) and are not shown because of triviality and in order to save space. Equations Equations (11) can be solved (in order to define the form of relationships $y_i(x)$, $y$=1,2,3,4) only numerically. Approximate solutions can be obtained by partially linearizing equation (11). To do this we need to make the following notations:

$$\frac{1}{h+y_2}\int_{-h}^{y_2}(c^- + d^- y)^{\frac{1}{2}}dy = \lambda^-;$$

$$\frac{1}{a-x_1}\int_{x_1}^{a}(l^+ + p^+ x)^{\frac{1}{2}}dx = \delta^+;$$

$$\frac{1}{a+x_2}\int_{-a}^{x_2}(l^- + p^- x)^{\frac{1}{2}}dx = \delta^- \quad , \tag{12}$$

in which $y_1$, $y_2$, $x_1$, $x_2$ – points, presented on Figure 5. In these notations after linearization, the linear equations are obtained for boundary lines, while actual lines become line segments. Equations for boundary lines acquire the following form:

$$(a^- + c^-\lambda^-) + (b^- + d^-\lambda^-)y = (m^+ + l^+\delta^+) + (n^+ + p^+\delta^+)x;$$
$$(a^+ + c^+\lambda^+) + (b^+ + d^+\lambda^+)y = (m^- + l^-\delta^-) + (n^- + p^-\delta^-)x;$$
$$(a^- + c^-\lambda^-) + (b^- + d^-\lambda^-)y = (m^- + l^-\delta^-) + (n^- + p^-\delta^-)x. \tag{13}$$

Solutions (13) are represented on Figure 5 as contour lines. In order to obtain values $x_1$ and $x_2$ (see Figure 5) the solutions of first two and the last two equations (13) should be equated in pairs. As a result, for $x_1$ and $x_2$ we get the following formulas:

$$x_2 = \frac{\dfrac{(m^- + l^-\delta^-) - (a^+ + c^+\lambda^+)}{b^+ + d^+\lambda^+} - \dfrac{(m^- + l^-\delta^-) - (a^- + c^-\lambda^-)}{b^- + d^-\lambda^-}}{\dfrac{n^- + p^-\delta^-}{b^- + d^-\lambda^-} - \dfrac{n^- + p^-\delta^-}{b^+ + d^+\lambda^+}} \tag{14}$$

Since $\lambda^{\pm}$ and $\delta^{\pm}$ are unknown, they should be determined by the equations (12) and (13), where values $y_1$ и $y_2$ should be defined as well. The system of equations for finding $\lambda^{\pm}$ and $\delta^{\pm}$ is nonlinear and is solved numerically.

For velocity profiles $v_z^i$ for which $y^*$ and $x^*$ lie correspondingly in intervals $(-h, h)$ and $(-a, a)$, the non-numeric solution can be obtained. The expressions for $v_z^i$ near the points $y = \pm h$ and $x = \pm a$ should be expanded in Taylor series up to and including the first term. The results of these expansions can be written in the following form:

$$v_z^-(y) = w_y^- + \left[\frac{\alpha_y}{2\beta_y} + \left(\frac{\alpha_y^2}{4\beta_y^{\,2}} + \frac{h-y^*}{\beta_y}\frac{dP}{dz}\right)^{1/2}\right](h+y),$$

$$v_z^+(x) = w_x^+ + \left[\frac{\alpha_x}{2\beta_x} - \left(\frac{\alpha_x^2}{4\beta_x^{\,2}} + \frac{a-x^*}{\beta_x}\frac{dP}{dz}\right)^{1/2}\right](a-x),$$

$$v_z^-(x) = w_x^- + \left[\frac{\alpha_x}{2\beta_x} + \left(\frac{\alpha_x^2}{4\beta_x^{\,2}} + \frac{a+x^*}{\beta_x}\frac{dP}{dz}\right)^{1/2}\right](a+x) \quad . \tag{15}$$

In order to save space we denote multipliers before $h \pm y$ and $a \pm x$ as $M$, $N$, $R$, $S$ correspondingly. The equations of boundary lines will then be written in a following way:

$$y = h - \frac{\left(w_x^+ - w_y^+\right) + R \cdot a}{M} - \frac{R}{M} x;$$

$$y = -h - \frac{\left(w_x^+ - w_y^-\right) + R \cdot a}{N} - \frac{R}{N} x;$$

$$y = -h + \frac{w_x^- - w_y^- + S \cdot a}{N} + \frac{S}{N} x. \tag{16}$$

Values for coordinates $y_1, x_1$ и $y_2, x_2$ are obtained from equations (16) from the following relations:

$$2h - \frac{w_x^+ - w_y^+ + S \cdot a}{M} - \frac{w_x^- - w_y^- + S \cdot a}{N} = \left(\frac{1}{M} + \frac{1}{N}\right) S \cdot x_2,$$

$$y_1 = h - \frac{w_x^+ - w_y^+ + R \cdot a}{M} - \frac{R}{M} x_1,$$

$$y_2 = h - \frac{w_x^+ - w_y^+ + S \cdot a}{M} + \frac{S}{M} x_2. \tag{17}$$

## Conclusion

The results that we received, allow us to construct the field of longitudinal flow in rectangular channel with moving borders. At the base of the construction is the solution of one-dimensional problem of slot flow. The composition of flows in slot channels with mutually perpendicular pairs of borders allows to receive the flow rate formula, which satisfies the principle of limit correspondence between flows in rectangular and slot channels [10]. We proposed the method of constructing the velocity field, which in essence, is the partition of the final cross-section of the channel into sections with different dependency on coordinates in a way that on some sections velocity depends only on one coordinate while on others – only on another coordinate. We established the equations for lines, which divide these regions. We suggested methods to define shapes of divider lines: one of them is the rectifications of these lines. For this method, the coordinates of divider lines intersection are fully defined by explicit formulas.

Analyzing formulas (5), which are received from equations (3) it should be noted that the reduction of the original problem with velocity dependency on two coordinates to two problems, in each of which velocity depends on only one coordinate, was based on assessment of relation of derivatives $\partial v_z/\partial y$ к $\partial v_z/\partial x$. Authors chose the simplest assessment. Objectively, this assessment is valid in the case when differences of velocities $w_y^+ - w_y^-$ and $w_x^+ - w_x^-$ do not differ very much one from another. The more accurate assessment leads to the following relation between derivatives:

$$\frac{\partial v_z / \partial y}{\partial v_z / \partial x} \approx x \frac{w_y^+ - w_y^-}{w_x^+ - w_x^-}. \tag{18}$$

This assessment is valid for such flows, velocity profiles of which are not too convex of bent. These flows are correspondent by values $y^*$ and $x^*$, which are outside the intervals $(-h, h)$ and $(-a, a)$ correspondingly. Formally, in this case, the formulas do not change their

appearance. For strongly convex or concave velocity profiles when the contributions of pressure exceeds the contribution of moving channel walls, the assessment of relation of derivatives can be written in a following way:

$$\frac{\partial v_z / \partial y}{\partial v_z / \partial x} \approx x \frac{c_1(w_y^+ - w_y^-) + c_2 v_{my} l\left(y^*\right)}{c_3(w_x^+ - w_x^-) + c_4 v_{mx} l\left(x^*\right)}, \qquad \begin{array}{l} v_{my} \equiv \max v_z\left(y\right), \\ v_{mx} \equiv \max v_z\left(x\right), \end{array} \qquad (19)$$

where $c_1$, $c_2$, $c_3$, $c_4$ are constants, which depend on flow characteristics.

In conclusion, it must be emphasized that the general form of the formulas in the present work does not depend on the choice of assessment of the relation of derivatives, because these assessments are included into values $\alpha_y$, $\beta_y$, $\alpha_x$, $\beta_x$ as factors.

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# Modeling of furnace work with recirculating of heating gases for tunnel baking ovens

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**Abstract**

**Introduction.** With the aim of increasing the efficiency of the sources of heat in the furnaces of tunnel ovens it was investigated the process of recycling of heating gases.

**Materials and methods**. In this work, we used CAE-software for modeling the motion of liquids, the principle of software is based on the method of finite elements. For calculation was used rheological and kinematic parameters of hot gases which were obtained for the real model experiments.

**Results and discussion**. It is found that the velocity distribution along the length of the furnace is divided into two sustainable region: the axis of the furnace and around the outer walls of the furnace. The mixing of the gases starts in the junction box.

The temperature of the gases at the center of the stream is reduced from 1900 to 600 °C is almost a linear relationship.

The temperature of the gases near the furnace walls varies throughout the length of the furnace ranging from 260 °C to 360°C because of its low turbulence flow.

On the contour of the region of maximum dissipation of kinetic energy the turbulence were observed in the two local areas. The first is the ring around the exit of products of combustion from the combustion chamber. The second area is at the area of narrowing of the furnace. It is near the exit to the junction box.

It was obtained for the first time the visual and the numerous information that reflects how the combination of local resistance in a gas path affects the value of the Reynolds criterion and the nature of the movement of the heating agent.

It is proposed to equip the furnace of this type for more details – ring washers. It is proven that they change the direction of the flow of recirculation gases so that there is an active mixing with the combustion products along the entire length of the furnace.

**Conclusions**. Computer modelling of furnace operation has allowed to detect and localize the flaws of the current baseline design and to propose ways of modernize of the system.

## Introduction

The bulk of the bread currently baked in tunnel baking ovens. They are universal, cost-effective, the possibility of widely varying temperature settings over the length of the baking chamber.

Main unit of work which depends on the stability of the Baking Ovens – a furnace. It combusted natural gas or fuel oil.

Its principle of operation is associated with recirculation of exhaust gases heating. Flue gas with a temperature of 350 ... 400 °C is partially removed from the stack and partially fed into the furnace, where they are mixed with fresh products of combustion, and sent in the heating channels.

From the quality of mixing of fresh and exhaust heating gas depends on the uniformity of the heating of the baking chamber.

Definition of furnace operating parameters in the factory – a complex experimental process. Research methods are laborious and have significant errors.

It is proposed to use the methods of computer simulation. They allow to visualize the flow of hot gases inside the furnace. These methods allow you to track changes in flow rate, temperature and pressure drops, the processes of dissipation of the kinetic energy of the gas stream.

## Materials and methods

In this paper FlowVision program Tesis company was used.

It is designed for the calculation of hydro – and gas dynamics problems (together with related processes warmly – and mass transfer) in a wide range of Reynolds numbers in an arbitrary three-dimensional domains.

The use of this program has allowed us to obtain unique scientific information in various sectors of the food industry [1–5]. Has been researched and proposed ways of modernization of equipment for the grinding of food [6, 7], for mixing food products [8–10], thermal processes in proofers [11–13], to transport products through pipes [14].

It was also studied the movement of heating gases in various zones of tunnel ovens [15].

The basic program is the Navier-Stokes equations, the flow continuity equation, the equation for the turbulent viscosity. In addition, the model includes equations for the turbulent energy k and the rate of turbulent energy dissipation $\varepsilon$.

In this paper, in the course of the simulation was used $k - \varepsilon$ model of turbulent flow of viscous fluid with slight changes in the density changes at high Reynolds number.

Numerical integration of the equations of the spatial coordinates is performed using a rectangular milled locally adaptive grids. This approach allows the solution of problems carry out a grid adapted to the peculiarities of the geometry near the borders.

In the calculation were used physical parameters obtained at the time when the actual model experiments:

- the temperature of the combustion products leaving the combustion chamber is 1900 °C;
- the temperature of the gases supplied to the recycling of 350 °C;
- pressure drop, which is available at the site exit gas distribution box, is 30 Pa.

The number and proportion of the feed gases was determined by the ratio of excess gas, equal to 2,15.

When referring to the boundary wall of the conditions of surface roughness has been set, which is characteristic of the material from which made the furnace.

In FlowVision package used several ways to visualize the results. Visualization of scalar field dissipation of kinetic energy, which is proportional to the gradient of the speed of deformation of the product, possible to determine the place of origin of turbulence in the flow. Namely education turbulence leads to a mixing of gases. Field dissipation visualized through the use of contour lines of the gradient.

Vector field visualization speed it possible to determine a change in the value of speed and change the direction of movement of the product.

Typical furnace design with mixing chamber is shown in Figure 1. It consists of a burner 1, combustion chambers 2, the tangential entry nozzle gas recirculation 3, 4, mixing, dispensing duct chamber 5. The latter also was simulated, as at the entrance to him to change direction of the heating gas, which affects the total gas-dynamic situation.

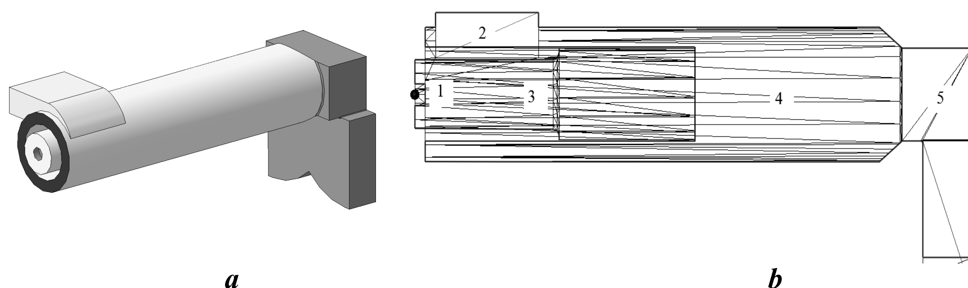


**Figure 1. The appearance of the furnace tunnel baking ovens (a) and its section (b):**
1 – burner; 2 – input pipe gas recirculation; 3 – combustion chamber;
4 – mixing chamber; 5 – distribution box

Examined type furnaces refers to a group of structures in which only the recycle gas is cooled outer surface of the combustion chamber and is widespread in the industry.

## Results and discussion

The efficiency of the furnace can be estimated by graphic materials. They are presented below.

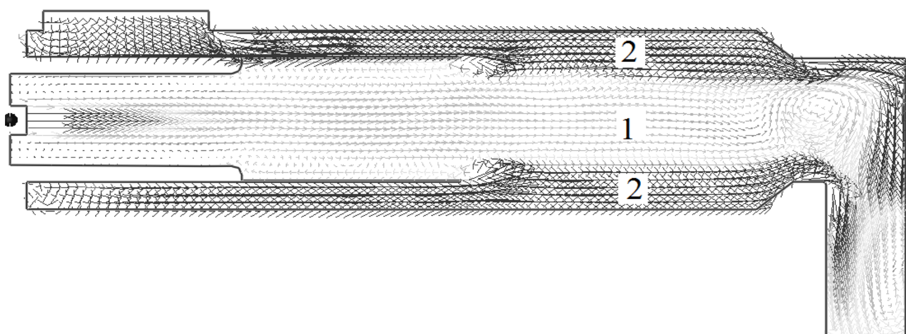


**Figure 2. The velocity vector along the length of the furnace**

Analyzing the velocity distribution along the length of the furnace (Figure 2), we can distinguish two stable regions. The first region – on the axis of the furnace – gases from the combustion chamber. The second area – cylindrical – recycle gases moving around the outer walls of the furnace.

Practically there is no mixing of gases in the furnace. It begins in the switch box. It is clear that such an operation unstable and does not give a good result.

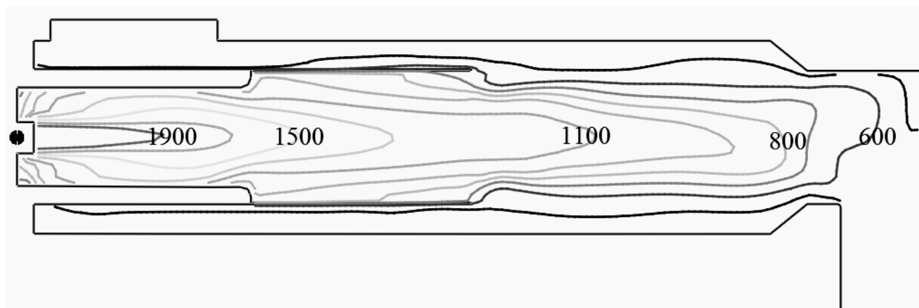


**Figure 3. Isolines changes in gas temperature in the furnace**

Consider the temperature drop along the length of the furnace. The flue pipes should go with the flow temperature of about 600 °C. Obtaining such temperature occurs at the end of the mixing chamber (Figure 3).

Any gas pressure drop across the burner, any change in the position of valves in the distribution boxes and heating ducts lead to the inability to obtain a stable flow set temperature.

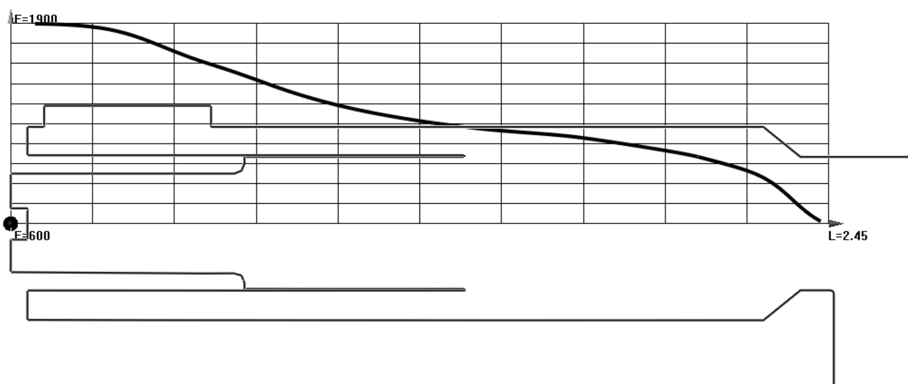


**Figure 4. Schedule of changes in temperature of the combustion products in the furnace center**

The temperature in the center of the flow changes from 600 to 1900°C virtually linear relationship (Figure 4). But efficient operation of the furnace, it should be at the beginning of the mixing zone to drop sharply.

