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Modelling the browning of bakery products during baking: a review

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Abstract

Introduction. It was reviewed the results of scientific studies on the presence of non-enzymatic browning compounds in bakery products, the mechanism and factors influencing their formation, as well as the prediction and control of the development of browning in baked goods using mathematical modelling.

Materials and methods. Analytical studies on the mechanism of browning on the surface of bakery products and the prediction and control of the development of browning in bakery products using mathematical modelling based on already available research articles.

Results and discussion. The formation of colour in bakery products during the baking phase is commonly known as browning. The brown colour on the surface of bakery products comes from melanoidins (an insoluble brown pigment) and caramel, which are products of non-enzymatic browning reactions (Maillard reactions and caramelization). These reactions can also form undesirable products with potentially mutagenic effects (acrylamide, hydroxymethylfurfural and furfural), resulting in a loss of nutritional value of the product. The change in the colour of the surface of the product is considered an essential parameter for determining the end of the baking process of bakery products. Efforts should be made to develop a fast, inexpensive, automated, reasonable and objective method to track colour change during baking. The development of a mathematical model of browning is essential to predict and control this phenomena during baking as a function of operating conditions and the product recipe. Kinetic models for the colour change of bakery products are divided into two groups. The first group consists of kinetic models of colour change where the independent variable is time. This group includes kinetic models of zero, first and second order reactions and the exponential empirical model. The second group consists of kinetic models of colour change where the independent variable is mass loss.

Conclusion. Since browning affects the overall quality of food and leads to changes in sensory and nutritional properties (reduction in bioavailability of proteins and amino acids, formation of acrylamide, hydroxymethylfurfural, and formation of substances with antioxidant activity), it is a topic of great interest to food technologists.

Introduction

Bakery products include foods whose main ingredient is flour and which undergo a baking process, such as bread, various pastries, cakes, crackers, pies, croissants, and many other products. The external appearance is the first characteristic of the quality of bakery products that the consumer perceives. Controlling the development of colour on the surface of bakery products directly affects their acceptance or rejection by consumers. The formation of colour on the surface of bakery products during baking is considered a desirable characteristic and is the result of non-enzymatic browning reactions (Hodge, 1953). By monitoring and controlling these reactions, it is possible to influence the desired colour of the final product.

The brown colour on the surface of bakery products comes from melanoidins (an insoluble brown pigment) and caramel, which are products of non-enzymatic browning reactions (Maillard reactions and caramelization). These reactions can also form undesirable products with potentially mutagenic effects (acrylamide (AA), hydroxymethylfurfural (HMF) and furfural), resulting in a loss of nutritional value of the product.

During baking of bakery products, a crust is formed and the change in the initial colour of the crust starts with the appearance of light yellow dextrins when a temperature of 110 to 120 °C is reached on the surface of the product (Wahlby and Skjoldebrand, 2002). A further increase in temperature leads to the formation of products of the Maillard reaction and caramelization (melanoidins and caramel) and then to the combustion of the products and the formation of a black porous mass. The speed of colour development on the surface of bakery products depends on the process conditions such as temperature and baking time. However, apart from the process conditions, colour development is also influenced by the amount of water, water activity, pH, amount of reducing sugars, etc.

Numerous researchers have developed various direct and indirect methods for measuring colour on the product surface. Direct methods aim at quantitative monitoring of the products of Maillard reactions and caramelization (AA, HMF and furfural) (Ramirez-Jimenez et al., 2000). While indirect methods are based on the principle of measuring the amount of light reflected from the surface of the analysed sample using devices such as colorimeter, chromameter (Gokmen et al, 2008a-b; Purlis and Salvadori, 2007), and a computer image analysis system (Brosnan and Sun, 2004, Du and Sun, 2004, 2005; Shahin and Symons, 2001).

All reactions proceed at a certain rate, which depends primarily on the temperature and concentration of the reacting substances. The rate of chemical reactions (chemical kinetics) is an area of interest for many scientists (Montgomery and Runger, 2003; Purlis and Salvadori, 2007, 2009a-c; Purlis 2010, 2011; van Boekel, 2008), whose research is related to the colour change of bakery products during baking (kinetic modelling). Furthermore, a good understanding of the kinetics of non-enzymatic browning reactions can inform how to improve the food product, preserve existing nutritional components during processing or minimise the occurrence of undesirable degradative changes. Therefore, the purpose of developing mathematical models is often to predict the behaviour of food ingredients during processing and storage and to optimise the process to obtain the highest quality product.

Considering that the external appearance is the most striking feature of the quality of bakery products, monitoring the kinetics of colour change during baking is important for optimising the quality of the final product. Good management of all processes in the production and distribution chain in the market leads to high quality food that is safe for the health of consumers. Among other things, it is necessary to prevent non-enzymatic browning reactions to reduce colour and flavour changes when these changes have a negative impact

on the quality of the final product. Changes caused by non-enzymatic browning may be desirable in some cases when a specific flavour is to be achieved during a thermal treatment such as baking, roasting or drying. Enzymatic browning reactions can contribute to the general acceptability of foods such as tea, coffee, cocoa and dried fruits. Despite much research on non-enzymatic browning, with a focus on Maillard reactions, the means to control these reactions during processing are not yet fully understood.

The change in the colour of the surface of the product is considered an essential parameter for determining the end of the baking process of bakery products. The development of a mathematical model of browning is essential to predict and control this phenomenon during baking as a function of operating conditions and the product recipe. Kinetic models for the colour change of bakery products are divided into two groups. The first group consists of kinetic models of colour change where the independent variable is time. This group includes kinetic models of zero, first and second order reactions and the exponential empirical model (Pedreschi et al., 2006; van Boekel, 2008). The second group consists of kinetic models of colour change where the independent variable is mass loss (Purlis and Salvadori, 2007).

The aim of this article is to present, based on the available literature, the results of scientific studies on the presence of non-enzymatic browning products in bakery products, the mechanism of their formation and the factors influencing their formation, as well as the prediction and control of the development of browning in bakery products using mathematical modelling.

Materials and methods

The review is based on already available research articles on the presence of non-enzymatic browning products in bakery products, the mechanism of their formation and the factors influencing their formation, as well as on the use of kinetic models of browning, which are essential for predicting and controlling this phenomenon during baking depending on the operating conditions and product formulation.

Literature referenced in this review article was obtained from bibliographic information in Google Scholar, Web of Science, Science Direct, Scopus, Springer Link, EBSCO host, Wiley online library, PubMed, DOAB (directory of open access books), Ovid SP database and CAB abstracts.

Results and discussion

Changes in bakery products during heat treatment

Products in which flour is the main ingredient and which undergo a baking process are called bakery products. Bakery products include bread, pastries, croissants, pies, cookies, cakes, and many other products, all of which differ in their composition and production methods. Baking is a heat treatment process in which heat is applied directly to the food and temperatures of up to 260 °C are reached. Due to the high temperatures used in baking and the low moisture content, the heat treatment of bakery products triggers a series of chemical reactions between food ingredients that affect the quality of the final product. The consequences of these chemical reactions are mainly improvements in the textural and organoleptic properties of the food. However, undesirable consequences may also occur, such

as the natural formation of potentially toxic products, which may also affect the final taste and appearance of the food. Baking can be defined as a process that transforms a base of flour and water or a dough into a food product with unique sensory characteristics. Therefore, the appearance and the colour of the surface of bakery products in general are very important quality parameters on which the consumer's decision to accept the product depends, as it is related to the taste and the degree of satisfaction (Pedreschi et al., 2006). As for the quality of bakery products, although the typical characteristics depend on the product itself, the surface colour, together with texture and taste, is the most important characteristic for consumer preference, so they can be used to evaluate the baking result (Abdullah, 2008). In addition, legal regulations may also set certain parameters for this aspect. For example, in Argentina, bread crust must have a uniform golden yellow colour (ANMAT, 2004). Therefore, understanding the evolution of colour on the product surface is a very important factor for the bakery industry.

Chemical processes that influence the colour development of bakery products

Baking is a complex process that involves a number of physical, chemical, and biological changes, such as water evaporation, creation of porous structures, volume expansion, denaturation of proteins, gelatinization of starch, crust formation, and others (Mondal and Datta, 2008). The consequence of the above changes is the development of certain characteristics of bakery products – colour, shape, size and texture, where the colour of the product surface has a significant impact on the evaluation of the quality of the food itself. During baking, a brown colour develops on the surface of bakery products, which is the result of non-enzymatic chemical reactions of the colorants present (Purlis, 2010).

Non-enzymatic browning reactions include several types of reactions: dehydration, degradation, fragmentation, condensation, and polymerization, whose chemistry and kinetics are complex. In many cases, non-enzymatic browning is a negative phenomenon that leads not only to a change in colour, but also to other changes such as the degradation of food components (amino acids, ascorbic acid), a decrease in protein digestibility and, in some cases, the formation of toxic compounds. Non-enzymatic browning involves a whole series of reactions that lead to the formation of brown pigments. However, non-enzymatic browning is not always a negative phenomenon, and work is often done to create the conditions for its occurrence. Non-enzymatic browning products are compounds with a certain colour and flavour, which in some cases are desirable and very important for consumer acceptance of some products (bakery products, roasted meat, roasted coffee, French fries, etc.).

When non-enzymatic browning reactions occur without the presence of nitrogen compounds, these reactions are called caramelization reactions, and when they occur in the presence of nitrogen compounds, they are called carbonyl-amine reactions or Maillard reactions. Maillard reactions and caramelization reactions are the main processes involved in the colouration of bakery products (Capuano et al., 2008).

The brown products of Maillard reactions, melanoidins, are formed when reducing sugars and amino acids, proteins, and/or other nitrogenous compounds are heated at specific temperatures. The process of caramelization involves complex groups of reactions that result from the direct heating of carbohydrates, particularly sucrose and reducing sugars (Bemiller et al., 1996). Maillard reactions occur under conditions corresponding to an average moisture content, a temperature above 50 °C, and a pH between 4 and 7 (Kroh, 1994).

