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Quality of Canadian commercial plain non-fat Greek-style yogurts produced only from natural dairy ingredients

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Abstract

Introduction. Article presents evaluation of quality of Canadian commercial plain non-fat Greek-style yogurts produced only from natural dairy ingredients.

Material and methods. Yogurts samples were purchased 10 days after their production and were stored at 5°C to obtain 18 or 35 days of storage. Dynamic rheological properties and surface whey-off were measured. An ordinal numerical scale was used in order to evaluate whey drainage and the presence and size of visible clusters.

Results and discussion. Greek-style yogurt is a typical weak viscoelastic gel whose elastic properties predominate over its viscous properties over the measured range. A structural degradation was observed in all samples at some point during the stress-amplitude range applied. B samples had a higher total solids content than A samples therefore, this might be the reason that B samples presented higher dynamic moduli than A samples, even for the same protein content. Both reference samples presented a significant increase in their viscous ($G''$) and elastic ($G'$) moduli upon storage at 5°C. This fact suggests that casein gels are dynamic by nature and that further development of the gel structure occurs during storage. At high amplitudes, A samples presented a significant increase in their tan $\delta$ values due to the rupture of their gel structures. A proportional increase in $G'$ and $G''$ during storage was observed; hence, the tan $\delta$ values for the same types of samples, after 18 and 35 days of storage, were similar. Samples did not differ in levels of whey drainage and in the size of visible clusters. Both market samples presented higher amounts of surface whey-off as storage time increased. Probably during storage, large scale rearrangements occurred in the gel network which increased the level of instability of the gel, resulting in the loss of the ability to entrap all the serum phase. Although none of the reference samples had visible whey drainage, all of them presented small visible clusters.

Conclusions. Protein is not the only ingredient shaping structure and rheological properties of Greek-style non-fat yoghurt. Properties of commercial plain non-fat Greek-style yogurts changes at the storage time. Samples becomes more solid, but with higher whey syneresis.

Keywords: Yoghurt, Greek-style, Whey, Syneresis, Rheology
Introduction

Greek-style yogurt (strained yogurt) is a semisolid dairy product obtained by removing part of the whey from regular yogurt [1–3]. As a result of this action, the total solids content and lactic acid concentration of the initial yogurt are increased (concentrated yogurt typically contains 22–23 g/100g total solids and has an acidity of around 1.60–1.80 g/100g lactic acid), giving the final product a much thicker consistency and a distinctive, slightly tangy taste [4, 5]. In addition, the product obtained has nutritional properties superior to those of regular yogurt, with higher protein and mineral contents and very low lactose content. It also has better keeping qualities due to the increased lactic acid concentration [6–8].

Health benefits associated with yogurt cultures and probiotics led to a sharp increase in the per capita consumption of yogurt in Canada and the United States during the last decades [9]. According to Chandan [10], yogurt sales in the U.S. have been spectacular, increasing from 1,837 million pounds in the year 2000 to 2,990 million pounds in 2005, and they continue to show remarkable growth. Overall yogurt category sales increased 12% year after year. Of those sales, 85% was driven by a 146% increase in Greek-style yogurt sales, while a 2% increase in traditional yogurt sales accounted for only 15% of category growth [9].

To take advantage of the current remarkable economic growth of Greek-style yogurt, the issue of good quality is very important. Some researchers work on development of an efficient formulation for the production of strained yogurt powder. It is believed that a dried type of concentrated yogurt will help to expand the economic boom of Greek-style yogurt in areas that have a limited indigenous dairy industry, or regions that suffer from seasonal deficiencies in milk supply [11]. Thus, this type of product is intended to open new markets to this highly valuable food commodity.

The pronounced economic growth of Greek-style yogurt has led to a noticeable diversification of the traditional product. Many mechanized systems based on modern techniques, such as membrane processes, centrifugation, and direct reconstitution, have been developed to manufacture strained yogurt in large volumes [1, 12–14]. Because the overall characteristics of concentrated yogurt depend on the method of production, the use of different manufacturing methods has led to the production of diverse varieties of commercial Greek-style yogurt which significantly differ in their composition [1, 15]. Tamime [16] and Tamime & Robinson [17] have reported about the difference in composition of various types of commercial concentrated yogurt that exist around the world. In order to respond to the increasing consumer preference for reduced fat and additive-free products, the current study will emphasize the production of a non-fat, additive-free type of yogurt [18].

Regardless of the production method and composition of the final product, one of the major concerns facing the Greek-style yogurt industry is the production and maintenance of a product with optimum consistency, stability and texture properties [19]. The overall visual appearance, microstructure, and rheological properties of acid milk gels are important physical attributes which contribute to the overall sensory perception and functionality of these products [20]. Textural attributes, including the desired oral viscosity, are very important criteria for quality and for consumer acceptance of yogurt [21]. Skriver et al. [22], Richardson et al. [23], and Stanley & Taylor [24] reported that sensory texture analyses are highly correlated with the rheological properties of stirred yogurt and other semi-solid foods. Thus, the objective of this experiment was to evaluate the quality of commercial Greek-style yogurt and its "on shelf" stability. Such stability is particularly difficult to preserve for natural non-fat yoghurts. Fat is an ingredient which stabilizes the structure and texture of yogurt and makes it more attractive for consumers. Different texture shaping ingredients added to many
commercial yogurts enable to obtain a product with any desired texture and stability. Much more difficult it to obtain product based only on non-fat milk ingredients.

The aim of this study was to investigate the structure and rheological properties of non-fat Greek-style yogurts with different dry matter content, produced only from natural dairy ingredients.

The research tasks:
1. To analyse the current state of production of Greek-style yogurts;
2. To investigate the quality indicators of commercial samples of non-fat Greek-style yogurts;
3. To identify patterns of changes in the structure of the protein gel of yogurt with different dry matter content during storage.

Materials & methods

Market reference samples
All types of commercial, plain Greek-style yogurts (0% M.F.) produced from natural dairy ingredients without added preservatives, emulsifiers or stabilizers (according to their labeling), that were commercialized in Edmonton (Canada) by three supermarket chains (Walmart, Superstore, and Safeway) were used as reference samples in this study. Two products that met these requirements were labeled as “A” and “B”. Reference samples were purchased 10 days after their production and were stored at 5 °C to obtain 18 or 35 days of storage. Specifications of these samples are shown in Table 1.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A Skim milk, live active cultures</th>
<th>B Skim milk, live active cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production method</td>
<td>Traditional/Stirred</td>
<td>Traditional/Stirred</td>
</tr>
<tr>
<td>Shelf life (Days)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>10.3</td>
<td>10.3</td>
</tr>
<tr>
<td>Carbohydrates content (%)</td>
<td>3.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Total Solids content (%)</td>
<td>13.7</td>
<td>17.1</td>
</tr>
</tbody>
</table>

| Specifications obtained from products labels. |
| Specifications obtained from customer services. |
| Total solids content was calculated based on the carbohydrates, fat and protein contents. |

Dynamic rheological measurements
Small amplitude oscillatory rheology (SAOR) tests were performed with a Paar Physica UDS200 MCR Rheometer. The evaluation method was adapted from Özer et al. [25–27]. The rheometer was set up with a parallel-plate geometry (10 mm plate radius and 1 mm gap setting). All samples were gently stirred with a spoon for 30 seconds before measurement in order to mix the potential free whey with the resultant gel. Each sample was loaded into the rheometer and allowed to relax and equilibrate to measuring temperature (25±0.1 °C) for 2
minutes prior to testing. The temperature of the samples inside the rheometer was maintained by a circulating cooling system. Rheological aspects of all samples were evaluated by conducting stress amplitude sweep tests. A sweeping amplitude from \(1.5 \times 10^{-2}\) to \(1.5 \times 10^{-1}\) mNm at 0.25 Hz was used and 25 measuring points were performed through the sweeping range. Storage (\(G'\)) and loss (\(G''\)) moduli were recorded. Three replications were conducted for each sample.

**Whey separation measurements**

**Surface whey-off (SWO).** The method used to quantify the amount of free whey present on top of the resultant gel was adapted from Lucey *et al.* [28]. Experimental samples were evaluated before and after applying homogenization during their production. Any free whey expelled on top or around the sides of the gel was gently sucked with a polyethylene transfer pipette and weighted. Once all the free whey was sucked from the surface, the gel was allowed to rest for 1 minute and any further surface whey was sucked and weighted. The degree of whey separation was expressed as a percentage of the total sample weight (% m/m). After quantification, free-whey was reintroduced into the samples.

**Whey drainage (WD).** To evaluate the degree of whey drainage present in the final samples, the resultant gels were broken with a spoon and the level of whey drainage was quantified using an ordinal numerical scale from 0 (no visible whey drainage) to 2 (high amount of whey drainage). Figure 1 illustrates the different levels that were used to classify the samples according to whey drainage.

![Figure 1. Ordinal levels used for the classification of samples according to the degree of whey drainage](image)

**Presence and size of visible clusters.** An ordinal numerical scale from 0 (no visible clusters) to 3 (big visible clusters) was used in order to evaluate the presence and size of visible clusters in the final products. All samples were gently stirred with a spoon for 30 seconds before the evaluation in order to mix the potential free whey with the resultant gel. Figure 2 shows the different levels that were used to classify the samples according to the presence and size of the clusters.
Figure 2. Ordinal levels used for the classification of samples according to the presence and size of visible clusters
Level 0 could not be assigned to any of the evaluated samples. All samples presented visible clusters.

Experimental design and statistical analysis

The market reference samples were evaluated for their rheological and physicochemical properties at day 18 and 35 after their production. All measurements were carried out in triplicate. According to the data collected from these analyses, a mean reference value and a two-sided confidence interval ($\alpha=0.05$) was established for each parameter tested. Reference confidence intervals at $P < 0.05$ were used for comparisons with experimental data.

Results and discussion

Dynamic rheological analyses

In particular, the weak viscoelastic nature of yogurt gel is well established and the rheological properties of yogurt can be explained by measuring its viscous ($G''$) and elastic ($G'$) moduli [25]. Figure 3 shows the dynamic moduli ($G'$ and $G''$) of the reference samples when applying a stress amplitude sweep test. Consistent with the data presented in Figure 3, it can be stated that Greek-style yogurt is a typical weak viscoelastic gel whose elastic properties predominate over its viscous properties over the measured range.
According to the previous figure, both fundamental dynamic parameters (G’ and G'”) showed a stress-amplitude dependence. Also, a linear viscoelastic region was evident for all samples. A structural degradation was observed in all samples at some point during the stress-amplitude range applied. These breakdown as a function of amplitude suggests that mechanical changes produced in the gels during the manufacturing process would lead to drastic rheological changes [20]. Özer et al. [25] stated that yogurt is a metastable gel and any change in its enthalpic/entropic nature creates irreversible deformation.

Although both types of reference samples had the same protein contents (Table 1), B sample had higher G’ and G’” moduli than A sample. As a result of more protein-protein interactions at higher protein levels, a much denser and stronger gel structure can be expected; however, the rheological properties of yogurt are not only dependent on the protein content, but are also highly dependent on total solids content and on the type of protein present in the gel matrix [29–31]. B samples had a higher total solids content than A samples (Table 1); therefore, this might be the reason that B samples presented higher dynamic moduli than A samples. Even though the spatial distribution of the protein-protein bonds over the gel network, the strength of the interaction forces between protein molecules and the structure of the protein particles themselves also defined the mechanical properties of a gel network [32, 33].

Both reference samples presented a significant increase in their G’ and G’” upon storage at 5°C. This matches the findings of Marafon et al. [34], Serra et al. [35] and Weidendorfer et al. [36], who reported an increase in G’ in stirred yogurts within storage. This fact suggests
that casein gels are dynamic by nature and that further development of the gel structure occurs during storage [37]. Özer et al. [26] affirms this point by stating that the number and/or strength of nonrelaxing and relaxing protein bonds in a protein gel matrix increases during storage. Upon storage, casein particles experience several large-scale rearrangements which result in the formation of new linkages to decrease the total free energy of the system and move to a more thermodynamically stable state [20, 35, 37].

Figure 4 presents the loss tangent (\(\tan \delta = \frac{G''}{G'}\)) values of the reference samples in days 18 and 35 after their production. Although B samples had higher \(G''\) and \(G'\) values than A samples, within the linear viscoelastic region, the \(\tan \delta\) values of these two samples were similar.

![Figure 4. Loss tangent (\(\tan \delta = \frac{G''}{G'}\)) values of reference samples at days 18 and 35 after their production](image)

Presented values are the means of triplicate measurements.

Loss tangent values are highly dependent on the nature of bonds between the particles integrating the gel network [25, 27, 38]. Thus, it can be stated that at low amplitudes, the nature of bonds between the particles integrating both gels was similar. However, at high amplitudes, A samples presented a significant increase in their \(\tan \delta\) values due to the rupture of their gel structures, which resulted in a non-proportional decrease in the number and/or strength of non-relaxing protein bonds and relaxing bonds. After the breakage point, the number and/or strength of non-relaxing bonds declined more rapidly than the number and/or strength of relaxing bonds; therefore, \(G'\) decreased more pronouncedly than \(G''\), indicating a partial breakdown of the elastic structure and a change to a relatively more viscous behavior. Due to this fact, at high amplitudes, A samples had a higher liquid-like behavior than B samples, and a significant difference in \(\tan \delta\) values was observed between both samples [39, 40].
Even though the $G'$ and $G''$ of both reference samples increased upon storage time (the number and/or strength of non-relaxing protein bonds and rapidly relaxing bonds increase with storage time), the $\tan \delta$ remained almost unchanged for each sample throughout the storage period, suggesting the formation of essentially similar network structures throughout storage time [26]. This fact led to a proportional increase in $G'$ and $G''$ during storage; hence, the $\tan \delta$ values ($\tan \delta = G''/G'$) for the same types of samples, after 18 and 35 days of storage, were similar. For comparative purposes, Table 2 provides the two-sided confidence interval limits ($\alpha = 0.05$) for the mean $G'$ and $\tan \delta$ values of the reference samples at points 1, 12 and 25 of the sweeping amplitude range applied. This table also presents the corresponding confidence interval limits ($\alpha = 0.05$) for the mean SWO value of the reference samples and the mean values of the ordinal measurements (WD; presence and size of clusters) that were carried out on these samples.

**Rheological and physicochemical values of the market reference samples**

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Market Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G'$ (Pa) [P1: 14.6 $\mu$Nm] †</td>
<td>22.358 – 16.226 a</td>
</tr>
<tr>
<td>$G''/G'$ [P1: 14.6 $\mu$Nm] †</td>
<td>0.257 – 0.249 a</td>
</tr>
<tr>
<td>$G'$ (Pa) [P12: 43.6 $\mu$Nm] †</td>
<td>22.418 – 15.374 a</td>
</tr>
<tr>
<td>$G''/G'$ [P12: 43.6 $\mu$Nm] †</td>
<td>0.283 – 0.260 a</td>
</tr>
<tr>
<td>$G'$ (Pa) [P25: 150 $\mu$Nm] †</td>
<td>16.304 – 0.000 a</td>
</tr>
<tr>
<td>$G''/G'$ [P25: 150 $\mu$Nm] †</td>
<td>5.324 – 0.040 a</td>
</tr>
<tr>
<td>SWO (%m/m) †</td>
<td>0.190 – 0.028 a</td>
</tr>
<tr>
<td>WD§</td>
<td>0.000 ± 0.000 b</td>
</tr>
<tr>
<td>Presence and size of clusters§</td>
<td>1.000 ± 0.000 b</td>
</tr>
</tbody>
</table>

† Scale measures.  
§ Ordinal measures.  
a 95% two-sided confidence intervals.  
b Mean values of reference samples ± SD.

As the level of WD and the size of visible clusters were measured using ordinal scales, confidence intervals could not be determined for this type of data. Therefore, the total mean values for these two measurements were considered as reference values for comparison with experimental data.

**Physicochemical analyses**

Table 3 shows the physicochemical results obtained from market reference samples at days 18 and 35 after their production. Samples did not differ in levels of WD and in the size of visible clusters. All tested samples presented significant levels of SWO. Both market samples presented higher amounts of SWO as storage time increased. Due to this fact, it can be stated that, during storage, large scale rearrangements occurred in the gel network which increased the level of instability of the gel, resulting in the loss of the ability to entrap all the serum phase [28]. This observation agrees with Al-Kadamany et al. [41] who reported that the level of free whey in concentrated yogurt produced by the traditional method increases upon storage. Additionally, Salvador & Fiszman [42] reported that the level of syneresis in whole and skimmed set types of yogurt increases with storage time.
Physicochemical properties of market reference samples

<table>
<thead>
<tr>
<th>Reference samples</th>
<th>Surface whey-off (% m/m)</th>
<th>Whey drainage</th>
<th>Size of visible clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>A – Day 18</td>
<td>0.050 ± 0.087</td>
<td>0.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
<tr>
<td>A – Day 35</td>
<td>0.203 ± 0.021</td>
<td>0.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
<tr>
<td>B – Day 18</td>
<td>0.030 ± 0.052</td>
<td>0.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
<tr>
<td>B – Day 35</td>
<td>0.153 ± 0.150</td>
<td>0.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
<tr>
<td>Total mean value</td>
<td>0.109 ± 0.083</td>
<td>0.000 ± 0.000</td>
<td>1.000 ± 0.000</td>
</tr>
</tbody>
</table>

Presented values are the means of 3 replicate trials ± SD

Although none of the reference samples had visible WD, all of them presented small visible clusters. According to Lee & Lucey [21], stirred yogurts are likely to have clusters of protein aggregates which are presumably created by the collisions and shearing during the mixing process involved in their production. Due to this mechanical process, the characteristic three-dimensional gel matrix of set yogurt is no longer visible in stirred products. Lee & Lucey [40] stated that stirred yogurt is a weak gel system and although “particle size” is sometimes reported for stirred yogurt it should be recognized that there are no individual particles; rather, there are weakly associated clusters of proteins that make up the network. The stirring action associated with the production of stirred yogurts disrupts the weak protein network and creates “particles”. It is important to remark that the damage done to the coagulum during the production of concentrated stirred yogurts has a major impact on the viscosity of the final products. The larger the undisturbed aggregations of casein, and the smaller the whey-filled spaces, the higher the viscosity of the final product [32].

Several researchers, such as Weidendorfer et al. [36], studied and continue to study the way to avoid or minimize visual particles in stirred yogurt. Kucukcetin [43] stated that numerous manufacturing parameters, such as high incubation temperatures, excessive whey protein to casein ratios, certain types of starter cultures and the use of excessive amounts of starter culture, are associated with textural defects of stirred yogurt, including graininess (particles) and surface roughness (irregularities in the yogurt matrix). Thus, to minimize visual clusters in the final product, it is very important to control these production parameters.

Conclusions

Protein is not the only ingredient shaping structure and rheological properties of Greek-style non-fat yoghurt. Other non-fat ingredients influence probably the structure of the protein particles and forces between protein molecules. Properties of commercial plain non-fat Greek-style yogurts change at the storage time. Samples become more solid, but with higher whey syneresis. After 35 days of storage commercial Greek-style non-fat yoghurt had good properties without visible whey drain drainage and small clusters.

References


Morphology of starch from *Araucaria angustifolia* seeds treated by HMT and studied by SEM, AFM, and XRD

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**Keywords:**
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*Pinhão*
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**Abstract**

**Introduction.** The aim of this study was to evaluate the main morphological and structural properties of native and modified starch by heat-moisture treatment (HMT) of “pinhão” seeds.

**Materials and methods.** “Pinhão” starch was extracted from seeds of *Araucaria angustifolia*. Amylose content was determined by iodine affinity. Humidity, ashes, protein and fats were determined by the Association of official analytical chemists (AOAC) methods. The microscopic techniques used were Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). For structural evaluation of the granules, X-ray Powder Diffraction (XRD) was used.

**Results and discussion.** The “pinhão” starch extracted from aqueous methodology showed an amylose content of 26.3%. The samples showed low moisture (<8.5%) content as well as ash content (<1.44%). By SEM and AFM, the largest average diameter (dₐ) was for untreated “pinhão” starch granules; 10.85 and 10.64 μm, respectively. The smallest granules were those treated with 10% humidity for 60 min and 120 °C. The medium roughness (rₐ) studied by AFM increased according to HMT, from 321.68 to 470.06 μm, respectively. A reduction in the relative crystallinity (Rc) was observed according to the HMT performed.

**Conclusions.** Round and oval shapes were found for the starch granules, with a flat face. Similarities were found in the mean diameter values (dₐ) between the techniques. The average diameter of the granules increased as humidity increased during the modification by HMT. The mean roughness (rₐ) increased with the HMT while the relative crystallinity (RC) decreased.
Introduction

Starch is a source of carbohydrates in nature. It is a natural biopolymer arranged as semi-crystalline granules. Their main fractions are macromolecules of linear amyllose and branched amyllopectin. Due the abundance it is main source of carbohydrates in human diet. Starches can be obtained from several sources as roots, seeds, stems, etc.

“Pinhão” are the seeds from *Araucaria angustifolia* tree. This is a pine tree and grows in south of Brazil and other countries of south America. The seed contain 68–72% starch which presents 26–28% amylose. Other compounds are proteins (≥3%), lipids (≥1%), soluble sugars (≥2.4%) and fibres, minerals, phenolic compounds (≥ 0.2%) [1–4].

Starch is useful in various industrial fields. However, in its native form has restricted limitations as low solubility in cold water, high viscosity, paste instability, etc. Talking in account this aspect, starch is frequently submitted to physical, chemical or enzymatic treatment, or their combinations in order to obtain some desirable properties to industrial applications [4, 5].

The heat-moisture treatment (HMT) of starches is a physical procedure that consists in add water upon starch (ratio <35%) and following by heating to temperatures above its gelatinisation temperature (90–120°C) and for some time (15 min – 16 hours). This hydrothermal modification is a technique considered natural and safe. It causes some features in starch granules, acts within the amorphous and crystalline regions and without destroying granular structure of starch granules [5–9].

The main microscopic techniques used in observation of starches are: optical microscopy – OM [2, 10, 11], scanning electron microscopy – SEM [5–11], atomic force microscopy – AFM [4, 12, 13]. In a previous review [14] has been reported the main characteristics (shape and size) of native starches from different sources obtained by SEM.

Many starches have been classified as A-type, B-type and C-type, by X-ray powder pattern diffractometry technique (XRD). This classification is in agreement with the position of diffraction angle (2θ). The A-type show higher peak intensity at 20 at 15°, 17°, 18° and 23°; the B-type with higher peak intensity for angles of refraction 20 at 5°, 6°, 15°, 17°, 22° and 23 °. The C-type is a blend of A and B with peaks at 20 at 15°, 17°, 22° and 23° [16]. On the other hand, with this technique it is possible to calculate the relative crystallinity of starch granules [11–13].

The “pinhão” is an unconventional source of starch, recognized for its nutritional value, and therefore encourages studies that enable its application. Thus, modifications can be tested to understand the behaviour of starch under different conditions employed. Physical modification, such as HMT, is widely valued as an environmentally friendly technology.

Therefore, the aim of this study was to evaluate the main morphologic and structural properties of untreated and modified starch by heat-moisture treatment (HMT) of “pinhão” seeds.

Materials and methods

Materials

The “pinhão” seeds were purchased from a popular market in the city of Ponta Grossa-PR-Brazil.
The starch was extracted in aqueous medium according to the methodology described by Bet et al., [1]. After extraction, the starch was filtered and dried in an oven at 40°C for 24 hours and maintained in a desiccator up to constant mass.

Methods

Amylose Content

The amylose content of “pinhão” starch was performed in agreement with literature [1]. A potentiometric titrator (702 SM Titrino, Brinkmann Instr., NY) was used in determination of iodine affinity (IA). The procedure was performed in triplicate. The calculation of the amylose content, the IA value of starch was divided by the IA according to the Equation 1:

\[
\text{Amylose (\%) = 100\% IA / 0.2}
\]  

Physicochemical Analysis

The moisture, ash, protein and fat content were determined as AOAC methodology [19].

Heat Moisture Treatment (HMT)

The heat-moisture treatment (HMT) of starches were performed in the following way [11]: an aliquot of 100 g “pinhão” starch was divided in four portions with 25 g. All samples were maintained in desiccator with anhydrous calcium chloride up to constant mass. Each sample show 8.1% moisture. One sample (untreated) was maintained in desiccator and labelled (a). Upon the second was added distilled water up to 10% (b); upon the third up to 15% (c) and upon the fourth up to 20%, respectively. The samples (b, c and d) were homogenised and transferred to three pressure flasks, sealed tightly with a hermetic lid. So, the flasks were maintained in an autoclave for 60 min at 120°C. After this time, the flasks were opened and maintained in desiccator up to constant mass.

Scanning Electron Microscopy (SEM)

The micro-images of each sample were observed with high resolution using a Scanning Electron Microscope (model VEGA 3 Tescan, Czech Rep.) [10]. Initially, the samples were sprayed on a carbon tape and metalized with gold and so observed. It was possible to observe the shape and measure its size as well as the average diameter (dₐ) of a number of granules.

Atomic Force Microscopy (AFM)

Micro-images of “pinhão” starches were observed with high resolution using an atomic force microscope SPM 9600 (Shimadzu, Japan), by the non-contact method [8]. The starch samples were spread directly onto an adhesive tape fixed in the AFM sample holder. The micro-images of samples were observed with high resolution in 2D or 3D. Measurements and average diameter “dₐ” can be performed. With the scanning of surface of a set of granules it is possible to observe that this surface has irregularities. Thus, more or less smooth parts can be observed with protrusions and depressions. The measurement parameter that corresponds to the arithmetic mean of the absolute values of the distance ordinates (y₁, y₂, y₃, ..., yₙ), divided by the number of measurements (yₙ) in the considered distance (dₐ), Figure 1.
The Equation 2 was used in the calculation of average roughness ($r_a$):

$$r_a = \frac{y_1 + y_2 + y_3 + \ldots}{n} = \frac{y_n}{n}$$

(X-ray Powder Pattern Diffractometry (XRD))

The X-ray diffractogram of each sample was obtained by the instrument Ultima IV (Rigaku, Japan). The following parameters were used: Cu K $\alpha$ radiation ($\lambda = 1.544\AA$), voltage 40 kV and current 30 mA; scanning speed 2 min$^{-1}$, step 0.02, Bragg-Brentano geometry of $5^\circ < \theta < 80^\circ$ (20). The main degrees of diffraction angles were observed and registered. The relative crystallinity was calculated in agreement with Equation 3, as described in literature [7, 12].

$$R_c = \frac{A_p}{A_p + A_b} \cdot 100$$

where: $R_c$ = relative crystallinity; $A_p$ = peak área; $A_b$ = base area

Results and discussion

The amylose content in the studied “pinhão” starch was 26.3%. Similar values were found by Bello-Pérez et al. and Costa et al. [10, 12].

The moisture content for untreated and HMT modified starches were, 8.5%, 6.4%, 7.0% and 7.4%, respectively.

The ashes were determined quantitatively and the values obtained were: 1.44%, 1.08%, 1.39% and 0.85%, respectively.

The protein (2.12%) and fat (0.44%) content were determined only for untreated "pinhão" starch, due to the changes that these macronutrients can undergo during HMT.

Scanning Electron Microscopy (SEM)

The microimages obtained by SEM are collected in Figure 2.
Figure 2. Scanning electron microscopy (SEM) of “pinhão” starch granules (a) untreated; (b), (c), and (d), treated by HMT (magnification 1200 X, bar 10 µm)

According to the micrographs observed by SEM, the starch granules of the "pinhão" presented a rounded or oval shape, besides a flat face with some protuberances and depressions. No aggregation of granules was found in this investigation. Pinto et al., 2015, found aggregation in pinion starch granules when they were modified with moisture ≥25% [6]. The mean diameter of untreated and modified starches measured by SEM was 14.65 µm; the smallest granules had a diameter of 8.1 µm and the largest 23.6 µm. Conto et al., 2011 [15], found values of 10-25 µm. The measurement of the average diameter (dₐ) by SEM is shown in Table 1.

**Atomic Force Microscopy (AFM)**

This tool was used as the contactless method (AFM-NC). The samples were spread directly on an adhesive tape fixed on the sample holder, and this was enough to immobilize the granules and avoid contamination of the starch surface. In Figure 3(a) scan size (24 x 24 µm), a 3D acquisition image of the typical untreated “pinhão” starch granule demonstrating an oval shape is shown. On the surface we can see that it is relatively smooth with depressions and shallow grooves. Below Figure 3(b), the same 2D image of the starch granule with the measurements and next to the segments A-B, C-D, E-F, G-H that represents the height profile
of the morphological surface. Considering the values found for the cross-section segments: A-B = 20.67 µm, C-D = 15.01 µm, E-F = 16.65 µm and G-H = 19.46 µm, the average diameter ($d_a$) found for this granule was 17.95 µm. However, at least three similar measurements must be made and the mean as well as a larger number of granules must be calculated. The graph of the section in Figure 3(b), right, corresponds to the measurement of the surface topography from different angles of the granule and is used with the software of the instrument to calculate the average roughness ($r_a$), which was 302.4 µm for this granule.

In Figure 4, with the resolutions of 120 x 120, 50 x 50 µm, as in Figure 3, the 2D microimage of “pinhão” starch granules is represented. Here each segment A-B, C-D, E-F, G-H, displayed in a group of granules in each Figure, corresponds to a section measurement (in µm) that can be used to calculate the average diameter of each granule. On the right side of Figure 4-b, the segments displayed correspond to the surface of the starch granules and are used to calculate the average roughness ($r_a$), according to Equation 2.

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**Figure 3.** NC-AFM, 3D (a) and 2D (b) topographic micro-images of untreated “pinhão” starch (scan size 24 x 24 µm), height difference 2.02 µm, and the cross section.
Since in a large amount of starch granules, they displayed different sizes, with each segment it is possible to measure different granules. Thus, other measurements are made in resolutions 20 x 20, 50 x 50, 80 x 80, 100 x 100 and 120 x 120 µm and in each one of them at least 4 measurements are made with 3 repetitions. Therefore, as shown in Figure 3, the measurements were made using the software of the instrument and it was possible to determine the average diameter ($d_a$) of the granules and the average roughness ($r_a$) of the surface granules.

In Figure 5 we can observe the 3D microimages of each sample of “pinhão” starch granules (untreated and after HMT with 10, 15 and 20% moisture), under a scan size of 50 x 50 µm.

Figure 4: NC-AFM, 2D micro-image of untreated “pinhão” starch
(a) and (b) – scan size 120 x 120 µm, height difference 4.29 µm;
(c) and (d) – scan size 50 x 50 µm, height difference 2.61 µm.
Figure 5. NC-AFM 3D micro-images of:
(a) – untreated “pinhão” starch granules;
(b) – “pinhão” starch granules with 10% moisture and after 60 min at 120 ºC;
(c) – “pinhão” starch granules with 15% moisture and after 60 min at 120 ºC;
(d) – “pinhão” starch granules with 20% moisture and after 60 min at 120 ºC.

Visually it is possible to verify that some changes occurred in the structure, mainly in the roughness, which increased proportionally with the increase of moisture in the HMT and heating for 60 minutes at 120 ºC. The roughness was calculated according to Equation 2 and the values are represented in Table 1.

Table 1
Average diameter ($d_a$) of “pinhão” starch granules measured by SEM and AFM and average roughness ($r_a$) measured by AFM
(a) – untreated “pinhão” starch granules;
(b) – “pinhão” starch granules with 10% moisture and after 60 min at 120 ºC;
(c) – “pinhão” starch granules with 15% moisture and after 60 min at 120 ºC;
(d) – “pinhão” starch granules with 20% moisture and after 60 min at 120 ºC.

<table>
<thead>
<tr>
<th>Samples</th>
<th>SEM – $d_a$ (µm)</th>
<th>AFM – $d_a$ (µm)</th>
<th>AFM – $r_a$ (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>10.85</td>
<td>10.64</td>
<td>321.68</td>
</tr>
<tr>
<td>(b)</td>
<td>9.56</td>
<td>9.29</td>
<td>426.15</td>
</tr>
<tr>
<td>(c)</td>
<td>9.67</td>
<td>9.37</td>
<td>439.72</td>
</tr>
<tr>
<td>(d)</td>
<td>10.48</td>
<td>10.26</td>
<td>470.06</td>
</tr>
</tbody>
</table>

X-ray Diffractometry (XRD)
The X-ray diffractometer pattern of untreated and modified “pinhão” starches is shown in Figure 6. The values of the main peaks in the diffraction angle 2θ are shown in Table 2. The analysis of these figures and values reveals that “pinhão” starches are semicrystalline solids, with main peaks (2θ) around 15, 17, 18 and 23º, characteristic of C-type.

![Figure 6. X-ray Diffractograms (XRD) of:](image)

(a) – untreated “pinhão” starch granules;
(b) – “pinhão” starch granules with 10% moisture and after 60 min at 120 ºC;
(c) – “pinhão” starch granules with 15% moisture and after 60 min at 120 ºC;
(d) – “pinhão” starch granules with 20% moisture and after 60 min at 120 ºC.

According to the literature [17], the C type starch pattern is a semicrystalline polymorph characteristic of cereal and tuber starches, being a mixture of A and B polymorphs.

The relative crystallinity (RC) of untreated “pinhão” starch and modified HMT was calculated. The results obtained are in Table 2, and a decrease in RC with an increase in HMT was observed. This behaviour was observed by other authors [7]. The differences in values may be associated with the starch extraction method, which in this study was performed by aqueous methodology.

| Table 2 |
XRD values of: (a) untreated “pinhão” starch granules; (b) “pinhão” starch granules with 10% moisture and after 60 min at 120 ºC; (c) “pinhão” starch granules with 15% moisture and after 60 min at 120 ºC; (d) “pinhão” starch granules with 20% moisture and after 60 min at 120 ºC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peaks (2θ)</th>
<th>Peaks (2θ)</th>
<th>Peaks (2θ)</th>
<th>Peaks (2θ)</th>
<th>Peaks (2θ)</th>
<th>Relative crystallinity (Rc) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>15.09</td>
<td>17.05</td>
<td>18.10</td>
<td>18.75</td>
<td>23.30</td>
<td>20.94</td>
</tr>
<tr>
<td>(b)</td>
<td>15.11</td>
<td>17.12</td>
<td>18.05</td>
<td>18.60</td>
<td>23.11</td>
<td>19.89</td>
</tr>
<tr>
<td>(c)</td>
<td>15.23</td>
<td>17.10</td>
<td>18.01</td>
<td>18.66</td>
<td>23.00</td>
<td>19.02</td>
</tr>
<tr>
<td>(d)</td>
<td>15.80</td>
<td>17.22</td>
<td>18.00</td>
<td>18.62</td>
<td>22.94</td>
<td>17.76</td>
</tr>
</tbody>
</table>

Conclusion

Untreated and modified HMT “pinhão” starch granules were investigated by SEM and AFM microscopy techniques, and the results were similar between the two techniques. Some changes could be observed in the surface of the granules when analyzed by AFM, mainly in the average roughness that increases with heat-moisture treatment. The XRD technique allowed to determine the characteristics of type C and to calculate the relative crystallinity that decreased with the treatment performed.

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References


Evaluation of visual characteristics of beer using the computer vision method

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Abstract

Introduction. The aim of this study was to monitor the colour and stability of foam of different types of beer (light and dark beer) using a non-destructive method - computer vision and digital image analysis.

Materials and methods. Beer colour and beer foam stability of different beer types (declared as dark and light beer type) were measured using computer vision method. Beer foam stability, expressed as change in foam height over time, is modelled using an exponential decay model. Measurement of foam decay generally involves measuring beer drainage or the decreasing height of the head.

Results and discussion. The dark beer was less bitter (16.75 IBU), with higher polyphenol content (181.80 EBC), compared to the light beer style (bitterness was 26.50 IBU, total polyphenol content 103.50 EBC). Alcohol content was mostly below 5% (4.82% for the pale beer and 4.90% for the dark beer), and pH was 4.39 for the pale beer and 4.43 for the dark beer. Beer colour was expressed in the CIEL*a*b* colour system, with darker beers having lower values for lightness ($L^*$ = 28.7), higher values for $a^*$ = 10.4, and lower values for $b^*$ = 4.4. In contrast, light beers were brighter ($L^*$ = 65.5), with lower values of $a^*$ = 7.7 and higher values of $b^*$ = 4.4. Dark beers had higher EBC, lower $L^*$, and higher $a^*$ values than pale beers due to the use of colouring malts that were kilned and roasted at a higher temperature at which Maillard reaction products were formed. The change in beer foam over time is a combination of liquid removal and bubble decay. The dark style of beer showed much more stable foam than the light beer. Beer foam height was statistically different ($p< 0.05$) between light and dark beer, with dark beer samples having higher initial foam height (66.1 mm) compared to light beer (48.7 mm). Dark beer samples had lower values of rate constant (0.0091 s$^{-1}$) and higher values of foam half-life time (76.1 s), implying that dark beer had more stable foam than light beer. In contrast, light beer samples had higher values of the rate constant (0.0110 s$^{-1}$) and lower values of the foam half-life time (63.2 s). The applied mathematical model (exponential decay model) was found to be suitable for predicting the change in foam decay rate and foam stability of light and dark beer samples. Moreover, the values of foam height of dry and wet part of foam were well predicted by the exponential decay model.

Conclusions. Computer vision has been shown to be a suitable, objective, reproducible, and reliable method for measuring the colour and stability of beer foam (total foam, wet foam, and dry foam) for light and dark beer varieties.

Keywords:
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Introduction

Among the important parameters of beer quality are the colour of beer, its clarity, bitterness and the volume fraction of alcohol. The colour and clarity of beer are expected to be constant after production and during storage and sale [1]. According to the colour of beer, we divide it into light, red, dark and black. The colour of beer conveys an important message to the consumer, so the darker colour of beer suggests a stronger taste and aroma, a higher percentage of alcohol and a richer fullness of beer, while the lighter colours of beer are just the opposite. [1,2] There are several methods for measuring beer colour like the Lovibond comparison system method, the spectrophotometric method, the tristimulus colorimetric method and the computer vision method [3].

The quality of the foam influences the overall perception of the consumer to a great extent. Therefore, the control of foam behaviour is essential. The physical properties responsible for beer foam behaviour are: foam or head formation, foam collapse, foam drainage, or head retention, bubble collapse, and bubble cling, lacing, or foam adhesion. [2,4] Numerous methods and procedures have been proposed to determine foam characteristics. Some of them are based on the rate of the drainage of liquid from the foam, like the Blom or Rudin method. The Blom method [5] is based on the rate of liquid drainage from the foam, where the rate of drainage is described as first order kinetic. After a short lag period, the logarithm of the weight of the foam is proportional to time. The Rudin tube method [6] uses a long tube of small diameter with the CO$_2$ injection for foam formation in the degassed samples. Some methods are based on the measurement of foam collapse, where the foam is measured by focusing a microscope onto the foam surface [3]. The most popular method to measure foam collapse is the NIBEM foam stability tester where foam collapse is measured with a conductivity probe, which follows the upper foam level as a function of time. The time elapse from 1 – 4 cm below the top of the glass is measured every cm and taken as a measure for foam behaviour. Other methods are based on the measurement of the conductivity of a foam or methods based on determining bubble-size distribution in aqueous foams. In general, it is possible to categorize foam measurement analysis methods by means of foam generation into two groups. [7]

The first group generates the foam using “natural” pouring techniques (Constant method, the foam collapse time by Yasui, the cylinder pour test by Vundla) [8,9]. The second group is artificial methods, which assess the foam stability using beer that is not carbonated. These foams are generated by gassing through porous frits, (Ross and Clark and Rudin) [6,10], passing through nozzles (Steinfurth Foam Stability Tester and Lg Foam tester (MEBAK)) [11], or by employing other methods such as shaking [12,13] or flashing (NIBEM) [8].

Most of the standard methods for measuring beer foam stability are based on measurements of the weight or volume of the liquid collapsed from the foam [5,6,14,15] and they have several limitations (Foam is generated in an atypical way, so the resulting foam are different from those of foam produced by typical beer-pouring methods; Liquid drainage is only one of the factors related to foam stability). Evans et al. [16] concluded that the ideal foam measurement method would be to assess beer foam quality with a combination of digital camera and image analysis software.

Given the need for objective instrumental measurements of colour in the coloured and almost translucent samples, the purpose of this paper was to find the method that can simultaneously determine the colours of both light and dark beer. In this study the same mathematical model (exponential decay model) was applied to model of the wet and dry parts of beer foam. Furthermore, the same method was applied for the purpose of determining the
foam stability of these two different types of beer. So far, there has been no research on the stability kinetics of beer foam, which would include both dark and light beer style. The beer model was improved by separating the wet part of the beer foam from the dry part of the foam, and by distinguishing between the contribution of drainage and condensation to the beer foam collapse. The basic concept was to develop a customer-oriented approach, so that values are measured in a consumer-use situation.

The aim of this study was to monitor the colour and stability of the foam of different types of beer (light and dark beer) using a non-destructive method – computer vision and digital image analysis.

Materials and methods

Materials

For this study, two bottled lager beer style sample sourced from Osječka pivovara d.d. (Osijek, Croatia) were used one declared as dark beer and the other as light beer. Six bottles per beer sample from two different batches (purchased in 2018 and 2019) were used for all the analyses. All measured parameters were made in four replicates, and average values were used for data analysis.