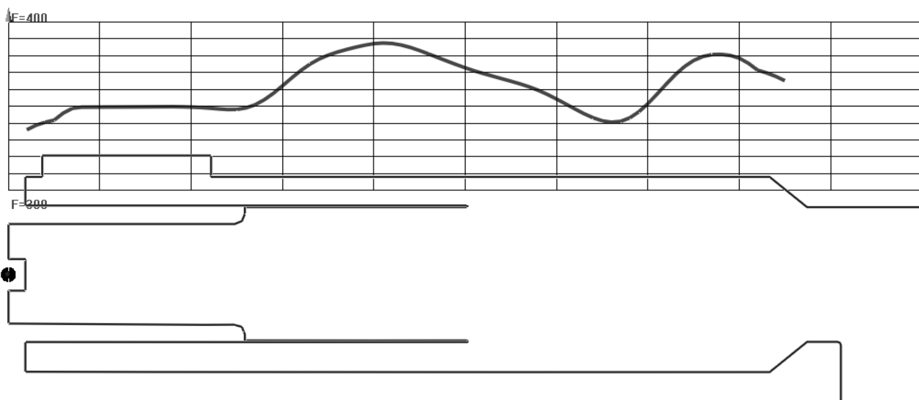


**Figure 5. Schedule changes recirculation gas temperature at the outer walls of the furnace**

Neither Figure 5 is a gas temperature near the furnace wall (the abscissa axis of the graph is in the middle of the annular gap through which recirculation gases move). The range of temperature fluctuations across the furnace length of about 100 $^{o}$C, which is clearly insufficient.

The dissipation of the kinetic energy of gases is due to internal friction between layers of flow, which are moving at different speeds. It shows the place of occurrence of turbulence, thanks to which there is a mixing of hot and cold gases.

Isolines of maximum dissipation area is shown in Figure 6. It may be noted swirls in two local areas. The first area – a ring around the exit of combustion products from the combustion chamber. The second area – in the narrowing of the furnace. It is already at the outlet to the distribution box.

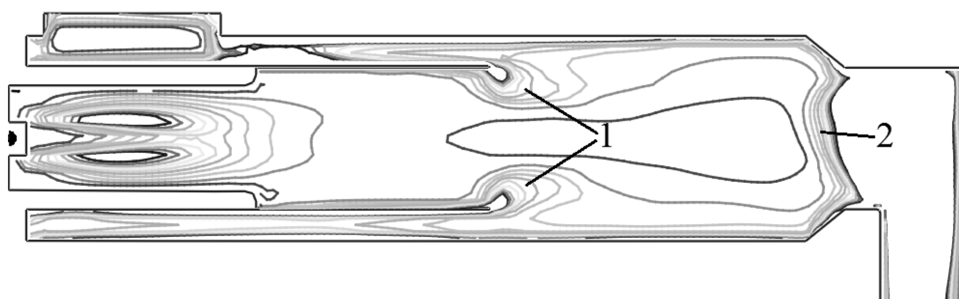


**Figure 6. Areas of active mixing of gases along the length of the furnace**

The above analysis allowed the furnace operation to detect and localize the shortcomings of the existing basic structure. This lack of turbulence in the gas flow.

The method of computer modeling allows us to offer solutions to the problem. Check the correctness of the proposed solutions can be directly, without the creation of pilot plants, stands and full-scale experiments.

Consider one of the solutions to the problem.

As gas flow path is necessary to create an artificial local resistance. It will change the speed and direction of flow. This will lead to their collision and active mixing.

local resistance structure can be set. Also, there may be many places of its location. In the present embodiment, the test – **A** washer ring is mounted on the inner wall of the mixing chamber in the middle of its length.

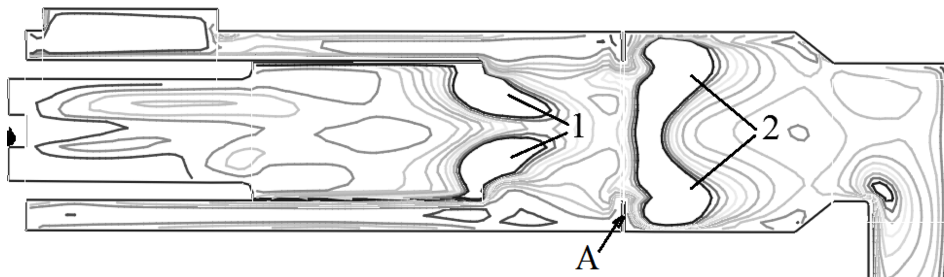


**Figure 7. Changes in the activity of dissipative processes after the furnace modernization**

As follows from Figure 7, activity increased mixing streams. Formed two large area – at the beginning of the mixing chamber and in the middle of it. Main processes now occur in the furnace itself, rather than leaving it. This allows more precise adjustment of processes of heat exchange and mass transfer between gas flows.

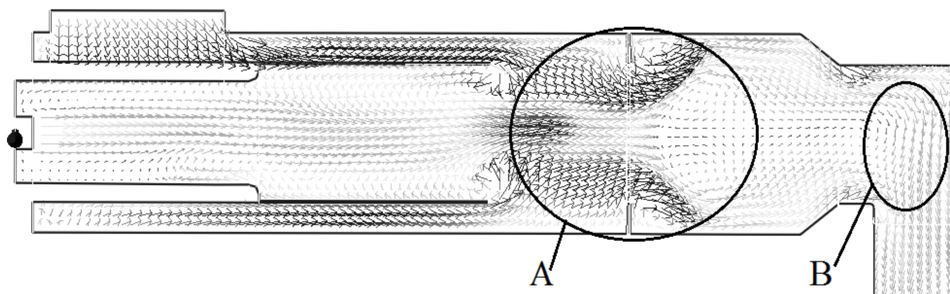


**Figure 8. Velocity vector gas streams after modernization**

The most visually look the field of active mixing in Figure 8. In the field of A the vector flow rates actively change direction. Streams collide and there is reduction in temperature to a predetermined value. On the contrary B – in the control box – flows have already mixed and a uniform temperature.

## Conclusions

Computer modeling of complex heat exchange processes provides a unique scientific information on the furnaces ovens.

Gas of recycling, which are introduced into the furnace tangentially to form a stable rotating flow around the chamber walls. This prevents them from mixing with fresh products of combustion.

Gas of recycling, which are introduced into the furnace tangentially, effectively cooling the outer surface of the device. Also recirculation gases effectively cooled combustion chamber surface.

The greatest area of turbulence in which the mixing gas are located at the beginning of the mixing chamber and the outlet the refrom.

It is proposed to equip the furnace of this type with additional details. They must change the direction of flow of recirculation gases so that their active mixing occurred with the combustion products over the entire length of the furnace.

Computer modeling allows you to quickly check the correctness of the proposed technical solutions for the modernization of structures of thermal devices baking ovens.

The proposed method of investigation baking ovens can be used to develop new and effective structures of this kind of baking equipment and to upgrade existing designs.

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# Are public perceptions precise towards the status of quality and safety of commercial brands of noodles?

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## Abstract

**Introduction**. Consumers are more conscious about the food safety and food quality which are the most important challenges throughout the world. In the aspect of food quality and safety, the study was conducted to assess quality in some commercial brands of Bangladeshi noodles.

**Materials and methods**. The study was carried out in two phases. In phase I, a comprehensive baseline survey was completed to know consumer attitude towards noodles covering the peoples of different areas in Bangladesh. Consumers alleged that all commercial branded noodles are adulterated by the use of low grade flour and harmful food colors that are hazardous for health. In phase II, four commercial branded noodles samples were collected from local market for assessing chemical composition, cooking characteristics, microbial profile and sensory evaluation (descriptive and preference test).

**Results and discussion**. Noodles samples were found in the range as moisture 7.07–10.70%, ash 1.01–2.70%, fat 0.40–0.73%, crude protein 11.00–11.17%, crude fibre 0.05–0.30%, total carbohydrate 74.19–80.34%, starch content 61.09–66.35% and pH 8.15–8.88. The degree of gelatinization, acid value and energy content was found as 96.55–99.85%, 0.79–0.85 mg/g and 344.52 kcal/100g – 370.53 kcal/100 g respectively. Microbial profile indicates, noodles industries maintained good hygienic conditions during noodles production according to GMP and there was no fecal contamination and any microbial hazards. The cooking characteristics of all noodles samples were varied but all had acceptable quality. There was significant difference ($p<0.05$) in the smoothness, yellowness, whiteness and shininess except brightness and textural attributes examined among the cooked noodles samples. Although descriptive score were varied, but were obtained satisfactory score. There was significant difference ($p<0.05$) in all the sensory attributes (color, flavor, taste, texture and overall acceptability) among the noodles but were achieved satisfactory score for all noodles samples.

**Conclusions**. Commercial brands noodles samples fulfilled the requirement of Bangladesh Standards and Testing Institution (BSTI) standards. So the concept of peoples about commercial brands noodles is not precise.

## Introduction

In Asia, noodles are consumed extensively as a traditional food. In the ancient period, they were prepared and consumed within the home. Now-a-days the new technology developed to improve it's quality with different variety, continue a staple of Asian intakes. Noodles become very available and can be obtained with different forms including fresh, cooked or processed for longer shelf life. Now noodles are reflected as a convenient fast food because of easy to prepare [1].

According to World Instant Noodles Association (2013), noodles are consumed in more than 80 countries and have become worldwide renowned foods. The world intake of noodles was 101.42 billion packets in 2013 and most of the intake took place in Asia. This included 44.03 billion in China, 14.10 billion in Indonesia, 5.41 billion in Japan, 4.36 billion in India and 0.16 billion in Bangladesh. However, North America also consumed 4.34 billion packets of noodles [2].

Factors to assess Asian noodle quality are appearance, color, eating quality and texture. Cooking characteristics and shelf life belongs to additional quality factors. Appearance can be assessed by three parameters such as brightness, yellowness and discoloration, although additionally glossiness, luster and geometry also consider as quality. Sensory evaluation and instrumental testing are the ways to assess eating quality and textural attributes. Trained panels frequently accomplish for sensory evaluation of Asian noodle texture and terminologies used are often subjective [3].

However, in the developing country like Bangladesh, it is very essential to assess the quality of commercial brands of noodles because it has very insignificant information in it and consumer are very worried about the quality and safety of foods. Hence, this experiment is crucial to meet a void in the scientific field as well as to meet up consumer demand.

The current study was carried out in two phases. In the first phase, the main goal was to complete a comprehensive baseline survey to know consumer attitude towards noodles covering the people of different sections of society. In the second phase, some commercial brands of Bangladeshi noodles quality were evaluated.

## Materials and methods

**Phase-I: Method of Base Line Survey.** A comprehensive Baseline Survey was completed covering the people of different sections of society. 1920 questionnaires were distributed among the respondents from where 951 complete questionnaires were received. Quantitative and qualitative data were transformed into scoring.

**Phase-II: Laboratory experiment.** The study was conducted in the laboratory of the department of Food technology and Rural Industries under the faculty of Agricultural Engineering and Technology and the laboratory of the department of Microbiology and Food Hygiene, Bangladesh Agricultural University and the laboratory of Bangladesh Standards and Testing Institution (BSTI).

**Sampling.** In order to carry out experiment, noodles samples of different commercial brands (coded with A, B, C and D to overcome the sampling biasness) were purchased from local market, Bangladesh, whose manufacture date were same. Control sample (coded with E) was made by using wheat flour (100g), salt (2g), gelatin (0.5g) and water (40g) with standard procedure.

**Chemical analysis of noodles.** All noodle samples were analyzed for its moisture content, ash content, fat content, crude protein content, crude fibre content, total carbohydrate content, starch content, pH of cooked noodles, acid value and degree of gelatinization, to determine important chemical parameters to assess the quality according to AOAC method [4]. All tests were executed in triplicate and the results averaged.

**Microbial profile.** The standard plate count and yeast and mold count were done according to the method described in "Recommended Method for Microbiological Examination of Food" [5]. Coliform count of selected brands noodles was done [6]. All tests were executed in triplicate and the results averaged.

**Cooking Quality Attributes of Noodles.** Cooking time, cooking yield, cooking loss and water absorption of all noodle samples were examined using standard method [7]. All tests were executed in triplicate and the results averaged.

**Sensory Assessment.** Samples were presented to a panel of 30 panelists selected from department. The descriptive test using a 5-point attribute scale and preference test using a nine-point hedonic scale were conducted consecutively. All the panelists were briefed before evaluation. Panelists evaluated the appearance of uncooked and cooked noodles under daylight illumination. The samples were coded with letters and served to the panelists at random to guard against any bias. Each sample was simulated triplicate by the full panel over three consecutive days during the course of evaluation.

**Statistical analysis.** The data obtained from the experiments were statistically analyzed for analysis of variance (ANOVA) and consequently Duncan's Multiple Range Test (DMRT) was used to determine significant difference among the various samples in triplicate. Data were analyzed using the software, Statistical Package for Social Sciences (SPSS) version SPSS 16.0.2 at the 0.05 level [8].

## Results and discussion

### Phase-I: Baseline Survey Results

**Status of Adulteration.** This survey was performed to know the opinions of general people of our society. 1920 questionnaires were distributed among the respondents and 951 complete questionnaires were received from them and most of the respondent's opinion is shown in table 1.

**Table 1**

**Status of Adulteration in Noodles Available in Local Market**

| Total respondent (n) | Responses | |
|---|---|---|
| | Adulterated | Not-Adulterated |
| 951 | 85.40% | 14.60% |

From Table 1, it is clear that 85.40% people of our society think that noodles available in local market are adulterated.

**Adulterants Used in Noodles.** According to the survey results, about six out seven of respondents opined that the processed noodles are being adulterated with different types of

adulterant like high moisture, excessive amounts of permitted preservatives, harmful food colors, low grade flour and harmful preservatives. The consumer response about the type and the level of individual adulterants in noodles has been shown in Figure 1.

According to the public opinion, 41.66% among adulterated noodles are adulterated by the use of low grade flours.

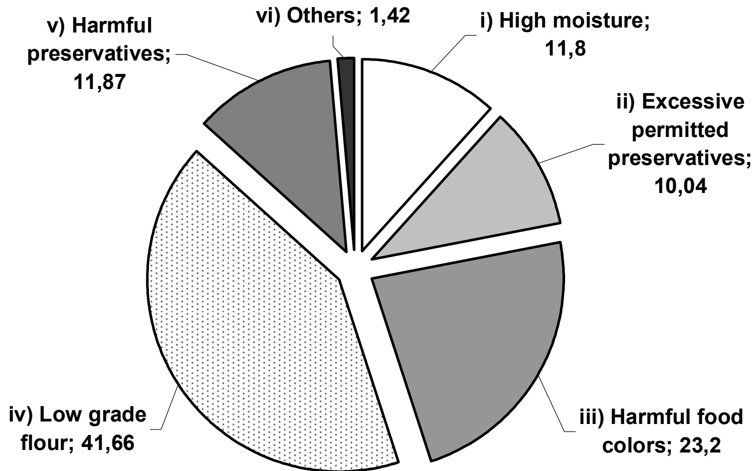



**Figure 1. Consumer Response about the Individual Adulterants Present in Noodles (Percent Respondent)**

**Status of Quality and Safety of Noodles.** Different respondent interpreted from different points on quality and safety issue of noodles avail in the market. The present respondent on quality and safety issue of noodles has been shown in Figure 2.

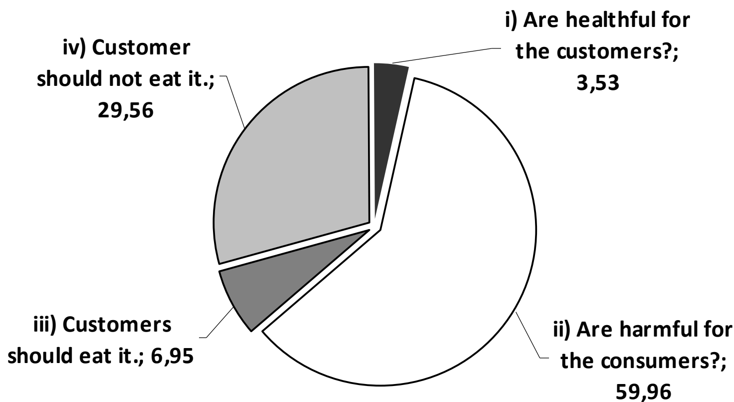



**Figure 2. Consumer Response about the Status of Quality and Safety of Noodles (Percent Respondent)**

From Figure 2, about six tenth people of our society believed noodles avail in market are harmful for consumers where three out of ten respondents stated that customer should not eat it. According to the survey results, it was concluded that public thought noodles available in market are unsafe for consumers to eat.

**Preventive Measures to Control Adulteration in Noodles.** The preventive measure need to be undertaken to control adulteration in commercial brands noodles are shown in Figure 3.

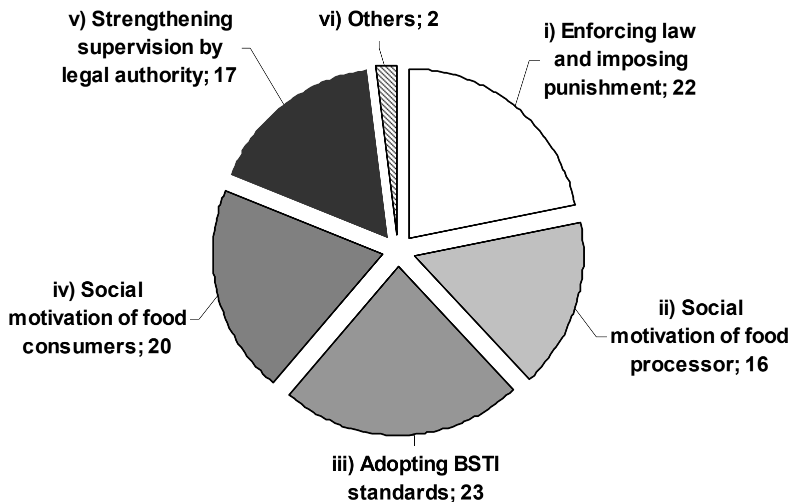



**Figure 3. Consumer Response about the Status of Preventive Measures to Control Adulteration (Percent Respondent)**

According to the respondents, preventive measure should be "Adopting BSTI standard" (23%), "Enforcing law and imposing punishment" (22%) and "Social motivation of food consumers" (20%). On the other hand 17% people believed that preventive measure should be "Strengthening supervision by legal authority". "Social motivation of food processor" (16%) is also a preventive measure need to be undertaken to control adulteration in commercial brands noodles.