Caramelization and Maillard reaction

Caramelization, which depends on direct degradation of sugar, requires stronger conditions, such as temperatures above 120 °C, pH between 3 and 9, and low water activity (Kroh, 1994). During baking, starch and sucrose can be hydrolysed to reduce sugars, which can then participate in both reactions, usually allowing the Maillard reaction and the caramelization reaction to occur simultaneously (Villota and Hawkes, 2007). In caramelization reactions in many cases, although not necessarily, sugars are the main reactants. These reactions involve the conversion and degradation of sugars without the presence of amino compounds. The process of caramelization includes the following reactions: enolization, isomerization, dehydration, fragmentation and polymerization, forming light yellow to black pigments. During caramelization of sucrose at 200 °C, three endothermic processes were observed:

- After melting of sucrose, foaming of the mass begins and there is a loss of one molecule of water per molecule of sucrose, resulting in the formation of isosucrose.
- Further heating with loss of mass produces caramel ($C_{24}H_{36}O_{18}$). The isolated caramel dissolves in water and ethyl alcohol and has a bitter taste.
- The third stage occurs after foaming, with prolonged heating, and the caramel pigment is formed.

Further heating of sucrose leads to the formation of humin, i.e. caramelin, a dark substance with high molecular weight. The mechanism of caramel pigment formation involves a polymerization reaction that produces coloured polymers with high molecular mass, in which the number of C atoms increases in proportion to the degree of dehydration, i.e. the temperature and duration of their exposure. In systems subject to caramelization reactions, this leads to further parallel reactions with a very complex mechanism and to the formation of red, brown and dark brown pigments of different composition and properties, which differ significantly from Aldo-caramel in structure, composition and properties (Nursten, 2005).

Maillard reactions are named after the French chemist Louis-Camille Maillard, who was the first to describe the changes in flavour and colour when reducing sugars are heated with amino acids (Nursten, 2005). These reactions are of great importance in food technology and chemistry, as well as in medicine and nutrition (Tomasik, 2004). Maillard reactions are one of the main reactions that cause protein degradation during food processing and storage. They can also cause other undesirable nutritional changes, such as loss of essential amino acids (lysine, arginine, cysteine, and methionine) or reduction of protein digestibility and amino acid availability or changes in flavour. The whole process of forming Maillard reaction products can be divided into three main stages depending on the colour formation (Figure 1). In the first stage, sugars and amino acids condense, and after condensation, the Amadori rearrangement and 1-amino-1deoxy-2-ketose are formed. In the second stage, the product may be slightly yellow or colourless, and dehydration and fragmentation of the sugar molecules occur. Amino acids are also broken down at this stage. In this intermediate stage, HMF cleavage products such as pyruvaldehyde and diacetyl are formed. In the last stage, aldol condensation takes place and finally the heterocyclic nitrogenous compounds, the melanoidins, which are strongly coloured, are formed (Nursten, 2005).

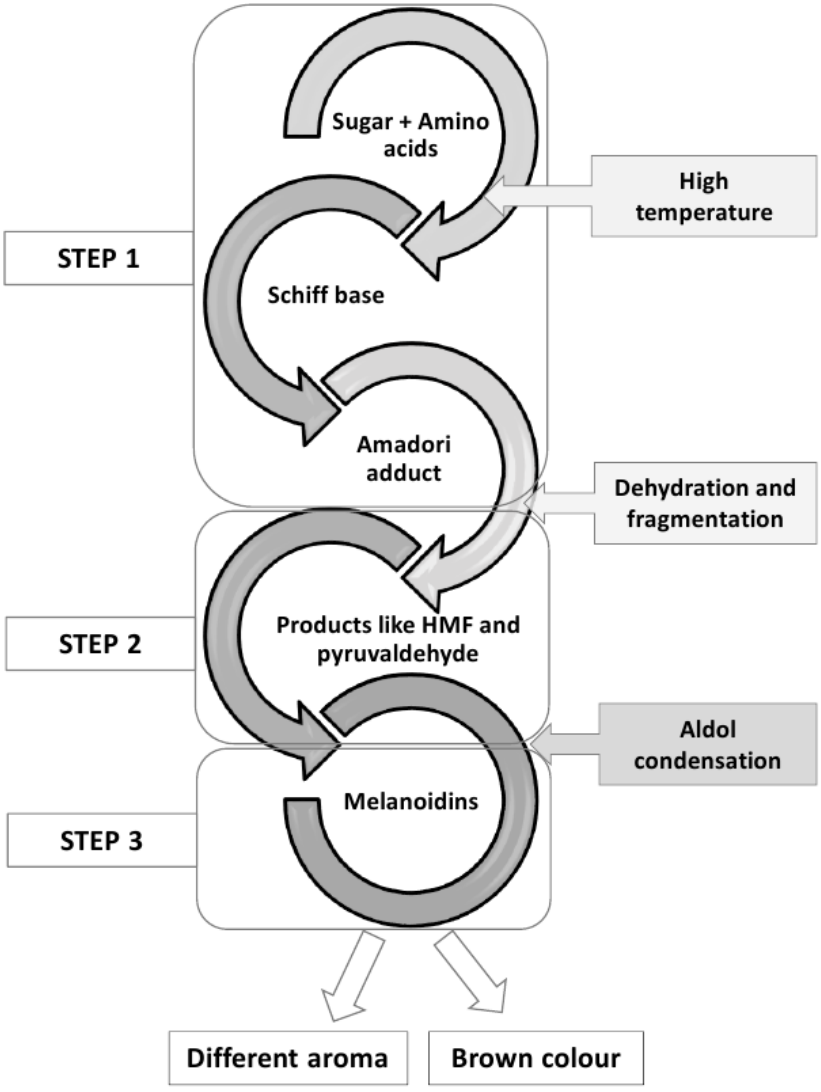

Figure 1. The process of Maillard reaction products formation

The Maillard reaction is the main reaction for colour formation. The formation of melanoidins by the Maillard reaction follows a zero order kinetic (Bates et al. 1998; Martins and van Boekel, 2003; Morales and van Boekel, 1998). The formation of melanoidins in biscuits is faster than in bread. The low water content and small size cause the water to evaporate quickly and the product to dry out faster. The nature of these reactions and the nature of the resulting products are influenced by the properties of the medium itself, more specifically the food (e.g. water activity, pH, chemical composition of the food, temperature). The type of reducing sugar is also very important, for example, pentoses react much faster than hexoses, monosaccharides faster than disaccharides (Tomasik, 2004; Hui et al., 2006).

Because of the chemical characteristics (i.e., reactants and products) of the Maillard and caramelization reactions, the importance of colour development during roasting is not only related to sensory characteristics such as the formation of a desirable hue and flavour, but also to changes in nutrient composition. In this sense, Maillard reactions affect the content and bioavailability of amino acids and proteins (Morales et al., 2007), which is associated with the formation of harmful compounds such as AA and HMF (Stadler et al., 2016). The formation of AA begins with the condensation of reducing sugars and the amino acid asparagine in the first stage of Maillard reactions (De Vleeschouwer et al., 2009). The formation of AA correlates strongly with the temperature and duration of the baking process, the amount of asparagine and reducing sugars, and starts at a temperature of 120 – 130 °C (Ahrné et al., 2007). The formation of AA is also related to the development of surface colour of bakery products (Gökmen et al., 2008a, b; Mesias and Morales, 2016).

Factors influencing the non-enzymatic browning reaction of bakery products

Important parameters in non-enzymatic browning reactions are temperature, pH of the environment, water activity, type and concentration of reactants, reaction time and water content. Depending on these factors, the reaction proceeds with different qualitative changes and at different rates.

The **temperature** dependence of the reaction is often expressed by the activation energy. Activation energy data for Maillard reactions range from 10–160 KJ/mol, depending on which effect of which reaction was measured. The activation energy is highly dependent on pH and reactant structure, making it difficult to isolate the effect of temperature as an independent variable. For all model systems, the rate of browning, as measured by colour development, increases two- to threefold for every 10 °C increase in temperature. As temperature increases, compounds are formed that may participate in or inhibit browning reactions. Sucrose is inert at relatively low temperatures, but when reaction conditions are suitable for its hydrolysis to glucose and fructose, the newly formed compounds are readily involved in caramelization reactions or in the carbonyl-amine reaction. Amino acids catalyse the reaction of sucrose at neutral pH, while formaldehyde formed by the Strecker degradation of glycine can effectively block the involvement of unreacted glycine or other amino acids in the non-enzymatic browning reaction.

Changing the **pH** of a model system results in qualitatively different browning reactions. The browning reactions show a decrease in reaction rate at low pH values, i.e., pH values with optimal stability of the reducing sugars present. The browning reactions themselves affect pH, making it difficult to assess the effect of pH on the overall system. Tests have shown that browning in aqueous solutions is a consequence of caramelization, while in the almost dry state of the reactants or at alkaline pH values, the Maillard reactions predominate.

Water and concentration of reactants (sugar and protein) – water catalyses the enolization of reducing sugars and the enol forms readily undergo fragmentation and dehydration reactions. At the beginning of the carbonyl-amine reaction, an aldose or ketose sugar reacts with a primary or secondary amine or amino acid to form a glycosylamine, and the reaction is reversible. The influence of water content is important for glycosylamine yield. At low water content, there is a significant accumulation of these compounds, which is why non-enzymatic browning of carbonylamine is pronounced in dehydrated and concentrated foods.

Mathematical model for browning of bakery products during baking

Determining the colour of bakery products

The first step in predicting and controlling the development of browning is its quantification. Numerous researchers have developed various methods to determine the colour on the surface of bakery products. Generally, methods can be classified in:

- Direct (chemical, objective) methods,
- Indirect (sensory, subjective) methods.

Direct methods aim at the quantitative monitoring of the products of Maillard reactions and caramelization (AA, HMF and furfural) (Ramirez-Jimenez, 2000), while indirect methods are based on the principle of measuring the amount of light reflected from the surface of the analysed sample with different measuring devices. Many different devices for indirect colour determination are available on the market. Most of them are designed in such a way that the colour determination is done by direct contact between the instrument and the sample. Instruments for indirect colour determination that are frequently used in practise are: Colourimeter, Chromameter, Spectrophotometer, Densiometer (Gokmen et al., 2008a; Purlis and Salvadori, 2007) and more recently a computer vision system (Brosnan and Sun, 2004; Zeng et al., 2007; Lukinac et al., 2018).