Physical-chemical analyses

Beer alcohol content was measured on an Alcolyzer (Anton Paar GmbH, Austria). Standard beer analyses and determination of colour, bitterness, total polyphenols and pH were carried out according to EBC methods 9.6, 9.8, 9.11 and 9.35 (Analytica-EBC®, 2010) [17]. Standard method for foam stability evaluation were conducted according to the MEBAK method 2.18.2 (MEBAK®, 2012) [11], using the NIBEM-TPH foam stability tester with the Inpack 2000 Sampling Device, type ISD (Haffmans, Holland).

Analysis of beer colour with computer vision

The colour stability of beer samples was evaluated with tristimulus analysis by using computer vision. The computer vision method is implemented in several steps: image acquisition, image analysis and extracting the features of interest.

Image acquisition. To acquire an image of beer sample, a digital camera was used (Canon EOS 1100D). Before shooting, the camera was calibrated with a Datacolor SpyderCHECKR™ calibration plate. Before the experiment, the beer samples were degassed, so that gas bubbles did not affect the colour measurements. Beer samples were held at 6 °C, and analyses were performed at ambient temperature.
(20 °C). Before analysis, the beer samples were degassed by gently stirring with a magnetic stirrer and the sample was filtered through a 0.45 μm membrane filter. A 100 ml of each beer sample was poured into a cylindrical pilsner beer glass, and placed inside the photographing chamber at a distance of 50 cm from the camera lens. The 24-bit coloured images was captured in TIFF format and sRGB colour model. The cylindrical pilsner beer glass used in the analysis is shown in Figure 1. The photographing chamber was illuminated by four LED lamps with a diffuser located on the outside.

**Image analysis.** After photographing, the colour of the samples was determined using the digital image analysis method. A digital image processing software, ImageJ™ (Wayne Rasband, National Institute of Health, Maryland, USA), was used to analyse the images obtained during colour tests. The results of colour measurement were expressed as values of red (R), green (G) and blue (B) in the RGB colour system. The obtained colour values were converted to the CIEL*a*b* colour model.

**Analysis of foam collapse rate and foam stability using the computer vision**

Analysis of beer foam was performed using the computer vision method in three steps: foam generation, measurement of foam height and foam collapse time, and calculation of foam stability value.

Before analysis beer samples were held at 6 °C, and analyses were performed at ambient temperature (20 ±1 °C). Foam is generated by natural pouring using the adopted method reported by Yasui [9], where a volume of 100 ml of beer was poured freehand into a 400 mL cylindrical pilsner beer glass from a precisely defined height. The angle between the bottle and the glass was adjusted to ensure that the beer struck the bottom of the glass and was poured as a steady stream for the duration of the pour.

![Figure 2. Beer foam collapse determined using the image analysis method](image-url)
The profile of the beer in a glass was recorded by a CCD-camera every 5 s for 10 min (600 s) period. After the image of foam profile was captured, photographs were subjected to digital image analysis, and the percentage of foam layer was measured using a computer software program ImageJ™. The 24-bit coloured foam images captured during tests were converted into an 8-bit format (Figure 2). After that, the images were converted to binary images using a procedure called thresholding and then the height of foam was calculated [20].

The beer height measurements were taken from the inside bottom of the glass to the beer/foam interface. The foam measurements were calculated according to Equation 1-2 [21]. The values for both the liquid and wet part of the foam were used for further mathematical modelling procedure.

\[ H_t(DFP) = H_t(F) + H_t(L) - H_{max}(L) \]  
\[ H_t(WFP) = H_t(F) - H_t(DFP) \]

where:
- \( H_t(DFP) \) – height of the dry part of the foam after a certain time \( t \),
- \( H_t(WFP) \) – height of the wet part of the foam after a certain time \( t \),
- \( H_t(F) \) – foam height after a certain time \( t \),
- \( H_t(L) \) – height of the liquid after a certain time \( t \),
- \( H_{max}(L) \) – maximum height of the liquid, at time \( t = 600s \).

Several parameters were extracted from the images: foam height, liquid height (beer beneath foam), and some parameters were calculated (foam collapse rate and foam half-life). The results of the image analysis showed the change in the height of the foam column, over time (Figure 3).

Modelling the change in foam column height over time

The method is based on the simultaneous measurement of the level of the foam-liquid interface and the foam height as a function of time. The height of the foam, the height of the wet part of foam and dry part of the foam, rate constant and foam half-life time (or head retention value) was thus obtained. The exponential decay model was used to predict the foam stability of different types of beer. Foam collapse follows an Exponential Decay Law (EDL) [5], where the foam height at time can be expressed as the following function of time (or first-order reaction model):

\[ H_t = H_0 \times e^{-kt} \]
\[ t_{1/2} = \frac{ln (2)}{k} \]

where:
- \( H_0 \) – initial height of the foam at time \( t=0 \),
- \( H_t \) – height of the foam after a certain time \( t \),
- \( k \) – rate constant (s⁻¹),
- \( t \) – time (s),
- \( t_{1/2} \) – foam half-life time (s) or Head Retention Value (HRV).
Figure 3. Dynamics of beer foam stability measured using digital image analysis

The beer model was improved by separating the wet part of the beer foam from the dry part of the foam, and by distinguishing between the contribution of drainage and condensation to the beer foam collapse. In this study the same mathematical model (exponential decay model) was applied to model the wet and dry parts of the beer foam [21].

The XLSTAT plugin in MS Excel was used to process the experimental data, and the model parameters were calculated using regression analysis. The success of the approximation of experimental data by mathematical models was evaluated on the basis of several statistical criteria:

Coefficient of determination, \( R^2 \)

Mean square error, \( RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left( H_{pre,i} - H_{eks,i} \right)^2} \)

Statistical analysis

Data were expressed as means ± standard. Analysis of variance (ANOVA) was performed using the XLSTAT add-in within the MS Excel program (Addinsoft, NewYork, USA). Differences were considered to be significant at validity of \( \alpha = 0.95 \).
Results and discussion

Quality indicators such as foam stability, alcohol content, pH value, bitterness, polyphenols content and colour are important for consumers and reveal much about the beer type. The results of the physical–chemical analyses are shown in Table 2.

The dark beer style showed much stabile foam than light. Alcohol content was mostly below 5% as well as pH value, which was almost the same for all beer styles (below 4.4). The ethanol content of beer is very important, from both an economic and sensory points of view, as it is used to classify beers in terms of taxes and taste. The dark beer type was less bitter, with higher polyphenolic content and EBC colour value, in comparison to the light beer style (Table 2). Typically, the colour of the beer is due to the malt and other raw materials that were used in the brew house [7] and is largely due to the melanoidins and caramel present in the malt, although further caramelization can take place during wort boiling.

<table>
<thead>
<tr>
<th>Beer type</th>
<th>Foam stability (min)</th>
<th>Alcohol v/v (%)</th>
<th>pH value</th>
<th>Bitterness (IBU)</th>
<th>Total Polyphenols (EBC)</th>
<th>Colour (EBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>7.00±0.08&lt;sup&gt;b&lt;/sup&gt; 4.82 ±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.39 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.50 ±3.00&lt;sup&gt;a&lt;/sup&gt; 103.50 ±7.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.80 ±2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>10.00±0.09&lt;sup&gt;a&lt;/sup&gt; 4.90 ±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.43 ±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.75 ±3.86&lt;sup&gt;b&lt;/sup&gt; 181.80 ±6.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.14 ±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means of four replications ± SD.
Values in the same column with different superscripts (a-b) are significantly different ($p<0.05$).

Colour formation occurs in beer during caramelization and Maillard reactions. Caramelization can produce hundreds of different chemical products in different colours, but most of them are brown. Maillard’s reactions contribute to the colour of the beer by forming melanoidins during them, which give the beer a darker colour. Also, some products of Maillard’s reactions, such as furans, furanosines, pyrroles, and pyrazines affect the colour of beer. The increased concentration of these compounds in beer will cause beers to be darker, while a smaller amount of these compounds yields lighter beers [22]. Furthermore, the colour of beer is influenced by the polyphenols found in barley, and when malt is cooked, polyphenolic components are released, and some new components are formed, and colour is formed as well. During the storage and/or aging of beer, polyphenol oxidation can occur, which is very noticeable in light beers [23]. In darker beers, colour change due to polyphenol oxidation is masked by the colour of coloured and roasted malt. Furthermore, oxidation of the polyphenol can lead to an enhanced protein-polyphenol interaction and the formation of haze of non-biological origin. The colour change of beer due to a polyphenol oxidation is most noticeable in light beers during storage and/or aging of beer [1,24].

Low EBC values denotes (light) pale beer, and higher EBC values describe darker beers. Figures 4–6 show the colour measurement results of two different beer samples declared as dark and light lager beer. The colour of the samples was measured using the computer vision method and represented as CIE $L^*$, $a^*$ and $b^*$ values. The lightness ($L^*$) is achromatic component of CIE colour space in the range 0-100, the higher the $L^*$, the lighter the sample. The $a^*$ and $b^*$ values are chromatic component of the CIE colour space, and can be between –127 and +127. The chromatic component $a^*$ indicates the presence of a green-red colour.
(smaller $a^*$ means green, and the higher $a^*$ denotes red colour of sample). The chromatic component $b^*$ indicates the presence of a blue-yellow colour (smaller $b^*$ means blue, and the higher $b^*$ denotes yellow colour of sample).

**Figure 4. Results of the brightness value for different beer samples**

Values are means of four replications ± SD. Values with different letters (a-b) are significantly different ($p<0.05$)

**Figure 5. Results of the green-red chromatic component value for different beer samples**

Values are means of four replications ± SD. Values with different letters (a-b) are significantly different ($p<0.05$)

**Figure 6. Results of the blue-yellow chromatic component value of different beer samples**

Values are means of four replications ± SD. Values with different letters (a-b) are significantly different ($p<0.05$)

As can be seen in Table 2 and on Figures 4–6, light beer had the lower EBC values, the higher $L^*$ values and lower $a^*$ values than a dark beer. That indicates that these are the palest...
samples as they do not contain or contain a very low amount of special malts, which can contribute to their colour. Dark beer had higher EBC, lower $L^*$ and higher $a^*$ values than pale beer, due to the use of colouring malts which were kilned and roasted at a higher temperature where Maillard reaction products were formed [25].

**Results of foam stability measurements of different beer samples using the computer vision system and image analysis**

The foam stability of different types of beer was analysed by CVS and image analysis and expressed as the change in the height of the foam column ($H_0$) over time ($t$). Figures 7 – 8 shows the collapse of the head of a beer poured into a glass.

![Graph showing foam stability measurements of dark beer](image)

Values are means of four replications ± SD.

**Figure 7. The height of the beer foam as a function of time (dark beer)**
Values are means of four replications ± SD.

**Figure 8. The height of the beer foam as a function of time (light beer)**

The decay of beer foam takes place in three phases, i) the initial phase – it takes about 300 seconds, where the liquid beer drainage is driven by gravity, ii) the consolidation phase – characterised by an increase of the concentration of polypeptide material in the foam leading to bubble coalescence and foam collapse, iii) the residual phase.

When a beer is poured into a glass the initial burst of CO\textsubscript{2} creates foam with a high liquid fraction. There are actually two types of foam: "wet foam" consisting’s of spherical bubbles with liquid beer between them and "dry foam" consisting of polyhedral bubbles with no liquid between them. Liquid foam slips and slides while dry foam is stiff and sticky. The drainage of beer from the foam begins as soon as the pour is complete. The rate of foam collapse depends on the progress of three physical processes involved in the breakdown of the foam: drainage, coalescence and disproportionation. When beer is poured into a glass, initially, drainage is the main process, and at a later stage, coalescence and disproportionation become more important. Creaming, also called beading, is the continuous formation of new bubbles. Creaming is how foam forms from bubbles rising out of a glass of beer. Drainage is the liquid flow from a foam to the liquid underneath. It is not well-defined where creaming
stops and drainage begins. The main driving force for both processes is gravity. Drainage occurs if the bubbles become more densely packed. The foam becomes dryer, and the bubbles become deformed. Coalescence in foam is the merge of two bubbles caused by the rupture of the film between the bubbles. Two smaller bubbles become one larger bubble [26].

Figures 7–8 show the experimentally obtained and model-predicted values of the height of the foam column change as a function of time for the dark and light beer style. The values of the height of the beer foam $H_0$ of the dry and wet part of the foam was well predicted with the exponential decay law model, in contrast to the predicted values of the height of the beer foam for the initial foam. The diagram shows the exponential decay of the beer head associated with the measurement. The regression curves match the measured data well, which means that the beer foam collapsed quickly, as does the beer drainage. Correspondingly, the model of beer height estimate well the real height. This was expected because at the beginning of the foam decay, the packing density of the bubbles in the foam was much less than later in the experiment (which leads to a rapid loss of beer in the foam in the first 30 seconds following the pour) [27].

Using regression and curve fitting techniques, the generalized parameters for each foam sample was obtained, which is shown in Tables 3–5. The success of approximation of experimental data by the selected mathematical model was analysed on the basis of several statistical criteria. In this paper, the coefficient of determination ($R^2$) is presented, which would ideally have a value of 1, and the mean square deviation (RMSE) at which smaller values indicate a more successful approximation of the data by the applied model. In Tables 3–5, a comparison of $R^2$ for the least squares regression of the change in the height of the foam column over time indicates that $R^2$ was higher in the dry foam set, (dark beer $R^2 = 0.9972$, light beer $R^2 = 0.9765$) than in the wet part of the foam (dark beer $R^2 = 0.9595$, light beer $R^2 = 0.9201$) or in the total foam (dark beer $R^2 = 0.9893$, light beer $R^2 = 0.9695$). This is a consequence of in-creasing the variance in the pour value for dry foam since it contains the variance from three measurements [28].

Table 2

<table>
<thead>
<tr>
<th>Beer type</th>
<th>$H_0$ (mm)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$t_{1/2}$ (s)</th>
<th>$R^2$</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>66.1±2.0</td>
<td>0.0091±0.0004</td>
<td>76.1±3.4</td>
<td>0.9893</td>
<td>1.7242</td>
</tr>
<tr>
<td>Light</td>
<td>48.7±1.4</td>
<td>0.0110±0.0000</td>
<td>63.2±0.1</td>
<td>0.9695</td>
<td>2.2525</td>
</tr>
</tbody>
</table>

$p$-value 0.0194* 0.0465* 0.0640

Values are means of four replications ± SD. Values marked with *are statistically significant ($p < 0.05$).

The effect of drainage time on the amount of beer in the foam was similar to curves found by Blom [5], who suggested a first-order reaction model, i.e., the rate of collapse decreases logarithmically with time. After the mathematical modelling of the beer foam stability, the parameters of the exponential decay model were obtained: the rate constant $k$ and the foam half-life time $t_{1/2}$. If the values of the rate constant are lower and the foam half-life time is higher, then the foam of the analysed samples is more stable.

Considering the obtained results of the height of the beer foam, there was a statistically significant difference ($p<0.05$) between the light and dark beer, where the samples of dark
beer showed a higher initial height of foam (Table 3). There was a statistically significant difference between the values of rate constant and no statistically significant difference between the values of foam half-life time in the observed beer samples. Dark beer samples had lower rate constant (0.0091 s\(^{-1}\)) values and higher foam half-life time values (76.1 s), which means that dark beer had more stable foam than light beer. The foam height of the light beer was less stable than that of dark beer (Table 3). This means that the growth of the bubble size in the case of the light beer type mentioned leads relatively quickly to bursting. The foam-forming proteins of the dark beer type on the other hand, appear to stabilize the large foam lamellae well so that its fluffy, large-pored foam remains intact for a long time [29].

Table 3

Statistical analysis and parameters of an exponential decay mathematical model for wet foam stability

<table>
<thead>
<tr>
<th>Beer type</th>
<th>(H_0) (mm)</th>
<th>(k) (s(^{-1}))</th>
<th>(t_{1/2}) (s)</th>
<th>(R^2)</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>24.6±0.7</td>
<td>0.0342±0.0012</td>
<td>20.3±0.7</td>
<td>0.9595</td>
<td>0.7659</td>
</tr>
<tr>
<td>Light</td>
<td>19.7±1.2</td>
<td>0.0279±0.0029</td>
<td>25.1±2.6</td>
<td>0.9201</td>
<td>0.9383</td>
</tr>
</tbody>
</table>

\(p\)-value 0.0771 0.1786 0.2122

Values are means of four replications ± SD. Values marked with *are statistically significant (\(p < 0.05\)).

Table 4 shows the results of the stability of the wet part of the foam of light and dark beer. According to the values of statistical criteria it is evident that the exponential decay model fits well the experimental data (low values of RMSE for all analysed beer type) and can be used to predict the stability of the wet part of foam for the light and dark beer types [21]. Given the higher \(R^2\) values for dark beer samples, the model better predicts the stability of the wet part of the foam in dark beer samples. Considering the obtained results of the height of the beer foam of the wet part of the foam, there was no statistically significant difference between light and dark beer, where the samples of dark beer showed a higher initial height of the wet part of the foam (24.6 mm), higher rate constant (0.0342 s\(^{-1}\)) values and lower foam half-life time values (20.3 s) which means that the dark beer type has a less stable wet part of the foam than the light beer type.

Table 4

Statistical analysis and parameters of an exponential decay mathematical model for dry foam stability

<table>
<thead>
<tr>
<th>Beer type</th>
<th>(H_0) (mm)</th>
<th>(k) (s(^{-1}))</th>
<th>(t_{1/2}) (s)</th>
<th>(R^2)</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>51.5±2.6(^{bc})</td>
<td>0.0078±0.0005</td>
<td>88.1±5.6</td>
<td>0.9972</td>
<td>0.6800</td>
</tr>
<tr>
<td>Light</td>
<td>34.1±1.5(^{ef})</td>
<td>0.0091±0.0001</td>
<td>76.2±0.9</td>
<td>0.9765</td>
<td>1.4704</td>
</tr>
</tbody>
</table>

\(p\)-value 0.0289* 0.1325 0.1590

Values are means of four replications ± SD. Values marked with *are statistically significant (\(p < 0.05\)).
The values of the rate constant and the foam half-life time indicated a higher stability of the dry part of the foam in samples of dark beer. They had lower values rate constant (0.0078 s\(^{-1}\)) and higher values of the foam half-life time (88.1 s) of the loss of the dry part of the beer foam. Given the obtained results, it is evident that there was a statistically significant difference in the stability of the dry part of the foam between light and dark beer.

The dark beer samples showed higher values of the height of the beer foam and longer foam half-life time of the dry part of the foam (Table 5). According to the values of statistical criteria for the success of approximation of experimental data by an exponential decay model, it can be seen that the used model fit well the experimental data (\(R^2\) values are high) and can be used to predict foam stability for both beer types, light and dark.

**Conclusion**

The computer vision system and image analysis have been shown to be a suitable, objective, reproducible, and reliable method for measuring the colour, foam breakdown rate, and foam stability of beer (namely, total foam, wet foam, and dry part of the foam). Furthermore, the method is sensitive enough that it can be applied to analyse different types of beer (light and dark). The parameters of colour coordinates (defined in CIEL*a*b*\(a\text{,}1976\) colour space) measured in the entire visible wavelength range can distinguish beers more objectively than methods based on absorbance.

The concept of using a simple technique (naked eye assessment of foam quality), has a high correlation with consumer perception of beer foam quality and can be improved by using computer vision technologies for objective assessment of foam quality parameters.

The change in beer foam over time is a combination of liquid drainage and bubble decay. Measurement of foam decay generally involves measuring beer drainage or decreasing head height. From the foam stability results, dark beers have a more stable foam. The mathematical model used (exponential decay model) was found to be suitable for predicting the change in foam collapse rate and foam stability of light and dark beers. This model can be used as a cost-effective method for rapid screening of beers during processing to evaluate acceptability more efficiently.

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**References**


Antioxidant ability of alcoholic infusions from vegetable raw materials

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Alcohol
Beverage
Antioxidant
Redox
Infusion

Abstract

Introduction. The aim of the study is to develop the scientific bases of antioxidant activity of water-alcohol infusions from vegetable raw materials and to identify the most promising plants as sources of natural antioxidants.

Materials and methods. Alcohol infusions from vegetable raw materials, which were considered on morphological grounds: herbs (35 samples); roots and rhizomes (9 samples); flowers (7 samples); tree bark (2 samples); dried fruits (18 samples); juicy fruits (29 samples). Methods of investigation: redoxmetry – determination of antioxidant capacity of from plant raw materials; pH-metry.

Results and discussion. Infusions of alcohol from vegetable raw materials have a hydrogen index (pH) of 3.13 (Sudanese rose) to 8.17 units pH (Stinging nettle).

The minimum theoretical value of redox potential (RP) (Eh min) has a value from 159.1 mV (Stinging nettle) to 370.8 mV (Sudanese rose), and the actual value of RP (Eh act) from 8.0 mV up to 308.5 mV (Quince oblong).

The minimum value of antioxidant (restorative) ability of alcohol infusions (RE inf) is for Lemon fruits – 18.8 mV, the maximum value of restorative ability – 209.0 mV – for alcohol infusion of Wild strawberry.

The energy of reduction/oxidation of plant raw materials (RE plant) relative to the alcohol mixture is in the range of reduction values from 124.5 mV (Wild strawberry leaves) to oxidative values of -65.7 mV (Lemon fruit).

Alcohol infusions, depending on the activity of plant raw materials have a reducing ability (over 0 mV) – 65% of samples and oxidizing capacity (less than 0 mV) – 35% of samples.

The creation of alcoholic tinctures with antioxidant action allows you to bring to market new products that favorably distinguish the range of manufacturers from the range of competitors, creating a positive image of the company.

Conclusion. The expansion of the range of alcoholic beverages with the use of alcoholic infusions from plant raw materials to enhance the antioxidant effect is justified.
Introduction

One of the promising ways to form consumer properties and expand the range of alcoholic beverages is the use of various compositions of ingredients, food additives and biologically active substances [1–10]. Such substances are designed to improve the organoleptic properties of alcoholic beverages, give them stable therapeutic [11] and prophylactic properties [12].

It is especially important to reduce the toxicity of ethyl alcohol and its impurities, alleviate or eliminate the hangover syndrome, by affecting the metabolism of ethyl alcohol and its impurities in the body [4, 5]. Creating alcoholic beverages with low toxicity [3, 13–15] allows you to bring to market new products that distinguish the manufacturer's range from the range of competitors, creating a favorable image of the company [16], which cares about protecting consumers from the negative effects of alcoholic beverages [3].

Currently, the use of vegetable raw materials in the technology of alcoholic beverages is very relevant [17–19]. Herbal beverages commonly consumed worldwide contain different chemical substances that display a broad spectrum of biological activities [20–22]. They have gained growing interest among scientists and consumers due to their antioxidant properties [23]. The ability of plant phenolics to act as free radical scavengers has led to increased interest in their ability to act as antioxidants [24, 25]. At present, the antioxidant characteristics of all prescription components, food additives, biologically active substances and their combinations have not been sufficiently studied [26, 27, 28].

$RP$ is an important indicator of the biological activity of solutions [29]. It characterizes the deviation from the ionic balance of free electrons in a liquid medium. Changing the concentration of free electrons leads to a change in its electron charge and, accordingly, the $RP$. If the $RP$ is positive, it indicates the oxidizing ability of the solution, negative indicates recovery ability. The value of $RP$ allows to estimate the energy of processes, that is, characterizes the activity of ions in redox reactions [30, 31]. Redox reactions affect the ratio of energy to support homeostasis – relativity of dynamic constancy of composition and properties of internal environment and stability of basic physiological functions of an organism. This ensures the vital activity of any organism. The magnitude of this rate depends on the ratio and concentration of oxidized and reduced substances in the body, including substances coming from food and beverages, so one of the main factors in the regulation of redox reactions is the $RP$ [30, 31].

These circumstances determine the relevance of this work, which is to develop water-alcohol extracts of vegetable raw materials in the technology of alcoholic beverages. Creating alcoholic beverages with reduced toxicity through the introduction of water-alcohol infusions from vegetable raw materials antioxidant properties, allows to create products, which favorably differentiates them from competitors, creating a favorable image of the institution, which cares for the protection of consumers.

At present, the antioxidant characteristics of all prescription components, food additives, biologically active substances and their combinations have not been sufficiently studied.

The aim of the study is to develop the scientific bases of antioxidant activity of water-alcohol infusions from vegetable raw materials and to identify the most promising plants as sources of natural antioxidants.

When achieving this goal, it is necessary to solve the following problems:
- To substantiate the prospect of using water-alcohol infusions from vegetable raw materials in the production of alcoholic beverages;
- To establish the value of the restorative capacity of water-alcohol infusions from vegetable raw materials;
- Identify the most promising sources of natural antioxidants for use in alcoholic beverage technology.
Materials and methods

Materials

100 plant samples were used as objects, which were considered on morphological grounds: herbs (35 samples); roots and rhizomes (9 samples); flowers (7 samples); tree bark (2 samples); dried fruits (18 samples); juicy fruits (29 samples). As a control sample used a water-alcohol mixture with a strength of 40% vol.

Methods of obtaining water-alcohol infusions [3, 15]

Vegetable raw materials were ground with scissors to a size of 3×3 mm, samples weighing 4 g were placed in bottles of dark composition, poured 100 ml of a water-alcohol mixture with a strength of 40% vol. The vials were capped and placed in a Durocell dry air thermostat for 48 hours at 40 ºC. The resulting infusions were cooled to room temperature [3, 15].

Methods for determining active acidity and RP [3, 15, 30]

The active acidity index was measured on a pH-meter «pH-150 MA» with a combined glass electrode «ESC 10601/4». RP was measured on the pH-meter «pH-150M», in the mode of measuring the potential, with a redoxmetric platinum electrode «ERP-105».

To evaluate the antioxidant properties of the obtained water-alcoholic plant extracts, the method [30], based on the difference of RP in inactivated inorganic solutions and complex biochemical media. The main criteria of this method were its clarity, simplicity, specificity, reproducibility of results and efficiency. A number of researchers also emphasize that method allows to determine the total antioxidant activity of liquid products, including in total in a complex mixture, and multifunctional antioxidants [3, 15].

Formula (1) holds for inactivated inorganic solutions in equilibrium. This formula links the active acidity of the pH and the RP [30]:

$$Eh_{\text{min}}=660-60\cdot pH, \text{mV}$$

(1)

where $Eh_{\text{min}}$ – the minimum theoretically expected value of the RP;

$pH$ – active acidity of the test solution.

Acquired meanings of $Eh_{\text{min}}$ were compared with the actual measurements of $Eh_{\text{act}}$ of solution. The shift of RP to the side of the recovered meanings – recovery energy (RE) was determined by the formula [30]:

$$RE = Eh_{\text{min}}-Eh_{\text{act}}, \text{mV}$$

(2)

where $RE$ – the shift of RP to the side of recovered meanings (resilience);

$Eh_{\text{min}}$ – minimal theoretically expected meaning of RP;

$Eh_{\text{act}}$ – actual measured RP.

The value of RP allows you to estimate the energy of the processes, i.e. characterizes the activity of ions in redox reactions.
Research of RP from hydrogen display in infusions of alcohols from vegetable raw materials

For water-alcohol mixture, the relationship between hydrogen (pH) and RP (Eh) was experimentally determined. It is proved that the change of the hydrogen index of the water-alcohol mixture by 1 unit, pH leads to a change in RP by 42 mV:

\[ Eh_{min} = 502 - 42 \cdot \text{pH}, \text{ mV} \]  

(3)

In the range of values of the hydrogen index of pH 2.0-11.0 pH units for the water-alcohol mixture of RP is in the range of \( Eh_{min} \) 418.0-40.0 mV.

The obtained values of the RP of the water-alcohol mixture \( Eh_{min} \) were correlated with the actually measured values of the RP of alcohol infusions from vegetable raw materials by the platinum electrode (\( Eh_{act} \)), which characterizes the difference raw materials (\( RE_{inf} \)):

\[ RE_{inf} = Eh_{min} - Eh_{act}, \text{ mV} \]  

(4)

The energy of reduction/oxidation of vegetable raw materials (\( RE_{plant} \)) is determined by the difference between the RP of infusions of alcohol from vegetable raw materials (\( RE_{inf} \)) and solvent (control):

\[ RE_{plant} = RE_{inf} - RE_{sol}, \text{ mV} \]  

(5)

According to the results of research, an improved method for assessing the antioxidant capacity of infusions of alcohol from vegetable raw materials for the technology of alcoholic beverages.

Results and discussions

Expansion of the range of alcoholic beverages with the use of alcoholic infusions from vegetable raw materials with increased antioxidant action is substantiated.

Alcohol infusion – a semi-finished product, which is prepared by infusion of vegetable raw materials (both aromatic and non-aromatic) in a water-alcohol mixture [32] with a strength of 40 to 90% vol.

For the preparation of alcohol infusions used: rectified ethyl alcohol [33-34]; water [26]; vegetable raw materials, which are allowed for use in alcoholic beverages; filter cardboard.

The studies used 100 samples of vegetable raw materials by morphological characteristics [35]: herbs; roots and rhizomes; flowers; tree bark; dried fruits; juicy fruits. As a solvent used a water-alcohol mixture with a strength of 40%: pH 5.63 units pH; \( Eh_{min} \) 265.5 mV; \( Eh_{act} \) 181.0 mV; \( RE_{inf} \) 84.5 mV; \( RE_{plant} \) 0.0 mV. Sensory evaluation of the control sample are as follows: color – colorless; aroma – alcohol; taste – moderately burning, empty.
Study of alcoholic infusions of herbs

35 samples of alcohol infusions from herbs were studied (Figure 1, 2). The pH level for alcohol infusions of herbs ranges from 4.53 (Wild strawberry) to 8.17 (Stinging nettle), which have a reaction environment – from acidic to alkaline. The minimum theoretically expected RP (Eh\text{min}) of alcohol infusions of herbs ranges from 159.1 mV (Stinging nettle) to 312.0 mV (Wild strawberry).

Figure 1. Characteristics of alcohol infusions of herbs

Figure 2. Characteristics of alcohol infusions of herbs
Actually measured $RP$ ($E_{\text{act}}$) from 8.0 mV (Stinging nettle) to 201.5 mV (Common cherry). At the same time, the minimum value of energy of reduction/oxidation of alcohol infusions ($E_{\text{act}}$) – 35.4 mV is set for Curly parsley, and the highest – 209.0 mV – for Wild strawberry. The minimum value of energy of reduction/oxidation of vegetable raw materials ($RE_{\text{plant}}$) is set for Curly parsley $\triangleleft$49.1 mV (oxidative values), the maximum value of 124.5 mV – for Wild strawberry (reduction values).

5 samples of herbs, depending on the energy of reduction/oxidation, have oxidizing characteristics from $\triangleleft$49.1 mV (Parsley curly) to $\triangleleft$5.6 mV (Violet tricolor), which is 14% of all herbs. 30 samples of herbs have regenerative characteristics from 1.0 mV (Basil eugenol) to 124.5 mV (Wild strawberry), which is 86%.

According to sensory evaluation, infusions of alcohol from herbs [47–49] are recommended for use in the production of tinctures, aromatic alcohols, balms, liqueurs [17–19, 21, 36].

Infusions of alcohol from Wild strawberry have received the greatest value of antioxidant activity; sensory evaluations of the product: color – deep brown; aroma – herbal; taste – soft, tart, sour, which is recommended for use in alcoholic beverages for tinctures and balms.

**Research of alcoholic infusions from roots and rhizomes**

9 samples of alcohol infusions from roots and rhizomes were studied. The $pH$ level for infusions of alcohol from the roots and rhizomes is from 3.87 (Garden onion) to 6.86 (Carrot), i.e. infusions of alcohol have an acidic reaction, which is close to neutral (Figure 3).

![Figure 3. Characteristics of alcohol infusions from the roots and rhizomes](image)
The minimum theoretically expected RP \((Eh_{min})\) for alcohol infusions from roots and rhizomes ranges from 214.1 mV (Carrot) to 339.7 mV (Garden onion), and the actually measured RP \((Eh_{act})\) – from 98.0 mV (Naked licorice) to 199.0 mV (Elecampane high). At the same time, the minimum value of energy of reduction/oxidation of alcohol infusions \((RE_{inf})\) is characteristic of Allspice celery and is equal to 44.0 mV, and the highest value – 148.2 mV has an alcoholic infusion of Garden onion. The minimum value of energy of reduction/oxidation of vegetable raw materials \((RE_{plant})\) is set for Allspice celery \(\Leftrightarrow\) 40.5 mV (oxidative values), the maximum value of 63.7 mV – for Garden onion (reducing values).

In the group of plant raw materials roots and rhizomes were identified: plants with oxidative values of 5 samples (56%); plants with restorative values – 4 samples (44%).

When assessing the sensory evaluations of alcohol infusions from the roots and rhizomes of 3 samples (Elecampane high, Valerian officinalis, Garden onion) – it is not recommended to use for alcoholic beverages, due to their aroma and taste [37, 38]. Thus, Elecampane high has an unpleasant taste (soapy, sour-bitter, obsessive), alcohol infusion of valerian has an unpleasant (Valerian officinalis) aroma and tastes burning, sour, with an unpleasant aftertaste; infusion of alcohol from Garden onion has an unpleasant onion (nitrogen-containing compounds) aroma and tart taste. Sensory evaluations of the proposed (possible) options for the use of infusions of alcohol from the roots and rhizomes gives grounds not to offer for use in alcoholic beverages samples even with maximum antioxidant characteristics (for example, Garden onion with a high regenerative activity of 63.7 mV).

**Research of infusions of alcohol from flowers**

From the group flowers 7 samples were studied. The \(pH\) level for infusions of alcohol from flowers has a reaction of the environment from 3.13 (Sudanese rose) to 5.98 (Chamomile officinalis), i.e. infusions of alcohol from flowers have an acid reaction (Figure 4).

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**Figure 4. Characteristics of alcoholic infusions of flowers**
The minimum theoretically expected RP (Eh\textsubscript{min}) for infusions of alcohol from the flowers is from 251.1 mV (Chamomile officinalis) to 370.8 mV (Sudanese rose), and the actually measured RP (Eh\textsubscript{act}) – from 93.5 mV (Chamomile officinalis) to 239.0 mV (Sudanese rose). The minimum value of the energy of reduction/oxidation of alcohol infusions (RE\textsubscript{inf}) is characteristic of Calendula officinalis and is equal to 86.0 mV, and the highest value – 157.6 mV has an alcoholic infusion of Chamomile officinalis. The minimum value of energy of reduction/oxidation of vegetable raw materials (RE\textsubscript{plant}) is set for Calendula officinalis 1.5 mV (reducing values), the maximum value of 73.1 mV – for Chamomile officinalis (reducing values) [38, 39].

According to sensory evaluations, infusions of alcohol from flowers are recommended for use in the production of tinctures, aromatic alcohols, balms, liqueurs.

**Research of alcohol infusions from tree bark**

From the group tree bark 2 samples were studied. The pH level for infusions of alcohol Cinnamon (Cinnamon bark) is 5.34 and Rooibus – 5.52 units pH. The minimum theoretically expected RP (Eh\textsubscript{min}) is 277.9 mV (Cinnamon) and 270.2 mV (Rooibus), the actually measured RP (Eh\textsubscript{act}) is 208.5 mV and 155.0 mV, respectively. At the same time, the minimum value of energy of reduction/oxidation of vegetable raw materials (RE\textsubscript{plant}) is equal to 69.4 mV and 115.0 mV, respectively (Figure 5).

According to sensory evaluations, infusions of alcohol from the bark are recommended for use in the production of tinctures, aromatic alcohols, balms.
Research of infusions of alcohol from dried fruits

In the group dried fruits 18 samples were studied. The pH level of infusions of alcohol from dried fruits (Figure 6) ranges from 4.48 (Badian real) to 6.82 (Horse chestnut), i.e. infusions of alcohol from dried fruits has an acidic environment that is close to neutral.

![Figure 6. Characteristics of alcoholic infusions of dried fruits](image)

The minimum theoretically expected RP (Eh_{min}) for infusions of alcohol from dried fruits has a value from 215.6 mV (Horse chestnut), to 314.1 mV (Badian real), the actually measured RP (Eh_{act}) – from 83.5 mV (Flax ordinary) to 231.5 mV (Badian real). The minimum value of the energy of reduction/oxidation of alcohol infusions (RE_{inf}) is typical for Walnuts [40] – 44.5 mV, and the highest value – 158.9 mV has an alcoholic infusion of Cocoa. The minimum value of energy of reduction/oxidation of vegetable raw materials (RE_{plant}) is set for Walnut «–» 40.0 mV (oxidative values), the maximum value of 74.4 mV – for Cocoa (reduction values).

9 samples of dried fruits, depending on the energy of reduction/oxidation, have oxidizing characteristics from «–»40.0 mV (Walnut) [41] to «–»1.9 mV (Badian real), which is 50% of all dry fruits. 9 samples of dried fruits have restorative characteristics from 1.4 mV (Scots pine) [42] to 74.4 mV (Cocoa), which is 50%.

According to the sensory evaluations of 18 studied samples of alcoholic infusions of dried fruits – 1 sample (Flax ordinary) – is not recommended for use in the production of alcoholic beverages due to the aroma and taste (aroma of vegetable oil with a bitter taste).

Research of alcohol infusions from juicy fruits

In the group juicy fruits [33, 34] 29 samples were studied (Figure 7, 8). The pH level of infusions of alcohol from juicy fruits has an acidic environment, approaching neutral and varies from 3.60 (Black currant) to 6.77 (Sowing cucumber).
The minimum theoretically expected RP ($E_{\text{hmin}}$) for alcoholic infusions of juicy fruits is from 217.9 mV (Sowing cucumber), to 351.0 mV (Black currant), the actually measured RP ($E_{\text{hact}}$) – from 138.5 mV (Mountain ash) to 308.5 mV (Quince oblong). The minimum value of the energy of reduction/oxidation of alcohol infusions ($RE_{\text{inf}}$) is typical for Lemon 18.8 mV, and the highest value – 174.8 mV has an infusion of alcohol Grapefruit. The minimum value of energy of reduction/oxidation of vegetable raw materials ($RE_{\text{plant}}$) is set for Lemon $\approx$65.7 mV (oxidative values), the maximum value of 90.3 mV – for Grapefruit (reduction values).

15 samples of juicy fruits, depending on the energy of reduction/oxidation, have oxidizing characteristics from $\approx$ 65.7 mV (Lemon) to $\approx$ 2.2 mV (Grapes), which is 52% of all juicy fruits. 14 samples of juicy fruits have restorative characteristics from 3.5 mV (Grapes – raisins) to 90.3 mV (Grapefruit), which is 48%.

According to sensory evaluations, all infusions of alcohol from juicy fruits except infusion of Figs and Raisins are recommended for use in the production of tinctures, aromatic alcohols, balms, liqueurs. When examined in juicy fruits, a negative aroma of sulfur dioxide was detected, which is used for processing, in order to increase their shelf life.

It can be concluded that infusions of alcohol from vegetable raw materials have a hydrogen index of 3.13 (Sudanese rose) to 8.17 units pH (Stinging nettle). The minimum theoretical value of RP ($E_{\text{hmin}}$) has a value from 159.1 mV (Stinging nettle) to 370.8 mV (Sudanese rose), and the actual value of RP ($E_{\text{hact}}$) from 8.0 mV (Stinging nettle) up to 308.5 mV (Quince oblong). The minimum value of antioxidant (restorative) ability of alcohol infusions ($RE_{\text{inf}}$) is for lemon fruits – 18.8 mV, the maximum value of restorative ability – 209.0 mV – for alcohol infusion of Wild strawberry. It is proved that the energy of reduction/oxidation of plant raw materials ($RE_{\text{plant}}$) relative to the alcohol mixture is in the range of reduction values from 124.5 mV (Wild strawberry leaves) to oxidative values of $\approx$65.7 mV (Lemon fruit). It was found that alcohol infusions, depending on the activity of plant raw materials have a reducing ability (over 0 mV) – 65% of samples and oxidizing capacity (less than 0 mV) – 35% of samples.

**Conclusions**

1. The expansion of the range of alcoholic beverages with the use of alcoholic infusions from plant raw materials to improve sensory evaluations and enhance the antioxidant effect is justified. A study of alcohol infusions from vegetable raw materials, which were considered on morphological grounds: herbs (35 samples); roots and rhizomes (9 samples); flowers (7 samples); tree bark (2 samples); dried fruits (18 samples); juicy fruits (29 samples).

2. Infusions of alcohol from vegetable raw materials have a hydrogen index from 3.13 (Sudanese rose) to 8.17 units pH (Stinging nettle). The minimum theoretical value of RP ($E_{\text{hmin}}$) is from 159.1 mV (Stinging nettle) to 370.8 mV (Sudanese rose) and the actual value of RP ($E_{\text{hact}}$) from 8.0 mV (Stinging nettle) up to 308.5 mV (Quince oblong). The minimum value of antioxidant (restorative) ability of alcohol infusions ($RE_{\text{inf}}$) is for Lemon fruits – 18.8 mV, the maximum value of restorative ability – 209.0 mV – for alcohol infusion of Wild strawberry. It is proved that the energy of reduction/oxidation of vegetable raw materials ($RE_{\text{plant}}$) relative to water-alcohol mixtures is in the range of reduction values from 124.5 mV (Wild strawberry leaves) to oxidative values of -65.7 mV (Lemon fruit).
Alcohol infusions, depending on the activity of plant raw materials have a reducing ability (over 0 mV) – 65% of samples and oxidizing capacity (less than 0 mV) – 35% of samples. The creation of alcoholic beverages with antioxidant action allows you to bring to market new products that favorably distinguish the range of manufacturers from the range of competitors, creating a positive image of the company.