**Phase-II: Chemical Analysis of Noodles**

The results of chemical analysis of noodles samples are tabulated in Table 2. The higher moisture content was obtained in sample A (10.70%) followed by B (8.35%), C (7.71%), E (7.31%) and D (7.07%). According to Bangladesh Standard and Testing Institution [9], the maximum moisture content of noodles should be 10%. The analysis showed that sample A had little bit higher moisture content than standard limits. The moisture content of all noodles samples varied. This variation might be due to the different drying time, drying condition and temperature.

**Table 2**

**Chemical Analysis of Noodles**

| Parameter | Noodles Samples | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Moisture content (%) | 10.70[a] | 8.35[b] | 7.71[bc] | 7.07[c] | 7.31[c] |
| Ash content (%) | 2.70[a] | 1.78[bc] | 2.25[ab] | 1.01[c] | 2.18[b] |
| Fat content (%) | 0.40[ab] | 0.73[a] | 0.67[ab] | 0.57[ab] | 0.49[ab] |
| Crude Protein Content (%) | 11.04[a] | 11.00[a] | 11.03[a] | 11.01[a] | 11.17[a] |
| Crude Fibre Content (%) | 0.30[a] | 0.29[a] | 0.05[b] | 0.05[b] | 0.24[a] |
| Total Carbohydrate (%) | 74.19[c] | 78.14[b] | 78.33[b] | 80.34[a] | 77.95[bc] |
| Energy content (kcal/100 g) | 344.52[d] | 363.13[bc] | 363.51[bc] | 370.53[a] | 360.89[cd] |
| Starch (%) | 66.35[a] | 61.85[d] | 64.23[c] | 61.09[d] | 65.21[bc] |
| pH of Cooked Noodles | 8.25[ab] | 8.20[ab] | 8.88[a] | 8.10[ab] | 8.81[a] |
| Degree of Gelatinization,% | 96.55[c] | 97.00[bc] | 97.75[bc] | 99.85[a] | 98.36[ab] |
| Acid Value (as KOH), mg/g | 0.86[a] | 0.82[ab] | 0.79[ab] | 0.84[a] | 0.85[a] |

Sample A had higher ash content (2.70%) followed by C (2.25%), E (2.81%), B (1.78%) and D (1.01%). The ash content depends on the quality of the flour and thus corresponds to the higher mineral content. According to Miskelly (1996), ash levels should be between 0.35% – 0.4% for high quality noodles [10]. The analysis showed that all noodles samples had higher ash content than Miskelly reported. This variation might be due to flour quality and use low grade ingredients that contained heavy metal. In our previous study, we found that commercial brands noodles contain some heavy metals but safe level [11].

The maximum fat content of noodles should be 2% [9]. The analysis showed that all commercial brands had lower fat content than maximum limits. The higher protein content was obtained in sample E (11.17%) among all commercial brands of noodles. Fu (2008) studied on the protein content of noodles showed that the protein levels ranged from 8.5 to 12.5% [12]. The analysis showed that all noodles samples almost similar within the range reported by Fu. But protein content of all noodles samples varied. This variation of protein content might be due to the different classes of wheat and milling procedures used by the various flour mills and use of ingredients in noodles that contained protein.

Hou (2001) expressed that noodles contained 0.05–0.50% crude fibre [13]. The analysis showed that crude fibre content of all commercial brands of noodles was almost similar within the range reported by Hou (2001). Total carbohydrate content was higher in sample D (80.34%) among the other commercial brands of noodles. The higher energy content obtained from sample D (370.53 Kcal/100g) due to higher carbohydrate content. Sample A had higher starch content (66.35%).

The pH of commercial brands of noodles and one lab made noodles after cooking was found in the range as 8.10–8.88 where highest pH was found 8.88 in sample C. Asenstorfer *et al.* (2006) found that noodles are yellow in color due to the detachments of flavone-C-diglycosides from starch under alkaline pH. The amount of pH increased the appearance of the raw sheet and the cooked noodles grew darker [14]. From Figure 4 (b), the color of Sample C was more yellow followed by sample E, A, B and D and from Table 2, the pH of

Sample C was highest followed by sample E, A, B and D. So the relationship between pH and color of noodles found similar that reported by Asenstorfer *et al.* (2006).

The degree of gelatinization of all commercial brands of noodles were obtained in the range as 96.55–99.85%. According to BSTI (2001), the minimum requirements of degree of gelatinization for noodles should be 80% [9]. The analysis showed that all commercial brands of noodles had higher degree of gelatinization than minimum limits.

Acid value was found in the range as 0.79–0.86 mg/g where the reference values are maximum 2mg/g prescribed in relevant BSTI standards [9]. The analysis showed that all noodles samples had lower acid value than maximum limits.

**Table 3**

**Microbial Profile of Noodles**

| Parameters (cfu/g) | Noodles Samples | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| Total Plate Count | <10 | <10 | <10 | <10 | <10 |
| Total Fungi Count | ND | ND | ND | ND | ND |
| Coliform Count | ND | ND | ND | ND | ND |
| *Bacillus cereus* | ND | ND | ND | ND | ND |
| Staphylococcal | ND | ND | ND | ND | ND |

Results are expressed as mean values of three replicates; ND – Not Detected

**Microbial profile of noodles.** The microbiological profile of the all commercial brands of noodles along with control noodles are presented on Table 3.

Total plate count of the all commercial brands of noodles and control noodles was observed to be below 10 cfu/g. Total fungi count, coliform count, *Bacillus cereus* and staphylococcal were not detected in all commercial brands of noodles samples and control noodles.

It indicates, noodles industries maintained good hygienic conditions during noodles production according to GMP and there was no fecal contamination. But continuous inspection should be needed for maintaining proper hygienic condition in noodles production floor.

**Cooking Characteristics of Noodles.** Table 4 shows the mean values of the cooking characteristics of commercial brands of noodles samples along with control noodles.

The optimum cooking time of all commercial brands of noodles samples were found in this study ranged from 6 – 9 minutes. Sample A had higher cooking yield 484.62% because of high amount of starch content among all commercial brand noodles samples (shown in Table 2). The cooking yield varied for all noodles samples. This variation of cooking yield might be due to flour quality, starch content, water holding capacity, water absorption rate and use of ingredients for example gum that increase water holding capacity.

Cooking loss is undesirable and according to Wu *et al.* (1987), it should not exceed 10% [15]. From Table 4, sample C had higher cooking loss. The significantly lowest value of the cooking loss occurred with all noodles samples than Wu *et al.* (1987) reported. The differences observed in cooking loss might be due to fineness of granulation of flour and starch damage used in noodles making and free lipids content in noodles.

Table 4

**Cooking Characteristics of Noodles**

| Noodles Sample | Cooking Time (Min.) | Cooking Yield (%) | Cooking Loss (%) | Water Absorption (%) | Moisture content after cooking (%) |
|---|---|---|---|---|---|
| A | $6^{a}$ | $484.62^{a}$ | $6.11^{b}$ | $384.62^{a}$ | $84.36^{a}$ |
| B | $7^{ab}$ | $388.64^{bc}$ | $7.90^{bc}$ | $288.64^{bc}$ | $77.24^{bc}$ |
| C | $9^{c}$ | $426.75^{ab}$ | $9.35^{cd}$ | $326.75^{ab}$ | $79.67^{ab}$ |
| D | $7^{ab}$ | $380.64^{bc}$ | $5.30^{ab}$ | $280.64^{bc}$ | $77.09^{bc}$ |
| E | $6^{a}$ | $481.02^{ab}$ | $4.01^{a}$ | $381.02^{ab}$ | $80.83^{ab}$ |

The water absorption for all noodles ranged from 280.64 – 384.62% where moisture content varied from 77.09–84.36% and varied might be due to flour quality, starch content, water holding capacity and use of ingredients for example gum that increase water holding capacity.

The better cooking quality was observed in sample A, C and E noodles samples. But the quality of sample B and D was also acceptable.

**Sensory Assessment of Noodles**

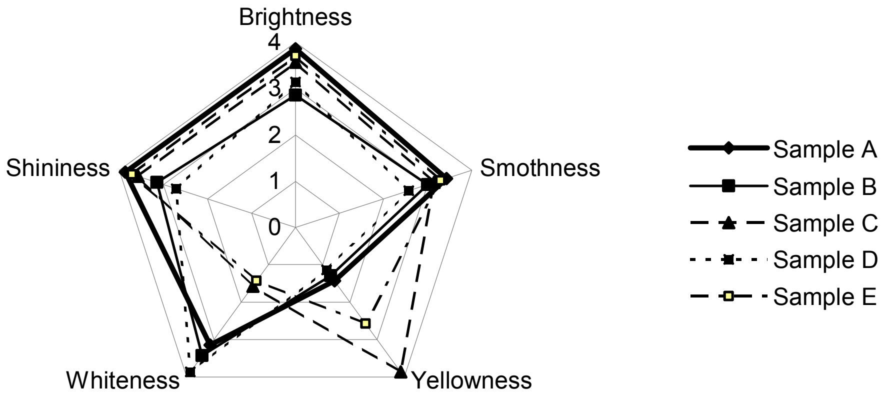
**Descriptive Test.** Figure 4 represents the Spider web plot of the sensory attributes of brightness, smoothness, yellowness, whiteness and shininess of the all uncooked (a) and cooked noodles (b).

The one way analysis of varience indicated that there was no significant difference ($p>0.05$) in the brightness, smoothness and shininess except yellowness and whiteness of the uncooked noodles (values are not shown but mean score was varied. In terms of brightness, smoothness and shininess, the range of mean score was 2.86–3.86, 2.57–3.43 and 2.71–3.86 respectively. But sample C (3.86) was declared to be very yellow among the noodle samples while Sample D had higher score (3.86) for whiteness.

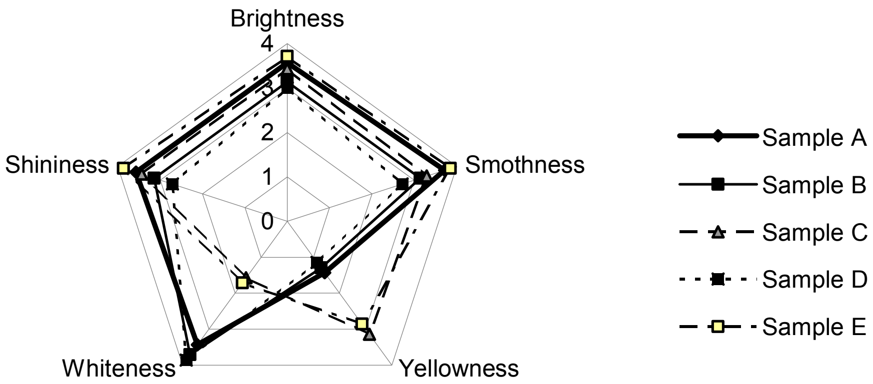
For cooked noodles samples, there was significant difference ($p<0.05$) in the smoothness, yellowness, whiteness and shininess except brightness examined (Figure 4(b)). The range of mean scores for brightness was (3.00–3.71), smoothness (2.71–3.86), yellowness (1.14–3.14), whiteness (1.57–3.86) and shininess (2.71–3.86).

Figure 4(c) represents the Spider web plot of the textural attributes according to smoothness, softness, chewness and ease of swallowing of the all cooked noodles. The one way analysis of varience indicated that there was no significant difference ($p>0.05$) in the textural attributes according to smoothness, softness, chewness and ease of swallowing of the all cooked noodles. The range of mean scores for smoothness was (3.14–3.86), softness (2.86–3.71), chewiness (2.29–2.86) and ease of swallowing (2.71–3.71).

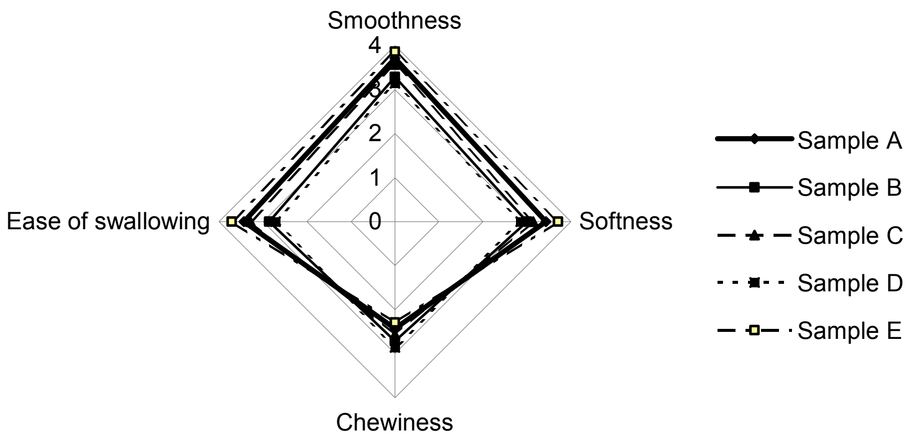
**Preference Test.** The means sensory liking scores for color, flavor, texture, taste and overall acceptability of cooked noodle samples are shown in Table 5.




**(a) Uncooked Noodles Appearance**




**(b) Cooked Noodles Appearance**




**(c) Cooked Noodles Texture**

**Figure 4. Spider Web Plot on Sensory Descriptive Profiling of Noodles Samples**

Table 5

**Mean Sensory Score of Noodle Samples**

| Sample | *Mean Scores on sensory attributes | | | | |
|---|---|---|---|---|---|
| | Color | Flavor | Texture | Taste | Overall Acceptability |
| A | 7.57 ± 0.68[a] | 7.60 ± 0.67[a] | 7.67 ± 0.99[a] | 7.63 ± 0.99[a] | 7.67 ± 0.66[a] |
| B | 6.53 ± 0.94[b] | 6.77 ± 1.01[b] | 6.80 ± 1.19[b] | 6.97 ± 0.96[b] | 6.80 ± 0.96[b] |
| C | 7.27 ± 1.01[a] | 7.40 ± 0.86[a] | 7.60 ± 0.97[a] | 7.67 ± 1.24[a] | 7.63 ± 0.81[a] |
| D | 6.50 ± 1.22[b] | 6.57 ± 1.06[b] | 6.93 ± 1.05[b] | 6.60 ± 0.93[b] | 6.63 ± 0.85[b] |
| E | 7.63 ± 0.81[a] | 7.80 ± 0.55[a] | 7.97 ± 0.85[a] | 8.07 ± 0.74[a] | 7.90 ± 0.85[a] |
| LSD (p<0.05) | 0.4853 | 0.4277 | 0.5180 | 0.5043 | 0.4281 |

*Mean value ± standard deviation (n = 30). Means with different superscripts within a column are significantly different and the same superscripts do not significantly different (NSD) at p<0.05

The one-way analysis of variance indicated that there was significant difference (p<0.05) in all the sensory attributes examined among all noodles samples. The range of mean scores for color was (6.5–7.63). In term of flavor, sample E had the highest mean score (7.8) followed by samples A (7.6) and C (7.4), while samples D (6.57) and B (6.77) had acceptable but slightly lower score. There was no significant difference in texture acceptability among samples A (7.67), C (7.60) and E (7.97). The range of mean score for taste was (6.60–8.07). Based on the scores for overall acceptability, there was no significant difference in overall acceptability among samples A (7.67) and C (7.63) over control samples (7.90). Also sample B and D also got satisfactory overall acceptability score. All the noodles samples obtained satisfactory score so they may be recommended as preferred for consumption.

## Conclusions

The assurance and protection of food quality has always been important to consumers. Governments over many centuries have endeavored to provide for the safety and wholesomeness of food by legal provisions. Though commercial brands of noodles samples were varied in their quality characteristics but all noodles samples fulfilled the requirement of Bangladesh Standards and Testing Institution (BSTI) standards. However, this study was useful in differentiating the quality characteristics among the Commercial brands noodles. So public perceptions to judge the quality and safety of commercial brands of noodles is erroneous might be due to lack of familiarity to evaluate quality.

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# System of quality labels in the European Union

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**Abstract**

**Introduction.** The paper deals with European Union schemes of Protected Designation of Origin, Protected Geographical Indication and Traditional Speciality Guaranteed labels used in agricultural and food products sector.

**Materials and methods.** Secondary data from the Database of Origin and Registration are used. Sample consists of 1,356 labels registered in this database to the 18th July 2016. The frequency of labels utilization is analysed according to country, type of label, and product classes.

**Results and discussion.** The task of the research is to analyse the utilization of these labels in European Union market according to selected criteria including type of label registered in each country, and number of agricultural products and foodstuffs registered as quality labels in total. As results show, the highest number of product names is registered under PDO label followed with slight difference by PGI label. There are only minimum product names registered as TSG. Dominating country is Italy followed other Mediterranean countries like France, Spain, Portugal and Greece. Based on product class, the most common classes are Fruits, vegetables and cereals (for PGI, PDO), Cheeses (PDO) and Meat products (TSG, PGI). This is confirmed by Pearson´s chi-square test of independence in order to determine if significant differences do exist between frequency of using the labels and mentioned criteria. It is confirmed weak dependence between number of product names registered as PDO, PGI and TSG and country of origin, middle strong dependence between type of label and product classes where the label is used, and strong dependence between country of origin and the most often registered product class.

**Conclusion.** The PDO, PGI and TSG schemes bring benefits to consumers as well as to producers. Consumers are buying a product with specific value-adding qualities.