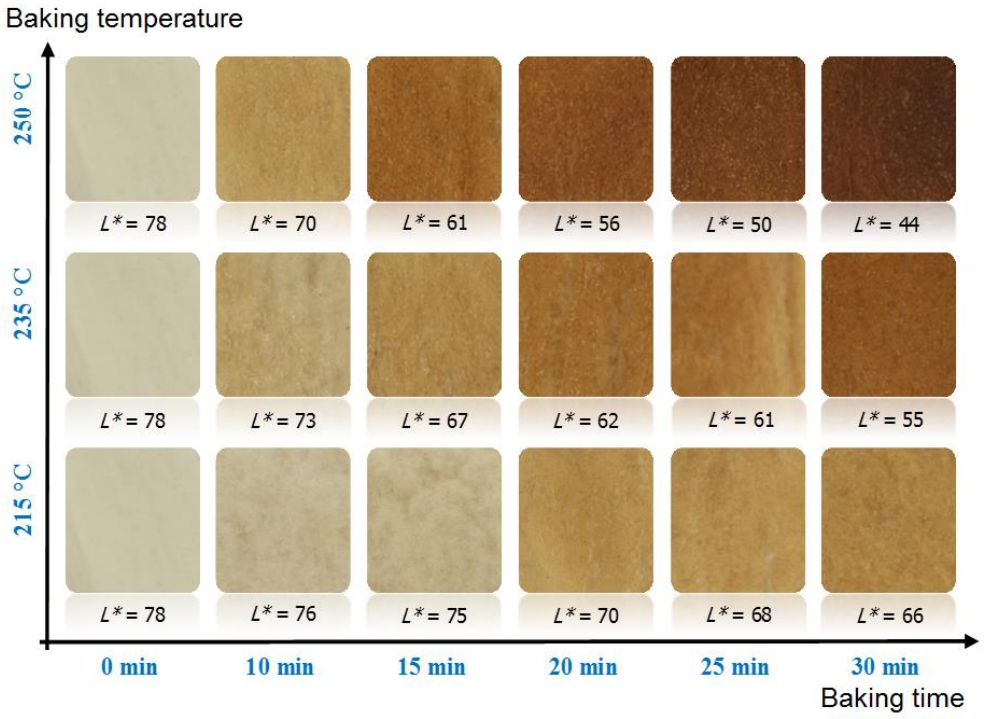

Figure 2. Browning development at surface of bakery products during baking at different baking temperatures

The computer vision system can cover the entire surface of the sample, making it a more objective and precise method, in contrast to the colorimeter, which analyses the surface of only a few centimetres (about 2 cm^2) (Mendoza et al., 2007a, b). This method of colour measurement can be used as a tool for automatic process control in industry (for a visual overview of the production process), improving the overall quality of the product. The advantage of the computer vision system over colour assessment with the human eye is the objectivity and continuity in colour assessment (Zheng et al., 2006).

In food research, colour is often represented using the CIE *L*a*b** colour space, which is an international standard for colour measurement (Mendoza et al., 2007a, 2007b). The three parameters of this model represent the lightness of the colour (*L**), which is between 0 and 100 (0 = black, 100 = white), its position between red and green (*a**, values between -120 and +120) and its position between yellow and blue (*b**, values between -120 and +120) (Yam and Papadakis, 2004).

Some typical values of lightness (*L**) of the bread crust at different baking conditions are shown in Figure 2, where the influence of the oven temperature on the colour development can be clearly seen. The intensity of the colour of the samples increases with baking time, which is to be expected and is also confirmed by the lower values of *L**. In addition, increased temperatures and a low water content influence the formation of the yellow-brown colour of the bread crust. Browning occurs only after baking for 10 minutes at 250 °C, 15 minutes at 235 °C and 20 minutes at 215 °C oven temperature.

Modelling the crust browning of bakery products based on the measurement of lightness and total colour change

According to Haefner (2005), modelling has three goals: understanding, predicting and controlling the process, while other authors also mention optimisation (Montgomery and Runger, 2003). In terms of understanding, modelling is a tool in science that uses mathematical models to describe the physical and chemical changes that take place in food. The difference between prediction and control is that prediction is a quantitative prediction of the future properties of a food based on knowledge of the food and the processing it will undergo. Control, on the other hand, is about checking the process conditions during production with the aim of achieving the desired quality. It can be concluded that mathematical models can be used to predict and control the qualitative properties of food and other possible changes in these properties. Mathematical models are mostly linear, polynomial, and exponential or power expressions (van Boekel, 1996, 2008; Dolan, 2003).

Although a group of complex chemical reactions causes colour formation, it can be simplified for technological purposes by assuming a general mechanism of browning and then using colour models based on reflectance methods. It has been found in the literature that the development of browning during baking can be well described by a first-order kinetic model whose parameters depend on the local temperature and the water activity of the product. Moreover, the kinetic parameters should be estimated from experiments that are close to the actual baking conditions, i.e. a non-isothermal process occurring in a non-ideal system, to obtain better predictive performance (Dolan, 2003). The kinetic models used to describe the colour change of bakery products can be divided into two groups.

- The first group consists of kinetic models of colour change in which time is the independent variable (Eq. (2-14)), kinetic models of zero-, first- and second-order reactions and the empirical exponential model (Pedreschi, 2007; van Boekel, 2008).
- The second group consists of kinetic models of colour change of bakery products where the independent variable is mass loss (Eq. (15–18)) (Purlis and Salvadori, 2007).

Hermann and Nour (1977) studied the kinetics of surface browning in dough made of flour and water during baking at 150, 170 and 190 °C. They found that the surface browning of dough made of flour and water is due to Maillard reactions, which are described as sequential reactions Eq. (1):

$$A + K \ldots \overset{k_1}{\rightarrow} Z \overset{k_2}{\rightarrow} P \overset{k_3}{\rightarrow} M \quad (1)$$

The reaction between amino acids (A) and reducing sugars (K) leads to intermediates (Z). These lead to water-soluble coloured products (P), which turn into insoluble coloured compounds, namely melanoidins (M). Experimental tests have shown that the kinetics of browning are characterised by three phases: a lag phase (found at 150°C), followed by an exponential phase (found at the three temperatures) and an asymptotic phase (found at 190°C). The browning reaction after the lag phase can be described by first order kinetics for the formation of compounds P (i.e. exponential phase) when $k_2 > k_3$, and then by the combination of kinetics for the formation of compounds P and M when $k_2 \sim -k_3$ (i.e. asymptotic phase).

Based on the general rate law, the disappearance of a compound in a closed system with only one compound reacting can be written in Eq. (2). By including the surface lightness (L^*) in Eq. (2) and choosing the surface lightness as the browning index, a general model for the colour development of the surface of bakery products during baking can be given as follows (Eq. (3)):

$$r = \frac{dc_A}{dt} = k \cdot c^n \quad (2)$$

$$\frac{dL^*}{dt} = k \cdot (L^*)^n \quad (3)$$

where r = reaction rate (mol/dm^3·s);
c_A = concentration of reactants A (mol/dm^3);
t = baking time (s);
k = reaction rate constant (s^{-1});
c = concentration (mol/dm^3);
n = order of reaction ($0 \leq n \leq 2$)
L^* = CIE colour component of surface lightness.

The temperature dependence of the reaction rate constant for browning is generally explained by the Arrhenius equation (Eq. (4)), and isothermal and non-isothermal methods have been used to determine the kinetic parameters.

$$k = k_0 \cdot exp\left(-\frac{E_a}{RT}\right) \quad (4)$$

where k_0 = Arrhenius constant (s^{-1});
E_a = activation energy (J/mol);
R = universal gas constant (8.314 J/mol/K);
T = (absolute) temperature (K).

The kinetics of the browning reaction in food is generally considered to be a zero-order or first-order reaction, with first-order kinetics being the most commonly used in the literature. First order kinetics was frequently used for describing the browning reactions in terms of the colour change indicated in CIE*L*a*b** colour model.

Shibukawa et al (1989) studied the effect of heating by convection and radiation in an oven at different baking temperatures (180 – 240 °C) on the surface colour of biscuits. This colour was compared to the browning of a model solution of monosodium glutamate and glucose, which followed first order kinetics. Mundt and Wedzicha (2007) proposed a first-order kinetic model based on the measurement of the surface colour of biscuits in R, G, B colour values during baking at temperatures of 105–130 °C. Ait Ameur et al., (2006, 2007) showed that the formation of HMF in cookies follows first-order kinetics, as does colour development, and that water activity strongly influences the production of coloured compounds. The rates of HMF formation during baking were 0.0028, 0.0067 and 0.0082 s^1 at 200, 250 and 300 °C, respectively. Furthermore, Hadiyanto et al. (2007) proposed a zero-order kinetic model for the formation of melanoidins (by the Maillard reaction) during the baking of bakery products, taking into account the influence of temperature and water activity.

To obtain a model for browning development, parameter estimation is required. If a non-isothermal approach is applied, the model will include the thermal history of the product during baking (the same analysis is valid for water activity or water content). To describe the dependence of rate constant (k) with temperature, the Arrhenius' law is commonly used (Eq. (5):

$$k = A \cdot exp\left(-\frac{E_a}{RT}\right) \tag{5}$$

where k = reaction rate constant (s^{-1});
A = pre-exponential factor;
E_a = activation energy (J/mol);
R = universal gas constant (8.314 J/mol/K);
T = (absolute) temperature (K).

The kinetic constants of the browning reaction during baking (k) are different for different products. They depend on the composition, especially on the concentration of reducing sugars and amino groups.

Zanoni et al. (1995) proposed a first-order kinetic reaction model for prediction of crust browning of bread during the baking process. The experimentally determined colour values of samples of grinded bread crust by heating in the range of 140 – 250 °C served as the basis for building the model. In this model (Eq. (6)); the reaction rate constant depends on surface temperature according to the Arrhenius equation:

$$k = k_0 \cdot exp\left(-\frac{E_a}{RT}\right) \tag{6}$$

with $k_0 = 42000\ (s^{-1})$, E_a= 64.151 (kJ/mol)

where k = reaction rate constant (s^{-1});
k_0 = pre-exponential factor;
E_a = activation energy (J/mol);
R = universal gas constant (8.314 J/mol/K);
T = (absolute) temperature (K).