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Identification, biochemical and technological properties of *Enterococcus* species isolated from raw milk and traditional dairy products

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**Abstract**

**Introduction.** The purpose of research was isolation and identification of *Enterococcus* species from raw milk and traditional dairy products and their biochemical properties were examined. Some biochemical and technological properties of the strains were determined.

**Materials and methods.** Raw milk and some dairy product samples (Izmir Tulum cheese, Koy cheese, White cheese, butter, Ezine cheese, kefir grain, kefir drink, Armola, Tire Camur cheese, Herby cheese, Goat cheese and Cecil cheese) were collected in different regions of Turkey and samples were inoculated in Kanamycin Aesculin Azide Agar, Slanetz Bartley and M-17 Agar media and *Enterococcus* spp. were isolated. Acidification properties, exopolysaccharide (EPS) production, lipolytic and proteolytic activity, and decarboxylation activity of *Enterococcus* strains, which were diagnosed phenotypically and biochemically with different techniques were investigated.

**Results and discussion.** 167 lactic acid bacteria were identified after Gram staining and catalase tests. Due to the analysis, 122 of these isolates identified as *Enterococcus faecium*, 18 as *Enterococcus durans*, 17 as *Enterococcus faecalis*, 8 as *Enterococcus faecium* var. and 2 as *Enterococcus hirae*. Some biochemical and technological properties of these species were studied. It was determined that *E. faecium* and *E. faecalis* strains produced higher acidity compared to *E. durans* and *E. hirae*, a total of 19 strains were capable of producing EPS, while 9 strains showed poor EPS production. Also, it was performed 17 *E. faecium*, 2 *E. faecalis*, 1 *E. durans* and 1 *E. hirae* strains showed lipolytic activity and 95 *E. faecium*, 12 *E. durans*, 5 *E. faecalis* var., 3 *E. faecalis* and 2 *E. hirae* decarboxylated to lysine and ornithine amino acids. It was observed that *Enterococcus* spp. isolated from raw milk and traditional dairy products showed differences especially technological characteristics according to the source, species and strain.

**Conclusions.** Raw milk and dairy products are important source for isolation of enterococci species. The characteristics of *Enterococcus* species such as acidification, exopolysaccharide production ability, proteolytic and lipolytic activity, decarboxylase activity differed by species and strain.
Introduction

Lactic acid bacteria (LAB) are considered to be industrially important microorganisms because of their benefits in human health and nutrition and their fermentation ability. LAB are Gram-positive, prokaryotic bacteria identified as lactic acid productions as the final product. These bacteria are characterized by turning glucose into lactic acid. LAB generally include Lactobacillus, Pediococcus, Leuconostoc and Streptococcus species [1, 2]. Unlike other lactic acid bacteria, enterococci are not GRAS (generally recognized as safe) status, and their presence in water is considered to be an indicator of faecal contamination. However, enterococci are one of the lactic acid bacteria that have an important place in the fermented food industry due to their functional properties, production of lactic acid, proteolysis and lipolytic activities, citrate metabolism, probiotic properties and their ability to synthesize proteins with antimicrobial activity such as bacteriocin production [3, 4]. Enterococci are Gram positive cocci found in single, double or short chains. Until recently, they have been classified in the genus Streptococcus. Streptococcus faecalis was identified by Andrewes and Horder in 1906, and Streptococcus faecium in 1919 by Orla-Jensen [5, 6].

As a result of molecular studies carried out by Schleifer and Kilpper-Balz in 1984, it was suggested that S. faecalis and S. faecium should be separated from streptococci and transferred to the genus Enterococcus. Then the bacteria in this genus are E. faecalis, E. faecium, E. durans, E. avium, E. casseliflavus, E. malodoratus, E. hirae, E. gallinarum, E. mundtii, E. raffinosus, E. pseudoavium and they are divided into several species such as E. flavescent, E. dispar, E. sulfureus, E. saccharolyticus, E. columbae and E. cecorum [7, 8]. In some literatures, it is reported that E. durans and E. faecium are the same species [9, 10]. Devriese and Pot [11] gave E. durans within the group of E. faecium species consisting of E. faecium, E. durans, E. hirae and E. mundtii. In recent identification studies, E. durans and E. faecium are mentioned as two separate species [12–15].

Although the use of Enterococcus species may be a controversial issue, certain Enterococcus species can be used as probiotic, starter, and protective cultures, even as food and feed supplements [3, 4]. Studies have generally been carried out by focusing on some species of Enterococcus genera. Although there are studies on Enterococcus species isolated from traditional dairy products, as in our study, there are limited studies mainly on those isolated from Izmir Tulum Cheese. Also, studies on examining the technological properties of different species and strains are limited.

The purpose of research is isolation and identification of Enterococcus species from raw milk and traditional dairy products and their biochemical and technological properties of the strains were determined.

Materials and methods

Isolation of Enterococcus species

In aseptic conditions, 10 g and / or 10 mL sample was weighed into a sterile stomacher bag and 90 ml of 0.1% sterile peptone water solution was added. It was homogenized for two minutes using Stomacher (Colworth 400, UK) and 1ml was taken from this mixture and added to 9ml of 0.1% sterile peptone water (Merck, Germany) solution and mixed. Dilution series were prepared by taking 1 ml of the previous dilutions. From the selected dilutions, inoculation was performed using Kanamycin Aesculin Azide Agar (Merck, Germany), Slanetz Bartley Medium (Merck, Germany) and M-17 Agar (Merck, Germany). Petri dishes
were incubated at 37 °C for 24–48 hours under aerobic conditions, and the colonies that were considered to be *Enterococcus* species, which were thought to be *Enterococcus* species, were taken with the help of the essence and each colony was transferred to Kanamycin Esulin Azide Broth (Lab M, UK). The medium was inoculated and left for incubation at 37 °C. At the end of the incubation, Kanamycin Esulin Azide Agar was transferred by using the line cultivation method using the medium. Thus, isolates were purified, and purified colonies were transferred to M-17 Broth (Merck, Germany). Gram staining and catalase tests were performed in all isolates before being included in the diagnosis. The isolates which were determined to be Gram positive catalase negative and classified according to their morphology were transferred to M-17 Broth (Merck, Germany) medium containing 20% glycerol and stored at -20 °C. For this, M-17 broth + 500 μl active bacteria culture containing 500 μl, 40% glycerol (AppliChem, Germany) was taken into the eppendorf tube. The medium and glycerol were autoclaved in separate bottles (121 °C 15min) and mixed sterile before use.

**Phenotypic and biochemical identification**

Phenotypic identification of *Enterococcus* species was carried out by modifying the diagnostic stages of Reuter [16]. *Enterococcus faecium* B-2354, *Enterococcus casseliflavus* B-3502, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* UWWE 3080, *Enterococcus hirae* UWWE 3102 and *Enterococcus durans* GE-66 were used as reference cultures for the identification of bacteria. In order to identify Gram-positive catalase negative cocci at the level of species, development at different temperatures (at 10, 40 and 45 °C), development at different salt concentrations (2, 4 and 6.5%), development at pH 9.6, formation of ammonia from arginine, and hydrolyse of esculin. In addition to tests such as shredding, various carbohydrates fermenting tests were performed. 13 different carbohydrates were used for this (Lactose, Fructose, Galactose, Maltose, Melibiose, Salicin, Sucrose, Sorbitol, Raffinose, Arabinose, Mannitol, Trehalose and Inulin). Diagnostic tests were carried out by inoculating 1% of each bacterial culture incubated at 37 °C for 18–24 hours on M-17 medium. Except for those for temperature tests, tubes were incubated at 37 °C at the temperature at which the inoculated bacteria were isolated, examined at 24 hours, 48 hours, days 5 and 7, reactions were recorded as positive or negative.

**Identification with API test kits**

Among the strains that could not be identified by traditional diagnostic stages, have problems or appear suspicious, diagnostics were made using API® 20 Strep test kits. API® 20 Strep (BioMérieux, France). API® 20 Strep is a standardized method that combines 20 chemical tests with a large capacity. It provides the identification of many streptococci and enterococci at the group or species level. The reactions that occur are read according to the "reading table" and the diagnosis is carried out using the analytical profile index or a computerized diagnostic program. The API® 20 Strep test was performed as follows: The prepared M-17 agar (Merck, Germany) was poured into petri dishes and solidified. Each isolate was streaked on the surface of the medium in a separate petri dish. Colonies developing in petri dishes incubated at 37 °C for 24 hours were inoculated into test strips by applying the procedure steps described in the user manual of API® 20 Strep. A separate strip was used for each isolate. Strips were incubated at 37 °C. They were checked at the 4th and 24th hours of the incubation as specified in the instructions for use. The resulting changes were recorded and the results were evaluated using the diagnostic program (Apiweb® stand-alone V 1.1.0, BioMérieux).
Determination of technological properties

Acidification. The amount of lactic acid and pH changes occurring as a result of 24-hour incubation in skim milk containing 10% non-fat dry matter (reconstituted skim milk) were determined at the 3rd, 6th, 9th and 24th hours after inoculation [17].

Exopolysaccharide (EPS) production. Using the method specified by Rasic and Kurmann [18], the ability of strains to produce EPS was determined. To this end, M-17 containing double-force lactose and MRS agar medium containing double-force glucose were extracted from active cultures, and streaked colonies were performed, and colonies that creep at the end of the 48-hour incubation period were examined.

Lipolytic activity. Lipolytic activities of the strains were determined using Tributyrin Agar (Merck, Germany) containing Neutral tributyrin (Glycerol tributyrate; Merck). 10µL cell suspension was spotted on the petri dish and the petri dishes were incubated at 30 °C for 5 days. Lipolysis was determined by pouring 10mL of saturated copper sulphate solution onto the petri dish, and around the colonies being green blue [19, 20].

Proteolytic activity. Proteolytic activity of strains was performed according to Church et al. [21]. 3 mL o-phthaldialdehyde (Sigma-Aldrich, USA) reagent was added to 150 µL TCA filtrate and 5 sec. mixed 2 minutes at room temperature. The absorbance at 340 nm wavelength was read in the spectrometer Spekol-1300 (Analytik Jena, Germany) until the waiting time. Values are given as OPA (o-phthaldialdehyde) equivalent.

Decarboxylase activity. The ability of amino acids to produce biogenic amines by decarboxylation was tested using the media specified by Bover-Cid and Holzapfel [22], which contain any of the lysine and ornithine amino acids. In order to promote decarboxylase activity, strains were sub-cultured 2 times in M-17 broth (Merck, Germany) containing 0.1% precursor amino acid [23]. Active cultures were inoculated with 0.5% of Moeller decarboxylase Broth containing control, lysine and ornithine. The yellow color decarboxylation (-), which was formed by comparison with the control sample at the end of the 48 hour incubation, was evaluated as purple color decarboxylation (+). Escherichia coli O157: H7 ATCC 8739 reference strain was used as positive control in the study [24].

Results and discussion

Physiological and biochemical diagnostic results of Enterococcus species

As a result of Gram staining, catalase test and morphological examinations, a total of 167 isolates with Gram positive, catalase negative and cocci appearance were taken into various physiological and biochemical tests. Traditional methods were compared with API test results and those that were compliant were analysed further. Strains were named by coding K: the source of isolation and E: the strain number (Table 1).
As a result of the comparison and evaluation, 122 isolates were *E. faecium* (73.05%), 18 isolates were *E. durans* (10.78%), 17 isolates were *E. faecalis* (10.18%), 8 isolates were *E. faecalis* (4.79%) and 2 isolates were *E. hirae* (1.19%). Sources of isolation are given in Table 1. In the differentiation of *Enterococcus* species, sugars such as sorbitol, arabinose, mannitol and raffinose and changes in different temperature and salt concentrations were taken into consideration. These saccharides are reported to be critical sugars in the separation of *E. faecium*, *E. durans* and *E. hirae* species. Besides these saccharides, fermentation tests using sugars such as trehalose, lactose, fructose, galactose, maltose, mellobiose, salicin, sucrose and inulin helped in definitive diagnosis [25, 26].

In some literatures, *E. durans* and *E. faecium* are reported as the same species [9, 10]. Devriese and Pot [11] gave *E. durans* within the group of *E. faecium* species, consisting of *E. faecium*, *E. durans*, *E. hirae* and *E. mundtii*. In recent identification studies, *E. durans* and *E. faecium* are mentioned as two separate species [12–14]. Although it is stated in many research and literature that *Enterococcus* species develop at 6.5% salt concentration, the results obtained in the study show that there are strains that can develop in maximum 4% salt concentration. Similar results have been revealed by Guley [15]. Only reference strains of *E. casseliflavus* inulin (+) reacted, while only 2 of the *E. faecium* isolates gave a weak inulin reaction. *E. faecium* isolates gave variable reactions in terms of melibiose, raffinose and sucrose. Their ability to ferment sugar in question varies from strain to strain. Some strains of *E. faecium*, on the other hand, suggested that there was a mistake in the diagnosis of bacteria since mannitol gave a negative reaction, and it was concluded that this species may be a variant of *E. faecium* by making a detailed literature scan as a result of repeated positive reactions in repeated controls [11, 15, 25, 28,29].

### Table 1

<table>
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<th>Isolate codes</th>
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Acidification

Enterococcus species showed different acidification results depending on isolation source, species and strain differences. While some species produced high acidity, some species showed low acidity. When the isolated species were examined, E. faecium and E. faecalis strains produced higher acidity compared to E. durans and E. hirae, but some E. faecium strains formed low acidity and some E. hirae strains had some E. faecium. It can be seen that it can produce higher lactic acid than strains. In the medium of E. faecium strains containing 10% skim milk powder, pH values changed between 5.80 and 6.64 hours at the 3rd hour, while the amount of lactic acid (LA) occurred in the lowest (0.108%) K69E2 strain and highest in the K80E4 strain (0.468%). At the end of the 24 hour incubation, pH values ranged from 4.10 to 5.12, while the lowest lactic acid amount was determined in the K1E1 strain with 0.230%, while the highest was determined in the K5E2 strain with 0.932%. The pH values of E. durans strains were changed between 6.07 and 6.55 at the 3rd hour, while the amount of lactic acid formed was determined in the K63E1 strain with the lowest amount of 0.120% LA, while the highest was determined in the strain of 0.257% LA K7E2. At the end of the 24-hour incubation, pH values ranged from 4.06 to 5.38, while the lowest LA amount was determined in the K40E4 strain with 0.293%, while the highest was determined in the K75E1 strain with 0.859%. When looking at the pH decreasing properties of E. faecalis strains at the 3rd hour, it is seen that the values vary between 6.12–6.57, the amount of lactic acid occurring is determined in the K41E2 strain with the lowest amount of lactic acid K5E1 with the lowest 0.112%. At the end of the 24 h incubation, pH values ranged from 4.16 to 4.94, while the lowest lactic acid amount was determined in the K64E2 strain with 0.315%, while it was observed in the K7E1 strain with the highest 0.99%.

The pH value of the isolated 2 E. hirae strains were determined as at 3rd hour 6.42 in K17E2 strain and 5.82 in K73E5 strain. The amount of lactic acid produced by the isolates was determined as 0.199% in K17E2 strain and 0.170% in K73E5 strain. At the end of the 24 h incubation, it was observed that the K17E2 strain was higher (5.95) and the K73E5 strain was lower (4.74) as in the 3rd hour. Lactic acid amounts were determined as 0.504% and 0.890% in these strains. Acidification is an important criterion in the selection of starter cultures to be used in cheese production. Tuncer [30] determined that E. faecium strains showed higher acidification than E. faecalis and E. durans in Enterococcus species isolated from Tulum Cheese. On the other hand, it has been revealed by some researchers such as Dagdemir and Ozdemir [31], Suzzi et al. [32] some E. faecalis strains have higher acidification power than E. faecium and E. durans.

Exopolysaccharide (EPS) production

Some lactic acid bacteria can form sticky, lysing agents. This feature of bacteria is revealed by mutation, that is, the permanent character and structure change that occurs in the organism. Sometimes there are development conditions that increase the production of these substances. These conditions can be listed as too high or too low development temperature, lack of nutrients in the culture of the culture, separation of strains in mixed cultures, high pH and lyophilization [9]. In the study, it was determined that a total of 19 strains were capable of producing EPS, while 9 strains showed poor EPS production. When the distribution of EPS producing Enterococcus species and strains is examined, there are 15 E. faecium, 2 E. durans and 2 E. faecalis has been in the formation. Any E. faecalis strains produced EPS. The distribution of the species and strains that have poor EPS production ability were 6 E. faecium, 1 E. durans and 2 E. faecalis. There are a limited number of studies conducted with
exopolysaccharide production of Enterococcus species. Jamaly [33] determined that all 23 E. durans strains isolated from Moroccan dairy products are capable of producing EPS. Omfuvbe and Enyioha [34] determined that 1 of the 2 strains of E. faecalis isolated from yogurts consumed in Nigeria is capable of producing higher EPS than Lactobacillus, Streptococcus and Lactococcus species.

**Lipolitic activity**

Lipolysis is the phenomenon of hydrolysis of lipids under the influence of lipolytic enzymes such as lipase and breaking down into fatty acids with glycerine, the building blocks. The lipase enzyme can be milk-specific, of natural or microbial origin. Generally, lactic acid bacteria have a weak level of lipolytic activity and are more effective on mono- and diglycerides. Lipolysis is a desired feature for some cheese types to gain their desired qualities [10].

Fatty acids directly affect the flavor of many cheese types. Especially C4 (butyric acid) – C10 (caproic acid) acids have strong flavor (rancid, sharp, goat, soapy, coconut-like). The amount of fatty acids varies widely between varieties. In addition to its direct effects on cheese flavor, fatty acids act as pioneers in the production of other volatile flavor compounds during ripening [35]. Among the Enterococcus species and strains in the study, 17 E. faecium, 2 E. faecalis, 1 E. durans and 1 E. hirae strains showed lipolytic activity. There are contradictory data and statements regarding the lipolytic activities of Enterococcus species in the literature. Suzzi et al. [32] reported that while strains of different species did not observe lipolytic activity, Durlu-Ozkaya [36] reported that E. faecalis hydrolysed milk triglycerides at a higher level than E. faecium and E. durans.

**Proteolytic activity**

Proteolytic activity is the hydrolysis of proteins by proteolytic enzymes of natural or microbial origin. Proteolytic activity is important both in terms of acid forming function of starter cultures and sensory properties of the product. In research, it has been determined that lactic acid production and proteolytic activity differ between genus, species and strains in lactic acid bacteria [37]. In the study, isolates with different characteristics and high activity under development conditions were evaluated, and strains that could not survive were excluded.

It is seen that E. faecium strains constitute the majority of the tested species and their proteolytic activities vary between 0.21–0.50 as OPA values, OPA values varied between 0.28–0.49 for E. durans strains and 0.21–0.48 for E. hirae strains. There is E. faecalis taken to the test. The value of the strain was determined as 0.41, while the reference was determined as 0.23 in the strain of E. casseliflavus. It has been revealed by many studies that the proteolytic activity of enterococci varies depending on the source, species and strain. Wallace and Harmon [38] studied the intracellular protease of an E. durans strain and determined that the protease produced was particularly active against casein and β-lactoglobulin and did not hydrolyse bovine serum albumin. Dovat et al. [39] studied the proteolytic activity of enterococci in milk and demonstrated that they exhibit low proteolytic activity. Carrasco de Mendoza et al. [40] found that Enterococcus strain used sodium caseinate as a substrate in determining the extracellular proteolytic activity, proteolytic activity may change depending on strain and time, and after 48 hours incubation, this situation became more evident after 120 hours of incubation. Wessels et al. [41] found that total 108 E. faecium, E. faecalis and E. durans strain isolated from various dairy products developed under cold conditions and
showed proteolytic activity. Villani and Coppola [42] examined the proteolytic activity of 24 E. faecium and 60 E. faecalis strains in skim milk at 37 °C for 6 hours after incubation, and all E. faecalis strains were more proteolytic compared to E. faecium strains. Andrighetto et al. [43] revealed in their study on 124 Enterococcus species isolated from traditional Italian cheeses, showing that they showed weak proteolytic activity in milk and 30 more strains including E. faecalis strains were proteolytic. Sarantinopoulos et al. [44] 129 E. faecium, E. durans and E. faecalis strains showed low extracellular proteolytic activity and E. faecalis strains were more proteolytic.

Decarboxylation activity

Decarboxylation of amino acids by bacteria has been linked to their ability to produce biogenic amines. It was performed on 95 E. faecium, 12 E. durans, 5 E. faecalis var., 3 E. faecalis and 2 E. hirae among the Enterococcus species, which were decarboxylated using lysine and ornithine amino acids. A total of 91 E. faecium strains were found to decarboxylated to ornithine of 21, 8 of them were lysine, and 5 of them were decarboxylated together with both amino acids. There are 5 E. faecalis and 12 E. durans strains, 2 of them are both lysine and ornithine, 2 strain decarboxylated only ornithine. None of the E. faecalis and E. hirae strains gave decarboxylation positive results. Sarantinopoulos et al. [44] determined that none of the strains decarboxylated lysine or ornithine in their study on 129 Enterococcus strains (E. faecium, E. faecalis and E. durans). Tuncer [30] found that none of the 39 Enterococcus species and strains isolated and identified from Turkish Tulum type cheese decarboxylated lysine or ornithine. Yousif et al. [45] revealed that none of the 22 E. faecium isolates isolated from African fermented sorghum products decarboxylated lysine and ornithine. Hassaïne et al. [46] determined that the Enterococcus species isolated from raw milk samples do not decarboxylased lysine and ornithine. Belgacem et al. [47] found that none of the 24 strains of E. faecium isolated from Gueddid, a traditional fermented meat product, decarboxylated lysine and ornithine. Omafuvbe and Enyioha [48] determined that 2 strains of E. faecalis isolated from yoghurts consumed in Nigeria decarboxylated lysine and ornithine.

Conclusions

As a result of the research, Enterococcus species and strain with different biochemical properties and technological characteristics were isolated and diagnosed. In the study, it was observed that Enterococcus spp. showed differences according to the source, species and strain from which the technological features were isolated. In future studies, it will also be useful to investigate whether enterococci species isolated from traditional dairy products have different aroma substances, their amount of forming exopolysaccharide and whether they have mutagenic effects. Thus, by using the culture catalogues of the species and strains that will not pose a problem for human health, there will be many Enterococcus species and strains with different characteristics in these catalogues, and their use in different types of dairy products can be easily tested.

Acknowledgements

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Proximate composition, physicochemical properties and sensory qualities of salad cream from corn and tigernut starch blends

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Abstract

Introduction. This study was carried out to determine the proximate composition, physicochemical properties and sensory qualities of salad cream from corn and tigernut starch blends

Materials and methods. Yellow variety of tigernut and corn was sorted from dirt, washed, soaked for 24hrs and was wet milled into slurry using a laboratory hammer milling machine. The resulting slurry was filtered through muslin cloth and allowed to sediment for 3hrs, after which the sediment was decanted, dried in a cabinet dryer and milled to obtain starch. Salad cream was produced from the blends of corn and tigernut starch with other ingredients.

Results and discussion. Moisture content, total ash, crude fat, crude protein and carbohydrate of the salad cream from the starch blends of corn and tigernut ranged from 51.50 to 59.58%, 0.33 to 0.58%, 23.05 to 24.78%, 0.65 to 1.16% and 14.31 to 24.41% respectively. The interaction effect of corn and tigernut starch does not have any significant (p>0.05) effect on the moisture content, total ash, crude fat, crude protein and carbohydrate of the salad cream from corn and tigernut starch. The moisture content of salad cream obtained in this study can affect its quality and its shelf stability. Salad cream from corn-tigernut starch at 90%:10% had the highest total ash and crude protein while salad cream from corn-tigernut starch at 10:90% had the lowest total ash and crude protein. However, a progressive increase in total ash content was observed with the addition of corn starch. The values for the crude fat content of salad cream from corn-tigernut starch were similar. The interaction effect of corn and tigernut starch had a significant (p<0.05) effect on the starch content and total titratable acidity. Sugar and Starch content ranged from 2.67 to 9.50% and 4.14 to 11.45% respectively, salad cream from corn-tigernut starch at 10%:90% had the highest value while salad cream from corn-tigernut starch at 70:30% had the lowest value for sugar and starch content. The pH, total titratable acidity and viscosity were 3.57–3.77, 0.32–0.69% and 472–1683 Pa·s respectively. The solution process to the optimization of salad cream from corn and tigernut starch blend is 90% corn starch and 10% tigernut starch. However, optimized salad cream of 90% corn starch and 10% tigernut starch and commercial salad cream (Heinz salad cream) was most preferred by the panelist in term of their sensory attributes.

Conclusion. Salad cream from corn and tigernut starch had desirable physicochemical properties and has a compared sensory attributes with the commercial salad cream. Therefore, an acceptable salad cream can be produced up to 10% substitution of tigernut starch with corn starch.
Introduction

Starch has been a key carbohydrate storage produce in all plants with green parts containing chlorophyll. It is economically accessible and commonly used in many facets of life for the manufacture of industrial products. The distinctive characteristic of starch which enhances its use basically includes biocompatibility, biodegradability, gelation and modification according to its potential usage [1, 2, 3]. Starch exists as the most essential carbohydrate in the human diet and in large quantity in many staple foods. Starch usually contains 20 to 25% amylose and 75 to 80% amylopectin by weight [4]. Starches are extracted from a number of different starchy raw materials, such as barley, maize, rice, sweetpotato, and cassava. Sweetpotato and cassava are two major starchy root and tuber crops used in many tropical countries [5]. However, information is still scanty on starch from tigernut. Tigernut (Cyperus esculentus L) is an underutilized crop which belongs to the division Magnoliophyta, Class-liliopsida, order-cyperales and family-cyperaceae and was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant. Other names of the plant are earth almond as well as yellow nut grass [6, 7]. It is locally called “aya” in Hausa; “akiawusa” in Igbo; “ofio” in Yoruba and “isipaccara” in Effik most especially in Nigeria. Tigernut tubers are often collected and eaten raw, baked as a vegetable, roasted or dried and ground to flour [8]. Tigernut tuber exist in different varieties in Nigeria, but the two major varieties include, yellow and brown. It is an essential food crop for some certain tribes in Africa, tigernuts are often collected and eaten raw, baked as a vegetable, roasted or dried and ground to flour [8]. Tigernut tubers are rich in carbohydrate, starch and dietary fiber [9]. According to Adama et al. [3], extraction of tigernut starch gives an odourless, brilliant white or off-white non-hygrosopic powder with yields changing from as low as 14 to 37% depending on the volume and flesh of the tubers or nut. Salad cream is a creamy, yellow condiment based on an emulsion of about 25–50% of oil in water, it is prepared from ingredients such as distilled vinegar, vegetable oil, water, sugar, mustard, salt, egg yolks, modified corn flour / starch, xanthan gum and guar gum as stabilizers and riboflavin for colouring [10]. It can also be described as a readymade creamy-white dressing with a flowing consistency and uniformity for eating salad, (mixture of raw vegetable). It can be prepared with various ingredients such as modified maize flour which serves as the starting raw material [11, 10]. In order to reduce the overdependence of corn which might not readily be available throughout the year due to seasonal variations, there is need to produce salad cream with or without the use of corn starch by substituting other local crop such as tigernut. Corn starch substituted with tigernut starch can be used in appropriate proportion thereby enhancing the nutritional quality and versatility of the salad cream. It can also increase its usefulness in food industry and serve as a better raw material for food products. However, there is dearth of information on salad cream from corn starch and tigernut starch.

The main objective of the study is to determine the proximate composition, physicochemical properties and sensory qualities of salad cream produced from corn and tigernut starch blends.

Materials and methods

Materials

The yellow variety of tigernut and corn, vegetable oil, egg yolk, vinegar, sugar, mustard paste, salt, flavoring and colouring was obtained at Eleweran market in Abeokuta, Ogun state, Nigeria.
Methods

Extraction of starch from corn

The corn starch was prepared according to the method described by Yao et al. [12]. Corn grains are free from dirt and other foreign materials like stones, sticks and leaves were weighed and cracked into grits. The grits were soaked in water for 24hrs with occasional changing of water to prevent fermentation. Then, the steeped grits was drained and wet milled using laboratory hammer mill (Fritsch, D-55743, Idar-oberstein-Germany) with warm water into fine slurry. The resulting slurry was sieved with muslin cloth and allowed to sediment for 3hrs. The filtered and sedimented starch was decanted, dewatered and dried in a cabinet drier (65 °C, 6h). The dried corn starch was milled using a laboratory hammer mill and also sieved using 400mesh sieves.

Extraction of starch from tigernut

The method described by Adama et al. [3] was used for extraction of tigernut starch. The dried tigernut tubers were sorted from dirt and washed. The washed tuber was soaked in sodium metabisulphite solution at room temperature (27 °C). Thereafter, the tuber was removed and wet milled into slurry using a laboratory hammer milling machine (Fritsch, D-55743, Idar-oberstein-Germany). The paste was then dispersed in a large volume of 1% sodium metabisulphite solution and filtered through muslin cloth. The suspension was centrifuged at 3500 revolution per minute for 10 mins to ease the removal of dirt. The supernatant was then carefully decanted and the mucilage scraped off. The process was repeated for the period of three times with the mucilage on the starch been scraped constantly until a pure starch is obtained. The resulting starch was dried in the sun and further dried at 60 °C in a hot air oven, milled, weighed and stored in an airtight bag.

Formulation of corn and tigernut starch blends for salad cream

D-optimal mixture design was used to produce the percentage of corn and tigernut starch blends by combining corn and tigernut starch at 10%:90%, 90%:10%, 50%:50%, 70%:30%, 90%:10%, 30%:70%, 90%:10%, 10%:90% and 50%:50% respectively.

Production of salad cream from corn and tigernut starch

The method described by Sanni et al. [13] was used for the preparation of salad cream from corn and tigernut starch. The salad cream was produced from nine blends formulation of corn-tigernut starch. The tigernut starch, corn starch, mustard, sugar, salt and water was weighed into a cooking pot, stirred and brought for heating at 90 °C then was cooled to temperature (8 °C). The egg yolk and oil was then stirred into the slurry followed by the vinegar which was added gradually and boiled for 5 min. The slurry of each blend was poured into labeled sterilized jars and stored in a refrigerated storage condition.

Proximate composition of salad cream made from corn and tigernut starch

Moisture content, total ash, crude fat and crude protein of salad cream from corn and tigernut starch was analyzed using the method described by AOAC [14], while carbohydrate content was determined using difference method.
Physico-chemical properties of salad cream from corn and tigernut starch

**pH determination.** The pH meter was standardized with standard buffer solution of 4.0. and 7.0. The pH was measured by inserting directly the electrodes into 10ml beaker containing the sample.

**Determination of titratable acidity.** Titratable acidity was determined according to the method described by Patterson et al. [15]. 1g of the portion of the sample was weighed and put into 50ml centrifuge tube respectively. 10ml of distilled water was then added to each tube to dissolve each respectively and then flitted. 1ml aliquot of each solution was taken into another 50ml centrifuge tube and 10ml of distilled water was added to dilute the sample because it is highly colored. 10ml of the diluent was titrated against 0.1N NaOH solution using phenolphthalein (2 drops) indicator, until a pink colour is observed then percentage titratable acidity was calculated.

**Determination of total sugar and starch contents.** Sugar and starch contents were determined with the method of Dubois et al. [16] as described by Eke-Ejiofor and Kiin Kabari [17]. Hot ethanol was used to extract starch and sugar from corn-tigernut salad sample. The extract (supernatant) and digest (from the residue) was quantified calorimetrically for sugar and starch respectively, using phenol-sulphuric acid as the colour developing reagent; and absorbance read at 490 nm wave length. Corn-tigernut salad sample (20 mg) was weighed into a centrifuge tube and wetted with 1 ml of 95% ethanol. 2 mL of distilled water was added followed by 10 mL of hot 95% ethanol. The content was vortexed and centrifuged (GALLENKOMP Centrifuge Model 90 – 1, USA) at 2000 rpm for 10 min. The supernatant was decanted and used for determining sugar content while the sediment was hydrolyzed with perchloric acid and used to estimate starch content. Phenol- sulfuric reagent was used for colour development and glucose standards were used for estimation of sugar. The absorbance was read with a spectrophotometer (Milton Spectronic 601, USA) at 490 nm.

\[
\% \text{ Sugar} = \frac{\text{Absorbance} - I(0.0044)}{\text{sample wtx 0.55}}
\]

\[
\% \text{Starch} = \frac{(\text{Absorbance} - 0.0044)4}{\text{sample wtx 0.55}}
\]

**Rheological properties of salad cream from corn and tigernut starch using Brookfield viscometer**

The method described by Muhammad and Sagir [18] was used for rheological properties of corn and tigernut starch. The viscosity of the salad cream samples was measured at a controlled temperature of 500 °C using a digital rotational Brookfield viscometer (Brookfield Engineering Laboratories, Middleboro, USA, Model DV – E). These readings were taken per samples at 20, 40 and 1 min rotation at each speed (30, 60 and 100 rpm). Spindle #4 was used for all measurements. A 600 ml beaker was used for the measurement with the viscometer guard leg on. The samples were poured into a beaker to reach a level that covers the immersion groove on the spindle shaft. The viscosities of the products were measured at temperature between 25–26 °C (±1).
Sensory quality of salad cream made from corn and tigernut starch

The method described by Iwe, [19] was used. Fifty Panelists from Federal University of Agriculture, Abeokuta (FUNAAB) was chosen for the sensory evaluation. The optimized sample of salad cream was presented to the panelists. The salad cream attribute that was evaluated were appearance, aroma, taste and spreadability. A 9 point Hedonic scale test was used to determine the overall acceptability of corn-tiger nut salad cream as 9= like extremely, 5= neither like nor dislike and 1= dislike extremely.

Statistical analysis

All experimental data obtained were subjected to statistical analysis. Means, Analysis of variance were determined using SPSS version 21.0 and the difference between the mean values were evaluated at p<0.05 using Duncan multiple range test. The effect of optimization procedure was investigated using Design Expert version (8.0) and significant effects of the independent variables were determined at 5% confidence level.

Results and discussion

Proximate composition of salad cream produced from corn and tigernut starch blends

The mean values of the proximate composition of salad cream produced from corn and tigernut starch blends presented in Table 1. Moisture content ranged from 51.50% to 59.58%, corn-tigernut starch (50%:50%) had the lowest value, while corn-tigernut starch (70%:30%) had the highest value. The interaction effect of corn and tigernut starch does not have a significant (p>0.05) effect on the moisture content of salad cream produced from corn and tigernut starch as shown in the regression coefficient of Table 2. The result obtained for moisture content of this study was higher than that reported by Babajide and Olatunde [20] in corn-cocoyam starch salad cream, who reported moisture content of 48.80 to 49.79%. Eke-Ejiofor and Owuno [21] also reported a moisture content of 57.84 to 64.88% for cassava and potato starch based salad cream, which is similar to the result observed in this study. The moisture content of any food also indicates its level of water activity and thus, be used to measure its stability and susceptibility to microbial contamination [22]. The high moisture content of the salad cream obtained in this study can affect its quality and its shelf stability and this implies that the salad cream cannot be kept for a longer period of time. It can also lead to hydrolytic rancidity, which can cause off flavor of the salad cream. The different in the moisture content obtained in this study, may be attributed to the difference in the starch origin of the corn and tigernut crops. Total ash content varied between 0.33 and 0.58% with corn-tigernut starch (90%:10%) having the highest value, while corn-tigernut starch (10%:90%) had the least value. The regression coefficient shown in Table 2 shows that the interaction effect of corn and tigernut starch does not have a significant (p>0.05) effect on the ash content of salad cream produced from corn and tigernut starch. The ash content of any given food sample is a measure of the mineral level that the food contains. There was a progressive increase in ash content with addition of corn starch in Table 1, which implies that salad cream with higher corn and tigernut starch (90%:10%) had more nutrients and this is beneficial to the health of the consumer. Total ash content obtained in this study was lower than the values of 0.50 to 4.10% and 1.74 to 2.75% reported by Oli et al. [23] and Ashaye et
al. [24] on salad cream from yellow corn flour and cassava starch respectively but also similar to the values of 0.59 to 0.79% reported by Babajide and Olatunde [20] for corn-cocoyam starch salad cream. Crude fat ranged from 23.05 to 24.78% with corn-tigernut starch (30%:70%) having the least value while corn-tigernut starch (90%:10%) had the highest value. The interaction of corn and tigernut starch were not significantly (p>0.05) affected as shown in the regression coefficient in Table 2 The fat content of corn-tigernut starch salad cream had a similar value irrespective of blends combination. Crude fat ranged from 23.05 to 24.78% with corn-tigernut starch (30%:70%) having the least value while corn-tigernut starch (90%:10%) had the highest value. The interaction of corn and tigernut starch were not significantly (p>0.05) affected as shown in the regression coefficient in Table 2 The fat content of corn-tigernut starch salad cream had a similar value irrespective of blends combination. Fat plays a significant role in the shelf life of food products and as such relatively high fat content could be undesirable in food products. Eke-Ejiofor and Owuno [21] reported a fat content of 25.17 to 28.15% in cassava and potato starch based salad cream, while Babajide and Olatunde [20] reported a fat content of 27.04 to 29.68% in corn-cocoyam starch salad cream. These values are comparable to the result obtained in this study. Until recently, lipids have been considered as functional foods to enhance their analysis, extraction procedures or to enrich the nutritional profile of traditional foods [25]. However, the higher the fat content, the more tendency to promote rancidity, leading to development of unpleasant and odorous compounds. The protein content ranged from 0.65 to 1.16%, salad cream with corn-tigernut starch (10%:90%) had the lowest value while salad cream with corn-tigernut starch (90%:10%) had the highest protein. Salad cream with higher proportion of corn and tigernut starch had higher protein and showed the influence of corn starch in the contribution of proteins. However, this should not be depended on because it does not determine the major source of protein. Proteins help to build and repair worn out tissues in the body. Studies of salad cream by Eke-Ejiofor, [26], Eke-Ejiofor and Owuno [21], Babajide and Olatunde [20], had lower protein values than what was obtained in this study. Thus values obtained in this study were far lower than the values of 1.40 to 8.75% reported by Oli et al. [23] and 1.55 to 1.80% reported by Naknaen et al. [27]. The carbohydrate content of the salad cream ranged between 14.31 and 23.41%. Carbohydrate is a major nutrient that provides energy and helps promote healthy digestive system. Although the values obtained in this study was lower than the value of 7.11 to 17.32% reported by Eke-Ejiofor and Owuno [21] on potato starch and cassava starch based salad cream and 13.99 to 37.18% reported by Eke-Ejiofor and Beleya [28] on cassava, sweetpotato and three leaf yam starches

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Mean values with different superscripts within the same column are significantly different (p <0.05);
CS- Corn starch, TS- Tigernut starch
Table 2
Regression coefficient of proximate composition of salad cream from corn and tigernut starch

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Moisture Content</th>
<th>Total Ash</th>
<th>Crude Fat</th>
<th>Crude Protein</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>58.20</td>
<td>0.58</td>
<td>24.83</td>
<td>1.15</td>
<td>14.75</td>
</tr>
<tr>
<td>B</td>
<td>56.59</td>
<td>0.34</td>
<td>23.02</td>
<td>0.65</td>
<td>19.32</td>
</tr>
<tr>
<td>AB</td>
<td>-16.51</td>
<td>-0.064</td>
<td>-0.71</td>
<td>0.24</td>
<td>17.54</td>
</tr>
<tr>
<td>F-VALUE</td>
<td>3.45</td>
<td>93.49</td>
<td>122.58</td>
<td>94.34</td>
<td>5.92</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.5348</td>
<td>0.9689</td>
<td>0.9761</td>
<td>0.9692</td>
<td>0.6639</td>
</tr>
</tbody>
</table>

A- Corn starch, B-Tigernut starch, AB-Interaction of corn and tigernut starch, $R^2$- Coefficient of determination

Physicochemical properties of salad cream produced from corn and tigernut starch blends

The mean values and the interaction effect on the physicochemical properties of salad cream produced from corn and tigernut starch blends is presented in Table 3 and 4. Sugar and starch content of salad cream from corn and tigernut starch ranged from 2.67 to 9.50% and 4.14 to 11.45% respectively with salad cream from corn-tigernut starch (10%:90%) having the highest value for sugar and starch content while salad cream from corn-tigernut starch (70%:30%) had the least value for sugar and starch content respectively. The interaction effect of corn and tigernut starch had no significant (p>0.05) effect on the sugar contents as shown in Table 4. Sugar content was higher in salad cream from corn-tigernut starch (10%:90%), this may be attributed to high sucrose level. Sugar may also act as sweetener; thereby contribute to the taste and flavour of the salad cream. The interaction effect of corn starch and tigernut starch significantly (p<0.05) affect the starch content of the salad cream negatively as shown in Table 4. The starch content of salad cream ranged between 4.14 and 11.45% with corn-tigernut starch (10%:90%) having the highest value, while corn-tigernut starch (70%:30%) had the least value for starch content. The starch content obtained in this study was in range with the value of 8.03 to 12.25% and 6.40 to 14.41% reported by Ashaye et al. [24] and Eke-Ejiofor and Owuno [21]. The starch obtained in this study will helps to bind and thicken the salad cream together. It also helps in increasing the paste viscosity, emulsion stabilizers and it lowers gelatinization temperature. The pH of a food is defined as the level of acidity or alkalinity of food content. The pH of the salad cream ranged from 3.57 to 3.77 with corn-tigernut starch (90%:10%) having the lowest pH while corn-tigernut starch (10%:90%) had the highest pH. Maximum desirable pH level for safety and optimum target pH should be 4.25 to ensure food safety [29]. pH of 4.5 is a desirable trait because it will halt the proliferation of micro-organism, thereby favoring the conservation of the in the final product [30, 31]. However, the pH of corn-tigernut starch salad cream in this study was acidic. The pH of the salad cream obtained in this study was higher than the value of 3.14 to 3.50 reported by Eke-Ejiofor and Owuno [21] on salad cream from cassava and potato starch but lower than the value of 3.78 to 3.91 reported by Gaikwad et al. [32] on physicochemical properties of flavoured mayonnaise. The total titratable acidity of corn and tigernut starch salad cream ranged from 0.32 to 0.69%, corn-tigernut starch (90%:10%) had the lowest value, while corn-tigernut starch (50%:50%) had the highest value of total titratable acidity. The total titratable acidity was significantly (p<0.05) affected by the interaction effect of corn and tigernut starch as shown in Table 4.Titratable acidity is a
measure of food products acidity [15]. The values of total titratable acidity obtained in this study was higher than the values of 0.02 to 0.89% reported by Eke-Ejiofor and Owuno [21] on cassava and potato starch based salad cream but lower than the values of 1.35 to 2.50% reported by Oli et al. [23] on salad cream from yellow corn flour. The difference obtained in total titratable acidity in this study could be due to the difference in starch properties between corn and tigernut crops. The viscosity of the salad ranged between 472 and 1683rpm with corn-tigernut starch (90%:10%) having the highest viscosity while corn-tigernut starch (10%:90%) had the lowest viscosity. However, the interaction effect of corn and tigernut starch on the viscosity of salad cream were significantly (p<0.05) affected. The high viscosity obtained in corn-tigernut starch (90%:10%) could be due to the addition of the salad ingredients used in its preparation.