## Introduction

The paper is focused on the specific problem area of so-called quality labels and presents results of marketing research focused on analysis of using selected quality labels in European Union (EU) agricultural and food products market. The production of food and agricultural products is an important part of the European Union economy. European food and beverages play a major role in the cultural identity of European citizens and regions. High quality of European food is a key advantage for European agriculture. [1] Many food and agricultural products exhibit special characteristics linked to their geographical area, traditional composition or traditional production method.

An integral part of EU agricultural policy is an effort to improve food quality and safety. Also consumers in EU countries show growing interest in the quality as well as traditional products. This generates a demand for food and agricultural products with identifiable specific characteristics, in particular those linked to their geographical origin. Producers offering products with the value-adding attributes should communicate them on the marketplace and highlight the characteristics of their products to consumers. Agricultural product quality policy therefore should provide producers with the right tools to better identify and promote such products and protect them against unfair practices. [2] Protection of original and traditional food from the EU is not only an important factor in preserving cultural and national traditions in the member states of the EU but also an important dimension of marketing for producers, taking into consideration the consumers´ interest and confidence. [3]

In order to allow producers to use the added value of their products as effectively as possible and to facilitate consumers' choice of food products, since 1992, the EU has established quality labels system known as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG) to protect the names of these products ([4]; [2]). These three schemes have built on a long history of regional and traditional specialities, especially in southern European countries. [5] The image of the region of origin and the specific product characteristics create a unique identity for food products bringing in this way added value. [6] The PDO, PGI and TSG labels aim to provide consumers with clear information on the product origin or speciality character, enabling them to make more informed purchases and the best possible choices. [7]

## Literature review

In the context of the topic, the terms "food quality" and "quality labels" are explained, followed by the specification of surveyed labels PDO, PGI and TSG. Quality label is a term for a symbol that can be put on a product or its packaging indicating that the product or the process to make the product complies with given standards and that this compliance has been certified. [8], [9] Quality labels guarantee compliance not only with current standards, but also with additional quality criteria determined in a corresponding certification system. A quality labels give added value to the products and are usually used in communication with end consumers. [10] According to [1], quality labels are an ambiguous category that covers many different things. They can be divided into obligatory (determined by legal rules and compulsory for all products in a given product category) and voluntary labels (bring competitive advantage for a product), into general (address all product quality characteristics) and specific labels (focused only on particular quality characteristics), or

into regional, national, international and global labels. They can cover quality, safety, organic origin and other characteristics of product. [1], [8], [11], [12] More about influence of quality labels on its regional distribution offers. [13], [14], [15] [16]

Food quality labels are determined to promote and protect food products and should be a guarantee of quality products, their geographical origin, specific characteristics and/or production methods. [8] They give a legal protection of a product against imitation throughout the market and eliminate the misleading of consumers by non-genuine products, which may be of inferior quality; they help producers obtain a premium price for their authentic products, and finally they should give clearer information to consumers about product characteristics and facilitate identification of food products with certified quality. [17]

Following text is focused on EU quality labels scheme that identifies agricultural products and foodstuffs farmed and produced to exacting specifications. It includes Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) for agricultural products and foodstuffs as well as for wine and spirits (there are different rules for wine and spirits and they are not included in our analysis), and Traditional Speciality Guaranteed (TSG) for agricultural farm products and foodstuffs. While a PDO covers agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how, a PGI indicates a link with the geographical area in at least one of the stages of production, processing or preparation. The link with the area is therefore stronger for PDO; PGI is a more flexible regulation. For PDO food products, management conditions are regulated by very strict rules with the aim of obtaining high quality process [18], the link with the area is stronger; PGI is a more flexible regulation. A TSG highlights a product´s traditional character, either in the composition or means of production. According to new Regulation on EU quality schemes for agricultural products and foodstuffs entered into force in the beginning of 2013, in order to be "traditional" proven usage on the market during at least 30 years (instead of 25) is now required [2], [19], [20].

## Material and methods

The main purpose of marketing research was to analyse the use of PDO, PGI and TSG label registered in DOOR database for agricultural products and foodstuffs in EU market. The DOOR database includes a list of product names (agricultural products and foodstuffs) registered as PDO, PGI or TSG as well as names for which registration has been applied. PDO and PGI cover also wine and spirits, however there are different rules for those products (in particular Regulation (EC) No 1234/2007 for wines, Regulation (EEC) No 1601/91 for aromatized wine products, and Regulation (EC) No 110/2008 for spirits) and they are not included in DOOR database as well as other products out of Regulation 1151/2012. [20] Geographical indications protected in the European Community for wines originating in Member States and third countries are registered in E-BACCHUS database, geographical indications for spirits are listed in E-SPIRIT-DRINKS database.

The DOOR database user can scan a list of product names registered as PDO, PGI or TSG (generally or in selected country), product names can be also sorted according to product classes. However, the user can always see just a list of products. Database does not offer a summarized data and their comparison. Research studies dealing with the topic are focusing only on partial aspects of quality labels like analysis of food products registered as PDO, PGI and TSG in selected countries [3], [21], analysis of customer loyalty and buying

intention for PDO label [22], consumer awareness and perception of labels [7], [23], consumer behaviour [14], customer satisfaction [24], or impact of labels on customer loyalty. [25] As criteria of analysis we set country of origin and type of label. Specific research purposes were as follows:

To identify the frequency of PDO, PGI and TSG labels utilization, to compare using of the labels according to EU countries and to compare using of the labels according to product classes.

The secondary data from the Database of Origin and Registration (DOOR) database were used. In July 2016, DOOR database includes total number of 1,514 items, but some of them are on waiting list and there is not sure if they would be accepted. [16], [26] therefore, in our analysis we have calculated with sample of registered items only, i.e. 1,356 items. We have to notice, in database are not only European countries, but also China represented with 10 own products certified with PDO and PGI labels, Thailand has three PGI labels, Vietnam, Colombia, and India have one registered product name. In the analysis, we proceeded descriptive statistics and contingency tables, where we tested relations with Chi-square test.

## Results and discussion

Research outcomes correspond with the date of 18th July 2016, when 1,356 product items certified with PGI, PDO or TSG label were registered in the DOOR database. Sample structure is presented in Table 1.

**Table 1**

**Sample Characteristics (n = 1,356, in per cent), first 90% of cases**

* Species, condiments, ciders, teas, etc.;

** eggs, honey, various milk products excluding butter etc.

| **Type of Label** | PGI | 45.05 | **Country** | Italy | 20.90 |
|---|---|---|---|---|---|
| | PDO | 50.88 | | France | 17.2 |
| | TSG | 3.98 | | Spain | 14.2 |
| **Product Class** | 1.6 Fruits, vegetables and cereals | 27.3 | | Portugal | 10.0 |
| | 1.3 Cheeses | 17.3 | | Greece | 7.6 |
| | 1.2 Meat products | 13.2 | | Germany | 6.5 |
| | 1.1 Fresh meat | 11.4 | | United Kingdom | 4.4 |
| | 1.5 Oils and fats | 9.5 | | Poland | 2.7 |
| | 2.4 Bread, pastry, cakes, other baker´s wares | 5.5 | | Czech Republic | 2.1 |
| | 1.8 Other products of Annex I* | 4.2 | | Slovenia | 1.6 |
| | 1.4 Other products of animal origin** | 3.3 | | Other | 12.8 |
| | Other | 9.25 | | | |

As we can see in Table 1, the highest share has PDO label followed by PGI label. Number of registered PGI (611, i.e. 45.05%) and PDO labels (691, i.e. 50.88%) is relatively

balanced, with the slight predominance of PDO. There are only minimum product names registered as TSG (54, i.e. 3.98%). Based on product class, the most of labels were awarded for fruits, vegetables and cereals. The most frequently certified products come from Italy (284 registered product names as PGI, PDO and TSG, i.e. 20.94% from all registered product names).

Frequency of PGI, PDO and TSG labels according to country is based on the list of all countries and product names registered in the DOOR database. The ranking of all countries according to total number of registered product names as PGI, PDO and TSG was created, see Table 2. Fields with the largest number of registered product names under PGI, PDO and TSG are highlighted in grey colour.

**Table 2**

**Numbers of product names registered as PGI, PDO and TSG according to countries (n = 1,356, in per cent)**

* The Czech Republic and Slovakia has registered four the same product names as TSG. Because of this duplicity, the sum of per cent for TSG is higher and also total sum of per cent is higher than 100%.

| Country | PGI | PDO | TSG | Total |
|---|---|---|---|---|
| 1. Italy | 8.63 | 12.17 | 0.15 | **20.94** |
| 2. France | 9.88 | 7.23 | 0.07 | **17.18** |
| 3. Spain | 6.49 | 7.45 | 0.29 | **14.23** |
| 4. Portugal | 5.24 | 4.72 | 0.07 | **10.03** |
| 5. Greece | 2.06 | 5.53 | 0.00 | **7.60** |
| 6. Germany | 5.60 | 0.88 | 0.00 | **6.49** |
| 7. United Kingdom | 2.43 | 1.77 | 0.22 | **4.42** |
| 8. Poland | 1.47 | 0.59 | 0.66 | **2.73** |
| 9. Czech Republic | 1.70 | 0.44 | 0.35 | **2.14** |
| 10. Slovenia | 0.81 | 0.59 | 0.22 | **1.62** |
| 11. Belgium | 0.74 | 0.22 | 0.37 | **1.33** |
| 12. Austria | 0.44 | 0.66 | 0.07 | **1.18** |
| 13. Hungary | 0.52 | 0.44 | 0.07 | **1.03** |
| 14. Netherlands | 0.37 | 0.44 | 0.07 | **1.03** |
| 15. Slovakia | 0.74 | 0.07 | 0.22 | **1.03** |
| 16. Finland | 0.15 | 0.37 | 0.22 | **0.74** |
| 17. Lithuania | 0.29 | 0.07 | 0.15 | **0.52** |
| 18. Sweden | 0.22 | 0.15 | 0.15 | **0.52** |
| 19. Bulgaria | 0.15 | 0.00 | 0.29 | **0.44** |
| 18. Denmark | 0.44 | 0.00 | 0.00 | **0.44** |
| 19. Ireland | 0.29 | 0.07 | 0.00 | **0.37** |
| 20. Latvia | 0.07 | 0.07 | 0.22 | **0.37** |
| 21. Cyprus | 0.29 | 0.00 | 0.00 | **0.29** |
| 22. Luxembourg | 0.15 | 0.15 | 0.00 | **0.30** |
| 24. Romania | 0.15 | 0.07 | 0.00 | **0.22** |
| Total | **50.96** | **45.06** | **3.69*** | **100.35*** |

As it is evident from Table 2, 24 from 27 EU member countries have registered their product names as PGI, PDO or TSG in DOOR database. Malta and Estonia have not yet used this type of protection. The first six countries of ranking in Table 2 (i.e. 22% of all EU countries) have obtained PDO, PGI and TSG labels for 80% product names registered in DOOR database (interestingly, the Pareto rule is shown here). The first three countries in the ranking, Italy, France and Spain, then have more than 50% of all registered product names. Italy has registered the highest number of product names as PDO; France is the first in number of PGI labels. It is interesting, that most of TSG labels belong to countries in weaker positions in the overall ranking, i.e. to Poland, Slovakia, Belgium and the Czech Republic.

In order to discover reciprocal dependences of tracked characters, we proceeded Chi-square test at significance level $\alpha = 0.05$, when sig F = 0, and we can confirm variables depend reciprocally. Thereby, we accept stated hypothesis about highest distribution of labels in three Mediterranean countries, Italy, France and Spain. Reasons for this could be well-known gastronomic specialities as well as national cuisine of these countries, which have built on a long history and are popular around the world. Overall, these countries have higher impact on global food marketplace, comparing with countries such Luxembourg, Ireland and Lithuania, whose food products are not so popular in customers' minds. Relations in the sample are described by Pearson contingency coefficient (0.520) and Cramer's contingency coefficient (0.430), thus there is weak dependence between number of product names registered in the database and country of origin. These two contingency coefficients are the most basic measure of association between two nominal-level variables. [27]

Comparison of selected EU countries according to numbers of product names registered in the individual product classes.

In the last step of analysis, we compare EU countries by numbers of product names registered in the individual product classes. We wanted to know which product class is the most typical in each country. Because of too high number of items in the DOOR database, we have decided to focus only on countries which have registered more than 15 product names. This is ten first countries mentioned in Table 2 which have registered 87% (1180) items as PGI, PDO and TSG (in July 2016).

The most common product class is 1.6 (Fruits, vegetables and cereals) which is dominant in Italy, Spain, Greece and Poland. Portugal and Slovenia have the highest number of registered products in class 1.2 (Meat products), France in class 1.1 (Fresh meat), UK in class 1.3 (Cheeses). As we expected, the Czech Republic excels in class 2.1 (Beers). Germany is the only country represented in the class 2.2 (Mineral and spring waters) and at the same time this product class is dominant for Germany.

In order to discover dependency between country of origin and the most often registered product class, we proceeded Chi-square test at significance level $\alpha = 0.05$, sig F = 0 and we can confirm, there are dependencies between these variables. In the next step, we measured tightness size between same variables with using of Pearson contingency coefficient. Its value is 0.707, which means strong positive dependence between country and the most often registered product class, i.e. each country has one important product class in which the most of domestic products is registered. Similar to the analysis of labels utilization by country of origin, we prove the most product types are registered in Italy, France and Spain. The most likely explanation is importance of these national food products at the global marketplace, where products like olive oils, cheeses, vegetable a fruit products play important role.

In the literature, PDOs, PGIs and TSGs are usually modelled as a signal of high quality in a vertical differentiation context. In some countries, importance of PDOs, PGIs and TSGs is very high. For instance, in leading countries such France, Italy and Spain a high share of wine is sold under PGIs and important factors for this are soil, climate and traditional know-how, then there is a high importance of geographical location. There also exists real impact on national economies because smaller producers of agricultural and food products can benefit from well-established reputation of EU-wide known quality labels and can manufacture products with added value and differ from their competitors. Furthermore, it can affect positive impact on regional development in particular region in form of new jobs.

Success of utilization of European Union quality schemes is influenced by perception of quality in certain European countries. In southern Europe, customers willing to pay more for acquiring a good from a particular origin [28], but in northern Europe quality is associated more with a set of rules on safety, integrity, or conformity to industrial processes and there is not needed to support traditional know-how of certain geographical origin. Another case are French wines, which names are so well-known, that further geographical protection is not needed, because there are well-promoted brands (for instance Chateau Margaux, Georges Duboeuf, Mouton-Cadet).

The reason why countries such France, Italy and Spain dominate, is also long tradition of protection their food products prior nowadays European Union Quality Schemes. For instance, Roquefort, famous French cheese is protected since 15th century, when King Charles VI granted a monopoly for producers in Roquefort-sur-Soulzon only. Perhaps France has the biggest experiences with protection of certain food brands by organization called Appellation d'origine controlee.

## Conclusion

Presented paper deals with analysis of European Union quality labels scheme known as PDO, PGI and TSG used in agricultural and food products sector. Data comes from the DOOR database and for obtaining outcomes, statistical methods have been used. The main contribution of the paper is a comprehensive view on the topic, the comparison of summarized data according to selected criteria including country of origin, type of label and product class, and statistical testing the relations between using of the labels and mentioned criteria. As results show, the highest number of product names is registered under PDO label followed with slight difference by PGI label. There is only minimum product names registered as TSG. Dominating country is Italy followed other Mediterranean countries like France, Spain, Portugal and Greece. The reason for this could be a long history of regional and traditional specialities in these countries and a higher importance of these products (such olive oil, cheeses, vegetable products and other) on the global marketplace. Based on product class, the most common classes are Fruits, vegetables and cereals (for PGI, PDO), Cheeses (PDO) and Meat products (TSG, PGI). In various countries dominate different product classes, but most frequent are, in consistency with previous results, classes Fruits, vegetables and cereals (Italy, Spain, Greece and Poland) and Meat products (Portugal and Slovenia). Beer is the most typical product class in the Czech Republic. Statistic testing has confirmed weak dependence between number of product names registered as PDO, PGI and TSG and country of origin, middle strong dependence between type of label and product classes where the label is used, and strong dependence between country of origin and the most often registered product class.

Original and traditional agricultural and food products can be perceived as an important part of tradition and image of the region. The PDO, PGI and TSG schemes bring benefits to consumers as well as to producers. Consumers are assured they are buying a genuine product with specific value-adding qualities. Producers benefits lies in fair competition, protection, and promotion of their products. To take full advantage of these benefits, producers should communicate their products with the value-adding attributes and highlight the specific character of their products to consumers, enabling them to make more informed purchases and the best possible choices. The aim of the communication campaign should be to build awareness, credibility and favourable perceptions about quality and distinctiveness of PDO, PGI and TSG products, and to stimulate consumers´ interest in such products.

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# Анотації

## Харчові технології

### Спектроскопічний аналіз меду

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**Вступ.** Основною метою даної статті є дослідження зв'язку хімічного складу, фізичних властивостей, ботанічного та географічного походження, а також віку меду з його спектральними параметрами, які можуть бути використані як таксономічні критерії та індикати його стану, якості та можливої фальсифікації.

**Матеріали і методи.** В даних дослідженнях були використані два методи неруйнівного контролю меду – спектроскопія в ближній інфрачервоній області спектра і флуоресцентна спектроскопія.

**Результати і обговорення.** Хімічний склад, фізичні властивості, ботанічне і географічне походження, а також вік меду тісно пов'язані з його спектральними параметрами, які можуть бути використані як таксономічні критерії або індикатори стану, якості меду та його можливої фальсифікації.

Інтенсивність спектрів поглинання було використано як критерій географічного походження і віку меду. Інтенсивність спектрів випромінювання флуоресценції залежить від географічного походження, віку й типу меду.