He gave the relationship between the reaction rate constant and the baking temperature by applying the Arrhenius law. The proposed model was tested on bread samples, at baking temperatures at 200 and 250 °C. According to the results, the model was applicable at a baking temperature of 250 °C, given that the experiment was conducted in non-isothermal conditions (real conditions).

However, this expression (Eq. (6)) for temperature dependence is relevant to chemical compounds such as HMF where energy activation occurs in the context of a reaction. In the case of lightness or any other colour variable that represents the change in colour intensity and is not directly related to chemical compounds, the concept of activation energy may not be applicable (van Boekel, 2008). Instead of the Arrhenius equation, the following expression can be used to describe the dependence of the browning rate constant on temperature equally well (Eq. (7)):

$$c = c_0 \cdot exp\left[-A\, exp\left(-\frac{E_a}{RT}\right) \cdot t\right] \tag{7}$$

where c = concentration (mol/dm^3);
c_0 = initial concentration at t =0 (mol/dm^3);
A = pre-exponential factor;
E_a = activation energy (J/mol);
R = universal gas constant (8.314 J/mol/K);
T = (absolute) temperature (K);
t = time (s).

Because of the strong correlation between A and E_a, it is desirable to reparametrize the Arrhenius equation (van Boekel, 1996). A very simple reparametrization is the introduction of the reference temperature (T_{ref}) in the Eq. (5):

$$k_1 = A \cdot exp\left(-\frac{E_a}{RT_1}\right) \tag{8}$$

$$k_2 = A \cdot exp\left(-\frac{E_a}{RT_2}\right) \tag{9}$$

where k = reaction rate constant (s^{-1});
A = pre-exponential factor;
E_a = activation energy (J/mol);
R = universal gas constant (8.314 J/mol/K);
T = reference temperature.

Besides temperature and baking time, colour development on the surface of bakery products is also influenced by water activity (a_w), or the amount of water in the crust (X_b). Broyart et al. (1998) proposed to define the parameters of the browning rate constant (k_0 and E_a in their model) as a function of water content (Eq. (10–12)). The kinetic model developed is a first-order reaction and is based on monitoring the lightness of the cracker (L^*) as a function of product temperature and moisture. The model is applicable for predicting the brightness change within the temperature range 180–330 °C. The model is also suitable to suggest how baking profiles should be changed in order to obtain products with a different final lightness. The authors (Broyart et al., 1998) have proposed a model (two multiparameter equations) for prediction of lightness variations during baking as a function of time, temperature and water content, that differs for the lightening (Eq. (10)) and darkening (Eq. (11-12)) phases of the biscuit surface during baking:

$$\frac{dL^*}{dt} = + k_1 \cdot L^*$$
$$k_1 = k_1^0 \cdot exp\left(-\frac{E_{a1}}{RT_{b(t)}}\right) \quad (10)$$

$$\frac{dL^*}{dt} = - k_2 \cdot L^*$$
$$k_2 = k_2^0 \cdot exp\left(-\frac{E_{a2}}{RT_{b(t)}}\right) \quad (11)$$

$$k_2^0 = k_2^1 + \left(\frac{k_2^2}{X_{b(t)}}\right)$$
$$\frac{E_{a2}}{R} = k_2^3 + \left(\frac{k_2^4}{X_{b(t)}}\right) \quad (12)$$

where L^* = CIE colour component of surface lightness.

k_1 = reaction rate constant of enlightenment reaction (min^{-1});

k_2 = reaction rate constant of darkening reaction (min^{-1});

k_1^0 = kinetic constant of enlightenment reaction (min^{-1});

k_2^0 = kinetic constant of darkening reaction (min^{-1});

k_2^1, k_2^2, k_1^0, k_1^0, = Kinetic parameters of darkening reaction (respectively in min^{-1}, g water/100 g dry matter/min, K, g water/K/ 100 g dry matter);

E_{a1} = activation energy of enlightenment reaction (kJ/mol);

E_{a2} = activation energy of darkening reaction (kJ/mol);

R = universal gas constant (8.314 J/mol/K);

t = baking time (min);

$T_{b(t)}$ = cracker temperature (°C)

$X_{b(t)}$ = water content (g water/100 g dry matter).

In addition to the temperature and baking time, the water activity in the bread also has significant influence on the colour. Considering this fact, Purlis and Salvadori (2009c) proposed another model for monitoring the colour development of the crust of bread. This approach to define the parameters of an Arrhenius-like expression for the rate constant (k) as a function of water activity (a_w) (Eq. (13–14)):

$$k_0 = k_1 + \frac{k_2}{a_w} \quad (13)$$

$$Ar = k_3 + \frac{k_4}{a_w} \quad (14)$$

where a_w = water activity;

k_0, k_1, k_2, k_3, k_4 and Ar are fit parameters.

Kinetic models for the colour change during bread baking as a function of product weight loss and baking temperature were reported by Purlis and Salvadori (2007) and are represented by the equations Eq. (15-16). They proposed a colour prediction model where

the total colour difference (ΔE) is a function of product mass loss (WL) and baking temperature (T_{oven}):

$$\Delta E = k \cdot WL \tag{15}$$

$$k = k_0 \cdot T_{oven} + k_1 \tag{16}$$

where ΔE = total colour difference;
k = reaction rate constant (s^{-1});
WL = product weight loss (%);
T_{oven} = baking temperature (°C);
k_0, and k_1 are fit parameters.

The experiment was conducted at temperatures of 180, 200 and 220 °C, with forced and natural convection, and the colour of the surface of the bread samples during baking was monitored by computer image analysis. The developed mathematical model predicted the colour change in non-ideal conditions, similar to the real conditions of bread production in the bakery industry. In this way, the evolution of browning was followed in a non-ideal system close to real baking conditions. Acceptable results for a general baking process were reported. Quevedo et al. (2017) investigated the browning kinetics of two types of pita bread based on computer vision colour measurement on the surface at four different baking temperatures (160, 180, 200 and 220 °C). They suggested that the fractal method can be used to record the browning of the pita bread and to calculate a fractal browning rate. In general, the fractal method can be considered as a new means to quantify the browning kinetics, where the formation of a heterogeneous colour on the surface is observed and where the traditional method is more difficult to apply. The method offers great potential for application not only to bread but also to other foods which show an inhomogeneous colour on the surface. Zhang et al. (2016) investigated the bread baking process using a miniature bread approach and modelled the browning kinetics of miniature bread during baking with spatial reaction engineering approach (S- REA). They found that the combination of S-REA and equations relating surface moisture content and temperature to overall colour changes modelled browning kinetics well. Golchin et al. (2020) developed a mathematical model for predicting the crust temperature and weight loss of toast bread at different oven temperatures and baking times. The predicted crust temperature and weight loss of the bread (control and with guar gum) agreed well with the experimental temperature with coefficients of determination of 0.98 and 0.99, respectively (Eq. (17–18)).

$$WL_{prc} = -0.0325 \cdot (WL_{Ex})^2 + 2.326 \cdot WL_{Ex} + 3.474 \tag{17}$$

$$WL_{prc} = -0.017 \cdot (WL_{Ex})^2 + 1.218 \cdot WL_{Ex} + 3.507 \tag{18}$$

where WL_{prc} = predicted weight loss of sample containing guar (%);
WL_{Ex} = Measured weight loss (%).

Conclusion

1. Chemically, non-enzymatic browning is mostly based on caramelization reactions and Millard reactions. Various factors such as temperature, concentration of reactants, water activity, pH and others influence the intensity of the colour change. These reactions occur when food is treated at elevated or high temperatures.
2. Efforts should be made to develop a fast, inexpensive, automated, reasonable and objective method to track colour change during baking. One possible approach would be to calibrate e.g. a computer vision system or a colorimeter) against a quantification of AA or HMF depending on the product recipe and finally express the colour in standardized units (e.g. using the CIE*L*a*b** model).
3. The formation of colour in bakery products during the baking phase is commonly known as browning. Understanding browning development provides the opportunity to control, optimize and design processes and equipment for the bakery industry as it affects the overall quality of bakery products, including their sensory and nutritional properties. For this purpose, a mathematical model for browning development is useful.
4. The development of a mathematical model of browning is essential to predict and control this phenomenon during baking depending on the operating conditions and the product recipe. A browning model cannot be developed from the actual mechanisms of colour formation, as these have not yet been clarified. However, the kinetic approach is a helpful alternative to describe colour changes during baking.
5. Since browning affects the overall quality of food and leads to changes in sensory and nutritional properties (reduction in bioavailability of proteins and amino acids, formation of AA and HMF, and formation of substances with antioxidant activity), it is a topic of great interest to food technologists.

References

Abdullah, M.Z. (2008), Quality evaluation of bakery products. In: Sun D.W., ed., *Computer vision technology for food quality evaluation, 2nd edition,* pp. 481–522.

Ahrné, L., Andersson, C.G., Floberg, F., Rosén, J., Lingnert, H. (2007), Effect of crust temperature and water content on acrylamide formation during baking of white bread: steam and falling temperature baking, *LWT – Food Science and Technology,* 40(10), pp. 1708–1715.

Ameur, L., Mathieu, O., Lalanne, V., Trystram, G., Birlouezaragon, I. (2007), Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures, *Food Chemistry*, 101(4), pp. 1407–1416.

Ameur, L.A., Trystram, G., Birlouez-Aragon, I. (2006), Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method, *Food Chemistry*, 98(4), pp. 790–796.

ANMAT (2004), Código Alimentario Argentino (Food Code of Argentina, chapter IX). ANMAT – La Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (The National Administration of Drugs, Foods and Medical Devices), http://www.anmat.gov.ar/codigoa/CAPITULO_IX_Harinas_actualiz_06-03.pdf (01.10.2022).