### Table 3

<table>
<thead>
<tr>
<th>CS:TS</th>
<th>Sugar (%)</th>
<th>Starch (%)</th>
<th>pH</th>
<th>Total titratable acidity (%)</th>
<th>Viscosity (Pa*s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:90</td>
<td>9.30c</td>
<td>11.43a</td>
<td>3.76c</td>
<td>0.57c</td>
<td>472a</td>
</tr>
<tr>
<td>90:10</td>
<td>3.39b</td>
<td>4.87b</td>
<td>3.57a</td>
<td>0.32a</td>
<td>1681e</td>
</tr>
<tr>
<td>50:50</td>
<td>6.56c</td>
<td>7.01c</td>
<td>3.68b</td>
<td>0.68d</td>
<td>1189cd</td>
</tr>
<tr>
<td>70:30</td>
<td>2.67a</td>
<td>4.14a</td>
<td>3.61a</td>
<td>0.38b</td>
<td>1412de</td>
</tr>
<tr>
<td>90:10</td>
<td>3.40b</td>
<td>4.88b</td>
<td>3.58a</td>
<td>0.33a</td>
<td>1683e</td>
</tr>
<tr>
<td>30:70</td>
<td>8.12d</td>
<td>9.74d</td>
<td>3.60ab</td>
<td>0.56c</td>
<td>990bc</td>
</tr>
<tr>
<td>90:10</td>
<td>3.41b</td>
<td>4.89b</td>
<td>3.58a</td>
<td>0.35a</td>
<td>1682e</td>
</tr>
<tr>
<td>10:90</td>
<td>9.50d</td>
<td>11.45a</td>
<td>3.77c</td>
<td>0.58c</td>
<td>474a</td>
</tr>
<tr>
<td>50:50</td>
<td>6.57c</td>
<td>7.02c</td>
<td>3.67b</td>
<td>0.69d</td>
<td>1190cd</td>
</tr>
</tbody>
</table>

Mean values with different superscripts within the same column are significantly different (p <0.05); CS- Corn starch, TS- Tigernut starch

### Table 4

Regression coefficient of physicochemical properties of salad cream from corn and tigernut starch

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sugar</th>
<th>Starch</th>
<th>pH</th>
<th>Total titratable acidity</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td></td>
<td>4.69</td>
<td>3.58</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9.59</td>
<td>75.03</td>
<td>3.75</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>-1.38</td>
<td>-5.36</td>
<td>-0.059</td>
<td>0.66*</td>
</tr>
<tr>
<td></td>
<td>F-VALUE</td>
<td>37.80</td>
<td>75.03</td>
<td>8.98</td>
<td>13.29</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.9265</td>
<td>0.9616</td>
<td>0.7495</td>
<td>0.8158</td>
</tr>
</tbody>
</table>

A- Corn starch, B- Tigernut starch, AB- Interaction of corn and tigernut starch, R²- Coefficient of determination
Optimization process of salad cream produced from corn and tigernut starch blends

The salad cream was optimized based on some important properties attributed to salad cream. Moisture, total ash, crude fat, crude protein, carbohydrate, sugar, starch, pH, total titratable acidity and viscosity were the main quality parameters studied in this work which were also the criteria based on desirability concept as well as their main quality parameters serving as the constraints to process optimization. Moisture, crude fat, carbohydrate, sugar, starch, pH, total titratable acidity and viscosity were maximized while total ash and crude protein were set at none. The solution to the optimized salad cream from corn and tigernut starch blends is 90% corn starch and 10% tigernut starch as shown in Table 5.

### Table 5
Solution process to the optimization of salad cream from corn and tigernut starch blends

<table>
<thead>
<tr>
<th>Number</th>
<th>CS</th>
<th>TS</th>
<th>Moisture</th>
<th>Crude Fat</th>
<th>CHO</th>
<th>Sugar content</th>
<th>Starch content</th>
<th>pH</th>
<th>Viscosity</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>10</td>
<td>58.20</td>
<td>24.83</td>
<td>14.75</td>
<td>3.154</td>
<td>4.685</td>
<td>3.58</td>
<td>1670.29</td>
<td>0.651</td>
</tr>
</tbody>
</table>

CS- Corn starch, TS- Tigernut starch, CHO-Carbohydrate

Sensory score of salad cream produced from corn and tigernut starch blends

The sensory score of salad cream obtained from corn and tigernut starch blends is shown in Table 6.

### Table 6
Sensory score of salad cream produced from corn and tigernut starch blends

<table>
<thead>
<tr>
<th>CS:TS</th>
<th>Appearance</th>
<th>Aroma</th>
<th>Taste</th>
<th>Spreadability</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>4.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>90:10</td>
<td>6.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.95&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Commercial (Heinz Salad Cream)</td>
<td>7.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values with different superscripts within the same column are significantly different (p<0.05); CS- Corn starch, TS- Tigernut starch

Consumer quality is a major factor for selecting a product and among the main characteristics related to quality are texture, taste, and surface color of foods [33]. Significant difference (p<0.05) was observed in all the sensory attribute of salad cream produced from corn and tigernut starch blends. Appearance, aroma, taste, spreadability and overall acceptability ranged from 4.14 to 7.10, 4.15 to 6.85, 4.40 to 6.90, 3.25 to 6.10 and 4.50 and
6.95 respectively. Optimized salad cream had the highest value for aroma, taste and overall acceptability, commercial salad cream had the highest for appearance and spreadability while salad cream produced from 100% corn starch had the least for all attributes. Aroma, taste and overall acceptability of optimized salad cream were most preferred over commercial salad cream and 100% corn starch salad cream with a sensory score of 6.85, 6.90 and 6.95 respectively. Commercial salad cream had higher sensory scores in terms of appearance and spreadability. Meanwhile, 100% corn starch salad cream showed a very poor sensory attributes and was rejected by the panelist.

Conclusions

1. Salad cream from corn and tigernut starch had desirable physicochemical properties and has a compared sensory attributes with the commercial salad cream, especially for appearance.
2. However, 100% corn starch based salad cream was rated low. Therefore, an acceptable salad cream can be produced up to 10% substitution of tigernut starch with corn starch thereby increasing the utilization of tigernut starch.

References


Polyphenol composition and technological characteristics of the coloured whey from various origin

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National University of Food Technologies, Kyiv, Ukraine

Abstract

Introduction. The aim of this work was to determine the polyphenol composition and technological characteristics of coloured whey from various origin – obtained by milk proteins coagulation with traditional berry raw materials and wild herbs.

Materials and methods. Coloured whey is obtained as a result of thermo acid coagulation of milk proteins by plant origin coagulants. The identification and quantitative determination of polyphenol compounds in the coloured whey samples has been carried out by high performance liquid chromatography. The solids content in the coloured whey has been investigated by the refractometric method and optical dense for the colouration and turbidity of the coloured whey – by the colorimetric method.

Results and discussion. The whey samples had violet and green shades, respectively, due to the presence of specific coloured substances, including various polyphenols, which are a component in black currant berries and chlorophyll in plantain juice. The content of the above-mentioned compounds in whey after milk proteins coagulation with Plantago major L. juice and cavitation processed blackcurrant paste are 324.43 and 265.49 mg/L, respectively. With an increase in the amount of plant coagulant for thermo acid coagulation of milk proteins from 5 to 11%, the optical dense value for turbidity of coloured whey decreases by 0.40 and 0.41 cond. units and for the colouration, on the contrary, increase by 0.17–0.19 cond. units for coloured whey after milk proteins coagulation with Plantago major L. juice and blackcurrant paste, respectively. Dry matters weight ratio in coloured whey has been fixed at the level of 6.80–8.55%. The protein content was 0.96–1.33%, depending on the amount of plant origin coagulant during thermo acid milk coagulation. The coloured whey, obtained after the milk proteins coagulation by plant coagulants in the amount of 8% and 11% had a lower protein content by 0.32–0.45%, compared to the control sample. This confirms the complex casein and milk whey proteins coagulation by organic acids of the cavitation processed blackcurrant paste and the active complex (proteases and organic acids) of plantain juice.

Conclusions. Coloured whey samples have improved taste, colour characteristics and increased nutritional value, which makes it widely used in whey beverages with or without additional processing.
**Introduction**

Research of various additional sources of milk raw materials, including milk whey, in order to develop full-fledged, available and safe drinks, is actual [1]. However, the use of whey for various drinks needs to be adjusted in the recipe to weaken whey tones in taste and smell [2].

In the beverage industry, whey is combined with fruit and vegetable juices, fruit and berry purees and herbal extracts to give the product not only a pleasant and refreshing effect, but also preventive properties. Depending on the beverage type, processed or unprocessed whey is used, as well as condensed or dry concentrates with the fruit and aromatic fillers addition. For example, in Germany, whey is used to prepare tasty beverages containing 80–90% whey and 10–20% strawberry and peach juice. Similar beverages are made with the addition from 7 to 20% grapefruit or other juice. The traditional Iranian beverage «Dough» is made from milk whey pasteurized at a temperature of 74–76 °C and then cooled to 40–42 °C with the addition of 2.5% dessert yogurt. In Austria, a popular drink with a long storage period is a mixture of 50% whey, 40% fermented milk and 10% fruit juice [3, 4].

The above-mentioned developments are focused on correcting the chemical composition and sensory characteristics of whey in order to enrich and increase the nutritional value in beverages based on it.

For the manufacturer, an important advantage of adding fruit and berry fillers is the bright taste and fresh flavor in the final product. In addition, the filler presence with an appropriate chemical composition eliminates the necessity to add flavoring matter, coloring agents and some types of stabilizers [5].

A promising direction in the technology for the enriched dairy products production with functional properties is using of coloured whey obtained as a result of complex milk processing into protein concentrates in the presence of plant coagulants. In particular, the plant using as technological components allows to change the sensory characteristics in the milk whey, due to different taste shades and colours, and also to regulate the composition of biologically active substances [6].

It is recommended to use intensely coloured herbal compositions in order to improve the sensory characteristics of whey-based products. This is due to the fact, that in an acidic environment caused by whey lactic acid, plant systems acquire changes in colour intensity caused by the biocatalysts dissociation [7].

There are no studies to determine the optical dense for colouration and turbidity, as well as coloured substances and polyphenol composition in the coloured whey obtained after the milk proteins coagulation by plant coagulants, which caused its natural color.

The use of the plant raw materials, both berry and herbal, as a coagulant for thermo acid coagulation of milk proteins will contribute to the addition of organic complex compounds in whey and the formation of original sensory characteristics in the separation products. This will exclude the use of artificial coloring agents and flavors in the future [8, 9].

The aim of this work was to determine the polyphenol composition and technological characteristics of coloured whey from various origin – obtained by milk proteins coagulation with traditional berry raw materials and wild herbs. In addition, sensory characteristics, dry matters weight ratio, including protein, and active acidity of from various origin have been determined.
Materials and methods

Materials

Coloured whey, obtained in the protein concentrates production by thermo acid coagulation of milk proteins with plant coagulants – berry paste and plantain juice. Coloured whey was obtained in two methods during thermo acid coagulation of milk proteins using as a coagulant – cavitation processed blackcurrant paste and Plantago major L. juice. According to the first method, a berry coagulant with an active acidity of 3.0±0.2 pH was added to heated milk at a temperature of (75±1) °C in an amount of 5 to 11%, slightly mixed and kept (2±1) min until a clot formed. The combined effect of high temperatures and acidic reagents on milk proteins leads to their maximum coagulation. The coagulation process was visually established by the intensive formation of protein flakes and whey release, that has a natural violet colour of the coagulant [10].

According to the second method, directly squeezed juice from the ground part of Plantago major L. was used as a coagulant in an amount from 5% to 11% with the following indicators: dry matters – 4.55±0.23%, pH – 5.85±0.18. The juice was added to milk at a coagulation temperature of 55–60 °C and held for 45–60 min. Then the temperature was increased to 90–95 °C, slightly mixed and held for 2–3 minutes until a clot was formed. Then the clot was pressed for 15±2 min to separate the whey [11].

As a result, about 80% of whey was obtained from volume of milk raw materials in the protein concentrates manufacture by both methods. Coloured whey has been sent to determine the content of polyphenol compounds, sensory, physical and chemical indicators.

Determination of the polyphenol composition in the coloured whey from various origin

The determination was carried out by high-performance liquid chromatography (HPLC) using system Prominence LC-20 Shimadzu (Japan). The substances identification in the coloured whey extracts was performed by comparing the retention time and spectral characteristics of the test substances with similar characteristics of the standards according to the method identification of polyphenols. Chromatography was performed at 225, 255, 286 and 350 nm [12]. For accurate identification of the test substances to specific polyphenols groups, the following regulatory documents were used: chlorogenic and caffeic acids (phenolic acids), catechin (catechins), flavonols myricetin, quercetin and rutin, flavanones naringenin, naringin, hesperidin, and proteost cyanidine (anthocyanins) (Sigma-Aldrich, Germany). The identification characteristics of these standards were obtained under the above-mentioned chromatography conditions [13]. The "peak area–standard content" calibration dependences had a linear form with an accuracy of at least \( r^2 = 0.994 \).

Determination of colouration and turbidity of the coloured whey from various origin

Samples of milk whey from various origin have a natural colour and turbidity, therefore, we used for research an adapted method for determining indicators in soft beverages. This method is based on optical dense determination and comparison with the corresponding standards: coloured solutions for colouration and suspension for turbidity.

The optical dense determination was carried out by the colorimetric method [14]. The value of optical dense \( D_{\text{gen}} \) consists of \( D \) due to the colouration caused by the coloring agents,
and D, due to the turbidity caused by the proteins presence that scatter the light flux. When colouration determination, it is necessary to have a total absence of foreign particles with a radius of 0.4–0.8 microns or more, which contribute to light scattering. Therefore, to prepare the samples for analysis, the coloured whey was filtered.

The research was carried out using a Helios Omega spectrophotometer (Thermo Scientific Spectronic, USA). The spectrophotometer is designed for measure in individual sections of the wave-length range 315-980 nm, which are formed by light filters, transmission coefficients, optical dense of liquid solutions, and measuring the substances concentration in solutions after preliminary determination of the calibration characteristic. Cuvettes with a test solution (coloured whey) and control solution (milk whey), in relation to which the measurements were carried out, were installed in the cuvette compartment. The necessary light filter and photodetector were installed, the sample compartment cover was closed, and the studies were carried out. On a digital display, values were obtained that correspond to the optical dense of coloured whey.

The content of dry matters in the coloured whey was investigated by the refractometric method according to the light refractive indices [15]. First, check the correctness of the refractometer readings for distilled water at a temperature of 20±0.1 °C. With one or two water drops applied to the prism, the refractometer reading should be zero. The refractometer prism is wiped off with a paper filter and one or two drops of a test whey sample are applied. On the right refractometer scale, the content of dry matters was found, which coincides with the distribution boundary of the dark and light fields.

The active acidity in the coloured whey was determined potentiometrically [16] on a Sartorius PB-20 universal pH meter. To determine the pH, 40 cm$^3$ of coloured whey was taken into the beaker, the electrodes are immersed in the beaker and after 10-15 seconds the readings of the device are recorded. The electrodes are rinsed with distilled water and wiped off with filter paper after each measurement.

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

Determination of sensory characteristics and polyphenol composition of the coloured whey from various origin

The sensory characteristics of the coloured whey obtained as a result of the thermo acid coagulation of milk proteins by plant coagulants – blackcurrant paste and Plantago major L. juice, is given in Table 1.
### Sensory characteristics of the coloured whey from various origin

<table>
<thead>
<tr>
<th>Indicator name</th>
<th>Amount of blackcurrant paste during thermo acid coagulation of milk proteins, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency and appearance</td>
<td>Homogeneous, flowing liquid, slight sediment is allowed</td>
</tr>
<tr>
<td>Taste and flavor</td>
<td>Clean, lactic, without foreign tastes and smells</td>
</tr>
<tr>
<td></td>
<td>Slightly acid, with a berry coagulant taste and flavor</td>
</tr>
<tr>
<td>Colour</td>
<td>Light violet, uniform in volume</td>
</tr>
<tr>
<td></td>
<td>Violet, uniform in volume</td>
</tr>
<tr>
<td></td>
<td>Intense violet, uniform in volume</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indicator name</th>
<th>Amount of Plantago major L. juice during thermo acid coagulation of milk proteins, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency and appearance</td>
<td>Turbid liquid, with visible protein inclusions, which in the time following coagulates</td>
</tr>
<tr>
<td></td>
<td>Homogeneous, clear liquid, with a small sediment amount</td>
</tr>
<tr>
<td>Taste and flavor</td>
<td>Lactic, with a barely there herbal flavor</td>
</tr>
<tr>
<td></td>
<td>Slightly bitter, with a pleasant herbal smell and taste</td>
</tr>
<tr>
<td>Colour</td>
<td>Mostly white, with a barely there greenish cast</td>
</tr>
<tr>
<td></td>
<td>Green, uniform in volume</td>
</tr>
<tr>
<td></td>
<td>Intense green, uniform in volume</td>
</tr>
</tbody>
</table>

The obtained whey samples had the corresponding shades, which is due to the presence of specific colour substances – flavonoid pigments and chlorophyll in black currant berries and plantain juice [17].

In order to determine the transition degree of colouring compounds, it was analyzed the polyphenol composition in plant coagulants – cavitation processed blackcurrant paste, *Plantago major* L. juice, protein–plant concentrates and coloured whey from various origin.

The total polyphenols content in the studied samples has been determined by summing up the substances content, that were found in the peaks range of flavonoids, non-flavonoids, and phenolic acids on chromatograms.

The flavonoids content in all studied samples was equal to the substances content that are similar to the flavonoids standards (phenolic acids, catechins, flavonoids, flavonones and flavones), with the exception of catechin-like substances. The polyphenol composition of whey correlates with the composition of specially processed blackcurrant paste and *Plantago major* L. juice, respectively. The research results have been analyzed in comparison with the control samples – plant coagulants. Their transition degree to separation products – protein concentrates and whey of various colours has been determined. The research results are presented in Table 2.
Table 2
Total composition and polyphenols content in samples of the coloured whey from various origin

<table>
<thead>
<tr>
<th>Polyphenols group</th>
<th>Polyphenols content, mg/l</th>
<th>Coloured whey after milk proteins coagulation by Cavitation processed blackcurrant paste</th>
<th>Plantago major L. juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechins, including certain substances:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− catechin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin-like *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonols, including certain substances:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− glycosides of myricin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− rutin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− quercetin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavanones, including certain substances:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− naringin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− hesperidin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavones, including certain substances:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− glycosides of luteolin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthocyanins, including certain substances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− delphinidin-3-O-glucoside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− cyanidin-3-O-galactoside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− delphinidin-3-O-arabinoside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of polyphenols</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* − catechin-like – polyphenols whose peaks are located outside the area of the catechin peaks, but with the spectral catechins characteristics.

According to the obtained results in Table 2, flavonols are represented by glycosides myricithin, rutin and quercetin; naringin and hesperidin are found in flavonones [18]. The flavones identified in the coloured whey after milk proteins coagulation by the Plantago major L. juice have the smallest amount and are represented by luteolin and its glycosides. The content of phenolic acids is fixed at 2.13 mg/l for coloured whey after milk proteins coagulation by blackcurrant paste and 0.07 mg/l for whey after milk proteins coagulation by Plantago major L. juice.

In total, 12 flavonoid class compounds were identified in the coloured whey obtained after the milk proteins coagulation by Plantago major L. juice, which are 10 less than their content in the plantain juice. This is probably due to the fact that a significant amount of polyphenol compounds from juice was transferred to milk-protein concentrate during denaturation.
The content of polyphenol compounds in coloured whey after the milk proteins coagulation by *Plantago major* L. juice and cavitation processed blackcurrant paste is 324.43 and 265.49 mg/l, respectively. As a comparison, according to literature data, the polyphenols content in plantain juice was within 1411.13 mg/l, and in blackcurrant paste – 690 mg/l [19].

The transition degree of polyphenol compounds into coloured whey after the milk proteins coagulation by *Plantago major* L. juice is 23% of their total amount, about 77% of polyphenols, including 74% of flavonoids, remain in protein–plant concentrates. Probably, this effect is likely due to the polyphenols interaction with proteins and their attachment to the globule surface at the unfolding time of the polypeptide chain due to the formation of a hydrogen bond between the polyphenol hydroxyl group and the carbonyl group of the protein molecule [5].

Similarly, the transition degree of polyphenol compounds into coloured whey after the milk proteins coagulation by cavitation processed blackcurrant paste has been calculated, which was fixed at 42%. About 53% of polyphenol compounds, including anthocyanins, remain in concentrates, which is due to the weight loss correlation of the concentrate during technological operations, such as pressing and forming.

By their chemical nature, anthocyanins are representatives of natural polyphenol compounds of the flavanoids class with antimicrobial activity [20, 21]. Therefore, their content in the coloured whey was additionally analyzed.

Coloured whey contains all known anthocyanins: delphinidin-3-O-galactoside, delphinidin-3-O-glucoside, cyanidin-3-O-galactoside, delphinidin-3-O-arabinoside, cyanidin-3-O-glucoside, petunidin-3-O-galactoside, peonidin-3-O-glucoside, malvidin-3-O-arabinoside, etc. and complete, respectively, 47% of the total polyphenols content in the samples.

**Determination of optical dense for colouration and turbidity of the coloured whey from various origin**

Optical dense indices for characterizing the turbidity and colouration of the coloured whey from various origin are important for determining the using ways for technological purposes. The optical dense, which characterizes the turbidity and colouration of the coloured whey is given in Table 3.

<table>
<thead>
<tr>
<th>Amount of plant coagulant during thermo acid coagulation of milk proteins, %</th>
<th>Coloured whey after milk proteins coagulation by Cavitation processed blackcurrant paste</th>
<th>Coloured whey after milk proteins coagulation by <em>Plantago major</em> L. juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D₀, cond. unit</td>
<td>D, cond. unit</td>
</tr>
<tr>
<td>5</td>
<td>1.877±0.037</td>
<td>1.065±0.021</td>
</tr>
<tr>
<td>8</td>
<td>1.515±0.045</td>
<td>1.169±0.023</td>
</tr>
<tr>
<td>11</td>
<td>1.466±0.044</td>
<td>1.259±0.025</td>
</tr>
<tr>
<td>Control samples*</td>
<td>1.511±0.045</td>
<td>1.204±0.024</td>
</tr>
</tbody>
</table>

*Coloured whey beverages "Aktual" (with watermelon-melon and neon mojito flavor) produced by LLC «Danone Dnipro»
The optical density of the coloured whey samples obtained after the milk proteins coagulation by blackcurrant paste during determination the turbidity and colouration of the whey ranged from 1.46 to 1.88 cond. units and 1.06–1.26 cond. units, respectively. Optical dense indices before and after filtration for coloured whey obtained as a result of milk coagulation by *Plantago major* L. juice is fixed at the level of 1.55–1.95 cond. units and 1.13–1.29 cond. Units.

According to the results in Table 3, with an increase in the amount of plant coagulant for thermo acid coagulation of milk proteins from 5 to 11%, the optical dense value for turbidity (Dt) of coloured whey from various origin decreases by 0.40 and 0.41 cond. units for coloured whey after milk proteins coagulation with *Plantago major* L. juice and blackcurrant paste, respectively. This effect is due to the mechanism of thermo acid coagulation of milk proteins and an increase in the casein transition degree and the maximum amount of whey proteins into protein-plant concentrates [22].

Optical dense indicators, which characterize the colouration, on the contrary, increase with an increase in the amount of coagulant from 5% to 11% during thermo acid coagulation by *Plantago major* L. juice and blackcurrant paste, respectively. This is, probably, due to an increase in the transition degree of specific coloured substances – polyphenolic compounds, including flavanoids and anthocyanins, of plant raw materials into a protein clot and whey [23]. However, almost all of these values have variance from the control samples – whey beverages of the corresponding colour up to 5%.

**Determination of the dry matters weight ratio and active acidity in the whey from various origin**

Whey contains 50% of the dry matters of whole milk, almost all milk sugar and about 30% of milk proteins. The change in the dry matters weight ratio in coloured whey depending on the amount and type of plant coagulant is shown in Figure 1.

![Graph](image_url)
Dry matters weight ratio in coloured whey ranges from 6.80 to 8.55%, including 0.96–1.33% of protein and depends on the amount of plant coagulant during thermo acid coagulation of milk proteins. Thus, the protein content in coloured whey was in average at 1.33±0.04% after thermo acid coagulation using 5% plant coagulant, 1.01±0.03% – 8% coagulant, and 0.96±0.04% with the addition of 11% plant raw materials. This indicates a more complete coagulation and transition of milk proteins into a protein-plant clot [24].

The control sample obtained as a result of the milk proteins coagulation by whey with titrated acidity not less than 160 °T had a protein content of 1.35%. Compared to the control sample, coloured whey obtained after thermo acid coagulation of milk proteins with plant coagulant in the amount of 8% and 11% had a lower protein content by 0.34–0.39%. This confirms the complex casein and whey proteins coagulation by organic acids of blackcurrant paste and an active complex of plantain juice.

Dependence of the active acidity in coloured whey on the amount and type of plant coagulant during thermo acid coagulation of milk proteins is shown in Figure 2.

The histogram shows the pH value in the whey samples decreases in proportion to the increase in the amount of plant coagulant for thermo acid coagulation of milk proteins. At this stage, it can be observed, the amount and type of plant coagulant affects the active acidity, changing it to the acidic side by 0.55 and 0.70 pH when using 11% milk protein coagulant in the form of Plantago major L. juice and blackcurrant paste, respectively.

The value decrease of active acidity occurred slowly during storage period of whey samples at a temperature of (4±2) °C for 72 hours. During 48 hours of whey storage period, active acidity in the control sample decreased by 0.32 pH compared to the initial value of 5.91 pH, in coloured whey after protein coagulation by Plantago major L. juice and
cavitation blackcurrant paste by an average of 0.20 and 0.25 pH, respectively. An active decrease was observed in the samples obtained after the milk proteins coagulation by blackcurrant paste in an amount of 11%, that for 72 hours of storage period was at 4.65 pH. Coloured whey from various origin retained its sensory characteristics, including the colour intensity, at all stages of storage period at a temperature of (4±2) °C [25].

Scope of whey applicability is usually limited due to high acidity, deficiencies in sensory characteristics (salty and sour taste, pronounced whey flavor). In practice, for the whey deoxidation, which has a 4.50–5.00 pH, various chemical substances are used (solutions of ammonia, sodium hydroxide, etc.) [26]. Soda solution (sodium bicarbonate) is chemically safe, available and traditionally used in the food industry to neutralize (deoxidize) whey.

Coloured whey obtained after the milk proteins coagulation by cavitation processed blackcurrant paste and Plantago major L. juice can be used with or without additional processing (filtration, deoxidation) as a base and prescription component for whey beverages, such as pasteurized whey, pasteurized whey with sugar, and etc. This makes it possible to completely exclude the use of food coloring agents and flavoring matter of artificial origin in their composition. These technologies are classical and do not require additional parameters specification.

**Conclusion**

1. Coloured whey obtained after milk proteins coagulation by Plantago major L. juice and cavitation processed blackcurrant paste is characterized by polyphenol compounds content at the level of 324.43 and 265.49 mg/l, respectively, which has a positive effect on its nutritional value and sensory characteristics. Whey had, respectively, green and violet colour, which is characteristic for raw materials that contain flavonoids and anthocyanins.

2. Optical dense which characterizes the turbidity and colouration for samples of coloured whey obtained after milk proteins coagulation by cavitation processed blackcurrant paste ranged within 1.46–1.88 cond. units and 1.06–1.26 cond. units, and for the coloured whey obtained as a result of milk coagulation by Plantago major L. juice was fixed at the level of 1.55–1.95 cond. units and 1.13–1.29 cond. units, respectively.

3. The obtained results on the dry matters content at the level (6.80–8.55%), including protein (0.96–1.33%) and active acidity (4.90–5.90 pH), indicate the practicability of using in beverage formulations – coloured whey obtained as a result of thermo acid coagulation of milk proteins by plant coagulant in an amount of 8% without additional processing. When using whey obtained after coagulation by a coagulant in the amount of 11% – expose it to deoxidation, and in the amount of 5% – to filtration.

**References**


Physical, physiological and minerals changes of different legumes types during the germination process

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Stefan cel Mare University of Suceava, Suceava, Romania

Abstract

Introduction. The aim of this study was to highlight the physical and physiological changes of different types of legumes and the variation of their mineral amount during the germination process, in order to establish the optimal germination period for their use in consumption.

Materials and methods. Legumes types such as chickpea, bean, lentil, lupine, and soybean were germinated in a plant growth chamber Binder KBW/KBWF 240. To highlight the physical and physiological changes of legumes during the germination process, a Motic SMZ-140 Stereomicroscope was used. In order to highlight the variation of the amount of mineral substances during the germination process, a Shimadzu EDX-900HS was used.

Results and discussion. The protein content of the analyzed legumes varied between 19.40 and 40.34% the highest amount being for soy and the lowest one for chickpea sample. All the samples presented good viability for germination, the highest one being for lentil of 90% for which was also recorded the highest germination energy value. The image obtained clearly showed the development of the component parts of the germs: the radicle and the plumule which increases during the germination process, when the seed began to synthesize chlorophyll and when the root began to develop. According to their development the maximum germination time were of 10 days for lentil and lupin and of 9 days for chickpea, bean and soybean. However, for their use in food consumption, the optimum germination period was established for 4 days for all the analyzed legumes samples for which except the bean sample the radicle was much higher than the legume grain size. In general, the availability of calcium and sulf was improved for all the samples in the four day of germination. The calcium increases most for chickpeas with almost six times and for lentil with three times. Reported to the ungerminated seeds the phosphor and iron increases for lentil seeds in the four day of germinations whereas for the rest of legumes decreases. Regarding potassium, magnesium and zinc elements, in general, their values decreases with the increase time of germination period.

Conclusions. By highlighting the physical and physiological changes of legumes during germination, it is easier to determine when the germination process should be stopped. The amount of many nutrients increases as a result of the germination process, and this process can have various applications in the food industry.
Introduction

Seed germination is a complex physiological process that has as its starting point the absorption of water by seeds, and as a final point, the appearance of the root, a component part of the future plant [1]. It can be said that the seed germination is the first phase of the plant development cycle [2].

Lately, there has been a lot of interest in the germination process, regarding the food field. This is explained by several considerations. First, researchers are studying the germination process because in some industries it is desirable to combat this process because it makes food unfit for consumption. In this sense, the use of gamma radiation to inhibit the germination of nuts [3] or wheat [4] can be exemplified as the results of various studies. Secondly, the study of the germination process is of interest due to the fact that recently there has been an increase in the consumption of germs of different seeds. This is desirable due to the positive effects of germination on the grains subjected to this process. Different studies show that germination leads to increase bioavailability of minerals in seeds [5, 6, 7, 8], the amount of phenolic compounds [9, 10], flavonoids [11, 12], the amount of amino acids (Gamma Aminobutyric Acid and essential amino acids) [13, 14, 15], vitamins [16, 17, 18]. It has also been shown that germination contributes to the activation of hydrolytic enzymes, which result in improved digestion of certain compounds, such as proteins and starch [19, 20, 21]. This is of particular interest to people who are suffering of with various diseases of the digestive system [22]. At the same time, germination is seen as a desirable process because it has the role of decreasing the amount of antinutrients in the grain (for eg. phytic acid, which combines with various minerals and result phytates) [23, 24, 25]. Also, the interest for seed germs today is great due to the fact that they can be incorporated into the recipe of manufacturing various foods, in order to improve their nutritional profile or sensory and quality characteristics [22]. In this sense, can be list: bakery products [26, 27, 28], yogurt [29, 30, 31], biscuits [32, 33, 34], cakes [35, 36, 37] and so on.

Regardless of the purpose pursued, it is necessary to carefully monitor the parameters of the germination process (temperature, humidity, aeration, lighting), so that the germs obtained to be of a superior quality, to contain an optimal amount of nutrients (in this regard, it is recommended that the germination time should not be prolonged too much, so that the grains not to be depleted in nutrients) [9, 10, 16], the sensory characteristics (color, appearance, smell, taste) should not be adversely affected and the microbial load should not exceed that indicated by standards in force [26, 28], so that the health of consumers not to be jeopardized by any microorganisms that have developed or by toxins that have been released along the way [22, 23].

Depending on the field of use of the germs obtained, the germination parameters must be chosen in such a way that what is desired to be obtained (optimal enzymatic activity or inhibition of antinutritive factors, for example) to be successful [37]. In this sense, it is necessary to carefully study the literature that indicates clear suggestions in this regard. Highlighting the physical and physiological changes of different types of vegetables during the germination process is desirable so that the germination process can be optimally conducted, so that the germs obtained to be of superior quality and thus can serve with success for the purpose for which it is intended to be used [20, 22].

According to our acknowledgment, no comparative study has been made on so many types of legumes seeds during the entire germination period on their physical and physiological changes on modern device such as Stereomicroscope one. Also, no other study has been made on a comparative analysis of minerals availability during the 0, 2 and 4 germination days between chickpea, bean, lentil, lupine, and soybean type.
The aim of this study was to highlight the physical and physiological changes of different types of legumes during the germination process, changes that are correlated with the determination of the size of the developed parts during germination (plumule and radicle) and to highlight the variation of mineral content during the germination process, of which amount was compared to those of the ungerminated seeds. Thus, will be highlighted the optimal germination time, so that the germs to present superior quality for consumption.

**Materials and methods**

**Materials**

The grain legumes seeds used were: chickpea (*Cicer arietinum L.*), bean (*Phaseolus vulgaris*), lentil (*Lens culinaris Merr.*), lupine (*Lupinus albus*) and soybean (*Glycine max L.*). All the grain legumes seeds were cultivated in Romania and were not genetically modified.

**Grain legumes seeds analysis**

The grain legumes seeds were analyzed for its physical-chemical properties according to the International standard methods as follows: the hectoliter weight was determined according to the ISO 7971-1:2009, the moisture content of legumes seeds were determined according to ISO 7700-1:2008, the seeds viability was determined by usual inspection of cut seeds through tetrazolium staining [38], the seeds germination capacity was determined according to ISTA (2006) standard [39], and the protein content was determined according to EN ISO 20483:2006.

**Grain legumes seeds germination process**

For legumes seeds germination, a Binder KBW/KBWF germination chamber was used. The germination process was carried out to a maximum germination period of 9 days for soybeans, beans and chickpeas and for 10 days for lupine and lentils in accordance with the conditions provided by the ISTA (2006) standard [39]. The germination was made on dark conditions to a temperature which varied between 20 and 25 °C and to a constant humidity value of 80%. The germination layer used was the filter paper.

**Grain legumes seeds analysis during the germination process**

In order to highlight the physical and physiological legumes seeds changes during the germination process was measured every day the size of the radicle and plumule of legumes seeds by using a Modelcraft Vernier Calliper of 125 mm [40]. Also, it was captured the physical and physiological changes that occur in legumes seeds during the germination process by using a Motic SMZ-140 Stereomicroscope device [41]. This allowed us to obtain detailed images that highlight the essential changes: increasing the volume of the grain due to the water absorption, degradation of the protective coating of the grain, the development of the component parts of the germs, etc.
Statistical analysis

Data were expressed as means ± standard deviations for triplicate determination. Statistical analysis was performed using XLSTAT statistical package (free trial version, Addinsoft, Inc. Brooklyn, NY, USA) at a significance level of p < 0.05 [42].

Results and discussion

Physical-chemical characteristics of the ungerminated legumes seeds

The data for the physical-chemical analysis are shown in Table 1. As it may be seen, the soybean presented the highest protein content value, whereas the chickpea the lowest one. From the hectolitre point of view this value varied between 70.0 and 84.5 kg/hl with the highest value for lentil and the lowest one for lupine seeds. The humidity value for the seeds sample was not higher than 10.9% whereas the legumes seeds capacity to germinate determined through viability and germination energy indicated the fact that lentil presented the highest capacity to germinate whereas the lupine and chickpea the lowest one.

Table 1

<table>
<thead>
<tr>
<th>Legumes seeds</th>
<th>Hectolitre weight [kg/hl]</th>
<th>Humidity [%]</th>
<th>Viability [%]</th>
<th>Germination energy [%]</th>
<th>Protein content [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>70.5±0.03</td>
<td>9.8±0.01</td>
<td>77±0.47</td>
<td>74±0.81</td>
<td>40.34±0.01</td>
</tr>
<tr>
<td>Lupine</td>
<td>70.0±0.02</td>
<td>7.7±0.02</td>
<td>52±0.81</td>
<td>51±0.47</td>
<td>39.90±0.03</td>
</tr>
<tr>
<td>Chickpea</td>
<td>73.5±0.01</td>
<td>10.3±0.01</td>
<td>50±0.81</td>
<td>52±0.47</td>
<td>19.40±0.04</td>
</tr>
<tr>
<td>Bean</td>
<td>70.8±0.04</td>
<td>10.9±0.01</td>
<td>73±0.47</td>
<td>70±0.81</td>
<td>22.60±0.02</td>
</tr>
<tr>
<td>Lentil</td>
<td>84.5±0.02</td>
<td>7.6±0.01</td>
<td>90±0.47</td>
<td>88±0.81</td>
<td>28.69±0.03</td>
</tr>
</tbody>
</table>

Physical, physiological and minerals changes during the germination process of different types of legumes seeds

The physical and physiological changes of different types of legumes were presented below, during every day of germination process (from day 1 to 9, respectively to 10 days of germination process depending on the legumes type). For 0, 2 and 4 days of germination process it was determined the amount of mineral elements such as calcium, iron, phosphorus, sulfur, zinc, manganese, and potassium from the legumes seeds by using a spectrometer Shimadzu EDX-900HS (Shimadzu Corporation, Kyoto, Japan) device. It was established the variation of legumes types during the 4 days of germination due to the fact that according to the physical changes during the germination process was the optimum day for their use in food consumption. Also different studies has been reported that at this time of germination process the enzymatic activity of legumes are in a high amounts [43, 44, 45] and if the germination period exceeded too much the amount of nutrients begin to decreases which is not a desirable fact [8, 46, 47, 48].
Physical and physiological changes of the lentil during germination

Figure 1 shows the changes that occur during germination process in the lentil seeds, during the 10 days of germination. At the same time, it can be seen the variation of the size of the component parts (radicle and plumule) during the ten days of germination. Thus, on the first day of germination there is an increase in the volume of the lentil seed, as a result of the absorption of water needed in the subsequent germination process; the absorption of water being done through free pores and capillaries. Water absorption is done to hydrate the grain, in order to stop the grain dormancy [49]. In the literature, it is considered that the optimum moisture content for germination is 75–80 g of water per 100 g of dry matter [50]. During the other 9 days of germination, it can be seen the changes that occur: on the second day the radicle begins to develop, and from the third day, it can be seen the plumule. The dimensions of these components increase from day to day.

![Figure 1. Physical and physiological changes of the lentil seeds during the germination process](image)

Images captured and presented in Figure 2 with the Motic SMZ-140 stereomicroscope showed the grain in a dorsal, facial, transverse, and cross-sectional position. In the literature, the stereomicroscope has been used successfully to study the germination phenotype [51]. From these images, it can be seen that the development of the root begins from day one. The chlorophyll is found in cellular organs called chloroplasts and has an important role in the process of photosynthesis. It helps to capture the light needed in the photolysis of water molecules, in order to assimilate carbon in the other stages of photosynthesis [52, 53]. In the other stages, on days 7-10, it is observed that the root of the future plant grows more and more, so that the plant can be prepared for the absorption of nutrients and water from the soil, a process in which the root has an essential role [54, 55].
Physical and physiological changes of the bean, during germination

Figures 3 and 4 showed the changes that occur during germination process in the bean seed, during the 9 days of germination.