Дослідження впливу температури на інтенсивність флуоресценції меду довело, що збільшення температури викликає зменшення інтенсивності флуоресценції. Була встановлена кореляція флуоресцентних параметрів меду з вмістом у ньому води.

Спектри відбивання меду в ближній інфрачервоній області спектра характеризуються наявністю смуг відбивання при 1779 нм, 1933 нм і 2290 нм; відносна інтенсивність смуг залежить від типу меду і віку зразків. Вельми інформативними є спектральні параметри меду в ближній інфрачервоній області спектра при неруйнівному виявленні фальсифікації меду.

**Висновок**. Методи інфрачервоної і флуоресцентної спектроскопії можуть бути використані в технології виробництва меду як неруйнівні, швидкодіючі і точні методи діагностики меду.

**Ключові слова**: *мед, географія, походження, якість, фальсифікація, спектроскопія.*

### Склад і властивості частково гідролізованих соняшникових білкових ізолятів

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**Вступ.** Частковий ферментативний гідроліз білків є загальноприйнятим методом модифікації їх функціональних властивостей. У статті представлено характеристику властивостей частково гідролізованих білків, одержаних із соняшникового шроту.

**Матеріали і методи.** Білки соняшникового шроту екстрагували протягом 40 хв за наявності двох протеаз, а саме: нейтральної протеази і Алкалази. Зразки частково гідролізованих білків були одержані шляхом ізоелектричного осадження та наступного висушування. Поліпептидний склад білкових ізолятів вивчали за допомогою електрофорезу в поліакриламідному гелі, визначено також їх ступінь гідролізу, амінокислотний склад, поверхневу активність і функціональні властивості.

**Результати і обговорення.** В поліпептидному профілі білків, частково гідролізованих обома протеазами, були відсутні високомолекулярні поліпептиди із молекулярними масами 45–54 і 32–35 кДа. В той же час вони були збагачені поліпептидами з молекулярними масами 14–16 кДа та нижчими.

Зразки частково гідролізованих білків мали вищий вміст білків, нижчий вміст золи та вуглеводів порівняно із контрольним зразком. Біологічна цінність соняшникових білкових ізолятів була обмежена трьома амінокислотами − сірковмісними метионіном і цистином та лізином. Вміст метіоніну, цистину та лізину збільшувався у зразках білків, одержаних із нейтральною протеазою, порівняно з контрольним зразком.

Диференціально скануючий калометричний аналіз білкових зразків показав, що частково гідролізовані зразки містили неденатуровані білки, проте їх ступінь денатурації був вищим порівняно з контрольним зразком. Частковий гідроліз білків насіння соняшнику збільшував їх розчинність в діапазоні pH від 2 до 8, волого- та жироутримувальну здатність, піноутворювальну, емульгувальну здатність і поверхневу активність.

**Висновки.** Зразки частково гідролізованих білків мали вищий вміст білка, більш світле забарвлення, нижчий ступінь денатурації та кращі функціональні властивості порівняно з традиційними білковими ізолятами.

**Ключові слова:** *соняшник, білок, ізолят, гідроліз, протеаза.*

## Дослідження органолептичних властивостей смаженого м’яса, засіяного закваскою культури *Pediococcus acidilactici* FLE07, і летких з’єднань, які впливають на його якість

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**Вступ.** Використання таких біологічних препаратів, як молочнокислі бактерії, може покращити органолептичні властивості смажених м’ясних продуктів.

**Матеріали і методи**. Смажений м’ясний продукт (Tsire) було засіяно 6 млн КУО/г закваскою культури Pediococcus acidilactici FLE07 (ОЗ) з метою встановлення кількості летких з’єднань, які спричиняють псування протягом чотирьох днів зберігання за температури 30 °С. Як контрольні були обрані незасіяні зразки (НЗ) смаженого м’ясного продукту (Tsire). Тверда фаза масової екстракції методом газової хроматографії-мас-спектрометрії (ТФМЕ-ГХМС) та дегустаційне випробування за гедонічною шкалою були використані для оцінювання змін та органолептичних якостей під час зберігання.

**Результати і обговорення.** В попередніх експериментах для виробництва органічних кислот були оцінені десять штамів Pediococcus. Найбільшу кількість молочної кислоти виробляють 34 gacid/107КУО і P. acidilactici FLE07, що були

обрані як закваска для засіювання смаженого м'ясного продукту (Tsire). Було визначено, що штам виробляє оцтову кислоту в концентрації менше, ніж 12 gacid/107 КУО. ТФМЕ-ГХМС дослідження смаженого м'ясного продукту підтвердили наявність 48 летких компонентів, а саме: кетонів (35,42%), кислот (8,33%), спиртів (25%), арома/циклічних (14,58%), азотних з'єднань (16,67%), які були визначені під час зберігання. Леткі компоненти включали ацетон, 2-бутанон, 2,3-бутандіон, 3-гідрокси-2-бутанон, 2-гексанон, 2-гептанон та 1-гідрокси-2-пропанон, визначені серед кетонових з'єднань, зафіксованих у зразках м'ясних продуктів (Tsire). Доведено значну різницю ($p<0{,}05$) між засіяними і контрольними зразками м'ясного продукту(ОЗ та НЗ). Було виявлено зниження летких з'єднань, що пов'язано із псуванням. Так, для гептаналу, 1-октен-3-олу, 3-метил-бутанової кислоти концентрація (мг/г) склала 0,57, 1,98, 0,93 та 1,39 відповідно в засіяних зразках (ОЗ) порівняно з 2,43, 3,21, 2,94 та 2,94 для необсіяних зразків (НЗ) на третій день. Органолептичні дослідження підтвердили кращу оцінку ($p<0{,}05$) аромату, зовнішнього вигляду, консистенції, смаку та загальних якостей ОЗ порівняно із НЗ.

**Висновки.** Культури Pediococcus acidilactici FLE07 та інші закваски можуть бути використані для покращення органолептичних параметрів і доступності продукту незалежно від дня виробництва. Такі дослідження для смаженого м'ясного продукту проведено вперше.

**Ключові слова:** *м'ясо, гриль, Pediococcus acidilactici, псування, органолептика.*

### Підвищення харчової цінності зерна шляхом біологічного активування

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**Вступ.** Зернові продукти є джерелом вуглеводів, білків, макро- і мікроелементів, вітамінів, ферментів, харчових волокон, фосфоліпідів. Пророщування зерна – один із методів біологічного активування.

**Матеріали і методи.** Досліджено зерно пшениці, тритикале та голозерного вівса. Білок визначали методом Бредфорда, вміст крохмалю – поляриметричним методом. Жир визначали методом вичерпного екстрагування хімічно чистим гексаном. Вітаміни $B_1$, $B_2$, $B_3$, $B_6$ визначали флуорометрично. Вітаміни РР і Е визначати колориметрично. Визначення вітаміну С проводили титрометричним методом.

**Результати і обговорення.** Важливим завданням підготовки сировини для виробництва оздоровчих продуктів є підвищення її харчової та біологічної цінності.

Нами запропоновано режим гідротермічного оброблення зерна за температури 12 – 16 ºС. За цих умов відбувається активізація ферментного комплексу, зниження густини зерна та підвищення його питомого об'єму; синтез вітамінів і вітаміноподібних речовин.

У процесі біологічного активування зерна підвищується біодоступність білкових речовин, вуглеводів, жиру, що зумовлено їх частковим гідролізом.

Досліджено, що вміст клітковини, природного харчового сорбенту в біологічно активованому зерні пшениці, голозерному вівсі й тритикале складає, відповідно, 2,68, 2,34, 2,62%.

Під час запропонованого оброблення зерна пшениці, тритикале та голозерного вівса кількість вітаміну С збільшується більш як у два рази. Вміст токоферолів зростає у десять разів, рутину – у 2,5–3 рази.

Загальна кількість колонієутворювальних одиниць мезофільних аеробних і факультативно-анаеробних мікроорганізмів у нативних і висушених зразках зерна після гідротермічного оброблення знаходиться у межах норм, встановлених стандартами.

**Висновки.** Отримані результати мають практичне значення, оскільки дозволяють рекомендувати використання біологічно активованого зерна пшениці, тритикале, голозерного вівса для виробництва продуктів оздоровчого, функціонального та лікувально-профілактичного призначення.

**Ключові слова:** *зерно, активування, пшениця, тритикале, овес.*

## Малинові і ожинові вичавки як потенційне джерело біологічно активних речовин

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**Вступ.** Причиною досліджень підвищення цінності побічних продуктів виробництва став інтерес до отримання продуктів із корисними для здоров'я споживача властивостями. У цьому значенні побічні продукти, які отримують у процесі обробки фруктових культур, є цінним джерелом біологічно активних речовин.

**Матеріали і методи**. Вичавки з малини й ожини отримували після відділення соку. В отриманих побічних продуктах (вичавках) були визначені загальний вміст фенольних сполук (TPC) методом Folin-Ciocalteu і загальний мономерний вміст антоціанів (TAC) за pH диференціальним методом. Антиоксидантну активність вичавок оцінювали за взаємодією із стабільними радикалами 1,1-дифеніл-2-пікрилгідразилу (ДФПГ)

**Результати і обговорення.** Малинові вичавки характеризуються значно вищим загальним вмістом фенольних сполук (10,1 мг/г) та антоціанів (6 мг/г) порівняно з ожиновими (8,2 та 3,6 мг/г, відповідно). Активність ДФПГ радикалів була однаковою, з дещо вищими значеннями для малинових вичавок (11,7 μmolTrolox/г), ніж для ожинових (10,9 μmolTrolox/г). Загальна сума розчинних сухих речовин становила 9,3 oBx у зразку з малинових вичавок, що значно нижче, ніж для ожинових (14.5 oBx).

Дані дослідження свідчать про те, що як малинові, так і ожинові вичавки, які є відходами виробництва соків, можна було б використовувати як дешеве джерело біологічно активних сполук із сильною антиоксидантною активністю. Таким чином, вичавки доцільно розглядати як сировину для виробництва цінних біологічно активних добавок і натуральних барвників під час розроблення нових харчових продуктів із підвищеною цінністю. Доступність і низька вартість побічних продуктів переробки фруктів робить їх привабливими для використання у харчових продуктах з метою підвищення біологічних властивостей. Фруктові вичавки можна застосовувати

для збагачення різних продуктів, таких як хлібобулочні вироби, бісквіти, печиво, макаронні вироби, морозиво, фруктові йогурти тощо. Окрім того, застосування етанолу, використаного у даному дослідженні для екстракції біологічно активних сполук, необхідно здійснювати з урахуванням відсоткового вмісту обраного розчинника, співвідношення рідина/тверда речовина, температури і часу екстракції.

**Висновок.** Малинові й ожинові вичавки, отримані в процесі виробництва соків, є цінним джерелам таких біологічно активних сполук, як фенольні антиоксиданти.

**Ключові слова**: *малина, ожина, вичавки, БАР, антиоксидант.*

## Хімічний склад ефірної олії з дамаської троянди (*Rosa Damascena* Mill.), вирощеної в нових регіонах Болгарії

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**Вступ**. Метою дослідження є визначення хімічного складу ефірної олії з дамаської троянди (*Rosa Damascena* Mill.), вирощеної в нових регіонах Болгарії

**Матеріали і методи**. Пелюстки троянди були зібрані в 2016 р. поблизу м. Відин (північно-західна Болгарія) на стадії цвітіння в два періоди – 10 травня (зразок 1) і 26 травня (зразок 2). Хімічний склад олії визначено методом хроматографії.

**Результати і обговорення.** Вологість рослин становила 82,70% (для зразка 1) і 79,04% (для зразка 2). Вихід ефірної олії становив, відповідно, 0,08% і 0,03%. В ефірній олії із зразків 1 і 2 визначено 39 компонентів. Дві групи з'єднань було визначено в гідродистильованій трояндовій ефірній олії, що характеризуються як носії та як фіксатори запаху. Терпенові спирти є основними компонентами, які відповідають за характерний запах трояндової олії і складають близько 56% від загальної кількості зазначених речовин. Високий вміст гераніолу з комбінацією цитронелолу, фамесолу і неролу забезпечує міцний, солодкий, квітковий свіжий розоцвітий характер виробленої олії.

Хроматографічний профіль трояндової олії виявив значну кількість аліфатичних вуглеводнів (31%), які є основними компонентами, що відповідають за стійкість запаху.

Основні з'єднання ефірних олій такі: β-цитронелол (30.24-31.15%); транс-гераніол (20.62-21.24%), n-хенеікосан (8.79-9.05%), n-нонадекан (8.51-8.77%), нонадекан (4.42-4.55%) і фенілетиловий спирт (4.04-4.16%).

**Висновки**. Ефірна олія з дамаської троянди, вперше отримана в новому регіоні Болгарії, має високі показники якості і рекомендується до масового виробництва.

**Ключові слова**: *троянда, ефір, олія, хімія, Болгарія.*

**Молочний жир у формуванні структури молочних продуктів**

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**Вступ.** Стаття присвячена вивченню впливу молочного жиру на формування структури молочних продуктів на основі опублікованої інформації за останні 15 років. Консистенція продукту є однією з важливих характеристик, які визначають сприйняття молочного продукту споживачами. При цьому консистенція є органолептичним відображенням структури продуктів, тому встановлення і підтримання необхідної структури має вирішальне значення для забезпечення високої якості продукту.

**Матеріали і методи.** Досліджено структуру масла, сиру, морозива, збитих вершків. Проведено аналіз наукових статей, дисертації й монографії вчених даної галузі науки. Методологія дослідження ґрунтується на використанні методів аналізу, порівняння й узагальнення.

**Результати й обговорення.** У молоці та вершках молочний жир формує емульсію «жир-вода», існуючи у вигляді жирових глобул, покритих захисними оболонками. З практичної точки зору молочний жир є важливим складовим компонентом, оскільки забезпечує характерні фізико-хімічні, структурні, органолептичні властивості молочних продуктів, таких як вершки, масло, морозиво, збиті вершки, сир.

Жировмісні продукти, наприклад, масло, являють собою емульсію типу «вода-жир», яка складається з кристалізованого молочного жиру, що виконує роль дисперсійного середовища, в якому дисперговані краплини вологи, незруйновані та частково дестабілізовані жирові глобули. Переважання у структурі масла значної кількості дрібних кристалів молочного жиру сприяє підвищенню його твердості до 20% порівняно із продуктом з незначною кількістю кристалізованого жиру. Проте наявність великих кристалів обумовлює зміну консистенції, крихкості та еластичності продукту.

Для формування стабільної структури збитих вершкових десертів і морозива наявність молочного жиру також є необхідною. При цьому формування структури залежить від взаємодії між жировими глобулами, повітряними бульбашками та компонентами плазми (особливо білками). У процесі збивання й охолодження жирові кульки частково об'єднуються та адсорбуються на повітряних бульбашках, покриваючи їхні поверхні. Таким чином відбувається стабілізація повітряної фази. Окрім того, жир відіграє важливу роль у забезпечені твердості структури морозива під час фризерування, а отже, й консистенції, зовнішнього вигляду і опору до танення.

Наявність молочного жиру у сирах обумовлена необхідністю формування характерних смакових особливостей цього продукту. Окрім того, глобули молочного жиру впливають на консистенцію сиру, пом'якшуючи її частковим руйнування казеїнової матриці продукту.

**Висновок.** Молочний жир є цінним компонентом завдяки приємним смаковим характеристикам, тоді як інші його властивості часто потребують змін для належного застосування у виробництві харчових продуктів, що, у свою чергу, помітно впливає на формування структури молочних продуктів, зокрема масла, збитих вершків, морозива і сиру.

**Ключові слова:** *молочний жир, структура, масло, морозиво, сир.*

## Використання біоінформаційного аналізу і комп'ютерної морфометрії для вивчення *Fusarium* spp, які викликають суху картопляну гниль

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**Вступ.** Завдання дослідження – оцінити можливість застосування методів комп'ютерної морфометрії і біоінформаційного аналізу для вивчення реактивних змін клітин грибів *Fusarium spp.,* які викликають суху картопляну гниль.

**Матеріали і методи.** Для проведення досліджень були відібрані бульби картоплі сорту «Невський» із зовнішніми ознаками фузаріозної сухої гнилі. Вивчено три зони рослинної тканини картопляних бульб: епіцентр контамінації пліснявих грибів роду Fusarium; тканини, прилеглі до вогнища ураження; тканини поза зоною осередку ураження. Препарати *Fusarium spp.* отримували методом «відбитка» з рослинної тканини, фіксовані мікроорганізми фарбували 1% розчином метиленового синього. Морфологічне дослідження і комп'ютерну морфометрію мікроорганізмів проводили при загальному збільшенні x400 і x1000 мікроскопа "Micros" (Austria), модель MS 100 (XP).

**Результати і обговорення.** Морфометричні характеристики макроконідій Fusarium spp. (площа, периметр, довжина, кількість) отримані із застосуванням комп'ютерної програми аналізатора зображень "Micros".

На підставі даних морфометричного аналізу клітин грибів розроблені алгоритм і комп'ютерна програма для вивчення біоінформаційних характеристик макроконідій *Fusarium spp.*

Для встановлення термодинамічної рівноваги клітинної системи обчислювалися такі інформаційні характеристики: H – інформаційна ентропія, що характеризує ступінь упорядкованості біосистеми; $H_{max}$ – інформаційна ємність, що характеризує максимальну структурну різноманітність біосистеми з такими, що втратили функціональний взаємозв'язок елементами; R – коефіцієнт надмірності (відносної організації біосистеми).