Bates, L., Ames, J. M., MacDougall, D. B., Taylor, P.C. (2006), Laboratory reaction cell to model maillard color development in a starch-glucose-lysine system, *Journal of Food Science*, 63(6), pp. 991–996.

Bemiller, J.N., Whistler, R.L. (1996), Carbohydrates. In: Fennema O.R., ed., *Food Chemistry, 3ed*, Marcel Dekker, New York, pp. 171–174.

Brosnan, T., Sun, D.W. (2002), Inspection and grading of agricultural and food products by computer vision system-a review, *Computers and Electronics in Agriculture*, 36, pp. 193–213.

Brosnan, T., Sun, D.W. (2004), Improving quality inspection of food products by computer vision-a review, *Journal of Food Engineering*, 61, pp. 3–16.

Broyart, B., Trystram, G., Duquenoy, A. (1998), Predicting colour kinetics during cracker baking, *Journal of Food Engineering*, 35, pp. 351–368.

Capuano, E., Ferrigno, A., Acampa, I., Alt–Ameur, L., Fogliano, V. (2008), Characterization of the Maillard reaction in bread crisps, *European Food Research and Technology*, 228, pp. 311–319.

De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., Hendrickx, M.E. (2009), Modelling acrylamide changes in foods: from single-response empirical to multiresponse mechanistic approaches, *Trends in Food Science and Technology*, 20(3–4), pp. 155–167.

Dolan, K.D. (2003), Estimation of kinetic parameters for nonisotermal food processes, *Journal of Food Science*, 68, pp. 728-741.

Du, C.-J., Sun, D.-W. (2004), Recent developments in the applications of image processing techniques for food quality evaluation, *Trends in Food Science & Technology*, 15, pp. 230–249.

Du, C.J., Sun, D.W. (2005), Comparison of three methods for classification of pizza topping using different colour space transformations, *Journal of Food Engineering*, 68, pp. 277–287.

Gökmen, V., Açar, Ö.Ç., Arribas-Lorenzo, G., Morales, F.J. (2008a), Investigating the correlation between acrylamide content and browning ratio of model cookies, *Journal of Food Engineering*, 87(3), pp. 380–385.

Gökmen, V., Açar Ö.C., Serpen, A., Morales, F.J. (2008b), Effect of leavening agents and sugars on the formation of hydroxymethylfurfural in cookies during baking, *European Food Research and Technology*, 226, pp. 1031–1037.

Golchin, M.F., Movahhed, S., Eshaghi, M., Ahmadi Chenarbon, H. (2020), Mathematical modeling of weight loss and crust temperature of toast bread containing guar gum during baking process, *Food Science & Nutrition*, 9(1), pp. 272–281.

Hadiyanto, A.A., Straten, G., van Boom, R.M., Esveld, D.C., Boxtel, A.J.B. (2007), Quality prediction of bakery products in the initial phase of process design, *Innovative Food Science & Emerging Technologies*, 8(2), pp. 285–298.

Haefner, J.W. (2005), *Modeling biological systems: principles and applications*, Springer, New York.

Hermann, J., Nour, S. (1977), Modelluntersuchungen zur Bildung von Carbonylverbindungen und Brfiunung durch die Maillard-Reaktion beim Backprozess, *Nahrung*, 21, pp. 319-330.

Hodge, J.E. (1953), Chemistry of browning reactions in model systems, *Agricultural and Food Chemistry*, 15, pp. 928–943.

Hui, Y.H., Wai-Kit N., Nollet, L.M.L., Paliyath, G., Simpson, B.K. (2006), *Food Biochemistry and Food Processing*, Blackwell Publishing, USA.

Kroh, L.W. (1994), Caramelization in food and beverages, *Food Chemistry*, 51, pp. 373–379.

Lukinac, J., Jukić, M., Mastanjević, K., Lučan, M. (2018), Application of computer vision and image analysis method in cheese-quality evaluation: a review, *Ukrainian Food Journal*, 7(2), pp. 192-214.

Martins, S.I.F.S., van Boekel, M.A.J.S. (2003), Melanoidins extinction coefficient in the glucose/glycine Maillard reaction, *Food Chemistry*, 83(1), pp. 135–142.

Mendoza, F., Dejmek, P., Aguilera, J.M. (2007a), Calibrated color measurements of agricultural foods using image analysis, *Postharvest Biology and Technology*, 41, pp. 285–295.

Mendoza, F., Dejmek, P., Aguilera, J.M. (2007b), Colour and image texture analysis in classification of commercial potato chips, *Food Research International*, 40(9), pp. 1146–1154.

Mesias, M., Morales, F.J (2016), Acrylamide in bakery products. In: Gökmen V., ed., *Acrylamide in Food: Analysis, Content and Potential Health Effects, 1st edition*, pp. 131–157.

Mondal, A., Datta, A.K. (2008), Bread baking – A review, *Journal of Food Engineering*, 86, pp. 465–474.

Montgomery, D.C., Runger, G.C. (2003), *Applied statistics and probability for engineers*, John Wiley & Sons Inc., New York.

Morales, F.J., Açar Ö.Ç., Serpen, A., Arribas-Lorenzo, G., Gökmen, V. (2007), Degradation of free tryptophan in a cookie model system and its application in commercial samples, *Journal of Agricultural and Food Chemistry*, 55(16), pp. 6793–6797.

Morales, F.J., van Boekel, M.A.J.S. (1998), A Study on advanced Maillard reaction in heated casein/sugar solutions: Colour formation, *International Dairy Journal*, 8(10-11), pp. 907–915.

Mundt, S., Wedzicha, B.L. (2007), A kinetic model for browning in the baking of biscuits: Effects of water activity and temperature, *LWT – Food Science and Technology*, 40(6), pp. 1078–1082.

Nursten, H. (2005), The chemistry of nonenzymic browning, In: *The Maillard Reaction*. Royal Society of Chemistry, UK.

Pedreschi, F., León, J., Mery, D., Moyano, P. (2006), Development of a computer vision system to measure the colour of potato chips, *Food Research International*, 39(10), pp. 1092–1098.

Purlis, E., Salvadori, V.O. (2007), Bread browning kinetics during baking, *Journal of Food Engineering*, 80, pp. 1107–1115.

Purlis, E., Salvadori, V.O. (2009a), Bread baking as a moving boundary problem. Part 1: Mathematical modelling, *Journal of Food Engineering*, 91(3), pp. 428–433.

Purlis, E., Salvadori, V.O. (2009b), Bread baking as a moving boundary problem. Part 2: Model validation and numerical simulation, *Journal of Food Engineering*, 91(3), pp. 434–442.

Purlis, E., Salvadori, V.O. (2009c), Modelling the browning of bread during baking, *Food Research International*, 42(7), pp. 865–870.

Purlis, E. (2010), Browning development in bakery products – A review, *Journal of Food Engineering*, 99, pp. 239–249.

Purlis, E. (2011), Bread baking: Technological considerations based on process modelling and simulation, *Journal of Food Engineering*, 103(1), pp. 92–102.

Quevedo, R., Rojas, R., Pedreschi, F., Bastias, J. M., Siché, R., Uquiche, E., Díaz, O. (2017), Quantification of the browning kinetic on pita bread using fractal method, *Food and Bioprocess Technology*, 11(1), pp. 201–208.

Ramirez-Jimenez, A., Guerra-Hernandez, E., Garcia-Villanova, B. (2000), Browning indicators in bread, *Journal of Agricultural and Food Chemistry*, 48, pp. 4176–4181.

Shahin, M.A., Symons, S.J. (2001), A machine vision system for grading lentil, *Canadian Biosystems Engineering*, 43, pp. 7.7–7.14.

Shibukawa, S., Sugiyama, K., Yano, T. (1989), Effects of heat transfer by radiation and convection on browning of cookies at baking, *Journal of Food Science*, 54, pp. 621-624.

Stadler, R.H., Studer, A. (2016) Acrylamide formation mechanisms, In: Gökmen V., ed., *Acrylamide in Food: Analysis, Content and Potential Health Effects, 1st edition*, Elsevier, pp. 1–17.

Tomasik, P. (2004), *Chemical and Functional Properties of Food Saccharides*, CRC Press LLC.

van Boekel, M.A.J.S. (1996), Statistical aspects of kinetic modeling for food science problems, *Journal of Food Science*, 61, pp. 477–489.

van Boekel, M.A.J.S. (2008), Kinetic modeling of food quality: A critical review, *Comprehensive Reviews in Food Science and Food Safety*, 7(1), pp. 144–158.

Villota, R., Hawkes, J.G (2007), Reaction kinetics in food systems, In: Heldman D. R., Lund D. B., Sabliov C., ed., *Handbook of Food Engineering, 2nd edition*, pp. 125–286.

Wahlby, U., Skjoldebrand, C. (2002), Reheating characteristics of crust formed on buns, and crust formation, *Journal of Food Engineering*, 53, pp. 177–184.

Yam, K.L., Papadakis, S.E. (2004), A simple digital imaging method for measuring and analyzing color of food surfaces, *Journal of Food Engineering*, 61(1), pp. 137–142.

Zanoni, B., Peri, C., Bruno, D. (1995), Modelling of browning kinetics of bread crust during baking, *LWT – Food Science and Technology*, 28, pp. 604–609.

Zhang, L., Putranto, A., Zhou, W., Boom, R. M., Schutyser, M. A. I., Chen, X. D. (2016), Miniature bread baking as a timesaving research approach and mathematical modeling of browning kinetics, *Food and Bioproducts Processing*, 100, pp. 401–411.

Zheng, C., Sun, D. W., Zheng, L. (2006), Recent developments and applications of image features for food quality evaluation and inspection – a review, *Trends in Food Science and Technology*, 17, pp. 642–655.

Pastry sauce with carob (*Ceratonia siliqua*) powder

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Abstract

Introduction. The present research discusses the carob pastry sauce production without sugar addition and highlighting its functional and physico-chemical properties.

Materials and methods. To evaluate the possibility of carob use in the production of pastry sauce, powder of carob pods and beans was introduced in the recipe of cocoa sweet pastry sauce. The functional and physico-chemical properties of the produced sauce were characterized in terms of rheology, chemical composition, sensory analysis, antioxidant activity, and total phenol content.