Figure 2. Physical and physiological changes of the lentil, captured with the Motic SMZ-140 Stereomicroscope

Figure 3. Physical and physiological changes of the bean seeds during the germination process
It can be seen that on day 1 of germination, as in the case of lentils, the grain increased in volume due to the absorption of water needed for the next steps. Water plays an important role in the germination process. During germination, it is very important to keep the humidity constant because a water deficit will cause the degradation of the germ membrane and their integrity [62]. As studies suggested, the presence of water is necessary to initiate the germination process [63]. In the next stages of germination, days 2-5, the development of the radicle is observed. Starting with the sixth day, the appearance of the root of the future plant is observed. It is also observed, starting with the fifth day, the synthesis of chlorophyll, a pigment of significant importance in the subsequent process of photosynthesis, a process that ensures the correct development of the plant.

Figure 4 highlights in more detail the physical and physiological changes that occurred during the germination process. Images captured using the Motic SMZ-140 stereomicroscope showed the grain in a dorsal, facial, transverse, and cross-sectional position.

Physical and physiological changes of lupine during germination

Figures 5 and 6 showed the changes that occur during germination in the lupine grain during the 10 days of germination period. In the case of lupine, it can be seen that on days 2, 3 and 4 takes place the development of the radicle, up to a size of 11 mm. From the 5th day onwards, chlorophyll is synthesized. From the 8th day, the appearance of the root of the future plant is observed.
Figure 5. Physical and physiological changes of the lupine seeds during the germination process

Figure 6. Physical and physiological changes of the lupine, captured with the Motic SMZ-140 Stereomicroscope

**Physical and physiological changes of chickpeas during germination**

The changes that occur during germination in the chickpea, during the 9 days of germination, can be seen in Figures 7 and in Figures 8. Thus, the development of the radicle in the case of chickpea begins on the second day. Starting with the sixth day, the appearance and development of the plumule and the synthesis of chlorophyll are observed. On the ninth day, the radicle has a length of 130 mm and the plumule, 58 mm. The images from figure 8 captured to the dorsal, facial and transverse seed position, facilities the observing in detail the development of the leaves and the root (days 6–9).
Figure 7. Physical and physiological changes of the chickpeas seeds during the germination process

Figure 8. Physical and physiological changes of the chickpea, captured with the Motic SMZ-140 Stereomicroscope

Physical and physiological changes of the soybean during germination

Figures 9 and 10 show how the soybean evolved during the 9 days of germination. Thus, it can be seen that the radicle has reached the size of 24 mm at the end of the fourth day of germination. Starting with the fourth day, the seed began to synthesize chlorophyll, and the root began to be more pronounced starting with the eighth day.
Figure 9. Physical and physiological changes of the soybean seeds during the germination process.

Figure 10. Physical and physiological changes of the soybean, captured with the Motic SMZ-140 Stereomicroscope.
Mineral changes of the legumes seeds during the 4 days of germination period

Figures 11–16 showed how the mineral content varies with the germination time, during day 0, 2 and 4 for all five types of legumes considered in this study (lentil, bean, lupine, chickpea and soybean). It was observed that the amount of most minerals decreases with increasing germination time. From Figure 11, it can be noticed that the amount of calcium increases with increasing germination time. This is probably due to the fact that, during germination, the amount of phytic acid decreases. This is a desirable thing because phytic acid forms phytates with the minerals. At the same time, the minerals that are in bound form are released during germination [11, 56, 57]. Another reason would be that, during germination, amylases are activated. Amylases are metalloenzymes with calcium in the composition. Studies show that calcium is sometimes found bound to α-amylases [58]. If calcium is completely lost from the structure of the enzyme, then the enzymatic activity is stopped [59]. Therefore, if the germs obtained are to be used for their enzymatic activity, the variation of the amount of calcium must be followed carefully. In the case of the enzyme, calcium has an important role in supporting the structure of α-amylase because it interacts with negatively charged amino acid residues (aspartic and glutamic) and thus stabilizes the structure of the enzyme [60]. Calcium also plays an important role in stopping the proteolytic degradation of α-amylase [61].

In Figure 12, it can be noticed, for example, that the sulfur content increases for almost all legumes types on the fourth day of germination compared to the control sample. Increasing the amount of sulfur could be attributed to the fact that plants need sulfur to grow properly [64]. Increasing the amount of sulfur in seed germs is desirable, as shown by various studies in the field [65, 66] because sulfur is an essential mineral in the human body and has been shown to act as an antioxidant and help fight atherosclerosis and cancerous tumors [67].
The variation of the potassium content during the germination process of legumes can be seen in figure 13. Hence, it is observed that the potassium content decreases for the sample in the four day of germination compared to the control sample. This process occurs because potassium is a very important element used to develop the component parts of the future plant [68].

---

**Figure 12. Variation in sulfur concentration during the germination time**

**Figure 13. Variation in potassium concentration during the germination time**
In general, during the germination process, compared to the control sample the amount of phosphorus decreased. This may be due to the fact that in the germination process phosphorus is consumed for the development of the seeds components (radicle and plumule).

![Figure 14. Variation in phosphorus concentration during the germination time](image)

Figures 15–17 show how the concentration of mineral substances such as Fe, Zn and Mn varied during the 4 days of germination process.

![Figure 15. Variation in iron concentration during the germination time](image)
A decrease in iron content may be noticed to soy, chickpea and lupine to the 4 day of germination. This data are similar with those reported by others [69]. However, an increase of iron may be noticed to beans and lentil in the 4 day of germination period. Also the zinc
and manganese increases or decreases depending on the legumes type. Due to the fact that germination increases the concentration of some mineral substances, it can be said that this process can be used successfully to improve the nutritional profile of germinated grains or foods in which the germs will be incorporated in some mineral elements depending on the legumes type [71–77].

Conclusions

1. The physical and physiological changes of legumes types chickpea, bean, lentil, lupine, and soybean shown that for all the analyzed samples the four days of germination period may be an optimum one for their use in food consumption.
2. During the germination period, the amount of mineral substances varies differently for each type of legume, but also for each type of mineral element. These facts are important for germ development but also for food consumers which are interested for healthy ingredients for consumption.
3. For all the analyzed samples, potassium decreases during the germination period whereas calcium increases in a lowest amount of 7.5% for beans and in a highest amount for chickpeas (almost six times) in the four day of germination. Regarding the other minerals elements analyzed (sulf, phosphor, iron, magnesium, zinc, manganese) they presented different variations during the germination period. The sulf presented higher values in the four day of germination for all the analyzed legumes types except chickpeas for which it decreases with 5.15%. The phosphor decreases for all legumes types except lentil for which increases with 25.87%. The iron decreases for soy, chickpea, lupin and increases for lentil and beans in the four day of germination. In general, zinc decreases for all analyzed samples during the germination period except lupine of which value increases with 50%. Manganese was not detected in lentil and beans meaning that in these legumes types were of a very low concentration. This mineral did not vary during germination in soy seeds but increases in lupine with 9.61% and decreases in chickpeas with 20.43% in the four day of germination period.

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Differences in the composition of vocable compounds in fresh and dried mixed heat supply of white rolled cabbage

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Abstract

Introduction. The work aims to study the chromatographic profiles of volatile compounds in fresh and dried mixed heat white cabbage with the definition of differences in qualitative and quantitative composition.

Materials and methods. Chromato-mass spectrometry (GC/TOF-MS) methods were used to study the profiles of volatile substances in fresh and dried heat-fed vegetables, in particular, Amager white cabbage, with differences in qualitative and quantitative composition. Fresh white cabbage was chosen as a control.

Results and discussion. During chromatographic studies of volatile aromatic compounds of fresh and dried white cabbage, 20 volatile substances were identified. Both samples contain the following components: 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(2.61 and 2.11%), guanosine (1.07 and 0.48%), oxirane, tetradecyl (1.71 and 0.90%), tetradecanal (2.89 and 2.72%), 2-pentadecanone (3.2 and 3.00%), 2-nonadecanone (3, 01 and 2.84%), formamide, N-methyl-N-[1-(pyrrolidinyl)-2-butynyl] (3.12 and 2.92%), n-hexadecanoic acid (5.8 and 5.76%), cis-acetic acid (4.43 and 4.44%), oleic acid (3.94 and 4.02%), oleic acid amide (3.12 and 3.2%), 1,2-benzene dicarboxylic acid diisooctyl ether (5.28 and 5.22%), 6-methyl-octadecane (1.96 and 1.16%), 2,6,10-trimethyltetradecane (2.69 and 1.84%), heptacosan (34.16 and 32.15%), 2-hexadecanol (2.25 and 1.76%), 1,2-epoxyhexadecane (11.11 and 10.65%), nonacosanone-15 (3, 83 and 3.45%).

Drying of raw cabbage by drying with mixed heat transfer did not cause a change in the qualitative composition, however, caused a decrease in the amount of volatile aromatic substances.

Decrease in content, % of components: heptacosane, (34.15→32.16), 1,2-epoxyhexadecane (11.11→10.659), octadecenamide (3.12→3.02), n-Hexadecanoic acid (5.80→5.76) to some extent eliminates the bitter note of raw cabbage, greasy taste, softens its aromatic sensations.

Conclusions. Comparison of volatile substances between fresh and dried samples of cabbage allows us to claim the preservation of valuable biological substances of fresh cabbage after drying with mixed heat and to spread this method of processing cabbage with maximum use of its useful properties.

Keywords: Cabbage, Drying, Chromatography, Volatile compound, Composition

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Introduction

The aroma of dried vegetable raw materials is one of the determining factors of the level of quality because evaporating from the raw material, moisture takes with it volatile components, resulting in some loss of taste and aroma [1, 2]. To obtain high-quality food products using dried vegetable food products obtained by drying with mixed heat, in particular common vegetables – white cabbage, it was advisable to study the complex of its flavoring substances.

The conversion of volatile aromatic compounds of cabbage during heat treatment has been studied to a greater extent at the technological stage of blanching [3, 4] and traditional drying methods – convective, conductive, etc. Drying with mixed heat supply is currently promising and economical among the methods that provide heated air as a drying agent [5, 6]. We did not find studies comparing the profiles of volatile compounds of samples of fresh and dried cabbage with mixed heat, which led to the relevance of the chosen direction of research.

The study aimed to study the chromatographic profiles of volatile compounds in fresh and dried mixed heat of white cabbage with the determination of differences in qualitative and quantitative composition.

Materials and methods

Samples and their preparation

Fresh white Amager cabbage was used for the study. After removing the outer leaves, the cabbage heads were cut into 2 mm thick strips with a shredder. Part of the shredded cabbage (5 kg) was mixed and the juice was squeezed from the pulp. Another portion of cabbage (5 kg) was dried by the method of mixed heat and ground to a powder.

The hardware implementation of drying with mixed heat supply is a chamber measuring 2.0 × 1.0 × 1.5 m, having double walls of sheet steel with a thickness of 10–3 m, between which there is a thermal insulator.

The camera is mounted on a frame that serves as a base for the fan and air nozzles. The bottom of the chamber is uninsulated. In the upper part of the chamber, there is a hatch for loading and unloading of products: fan, pipes, heater, and working chamber.

The fan motor is outside the camera. The airflow from the fan is directed to the heater, then through the rotary nozzles to the working chamber and the fan inlet, ie the airflow is recirculated in the chamber [7].

Samples of raw cabbage and dried by drying with mixed heat were distributed in portions of 1 g in glass vials, sealed to prevent loss of volatile compounds.

Qualitative and quantitative analysis of volatile substances

A combination of capillary gas chromatography and mass spectrometry (GC / TOF-MS) was used to measure the concentration of cabbage volatile substances in samples of fresh cabbage and dried cabbage [8].

Separation of the components was performed using a standard chromatographic capillary column from PerkinElmer with active phase "Elite-5MS". The diameter of the column was 250 μm and the length was 30 m. Helium was used as a carrier gas, the flow of which was 20 ml/min. The temperature regime is shown in table 1.
Table 1

<table>
<thead>
<tr>
<th>Temperature mode</th>
<th>Speed of temperature change, °C / min</th>
<th>Final temperature, °C</th>
<th>Retention time, min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.0</td>
<td>80.0</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>130.0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>240.0</td>
<td>4.50</td>
</tr>
<tr>
<td>3</td>
<td>20.0</td>
<td>280.0</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Individual retention signals were recorded, in particular, the retention time Rt, which indicates the location of each component on the chromatogram. The paper takes into account to a greater extent compounds with high truth,% with a signal-to-noise ratio (S/ N> 250).

As a result of the experiment, chromatograms of experimental samples of cabbage were obtained (Figure 1).

![Chromatograms of experimental samples of Amager cabbage:](image)

**Figure 1. Chromatograms of experimental samples of Amager cabbage:**  
a – fresh, b – dried by mixed heat dissipation
Calculation of the quantitative content of the components of the experimental samples

Conducted by the method of internal normalization with statistical data processing of three parallel experiments [9]. The mass fraction of the investigated components $m_R,\%$ was calculated as the ratio of the peak area of the component SR to the total area of all components. For the final result, we used the mean value with the calculation of the standard deviation $S$. The level of probability with a confidence level $p = 0.95$ did not exceed $\alpha = 0.05$, the critical Student’s criterion $= 5.47$. Average values were chosen as the result.

The registration of mass spectrometers was carried out in the mode of ionization of molecules by electron impact with an electron energy of 70 eV using the El + mode. The scan time of the mass spectra was 0.2 s, with a pause between scans of 0.01 s. The number of scans per averaged mass spectrum was 106. The ions of the studied molecules were fixed in the mass range of $45 \div 450$ m / z. The residual pressure in the ionization chamber was $\sim 2.6 \times 10^{-6}$ Pa. The temperature of the ion source was 300 °C. the Input temperature of the analyzer was 280 °C.

Results and discussion

The quantitative and qualitative composition of volatile components in samples of fresh and dried cabbage

The identified components and their quantitative content of substances are listed in Table 2.

<table>
<thead>
<tr>
<th>№</th>
<th>$t_R$, min</th>
<th>Name of components</th>
<th>CAS#, formula</th>
<th>Characteristic</th>
<th>$m_R,%$ in samples of cabbage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fresh</td>
</tr>
<tr>
<td>1</td>
<td>04:78</td>
<td>4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl</td>
<td>28564-83-2 C$_6$H$_8$O$_4$</td>
<td>Ketone. Makes a sweet aroma [18]</td>
<td>2,61</td>
</tr>
<tr>
<td>2</td>
<td>07:50</td>
<td>Guanosine</td>
<td>118-00-3 C$<em>{10}$H$</em>{13}$N$_3$O$_5$</td>
<td>Purine nucleoside. Makes a characteristic pungent aroma [18]</td>
<td>1,07</td>
</tr>
<tr>
<td>3</td>
<td>08:35</td>
<td>Oxirane tetradecyl</td>
<td>7320-37-8 C$<em>{16}$H$</em>{32}$O</td>
<td>Saturated three-membered heterocycle. Characteristic pungent odor [2]</td>
<td>1,71</td>
</tr>
<tr>
<td>№</td>
<td>t_R, min</td>
<td>Name of components</td>
<td>CAS#, formula</td>
<td>Characteristic</td>
<td>m_R, % in samples of cabbage</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
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<td>--------------</td>
<td>--------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fresh/ Dried</td>
</tr>
<tr>
<td>5</td>
<td>10:70</td>
<td>2-Pentadecanone</td>
<td>2345-28-0</td>
<td>Ketone. Weak pleasant smell [2]</td>
<td>3,2/3,00</td>
</tr>
<tr>
<td>6</td>
<td>11:51</td>
<td>Octadecanoic acid</td>
<td>57-11-4 C_{18}H_{36}O_{2}</td>
<td>Fatty acid. Included in the lipid composition of vegetable wax. Introduces a viscous aroma [18]</td>
<td>1,82/1,06</td>
</tr>
<tr>
<td>7</td>
<td>12:12</td>
<td>2-Nonadecanone</td>
<td>629-66-3 C_{19}H_{38}O</td>
<td>Oxygen hydrocarbons – ketone. Weak &quot;green&quot; aroma [18]</td>
<td>3,01/2,84</td>
</tr>
<tr>
<td>8</td>
<td>12:41</td>
<td>Formamide, N-methyl-N-4-[1-(pyrrolid inyl)-2-butynyl]</td>
<td>18327-40-7 C_{10}H_{16}N_{20}</td>
<td>Heterocyclic diazo compound. Has a weak specific odor [19]</td>
<td>3,12/2,92</td>
</tr>
<tr>
<td>9</td>
<td>14:32</td>
<td>n-Hexadecanoic acid</td>
<td>57-10-3 C_{16}H_{32}O_{2}</td>
<td>Saturated fatty acid of the direct chain [18]</td>
<td>5,80/5,76</td>
</tr>
<tr>
<td>10</td>
<td>15:60</td>
<td>9,12-Octadecadienoic acid</td>
<td>60-33-3 C_{18}H_{32}O_{2}</td>
<td>Double unsaturated fatty acid, which is widely found in plant glycosides with a pleasant specific odor [18]</td>
<td>2,04/1,83</td>
</tr>
<tr>
<td>11</td>
<td>15:97</td>
<td>cis-Vaccenic acid</td>
<td>506-17-2 C_{18}H_{34}O_{2}</td>
<td>Cis-isomer of vaccine acid, part of phospholipids. Is an omega-7 fatty acid [18,2 ]</td>
<td>4,43/4,44</td>
</tr>
<tr>
<td>12</td>
<td>16:25</td>
<td>Oleic Acid</td>
<td>112-80-1 C_{18}H_{34}O_{2}</td>
<td>Belongs to monounsaturated fatty acids. It belongs to the group of omega-9 unsaturated fatty acids. With a mild odor [2]</td>
<td>3,94/4,02</td>
</tr>
</tbody>
</table>
As a result of the conducted researches in the spectrum of volatile substances of samples of fresh and dried cabbage 20 compounds were identified. Both samples contain the

<table>
<thead>
<tr>
<th>№</th>
<th>t_R, min</th>
<th>Name of components</th>
<th>CAS#, formula</th>
<th>Characteristic</th>
<th>( m_R, % ) in samples of cabbage</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>17:51</td>
<td>9-Octadecanamide</td>
<td>C18H35NO</td>
<td>Fatty amide of oleic acid. Plant metabolite [17]</td>
<td>3,12 3,02</td>
</tr>
<tr>
<td>14</td>
<td>18:47</td>
<td>1,2- Benzenedicarboxylic acid diisooctyl ester</td>
<td>C24H38O4</td>
<td>Terpenoid, a derivative of phthalic acid, a complex ethereal odor [18,2]</td>
<td>5,28 5,22</td>
</tr>
<tr>
<td>15</td>
<td>19:49</td>
<td>Octadecane, 6-methyl-</td>
<td>C19H34O</td>
<td>Isoprenoid alkane (isoprene). Volatile compound of cabbage, which gives a characteristic fresh aroma [18]</td>
<td>1,92 1,16</td>
</tr>
<tr>
<td>16</td>
<td>20:24</td>
<td>Tetradecane, 2,6,10-trimethyl</td>
<td>C17H36</td>
<td>Isoprenoid alkane (isoprene) gives a characteristic fresh aroma [2]</td>
<td>2,69 1,84</td>
</tr>
<tr>
<td>17</td>
<td>21:15</td>
<td>Heptacosane</td>
<td>C27H56</td>
<td>Saturated hydrocarbon, alkane [17]</td>
<td>34,16 32,15</td>
</tr>
<tr>
<td>18</td>
<td>22:04</td>
<td>2-Hexadecanol</td>
<td>C16H34O</td>
<td>Alcohol of high molecular weight, is a part of esters of wax [17]</td>
<td>2,25 1,76</td>
</tr>
<tr>
<td>19</td>
<td>23:35</td>
<td>1,2-Epoxyhexadecane</td>
<td>C16H32O</td>
<td>Saturated heterocycle with a weak bitter tone of aroma [18]</td>
<td>11,11 10,65</td>
</tr>
</tbody>
</table>
following components: 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(2.61 and 2.11%), guanosine (1.07 and 0.48%), oxirane, tetradecyl (1.71 and 0.90%), tetradecanal (2.89 and 2.72%), 2-pentadecaneone (3.2 and 3.00%), 2-nonadecanone (3.01 and 2.84%), formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl] (3.12 and 2.92%), n-hexadecanoic acid (5.8 and 5.76%), cis-acetic acid (4.43 and 4.44%), oleic acid (3.94 and 4.02%), oleic acid amide (3.12 and 3.2%), 1,2-benzene diercarboxylic acid diisocet ether (5.28 and 5.22%), 6-methyl-octadecane (1.96 and 1.16%), 2,6,10-trimethyltetradecane (2.69 and 1.84%), heptacosan (34.16 and 32.15%), 2-hexadecanol (2.25 and 1.76%), 1,2-epoxyhexadecane (11.11 and 10.65%), nonacosanone-15 (3, 83 and 3.45%).

The spectrum of volatile substances is characterized by a wide range of chemical compounds. These are oxygen-containing and esterified compounds, saturated heterocycles, diazo compounds, terpenoids, isoprenoid alkanes.

At the same time, isoprenoid alkanes are among the most typical and biogenic components. This group includes several dozen hydrocarbons, a characteristic feature of the structure of which is the location of methyl groups in the linear carbon backbone in positions up to = 2, k + 4, k + 8, k + 12 [10].

Some compounds are part of vegetable wax [11, 12]. They are a complex, multicomponent mixture of relatively simple hydrocarbons (primarily alkanes), wax esters, as well as fatty acids, alcohols, and ketones.

To some extent, this composition of volatile compounds is associated with their sensory properties. Some of the identified components, in particular, high molecular weight alkanes and their oxygen-containing derivatives, can contribute to "Bitter", "Fresh", "Cabbage", "Fatty" and other notes of cabbage flavor [13, 14].

Also, a significant number of compounds from the volatile substances of cabbage, including bioactive isoprene, nitriles, aldehydes, and alcohols, phospholipids (cis-vaccenic acid), show functional properties [15].

**Comparative characteristics of changes in volatile substances in cabbage samples**

Comparing the differences in changes in volatile substances in the test samples, we can state the following:

1. The test samples do not differ in qualitative composition, however, have the different quantitative composition of components.
2. It should be noted that the drying of raw cabbage by drying with mixed heat transfer caused a decrease in volatile aromatic substances in the test samples.
3. Both samples have the same dominant components, in particular, heptacosane (34.15 and 32.16%), 1,2-Epoxyhexadecane (11.11 and 10.65%). Heptacosane is a volatile waxy substance with a pungent odor, is part of beeswax [16, 17]. 1,2-epoxyhexadecane is a waxy substance with an ethereal odor, n-hexadecanoic acid is a saturated fatty acid of the direct chain, found everywhere in nature in many plants [18, 19].
4. It can be noted that the decrease in the content, wt% of components: №17 – heptacosane, (34,15 → 32,16), №19 – 1,2-epoxyhexadecane (11,11 → 10,659), №13 – octadecenamide 3.12 → 3.02), №9 – n-Hexadecanoic acid (5.80 → 5.76) eliminates the bitter note of raw cabbage, greasy taste, softens its aromatic sensations.
5. In dried cabbage there was no decrease in the variety and a significant percentage of oxygen-containing compounds. All analyzed samples of cabbage contained ketones, aldehydes (15-Nonacosanone, 2-Pentadecanone, tetradecanal), which introduce aromas of "green tone", in particular, 15-Nonacosanone with a hint of cut hay.
Conclusions

1. Chromato-mass spectrometry (GC / TOF-MS) methods were used to study the profiles of volatile substances in fresh and dried mixed heat-fed vegetables, in particular, amager white cabbage to determine differences in qualitative and quantitative composition.

2. 20 volatile substances were identified, including oxygen-containing and esterified compounds, saturated heterocycles, diazo compounds, terpenoids, isoprenoid alkanes.

3. Comparison of volatile substances between fresh and dried samples of cabbage allows asserting the preservation of valuable biological substances of fresh cabbage after drying with mixed heat supply and to spread this method of processing cabbage with maximum use of its useful properties.

Therefore, changes in the number of phases and thermodynamic potentials of vegetable raw materials during mixed heat drying have led to minimal changes in functional volatile chemical compounds and maximum changes in substances that cause a bitter taste. Thus, the goal of work on studying the qualitative and quantitative composition of volatile substances in samples of fresh and dried white cabbage was achieved, which confirmed the economical conditions of drying with mixed heat.

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Natural alternatives of Sulphur dioxide used in wine and their effects on aromatic compounds

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Abstract

Introduction. The aims of this study were to determine the effects of different natural plant extracts used as an alternative of Sulphur dioxide on wine aroma compounds.

Materials and methods. The wine production was done according to the accepted conventional method of red wines (Cabernet Sauvignon). The experimental design was achieved by using different plant extracts (grape pomace, rosemary, black blueberry) at different concentrations. As the first control group was used wine samples processed without natural extracts and Sulphur dioxide treatments and as the second group was used wines produced with 25mg/L Sulphur dioxide addition.

Results and discussion. The highest total amount of volatile compounds was achieved by applying blueberry extract and grape pomace extract. The combined application of Sulphur dioxide and blueberry extract increased the wine volatile complexity. The best results related to higher alcohols synthesis and their accumulation in wines was obtained by using Sulphur dioxide (25 mg/L) and plant extracts (0.3 mL). The terpenes were dominated by geraniol. The highest value was obtained in sample treated with grape pomace.

The ester fraction was represented by 9 identified compounds. The highest total ester content (169.13 mg/L) was found in sample obtained with combined treatment of 25 mg/L Sulphur dioxide and rosemary extract 0.3 mL. The other three variants of rosemary treatments demonstrated quantitatively close ester content. In samples containing grape pomace extract was found the lowest total ester content compared to all others. From this group, only sample including 25 mg/L Sulphur dioxide and grape pomace extract 0.3 mL (40.62 mg/L) was distinguished. Methyl alcohol was found in all tested wines. The methyl alcohol levels were very low and not pose a risk to the consumer.

Conclusions. The study demonstrated the possibilities of optimization of Sulphur dioxide by using natural plant extracts.
Introduction

During wine production, SO$_2$ is used as an antioxidant and antimicrobial additive in wine production. It uses for preventing oxidation and the spread of unwanted organisms such as wild yeasts, acetic acid bacteria and lactic acid bacteria [1]. Aging process causes gradual loss of phenolic compounds due to some oxidation reactions with polysaccharides and tannins leading to formation of other stable anthocyanin-derived pigments. These reactions can result in some changes in the color, taste and flavor properties of red wines [2, 3]. Even these advantages, negative effects of SO$_2$ on human health have been subject to researches for many years [4].

A number of studies have been indicated as an alternative of SO$_2$. Most of them proposed non-thermal processes, or using of new chemicals. One of the most promising natural alternatives to sulphides in wine production are using of natural plant extracts [5]. The flavonoids, phenolic compounds and their derivatives, which are naturally found in the structure of these extracts, have been shown to be effective in preventing auto-oxidation [6,7]. It is emphasized that some phytochemicals such as terpenes, alkaloids, lactones, etc. found in the extract may contribute to the prevention from auto-oxidation of wine. The aroma is an essential characteristic determining the quality of the wine. It is due to the significant diversity of volatile compounds (over 800) and the variation in their total content (up to 800–1200 mg/L) [8]. Numerous factors influence the formation of the final wine aroma: the genetic ability of the vine variety to synthesize and accumulate volatile aromatic components in the grapes, climatic, soil and geographic characteristic of the vine growing area, agrotechnical measures and phytosanitary status of the vine, the technique and technology of wine making, metabolic potential of yeast and malolactic microflora, processes during wine aging [9, 10, 11, 12, 13, 14, 15].The final wine aroma contains various volatile compounds belonging to several major groups such as esters, higher alcohols, aldehydes, terpenes and methoxypyrazines [16, 17]. The ester compounds have a significant contribution to the wine aroma. This is due to the low threshold of aromatic perception of the esters. This group of compounds is also formed in the grapevine, accumulating in very low amounts (10–30 mg/L) in the grapes [18]. The subsequent organic yeast ester synthesis is realized during alcoholic fermentation. It leads to significant ester accumulation (up to 500 mg/L) in young wines [12]. The third phase of their accumulation takes place in the aging process called esterification which is due to the chemical bonding between the available alcohols and the acids of the wine. This stage proposed the significantly increased of the total ester content of the wine (792–800 mg/L) and formed wine bouquet [19]. The higher alcohols are a group of aromatic compounds with a lesser aromatic effect. This is due to their higher thresholds of aromatic perception. They are, however, an important factor of the aroma profile of wine, since they cause formation of various esters with the wine acids [11]. The higher alcohols are the product of yeast amino acid metabolism and accumulate in red wines up to 600 mg/L [12]. The important representatives are 3-methyl-1-butanol, phenyl ethanol, hexanol, isobutyl alcohol and others [20]. The terpenes are mainly represented by terpene alcohols – linalool, α-terpineol, β-citronellol, nerol and geraniol [21]. These compounds have a significant contribution to the wine aroma [22]. Then the common question, what is the effect of natural plant extracts on wine aromatic profile in case of application of different methods during production?

The aims of this study were to determine the effects of different natural plant extracts used as an alternative of SO$_2$ on wine aroma compounds.
Materials and methods

Plant material

As materials were used grapes of *Vitis vinifera* L. cv. origin var: Cabernet Sauvignon from the Menderes/ Gölçüklar region of Izmir (Sevilen Winery vineyards). 100 kg grapes were processed in Ege University Food Engineering Department (Izmir/ Turkey) within 24 h of hand-harvest.

The grape pomace (GP) extract was supplied as waste in the normal wine production process of Cabernet Sauvignon grapes. The blueberry (BB) and rosemary (R) extract used in the experimental groups belongs to *Rosmarinus officinalis* L. and *Vaccinium myrtillus* L. spices, respectively. These plants were obtained from the Aegean region in Izmir/ Turkey.

The selection of *Cabernet Sauvignon* pomace and blueberries was done on the base of our previous studies [23, 24, 25, 26, 27, 28, 29] in which higher total phenols and antioxidant activities were determined. The choice of rosemary was done after evaluation of our project results related to conservation of foods by using rosemary extracts (unpublished). All these plants even have a different origin possess similar properties related to the protection of food as materials with higher phenolic content and higher antioxidant activities.

Used plants in the experiment were evaluated on the wet Basses. They were not dried before using in experiments.

Wine processing method

The grapes transferred to the mill for separation from stems, wastes and foreign materials after weighing process. Crushed fruit and juices were collected in stainless steel tank. The density of the juice was determined as 1110 g/L, the average pH was 3.8 and the total acid amount was 5.48 g/L (as tartaric acid). *Saccharomyces cerevisiae* Fermivin strain was added in the tank as commercial yeast (20 g/L dose SIHA Active Dry yeast 10). Fermentation process was completed in 12 days at 20-22 °C. The must was stirred twice daily. All fermentation process was carried out at controlled conditions. The separation of must was done by a mechanical press machine. During fermentation some measurements were carried out regularly such as alcohol content, density and sugar content of the product. At the end of the fermentation, the final sugar content was < 2 g/L. When the fermentation was completed, the wine was transferred and stabilized in the cold. The wines were stored at 15 °C and the preparation of the extraction agents was started. With the addition of the extracts, the samples were bottled and stored during 3 months.

Experimental design and treatments

The *Cabernet Sauvignon* grapes were gowned in Aegean region of Turkey-İzmir. After processioning of wine the pomace was used for the experiment as described. Blueberry (BB) and rosemary (R) plants were supplied from the same region of Turkey- Aegean region-İzmir. The botanical evaluation of plants was done by an expert from Ege University. Natural extracts were prepared as following the path given in Figure 1. Different plant extracts such as grape pomace, rosemary and black blueberry were used at different concentrations for experimental design. As the first control group was used as wine samples processed without natural extracts and SO₂ treatments and as the second group was used wines produced with 25mg/L SO₂ addition.
The main reason of adding these extracts after the fermentation step is inhibition effect of these extract in the negative direction during alcohol fermentation by inhibiting the yeast strain in the must. The wine was divided into five batches, in which the different treatments were carried out. For each batches the treatments were created with addition of the extracts at different concentrations. The experimental groups are presented in Table 1.

**Analyses**

For each experimental group, wine samples were analyzed under three main topics; classic wine analyzes aromatic compounds and statistical analyzes. Basic must analyzes were carried out according to the OIV Compendium of International Methods of wine and must [30,31]. All analyzes were carried out in triplicate.

**Classic wine analyzes.** Classic oenological wine analyzes were determined according to recommended methods by International Organization of Vine and Wine (OIV). Alcohol content (% v/v), pH (direct measurement by using pH meter), total acidity (tartaric acid g/L), volatile acidity amount (g/L acetic acid), total and free SO₂ (mg/L), dry matter (g/L) and ash (g/L) analyzes were performed.

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**Figure 1. Natural extracts preparation**

1. 5 gram plant sample
2. Crushing
3. Addition of 50 mL distilled water
4. Mixing
5. 10 min. ultrasound heat treatment (40Hz)
6. 90 min wait
7. Formation of experiment groups
Table 1

<table>
<thead>
<tr>
<th>PLANT EXTRACTS</th>
<th>Group 1 GRAPE POMACE (GP)</th>
<th>Group 2 ROSEMARY (R)</th>
<th>Group 3 BLUEBERRY (BB)</th>
<th>Control Group 1</th>
<th>Control Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Set 1</strong></td>
<td>0 mg/L SO₂+ 1 mL extract (GP01)</td>
<td>0 mg/L SO₂+ 1 mL extract (R01)</td>
<td>0 mg/L SO₂+ 1 mL extract (BB01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0 mg/L SO₂+ 1 mL extract)</td>
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</tr>
<tr>
<td><strong>Set 2</strong></td>
<td>25 mg/L SO₂+ 0.7 mL extract (GP257)</td>
<td>25 mg/L SO₂+ 0.7 mL extract (R257)</td>
<td>25 mg/L SO₂+ 0.7 mL extract (BB257)</td>
<td>non SO₂ and extract addition (TKOO)</td>
<td>SO₂ treatment (TK25)</td>
</tr>
<tr>
<td>(25 mg/L SO₂+ 0.7 mL extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Set 3</strong></td>
<td>25 mg/L SO₂ +0.3 mL extract (GP253)</td>
<td>25 mg/L SO₂ +0.3 mL extract (R253)</td>
<td>25 mg/L SO₂ +0.3 mL extract (BB253)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(25 mg/L SO₂ +0.3 mL extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Set 4</strong></td>
<td>25 mg/L SO₂ +1 mL extract (GP251)</td>
<td>25 mg/L SO₂ +1 mL extract (R251)</td>
<td>25 mg/L SO₂ +1 mL extract (BB251)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(25 mg/L SO₂ +1 mL extract)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Notes:

Grape Pomace (GP)
- 0 mg/L SO₂+ 1 ml (GP01)
- 25 mg/L SO₂+ 0.7 ml (GP257)
- 25 mg/L SO₂+0.3 ml (GP253)
- 25 mg/L SO₂ +1 ml (GP251)

Rosemary (R)
- 0 mg/L SO₂ + 1 ml (R01)
- 25 mg/L SO₂ + 0.7 ml (R257)
- 25 mg/L SO₂ + 0.3 ml (R253)
- 25 mg/L SO₂ + 1 ml (R251)

Blueberry (Bb)
- 0 mg/L SO₂ + 1 ml (BB01)
- 25 mg/L SO₂ + 0.7 ml (BB257)
- 25 mg/L SO₂ + 0.3 ml (BB253)
- 25 mg/L SO₂ + 1 ml (BB251)

Control groups (TK)
- Control group without SO₂ (TK00)
- Control group containing 25mg/L SO₂ (TK25)

Aromatic content determination by GC-FID. The aromatic profiles of samples were determined by gas chromatographic technique equipped with FID. The content of major volatile aromatic compounds was determined on the basis of stock standard solution. The purity of standard solution used in this study was as > 99.0%. The sample quantity was determined to be 2 μl. For analyses was used gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25 μm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220 °C; detector temperature – 250 °C, initial oven temperature – 35 °C/retention 1 min, rise
to 55 °C with step of 2 °C/min for 11 min, rise to 230 °C with step of 15 °C/min for 3 min. Total time of chromatography analysis – 25.67 min. The identified retention times of the compounds in standard solution were: acetaldehyde (3.141), ethyl acetate (3.758), methanol (3.871), 2-propanol (5.170), isopropyl acetate (5.975), 1-propanol (6.568), 2-butanol (7.731), propyl acetate (9.403), 2-methyl-propanol (10.970), 1-butanol (11.509), isobutyl acetate (11.662), ethyl butyrate (12.710), butyl acetate (12.752), 2-methyl-1-butanol (13.054), 4-methyl-2-pentanol (13.629), 3-methyl-1-butanol (13.840), 1-pentanol (15.180), isopentyl acetate (15.965), pentyl acetate (16.033), 1-hexanol (16.276), ethyl hexanoate (16.376), hexyl acetate (16.510), 1-heptanol (16.596), linalool oxide (16.684), phenyl acetate (18.055), ethyl caprylate (18.625), α-terpineol (19.066), 2-phenyl ethanol (19.369), nerol (19.694), β-citronellol (19.743), geraniol (19.831), ethyl decanoate (19.904). An internal standard octanol was used. After determination of the retention times of aromatic compounds in the standard solution, we proceed to the identification and quantification of the volatile aromatic substances in the wines. The aromatic compositions of samples were determined by using 2 μl of samples.

**Statistical evaluation**

Significant differences between averages were obtained at a 95% significance level. The values were averaged and standard deviation, minimum, maximum and mean values of samples were determined. The least significant differences (LSD) and correlations were also performed. Statistical analysis was performed using the PC (SPSS 15) software package.

**Results and discussion**

**General evaluation of wines**

Wine quality is closely related to aroma content of wine. Many aromatic compounds contribute to the flavour, taste and odour of wines. Some studies have demonstrated the relation of aroma compounds related to flavour characters of wines [32,33].

The statistical evaluation of results indicated the significant differences among same samples (p<0.05). Correlation analysis was used to determine the relation between parameters and within groups. The mean values with their standard deviation, minimum and maximum values were analyzed. The highest pH and total acidity value were detected in BB01 (4.90) and GP253 (6.70 tartaric acid g/L) groups, respectively. The lowest value of pH and total acidity were detected in TK00 (3.70) and BB257 (3.90 tartaric acid g/L), respectively. The highest and the lowest value of volatile acidity of samples were detected in TK00 (0.84 acetic acid g/L) and TK25 (0.24 acetic acid g/L) groups, respectively. While there was no significant correlation detected between pH and total acidity in the groups. However, there were a significant correlation between the pH and the volatile acid values (r= 0.3511, p=0.023).

In our study 18 aromatic compounds were determined in order to evaluate the effects of treatment on characters of Cabernet Sauvignon. These compounds included methanol, 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 1-hexanol, 1-heptanol, 2-phenyl ethanol, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, ethyl butyrate, ethyl hexanoate, penty acetate and phenyl acetate.

The identified and quantified higher alcohols, esters and terpenes compounds by GC-FID are presented in Figures 2, 3, and Table 2 respectively. All established concentrations
corresponded to the quantitative ranges typical for young red wines. The data in this aspect were correlated with other studies [34].

Figure 2. Identified and quantified (by GC-FID) higher alcohols compounds of wines with different added extracts. Average ethanol and methanol value of samples were determined as vol. 13% and 16.7 mg/L respectively.
Figure 3. Identified and quantified (by GC-FID) terpenes compounds of wines with different added extracts

Effects of treatments on the total volatile content and total higher alcohols in wine samples

The highest total content of volatile compounds was found in samples BB251 (579.31 mg/L) and GP253 (579.00 mg/L). Compared to the TK00 (296.29 mg/L) and TK25 (311.91 mg/L) controls, they have almost a two-fold higher total amount of synthesized volatile compounds. The incorporated two extracts (blueberry and grape pomace) applied in the indicated amounts have a positive effect on the wine total volatile composition. The lowest amount of total volatile compounds was found in the sample without SO$_2$, but with the addition of 1 mL of rosemary – R01 (242.63 mg/L).

The complete absence of SO$_2$ in this sample has affected negatively the fermentation process. This resulted in decreased secretion of metabolic yeast products, which reflected in the low total volatile content of the wine. This can be explained by uncontrolled fermentation,
without added sulfur dioxide, which not inhibits the activity of wild yeasts and other microflora. It is known that SO\(_2\) has the responsible for inhibit the growth of harmful yeasts and bacteria and ensure the normal course of fermentation [18]. High total content of volatile components were also found in the samples with extracts of blueberry BB01 (545.78 mg/L) and rosemary R253 (453.92 mg/L). It is noteworthy that the blueberries extract was a positive effect on the wine volatile composition, even in the absence of sulfur dioxide – BB01 sample. However, the results shown that the combined effect of sulfur dioxide and blueberry extract (BB253) influenced the wine volatile composition with increasing of its complexity.

The lowest established total higher alcohols content (182.67 mg/L) was observed in wine R01, obtained with added rosemary extract (1 mL) and without SO\(_2\). In the other three variants (R251, R253 and R257), where SO\(_2\) and rosemary extract were added at different concentrations, higher total levels of higher alcohols were observed. This demonstrated an improved sulphitation efficiency of the rosemary extract when it was combined with low SO\(_2\) levels. This synergy reflected on increased yeast aromatic metabolites.