Для досліджених грибів роду *Fusarium* виявлені низькі значення інформаційної ентропії H (0002) щодо значень інформаційної ємності $H_{max}$ (0,5) на тлі підвищених значень коефіцієнта надмірності R (99,40% – 99,69%), що характеризують упорядкованість і високий рівень відносної організації в мікробних клітинах, що вражають бульби картоплі. Ця інформація може вказувати на максимальний ступінь адаптації клітин паразитуючих грибів роду *Fusarium* до умов їх біоценозу з рослинними клітинами в процесі мікробної контамінації бульб картоплі. Слід очікувати, що в разі реакції грибів роду *Fusarium* на вплив хімічних або біопрепаратів значення інформаційної ентропії H повинні зростати до значень інформаційної ємності $H_{max}$, а значення коефіцієнта надмірності R – зменшуватися. Зміни показників можна буде трактувати як результат дезінтеграції структурних і функціональних зв'язків в клітинній системі грибів роду Fusarium.

**Висновки.** Метод біоінформаційного аналізу при морфологічному дослідженні клітин грибів роду *Fusarium*, що викликають суху картопляну гниль, може бути рекомендований як тест-системи під час розробки нових видів препаратів для захисту рослин.

**Ключові слова:** *Fusarium spp., картопля, суха гниль, морфометричний аналіз, біоінформатика, тест-система.*

### Виробництво етанолу з продуктів переробки кавових плодів

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**Вступ.** Проведено дослідження з метою оцінки виробництва етанолу з продуктів переробки кавовмісної сировини. Як основні компоненти використано зовнішню оболонку та м'якоть плодів кави.

**Матеріали і методи.** В процесі дослідження сусло екстрагувалося з кавової оболонки і м'якоті та зброджувалося. Використання дріжджів було оптимізоване з метою збільшення концентрації редукувальних цукрів. У свою чергу, кислотний гідроліз призвів до перетворення складних полісахаридів у прості мономери. Невелика кількість меляси та залишків були використані для підсилення бродіння. Сухі дріжджі *Saccharomyces cerevisiae* були регідровані та доповнені поживними речовинами, які також були збагачені азотом, магнієм і вуглецем.

**Результати і обговорення.** Сировина після переробки бродінням була перегнана. Зміна редукувальних цукрів в субстанції показує хід процесу ферментації. Процес бродіння закінчується, коли густина зменшується нижче 6%. Виявлено, що під час процесу бродіння вміст цукру зменшується, що безпосередньо пов'язано з ефективністю бродіння. З 6,5 л сусла було виготовлено 2,3 л етанолу з абсолютним обсягом 45 аб.% що, у свою чергу, складає 35,5 % від загального обсягу сусла. Оскільки pH сусла для бродіння було оптимізовано до 4–5, результати показують, що виробництво етанолу може здійснюватися з використанням м'якоті плодів та оболонки кави як додаткових продуктів, що призведе до зменшення забруднення ними навколишнього середовища. Під час дослідження було оптимізовано pH і температурні режими на належному рівні для всіх зразків.

**Висновки.** Переробка побічних продуктів (зовнішньої оболонки та м'якоті плодів кави) в етанол може збільшити потенціал сировинних ресурсів підприємств шляхом виробництва етанолу.

**Ключові слова:** *кава, плід, м'якоть, спирт, бродіння, Saccharomyces cerevisiae.*

### Особливості технології застосування камедей для створення гелевих основ

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**Вступ.** Метою дослідження є вивчення технологічних, фізико-хімічних і реологічних властивостей гелевих основ на основі гідроколоїдів для подальшої розробки харчових продуктів гелевої форми.

**Матеріали і методи.** Об'єктом дослідження обрано камідь ксантану (ККС), камідь гуару (КГ) та камідь рожкового дерева (КРД), а також гелеві основи з різною концентрацією даних галактомананів. З метою вивчення технологічних, фізико-хімічних і реологічних характеристик гелевих основ було приготовлено зразки гелів за трьома способами. Вивчення структурно-механічних властивостей проводилось за допомогою ротаційного віскозиметра.

**Результати і обговорення.** Досліджено вплив концентрації гелеутворювача, температури води й тривалості набухання на в'язкість колоїдних розчинів і на граничне напруження зсуву. Гелеві основи, приготовлені за трьома способами, з обраними гідроколоїдами відносяться до структурованих систем.

Найбільше значення величини максимальної в'язкості, що відповідає практично незруйнованій системі, для зразків гелевих основ із каміддю ксантану, приготовлених за способом 3, складає 106,3 Па·с, із камеддю рожкового дерева - 1,97 Па·с, із камеддю гуару - 17,7 Па·с за концентрації 1%.

Розраховано структурно-механічні характеристики. Для камеді ксантану та гуару величина напруження зсуву є більшою за нуль, що свідчить про те, що досліджувані зразки є структурованими твердоподібними тілами. Для зразків з камеді рожкового дерева величина напруження зсуву дорівнює нулю, що вказує на те, що їх можна віднести до псевдопластичної рідини. Найбільша динамічна межа здатності до течії спостерігалась у зразків із каміддю ксантану, приготовлених за способами 2 та 3, із камеддю рожкового дерева та гуару - за 1-м та 3-м способами з концентрацією 1%.

Порівняння розчинів гідроколоїдів, одержаних різними способами приготування, показало, що найбільш доцільним є приготування гелів за 3-м способом, оскільки за цих умов в'язкість розчинів (за однакової концентрації гелеутворювача) була вищою, а структура гелю була більш однорідною.

**Висновки.** Для всіх експериментальних зразків гелів характерний неньютонівський псевдопластичний тип течії. Найбільш доцільним є приготування гелів за третім способом, який полягав у набуханні гідроколоїду протягом 40 хв у воді кімнатної температури, підігріві до 60 °С та охолодженні. Оптимальна концентрація гелеутворювачів – 0,5%.

**Ключові слова:** *реологія, гелеутворювач, камедь, ксантан, гуар, рожкове дерево.*

### Механізм антимікробної дії рослинних антибактеріальних препаратів

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**Вступ.** Метою цього дослідження є здійснення огляду інформації про механізм дії рослинних антибактеріальних препаратів, які можна використовувати у харчових продуктах.

**Матеріали і методи**. Для дослідження використано літературні джерела із таких бібліографічних баз даних: AGRICOLA, CAB Abstracts, EBSCO, FSTA, Global Health, Google Scholar, Index Copernicus, PubMed, Scopus, TUBITAK ULAKBIM Life Sciences Database, і Web of Science.

**Результати і обговорення.** Деякі фруктові і овочеві соки, трави та спеції були використані як регулятори кислотності й ароматизатори для традиційних страв,

салатів і закусок. Усі ці продукти, а також їх екстракти широко використовують в харчовій промисловості як природні антибактеріальні препарати. Антибактеріальна дія таких рослинні добавок в основному обумовлена наявністю у них органічних кислот або/та фенольних речовин. Органічні кислоти і фенольні речовини, які є основними сполуками у рослинах, спричинюють їхню антимікробну активність. Проте вплив антимікробного компонента змінюється з урахуванням генотипу, структури клітинної оболонки і вихідної популяції мікроорганізмів, а механізм дії – від способу від'єднання та приєднання у клітині. Інгібуюча дія рослинних антибактеріальних препаратів на різні мікроорганізми досліджувалася неодноразово. Але й дотепер немає чіткого розуміння механізму їх дії, що викликає фунгіцидний статичний вплив. Важливим є визначення особливостей дії цього механізму, що дасть змогу зробити його доступним і прийнятним для промислового застосування.

**Висновок**. Визначення особливостей дії механізму антибактеріальної активності є необхідним для того, щоб зробити антимікробні препарати доступними для застосування у харчовій промисловості.

**Ключові слова:** *антимікробний, рослина, кислота, фенол.*

## Процеси і обладнання харчових виробництв

### Тривимірна модель течії неньютонівскої рідини в прямокутному каналі

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**Вступ.** Запропоновано тривимірну математичну модель течії неньютонівської рідини з в'язкістю, що залежить від швидкості зрушення в прямокутних каналах технологічного обладнання харчової промисловості.

**Матеріали і методи**. Застосовано метод суперпозицій, що дозволяє побудувати поле поздовжньої течії неньютонівскої рідини в прямокутному каналі технологічного обладнання зі стінками, що рухаються, і в такий спосіб визначити величини швидкості й тиску в будь-якій точці усередині каналу.

**Результати і обговорення.** Отримано модель поздовжньої течії неньютонівської рідини в прямокутному каналі, на стінках якого задані різні поздовжні швидкості. В основу побудови покладено рішення одномірного завдання про куеттівську течію у каналі. Композиція течій у щілинних каналах із взаємно перпендикулярними парами стінок дозволяє отримати формулу витрати течії, яка задовольняє принцип граничної відповідності між течіями в прямокутному та щілинному каналах. Пропонується спосіб побудови поля швидкості, що являє собою розбивку кінцевого перетину каналу на ділянки з різною залежністю від координат так, що на одних ділянках швидкість залежить тільки від однієї координати, а на інших – тільки від іншої координати. Отримано рівняння ліній, що розмежовують ці ділянки та способи визначення форми розмежувальних ліній.

**Висновки.** Отримані аналітичні формули дозволяють визначити макрокінетичні характеристики в кожній точці каналу з довільним розподілом граничних

швидкостей течії неньютонівских рідин, в'язкість яких залежить від швидкості зрушення.

**Ключові слова:** *неньютонівска рідина, Куетт, течія, реологія, модель, канал.*

## Моделювання роботи топки з рециркуляцією гріючих газів для тунельної хлібопекарської печі

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**Вступ.** З метою підвищення ефективності роботи джерел теплоти в топках тунельних хлібопекарських печей досліджений процес рециркуляції в них гріючих газів.

**Матеріали і методи**. Застосована CAE-програма моделювання руху рідин FlowVision, принцип дії якої ґрунтується на методі кінцевих елементів У розрахунку були використані реологічні та кінематичні параметри гарячих газів, отримані при проведенні реальних модельних експериментів.

**Результати і обговорення**. Встановлено, що розподіл швидкостей по довжині топки поділяється на дві стійкі області: по осі топки та біля зовнішніх стінок топки. Змішування газів починається в розподільній коробці.

Температура газів по центру потоку знижується від 1900$^{o}$C до 600$^{o}$C практично по лінійній залежності.

Температура газів біля стінок топки змінюється по всій довжині топки в діапазоні від 260$^{o}$C до 360$^{o}$C, що пояснюється слабкою турбулізацією потоку.

На ізолініях областей максимальної дисипації кінетичної енергії відзначені завихрення в двох локальних областях. Перша область – кільце навколо виходу продуктів згоряння з камери згоряння. Друга область – у звуженні топки. Вона знаходиться при виході газів у розподільний короб.

Вперше отримана візуальна та чисельна інформація, яка відображає, як комбінація місцевих опорів різного типу в газовому тракті впливає на значення критерія Рейнольдса та на характер руху гріючого агента.

Запропоновано обладнати топки подібного типу додатковими деталями – кільцевими шайбами. Доведено, що вони змінюють напрям потоків газів рециркуляції так, що відбувається активне їх перемішування з продуктами згоряння по всій довжині топки.

**Висновки**. Проведене комп'ютерне моделювання роботи топки дозволило виявити та локалізувати недоліки існуючої базової конструкції, а також запропонувати способи модернізації пристрою.

**Ключові слова**: *піч, тунель, топка, газ, нагрівання, рециркуляція, змішування.*

# Економіка і управління

## Суспільне сприйняття рівня якості і безпечності комерційних брендів локшини

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**Вступ**. Споживачі стають більш свідомими щодо безпеки і якості харчових продуктів. В аспекті якості і безпеки харчових продуктів було проведене дослідження оцінки якості деяких комерційних брендів бангладеської локшини.

**Матеріали і методи.** Дослідження проводилось у два етапи. На першому етапі здійснено загальне опитування для визначення оцінки відношення споживача до локшини, яке охоплювало населення різних областей Бангладешу. Споживачі стверджують, що всі компанії з виробництва локшини фальсифікують продукцію шляхом використання борошна низького сорту і шкідливих харчових барвників, які є небезпечними для здоров'я. На другому етапі на місцевих ринках вибрано чотири зразки фірмових брендів локшини для оцінки хімічного складу, кулінарних особливостей, мікробного профілю і сенсорної оцінки.

**Результати і обговорення.** Зразки локшини відібрані в діапазоні: вологість – 7,07-10,70%, зола – 1,01-2,70%, жир – 0,40-0,73%, сирий протеїн – 11,00-11,17%, сира клітковина – 0,05-0,30%, загальний вміст вуглеводів – 74,19-80,34%, вміст крохмалю – 61,09 -66,35%, pH – 8,15-8,88. Ступінь клейстеризації, кислотність і енергетичну цінність відібрано в діапазоні 96,55-99,85%, 0,79-0,85 мг/г і 344,52-370,53 ккал/100 г відповідно. Мікробний профіль показує, що на підприємствах галузі підтримуються належні гігієнічні умови під час виробництва локшини відповідно до GMP, також не виявлено жодного фекального забруднення та будь-якої мікробіологічної небезпеки. Кулінарні характеристики всіх зразків локшини різноманітні, однак усі мають прийнятну якість. Виявлено істотну відмінність ($p < 0{,}05$) в шорсткості, жовтизні, білизні і блиску. Хоча описові оцінки різноманітні, але отримані результати задовільні. Виявлено істотну відмінність ($p < 0{,}05$) у всіх сенсорних атрибутах (колір, запах, смак, текстура і загальна прийнятність) серед локшини, проте для всіх зразків локшини досягнуті задовільні оцінки.

**Висновки.** Зразки торговельних марок локшини відповідають чинним вимогам. Отже, припущення споживачів щодо комерційних брендів локшини є помилковими.

**Ключові слова:** *споживач, поведінка, локшина, хімікат, мікроорганізм.*

## Система якості маркувань харчових продуктів у Європейському Союзі

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**Вступ.** У статті розглядаються моделі Європейського Союзу щодо захисту позначення походження, географічної приналежності й гарантування традиційних

особливостей маркувань, які використовуються в сільському господарстві та харчовій продукції.

**Матеріали і методи**. Використано вторинні дані з бази даних про походження та реєстрацію. Зразок складався з 1356 маркувань (етикеток), зареєстрованих у цій базі до 18 липня 2016 року. Частота використання маркувань аналізувалася з урахуванням країни, типу маркування і класів продуктів.

**Результати і обговорення**. Завданням дослідження є аналіз використання вказаних маркувань на ринку Європейського Союзу відповідно до обраних критеріїв, які включають тип маркування, зареєстрованого в кожній країні, кількість сільськогосподарської продукції та продовольчих товарів, зареєстрованих як якісні етикетки в цілому. Як показують результати, найбільшу кількість назв продуктів зареєстровано з маркуванням PDO, з невеликою різницею – з маркуванням PGI. Лише мінімум продуктів зареєстровані як TSG. Домінуючою країною є Італія, далі йдуть інші середземноморські країни – Франція, Іспанія, Португалія та Греція. Що стосується класу продукту, то найбільш поширеними класами є фрукти, овочі і зернові (для PGI, PDO), сири (PDO) і м’ясні продукти (TSG, PGI), що підтверджує аналіз квадрата критеріїв незалежності Пірсона для визначення суттєвих відмінностей між частотою використання маркувань і згаданих критеріїв. Підтверджено слабку залежність між кількістю назв продуктів, зареєстрованих як PDO, PGI і TSG, та країною походження, середню залежність між типом маркувань і класом продукції, де використовується маркування, й значну залежність між країною походження і класом продукту, який реєструється найчастіше.

**Висновок**. PDO, PGI і TSG схеми корисні як для споживачів, так і для виробників. Споживачі купують продукт з певними якостями доданої вартості.

**Ключові слова:** *маркування, якість, база даних, DOOR, ЄС, їжа.*

# Аннотации

## Пищевые технологии

### Спектроскопический анализ меда

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**Введение**. Основной целью данной работы является исследование связи химического состава, физических свойств, ботанического и географического происхождения, а также возраста меда с его спектральными параметрами, которые могут быть использованы в качестве таксономических критериев и индикаторов состояния, качества и возможной фальсификации .

**Материалы и методы**. В данных исследованиях были использованы два метода неразрушающего контроля меда – спектроскопия в ближней инфракрасной области спектра и флуоресцентная спектроскопия.

**Результаты и обсуждение**. Химический состав, физические свойства, ботаническое и географическое происхождение, а также возраст меда тесно связаны с его спектральными параметрами, которые могут быть использованы в качестве таксономических критериев или индикаторов состояния, качества меда и его возможной фальсификации.

Интенсивность спектров поглощения использовалась как критерий географического происхождения и возраста меда. Интенсивность спектров излучения флуоресценции зависит от географического происхождения, возраста и типа меда.

Исследование влияния температуры на интенсивность флуоресценции меда показало, что увеличение температуры вызывает уменьшение интенсивности флуоресценции. Была установлена корреляция флуоресцентных параметров меда с содержанием в нем воды.

Спектры отражения меда в ближней инфракрасной области спектра характеризуются наличием полос отражения при 1779 нм, 1933 нм и 2290 нм; относительная интенсивность полос зависит от типа меда и возраста образцов. Весьма информативными являются спектральные параметры меда в ближней инфракрасной области спектра при неразрушающем выявлении фальсификации меда.

**Заключение.** Методы инфракрасной и флуоресцентной спектроскопии могут быть использованы в технологии производства меда как неразрушающие, быстродействующие и точные методы диагностики меда.

**Ключевые слова**: *мед, география, происхождение, качество, фальсификация, спектроскопия.*

### Состав и свойства частично гидролизированных подсолнечных белковых изолятов

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**Введение.** Частичный ферментативный гидролиз белков является общепринятым методом модификации их функциональных свойств. В данной работе представлена характеристика свойств частично гидролизированных белков, полученных из подсолнечного шрота.

**Материалы и методы.** Белки подсолнечного шрота экстрагировали в течении 40 мин в присутствии двух протеаз, а именно: нейтральной протеазы и Алкалазы. Образцы частично гидролизированных белков были получены путем изоэлектрического осаждения и последующего высушивания. Полипептидный состав белковых изолятов изучали с помощью электрофореза в полиакриламидном геле, определен также их степень гидролиза, аминокислотный состав, поверхностная активность и функциональные свойства.