Results and discussion. The incorporation of carob morphological parts (beans or pod pulp) in the pastry sauce recipe in order to replace the sugar and cocoa reduced its energy value by 60% compared to the original recipe (with cocoa and sugar). The addition of carob pod powder in the composition of the pastry sauce increased the content of Ca and Fe by 2.9 and 5.1 times, respectively. The biological value of sauce with carob pod powder showed an increase in terms of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) inhibition antioxidant activity up to 95.97% compared to 60% for control, and total phenol content up to 29.12 mg gallic acid equivalent (GAE) per g compared to 5.11 GAE/g for control.

Addition of carob pod powder in sauce formulations has a positive influence on the rheological properties of the sauces, leading to the increase of their viscosity, as well as their stability to the increase of shear stress and shear rate.

The sensory evaluation of sauces prepared with the addition of carob pod powder or carob bean powder showed that all sauces had a fine and homogeneous consistency, a pleasant flavor and smell characteristics of the added ingredients: the pastry sauce with carob pod powder had a specific smell and flavor of dark chocolate, and the pastry sauce with carob bean powder had a hint of caramel flavor.

Conclusions. The incorporation of carob pod or bean powder in pastry sauces to replace cocoa and sugar, enhanced the quality and biological values of the sauce by increasing its mineral content, antioxidant activity, total phenol content, the consumer acceptance, decreasing at the same time energetical value of the product.

Introduction

Pastries include a vast variety of fat and sugar rich products (Ooms et al., 2016). Due to the high amount of sugar and fat, on one hand, pastries are viewed as a source of products related to happiness (Wahl et al., 2017), and, on the other hand, the consumption of pastry is often associated with an increase in obesity in children and adults (Karp et al., 2016). Often, at serving phase, pastry is associated with sauces in order to reveal the taste of the product. The sauce can complement the delicacy or be a full-fledged companion for it. Basic pastry sauces, represent mixtures of divers ingredients, the most common being sugar, chocolate, caramel, cream, fruits, and berries (Benković et al., 2019). The pastry sauces are widely used by not only catering establishments, but are also available in stores for individual consuming. Thus, pastry sauces have positive effects on the products commercial quality, in terms of flavor, colour and aspect. However, because of their composition, sauces increase the calorific value of the pastry products enrolling them in the category of obesogenic foods (McKerchar et al., 2020). Thus, numerous studies are being carried out in order to obtain new natural additives to food products, including for confectionery sauces, preventing an increase in their calorie content (Chidambaram, 2021; Gorodyska et al., 2018; Kwon et al., 2021; Selvasekaran and Souza et al., 2021; Stabnikova et al., 2021).

Currently, vegetable raw materials are increasingly used in the development of functional foods (Popovici et al., 2019, Covaliov et al., 2021), including sauces: in the manufacture of emulsified sauces (Mirzanajafi-Zanjani et al., 2019), tomato sauces (Ferro et al., 2021), and confectionery sauces (Abushal et al., 2021). They diversify the range of products, making them more attractive to consumers. In some cases, these ingredients increase the energy value of the products, especially when butter, oils, and sugar are included in their recipes, but the biological value of such products remain low (Lebedenko et al., 2021).

Carob (*Ceratonia Siliqua* L., tree of the pea family *Fabaceae*) is a fruit species for the Mediterranean climate attracts attention for its high biological and nutritional value. Carob fruits are rich in natural sugars – 48–56% of dry weight, especially sucrose, fructose and glucose. The syrup obtained from the carob pods is recommended to be used as a sweetener, together with bee honey (Atasoy, 2009; Lambert et al., 2018). Several studies were made in order to show the carob high biological potential (Fidan et al., 2020), its consumption in the form of powders or tinctures were advised due to high content of antioxidants (Ibrahim et al., 2020; Vitali Čepo et al., 2014). In addition, its glycemic index is low, and carob pods do not include caffeine in the composition (Nasar-Abbas et al., 2016; Papakonstantinou et al., 2017; Rodríguez-Solana et al., 2021). More than, it was found that carob having high antioxidant capacity may serve as an effective anti-obesity compound (Fujita et al., 2021) and, so, can be used in the production of food recommended to people suffered from obesity. Meanwhile obesity is a major risk factor for various chronic diseases such as diabetes, cardiovascular disease, and cancer. All of the above indicates that carob fruits can be considered as a functional ingredient can be used as a source of biologically active compounds for the production of functional food for special nutritional requirements (Ivanov et al., 2021).

The aim of the present research was to study physico-chemical and functional characteristics of carob pastry sauce.

Materials and methods

Carob fruit collection and dry powder preparation

Carob pods were harvested in the central region of the Republic of Moldova at the middle of October 2021. During this period they reach a good state of ripeness. Carob beans were carefully separated from the pod pulp. The raw materials were washed thoroughly, followed by a drying procedure in order to remove any moisture acquired during drying during 48 hours at 40°C. The dried carob pods pulp and beans were ground until powder was obtained.

Preparation of carob pastry sauce

For the functional pastry sauce production, a standard chocolate sauce formulation was used. The usual ingredients for the pastry sauce were as: cocoa powder, pasteurized milk (3.5% fat), butter (82.5% fat), powdered sugar, vanilla extract and processed drinking water. Carob (beans and pods) powder was added to produce the functional pastry sauce.

Two types of sauces by addition of two types of carob powder produced from different morphological parts of carob, namely, carob beans and carob pods pulp, were prepared. Technology includes the use of moderate heat treatment. During the technological process, it was found that carob powder serves as a thicker that is why in the carob pastry sauce formulation sugar was replaced with water.

Firstly, the mixture of pasteurized milk with vanilla essence was the prepared. The butter was melted at a temperature of 30 °C and put into prepared mixture. Then, the rest of the ingredients followed by powdered sugar (or water) and carob pods pulp or carob beans powder was added. A short (5 min) heat treatment at a temperature up to 80 °C under continuous mixing to obtain a homogeneous mass to prepare the sauce was done, and the mass was cooled to 20 °C.

Determination of protein, carbohydrates and lipids content

The standard methods adopted by the AOAC (Association of Official Analytical Chemists) were used to determine the protein (2001.11), carbohydrates (2020.07), and lipids (996.01) contents (Horwitz, 2007; McCleary and McLoughlin, 2021).

Determination of mineral content

Mineral content was determined by Atomic Absorption Spectrometry (AAS) official method. The content of Ca, Fe and K in the experimental samples was determined according to García and Báez (2012).

Determination of total polyphenol content (TPC)

Total polyphenol content was determined by Folin-Ciocalteu method described by *Lamuela-Raventós* (Lamuela-Raventós, 2017).

Determination of antioxidant activity (AA)

Antioxidant activity was measured using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) method (Nenadis and Tsimidou, 2017).

Rheological measurements

The rheological measurements were performed by using a DV-III Ultra Rheometer (Brookfield Inc., USA) at 25±0.01 °C equipped with Peltier Temperature Controller Unit. The measuring system consisted of a cone and plate sensor with a diameter of 2 cm and cone angle of 2°. Shear rate range was 0–300 s^{-1} within 600 s. For each measurement, 1 ml of sample was poured over the plateau of rheometer. Each measurement was done in triplicate. Rheological parameters (shear stress, shear rate, apparent viscosity) were obtained from the Bohlin CVOR 150 data analysis software (Lystopad et al., 2020).

Sensory test of carob pastry sauces

Sensory test was performed by method described by Rachel Byarugaba and ISO 6658:2017 (Byarugaba et al., 2020). The study of sensory properties of carob pastry sauces the scoring scale from 1 to 5 in two groups of developers was used. 5 basic parameters according to ISO 6658:2017 were assessed. The resulting score for each quality index was appreciated by tasters and entered in the individual sensory analysis sheet. Following the statistical processing of the grades, the quality of the experimental samples was assessed.

Statistical analysis

All experiments were carried out in triplicate. The results are given as mean±standard deviation (SD). Statistical analysis was performed using XLstat (2020 version) software.

Results and discussion

Preparation of carob pastry sauces

During the research, seven samples of sauces were prepared. All recipes contain pasteurized milk, 30 mL; butter, 15 g; vanilla essence, 0.1 mL. The difference in recipes was in the content of cocoa, carob pod powder, carob bean powder, and powdered sugar (Table 1).

Table 1

Functional carob pastry sauces formulations

N of sauce	Cocoa, g	Carob pod powder, g	Carob bean powder, g	Powdered sugar, g	Water, mL
1 (control)	10	-	-	45	-
2	-	10	-	45	-
3	-	-	10	45	-
4	-	5	5	45	-
5	-	15	-		40
6	-	-	15		40
7	-	7.5	7.5		40

Sauce 1 was used as control with 10% of cocoa. Sauces 2, 3 and 4 contained 45 g of powdered sugar and 10% of carob pod powder; 10% of carob bean powder; mixture of 5% carob pod powder and 5% carob bean powder, respectively.

After the primary sensory testing of the sauces 2, 3, and 4, it was found that these sauces were very viscous and the taste was extremely sweet. The consistency of these sauces was almost solid, probably due to the high pectin content in carob. That why the sauces 2, 3, and 4 were excluded from the future research. The sauces 5, 6, and 7 were prepared with replacement of 45 g sugar with 40 mL of water. Sauce 5 with carob pod powder had a more intense bitter taste specific to dark chocolate, while sauce 6 with carob bean powder had a sweet aroma and flavor specific to caramel. Sauce 7 contains a mixture of carob pod powder, 7.5%, and carob bean powder, 7.5%. The consistency of sauces 5, 6, and 7 was more appropriate to control, the traditional pastry sauces consistency. The obtained sauces were placed to sterilized glass vessels, sealed and refrigerated at 4-6 °C for 24 hours before being use for analysis.

Physico-chemical characteristics of carob pastry sauces

Physico-chemical characteristics of studied sauces are shown in Table 2.