### Table 2

Identified and quantified (by GC-FID) esters compounds and total volatile content of wines with different added extracts

<table>
<thead>
<tr>
<th>IDENTIFIED COMPOUNDS mg/L</th>
<th>WINES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R01</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>15.98</td>
</tr>
<tr>
<td>Propyl acetate</td>
<td>26.87</td>
</tr>
<tr>
<td>Isopropyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>0.05</td>
</tr>
<tr>
<td>Pentyl acetate</td>
<td>0.05</td>
</tr>
<tr>
<td>Phenyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Total esters</strong></td>
<td>42.95</td>
</tr>
<tr>
<td><strong>Total Volatile Content</strong></td>
<td>242.63</td>
</tr>
</tbody>
</table>

Table 2 (continue)

<table>
<thead>
<tr>
<th>IDENTIFIED COMPOUNDS mg/L</th>
<th>WINES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB01</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>37.72</td>
</tr>
<tr>
<td>Propyl acetate</td>
<td>0.05</td>
</tr>
<tr>
<td>Isopropyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>ND</td>
</tr>
<tr>
<td>Pentyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Phenyl acetate</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Total esters</strong></td>
<td>143.08</td>
</tr>
<tr>
<td><strong>Total Volatile Content</strong></td>
<td>545.78</td>
</tr>
</tbody>
</table>
Total higher alcohols in wines with rosemary extract was not differ significantly from those found in the control samples (TK00 and TK25). The best result for this group of experimental wines was observed in variant R253 (270.32 mg/L). The synergetic effect of 25 mg/L SO$_2$ + 0.3 mL rosemary extract had a positive effect on the accumulation of higher alcohols in the wine.

In the following variants (grape pomace extract) in GP253, the highest total content of higher alcohols (519.86 mg/L) of all the wines analyzed was found. The results obtained with this type of extract showed a trend of increased amounts of higher alcohols compared to the two control samples. It was noticed that the combined effect of SO$_2$ and grape pomace extract (GP253) caused increasing of the concentration of higher alcohols in the wine. It was interesting to noted that in the variant of treatment with grape pomace without SO$_2$ (GP01), a good effect on the total content of higher alcohols (321.88 mg/L) was observed. It was significantly better than the same variant but with the addition of rosemary extract (R01).

With the addition of blueberry extracts, the best quantitative accumulation of higher alcohols was found in sample coded as BB253 (492.94 mg/L). This confirmed the hypothesis that the combined effects of SO$_2$ and extract could improve the synthesis of higher alcohols in the wine. The results for total higher alcohols in the other three samples (BB01, BB251 and BB257) were almost comparable to those found in the control variants (TK00 and TK25). The results obtained for the total content of higher alcohols in wines with incorporated extracts found that in all variants the best result was obtained with a combination of SO$_2$ (25 mg/L) and extracts (0.3 mL) in samples coded as R253, GP253 and BB253.

**Effects of treatments on individual higher alcohols in wine samples**

Eight higher alcohols were identified in the experimental wines. The dominant were 1-butanol, 2-methyl-1-butanol (active amyl alcohol), 3-methyl-1-butanol (isoamyl alcohol) and 1-hexanol.

The 3-methyl-1-butanol was a major component of higher alcohols group. The lowest concentration (157.14 mg/L) was found in BB01 wine – with the addition of blueberry extract. The highest amount (262.22 mg/L) was identified in sample BB257, obtained with the addition of blueberry extract. The lowest amounts of this compound were observed in the samples obtained with the addition of rosemary extract.

The experimental wines obtained with the addition of grape pomace extract had higher amounts of isoamyl alcohol than the samples with rosemary. The highest amount of 3-methyl-1-butanol of this group (239.75 mg/L) was distinguished in experimental wine coded as GP253. In this case the positive effect of the combination of SO$_2$ + extract on synthesis of 3-methyl-1-butanol was observed. In the samples with addition of blueberry extract a gradual increase in the levels of established 3-methyl-1-butanol from BB01 (157.14 mg/L) to BB257 (262.22 mg/L) was realized. The result confirmed the effect of the extract with certain doses of SO$_2$.

The 3-methyl-1-butanol is an important aromatic compound in red wines. It was found to be a an important component of Californian and Australian red wines from Merlot and Cabernet Sauvignon varieties subjected to aging in stainless steel tanks [11,35]. This compound formed the malt and whiskey flavor in wines [20].

The 2-methyl-1-butanol (active amyl alcohol) was found in the lowest quantities in sample coded as R01 (38.43 mg/L) obtained with the addition of rosemary extract. Its highest amount (49.81 mg/L) was found in sample BB01 – with addition of blueberry extract. The active amyl alcohol was not identified in three of the samples: GP01, GP251 and GP253.
Another representative with a significant presence in the wines studied was 1-butanol. Its concentrations in all tested wines corresponded to those found in the two control samples. In two of the samples (GP251 and GP257) it was identified in very low amounts. In R253 and BB01 it was not established. In the remaining samples the content of this component ranged within 14.83 mg/L (BB251) to 26.99 mg/L (R257). The content of 1-butanol in wine ranges in the concentration range of 1.00 – 64.00 mg/L [12]. This study corresponded to these quantitative variations.

The 1-hexanol was found in all experimental wines. This compound gives a herbaceous tone of wine aroma. It accumulates when the leaves and bunches are affected by the crushing process [12]. In most samples analyzed in this study, elevated levels of this alcohol were observed. The added extracts affect the final content of 1-hexanol in the wine. Significantly high levels were observed in the samples treated with grape pomace extract. The observed concentration of 1-hexanol in these wines ranged from 15.69 mg/L (GP01) to 71.99 mg/L (GP257). The highest content was observed in BB01 wine (85.81 mg/L). The best in terms of this indicator were two of the wines containing rosemary extract – R01 (10.42 mg/L); R257 (11.57 mg/L) and blueberry extract – BB253 (0.05 mg/L); BB257 (0.05 mg/L).

The 1-heptanol was identified in a substantial amount (67.27 mg/L) only in the GP01 sample. The 2-phenylethanol (aromatic alcohol) was found in small quantities in the experimental wines coded as R01, BB251 and in the TK25 control. This compound closely related to rose aroma in wines [36].

Considering the 2-methyl-1-butanol, 3-methyl-1-butanol and butyl acetate compounds were determined significant differences between wines treated with grape pomace extract and rosemary extract (p<0.05). There were also determined significant differences between wines treated with blueberry and rosemary extract according related to butyl acetate content (p<0.05).

**Effects of treatments on esters in wine samples**

The ester fraction was represented by 9 identified compounds. The highest total ester content (169.13 mg/L) was found in sample coded as R253 with combined treatment of 25 mg/L SO₂ + rosemary extract (0.3 mL). The other three variants of rosemary treatments demonstrated quantitatively close ester content. In samples containing grape pomace extract was found the lowest total ester content compared to all others. From this group, variant GP253 (40.62 mg/L) was distinguished.

In variants with blueberry extract were found high concentrations of total ester content. The highest amount of esters was identified in a sample without SO₂ – BB01 (143.08 mg/L) obtained only with the blueberry extract. This result revealed the potential of blueberry extracts as an alternative approach for sulphitation with enough ester accumulation. A satisfactory result was also established with sample BB253 (67.32 mg/L), also. This confirmed the good synergic action between SO₂ and discussed extract.

The ethyl acetate was found in all samples tested. It presents have a positive influence by providing a pleasant fruity aroma (concentrations of 50.00 – 80.00 mg/L) [37]. At high concentrations it has a negative effect [38]. The concentrations of this ester found in our study met the criteria corresponding to its positive effect. This ester was found in the lowest amount (9.63 mg/L) in the control sample TK25. The highest content of ethyl acetate (37.72 mg/L) was found in the wine sample coded as BB01 variant.

Another representative of the ester fraction identified in all the wines examined was propyl acetate. In most samples, it was found in low amounts (0.05 mg/L). The highest amount was observed in the GP253 sample with quantity of 29.09 mg/L.
Butyl acetate was identified only in wine samples numbered as R251 (26.44 mg/L) and R253 (95.09 mg/L). This compound was observed only in wines produced with the addition of rosemary extract.

Isobutyl acetate was identified only in wine samples R253 (58.82 mg/L). The phenyl acetate was found in two of the wines samples: R01 (0.05 mg/L) and BB253 (47.44 mg/L). Phenyl acetate was another identified ester. It was only observed in variant BB01 (105.31 mg/L). This ester gives fruity and floral characters to the wine aroma [39].

### Effects of treatments on terpenes in wine samples

From the group of terpenes, were identified five representatives – α-terpineol, linalool oxide, nerol, β-citronellol and geraniol. A remarkable high total terpenic content (0.97 mg/L), significantly greater than the two control samples, was found in wine coded as R01 which was produced without SO₂ and treated with rosemary extract. The higher total terpenic content was probably due to the transition of terpene compounds to wine from the rosemary extract. Wines treated with grape pomace extract, sample coded as GP257 demonstrated the highest total terpene content (0.68 mg/L). When a blueberry extract was applying, higher values of total terpenes were observed in sample BB251 (0.42 mg/L). The dominant terpene was geraniol. It was found in all tested wines. The highest concentration (0.68 mg/L) was found in the sample GP257 obtained by sulphitation and addition of grape pomace extract. The concentrations of this terpene found in the remaining experimental wines were not significantly different from those of the TK00 and TK25 controls. Geraniol has a strong aromatic effect in the muscat grapes and was found to be dominant (24.2%) [40].

The α-terpineol was identified in two of the experimental wines numbered as R01 (0.76 mg/L) and R257 (0.05 mg/L). This terpene alcohol gives the wine melon and lily aromas [41].

Linalool oxide was identified only in the wine with blueberry extract BB251 (0.24 mg/L). The nerol was identified only in R251 sample and β-citronellol was detected in R253 and GP251 samples. The last two terpenes were found in very low concentrations.

### Effects of treatments on methanol content in wine samples

Methyl alcohol were found in all tested wines. It results from degradation of pectins in fruits with the action of the pectolytic enzyme complex [42]. In red wines, it is formed in concentrations ranging from 60.00 to 230.00 mg/L [18]. A remarkable quantitative presence of this alcohol (109.74 mg/L) was recorded in experimental wine sample coded as BB01 produced with addition of blueberry extract and without SO₂. This is explained by the added effect of the pectin content of the blueberry fruit. In the remaining samples the amount of methanol was not different from responding control groups. The methyl alcohol levels were very low and not pose a risk to the consumer.

Correlation analyses were used to determine the relation between parameters and within groups. In this study, the regression correlations of compounds were significantly different and those compounds contributed to the wine aroma content. This indicates that each aroma compounds have different flavor characteristics in wines.

While there was no positive correlation detected between pH and total acidity or volatile acidity in the groups, there were a positive correlation between the pH and 1-hexanol, ethyl acetate and phenyl acetate values (respectively, r=0.598, r=0.814 and r=0.9771, p<0.05). There were also determined correlations between volatile acidity and total SO₂ content
(r = -0.772, p <0.05), total SO$_2$ and free SO$_2$ value (r = 0.763, p <0.05), dry matter and 2-methyl-1-butanol (r = -0.547, p <0.05), ash and 1-butanol value (r = -0.576, p <0.05).

Considering the butyl acetate, there were detected positive correlation between isobutyl acetate and isopropyl acetate (r = 0.679, p <0.05). It was also determined significant correlations between 3-methyl-1-butanol and 2-methyl-1-butanol (r =-0.889), 1-hexanol and 1-butanol (r = -0.9084), ethyl acetate and 1-butanol (r = -0.544), isopropyl acetate and ethanol vol.% (r = -0.578), isobutyl acetate and ethanol vol.% (r = -0.578), 1-hexanol and ethyl acetate (r = -0.566), propyl acetate and ethyl acetate (r = -0.552), propyl acetate and 2-phenyl ethanol (r = 0.624), phenyl acetate and ethyl acetate (r = 0.862) and ethyl hexanoate and phenyl acetate (r = 0.679) (p <0.05).

Conclusions

The results of the study demonstrated the influence of different plant extracts on the aromatic profile of Cabernet Sauvignon wines. The highest total amount of volatile compounds (twice higher than the TK00 and TK25 controls) was achieved by applying blueberry extract and grape pomace extract treatments- variants BB251 (579.31 mg/L) and GP253 (579.20 mg/L). The blueberry extract affected positively the volatile composition, even when it was applied alone – BB01. The combined application of SO$_2$ and blueberry extract (sample BB253) increased the wine volatile complexity. The best effectivity on higher alcohols synthesis and their accumulation in wines was obtained by using SO$_2$ (25 mg/L) and plant extracts (0.3 mL) in samples coded as R253, GP253 and BB253 and eight higher alcohols have been identified. 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 1-hexanol were determined as dominant. The highest total ester content was found in sample R253 (169.13 mg/L). A high amount of individual esters was identified in BB01 sample (143.08 mg/L), obtained only with blueberry extract, without SO$_2$. This revealed the potential of blueberries as an alternative approach for sulphitation, realizing good ester accumulation in wines. The terpenes were dominated by geraniol. The highest value was obtained in sample GP257 with concentration of 0.68 mg/L. Methyl alcohol was found in all wines tested. Its quantity was within normal limits. Results demonstrated the importance of treatment of plant extracts and their concentrations in red wine. The study indicated the possibilities of optimization of SO$_2$ in wine production and wines quality by treatment with natural plant extracts.

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References


Phase transitions in food production technologies

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Abstract

Introduction. The article deals with information about the general state of technologies for the utilization of secondary energy resources and environmental resources.

Materials and methods. Energy-consuming processes are investigated from the point of view of using their potentials as isoenthalpy in malt dryers, fermenters, heat pumps and vacuum dryers in their classical design. Research methods are based on the principles of technical thermodynamics. The combination of these objects concerns the possibility of creating closed energy-material circuits on their basis by supplementing them with compensatory processes.

Results and discussion. The analysis of isoenthalpy drying processes led to the conclusion that it is expedient to keep the total potential of the vapor-gas mixture in closed circuit, but with the feature that the drying potential of the medium will be renewable. Since the extraction of the vapor fraction is possible only through its condensation, which is carried out using a heat pump, energy potential is returned in it to the gas flow during its passing through the condenser.

It is shown that such a system implements the tasks of drying the grain mass, transporting the vapor-gas mixture, drying the gas fraction and returning energy potential to it. The heat pump circuit in this system plays a regulatory role in relation to the steam-gas mixture circuit, and the compensation process is assigned to the heat pump compressor.

It was shown that the thermodynamic properties of phase transitions correspond to the isobaric-isothermal process and this has at least two advantages from the point of view of the interests of energy recovery and regeneration. Firstly, as a result of the phase transition, the enthalpy of the vapor fraction is approximately 5 times higher than the enthalpy of the liquid fraction. Secondly, the temperature of the steam or gas phase can be changed by mechanical or thermal compression, including for changing temperatures of the phase transitions. The consequence of such transformations is the ability to minimize the cost of primary energy resources for evaporation processes.

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

Prospects for the restoration of low-temperature thermal resources with the latest technical capabilities are widely reflected in the general provisions of thermodynamics, and in solving individual technological problems [1, 3]. The dynamics of the use of heat pumps is achieving the levels of industries [5]. However, in each case the problem of phase transitions of working media at the level of parameters of the spent thermal energy and temperature modes of heat streams at the exit from the system should be solved [6].

In each case the problem of phase transitions of working media at the level of parameters of the spent thermal energy and temperature modes of heat streams at the exit from the system should be solved. The total potential of energy resources to be recovered in the industries of the EU is 7% of the total spent heat resource [3] with the ability to reach source temperatures up to 150 °C. Authors [7] provided information on the creation of a cascade heat pump with a capacity of 20 kW with propane in the low-temperature cycle and butane in the high-temperature cycle with a range of heat flux recovery from 30 °C to 115 °C.

The creation of systems for the utilization of secondary energy resources required an initial analysis in terms of assessing the possibilities of local transformation of thermal waste and the optimal use or connection to existing power systems. The combination of heat pump, heat transfer system and heat storage system can meet the needs at the relevant time scale, spatial coordinates and energy level. In the publication [8] it was proved that the logical addition of heat flow transport systems were heat pipes.

The technical limitation of the implementation of significant temperature differences in phase transitions forced the use of systems with three independent cycles [9]. Comparison of such triplex systems of heat pumps with single-circuit analogues showed the advantage of the first ones.

In the study [5] the use of secondary energy resources was associated with the prospects of reducing the risks of global warming, the concept of industrial waste heat, potential sources of the latter were defined, available for disposal resources were highlighted, heat pumps, heat exchangers, power cycles, transportation systems were illustrated.

The study [10] presented options for heat recovery of heat pumps for drying equipment for the food industry. Heating of air as a drying agent by heat recovery was carried out using 'air-to-air' heat exchangers and heat pumps up to 200 °C. The temperature of wasted air was 76 °C with a dew point of 38.5 °C. At the evaporation temperature of 25–30 °C, up to 40% of the air heating load was provided with a 20% reduction in energy cost. The transcritical cycle with dehumidification of wasted air at the constant temperature and heating of the input air in the supercritical area is thermodynamically good for the drying process.

Another direction of implementation of the fresh air dehumidification system concerned the use of the heat pump with the heat exchanger covered with the dehumidifier [11]. This treatment was due to the fact that dehydration with the transition to the dew point led to increased levels of electricity consumption in traditional air conditioning. The proposed system was recognized as effective for the treatment of air with high relative humidity.


The combination of low-temperature and solar energy potentials was estimated to have significant potential for different applications. Publication [13] summarized various aspects of this technology, including system configurations, performance optimization, simulation models, and various applications.
Analysis of the course of a significant number of food technology processes led to the conclusion that it was possible to complete them with components which transform the system to the levels of closed circulation circuits. This applied to the processes of drying, concentration of solutions, aeration of culture media, germination of grain areas and determined the relevance of the research topic.

The purpose of the study was to assess the prospects for the implementation of proposals for the use of secondary energy resources in existing systems by supplementing them to the levels of closed energy circuits.

Materials and methods

Materials Energy-consuming processes are investigated from the point of view of using their potentials as isoenthalpy in malt dryers, fermenters, heat pumps and vacuum dryers in their classical design [1, 2]. The combination of these objects concerns the possibility of creating closed energy-material circuits on their basis by supplementing them with compensatory processes [1, 3, 4].

Methods. Research methods are based on the principles of technical thermodynamics.

Results and discussion

General provisions

Water and water vapor can be attributed to the most common working fluids and media in food technologies [1]. This is due to the fact that their physical and chemical properties correspond to the conditions of existence of the biological world, and are in sufficient quantities in the environment [2]. Water is quite cheap, non-aggressive to the materials of technological equipment and through phase transitions in the cycles of natural cycles restores its properties. However, the phase transitions of evaporation and condensation of water vapor are inherent in the majority of industries [3]. However, the list of used media is logically supplemented by other substances involved in refrigeration plants, heat pumps, in the production of alcohol, liquefied gases, in the processes of crystallization, drying. In this case, water often acts as the medium in which thermal, chemical, mass transfer or biochemical processes take place. It is obvious that such a set of possibilities for the application of known and not known yet properties is based on information in the form of the laws of physics, chemistry, thermodynamics.

According to its characteristics, water vapor is a typical representative of real working bodies (the presence of its own volume of molecules and the forces of interaction between them). Therefore, the application of the equation of state of ideal gases to water vapor is almost impossible [1], and engineering calculations are proposed to do using thermodynamic tables and entropy diagrams i-s and T-s, built on the basis of experimental data.

The processes of formation of the vapor fraction are isobaric-isothermal, so to determine the state of the system it was necessary to have information about the parameters of pressure p, temperature t and degree of dryness. In estimating secondary energy resources in thermodynamic systems, the relationship between the enthalpies of liquid and vapor fractions, the heat of vaporization and the values of entropies depending on the pressure and the corresponding temperature were important [1]. These ratios in a significant number of technological processes led to the possibility of recovery and regeneration of energy
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potentials. Such transformations are often based on the addition of steam, gas or steam-gas systems with mechanical or thermal energy [2].

Since in the isolated flow system the sum of all types of energy remained constant, the equation of energy balance of the system had the form [1]:

\[ E_s = \Delta E_g + E_r , \]  

(1)

where \( E_s \) and \( E_r \) – supplied and removed energy respectively, J; \( \Delta E_g \) – energy gain of the system, J. In the elementary process [1], the energy balance equation is represented by the formula:

\[ \dot{E}_s d\tau = dE_g + \dot{E}_r d\tau , \]  

(2)

where \( \dot{E}_s \) and \( \dot{E}_r \) – the flows of supplied and removed energy respectively, W; \( d\tau \) – elementary process time. For a stationary process within a unit of time we have:

\[ \dot{E}_s = \dot{E}_r . \]  

(3)

Transition to the balance of mass flows was made similarly:

\[ m_s = m_r . \]  

(4)

Double-circuit recuperative malt dryer

Conditions (1) – (4) corresponded to those processes which occurred in isolated or conditionally isolated thermodynamic systems of aeration of culture media of aerobic or anaerobic cultivation of microorganisms, germinated malt, mixing streams, heating-cooling. Thus, the drying of malt or other grain mass occurred due to the interaction of the latter with the drying agent, the result of which was the redistribution of moisture and energy potentials [1, 2]. However, the total energy potential remained constant, which defined the drying process as isopotential. The direction of the material flow in the direction from the grain to the drying agent and the recognition of the process as isopotential led to the conclusion about the feasibility of using system with regeneration of the drying agent by removing the vapor phase and parallel recovery of energy potential with increasing temperature. It was obvious that the technical possibility of extracting the vapor fraction was associated with its condensation due to cooling of the gas or steam-air mixture. This meant that the energy potential of the condensing heat must be absorbed by the refrigerant of the heat pump. Having been removed from the drying agent condensate was removed from the system, and the low-potential heat energy flow obtained in the evaporator was transformed in the heat pump circuit into the high-potential one, which was transferred to the drying agent in the latter condenser (Figure 1).

Regenerated in this way in terms of energy and material indicators drying agent moved in a closed circuit A with the corresponding transformations in the circuit B of the heat pump. The initial power supply of the system was due to the heater 3, and in steady state – due to the compressor of the heat pump and the potential of the drying agent. The role of the energy-mass regulator in the system was performed by the heat pump circuit, and the evaporator of the latter was also a condenser of the circuit A of the drying agent.

The dryer itself was the evaporator in circuit A.

A common feature of both circuits was the presence of phase transitions in the modes of evaporation and condensation, due to which the restoration of driving factors was achieved in accordance with the second law of thermodynamics.
Thus, the compression of gas by the compressor led to an increase in temperature due to the mechanical reduction of the volume with a constant value of entropy. In the reverse process, the expansion of the compressed gas compensated energy costs. This feature in the form of adiabatic processes was used in refrigeration cycles and cycles of heat pumps with a super-important effect to change the temperature of the phase transitions. The latter opened the possibility of efficient mass and energy-intensive processes with technological medium and environment, including due to phase transitions with a high value of heat output and heat transfer coefficients. The presence of a phase transition with the formation of vapor or gas phase meant the creation of powerful energy flows, which were analogous to energy-saturated areas like a heat pipe. In this case, the circuit A was closed and met the requirements of the process, and the compensation process in its structure corresponded to a set of processes of condensation of the vapor phase and subsequent heating of the drying agent. In the heat pump circuit, the compressor and the control valve corresponded to the compensation process in its execution. The presence of the latter in the circuit of the heat pump as in an isolated system allowed to create and update temperature differences in the evaporator and condenser, ensuring that the system was in terms of heat transfer in an unbalanced state. The heat flux perceived by the thermodynamic refrigerant from the medium of circuit A was replenished by the potential of the compressor. It is known that the most efficient reverse cycle is the Carnot cycle.

In the technological process corresponding to the circuit A, there were thermodynamic processes of condensation of the vapor phase with appropriate cooling and subsequent heating of the gaseous part of the medium. The performance of both tasks was entrusted to the heat pump.

The next process in circuit A was the evaporation of the wet fraction. Thus in circuit A there were operations of phase transitions, as well as in circuit B of the heat pump. However,
it was impossible to organize the work of the first on the basis of thermodynamic principles of the second due to the combined vapor-gas medium and the peculiarities of the interaction of the drying agent and the humid medium, which are shown in diagram I-d. However, the isenthalpy nature of the latter determined the possibility and feasibility of creating recovery modes based on closed circuits in parallel and synchronized material and energy flows. The material flow of circuit A in the area between the evaporator of the heat pump and the dryer was represented by air, and in the dryer and to the evaporator – by a vapor-gas mixture. In the evaporator of the heat pump, which simultaneously acted as a steam condenser of the drying agent, the interaction between which and the thermodynamic agent of the heat pump, the condensation of the vapor phase was completed and the condensate was removed from the circuit.

Condensation of the vapor phase of the drying agent in the evaporator of the heat pump was accompanied by active heat transfer of the coolant to the circuit B, which in the form of vapor phase entered the compressor, compressed with increasing temperature and energy potential, and with the transition to the condenser 6 of the heat pump due to its condensation the return of the energy potential of the flow of drying agent was carried out. Restoration of the drying properties of the latter and its initial thermodynamic parameters meant the creation of a second closed energy circuit based on the circuit of the heat pump. An important combination of such a double closed-loop system was the technical ability to perform material flow processing in a continuous mode, provided the required input initial thermodynamic parameters.

The closed circuit B of the heat pump was arranged on the basis of the classical Carnot reverse cycle, in which the compensation process was represented by mechanical compression of the vapor phase. Compression itself was a prerequisite for the creation of driving factors of heat transfer in combination with the throttling of the refrigerant. Synchronization of the corresponding processes in the circuits A and B was reflected in the sequence of their execution in Figure 2.

Figure 2. Synchronization of processes in the circuits A and B from Figure 1

The absence of compression in the circuit A was compensated by the heating of the drying agent and this change will be conventionally considered to be analogous to thermal compression.

Parallel synchronous operation of both circuits ensured the retention of energy potential in the system, which meant the possibility of full use of the features of drying processes as isenthalpy.
Fermentation apparatus with distillation function

A significant number of media in food technologies has relatively small temperatures, which allows them to be classified as low-potential [2, 5]. Methods of converting processes to high-potential include mechanical or thermal compression of steam, gas or steam-gas media [1, 7]. In most cases [10], such transformations are used for recuperative transformations, but in parallel with them, purely technological problems are solved. An example of such a dual purpose relates to the patent of Ukraine for the invention 107407 "Fermenting apparatus". Its schematic representation is shown in Figure 3.

The unit consists of a cylindrical body 1 with a cooling jacket 2, a conical bottom 3, supply pipes 4 and discharge of fermented medium 5, safety valve 6, medium circuit with pump 7, pipeline 8, vacuum chamber 9 with dispersing head 10 and sluice gate with a sealed actuator 11, a vacuum circuit with a vacuum pump 12, a heat exchanger-recuperator 13 of liquid and steam-gas flows and a condenser 14 of the steam mixture.

The invention is based on the task of combining the processes of fermentation and extraction of alcohol, reducing the osmotic pressure in the medium, increasing the average fermentation rate of sugars and productivity of the fermentation process, reducing energy consumption to ensure fermentation and subsequent distillation.

Figure 3. Scheme of the fermenter with a recuperative system of alcohol extraction
To stabilize the concentration of alcohol at a given minimum level, the circuit of the medium with the pump 7, the pipeline 8, the vacuum chamber 9 with the dispersing head 10 and the sluice gate with the sealed actuator 11 was turned on and the medium was provided into the vacuum chamber 9, in which the liquid phase was dispersed, the alcohol evaporated, and the sluice gate with a sealed actuator removed the liquid fraction into the medium of the fermentation apparatus. Due to the reduction of the pressure in the vacuum chamber to 70 mm Hg boiling and evaporation of alcohol were achieved at a temperature of 30 °C and the formation of a water-alcohol mixture with an alcohol concentration of 50–60% occurred. Compression of this mixture by a vacuum pump 12 led to an increase in temperature and pressure and it was provided to the heat exchanger-recuperator 13 of the liquid and vapor stream. The transfer of the latter to the condenser 14 provided complete condensation of the water-alcohol mixture and the return of thermal energy to the fermentation medium.

The condensed mixture was provided to the distillation and due to the high concentration of alcohol in it the energy saving effect of the system was achieved.

In connection with the latter, it is worth emphasizing that the double energy result of the system was due to the fact that the stabilization of the medium temperature at the nominal level was provided by the removal of fermentation heat, which in this case was involved in the vacuum distillation process. Due to the variable pressures of vapor-liquid mixtures, thermodynamic parameters of phase transitions with a temperature close to the nominal one for yeast with satisfactory differences on the heat exchange surfaces were achieved. In such a system, the external heat dissipation, which is presented in the classical schemes of fermenters [2, 9] and is an additional energy load, in this case was equivalent to the distillation potential and logically complemented the overall positive of the system. The sequence of processes in this system had the form:

An important technological result in this case was the stabilization of osmotic pressures in the fermentation medium at the nominal level due to the limitation of alcohol concentrations due to changes in physical pressures in certain parts of the system. In addition, this patent did not contain information regarding the solubility of carbon dioxide synthesized in the system. Its concentration reached saturation and this was accompanied by additional mass transfer resistance on the phase separation surface. The reduction of the pressure in the vacuum chamber in accordance with Henry's law was accompanied by a corresponding share of desaturation with subsequent removal of CO₂ and additional positive effects on the system.

Thus, the combination in the system of phase transitions within the given thermodynamic parameters was accompanied by the following provisions:

- The main technological result of reduction and stabilization of osmotic pressure at the nominal level;
- Use of thermal fermentation potential for distillation;
- Regenerative support of the general energy potential of the system;
- No energy costs for cooling the fermented medium;
- Desaturation of the liquid phase of the medium.
Earlier it was noted that the energy costs of compensation processes were determined by temperature differences in isothermal phase transition processes [11]. That is why the efficiency of heat pumps largely depended on the temperatures of the carriers of low-temperature potentials. At the same time, for use in thermodynamic cycles of ammonia or freons (CFCs), their condensation temperatures were also limited and amount to 35–40 °C [8]. Expansion of the thermodynamic range of use of heat pumps related to the patent of Ukraine for the invention 90919 (Figure 4) with the following formula: a heat pump consisting of series-connected compressor, condenser, control throttle and evaporator, which differs from the previous inventions by the fact that the condenser was made in the form of a sealed tanks with a heat transfer surface and equipped with an axial compressor and a hydraulic power shut-off valve.

![Figure 4. Heat pump circuit](image)

The design of the condenser with its axial compressor and hydraulic shut-off valve made it possible to create a vacuum in its volume, boil the intermediate heat agent and generate its vapor, compress the latter and increase its temperature, which meant the transition of the intermediate heat agent to high potential. The hydraulic power shut-off valve maintained the nominal level of the intermediate heat agent (water).

The heat pump works as follows: compressor 1 sucks the refrigerant vapor from the evaporator 4, compresses it with increasing temperature and provides with new parameters to the condenser 2. Condensation of the refrigerant is carried out by removing the heat of condensation from it by the boiling intermediate heat agent. The boiling point of the latter is regulated by evacuating the system by an axial compressor 5, the compression of steam which increases its thermodynamic parameters to the level of high-potential ones. The level of the intermediate heat agent (water) in the condenser is regulated by the hydraulic shut-off valve 6. The condensed refrigerant in the throttle 3 reduces the pressure and enters the evaporator 4, completing the refrigeration cycle.
The following relations correspond to the specified set of processes:

\[ q_c = q_0 + \ell \quad \text{and} \quad q_n = q_c + \ell_a + \ell_{a.e} = q_0 + \ell + \ell_{a.e}, \]  

where \( q_c \) – specific heat of condensation of a thermodynamic agent; \( q_0 \) – specific heat perceived by the thermal agent in the evaporator; \( \ell \) – compressor operation; \( \ell_{a.e} \) – operation of the axial compressor.

The technical result related to the possibility of obtaining an intermediate thermal agent with high potential thermodynamic parameters.

Adding to technological complexes with phase transitions and the formation of a steam or gas structure can be considered an important indication of the feasibility of creating a recovery circuit [11, 12]. This was all the more important because under such conditions there were opportunities for additional variations in the effects on the technological parameters of the systems. Thus, the limitation of temperatures while maintaining the dynamics of the process allowed the drying of thermolabile products, to store vitamin complexes and biologically active substances under low pressure [13].

Such conditions are met by a continuous vacuum dryer (Ukrainian patent for invention 112880), a scheme of which is shown in Figure 5.

**Figure 5. Scheme of a vacuum dryer of continuous action**

According to the invention, the vacuum chamber is equipped with a belt conveyor with loading and unloading sluice gates with hoppers and energy-permeable screen, and the primary power supply system is made in the form of infrared radiation sources located above the energy-permeable screen, and supplemented by the circuit of secondary recuperative resources with a
secondary steam pipeline, a vacuum pump and a conductive heating surface of the product, which leads to increased productivity, the implementation of a continuous process, stabilization of temperature conditions, preservation of biologically active substances.

The device works as follows: the wet product is provided to the surface 9 of the conductive heating, from which under the action of gravitational forces enters the loading hopper 5.

The product enters the belt of the conveyor 2 of the vacuum chamber 1 along the sluice gate 3. In the process of moving the belt, the product is irradiated through the energy-permeable screen 8 by infrared rays from sources 7.

When vacuuming the internal volume of the vacuum chamber by the vacuum pump 10, secondary steam (steam released from the product) is removed by pipeline 11. Compressed by the vacuum pump steam enters the surface of conductive heating, and the dried product is transferred from the belt of the conveyor to the sluice gate 4 by gravity, from which it is transferred to the unloading hopper 6, and is allocated for packaging or storage.

**Conclusion**

The thermodynamic properties of phase transitions corresponded to isobaric-isothermal processes, which from the point of view of the interests of energy recovery and regeneration had at least two advantages.

Firstly, as a result of the phase transition, the enthalpy of the vapor fraction was approximately 5 times higher than the enthalpy of the liquid. It was important that the generation and condensation of steam were characterized by the same heat of phase transitions.

Secondly, the temperature of the vapor or gas phase can be changed by mechanical or thermal compression, including to change the temperatures of the phase transitions. The latter opened up prospects for the implementation of several stage condensations and generations of steam fraction in the direction of decreasing and increasing temperatures and pressures of phase transitions. The ability to minimize the cost of primary energy resources for evaporation processes was the consequence of such transformations.

Energy-intensive drying processes, aeration of grain masses during germination, culture media in the synthesis of microorganisms or their derivatives were evaluated as isoenthalpy, which meant the feasibility of creating closed energy circuits, as the hardware design of technological devices often had components at the level of heat pumps.

The initial energy potentials of the systems were represented by the components of secondary steam and condensate, and the combination of phase transitions with technological processes has prospects in the processes of drying, evaporation, aeration, anaerobic fermentation.

**References**


Modeling heat transfer in down flowing annular weakly turbulent vapor-liquid flows during evaporation

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Abstract

Introduction. The predominant number of relations for turbulent viscosity in down flowing films has a discrete layered structure, and the solutions of the heat and momentum conservation equations using these relations are only numerical. A new model is proposed and on its basis the analysis of thermohydrodynamic processes in films of liquids during vaporization is carried out.

Materials and methods. Physical modeling was performed in pipes: \( d = 22 \times 1 \text{ mm} \), \( L = 1.8 \text{ m} \), and \( d = 33 \times 1.5 \text{ mm} \), \( L = 9 \text{ m} \). The bulk density of irrigation varied in the range of \( 0.05–0.55 \cdot 10^{-3} \text{ m}^2/\text{s} \) in the pipe \( d = 20 \text{ mm} \), and \( 0.05–1.9 \cdot 10^{-3} \text{ m}^2/\text{s} \) in the pipe \( d = 30 \text{ mm} \). Model liquids – water and sugar solutions with a concentration of up to 70% at atmospheric pressure and a vacuum of up to 0.86 bar. Heating was carried out with dry saturated steam.

Results and discussion. A model of turbulent viscosity in a film in the form of a beveled interfacial surface of a parabola is proposed. Analytical expressions for temperature and velocity profiles in the film and corresponding integral thermohydrodynamic characteristics for the heat transfer regime characterized by evaporation from the interfacial surface are obtained from the heat transfer and momentum conservation equations.

Analytical expressions for heat transfer coefficient and film thickness are expressed in inverse hyperbolic functions and are simply correlated with the corresponding experimental data on heat transfer in flowing films of water and thick sugar solutions in the area of undeveloped and developed turbulence during vaporization. Experimental data on heat transfer in the presence of interfacial tangential stress on the film surface correlate with the theoretical results of the proposed model only with the introduction of an additional function that takes into account the suppression of turbulence in the film due to its thinning, expressed in Weber numbers for the vapor phase. According to experimental data, the effect of bubble boiling on the intensity of heat transfer during the movement of the vapor-liquid core is manifested only outside the limiting temperature pressure, expressed by the Clapeyron-Clausius ratio, and is taken into account by introducing the parameter turbulent viscosity models.

Conclusions. Based on the proposed model of turbulent viscosity, the analysis of thermohydrodynamic processes of weakly turbulent film flows is performed, the corresponding analytical expressions for calculation of heat transfer to films of solutions during vaporization in pipes, including bubble boiling region are obtained.
Introduction

Boiling fruit juices and sugar solutions in down flowing films in long evaporation channels under vacuum allows to obtain high-quality food concentrates, so this direction is developing, and film evaporators are improving.

The processes of heat and momentum transfer in flowing films of liquid differ significantly from the processes of transfer in a continuous medium due to the presence of an elastic, due to surface tension, interfacial surface covered with a system of waves. Measurements of turbulent viscosity in films given in the works of D.C. Jepsen and B.G. Ganchev, indicate its rapid fall near the interfacial surface, and the shape of the turbulence curve is deformed depending on the orientation of the film relative to the direction of gravity. Thus, in films flowing on inclined (at an angle of 9°) surfaces in the region of Reynolds numbers up to 1800 turbulent viscosity has an almost parabolic profile, Figure 1.A., while in the films flowing down the vertical surface, there is a deformation of the parabola with a flat part near the wall and a rapid fall near the interfacial surface, Figure 1. B.

![Figure 1. The dependence of turbulent viscosity on the film thickness of water (t = 20 °C). A – flow along an inclined angle of 9° (D.C. Jepsen); 1 – Re = 1834; 2 – 1462; 3 – 1099; 4 – 732, (method of CO₂ absorption); B – flow along the vertical surface (B.G. Ganchev) Re = 1310, (method of stroboscoping of alumina microparticles).](image)

In single-phase continuous media, modeling of complex near-wall flows is carried out mainly by direct numerical modeling based on grid methods, or modeling based on ANSYS software using either differential and turbulence models [1, 2, 3, 4], or mixed – in the near-wall algebraic form, and in the external – differential. Works on the application k – ε and k – ω models for the reproduction of thermohydrodynamic characteristics of film flows with a wave structure have not been found in the literature. In addition, as a result of the application of differential models, only numerical solutions of the differential equations of motion and heat exchange are obtained, while artificial algebraic models [5, 6, 7, 8, 9], which with a certain approximation copy the shape of the turbulent viscosity distribution curve in films,
having a relatively simple form, give satisfactory analytical results on the reproduction of thermohydrodynamic processes in films flowing on vertical surfaces.

A successful algebraic relation for turbulent viscosity in films is the expression of M.D. Millionshchikov, which postulates the presence of a laminar layer thickness \( \delta^* = 7.8 \), and a turbulent nucleus in the region \( \frac{7.8}{\delta^*} \leq \eta \leq 1 \) with a parabolic profile \( \nu, l \nu \).

\[
\frac{\nu}{\nu} = 0.39 \left( \eta \delta^* - \delta^* \right) \left( 1 - \eta \right),
\]

where \( y \) – normal to the heat surface coordinate; \( \delta \) – film thickness; \( \eta = \frac{y}{\delta} \) – dimensionless transverse coordinates; \( \nu, \nu \) – turbulent and molecular cinematic viscosity coefficient, respectively; \( \rho \) – density of liquid; \( \tau \) – shear stress on the film interface; \( g \) – acceleration of gravity; \( \eta = \frac{\delta}{\delta^*} \); \( \delta^* = \frac{\delta_u^*}{\nu} = 7.8 \); \( \delta^* = \frac{\delta_u^*}{\nu} = \sqrt{\frac{\tau \rho g \delta}{\rho}} \); \( \delta_u^* = \frac{7.8 \nu}{u^*} \).

In the case of replacing the constant coefficient (0.39) by a function of the flow and mode parameters of the film, expression (1) becomes convenient for analysis and generalization of experimental results on heat transfer and hydrodynamics of film flows. At the same time, the abrupt increase in turbulent viscosity at the outer boundary of the laminar layer in expression (1) seems physically unreasonable. In addition, the model (1) has a layered change in viscosity, so when solving the heat transfer equations there is a need to join the solutions between the laminar and turbulent layers. Two-layer algebraic models of turbulence in films with ascending and descending branches of the turbulent viscosity function have become widespread [5, 6, 7, 8, 9]. At the same time, in the near-wall and interfacial regions a gradual continuous transition from the laminar to the turbulent mode of motion is postulated. But the profiles proposed in [5, 6, 7, 8, 9], in contrast to (1), do not allow to obtain solutions of the equations of motion and thermal conductivity in quadratures.