**Результаты и обсуждение.** В полипептидном профиле белков, частично гидролизованных с помощью обеих протеаз, отсутвовали высокомолекулярные полипептиды с молекулярными массами 45–54 і 32–35 кДа. В то же время они были обогащены полипептидами с молекулярными массами 14–16 кДа и ниже.

Образцы частично гидролизированных белков имели более высокое содержание белка, низшее содержание золы и углеводов в сравнении с контрольным образцом. Биологическая ценность подсолнечных белковых изолятов была ограничена тремя аминокислотами − серосодержащими метионином и цистином, а также лизином. Содержание метионина, цистина и лизина увеличивалось в образцах, полученных с нейтральной протеазой, относительно контрольного образца.

Дифференциально сканирующий калометрический анализ белковых образцов показал, что частично гидролизированные образцы содержали неденатурированные белки, но степень денатурации бул выше относительно контрольного образца Частичный гидролиз белков семян подсолнечника увеличивал их растворимость в диапазоне pH от 2 до 8, влаго- и жироудерживающую способность, пенообразовательную, эмульгирующую способность и поверхностную активность.

**Выводы.** Образцы частично гидролизированных белков имели висшее содержание белка, более светлый цвет, низшую степень денатурации и улучшенные функциональные свойства в сравнении с традиционными белковыми изолятами.

**Ключевые слова:** *подсолнечник, белок, изолят, гидролиз, протеаза.*

## Исследование органолептических свойств жареного мяса, обсемененного закваской культуры *Pediococcus acidilactici* FLE07, и летучих соединений, влияющих на его качество

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**Введение.** Использование таких биологических препаратов, как молочнокислые бактерии, может улучшить органолептические свойства жареных мясных продуктов.

**Материалы и методы.** Жареный мясной продукт (Tsire) был обсеменен 6 млн КОЕ/г закваской культуры Pediococcus acidilactici FLE07 (ОО) с целью определения количества летучих соединений, которые вызывают порчу в течении четырех дней хранения при температуре 30 °С. В качестве контрольных были избраны необсемененные образцы (НО) жареного мясного продукта (Tsire). Твердая фаза

массовой экстракции методом газовой хроматографии-масс-спектрометрии (ТФМЭ-ГХМС) и дегустационное испытания по гедонической шкале были использованы для оценки изменений и органолептических качеств при хранении.

**Результаты и обсуждение**. В предыдущих экспериментах для производства органических кислот были оценены десять штаммов Pediococcus. Наибольшее количество молочной кислоты производят 34 gacid/107КУО и *P. acidilactici* FLE07, которые и были выбраны в качестве закваски для обсеменного жареного мясного продукта (Tsire). Было определено, что штамм производит уксусную кислоту в концентрации менее 12 gacid/107 КОЕ. ТФМЭ-ГХМС исследования жареного мясного продукта подтвердили присутствие 48 летучих компонентов, а именно: кетонов (35,42%), кислот (8,33%), спиртов (25%), арома/циклических (14,58%), азотных соединенией (16.67%), которые были определены при хранении. Среди кетоновых соединений, которые наблюдались в образцах мясных продуктов (Tsire), летучие компоненты включали ацетон, 2-бутанон, 2,3-бутандион, 3-гидрокси-2-бутанон, 2-гексанон, 2-гептанон и 1-гидрокси-2-пропанон. Существует значительная разница ($p<0,05$) между обсемененными и контрольными образцами мясного продукта (ОС и НЗ). Было выявлено снижение летучих соединений, связанных с порчей. Для гептанала, 1-октен-3-ола, 3-метил-бутановой кислоты концентрации (мг/г) составили 0,57, 1,98, 0,93 и 1,39 соответственно в обсемененных образцах (ОО) по сравнению с 2,43, 3,21, 2,94 и 2,94, полученных для необсемененных образцов (НО) на третий день. Органолептические исследования подтверждают более высокую оценку ($p<0,05$) аромата, внешнего вида, консистенции, вкуса и общих качеств ОО сравнительно с НО.

**Выводы**. Культуры *Pediococcus acidilactici* FLE07 и другие закваски могут быть использованы для улучшения органолептических параметров и доступности продукта независимо от дня производства. Такие исследования для жареного мясного продукта проведены впервые.

**Ключевые слова:** *мясо, гриль, Pediococcus acidilactici, порчи, органолептика.*

## Повышение пищевой ценности зерна путем биологического активирования

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**Введение.** Зерновые продукты – источник углеводов, белков, макро- и микроэлементов, витаминов, ферментов, пищевых волокон, фосфолипидов. Проращивания зерна является одним из методов биологического активации.

**Материалы и методы.** Исследовано зерно пшеницы, тритикале и голозерного овса. Белок определяли методом Бредфорда, содержание крахмала – поляриметрическим методом. Жир определяли методом исчерпывающего экстрагирования химически чистым гексаном. Витамины $B_1$, $B_2$, $B_3$, $B_6$ определяли флуорометрично. Витамины РР и Е определяли колориметрически. Определение витамина С проводили титрометрическим методом.

**Результаты и обсуждение.** Важной задачей при подготовке сырья для производства оздоровительных продуктов является повышение его пищевой и биологической ценности.

Нами предложен режим гидротермической обработки зерна при температуре 12 – 16 ° С. В этих условиях происходит активизация ферментного комплекса, снижение

плотности зерна и повышение его удельного объема; синтез витаминов и витаминоподобных веществ.

В процессе биологического активирования зерна повышается биодоступность белковых веществ, углеводов, жира, что обусловлено их частичным гидролизом. Доказано, что содержание клетчатки, природного пищевого сорбента в биологически активированном зерне пшеницы, голозерного овса и тритикале составляет, соответственно, 2,68, 2,34, 2,62%.

В процессе предложенной обработки зерна пшеницы, тритикале и голозерного овса количество витамина С увеличивается более чем в два раза. Содержание токоферолов возрастает в десять раз, рутина – в 2,5–3 раза.

Общее количество колониеобразующих единиц мезофильных аэробных и факультативно-анаэробных микроорганизмов в нативных и высушенных образцах зерна после гидротермической обработки находится в пределах норм, установленных стандартами.

**Выводы.** Полученные результаты имеют практическое значение, поскольку позволяют рекомендовать использование биологически активированного зерна пшеницы, тритикале, голозерного овса для производства продуктов оздоровительного, функционального и лечебно-профилактического назначения.

**Ключевые слова:** *зерно, активирование, пшеница, тритикале, овес.*

## Малиновые и ежевичные выжимки как потенциальный источник биологически активных веществ

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**Введение.** Интерес к получению продуктов с полезными для здоровья потребителя свойствами был причиной исследования повышения ценности побочных продуктов производства. В этом значении, побочные продукты, которые получают в процессе обработки фруктовых культур, являются ценным источником биологически активных веществ.

**Материалы и методы**. Выжимки из малины и ежевики получали после отделения сока. В полученных побочных продуктах (выжимки) были охарактеризованы общее содержание фенольных соединений (TPC) методом Folin-Ciocalteu и общее мономерное содержание антоцианов (TAC) по pH дифференциальному методу. Антиоксидантную активность выжимок оценивали по взаимодействию со стабильными радикалами 1,1-дифенил-2-пикрилгидразила (ДФПГ)

**Результаты и обсуждение.** Малиновые выжимки характеризуются значительно высшим общим содержанием фенольных соединений (10,1 мг/г) и антоцианов (6 мг/г) сравнительно с ежевичными (8,2 и 3,6 мг/г соответственно). Активность ДФПГ радикалов была одинаковой, с несколько высшими значениями для малиновых выжимок (11,7 ммolTrolox/г), чем для ежевичных (10,9 ммolTrolox/г). Общая сумма растворимых сухих веществ составляла 9,3 oBx в образце из малиновых выжимок, что значительно ниже, чем для ежевичных (14,5 oBx).

Данные исследования свидетельствуют о том, что как малиновые, так и ежевичные выжимки, которые являются отходами производства соков, можно использовать в качестве дешевого источника биологически активных соединений с сильной антиоксидантной активностью. Таким образом, выжимки целесообразно рассматривать как сырье для производства ценных биологически активных добавок и натуральных красителей при разработке новых пищевых продуктов с повышенной ценностью. Доступность и низкая стоимость побочных продуктов переработки фруктов делает их привлекательными для использования в пищевых продуктах с целью повышения биологических свойств. Фруктовые выжимки можно применять для обогащения разных продуктов, таких как хлебобулочные изделия, бисквиты, печенье, макаронные изделия, мороженое, фруктовые йогурты и так далее. Кроме того, применение этанола, использованного в данном исследовании для экстракции биологически активных соединений, необходимо осуществлять с учетом процентного содержания избранного растворителя, соотношения жидкость/твёрдое вещество, температуры и времени экстракции.

**Вывод.** Малиновые и ежевичные выжимки, полученные в процессе производства соков, являются ценными источниками таких биологически активных соединений, как фенольные антиоксиданты.

**Ключевые слова**: *малина, ежевика, выжимка, БАВ, антиоксидант.*

## Химический состав эфирного масла из дамасской розы (*Rosa Damascena* Mill.), выращенной в новых регионах Болгарии

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**Введение**. Целью исследования является определение химического состава эфирного масла из дамасской розы (*Rosa Damascena* Mill.), выращенной в новых регионах Болгарии

**Материалы и методы**. Лепестки рози были собраны в 2016 г. вблизи г. Видин (северо-западная Болгария) на стадии цветения в два периода – 10 мая (образец 1) и 26 мая (образец 2). Химический состав масла определен методом хроматографии.

**Результаты и обсуждение.** Влажность растений составляла 82,70% (для образца 1) и 79,04% (для образца 2). Выход эфирного масла составлял, соответственно, 0,08% и 0,03%. В эфирном масле из образцов 1 и 2 определено 39 компонентов. Две группы соединений были определены в гидродистилированном розовом эфирном масле, что характеризуются как носители и как фиксаторы запаха. Терпеновые спирты являются основными компонентами, ответственными за характерный запах розового масла, и составляют около 56% от общего количества отмеченных веществ. Высокое содержание гераниола с комбинацией цитронеллола, фамесола и нерола обеспечивает крепкий, сладкий, цветочный свежий розоцветный характер выработанного масла.

Хроматографический профиль розового масла обнаружил значительное присутствие алифатических углеводородов (31%), которые являются основными компонентами, отвечающими за стойкость запаха.

Основные соединения эфирных масел были следующими: в-цитронеллол (30,24-31,15%); транс-гераниол (20,62-21,24%), n-хенеикосан (8,79-9,05%), n-нонадекан (8,51-8,77%), нонадекан (4,42-4,55%) и фенилэтиловий спирт (4,04-4,16%).

**Выводы.** Эфирное масло из дамасской розы, впервые получено в новом регионе Болгарии, имеет высокие показатели качества и рекомендуется к массовому производству.

**Ключевые слова**: *роза, эфир, масло, химия, Болгария.*

## Молочный жир в формировании структуры молочных продуктов

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**Введение.** Статья посвящена изучению влияния молочного жира на формирование структуры молочных продуктов на основании опубликованной информации за последние 15 лет. Консистенция продукта является одной из важных характеристик, которые определяют восприятие молочного продукта потребителями. При этом консистенция является органолептическим отражением структуры продуктов, поэтому установление и поддержание необходимой структуры имеет решающее значение для обеспечения высокого качества продукта.

**Материалы и методы.** Исследовалась структура масла, сыра, мороженого, взбитых сливок. Проведен анализ научных статей, диссертации и монографии ученых данной отрасли науки. Методология исследования основана на использовании методов анализа, сравнения и обобщения.

**Результаты и обсуждение.** В молоке и сливках молочный жир формирует эмульсию «жир-вода», существуя в виде жировых глобул, покрытых защитными оболочками. С практической точки зрения молочный жир является важным составляющим компонентом, поскольку обеспечивает характерные физико-химические, структурные, органолептические свойства молочных продуктов, таких как сливки, масло, мороженое, сбитые сливки, сыр.

Жиросодержащие продукты, например, масло, представляют собой эмульсию типа «вода-жир», которая состоит из кристаллизованного молочного жира, выполняющего роль дисперсионной среды, в котором диспергированные капли влаги, неразрушенные и частично дестабилизированные жировые глобулы. Преобладание в структуре масла значительного количества мелких кристаллов молочного жира способствует повышению твердости до 20% по сравнению с продуктом с незначительным количеством кристаллизованного жира. Однако наличие крупных кристаллов обусловливает изменение консистенции, хрупкости и эластичности продукта.

Для формирования стабильной структуры взбитых сливочных десертов и мороженого наличие молочного жира является необходимым. При этом формирование структуры зависит от взаимодействия между жировыми глобулами, воздушными пузырьками и компонентами плазмы (особенно белками). В процессе взбивания и охлаждения жировые шарики частично объединяются и адсорбируются

на воздушных пузырьках, покрывая их поверхности. Таким образом происходит стабилизация воздушной фазы. Кроме того, жир играет важную роль в обеспечении твердости структуры мороженого во время фризерования, а следовательно, и консистенции, внешнего вида и сопротивления к таянию.

Наличие молочного жира в сырах обусловлено необходимостью формирования характерных вкусовых особенностей этого продукта. Кроме этого, глобулы молочного жира влияют на консистенцию сыра, смягчая ее частичным разрушением казеиновой матрицы продукта.

**Вывод.** Молочный жир является ценным компонентом благодаря его приятным вкусовым характеристикам, тогда как другие его свойства часто требуют изменений для надлежащего применения в производстве пищевых продуктов, что, в свою очередь, заметно влияет на формирование структуры молочных продуктов, в частности масла, взбитых сливок, мороженого и сыра.

**Ключевые слова:** *молочный жир, структура, масло, мороженое, сыр.*

## Использование биоинформационного анализа и компьютерной морфометрии для изучения *Fusarium* spp., вызывающих сухую картофельную гниль

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**Введение.** Задача исследования – оценить возможность применения методов компьютерной морфометрии и биоинформационного анализа для изучении реактивных изменений клеток грибов *Fusarium* spp., вызывающих сухую картофельную гниль.

**Материалы и методы.** Для проведения исследований были отобраны клубни картофеля сорта «Невский» с внешними признаками фузариозной сухой гнили. Изучены три зоны растительной ткани картофельных клубней: эпицентр контаминации плесневыми грибами рода *Fusarium;* ткани, близлежащие к очагу поражения; ткани вне зоны очага поражения. Препараты *Fusarium* spp. получали методом «отпечатка» с растительной ткани, фиксированные микроорганизмы окрашивали 1% раствором метиленового синего. Морфологическое исследование и компьютерную морфометрию микроорганизмов проводили при общем увеличении x400 и x1000 микроскопа "Micros" (Austria), модель MS 100 (XP).

**Результаты и обсуждения**. Морфометрические характеристики макроконидий *Fusarium* spp. (площадь, периметр, длина, количество) были получены с применением компьютерной программы анализатора изображений "Micros".

На основании данных морфометрического анализа клеток грибов были разработаны алгоритм и компьютерная программа для изучения биоинформационных характеристик макроконидий *Fusarium* spp.

Для установления термодинамического равновесия клеточной системы вычислялись следующие информационные характеристики: H – информационная

энтропия, характеризующая степень упорядоченности биосистемы; $H_{max}$ – информационная емкость, характеризующая максимальное структурное разнообразие биосистемы с утратившими функциональную взаимосвязь элементами; R – коэффициент избыточности (относительной организации биосистемы).

Для исследованных грибов рода *Fusarium* выявлены низкие значения информационной энтропии H (0,002) относительно значений информационной емкости $H_{max}$ (0,5) на фоне повышенных значений коэффициента избыточности R (99,40%- 99,69%), которые характеризуют упорядоченность и высокую степень относительной организации в микробных клетках, поражающих клубни картофеля. Эта информация может указывать на максимальную степень адаптации клеток паразитирующих грибов рода *Fusarium* к условиям их биоценоза с растительными клетками в процессе микробной контаминации клубней картофеля. Следует ожидать, что в случае реакции грибов рода *Fusarium* на воздействие химических или биопрепаратов значения информационной энтропии H должны возрастать до значений информационной емкости $H_{max}$, а значения коэффициента избыточности R – уменьшаться. Изменения показателей можно будет трактовать как результат дезинтеграции структурных и функциональных связей в клеточной системе грибов рода *Fusarium.*

**Выводы.** Метод биоинформационного анализа при морфологическом исследовании клеток грибов рода *Fusarium*, вызывающих сухую картофельную гниль, может быть рекомендован в качестве тест-системы при разработке новых видов препаратов для защиты растений.

**Ключевые слова:** *Fusarium* spp., *картофель, сухая гниль, морфометрический анализ, биоинформатика, тест-система.*

## Производство этанола из продуктов переработки кофейных плодов

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**Введение.** Проведено исследование с целью оценки производства этанола из продуктов переработки кофесодержащего сырья. В качестве основных компонентов использовали внешнюю оболочку и мякоть плодов кофе.

**Материалы и методы**. В процессе исследования сусло экстрактировалось из кофейной оболочки и мякоти и сбраживалось. Использование дрожжей было оптимизировано с целью увеличения концентрации редуцирующих сахаров. В свою очередь, кислотный гидролиз привел к превращению сложных полисахаридов в простые мономеры. Небольшое количество мелассы и остатков было использовано для усиления брожения. Сухие дрожжи *Saccharomyces cerevisiae* были регидратированы и дополнены питательными веществами, которые также были обогащены азотом, магнием и углеродом.