Table 2

Physico-chemical characteristics of carob pastry sauces

Components	**Sauces**			
	1 (control)	**5**	**6**	**7**
Protein, g/100 g	3.60±0.06	2.90±0.02	3.10±0.04	3.00±0.02
Carbohydrates, g/100 g	22.10±0.23	6.90±0.17	7.20±0.24	7.05±0.11
Lipids, g/100g	14.40±0.21	5.10±0.09	5.30±0.12	5.20±0.15
Energy value, kcal	230.70±1.32	85.10±0.45	88.90±0.76	87.00±0.54
Ca, mg/100 g	69.60±0.72	159.60±0.98	155.50±1.21	157.50±1.32
Fe, mg/100 g	0.13±0.01	1.17±0.02	0.91±0.02	1.04±0.01
K, mg/100 g	110.00±1.03	171.75±1.15	162.40±1.13	167.07±1.43

According to the results all sauces with carob powder had a significantly increasing content of mineral components, namely calcium (Ca), iron (Fe), and potassium (K) in comparison with traditional sauce (control 1) prepared with cocoa and powdered sugar. In the sauce with carob powder the calcium content increased more than 2 times, iron up to 9 times, and potassium up to 1.5 times. The content of protein almost did not change, meanwhile the content of carbohydrates and lipids significantly decreased. One of the most important characteristics of the carob pastry sauces is the energy value, which was reduced from 230.7 kcal to 88.9–85.1 kcal that is 2.7 times less than energy value of control (sauce with cocoa and sugar). The obtained data demonstrate the increased biological value of the experimental samples, but a lower energy value, which allows the recommendation of these products as a functional sauce.

Following the research of the functional potential of carob, it has been found that it is an important source of polyphenols, which show a strong antioxidant activity (Rtibi et al., 2015; Stavrou et al., 2018). In the present research, the total content of polyphenols was determined, as well as the antiradical activity of DPPH and in experimental sauces, in order to establish the effect of incorporating carob in the elaborated products (Table 3).

Table 3

Total polyphenol content and antioxidant activity of carob pastry sauces

Sauces	Total polyphenol content mg GAE/ g	DPPH, %
1 (control)	5.11±0.12	60.04±0.26
5	29.12±0.24	95.97±1.08
6	22.15±0.11	88.08±0.98
7	26.09±0.08	93.75±1.05

A positive correlation between antioxidant activity and total phenols content was found for studied sauces. Sauce 5 prepared with carob pod powder has a higher content of polyphenols than sauce 6 with carob bean powder contributing to a higher antioxidant activity of sauces 5. The highest total phenol content, 29.12 mg GAE/g, was determined for the sauce 5 also. According to Turhan et al. (2006), the total polyphenol content of carob pods is 17.50 mg/g. On the other hand, in their research, Mahtout et al. (2016) states that the total phenol content in carob pods reaches the value of 10.53 mg/g, while in beans this content is 17.23 mg/g (Mahtout et al., 2016). According to Cavallaro et al. (2021), the difference in the phenol amounts can be explained by the genotype, originating region, soil type, amount of precipitation (Cavallaro et al., 2021). The lowest total polyphenol content and antioxidant activity was in control, prepared with cocoa powder, 5.11 mg GAE/g and 60%, respectively, although according to Urban Urbańska and Kowalska (2019) the total content of polyphenols in fresh cocoa beans varies between 50–60 mg/g and can decrease up to 9.96–37.81 mg/g depending on the origin of the beans and the roasting treatment parameters.

Rheological properties of carob pastry sauces

The study of the rheological characteristics of foods allows to give characteristic basic quality indicators according to the values of structural and mechanical characteristics (Gonzalez-Gutierrez and Scanlon, 2018). Determining the structural and mechanical indicators of confectionery sauces, such as viscosity, allows obtaining data to improve properties: structure, texture, and shape. In order to investigate the rheological stability of the investigated sauces, the samples were subjected to research to increase shear stress and shear rate. For functional confectionery sauces, the rheological properties are determined by the value of the actual viscosity. Analyzing the rheograms for initial samples and after 4 months of storage, it was found that when increasing shear stress and shear rate, the viscosity of the emulsions decreases significantly, which can be explained by destroying their structure (Figure 1).

Comparing with control the sauces with carob powder were more stable and withstood a shear rate up to 4000–5200 s^{-1}. It was observed that the actual viscosity is directly dependent on the nature and composition of the studied pastry sauces. When the carob bean and pod powder is incorporated in sauce, the effective viscosity increases. For the control sauce 1 the value of this index changed from 11.0 to 10.7 Pa·s; for the sauce with carob bean powder from 16.5 to 16.0 Pa·s; for the sauce 6 with the bean powder from 18.1 to 17.6 Pa·s for fresh samples and after 4 months of storage, respectively.

After 4 months of storage, a non-essential change of the effective viscosity was observed, which proved the stability of the carob pastry sauces. The results of the investigations regarding the rheological properties of the sauces allowed us to state that the carob powders have a positive influence, lead to the increase of the viscosity of the sauces, as well as their stability to the increase of shear stress and the shear rate.

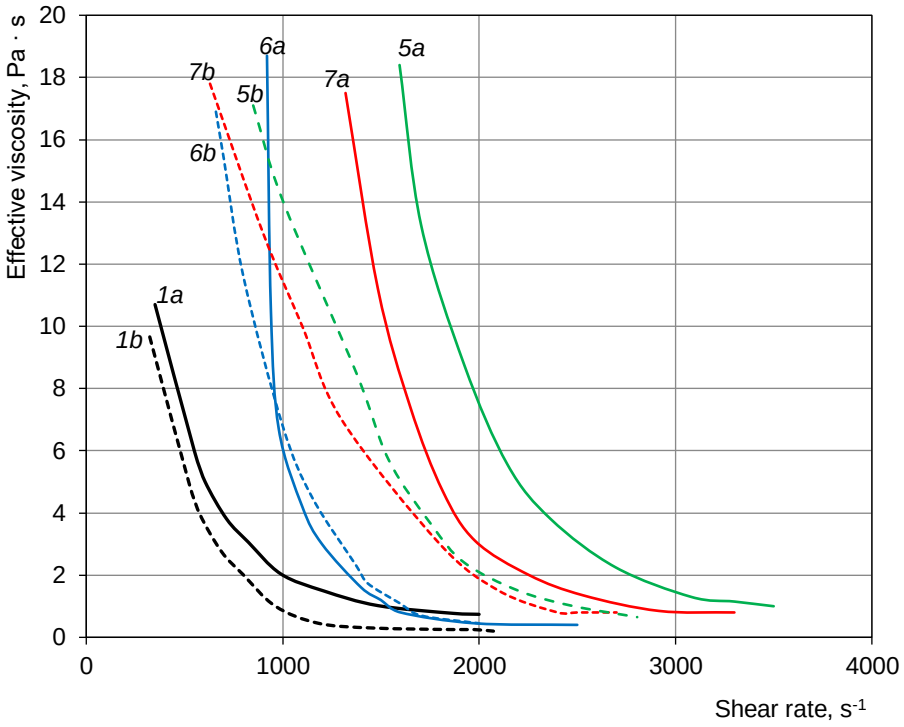


Figure 1. Variation of the effective viscosity according to the tangential stress of the carob pastry sauces:

1a – sauce 1 (control); 1b – sauce 1 (control) after 4 months of storage
5a – sauce 5; 5b – sauce 5 after 4 months of storage
6a – sauce 6; 6b – sauce 6 after 4 months of storage
7a – sauce 7; 7b – sauce 7 after 4 months of storage

Sensory test of carob pastry sauces

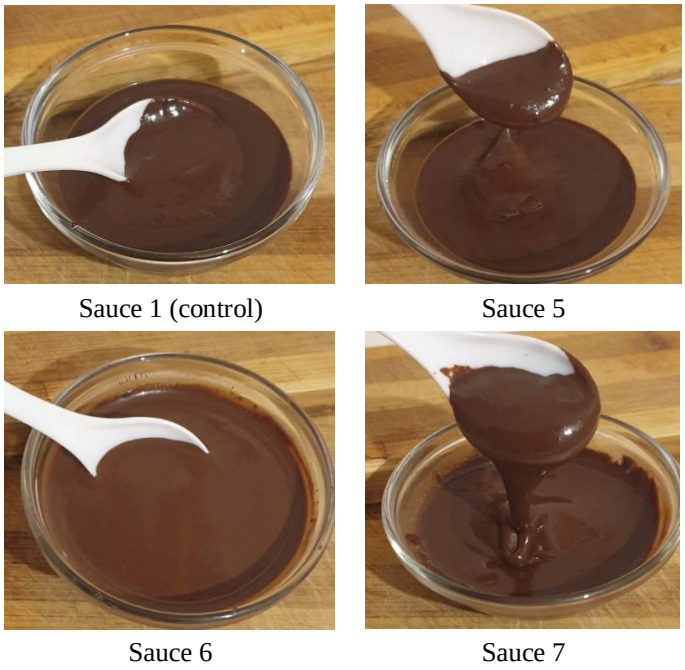
The evaluation of the sensory properties of carob pastry sauces was done using the scoring scale from 1 to 5 in two groups of developers. The results of the evaluation of sensory properties of the functional confectionery sauces are presented in Table 4.

Table 4

Sensory indices of the carob pastry sauces

Sensory properties	1 (control)	5	6	7
Flavor	4.25±0.03	4.46±0.02	4.78±0.03	4.54±0.01
Aroma	4.21±0.05	4.67±0.02	4.86±0.05	4.59±0.02
Color	4.32±0.01	4.43±0.01	4.79±0.03	4.45±0.04
Aspect	4.78±0.05	4.58±0.01	4.69±0.04	4.54±0.05
Consistency	4.56±0.03	4.45±0.03	4.87±0.01	4.62±0.03
Average score	4.42±0.03	4.52±0.02	4.80±0.03	4.55±0.03

The photos of sauces were shown in Figure 2.



Sauce 1 (control) Sauce 5

Sauce 6 Sauce 7

Figure 2. Images of experimental functional sauces

After evaluation of the sensory properties, it was found that all the sauces had a pleasant flavor and smell, and they had a characteristic consistency for each individual confectionery sauce. Based on the sensory evaluation, the sauce 6 with carob bean powder had better appearance and good consistency, as well as in a more expressive, a fine and pleasant flavor of caramel, obtaining an average appreciation score of 4.80. The sauce 5 with carob pod powder had a pronounced dark chocolate flavor and was highly appreciated by tasters with an average score of 4.52. It should be mentioned that the sauce made from a mixture of carob pod and bean powder was evaluated as one of high quality, obtaining an average score of 4.55. Taking into account the average scores, all carob pastry sauces were considered as acceptable.