The continuous distribution function of turbulent viscosity over the entire thickness of the free-flowing film with a gradual, in the form of Van Drist correction, transition from laminar to turbulent mode in the near-wall region and within the interfacial surface of the film was proposed by Mudawwar [10]. Later, this model of turbulence was developed for flows with concomitant steam flow over the surface of the film [11].

\[
\frac{\nu}{\nu} = -\frac{1}{2} + \frac{1}{2} \left( 1 + l^2 \frac{\tau_i + \rho g \left( \delta - y^* \right)}{\tau_i + \rho g \delta} \right)^{0.5},
\]

where \( y^* = \frac{y u^*}{\nu} \); \( l = 0.4 \); \( y^* = \left[ 1 - \exp \left( -\frac{y^*}{26} \right) \right] \left[ 1 - \exp \left[ n \left( \frac{y^*}{\delta^*} - 1 \right) \right] \right] \); \( n = 19, 435 \text{ Re}^{-0.345} \text{ Pr}^{-0.34} \left( 1 + \tau_i^* \right)^{0.163} \); \( \text{Re} = \frac{4 G_i}{\nu} \) – the Reynolds number; \( \text{Pr} \) – the Prandtl
number; \( G_v = \frac{G}{\rho \pi d} \) – volumetric liquid flux; \( G \) – mass flow rate; \( \tau_i^* = \frac{\tau_i}{\rho g \left( \frac{v^2}{g} \right)^{1/3}} \) – dimensionless shear stress on the film interface; \( d \) – pipe diameter.

Graphically, the dependences (1, 2) are shown in Figure 2.

Under the conditions of interfacial shear stress, according to (2), there is a shift of the maximum of the turbulence function from the middle of the film to the interfacial surface with its simultaneous growth, which corresponds to the physical idea of the turbulence process in the film, but obviously only to a certain extent, since with increasing interfacial tangential voltage, the film thickness, as well as the scale of turbulence, decrease.

Given the imperfections of existing models, the algebraic model of turbulence seems to be effective, which, firstly, with some approximation will reproduce the real distribution of turbulent viscosity in the film, and secondly, will perform analytical solutions of transfer equations for film flows.

Purpose of the study - on the basis of the proposed new algebraic model of turbulent viscosity to perform the analysis of thermohydrodynamic processes in the descending annular flows of liquids and solutions during vaporization.

Materials and methods

The object of research – film flows of saturated weakly turbulent liquids with concomitant steam flow.

Research methods
Physical modeling of heat transfer processes was performed in a stainless steel pipe with a diameter of 22 x 1 mm and a length of 1.8 m, divided into a stabilization section with a length of 1.5 m and a measurement section. Heating was carried out with dry saturated steam, the boiling of the film was simulated in the range of absolute pressures 10^5–1.4 x 10^4 Pa. The volumetric liquid flux varied in the range of 0.05–0.55 x 10^{-3} m^3/s. The model liquids were water and sugar solutions with a concentration of 20–70%. Temperatures were measured with copper-constantan thermocouples. In addition, we used experimental data obtained on a model installation of a stainless steel heat exchange tube with a length of 9 m and an inner diameter of 30 mm, partitioned into 20 sections with a length of 440 mm with drainage of each formed condensate into separate adiabatic measuring cups, and data [12], obtained on an installation with a pipe length of 3.9 m and a diameter of 32 mm to simulate the process of concentrating apple juice under vacuum. The volumetric liquid flux in a pipe with a diameter of 30 mm varied in the range of 0.05–1.9 x 10^{-3} m^3/s.

The simulation of the down flowing annular two-phase steams-liquid flow with the shear stress on the film interface was carried out by the method of forced independent introduction of steam in the upper part of the stabilization section of the experimental channel. The introduction of liquid into the pipe is performed by the method of transfusion through the edge, which corresponds to the real conditions of film formation in the pipes of film evaporators. Measurement of heat flux was performed by the method of collecting condensate from the model area in adiabatic measuring cups. Thermocouples were connected to the I-7018P analog input modules, which were connected to one I-7520 module with computer output for data storage. Survey of thermocouples during experiments 0.1–2 s.

Results and discussion

It is known that when the film of liquid flows down the vertical surface, even at low irrigation density, a wave structure is formed on its surface. At a distance of 2–2.5 m from the film-forming device comes the mode of saturation of wave motion [14] with the formed structure of low-frequency large waves, which "roll" on the interfacial surface covered with high-frequency waves. The movement of large waves on the surface of the film is accompanied by mixing of the liquid, and, accordingly, the deformation of velocity, temperature and concentration. Analysis of heat transfer processes in films with a developed wave structure based on the model of cyclic perturbation of the film by large waves was performed in [13], but the obtained results are difficult for engineering calculations. Therefore, a simplified heat transfer model based on the averaged thermohydrodynamic parameters of the film flow and the average turbulence parameters for the quasi-stationary film flow regime is considered. The film is considered conditionally flat, and surface waves act as turbulentizers.

Modeling of heat transfer in free-flowing weakly turbulent evaporating films

To model the turbulence and, accordingly, the transfer processes in a vertically flowing film, consider the expression

$$\frac{\nu_j}{\nu} = e \eta^2 \left(1 - \eta^2\right),$$  \hspace{1cm} (3)
where $\varepsilon$ – the maximum value of the function at the vertex of the deformed parabola. The graph of dependence (3) is shown in Figure 3.

![Figure 3: Graph of dependence (3) at $\varepsilon = 1$.](image)

The shape of the curve in Figure 3 seems to be the most adequate to the real distribution of turbulent viscosity in films in vertical channels with free flow, (Figure 1 b.). The maximum value of function (3) at the vertex of the deformed parabola depends on the flow parameters of the film, the degree of development of the wave structure, etc., and can be determined by comparing the calculated and experimental values of thermohydrodynamic parameters of the film flow.

Given the simplifications regarding the mode of motion of the film, the heat transfer process can be provided as

$$q = - \left( \frac{\lambda}{\delta} \right) \left( 1 + \frac{\text{Pr} \, \nu}{\text{Pr}_t \, \nu} \right) \frac{dt}{d\eta},$$

(4)

where $q$ – heat flow; $\lambda$ – heat conduction of liquid; $t$ – temperature; $\text{Pr}_t$ – turbulent Prandtl number.

Accepting $\text{Pr}_t = 1$, taking into account (3) under boundary conditions $\eta = 0, \ t = t_w$, we obtain the temperature profile in the film

$$t = t_w - \frac{q \delta}{\lambda} \sqrt{2H} \left[ \frac{1}{R} \text{Arth} \left( \frac{\sqrt{2} \, \text{Pr}}{R \, \eta} \right) - \frac{1}{A} \text{Arth} \left( \frac{\sqrt{2} \, \text{Pr}}{A \, \eta} \right) \right] ,$$

(5)

where $H = \sqrt{4 \epsilon \, \text{Pr} + \epsilon^2 \, \text{Pr}^2}$; $A = \sqrt{\epsilon^2 \, \text{Pr}^2 - \epsilon \, \text{Pr} \, H}$; $R = \sqrt{\epsilon^2 \, \text{Pr}^2 + \epsilon \, \text{Pr} \, H}$.

From equation (5) provided $\eta = 1, \ t = t_i$, the temperature on the interfacial surface

$$t_i = t_w - \frac{q \delta}{\lambda} \sqrt{2H} \left[ \frac{1}{R} \text{Arth} \left( \frac{\sqrt{2} \, \text{Pr}}{R} \right) - \frac{1}{A} \text{Arth} \left( \frac{\sqrt{2} \, \text{Pr}}{A} \right) \right]$$

(6)

where, $t_w, t_i$ – wall temperature and liquid film saturation temperature on the interface, respectively.

Expressing the heat transfer coefficient as $\alpha = \frac{q}{t_w - t_i}$, with (6) we obtain
\[ a = \frac{\lambda}{\delta} \left( \frac{4 + \varepsilon \text{Pr}}{\sqrt{2 \text{H} N}} \right), \tag{7} \]

where \( N = \left[ \frac{1}{R} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{R} \right) - \frac{1}{A} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{A} \right) \right]. \]

In expression (7) there is a film thickness, which is determined from the equation of motion using function (3)

\[ \frac{\tau \delta}{\rho v} + \frac{g \delta^2}{v} (1 - \eta) = \left[ 1 + \varepsilon \left( \eta^2 - \eta^4 \right) \right] \frac{du}{d\eta}, \tag{8} \]

where \( u \) – velocity.

From (8) under boundary conditions \( \eta = 0, u = 0 \), we obtain the velocity profile

\[ u = \left( \frac{\tau \delta}{\rho v} + \frac{g \delta^2}{v} \right) \frac{\sqrt{2h}}{(4 + \varepsilon)} \left[ \frac{1}{r} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{r} \eta \right) - \frac{1}{a} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{a} \eta \right) \right] - \frac{g \delta^2}{v h} \left[ \text{Arth} \left( \frac{\varepsilon}{2 h} \right) \left( \frac{2 \eta^2 - 1}{h} \right) + \text{Arth} \left( \frac{\varepsilon}{h} \right) \right], \tag{9} \]

where \( h = \sqrt{4 \varepsilon + \varepsilon^2}; \ a = \sqrt{\varepsilon^2 - \varepsilon h}; \ r = \sqrt{\varepsilon^2 + \varepsilon h}; \)

\[ n = \left[ \frac{1}{r} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{r} \right) - \frac{1}{a} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{a} \right) \right]. \]

The average speed \( \overline{u} = \int_0^1 u d \eta \) is obtained from (9)

\[ \overline{u} = \left( \frac{\tau \delta}{\rho v} + \frac{g \delta^2}{v} \right) \frac{\sqrt{2h}}{(4 + \varepsilon)} \left[ n - \frac{\sqrt{2}}{4 \varepsilon} \ln \left( \frac{a^2 - 2 \varepsilon^2}{r^2 - 2 \varepsilon^2} \right) - \frac{\sqrt{2}}{2 \varepsilon} \ln \left( \frac{r}{a} \right) \right] - \frac{g \delta^2}{v h} \left[ 2 \text{Arth} \left( \frac{\varepsilon}{h} \right) - \frac{\sqrt{2h}}{2} \left( \frac{1}{r} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{r} \right) + \frac{1}{a} \text{Arth} \left( \frac{\sqrt{2 \varepsilon \text{Pr}}}{a} \right) \right) - \frac{\sqrt{2 \varepsilon}}{2} \right] \tag{10} \]

Given that the film thickness \( \delta \) and the average velocity \( \overline{u} \) are related to the volumetric liquid flux \( G_v \) dependence \( \delta = \frac{G_v}{\overline{u}} \), we obtain the expression for the average film thickness in the form of a cubic equation

\[ G_v = \left( \frac{\tau \delta}{\rho v} \right) D + \frac{g \delta^3}{v h} (D h - B), \tag{11} \]
where

\[
B = \left[ 2 \text{Arth} \left( \frac{e}{h} \right) - \frac{\sqrt{2} h}{2} \left( \frac{1}{r} \text{Arth} \left( \frac{\sqrt{2} e}{r} \right) + \frac{1}{a} \text{Arth} \left( \frac{\sqrt{2} e}{a} \right) \right) - \frac{\sqrt{2} e}{2} \right],
\]

\[
D = \frac{\sqrt{2} h}{(4 + e)} \left[ n - \frac{\sqrt{2} h}{4 e} \ln \left( \frac{a^2 - 2 e^2}{r^2 - 2 e^2} \right) - \frac{\sqrt{2} h}{2 e} \ln \left( \frac{r}{a} \right) \right].
\]

In the case of free flow \( (\tau_i = 0) \), the film thickness is directly from (11)

\[
\delta = \sqrt{\frac{G v h}{g (D h - B)}},
\]

and the expression for the heat transfer coefficient for free flow conditions is written as

\[
\alpha = \frac{\lambda}{\sqrt{\frac{G v h}{g (D h - B)}}} \left( \frac{4 + e \text{Pr}}{\sqrt{2} h N} \right).
\]

In expression (13) for the condition of free flow, the only unknown parameter is the function of the maximum turbulent viscosity at the vertex of the parabola in expression (3) \( e \), which is essentially a correlation parameter. The function is found by comparing the experimental and calculated (13) values of the heat transfer intensity to the film in the mode of evaporation from the interfacial surface. For water and sugar solutions during vaporization in a pipe with a diameter of 20 mm (Figure 4) the relation for the function \( e \) in (3) is obtained in the form, (Figure 4)

\[
e = 5 \cdot 10^{-3} \text{Re}^{1.4},
\]

\[1\text{– water; } 2, 3 \text{– sugar solutions. } 2 \text{ – DM = 40%; } 3 \text{ – 50%}.\]
Since wave formation at the same volumetric liquid flux develops differently in channels of different diameters, then, accordingly, the turbulence in the film should depend on the geometry of the channel. The effect of surface curvature on heat transfer in the form (13) can be estimated by comparing experimental data on heat transfer to films in pipes of different diameters with the results of calculation by (13, 14). The result is a function of dependence $\varepsilon$ on the diameter of the pipe in the form of a factor to (14), which has the form of an exponential function

$$
\varepsilon = 5 \cdot 10^{-5} \left( \frac{d}{d_0} \right)^{1.4} \left[ 1 + 3.6 \left( 1 - \exp \left( 1 - \frac{d}{d_0} \right) \right) \right],
$$

where $d_0 = 0.02 \text{ m}$.

Graphical interpretation of the results of calculations by (13, 15) for free-flowing films of water in the mode of evaporation from the interfacial surface in pipes of different diameters and comparison with experimental data is shown in Figure 5.

![Figure 5. Dependence $\alpha = f(Re)$ for pipes of different diameters.](image)

1 – data of the authors, $d = 20 \text{ mm}$; 2 – $d = 30 \text{ mm}$; 3 – data [12], $d = 32 \text{ mm}$, water $t = 100 ^\circ \text{C}$.

Simulation of heat transfer in flowing weakly turbulent films in the presence of interfacial the shear stress

In the presence of the vapor velocity above the surface of the film and, accordingly, the shear stress on the film interface, the film thickness decreases, and the parameters of the wave structure of the film change. Thus, according to [14], the flow of gas over the surface of the vertically flowing film leads to a decrease in the amplitude of the waves and an increase in their frequency. These factors also affect the parameters of turbulence. In the absence of direct measurements of the turbulence intensity profile in the films during the movement of the gas flow (vapor) over its surface, it is convenient to make an assumption that only the parameter changes, while the shape of the turbulent viscosity profile curve (3) is slightly...
deformed. The new value of the turbulence function at the vertex of the parabola shifted relative to the middle of the film $\varepsilon$ is given in the form of the product (13) with the function of turbulence suppression by steam flow due to the reduction of the film thickness $f_u$.

Comparing the experimental data on heat transfer to the films of liquids in the presence of shear stress on the film interface with the calculated ones (7, 11, 13), we obtain the expression $f_u$ for in the region $We \leq 250$

$$f_u = S \left[ 1 - 0.1 \exp \left( -1.1086 \sqrt{We} \right) \right],$$

where

$$S = 1.119 - 0.122 \sqrt{We} + \left( 0.07424 + \frac{Re}{9.153 \cdot 10^6} \right) \left( \sqrt{We} \right)^2 - 0.01808 \left( \sqrt{We} \right)^3 +$$

$$+1.775 \cdot 10^{-3} \left( \sqrt{We} \right)^4 - 7.8 \cdot 10^{-5} \left( \sqrt{We} \right)^5 + 1.28 \cdot 10^{-6} \left( \sqrt{We} \right)^6,$$

$$We = \frac{\rho_2 u_2^2 d_u}{\sigma}$$ – the Weber number; $u_2$ – steam core velocity; $\rho_2$ – density of steam; $\sigma$ – surface tension.

Graphic interpretation (16) is shown in Figures 6, 7.

**Figure 6.** Dependence $f_u = f(u_2)$ on atmospheric pressure:

1 – $Re = 1000$, 2 – 3000, 3 – 6000, 4 – 9000.

**Figure 7.** Dependence $f_u = f(u_2)$ at $Re = 6000$ in the region of rarefaction:

1 – $\rho_2 = 0.6 \, kg/m^3$; 2 – 0.4; 3 – 0.2.
The nature of the curves in Figure 7 is due to the weakening of the dynamic action of the flow core on the film in the vacuum region, where the vapor density decreases, and hence the action of the flow core on the film, and, accordingly, on the turbulence parameters in it.

Finally, the algebraic function of the distribution of turbulent viscosity in flowing films with a concomitant steam flow in the range \( We \leq 250 \), \( G_s \leq 0.6 \times 10^{-3} \text{ m}^2 \text{/s} \) for pipes with diameters from 20 to 32 mm (investigated range) takes the form

\[
\nu_t = 5 \times 10^{-5} \text{Re}^{1.4} \left\{ 1 + 3.6 \left[ 1 - \exp \left( 1 - \frac{d}{\nu} \right) \right] \right\} \cdot f_s \eta^2 \left( 1 - \eta^2 \right)
\]  

Graphical interpretation (17) for different phase costs is shown in Figure 8.

Figure 8. Dependence \( \frac{\nu_t}{\nu} = f(\eta) \) on the ratio (17) for water at \( t = 100 \degree C, d = 0.02 \text{ m} \).

a – \( u_2 = 10 \text{ m/s}, 1 – \text{Re} = 1356; 2 – 4068; 3 – 6780. \)

b – \( \text{Re} = 6780, 1 – u_2 = 35 \text{ m/s}; 2 – 20; 3 – 0. \)

The coefficient of hydraulic friction of steam on the surface of the film \( \xi \), which is included in the ratio for the shear stress on the film interface \( \tau_i = \xi \rho_s \frac{u_2^2}{8} \), is calculated by the ratio

\[
\xi = \xi_1 + \frac{627 (d_{11} / d)}{Fr_{2,126}^{1.26} \left\{ \exp \left\{ 1,25 \times 10^{-2} K_{s}^{1.5} \left( Fr - H_{d}^{1.4} \sqrt{d / d_{11}} \right) \right\} - 1 \right\} },
\]

where \( \xi_1 = 3 \times 10^{-3} + 4 \times 10^{-2} K_{s} \) – is the coefficient of hydraulic friction for the first zone – the zone of the mode of weak interaction; \( \xi_2 = \frac{0.316}{\text{Re}_{2}^{0.25}} \) – coefficient of hydraulic friction on a dry wall; \( K_{s} = \sqrt{\frac{G_s \rho}{g \sigma}} \); \( H_{d} = \sqrt{\frac{\rho \sigma}{g d^3 \rho_s^2}} \); \( Fr_{2} = \frac{u_2^2}{g d} \) – the Froude number;
Re₂ = \frac{u₂d \rho₂}{\mu₂} – the Reynolds number for steam; \( \mu₂ \) – steam dynamic viscosity;

\( d_{\text{air}} = 0.013 \text{ m} \). The transition to a zone of strong phase interaction is carried out under the condition 

\[ Fr₂ - H_{\text{air}}^{1.3} \sqrt{d / d_{\text{air}}} \geq 0. \]

A comparison of the results of calculating the heat transfer intensity in the presence of a concomitant steam flow with experimental data obtained in a pipe with a diameter of 20 mm for films of water and sugar solution at atmospheric pressure and vacuum of 0.84 bar is shown in Figure 9.10

\[ Rw = \frac{u \cdot d \cdot \rho \cdot \mu}{\eta} \]

\( \eta \) – kinematic viscosity.

**Figure 9.** Dependence \( \alpha = f(u₂) \) for water and sugar solutions for the pipe, \( d = 20 \text{ mm} \).

1, 2 – water, \( t = 100 ^{\circ} \text{C}, 1 - G_v = 0.5 \times 10^{-3} \text{ m}^2/\text{s}; 2 - 0.3 \times 10^{-3}; \text{Pr} = 1.79. \\
3, 4 – sugar solution, \( t = 100 ^{\circ} \text{C}, DM = 40\%; \text{Pr} = 5.58. \\
Lines – calculation for (7, 11, 17, 18)

\[ \alpha = f(u₂) \]

**Figure 10.** Dependence \( \alpha = f(u₂) \) for sugar solution with a concentration of 40\% in a pipe with a diameter of \( d = 20 \text{ mm} \) in the vacuum of 0.86 bar.

1 – \( G_v = 0.1 \times 10^{-3} \text{ m}^2/\text{s}; 2 - 0.3 \times 10^{-3}; 3 - 0.45 \times 10^{-3}. \) The lines correspond to the calculation of (7, 11, 17, 18) at the corresponding irrigation densities.
Modeling of heat transfer processes in films under bubble boiling conditions

Equations (7, 11, 17) reflect the process of heat transfer to the films flowing in the pipes in the mode of evaporation from the free surface in the absence of bubble boiling in the region of temperature differences that do not exceed the limit value

$$\Delta t_{\text{min}} = \frac{2\sigma T_{\text{sat}}}{r_f \rho_z R_m} + \Delta \mu,$$

where $T_{\text{sat}}$ – saturation temperature, $K$; $R_m$ – roughness of the heat exchange surface (for new pipes $R_m = 0.5 \cdot 10^{-5} m$), $\Delta \mu$ – physicochemical temperature depression of the solution, $r_f$ – the heat of the phase transformation.

In the case $\Delta t \geq \Delta t_{\text{min}}$ of the surface of heat exchange, depending on the nature of the distribution of microcracks, there are foci of bubble boiling, which leads to intensification of heat transfer in proportion to the increase in temperature pressure $\Delta t = t - t_{\text{sat}}$, which, by analogy with the conclusions of [15], is taken into account by (7) $K_{\text{boil}}$

$$K_{\text{boil}} = 1 + 0.4 \left( \frac{\Delta t - \Delta t_{\text{min}}}{\Delta t_{\text{min}}} \right)^{1.2}.$$

Then the ratio for calculating the intensity of heat transfer when it $(\Delta t - \Delta t_{\text{min}} \geq 0)$ takes the form

$$\alpha = \frac{\lambda}{\delta \sqrt{2} \left( 4 + \varepsilon \text{Pr} \right)} \frac{H N}{K_{\text{boil}}}.$$

At $(\Delta t \leq \Delta t_{\text{min}})$ $K_{\text{boil}} = 1$.

A comparison of the results of the calculation of heat transfer intensity in the presence of bubble boiling with experimental data in a pipe with a diameter of $20 \text{ mm}$ for films of water and sugar solutions at atmospheric pressure at free flow and flow with concomitant steam flow is shown in Figures 11, 12.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure11.png}
\caption{Dependence $\alpha = f(q)$ on free flow of films of water and sugar solutions, $(t = 100 \ ^{\circ}\text{C}, R = 0.5 \cdot 10^{-5} \text{ m})$.
\begin{itemize}
\item a – Water, $1 - G_v = 1 \cdot 10^{-4} \text{ m}^2 / \text{s}; 2 - 2 \cdot 10^{-4}; 3 - 3 \cdot 10^{-4}; 4 - 5.5 \cdot 10^{-4};$
\item b – sugar solution, $DM = 70\%$, $1 - G_v = 0.5 \cdot 10^{-4} \text{ m}^2 / \text{s}; 2 - 2 \cdot 10^{-4}; 3 - 3 \cdot 10^{-4}; 4 - 5.5 \cdot 10^{-4}$.
\end{itemize}
The lines correspond to the calculation of (20) at the appropriate irrigation densities.}
\end{figure}
The lines correspond to the calculation of (20) at the appropriate irrigation densities.

As can be seen from the graphs shown in Figures 11, 12, the transition to the boiling mode with increasing steam velocity is shifted to the region of larger heat fluxes. During the boiling of solutions, the temperature of the film is higher than the saturation temperature by the amount of physicochemical depression \( \Delta_{fc} \), which is proportional to the mass concentration. Given the non-uniformity of the distribution of concentration over the film thickness and the lack of information about the concentration on the interfacial surface, the value \( \Delta_{fc} \) is calculated from the average concentration. Therefore, the experimental heat transfer coefficients to the film solutions are defined as the ratio of heat flux to the temperature difference between the wall temperature and the average mass temperature of the film. In the case of free flow, the experimental average mass temperature of the film and the calculated coincide.

But in the presence of steam flow over the film with increasing steam velocity, the film cools and its average mass temperature becomes less than the design. Therefore, the calculation of the heat flux to the boiling films of solutions in the presence of the vapor velocity above its surface must be performed taking into account the suppression of physicochemical temperature depression by the vapor flow, namely as

\[
q = \alpha \left( t_w - t_{sat} - \Delta_{fc} f_{fc} \right),
\]

where \( f_{fc} = \exp \left( -1.07 \cdot 10^{-2} \sqrt{We} \sqrt{Pe} \right) \) – the function of suppression of physicochemical temperature depression by steam flow, \( Pe = \frac{4 G}{a_m} \) – the Peclet number, \( a_m \) – temperature conductivity.
Conclusions

1. An algebraic model of turbulent viscosity is proposed, which qualitatively reproduces the real distribution of turbulent viscosity over the entire thickness of the film flowing down the vertical surface.
2. Based on the proposed model, analytical expressions for temperature and velocity profiles in the film and the corresponding integral thermohydrodynamic characteristics for both free flow and motion with a concomitant flow of steam (gas) are obtained.
3. On the basis of the received decisions and the corresponding experimental data the analysis of influence of factors on intensity of heat transfer is executed and the generalizing equations for calculation of coefficients of heat transfer to films of solutions during evaporation are received.

References

Methodical approaches to the determination of readiness of introduction of «blue ocean» strategy on the enterprises of food industry

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Abstract

Introduction. The aim of the research is the development of methodical approach to the determination the readiness of introduction of blue ocean strategy on the enterprise.

Materials and methods. The methods of systematization for finding out the factors that carry out influence on the position of enterprise on a way to introduction of «blue ocean» strategy were used. For their distribution on success and challenges the method of comparison was used. The evaluation of factors of success-challenges was carried out by expert methods. The state of industry/enterprise was determined by matrix method.

Results and discussion. Methodology that allows to define readiness of realization of «blue ocean» strategy is offered. General factors that stipulate the necessity of such strategy realization are outlined. Among them are the most substantial ones and qualificatory for industry/enterprise are elected. They can be both positive (success) and negative (challenges). Their measuring comes true by finding intersection of average estimation (it is determined by expert method) on a weighting coefficient (it is set on the basis of experience of researchers depending on the degree of factors meaningfulness and their influence on the development of industry). With the aim of interpretation of the given results the estimation of industry/enterprise situation, the use of offered matrix «success-challenges» that has 4 quadrants is offered.

Depending on what field the values of estimations got after success and challenges, drawn conclusion in relation to that, prepared enterprise to introduction of «blue ocean» strategy or not. There actions that can improve the position of the enterprise on a way to application of this strategy were offered. The worked out methodical approaches on the example of brewing industry of Ukraine and its leading company Private joint stock company «Obolon» were approved and successfully exports the products. The Ukrainian market of brewing industry is the typical representative of environment of red ocean, which is characterized by difficult competition situation with the high level of monopolization, and so for which the application of free market space strategy is actual.

Conclusion. Application of the methodology worked out by authors will allow to define the readiness to the realization of «blue ocean» strategy.
Introduction

The characteristic for the whole world strengthening of competition stipulates the necessity to search new decisions, disengage oneself from existing ones, challenge to fundamental principles of business and redistribute borders, creating new markets and industries [1, 2]. Many researches of scientists from different countries are sanctified to the idea to create such market space where competition will not dominate [11, 33]. It is the best method of development and search of non-standard approaches for business that wants to develop successfully [3, 4, 5].

One of decisions there can be application of «blue ocean» conception as alternatives of «red ocean» strategy (existent business-environment with exhausting competitive activity) [7, 8, 9, 10]. The new market space («blue ocean») can create any type of business, regardless of specific and sizes [33]. The main in the marked conception is persistent aspiration to avoid influence of competitors, to carry conviction in the future [11, 12, 13, 14]. It opens wide possibilities, what the results of activity of numerous companies are testifies [15, 16].

Without regard to advantages, that gives the use of «blue ocean» strategy, its introduction is related to certain difficulties. The unsolved are remained the questions of readiness of the enterprise to this strategy introduction, presence necessary terms for this purpose [15, 17]. Especially sharp is this problem for the enterprises of food industry, the competition dominates on these markets [24]. They are interested in that even on some time to have favourable operating conditions [17, 18].

Therefore, the research aim is the development of the methodical approach determination the readiness of introduction of blue ocean strategy on the enterprise.

For achievement of this aim, it is necessary to decide such tasks:

− To outline general factors that stipulate the necessity of «blue ocean» creation and to go into details taking into account the specific of the enterprises and industries of food industry and market situation;
− To systematize and divide certain factors into positive (success) and negative (challenges);
− To carry out the evaluation of factors of success and challenges;
− To define position of industry/enterprise on matrix «success-challenges» for finding out the readiness to introduction of «blue ocean» strategy;
− To work out the actions sent to the assistance for creation of new market space.

Methods and materials

Materials

The object of the research are the factors that determine readiness of introduction of «blue ocean» strategy on the enterprises of food industry. The subject of the research is: finding out and evaluation of factors of success and challenges that will allow to define the degree of readiness of the enterprise to realization of this strategy; brewing industry of Ukraine and its state; Private joint stock company «Obolon» as the enterprise that works at the market of brewing industry.
Characteristics of Private joint stock company «Obolon» as the subject of the research

Private joint stock company «Obolon» is in five of leaders of brewing industry. It is the most progressive private enterprise of beer production, by the national company that is known in the world. The brand portfolio counts over 16 sorts of beer.

Private joint stock company «Obolon» at the Ukrainian beer market is the founder of packing segment by PAT by capacity of 1 l and 2 l and 30-l kegs with the capacity of one liter for restaurant business. Three basic competitors of the company are: Private joint stock company «Carlsberg Ukraine», and Limited Liability Company «Persha Pryvatna Broviarnia».

Private joint stock company «Obolon» exports the products to many foreign countries: Canada, Panama, Chile, Great Britain, Japan, Singapore, Vietnam, UAE, Turkey, Germany, France, Switzerland and others [23, 24].

Infobase scientific sources served as [7, 8, 9, 11, 16, 18] and the results of brewing industry market researches [19, 20, 21, 22, 23, 24].

Methods of research

Scientific and special methods were used in researches [25]. With the aim, finding out the factors that stipulate the necessity of «blue ocean» creation, that have the influence on industry and enterprise, determine readiness to strategy realization, conducted review of scientific sources. In combination with the method of systematization and comparison, it allowed to distinguish exactly from many possible factors only those that provide success of industry or challenges for it [26, 27, 28].

Method of expert estimations

Expert methods for evaluation of factors that represented the state of industry and enterprise were used. For all factors there was a certain estimation at 5 scale: «5» - very high, «4» - high, «3» - moderate, «2» - weak, «1» - very weak.

In the role of experts leading specialists in this field were invited. The choice of circle and amount of experts came true by determination of establishments that engage in the development of alike questions, and acquaintance, with the publications of leading specialists on issue that is examined.

We made list of specialists, that acquaintances with the state of industry and have corresponding researches. According to this list were selected those who were qualified [29, 30].

Matrix method

Matrix methods are taken to the construction of two- or multivariable matrices [32]. They were used for finding out of brewing industry position and the enterprise in the system of coordinates «challenges-success». In the offered matrix on vertical wasp the values of quantitative estimation index of success are put aside, and on horizontal are sizes of quantitative estimation index of challenges. Every quadrant of matrix answers certain position of industry/enterprise. With the help of this matrix recommendations were developed to introduction of «blue ocean» strategy.
Methodology of determination the readiness of introduction of blue ocean strategy on the enterprises of food industry

Detailed analysis of existent theoretical researches in relation to introduction of «blue ocean» conception educed an urgent requirement in the lineation of factors, that inform about inevitability of changes for business, and practical actions that will assist creation of new market space [2, 3, 4, 5, 6]. The basic methodical approaches determination the readiness of realization of blue ocean strategy on the enterprises of food industry, that are based on marketing researches were offered by us. Within the framework of this methodology we suggest to carry out such steps.

The first step provides the quality of questioning conditions to define factors that predetermine the necessity of introduction of «blue ocean» strategy. To the number of such factors belong: deceleration or reduction the potential for further development of business; threatening changes in consumer environment; strengthening of competition; exceeding supply of commodities/services above demand; distribution and strengthening of processes of globalization; development of technologies for production of plenty of commodities/services; strengthening of price wars; tendency to the increase of production charges [3, 4, 6, 11, 15, 16]. Presence even one of them signals about the necessity of development and passing to new market space. Going out from the situation that was folded in industry and on the enterprise, the list of such factors is formed. They must be gone into detail thus, to take into account the specific of the enterprises and industries of food industry and market situation.

Next step is systematization and division of certain factors on positive and negative that in future will name accordingly as success and challenges. The high level of authenticity of the conducted analysis must be provided by taking into account of greater amount of terms as possible [10, 15, 22].

By success/ challenges for industry and enterprise it is possible to consider: political stability/instability in the country; favourable/difficult macroeconomic situation; increase/falling of production and consumption of products volumes; stability/instability of national currency; presence/absence of government control of market; small/large concentration of market; subzero/high entrance barriers in industry; large/small prospects of business development; presence/absence of information technologies; large/small stake of innovative products in general production of industry volume; presence/absence of new technologies of products making; stable/unstable demand on the products of industry; falling/increase of price on raw material and others [22, 23, 24].

On the next stage for determination the readiness to realization of strategy, position of industry/company through the quantitative measuring of success - cahllenges by the method of expert estimations is determined.

Leading branch workers that know problems and situation at the corresponding market are attracted for this purpose. Experts estimate influence of every factor on the state of industry/enterprise at 5 scale from «1» (very weak) to «5» (very strong). The average value accounts on the basis of the proposed expert estimations [29, 30].

Depending on the degree of meaningfulness of factors and their influence on development of industry (negative or positive), the coefficients of importance (matter from 0,01 to 0,99, and their sum must be equal 1) are determined. Thus, the special operating of industry conditions, state of economy of the country and political situation, are taken into account [22, 23, 24].

Finding the sum of products of average experts’ estimation on the coefficient of its importance the general estimations of of challenges and success factors are determined.
With the aim of interpretation of the got results of assessing the situation of industry and enterprise it was worked out and offered the matrix «success-challenge» (Figure 1).

At the place of location of industry/company on the field of matrix determine their position in accordance with offered descriptions of each of four zones of matrix «success-challenges».

Quadrant of matrix «Calm» is the position that is characterized by insignificant success of industry/company at small challenges. Specifies on weak development status and necessity of defence of existent position at the market. For industries/companies that got to the zone «Calm», selective development of those directions that can be effective in the future is offered. It can be strengthening of possibilities for rendering of challenges, but efficiency of these actions is small. The necessity of changes and readiness to them are estimated as remote.

Zone of matrix «Breeze» characterizes position with great successes at insignificant challenges. It is necessary to be concentrated on those prospects that is given by industry/company, and to develop them. At these favourable terms, passing to the «blue ocean» is estimated as the most comfortable. Besides, success in course of time can pass and lot upon them is impossible.

The quadrant of matrix «Hurricane» specifies on the high achievements of industry/company at very large challenges. It warning that maximal success that it attained, is under threat. Changes must be taken place necessarily. It is time, when future position depends on the produced actions. Operating is necessary immediately, while operating conditions are yet comfortable, but they can be quickly finished. And then passing to new reality can be riskier.

Zone of matrix «Gale» is positions that are characterized by very large challenges at small success. The marked position of industry/company abandons not enough chances on providing of comfort transition from the existent state to more perspective. Even at such difficult situation it is needed to search the ways of improvement of development. Passing to the «blue ocean» will take place, however, more time will be passed and terms will be not such advantageous that they could be on the stage «Breeze» or «Hurricane».

It should be noted that at the place of location on matrix «success-challenges» position of industry, by comparison with position of company, can substantially differ. Accordingly, actions that is needed for successful transition from «red» to «blue ocean» will differ (Table 1).
Table 1

Recommended actions for introduction of «blue ocean» strategy after the matrix «success-challenge, developed by authors

<table>
<thead>
<tr>
<th>Position of industry/company</th>
<th>Recommended actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position of industry is stronger than company position</td>
<td>Search of ways of improvement of enterprise’s position and its market attractiveness. Concentration of efforts on those possibilities, that is given by industry, and avoidances of those challenges, into it runs. The use of industry potential and internal resources of business for further development of company.</td>
</tr>
<tr>
<td>Position of industry is approximately identical to company position</td>
<td>Identical position of industry and enterprise. Using success of both industry and company for future increase.</td>
</tr>
<tr>
<td>Position of industry is more weak than company position</td>
<td>Attempt to avoid those challenges that threaten the development of industry. Exposure of own ways of increase, maintenance and strengthening of advantages. Strengthening of the personal success.</td>
</tr>
</tbody>
</table>

The observance of actions, corresponding to certain position will allow to provide successful realization of «blue ocean» strategy.

Results and discussions

Determination of factors of success and challenges

Approbation of offered methodology came true on the example of brewing industry of Ukraine, but can be used for any industry in other countries.

For determination of factors that have an influence on industry, were taken for basis reviews of brewing products markets and statistical information on their development [19, 20, 21]. The conducted analysis allowed to define challenges and success, and also execute their quantitative evaluation [19, 20, 21, 22, 24]. It is appeared, that industry had 13 factors of success and 19 factors that stipulated challenges for it (Table 2).

The analogical method the factors of success and challenges for Private joint stock company «Obolon» was determined [19, 20, 21]. Conducting the analysis of activity of the enterprise at the market, 15 factors that belong to the challenges of the company, and 8 factors of success, were educed.

Factors that stipulate challenges for Private joint stock company «Obolon» are: falling of production and consumption of beer volumes in Ukraine; difficult macroeconomic situation in the country; devaluation of national currency; absence of political stability; small social guarantees for highly skilled specialists; low level of integration of science and production; decline of purchasing power of population; reduction of consumption level of beer of average price segment; increase of beer consumption in the economy segment; large concentration of market; increase of excise on beer; including of beer to the list of alcoholic beverages in accordance with changes in the Internal Revenue Code; competition with the producers of strong alcohol; reduction of beer export to Russia; increasing of price on raw material [19, 20, 21, 22].
Table 2

Challenges and success of brewing industry of Ukraine

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Falling of production and consumption of beer volumes in Ukraine</td>
<td>1. Presence of foreign investments in industry</td>
</tr>
<tr>
<td>2. Difficult macroeconomic situation in the country</td>
<td>2. Introduction of progressive technologies of production of new types of products – unfiltered, icy, fruit, «living» beer</td>
</tr>
<tr>
<td>4. Absence of political stability</td>
<td>4. Increase of popularity of «authorial» products of small brewing plants</td>
</tr>
<tr>
<td>5. Presence of local conflict</td>
<td>5. Noticeable increase of beer sale in the restaurant business</td>
</tr>
<tr>
<td>6. Small social guarantees for scientists and highly skilled specialists</td>
<td>6. Change of consumers’ tastes to the consumption of the unpasteurized beer in kegs</td>
</tr>
<tr>
<td>7. Low level of integration of science and production</td>
<td>7. Increase of popularity of the «craft» brewing</td>
</tr>
<tr>
<td>8. Decline of purchasing power of population</td>
<td>8. Change in the culture of alcohol consumption: increase of beer consumption and reduction of vodka consumption, and also consumption in the place of its production</td>
</tr>
<tr>
<td>9. Reduction of consumptions level of trade marks, that is presented in a middle price segment</td>
<td>9. Increase of beer consumption in premium segment</td>
</tr>
<tr>
<td>10. Increase of consumption of beer in the economy segment</td>
<td>10. Adaptation of enterprises to the work on foreign malt and hop</td>
</tr>
<tr>
<td>11. Large concentration of market</td>
<td>11. Introduction of energy saving technologies of beer production</td>
</tr>
<tr>
<td>12. High entrance barriers in industry</td>
<td>12. Appearance of new harmless for nature types of packing</td>
</tr>
<tr>
<td>14. Increase of excise on beer</td>
<td></td>
</tr>
<tr>
<td>15. Including of beer to the list of alcoholic beverages in accordance with the changes in the Internal Revenue Code</td>
<td></td>
</tr>
<tr>
<td>16. Competition with the producers of strong alcohol</td>
<td></td>
</tr>
<tr>
<td>17. Reduction of beer export to Russia</td>
<td></td>
</tr>
<tr>
<td>18. Low level of introduction of information technologies in industry</td>
<td></td>
</tr>
<tr>
<td>19. Increasing of price advance on raw material</td>
<td></td>
</tr>
</tbody>
</table>

It is made on the basis of own researches and the sources [19, 20, 21, 22, 23, 24].
Success for Private joint stock company «Obolon» are: introduction of progressive technologies of production of new types of products – unfiltered, icy, fruit, «living» beer; noticeable increase of sale of beer is in the restaurant business; change in the culture of alcohol consumption; increase of beer consumption and reduction of vodka consumption, and also consumption in the place of its production; increase of beer consumption in premium segment; introduction of energy saving technologies of beer production; appearance of new harmless for nature types of packing; improving the new markets of sale [22, 23, 24].

**Evaluation of factors of successes and challenges**

According to the factors of industry, given in table 2 and by factors recommended for the enterprise, the state of each factors by questioning of experts was appraised. 5 leading specialists of brewing industry and Private Joint Stock «Obolon» came forward is the state of factors.

The experts’ opinions got as the result of questioning were average, and also checked for coordination by means of coefficient of variation, that in relation to the challenges of industry is confirmed by Table 3.