**Результаты и обсуждение**. Сырье после переработки брожением было перегнано. Изменение редуцирующих сахаров в субстанции показывает ход процесса ферментации. Процесс брожения заканчивается, когда плотность уменьшается ниже 6%. Выявлено, что в процессе брожения содержание сахара уменьшается, что непосредственно связано с эффективностью брожения. С 6,5 л сусла было изготовлено 2,3 л этанола с абсолютным объемом 45 аб.%, что, в свою очередь, составляет 35,5% от общего объема сусла. Поскольку pH сусла для брожения было

оптимизировано до 4–5, результаты показывают, что производство этанола может осуществляться с использованием мякоти плодов и оболочки кофе в качестве дополнительных продуктов, что приведет к уменьшению загрязнения ими окружающей среды. В ходе исследования было оптимизировано pH и температурные режимы на должном уровне для всех образцов.

**Выводы**. Переработка побочных продуктов (внешней оболочки и мякоти плодов кофе) в этанол может увеличить потенциал сырьевых ресурсов предприятий путем производства этанола.

**Ключевые слова**: кофе, плод, мякоть, спирт, брожения, *Saccharomyces cerevisiae.*

## Особенности технологии применения камедей для создания гелевых основ

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**Введение.** Целью данной работы является изучение технологических, физико-химических и реологических свойств гелевых основ на основе гидроколоидов для дальнейшей разработки пищевых продуктов гелевой формы.

**Материалы и методы.** В качестве объектов исследования выбраны камедь ксантана (ККС), камедь гуара (КГ) и камедь рожкового дерева (КРД), а также гелевые основы с разной концентрацией данных галактомананов.

С целью изучения технологических, физико-химических и реологических характеристик гелевых основ приготовлены образцы гелей за тремя способами. Изучение структурно-механических свойств проводилось с помощью ротационного вискозиметра.

**Результаты и обсуждение.** Исследовано влияние концентрации гелеобразователя, температуры воды и продолжительности набухания на вязкость коллоидных растворов и на предельное напряжение сдвига. Гелевые основы, приготовленные за тремя способами, с выбраными гидроколлоидами относятся к структурированным системам.

Наибольшее значение величины максимальной вязкости $\eta_0$, что соответствует практически неразрушеной системе, для образцов гелевых основ с камедью ксантана, приготовленных по способу 3, составляет 106,3 Па·с, с камедью рожкового дерева - 1,97 Па·с, с камедью гуара - 17,7 Па·с при концентрации 1%.

Рассчитаны структурно-механические характеристики. Для камеди ксантана и гуара значение напряжения сдвига больше ноля, что свидетельствует о том, что исследуемые образцы являются структурированными твердоподобными телами. Для образцов из камеди рожкового дерева значение напряжения сдвига равно нулю, что указывает на то, что их можно отнести к псевдопластическим жидкостям. Самый большой динамический предел способности к течению наблюдался у образцов с камедью ксантана, приготовленных по второму и третьему способу, с камедью рожкового дерева и гуара - первым и третьим способами с концентрацией 1%.

Сравнение растворов гидроколоидив, полученных при различных способах приготовления, показало, что наиболее целесообразным является приготовление гелей третьим способом, поскольку при этих условиях вязкость растворов (при одинаковой концентрации гелеобразователя) была выше и структура геля была более однородной.

**Выводы.** Для всех экспериментальных образцов гелей характерный неньютоновской псевдопластический тип течения. Наиболее целесообразным является приготовление гелей третьим способом, который заключался в набухании гидроколоидов в течение 40 мин в воде комнатной температуры, подогреве до 60 °С и охлаждении. Оптимальная концентрация гелеобразователей – 0,5%.

**Ключевые слова:** *реология, гелеобразователь, камедь, ксантан, гуар, рожковое дерево.*

## Механизм антимикробного действия растительных антибактериальных препаратов

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**Введение.** Целью этого исследования является осуществление обзора информации о механизме действия растительных антибактериальных препаратов, которые можно использовать в пищевых продуктах.

**Материалы и методы**. Поиск и использование литературных источников осуществлен из библиографических баз данных AGRICOLA, CAB Abstracts, EBSCO, FSTA, Global Health, Google Scholar, Index Copernicus, PubMed, Scopus, TUBITAK ULAKBIM Life Sciences Database, и Web of Science.

**Результаты и обсуждение.** Некоторые фруктовые и овощные соки, травы и специи были использованы в качестве регуляторов кислотности и ароматизаторов для традиционных кушаний, салатов и закусок. Все эти продукты, а также их экстракты широко используют в пищевой промышленности как естественные антибактериальные препараты. Антибактериальное действие растительные добавок в основном обусловлено наличием в них органических кислот или/и фенольных веществ. Органические кислоты и фенольные вещества, которые являются основными соединениями в растениях, вызывают их антимикробную активность. Однако влияние антимикробного компонента изменяется в зависимости от генотипа, структуры клеточной оболочки и исходной популяции микроорганизмов, а механизм действия – от способа отсоединения и присоединения в клетке. Ингибирующее действие растительных антибактериальных препаратов на разные микроорганизмы исследовалась неоднократно. Но и доныне нет четкого понимания механизма их действия, которое вызывает фунгицидное статическое влияние. Важным является определение особенностей действия этого механизма, чтобы сделать его доступным и приемлемым для промышленного приложения.

**Вывод**. Определение особенностей действия механизма антибактериальной активности является необходимым для того, чтобы сделать антимикробные препараты доступными к применению в пищевой промышленности.

**Ключевые слова:** *антимикробный, растение, кислота, фенол.*

# Процессы и оборудование пищевых производств

## Трехмерная модель течения неньютоновской жидкости в прямоугольном канале

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**Введение.** Предложена трехмерная математическая модель течения неньютоновской жидкости с вязкостью, зависящей от скорости сдвига в прямоугольных каналах технологического оборудования пищевой промышленности.

**Материалы и методы.** Применен метод суперпозиций, позволяющий построить поле продольного течения неньютоновской жидкости в прямоугольном канале технологического оборудования с движущимися стенками, и таким образом определить величины скорости и давления в любой точке внутри канала.

**Результаты и обсуждение.** Получена модель продольного течения неньютоновской жидкости в прямоугольном канале, на стенках которого заданы различные продольные скорости. В основе построения лежит решение одномерной задачи о куэттовском течении в канале. Композиция течений в щелевых каналах с взаимно перпендикулярными парами стенок позволяет получить формулу расхода течения, удовлетворяющую принципу предельного соответствия между течениями в прямоугольном и щелевом каналах. Предлагается способ построения поля скорости, который представляет собой разбиение конечного сечения канала на участки с различной зависимостью от координат так, что на одних участках скорость зависит только от одной координаты, а на других – только от другой координаты. Получены уравнения линий, разграничивающих эти участки и способы определения формы разграничительных линий.

**Выводы.** Полученные аналитические формулы позволяют определить макрокинетические характеристики в каждой точке канала с произвольным распределением граничных скоростей течения неньютоновских жидкостей, вязкость которых зависит от скорости сдвига.

**Ключевые слова:** *неньютоновская жидкость, Куэтт, течение, реология, модель, канал.*

## Моделирование работы топки с рециркуляцией греющих газов для тоннельной хлебопекарной печи

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**Введение.** С целью повышения эффективности работы источников теплоты в топках туннельных хлебопекарных печей исследован процесс рециркуляции в них греющих газов.

**Материалы и методы.** В данной работе была использована CAE-программа для моделирования движения жидкостей FlowVision, принцип действия которой основан на методе конечных элементов. При рассчете были использованы реологические и кинематические параметры горячих газов, полученные в свое время при реальных модельных экспериментах.

**Результаты и обсуждение.** Установлено, что распределение скоростей по длине топки делится на две устойчивые области: по оси топки и около внешних стенок топки. Смешивание газов начинается в распределительном коробе.

Температура газов по центру потока снижается от 1900 до 600 °C практически по линейной зависимости. Температура газов возле стенок топки изменяется по всей длине топки в диапазоне от 260 до 360 °C, что объясняется слабой турбулизацией потока.

На изолиниях областей максимальной диссипации кинетической энергии отмечены завихрения в двух локальных областях. Первая область – кольцо вокруг выхода продуктов сгорания из камеры сгорания. Вторая область – в области сужения топки. Она находится уже при выходе в распределительный короб.

Впервые получена визуальная и численная информация, которая отражает, как комбинация местных сопротивлений разного типа в газовом тракте влияет на значение критерия Рейнольдса и характер движения греющего агента.

Предложено оборудовать топки подобного типа дополнительными деталями – кольцевыми шайбами. Доказано, что они изменяют направление потоков газов рециркуляции так, что происходит активное их перемешивание с продуктами сгорания по всей длине топки.

**Выводы.** Проведенное компьютерное моделирование работы топки позволило обнаружить и локализовать недостатки существующей базовой конструкции, а также предложить способы модернизации устройства.

**Ключевые слова**: *печь туннельная, топка, газ, нагрев, рециркуляция, смешивание.*

## Экономика и управление

### Общественное восприятие уровня качества и безопасности коммерческих брендов лапши

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*2 - факультет пищевой технологии и перерабатывающей промышленности, Бангладешский аграрный университет, Мименсингх, Бангладеш.*

*3 - факультет биофункциональной науки и технологии, Высшая школа биосферной науки, Хиросимском университет, Хиросима, Япония*

**Введение.** Потребители становятся более сознательными к безопасности и качеству пищевых продуктов. В аспекте качества и безопасности пищевых продуктов было проведено исследование оценки качества некоторых коммерческих брендов бангладешских лапши.

**Материалы и методы**. Исследование проводилось в два этапа. На первом этапе осуществлено общий опрос для определения оценки отношение потребителя к

лапше, которое охватывало населения разных областей Бангладеша. Потребители утверждают, что все компании по производству лапши фальсифицируют продукцию путем использования муки низкого сорта и вредных пищевых красителей, которые опасны для здоровья. На втором этапе на местных рынках выбрано четыре образца фирменных брендов лапши для оценки химического состава, кулинарных особенностей, микробного профиля и сенсорной оценки.

**Результаты и обсуждение**. Образцы лапши отобраны в диапазоне: влажность – 7,07-10,70%, зола – 1,01-2,70%, жир – 0,40-0,73%, сырой протеин – 11,00-11,17%, сырая клетчатка – 0,05-0,30%, общее содержание углеводов – 74,19-80,34%, содержание крахмала – 61,09 -66,35%, pH – 8,15-8,88. Степень клейстеризации, кислотность и энергетическую ценность отобраны в диапазоне 96,55-99,85%, 0,79-0,85 мг/г и 344,52-370,53 ккал/100 г соответственно. Микробный профиль показывает, что на предприятиях отрасли поддерживаются надлежащие гигиенические условия при производстве лапши в соответствии с GMP, также не выявлено ни одного фекального загрязнения и любой микробиологической опасности. Кулинарные характеристики всех образцов лапши были разнообразны, но все имели приемлемое качество. Выявлено существенное отличие ($p < 0,05$) в гладкости, желтизне, белизне и блеске. Несмотря на разнообразность описательных оценок, полученные результаты оказались удовлетворительными. Выявлено существенное различие ($p < 0,05$) во всех сенсорных атрибутах (цвет, запах, вкус, текстура и общая приемлемость) среди лапши, но удовлетворительные оценки достигнуты для всех образцов лапши.

**Выводы**. Образцы торговых марок лапши соответствуют действующим требованиям. Следовательно, предположение потребителей в отношении коммерческих брендов лапши являются ошибочными.

**Ключевые слова**: *потребитель, поведение, лапша, химикат, микроорганизм.*

## Система качества маркировки пищевых продуктов в Европейском Союзе

Томаш Садилек

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**Введение.** В статье рассматриваются модели Европейского Союза по защите обозначения происхождения, географической принадлежности и обеспечения традиционных особенностей маркировок, которые используются в сельском хозяйстве и пищевой продукции.

**Материалы и методы**. Использованы вторичные данные из базы данных о происхождении и регистрации. Образец состоял с 1356 маркировок (этикеток), зарегистрированных в этой базе до 18 июля 2016 года. Частота использования маркировок анализировалась в зависимости от страны, типа маркировки и классов продуктов.

**Результаты и обсуждение**. Задачей исследования является анализ использования указанных маркировок на рынке Европейского Союза в соответствии с выбранными критериями, которые включают тип маркировки, зарегистрированный в каждой стране, количество сельскохозяйственной продукции и продовольственных товаров, зарегистрированных как качественные этикетки в целом. Как показывают результаты, наибольшее количество названий продуктов зарегистрировано с маркировкой PDO, с небольшой разницей – с маркировкой PGI. Только минимум продуктов зарегистрирован как TSG. Доминирующей страной является Италия, далее следуют другие средиземноморские страны - Франция, Испания, Португалия и

Греция. С учетом класса продукта наиболее распространенными классами являются фрукты, овощи и зерновые (для PGI, PDO), сыры (PDO) и мясные продукты (TSG, PGI), что подтверждает анализ квадрата критериев независимости Пирсона для определения существенных различий между частотой использования маркировок и упомянутых критериев. Подтверждена слабая зависимость между количеством названий продуктов, зарегистрированных как PDO, PGI и TSG, и страной происхождения, средняя зависимость между типом маркировок и классом продукции, где используется маркировка, и значительная зависимость между страной происхождения и классом продукта, который чаще всего регистрируется.

**Вывод.** PDO, PGI и TSG схемы полезны для потребителей и производителей. Потребители покупают продукт с определенными качествами добавленной стоимости.

**Ключевые слова:** *маркировка, качество, база данных, DOOR, EC, еда.*

# Instructions for authors

**Dear colleagues!**

The Editorial Board of scientific periodical
«**Ukrainian Food Journal**»
invites you to publication of your scientific research.

Requirements for article:
Language – English, Ukrainian, Russian
Size of the article – 8–15 pages in Microsoft Word 2003 and earlier versions with filename extension *.doc (!)
All article elements should be in Times New Roman, font size 14, 1 line intervals, margins on both sides 2 cm.

The structure of the article:
1. The title of the article
2. Authors (full name and surname)
3. Institution, where the work performed.
4. Abstract (2/3 of page). The structure of the abstract should correspond to the structure of the article (Introduction, Materials and methods, Results and discussion, Conclusion).
5. Key words.

Points from 1 to 5 should be in English, Ukrainian and Russian.

6. The main body of the article should contain the following obligatory parts:
   - Introduction
   - Materials and methods
   - Results and discussing
   - Conclusion
   - References

If you need you can add another parts and divide them into subparts.

7. The information about the author (Name, surname, scientific degree, place of work, email and contact phone number).

All figures should be made in graphic editor, the font size 14.

The background of the graphs and charts should be only in white color. The color of the figure elements (lines, grid, text) – in black color.
Figures and EXCEL format files with graphs additionally should submit in separate files.
Photos are not appropriate to use.

**Website of Ukrainian Food Journal: http://ufj.ho.ua**

**Extended articles should be sent by email to: ufj_nuft@meta.ua**

## Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

### Вимоги до оформлення статей

Мови статей – англійська, українська, російська
Рекомендований обсяг статті – **8–15 сторінок** формату А4**.**
Стаття виконується в текстовому редакторі Microsoft Word 2003, в форматі *.doc.
Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.
Всі поля сторінки – по 2 см.

### Структура статті:

1**.** УДК**.**
2. **Назва статті.**
3. Автори статті (ім'я та прізвище повністю, приклад: Денис Озерянко).
4. *Установа, в якій виконана робота.*
5. Анотація. Обов'язкова структура анотації:

- Вступ (2–3 рядки).
- Матеріали та методи (до 5 рядків)
- Результати та обговорення (пів сторінки).
- Висновки (2–3 рядки).

6. Ключові слова (3–5 слів, але не словосполучень).

**Пункти 2–6 виконати англійською, українською та російською мовами.**

7. Основний текст статті. Має включати такі обов'язкові розділи:

- Вступ
- Матеріали та методи
- Результати та обговорення
- Висновки
- Література.

За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім'я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії бажано не використовувати.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

В списку літератури повинні переважати статті та монографії іноземних авторів, які опубліковані після 2000 року.

## Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

**1. Посилання на статтю:**
**Автори А.А. (рік видання), Назва статті, Назва журналу (курсивом), Том (номер), сторінки.**

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

1. Приклад:
   Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (Juglans regia L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108.

**2. Посилання на книгу:**
**Автори (рік), Назва книги (курсивом), Видавництво, Місто.**

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

**Посилання на електронний ресурс:**

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова ***available at:*** та вказується електронна адреса.

Приклади:

1. (2013), *Svitovi naukovometrychni bazy*, available at: http://www1.nas.gov.ua/publications/q_a /Pages/scopus.aspx
2. Cheung T. (2011), *World's 50 most delicious drinks [Text]*, available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт, а з російської – стандарт МВД (в цих стандартах використовуються символи лише англійського алфавіту, без хвостиків, апострофів та ін).

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З російської мови – http://ru.translit.net/?account=mvd

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**http://ufj.ho.ua**

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УДК 663/664

**Ukrainian Food Journal** публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

**Тематика публікацій в Ukrainian Food Journal:**

Харчова інженерія
Харчова хімія
Мікробіологія
Фізичні властивості харчових продуктів
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Процеси та обладнання
Нанотехнології
Економіка та управління
Автоматизація процесів
Упаковка для харчових продуктів
Здоров'я

**Періодичність виходу журналу** 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

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Index Copernicus (2012)
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**Рецензія рукопису статті.** Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

**Авторське право.** Автори статей гарантують, що робота не є порушенням будь-яких авторських прав, та відшкодовують видавцю порушення даної гарантії. Опубліковані матеріали є правовою власністю видавця «**Ukrainian Food Journal**», якщо не узгоджено інше.

**Політика академічної етики.** Редакція «Ukrainian Food Journal» користується правилами академічної етики, викладених в роботі Miguel Roig (2003, 2006) "Avoiding plagiarism, self-plagiarism, and other questionable writing practices. A guide to ethical writing". Редакція пропонує авторам статей і рецензентам прямо слідувати цьому керівництву, щоб уникнути помилок у науковій літературі.

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