**Table 3**

<table>
<thead>
<tr>
<th>№</th>
<th>Experts estimations</th>
<th>Coefficient of variation,%</th>
<th>Average value of expert estimations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,4,5,5,5</td>
<td>8,33</td>
<td>4,8</td>
</tr>
<tr>
<td>2</td>
<td>4,5,4,4,4</td>
<td>9,52</td>
<td>4,2</td>
</tr>
<tr>
<td>3</td>
<td>3,3,2,4,3</td>
<td>21,08</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2,2,4,3,3</td>
<td>26,73</td>
<td>2,8</td>
</tr>
<tr>
<td>5</td>
<td>2,2,2,2,2</td>
<td>18,18</td>
<td>2,2</td>
</tr>
<tr>
<td>6</td>
<td>1,2,2,2,2</td>
<td>22,22</td>
<td>1,8</td>
</tr>
<tr>
<td>7</td>
<td>2,3,3,3,3</td>
<td>14,29</td>
<td>2,8</td>
</tr>
<tr>
<td>8</td>
<td>5,5,5,5,5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>2,4,4,3,3</td>
<td>23,39</td>
<td>3,2</td>
</tr>
<tr>
<td>10</td>
<td>4,4,3,3,3</td>
<td>14,41</td>
<td>3,4</td>
</tr>
<tr>
<td>11</td>
<td>3,4,4,5,5</td>
<td>17,82</td>
<td>4,2</td>
</tr>
<tr>
<td>12</td>
<td>4,4,3,4,4</td>
<td>10,53</td>
<td>3,8</td>
</tr>
<tr>
<td>13</td>
<td>2,4,4,4,4</td>
<td>22,22</td>
<td>3,6</td>
</tr>
<tr>
<td>14</td>
<td>4,4,3,3,3</td>
<td>14,41</td>
<td>3,4</td>
</tr>
<tr>
<td>15</td>
<td>3,4,4,4,4</td>
<td>10,53</td>
<td>3,8</td>
</tr>
<tr>
<td>16</td>
<td>3,3,2,4,3</td>
<td>21,08</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>1,1,2,2,2</td>
<td>30,62</td>
<td>1,6</td>
</tr>
<tr>
<td>18</td>
<td>3,2,2,3,3</td>
<td>18,84</td>
<td>2,6</td>
</tr>
<tr>
<td>19</td>
<td>3,3,3,3,3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

By analogical method the evaluation and checking for homogeneity for the factors of industry success was conducted (Table 4).
Table 4
Results of experts’ opinions verification on coordination and their middle values after the factors of successes of brewing industry

<table>
<thead>
<tr>
<th>№</th>
<th>Experts estimation</th>
<th>Coefficient of variation, %</th>
<th>Average value of expert estimations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 5 5 4 5</td>
<td>10,65</td>
<td>4,6</td>
</tr>
<tr>
<td>2</td>
<td>5 5 5 5 5</td>
<td>0</td>
<td>5,0</td>
</tr>
<tr>
<td>3</td>
<td>5 4 5 5 3</td>
<td>18,18</td>
<td>4,4</td>
</tr>
<tr>
<td>4</td>
<td>2 3 3 3 3</td>
<td>14,29</td>
<td>2,8</td>
</tr>
<tr>
<td>5</td>
<td>2 1 2 2 1</td>
<td>30,62</td>
<td>1,6</td>
</tr>
<tr>
<td>6</td>
<td>3 2 3 3 3</td>
<td>14,29</td>
<td>2,8</td>
</tr>
<tr>
<td>7</td>
<td>2 3 3 2 3</td>
<td>18,84</td>
<td>2,6</td>
</tr>
<tr>
<td>8</td>
<td>3 4 4 4 4</td>
<td>10,53</td>
<td>3,8</td>
</tr>
<tr>
<td>9</td>
<td>4 4 4 5 4</td>
<td>9,52</td>
<td>4,2</td>
</tr>
<tr>
<td>10</td>
<td>4 3 3 4 3</td>
<td>14,41</td>
<td>3,4</td>
</tr>
<tr>
<td>11</td>
<td>3 3 3 4 4</td>
<td>14,41</td>
<td>3,4</td>
</tr>
<tr>
<td>12</td>
<td>5 5 4 5 5</td>
<td>8,33</td>
<td>4,8</td>
</tr>
<tr>
<td>13</td>
<td>4 3 4 4 3</td>
<td>13,61</td>
<td>3,6</td>
</tr>
</tbody>
</table>

For every factor (both for group of challenges and success) the coefficient of variation is less than normative value (33%), thus totality of opinions of this group of experts can be considered homogeneous and use for further researches [29, 30].

Grouping of factors was farther carried out after the degree of importance (table 5). Determination of coefficients of importance was based on own researches of the state of brewing industry and results of working corresponding sources [19, 20, 21, 23, 24].

Table 5
Grouping of factors after the degree of importance and distribution of coefficients of importance

<table>
<thead>
<tr>
<th>Groups of challenges factors of brewing industry</th>
<th>Coefficient of importance</th>
<th>Groups of success factors of brewing industry</th>
<th>Coefficient of importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 8</td>
<td>0,08</td>
<td>1, 2, 8, 11</td>
<td>0,09</td>
</tr>
<tr>
<td>3, 4, 11</td>
<td>0,07</td>
<td>3, 10, 12, 13</td>
<td>0,08</td>
</tr>
<tr>
<td>6, 10, 12</td>
<td>0,06</td>
<td>4, 7</td>
<td>0,07</td>
</tr>
<tr>
<td>13, 14</td>
<td>0,05</td>
<td>5, 6, 9</td>
<td>0,06</td>
</tr>
<tr>
<td>7, 15, 16, 19</td>
<td>0,04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9, 17, 18</td>
<td>0,03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0,02</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*It is made on the basis of own researches and source [19, 20, 21, 23, 24].*
Using these tables 3, 4, 5 it was the certainly generalized average estimation of factors of challenges and success of brewing industry.

On counts, success of industry got a general estimation that equals 3,72 points. The quantitative value of challenges presented 3,48 points. Such estimation can be considered moderate, because it is considerably less than 5 points. It testifies that industry has large backlogs for development.

By the analogical method the evaluation of certain factors of Private joint stock company «Obolon» was conducted by bringing workers of higher and middle ranks of management of the enterprise. Calculations showed that the quantitative estimation of success of the enterprise at the market had laid down 4,55, and challenges are 3,11 points.

The got results are quite good enough. Although after the amount of success less than challenges, but their estimation is approached to 5 points. Challenges in number anymore and measuring results testify to their danger for the enterprise. Desirable is the value that is approached to 1.

**Determination of position of industry/ enterprise for finding out of readiness of Private joint stock company «Obolon» to introduction of «blue ocean» strategy**

For comfort presentation of analysis results of industry position and enterprise the «success-challenges» (Figure 2) shown on matrix by the mark of IP (Industry Position) and CP (Company Position) accordingly.

![Figure 2. Position of brewing industry and Private joint stock company «Obolon» on matrix «success-challenges», developed by authors](image)

On results of research position of industry and company on the field of matrix answers a quadrant «Hurricane». It testifies the necessity of immediate cardinal changes, while for this purpose there are comfortable terms at a minimum risk. In the near future appearance of urgent requirement is expected in the search of the non-standard approach development of industry and enterprise (for example, introduction of new types of products with unique...
descriptions, new technologies, non-standard methods of communications and others). But there are no obstacles to introduction of strategy of blue ocean.

Position of company (CP) on the field of matrix «success-challenges» (Figure 2) also «Hurricane» answers a quadrant and testifies to the large challenges. Therefore, passing to introduction of «blue ocean» strategy is desirable.

Without regard to that position of both industry and company got to one to the quadrant, it is necessary to mark that position of Private joint stock company «Obolon» is better than position of brewing industry on the whole: the quantitative estimation of success of the enterprise presents 4,55, and industries are 3,72 points. Challenges for the enterprise far fewer (3,11) in comparing to industry (3,48). There was a situation that answers two terms: from one side, strategic position of industry is approximately identical to position of company, from other - position of the enterprise looks more successful in comparing to industry. Thus, Private joint stock company «Obolon» can be considered ready to introduction of «blue ocean» strategy.

Conclusions

Thus, as the result of undertaken study, the methodical approach to the determination of readiness of introduction of «blue ocean» strategy on the enterprise, is firstly given, the use of that will allow:

− To make more balanced decision about creation of free market space;
− To elect the most optimal direction of introduction strategy;
− To form reasonably the program of actions and events for realization of «blue ocean» strategy.

The scientific value of the worked out methodology consists in possibility of determination of strategic position of industry/company by establishment and quantitative measuring of factors, that have both positive and negative influence, and also research of the level of company’s readiness to perception of «blue ocean» strategy. Thus, select factors go into details into of three directions: features of the enterprise, specific of industry and market situation. Offered methodology is enriched by authorial matrix «success-challenges» (each of its quadrants is characterised).

Unlike existing, the presented methodology is based on marketing researches, factor analysis, use of method of expert estimations and allows not only to define readiness of the enterprise to introduction of «blue ocean» strategy but also envisage actions in direction of motion to such space.

Application of offered methodical approaches will assist more comfort passing to the menage on principles of «blue ocean» conception, that will allow to avoid complete or partial influence of competition on activity of industry or company and will give the opportunity to free the financial resources related to realization of marketing researches of competitors and competition situation. The further prospect of research can be consideration and choice of additional analytical instruments and models that will help the enterprise to form new own market space.

References


Анотації

Харчові технології

Якість канадських комерційних нежирних йогуртів грецького типу, виготовлених з натуральних молочних інгредієнтів

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Вступ. У статті представлена порівняльна оцінка якості зразків канадських комерційних нежирних йогуртів грецького типу, виготовлених з натуральних молочних інгредієнтів.

Матеріали і методи. Зразки йогурту нежирного грецького через 10 днів після їх виготовлення зберігали при температурі 5 °C упродовж 18 та 35 днів. Вимірювали динамічні реологічні властивості йогурту та об’єм сироватки, що відділялася над поверхнею згустку. Для оцінки ступеня дренажу сироватки та структури зруйнованого згустку було використано порядкову числову шкалу.

Результати і обговорення. Йогурт у грецькому стилі – це типовий слабкий в’язкопружний гель, еластичні властивості якого переважають його в’язкі властивості у межах вимірюваного діапазону. Структурна деградація спостерігалась у всіх зразках у певний момент протягом діапазону амплітуд напруження. Зразки B за більшого загального вмісту сухих речовин, порівняно зі зразками A, характеризувалися вищим динамічними модулями, навіть за однакового вмісту білка. Обидва еталонних зразки показали значне збільшення їх в’язкого (G”) та еластичного (G’) модулів при зберіганні за температури 5 °C. Цей факт свідчить про те, що казеїнові гелі є динамічними за своєю природою, а подальший розвиток структури гелю відбувається під час зберігання. При високих амплітудах зразки A суцільно збільшували значення tan δ унаслідок розриву їхніх гелевих структур. Спостерігалося пропорційне збільшення G’ і G ”під час зберігання. Отже, значення tan δ для тих самих типів зразків після 18 та 35 днів зберігання були подібними. Зразки не відрізнялись за рівнем дренажу сироватки та за розмірами грудочок перемішаного згустка. Обидва ринкові зразки показали більшу кількість поверхневої сироватки за збільшення часу зберігання. Ймовірно, під час зберігання в структурі гелів відбулися масштабні перебудови, які збільшили рівень їхньої нестабільності, що призвело до втрати здатності захоплювати всю сироваткову фазу. Однак жоден із еталонних зразків не мав видимого дренажу сироватки, всі вони характеризувалися дрібнозернистою структурою.

Висновки. Білок – не єдиний інгредієнт, що формує структуру та реологічні властивості нежирного йогурту грецького типу. Властивості таких йогуртів змінюються під час зберігання – за зміцнення структури підвищується відділення сироватки зі згустку.

Ключові слова: йогурт, грецький тип, сироватка, синерезис, гелі, реологія.
Морфологічні і структурні властивості модифікованого крохмалю з насіння араукарії бразильської

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Вступ. Метою даного дослідження є оцінка основних морфологічних і структурних властивостей модифікованого крохмалю шляхом термоволого оброблення (ТВО) насіння араукарії бразильської.


Результати і обговорення. Крохмаль з насіння араукарії, витягнутий за осадовою методикою, мав вміст амілози 26,3%. Зразки демонстрували низький вміст вологи (<8,5%) і зольності (<1,44%). За даними SEM та AFM, найбільший середній діаметр був для необроблених гранул крохмалю з насіння араукарії: 10,85 та 10,64 відповідно. Найменшими гранулами були ті, які оброблені за вологостю 10% протягом 60 хв за температури 120 ºC. Середня шорсткість, досліджена методом AFM, зросла під час ТВО відповідно з 321,68 до 470,06 мкм. Після проведеного НМТ відбулося зменшення відносної кристалічності.

Висновки. Круглі та овальні форми були визначені для гранул крохмалю з плоскою поверхнею. Знайдено подібність середніх значень діаметра між методами. Середній діаметр гранул збільшувався із збільшенням вологості під час модифікації ТВО. Середня шорсткість зросла під час ТВО, тоді як відносна кристалічність зменшилась.

Ключові слова: крохмаль, араукарія бразильська, модифікація, морфологія.

Оцінка візуальних характеристик пива методом комп’ютерного зору

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Вступ. Метою цього дослідження був моніторинг кольору та стійкості пень під час імутки пивних видів пива (світлого й темного) за допомогою неруйнівного методу – комп’ютерного зору і цифрового аналізу зображень.

Матеріали і методи. Колір пива та стійкість пивної піни для різних типів пива (декларованих як темне та світле пиво) вимірювали методом комп’ютерного зору. Стійкість пивної піни, виражена як зміна висоти піни з плином часу, моделюється з використанням моделі експоненціальної спаду. Вимірювання розпаду піни, як правило, включає вимірювання стоку пива або зменшення висоти піни.

Результати і обговорення. Темне пиво було менш гірким (16,75 IBU), з більшим вмістом поліфенолу (181,80 EBC), порівняно зі світлим пивом (гіркота становила 26,50...
ІВУ, загальний вміст поліфенолу 103,50 ЕВС). Вміст алкоголю в основному був нижче 5% (4,82% для світлого пива і 4,90% для темного пива), а рН становив 4,39 для світлого і 4,43 для темного пива. Колір пива виражався в кольоровій системі CIEL*a*b*, причому темні сорти пива мали нижні значення легкості (L* = 28,7), вищі значення для a* = 10,4 і нижні значення для b* = 4,4. На відміну від цього, світле пиво було яскравішим (L* = 65,5), з нижчими значеннями a* = 7,7 і вищими значеннями b* = 4,4. Темні сорти пива мали вищі значення ЕВС, нижчі значення L* і вищі значення a*, ніж світлі сорти пива, завдяки використанню фарбувального солоду, який випалювали і смажили при більш високій температурі, при якій утворювалися продукти реакції Маяра. Зміна пивної піни з часом являє собою поєднання видалення рідини та розпаду будь-башок. Темне пиво мало набагато стабільнішу піну, ніж світле. Висота піни у світлому і темному пиві статистично відрізнялася (р <0,05), причому зразки темного пива мали вищу початкову висоту піни (66,1 мм) порівняно зі світлим пивом (48,7 мм). Темні зразки пива мали нижчі значення константи швидкості (0,0091 с\(^{-1}\)) і вищі значення періоду напіввиведення піни (76,1 с). Це означає, що темне пиво має більш стійкій піні, ніж світле пиво. На відміну від цього, зразки світлого пива мали вищі значення константи швидкості (0,0110 с\(^{-1}\)) та нижчі значення періоду напіввиведення піни (63,2 с). Встановлено, що застосована математична модель (модель експоненціального розпаду) підходить для прогнозування зміни швидкості розпаду піни та стабільності піни зразків світлого та темного пива. Значення висоти піни сухої та вологої частини піни передбачені моделлю експоненціального спаду.

Висновок. Показано, що комп’ютерна візуалізація є придатним, об’єктивним, відтворюваним і надійним методом вимірювання кольору й стійкості пивної піни (загальної піни, мокрої піни та сухої піни) для світлих і темних сортів пива.

Ключові слова: пиво, колір, піна, комп’ютерний зір, моделювання.

Антиоксидантна здатність настоїв спиртових із рослинної сировини

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Вступ. Метою дослідження є визначення антиоксидантної здатності водно-спиртових настоїв рослинної сировини та ідентифікація найбільш перспективних джерел природних антиоксидантів.

Матеріали і методи. Настрої спиртові з рослинної сировини за морфологічними ознаками: трави (35 зразків); коріння і кореневища (9 зразків); квіти (7 зразків); деревна кора (2 зразки); сухі плоди (18 зразків); соковиті плоди (29 зразків). Методи дослідження: редоксметрія – визначення антиоксидантної здатності рослинної сировини; pH-метрія; експертний метод визначення органолептичних показників.

Результати і обговорення. Настої спиртові з рослинної сировини мають водневий показник від 3,13 (суданська троянда) до 8,17 од. pH (кропива дводомна). Мінімальне теоретичне значення окисно-відновного потенціалу (Eh\text{min}) має значення від 159,1 мВ (кропива дводомна) до 370,8 мВ (суданська троянда), а фактичне значення окисно-відновного потенціалу (Eh_{\text{факт}}) складає від 8,0 мВ (кропива дводомна) до 308,5 мВ (айва довгаста).
Мінімальне значення антиоксидантної (відновної) здатності настоїв спиртових (EB\textsubscript{нaкт}) для плодів лимона – 18,8 мВ, максимальне значення відновної здатності – 209,0 мВ (для настою спиртового із сушки лісової).

Енергія відновлення/окиснення рослиної сировини (EB\textsubscript{росл}) щодо водно-спиртової суміші (розчинника) знаходиться в межах відновних значень від 124,5 мВ (листя сушки лісової) до окисних значень -65,7 мВ (плоди лимона).

Настрої спиртові залежно від активності рослиної сировини мають відновну здатність (понад 0 мВ) – 65% зразків, окислювальну здатність (менше 0 мВ) – 35% зразків.

Створення алкоголних напоїв з антиоксидантною дією дає змогу вводити на ринок нові види продукції, які вигідно відрізняють асортимент виробника від асортименту конкурентів, створюючи позитивний імідж підприємству.

Висновки. Обґрунтовано розширення асортименту алкоголних напоїв із застосуванням спиртових настоїв з рослиної сировини для посилення антиоксидантної дії.

Ключові слова: рослинна сировина, антиоксидант, окисно-відновний потенціал, настій, алкоголь.

Ідентифікація, біохімічні та технологічні властивості видів ентерококів, виділених із сирого молока і традиційних молочних продуктів

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Вступ. Метою дослідження є виділення та ідентифікація видів Enterococcus із сирого молока та традиційних молочних продуктів і вивчення їхніх біохімічних властивостей. Визначено деякі біохімічні і технологічні властивості штамів.

Матеріали і методи. Сире молоко та деякі зразки молочних продуктів (сир Ізмір Тулум, сир Кой, білий сир, масло, сир Еліне, кефірне зерно, кефірний напій, Армала, сир Тир Камур, сир Хербі, козячий сир, сир Сесіл) були зібрані в різних регіонах Туреччини. Зразки інокулювали в анамід канаміцину ескулін-азид, середовище Slanetz Bartley та M-17 Agar та Enterococcus spp. були ізольовані. Досліджено властивості підкислення, виділення екзополісахаридів (EPS), ліполітичну та протеолітичну активність та активність декарбоксилювання штамів Enterococcus, які діагностувались фенотипово і біохімічно різними методами.

Результати і обговорення. Після фарбування за Грамом і тестів на каталазу було виявлено 167 молочнокислих бактерій. Завдяки аналізу 122 з цих ізолятів були ідентифіковані як Enterococcus faecium, 18 як Enterococcus durans, 17 як Enterococcus faecalis, 8 як Enterococcus faecium var. і 2 як Enterococcus hirae. Також вивчені деякі біохімічні і технологічні властивості цих видів. Установлено, що штами E. faecium і E. faecalis підвищують кислотність порівняно з E. durans та E. hirae. Загалом 19 штамів здатні продукувати EPS, тоді як 9 штамів продукують EPS досить повільно. Також було проведено 17 E. faecium, 2 E. faecalis, 1 E. durans і 1 E. hirae. (штами продемонстрували ліполітичну активність) і 3 E. faecalis var., 3 E. faecalis і 2 E. hirae, декарбоксильовані до амінокислот лізину та орнітину. Було помічено, що Enterococcus spp., ізольовані із сирого молока і традиційних молочних продуктів, відрізняються технологічними характеристиками (за джерелом, видом і штамом).

Висновок. Сире молоко і молочні продукти є важливим джерелом для виділення видів ентерококів. Такі характеристики видів Enterococcus, як підкислення, здатність
Склад, фізико-хімічні властивості та органолептичні показники салатних соусів із кукурудзяних і чуф'яних крохмальних сумішей

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Вступ. Визначено склад, фізико-хімічних властивостей і органолептичних показників салатного соусу з кукурудзяних і чуф'яних крохмальних сумішей.

Матеріали і методи. Жовтий сорт чуфи та кукурудзи сортували від бруду, промивали, замочували протягом 24 год і розтирали в суспензію за допомогою лабораторного молоткового фрезерного верстат. Отриману суспензію фільтрували через муслинову тканину й осаджували протягом 3 годин. Після цього осад декантували, сушили в шафі-сушарці та подрібнювали для отримання крохмалю.

Соус для салату виготовляли із сумішей кукурудзяного і чуф'яного крохмалю з іншими інгредієнтами.

Результати і обговорення. Вміст вологи, загальної золи, сирого жиру, сирого протеїну і вуглеводів салатного крему з крохмальних сумішей кукурудзи та чуфи коливалося від 51,50 до 59,58%, 0,33 до 0,58%, 23,05 до 24,78%, 0,65 до 1,16% та 14,31 до 24,41% відповідно. Суміш кукурудзи та чуфи не має значного (р>0,05) впливу на масові частки вологи, золи, жиру, білка та вуглеводів салатного соусу з кукурудзяного і чуф'яного крохмалю.

Салатний соус з кукурудзяно-чуф'яного крохмалю на 90:10% мав найвищий вміст золи та сирого білка. Однак, загальна зольність спостерігалась із додаванням кукурудзяного крохмалю. Жирність салатних соусів з кукурудзяно-чуф'яного крохмалю була подібною. Ефект взаємодії кукурудзи та чуф'яного крохмалю мав значний (р<0,05) вплив на вміст крохмалю та загальну титровану кислотність. Вміст цукру та крохмалю коливався від 2,67 до 9,50% та від 4,14 до 11,45% відповідно, салатний соус із кукурудзяно-чуф'яного крохмалю. РН, загальна титрована кислотність і в'язкість становили 3,57–3,77, 0,32–0,69% та 472–1683Па·с відповідно.

Висновок. Соус із кукурудзяного і чуф'яного крохмалю має бажані фізико-хімічні властивості і схожі сенсорні характеристики порівняно з комерційними салатними соусами. Отже, прийнятний салатний соус можна виробляти із 10-відсотковою заміною чуф'яного крохмалю кукурудзяним.

Ключові слова: кукурудза, чуфа, крохмаль, соус.
Поліфенольний склад і технологічні характеристики молочної сироватки забарвленої різного походження

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Вступ. Метою дослідження є визначення поліфенольного складу і технологічних характеристик молочної сироватки забарвленої різного походження, отриманої при осадженні білків молока традиційною ягідною сировиною та дикоросами.

Матеріали і методи. Сироватку забарвлену отримано в результаті термокислотного осадження білків молока коагулянтами рослинного походження. Ідентифікацію та кількісне визначення поліфенольних сполук у зразках молочної сироватки забарвленої здійснювали методом високоекстрактивного рідинного хроматографії. Вміст сухих речовин у сироватці забарвленій досліджували рефрактометричним методом, а оптичну густину кольоровості та каламутності – колориметричним методом.

Результати і обговорення. Зразки сироватки мали відтінки фіолетового та зеленого кольору, що обумовлено наявністю специфічних барвних речовин, зокрема різних поліфенолів, які є складовою ягід чорної смородини та хлорофілу соку подорожника. Вміст вищезазначених сполук у молочній сироватці після осадження білків молока соком Plantago major L. та кавітаційно обробленою пастою чорносмородиновою становить, відповідно, 324,43 і 265,49 мг/л. Зі збільшенням кількості коагулянту для термокислотного осадження білків молока від 5 до 11% значення оптичної густини для каламутності сироватки забарвленої зменшуються на 0,40 та 0,41 ум. од., а для кольоровості, навпаки, збільшуються на 0,17–0,19 ум. од., відповідно, для сироватки забарвленої після осадження білків молока соком Plantago major L. та пастою чорносмородиновою. Масова частка сухих речовин сироватки забарвленої зафіксована на рівні 6,80–8,55%. Вміст білка складав 0,96–1,33% відповідно, від контроельних зразків, сироватка забарвленна, отримана після осадження білків молока рослинним коагулянтом у кількості 8 та 11%, мала менший вміст білка на 0,32–0,45%, що підтверджує комплексне осадження казеїну та сироваткових білків молока органічними кислотами кавітаційно обробленої пасти чорносмородинової та активним комплексом (протеазами та органічними кислотами) соку подорожника.

Висновки. Зразки сироватки забарвленої мають покращені смакові й колірні характеристики та підвищену харчову цінність, що уможливлює широке використання її у складі сироваткових напоїв без додаткового оброблення.

Ключові слова: сироватка забарвлена, коагулювання, кольоровість, поліфенол.

Фізичні, фізіологічні і зміни та вміст мінеральних речовин різних типів бобових у процесі проростання

Деніса Атудорей, Сільвіу-Габріел Строе, Георгіна Кодіна
УНКИ, Сучава, Румунія

Вступ. Досліджено фізичні та фізіологічні зміни різних типів бобових культур у процесі проростання з метою визначення оптимального вмісту мінеральних речовин.

—— Abstracts ——

Фізичні, фізіологічні і зміни та вміст мінеральних речовин різних типів бобових у процесі проростання

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Вступ. Досліджено фізичні та фізіологічні зміни різних типів бобових культур у процесі проростання з метою визначення оптимального вмісту мінеральних речовин.
Матеріали і методи. Такі бобові культури, як нут, квасоля, сочевиця, люпин і соя, пророщували в камері для росту рослин Binder KBW/KBWF 240. Для вивітрування фізичних і фізіологічних змін бобових культур у процесі проростання використаний стереомікроскоп Motic SMZ-140. Для підкреслення варіації кількості мінеральних речовин під час процесу проростання використано Shimadzu EDX-900HS.

Результати і обговорення. Вміст білка в аналізованих бобових варіюється від 19,40 до 40,34%, найбільша кількість — у сої, а найменша — у зразку нуту. Усі зразки показали хорошу життєздатність для проростання, найвища — у сочевиці (90%), для якої також було зафіксовано найвищу енергетичну цінність проростання. Отримане зображення чітко продемонструвало розвиток складових частин зародку: корінця і плюмули, які збільшуються в процесі проростання, коли насіння почало синтезувати хлорофіл, а корінь почав розвиватися. Відповідно до їхнього розвитку максимальний час проростання становив 10 днів для сочевиці та люпину і 9 днів для нуту, квасолі та сої. Однак для їх вживання в їжу було встановлено оптимальний період проростання протягом 4 днів для всіх аналізованих зразків бобових, у яких, крім зразку квасолі, корінць був набагато більшим за розмір зерна бобових. Загалом, вміст кальцію та сульфату був найвишим у всіх зразках за четвертий день проростання. Вміст кальцію для нуту зростає майже в шість разів, а для сочевиці — втричі. У непророщеному насінні вміст фосфору і заліза збільшується для насіння сочевиці протягом чотирьох днів проростання, тоді як для решти бобових зменшується. Що стосується калію, магнію та цинку, то загалом їхні значення зменшуються із збільшенням часу проростання.

Висновок. Дослідження фізичних і фізіологічних змін бобових під час проростання дає змогу визначити, коли слід зупинити процес проростання. Кількість багатьох поживних речовин збільшується в результаті процесу проростання, тож цей процес може мати різне застосування в харчовій промисловості.

Ключові слова: бобові, проростання, насіння, мінерали.

Відмінності у складі летких сполук у свіжій і висушених змішаним теплопідведенням білокачанній капусті

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Вступ. Метою дослідження є вивчення хроматографічних профілів летких сполук у свіжій і висушений змішаним теплопідведенням білокачанній капусти і зокрема білокачанний капусті сорту Амагер, із визначенням відмінностей в якісному та кількісному складі. Як контроль обрано капусту білокачанну свіжу.

Матеріали і методи. Методом хроматомас-спектрометрії (GC/TOF-MS) досліджено профілі летких речовин у свіжих і висушених змішаним теплопідведенням овочах, зокрема білокачанній капусті сорту Амагер, із визначенням відмінностей в якісному та кількісному складі. Як контроль обрано капусту білокачанну свіжу.

Результати і обговорення. В ході хроматографічних досліджень летких ароматичних сполук свіжої і висушеної білокачанній капусти ідентифіковано 20 летких речовин. В обох зразках містяться такі компоненти: 4H-піран-4-он, 2,3-дигідро-3,5-дигідрокси-6-метил-(2,61 та 2,11%), гуанозин (1,07 та 0,48%), оксиран, тетрадецил (1,71 та 0,90%, тетрадеканал (2,89 та 2,72%)), 2-пентадеканон (3,2 та 3,00%), 2-надеканон (3,01 та 2,84%), формамід, N-метил-N-[1-(пірролідоніл)]-2-бутиніл] (3,12 та 2,92%), н-гексадеканова кислота (5,8 та 5,76%), цис-ваценова кислота (4,43 та 3,88%), лаурієвий, пальмієвий, жирні кислоти.
4,44%), оліцьова кислота (3,94 та 4,02%), амід оліцьової кислоти (3,12 та 3,2%), 1,2-бензодікарбонова кислота діізоктиловий ефір (5,28 та 5,22%), 6-метил- октадекан (1,96 та 1,16%), 2,6,10-триметилпентадекан (2,69 та 1,84%), гептакозан (34,16 та 32,15%), 2-гексадеканол (2,25 та 1,76%), 1,2-епоксигексадекан (11,11 та 10,65%), нонакозан-15 (3,83 та 3,45%).

Висушування сирої капусти сушінням зі змішаним теплопідведенням не спричиняло зміни за якісним складом, проте зумовлювало зменшення кількості летких ароматичних речовин.

Зменшення вмісту,% компонентів: heptacosane, (34,15 → 32,16), 1,2-epoxyhexadecane (11,11 → 10,659), octadecenamide (3,12 → 3,02), n-Hexadecanoic acid (5,80 → 5,76) певною мірою нівелює гіркувату ноту сирої капусти, жирний присмак, пом'якшує її ароматичні відчуття.

**Висновки.** Порівняння летких речовин у свіжих і висушених зразках капусти підтверджує збереження цінних біологічних речовин сіркої капусти після сушіння зі змішаним теплопідведенням. Цей спосіб оброблення капусти максимально зберігає її корисні властивості.

**Ключові слова:** капуста, сушіння, хроматографія, леткі сполуки.

**Природні альтернативи діоксиду сірки, що використовуються у вині, та їх вплив на ароматичні сполуки**

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**Вступ.** Метою цього дослідження є визначити вплив різних природних рослинних екстрактів, що використовуються як альтернатива діоксиду сірки, на ароматичні сполуки вина.

**Матеріали та методи.** Виробництво вина проводилось за загальноприйнятою методикою для червоних вин (Каберне Совіньйон). Експериментальні зразки виготовлені із використанням різних рослинних екстрактів (виноградних вичавок, розмарину, чорниці чорниці) за різних концентрацій. У першій контрольній групі використовувались зразки вин без оброблення діоксидом сірки та рослинними екстрактами, у другій групі – вина, вироблені з додаванням діоксиду сірки.

**Результати та обговорення.** Найвища загальна кількість летких сполук була досягнута застосуванням екстракту чорниці та екстракту розмарину виноградних вичавок. Комбіноване застосування діоксиду сірки та екстракту чорниці збільшило кількість вина. Найкращі результати, пов’язані з синтезом вищих спиртів та їх накопиченням у винах, були отримані з використанням діоксиду сірки (25 мг/л) і рослинних екстрактів (0,3 мл). Найвище значення отримано у зразку, обробленому виноградними вичавками.

Ефірна фракція була представлена 9 ідентифікованими сполуками. Найвищий загальний вміст ефіру (169,13 мг/л) був виявлений у зразку, отриманому при комбінованій обробці 25 мг/л діоксиду сірки та екстрактам розмарину 0,3 мл. Інші три варіанти оброблення розмарином продемонстрували кількісно близький вміст ефіру. У зразках, що містять екстракт виноградних вичавок, виявлено найвищий загальний вміст ефіру порівняно з усіма іншими. З цієї групи було виділено лише пробу, що
включає 25 мг/л діоксиду сірки та екстракт виноградних вичавок 0,3 мл (40,62 мг/л). Метиловий спирт був знайдений у всіх досліджених винах. Рівень метилового спирту був дуже низьким і не створював ризику для споживача.

Висновок. Дослідження продемонструвало можливості оптимізації діоксиду сірки з використанням натуральних рослинних екстрактів.

Ключові слова: вино, екстракт, діоксид сірки, червоне вино, аромат.
Процеси і обладнання

Фазові переходи в технологіях харчових виробництв
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Вступ. Стаття стосується загального стану технологій утилізації вторинних енергетичних ресурсів і ресурсів зовнішнього середовища.
Матеріали і методи. Досліджуються енерговитратні процеси з точки зору використання їхніх потенціалів як ізоентальпійних у солодосушарках, бродильних апаратах, теплових насосах і вакуумних сушарках в їх класичному виконанні. Методи дослідження ґрунтуються на положеннях технічної термодинаміки. Поєднання названих об’єктів стосується можливостей створення на їх основі замкнutyх енергоматеріальних контуrів за рахунок доповнення їх компенсаційними процесами.
Результати і обговорення. Аналіз ізоентальпійних процесів сушіння привів до висновку про доцільність утримання загального потенціалу парогазової суміші в замкнутому контурі, але з особливістю, що осушувальний потенціал середовища буде поновлювальним. Оскільки вилучення парової фракції можливе лише через її конденсацію, яка здійснюється використанням теплового насоса, то в ньому ж енергетичний потенціал повертається газовому потоку за проходження його через конденсатор. В такій системі реалізуються завдання суціння зарозовної маси, транспортування парогазової суміші, осушування газової фракції та повернення її енергетичного потенціалу. Контур теплового насоса в цій системі виконує регуляторну роль щодо контuru парогазової суміші, а компенсаційний процес покладено на компресор теплового насоса.

Наведено інформацію щодо влаштування бродильного апарату з рекуперативною системою вилучення спирту. Технологічне завдання стосується стабілізації осмотичних тисків у культуральному середовищі шляхом суміщення процесів бродіння і перегонки.
Розширення діапазонів застосування теплових насосів стосується пропозиції їх двоступеневої структури, а варіант схеми вакуумної сушарки безперервної дії пропонується з рекуперативною системою.
Термодинамічні властивості фазових переходів відповідають ізобарично-ізотермічним процесам, що з точки зору інтересів енергетичної рекуперації і регенерації надає, як мінімум, дві переваги. По-перше, в результаті фазового переходу енталпія парової фракції приблизно у 5 разів перевищує енталпію рідинної. По-друге, температуру парової або газової фази можливо змінювати з рахунок механічної або термокомпресії, зокрема для зміни температур фазових переходів. Наслідком таких трансформацій є можливість мінімізувати витрати первинних енергоресурсів на процеси випарювання середовищ.

Висновки. Енергоемні процеси сушіння, аерalu зернових мас при пророщуванні, культуральних середовищ при синтезі мікроорганізмів або їхніх похідних оцінюються як ізоентальпійні, що означає доцільність створення замкнутих енергетичних контурів, оскільки апаратура оформлення технологічних апаратів часто має складові на рівні теплових насосів.

Ключові слова: перехід, трансформація, контур, теплообмін, конденсація.
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Вступ. Переважна кількість співвідношень для турбулентної в'язкості в стікаючих плівках має дискретну пошарову структуру, а розв'язки рівнянь збереження теплоти та імпульсу з використанням цих співвідношень лише чисельні. Пропонується нова модель, на основі якої здійснено аналіз теплогідродинамічних процесів в плівках рідин під час пароутворення.

Матеріали і методи Фізичне моделювання виконано в трубах: \( d = 22 \times 1 \text{ mm}, \ L = 1,8 \text{ m}, \) та \( d = 33 \times 1,5 \text{ mm}, \ L = 9 \text{ m}. \) Об'ємна жілість зрошування змінювалась в діапазоні \( 0,05–0,55 \times 10^{-3} \text{ m}^2/\text{s} \) в трубі \( d = 20 \text{ mm}, \) та \( 0,05–1,9 \times 10^{-3} \text{ m}^2/\text{s} \) – в трубі \( d = 30 \text{ mm}. \) Модельні рідини – вода і цукрові розчини концентрацією до 70% під атмосферним тиском і розрідженням до 0,86 бар. Нагрівання здійснювалось сухою насищеною парою.

Результати і обговорення. Запропонована модель турбулентної в'язкості в плівці у формі скосеної вбік міжфазної поверхні параболи. За рівнянь теплоперенесення та збереження їмпульсу отримано аналітичні вирази для температурного і імпульсного профілів в плівці, та відповідні інтегральні теплогідродинамічні характеристики для режиму тепловіддачі, що характеризується пароутворенням із міжфазної поверхні.

Аналітичні вирази для коефіцієнта тепловіддачі та товщини плівки виражені через обернені гіперболічні функції та просто корелюються з відповідними експериментальними даними з теплообміну в стікаючих плівках води та густих цукрових розчинів в області нерозвинутої та розвинутої турбулентності під час пароутворення. Експериментальні дані з теплообміну за наявності міжфазної дотичної напруги на поверхні плівки корелюються з теоретичними результатами запропонованої моделі лише за умови введення додаткової функції, що враховує пригнічення турбулентності в плівці внаслідок її потонення, виражену через числа Вебера для парової фази. Згідно експериментальних даних, вплив бульбашкового кипіння на інтенсивність тепловіддачі під час руху паро-рідинного ядра спостерігається лише за межами граничного температурного напору, вираженого через співвідношення Клапейрона-Клаузіуса, і враховано введенням параметра початку кипіння як співмножника до аналітичного виразу з інтенсивності тепловіддачі, отриманого на основі запропонованої моделі турбулентної в'язкості.

Висновки На основі запропонованої моделі турбулентної в'язкості виконано аналіз теплогідродинамічних процесів слаботурбулентних плівкових течій, отримано відповідні аналітичні вирази для розрахунку тепловіддачі до плівок розчинів під час пароутворення в трубах, включаючи область бульбашкового кипіння.

Ключові слова: тепловіддача, плівка, турбулентна в'язкість, швидкість, випаровування.
Економіка

Методичні підходи до визначення готовності впровадження стратегії «блакитного океану» на підприємствах харчової промисловості

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Вступ. Дослідження полягає у розробленні методичних підходів до визначення готовності впровадження стратегії блакитного океану на підприємстві.

Матеріали і методи. Застосовувалися методи систематизації для з'ясування чинників, які здійснюють вплив на стан підприємства на шляху до впровадження стратегії «блакитного океану». За даним розподілу значень використовувається мето дій по розрізнянню успіхів та виклику. Оцінювання чинників успіхів-викликів містить здійснено експертними методами. Стан галузі/підприємства визначався матричним методом.

Результати і обговорення. Пропонується методика, яка дає змогу визначити готовність реалізації стратегії «блакитного океану». Окреслюються загальні чинники, які обумовлюють необхідність реалізації такої стратегії. Слідством обираються такі, які є найбільш суттєвими для галузі/підприємства. Вони можуть бути як позитивними (успіхи), так і негативними (виклики). Здійснюється їх вимірювання шляхом знаходження добутку середньої оцінки (визначається експертним методом) на ваговий коефіцієнт (ставляється на основі досвіду дослідників залежно від ступеня значущості чинників та їхнього впливу на розвиток галузі). З метою інтерпретації отриманих результатів оцінювання стану галузі/підприємства пропонується використання запропонованої матриці «успіхи-виклики», яка має 4 квадранти.

Висновки. Застосування розробленої авторами методики дасть змогу визначити готовність до реалізації стратегії «блакитного океану».

Ключові слова: успіх, виклик, матриця, стратегія, конкуренція.
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The Editorial Board of scientific periodical “Ukrainian Food Journal” invites you for publication of your research results.

Requirements to all texts:
Language – English.
Recommended size of the article – 15–20 pages.
Font – Times New Roman, font size – 14, line intervals – 1, margins on both sides – 2 cm.

The structure of the article:
1. The title of the article
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3. Institution, where the work has been performed.
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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.
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Вимоги до оформлення статей

Мова статей – англійська.
Мінімальний обсяг статті – 10 сторінок формату A4 (без врахування анотацій і списку літератури).
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.
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6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

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Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути співрозмірним (!) тексту статті. Фотографії можна використовувати лише за їх значної наукової цінності.

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 СI.
У списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.
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В Ukrainian Food Journal взято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються лише комами.

1. Посилання на статтю:
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Приклади:
1. (2013), Svitovi naukovometrychni bazy, Available at: http://www1.nas.gov.ua/publications/q_a/Pages/scopus.aspx

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт, в російської – стандарт МВД (в цих стандартах використовуються символи лише англійського алфавіту, без хвостиків, апострофів та ін).

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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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**Processes and Equipment**

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