Conclusion

- The addition of carob pod or bean powder in pastry sauces is a good way to reduce the amount of sugar in the product. The substitution of cocoa powder and sugar with carob pod or bean powder reduces the caloric value of pastry sauces more than 60 % of initial value.
- Including carob pod or bean powder in pastry sauces increased their biological value, particularly, of total phenol and calcium, iron, potassium contents, and antioxidant activity, being in the same time an alternative for the consumers of decaffeinated products.

- The addition of carob pod or bean powder in pastry sauces has a positive influence on their rheological properties increasing the viscosity of the sauces, as well as their stability during storage.

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References

Abushal S. A., Elhendy H. A., Abd El Maged E. M., Darwish A. M. G. (2021), Impact of ground Ajwa (*Phoenix dactylifera* L.) seeds fortification on physical and nutritional properties of functional cookies and chocolate sauce, *Cereal Chemistry*, 98(4), pp. 958–967, DOI: 10.1002/cche.10437.

Atasoy A. F. (2009), The effects of carob juice concentrates on the properties of yoghurt, *International Journal of Dairy Technology*, 62(2), pp. 228–233, DOI: 10.1111/j.1471-0307.2009.00465.x.

Benković M., Bosiljkov T., Semić A., Ježek D., Srečec S. (2019), Influence of carob flour and carob bean gum on rheological properties of cocoa and carob pastry fillings, *Foods*, 8(2), p. 66, DOI: 10.3390/foods8020066.

Byarugaba R., Nabubuya A., Muyonga J. (2020), Descriptive sensory analysis and consumer preferences of bean sauces, *Food Science & Nutrition*, 8(8), pp. 4252–4265, DOI: 10.1002/fsn3.1721.

Cavallaro V., Maucieri C., Patanè C., Fascella G., Pellegrino A., Barbera A. C. (2021), Polyphenols leaching and seed dormancy in carob (Ceratonia siliqua L.) in relation to hot water treatment, *Acta Physiologiae Plantarum*, 43(11), p. 141, DOI: 10.1007/s11738-021-03308-z.

Covaliov E., Grosu C., Popovici V., Capcanari T., Siminiuc R., Resitca V. (2021), Impact of sea buckthorn berries (hippophae rhamnoides) on yoghurt biological value and quality, *The annals of the university Dunarea de Jos of Galati*, 45(2), pp. 62-76, DOI:10.35219/foodtechnology.2021.2.05

Ferro Y., Mazza E., Angotti E., Pujia R., Mirarchi A., Salvati M. A., Montalcini T. (2021), Effect of a novel functional tomato sauce (OsteoCol) from vine-ripened tomatoes on serum lipids in individuals with common hypercholesterolemia: tomato sauce and hypercholesterolemia, *Journal of Translational Medicine*, 19(1), p. 19, DOI: 10.1186/s12967-020-02676-3.

Fidan H., Stankov S., Petkova N., Petkova Z., Iliev A., Stoyanova M., Ivanova T., Zhelyazkov N., Ibrahim S., Stoyanova A., Ercisli S. (2020), Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua* L.) seeds, *Journal of Food Science and Technology*, 57(7), pp. 2404–2413, DOI: 10.1007/s13197-020-04274-z.

Fujita K., Norikura T., Matsui-Yuasa I., Kumazawa S., Honda S., Sonoda T., Kojima-Yuasa A. (2021), Carob pod polyphenols suppress the differentiation of adipocytes through posttranscriptional regulation of C/EBPβ, PLoS ONE, 16(3), e0248073, DOI: 10.1371/journal.pone.0248073.

García R., Báez A. P. (2012), Atomic absorption spectrometry (AAS), In*: Atomic Absorption Spectroscopy*, Croatia, INTECH Open Access Publisher, pp. 1–12.

Gonzalez-Gutierrez J., Scanlon M. G. (2018), Rheology and mechanical properties of fats, In *Structure-Function Analysis of Edible Fats*, *Elsevier*, pp. 119–168, DOI: 10.1016/B978-0-12-814041-3.00005-8.

Gorodyska O., Grevtseva N., Samokhvalova O., Gubsky S., Gavrish T., Denisenko S., Grigorenko A. (2018), Influence of grape seeds powder on preservation of fats in confectionary glaze, *Eastern-European Journal of Enterprise Technologies*, 6(11), pp. 36–43, DOI: 10.15587/1729-4061.2018.147760.

Horwitz W. (2007), *Official methods of analysis of AOAC International: Gaithersburg*, MD, AOAC International.

Ibrahim R. M., Abdel-Salam F. F., Farahat E. (2020), Utilization of carob (*Ceratonia silique* L.) extract as functional ingredient in some confectionery products, *Food and Nutrition Sciences*, 11(8), pp. 757–772, DOI: 10.4236/fns.2020.118054.

Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7-36, DOI: 10.24263/2304-974X-2021-10-1-3.

Karp S., Wyrwisz J., Kurek M., Wierzbicka A. (2016), Physical properties of muffins sweetened with steviol glycosides as the sucrose replacement, *Food Science and Biotechnology*, 25(6), pp. 1591–1596, DOI: 10.1007/s10068-016-0245-x.

Kwon H. C., Shin D. M., Yune J. H., Jeong C. H., Han S. G. (2021), Evaluation of gels formulated with whey proteins and sodium dodecyl sulfate as a fat replacer in low-fat sausage, *Food Chemistry*, 337, p. 127682, DOI: 10.1016/j.foodchem.2020.127682.

Lambert C., Cubedo J., Padró T., Vilahur G., López-Bernal S., Rocha M., Hernández-Mijares A., Badimon L. (2018), Effects of a carob-pod-derived sweetener on glucose metabolism, *Nutrients*, 10(3), p. 271, DOI: 10.3390/nu10030271.

Lamuela-Raventós R. M. (2017), Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity, In R. Apak, E. Capanoglu, F. Shahidi, eds., *Measurement of Antioxidant Activity & Capacity*, Chichester, UK, John Wiley & Sons, Ltd, pp. 107–115, DOI: 10.1002/9781119135388.ch6.

Lebedenko T., Krusir G., Shunko H., Korkach H. (2021), Development of technology of sauces with functional ingredients for restaurants, *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*, 23(95), pp. 57–64, DOI: 10.32718/nvlvet-f9510.

Lystopad T., Deinychenko G., Pasichnyi V., Shevchenko A., Zhukov Y. (2020), Rheological studies of berry sauces with iodine-containing additives: *Ukrainian Food Journal*, 9(3), pp. 651–663, DOI: 10.24263/2304-974X-2020-9-3-13.

Mahtout R., Zaidi F., Saadi L. O., Boudjou S., Oomah B. D., Hosseinian F. (2016), Carob (*Ceratonia siliqua* L.) (pod, pulp, seed) flours and pulp mucilage affect kefir quality and antioxidant capacity during storage, *International Journal of Engineering and Technology*, 2(2), p. 14.

McCleary B. V., McLoughlin C. (2021), Measurement of available carbohydrates in cereal and cereal products, dairy products, vegetables, fruit, and related food products and animal feeds: First action 2020.07, *The Journal of AOAC International*, 104(6), pp. 1465–1478. DOI: 10.1093/jaoacint/qsab019.

McKerchar C., Smith M., Gage R., Williman J., Abel G., Lacey C., Ni Mhurchu C., Signal L. (2020), Kids in a Candy Store: An objective analysis of children's interactions with food in convenience stores, *Nutrients*, 12(7), p. 2143, DOI: 10.3390/nu12072143.

Mirzanajafi-Zanjani M., Yousefi M., Ehsani A. (2019), Challenges and approaches for production of a healthy and functional mayonnaise sauce, *Food Science & Nutrition*, 7(8), pp. 2471–2484, DOI: 10.1002/fsn3.1132.

Nasar-Abbas S. M., e-Huma Z., Vu T.H., Khan M. K., Esbenshade H., Jayasena V. (2016), Carob kibble: A bioactive-rich food ingredient, *Comprehensive Reviews in Food Science and Food Safety*, 15(1), pp. 63–72, DOI: 10.1111/1541-4337.12177.

Nenadis N., Tsimidou M. Z. (2017), DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) radical scavenging mixed-mode colorimetric assay(s), In: R. Apak, E. Capanoglu, and F. Shahidi, eds., *Measurement of Antioxidant Activity & Capacity*, Chichester, UK, John Wiley & Sons, Ltd, pp. 141–164, DOI: 10.1002/9781119135388.ch8.

Ooms N., Pareyt B., Brijs K., Delcour J. A. (2016), Ingredient functionality in multilayered dough-margarine systems and the resultant pastry products: A review, *Critical Reviews in Food Science and Nutrition*, 56(13), pp. 2101–2114, DOI: 10.1080/10408398.2014.928259.

Papakonstantinou E., Orfanakos N., Farajian P., Kapetanakou A. E., Makariti I. P., Grivokostopoulos N., Ha M.A., Skandamis P. N. (2017), Short-term effects of a low glycemic index carob-containing snack on energy intake, satiety, and glycemic response in normal-weight, healthy adults: Results from two randomized trials, *Nutrition*, 42, pp. 12–19, DOI: 10.1016/j.nut.2017.05.011.

Popovici V., Radu O., Hubenia V., Covaliov E., Capcanari T., Popovici C. (2019), Physico-chemical and sensory properties of functional confectionery products with Rosa Canina powder. *Ukrainian Food Journal* 8(4), pp. 815–827. DOI 10.24263/2304-974X-2019-8-4-12

Rodríguez-Solana R., Romano A., Moreno-Rojas J. M. (2021), Carob pulp: A nutritional and functional by-product worldwide spread in the formulation of different food products and beverages. A review, *Processes*, 9(7), pp. 1146, DOI: 10.3390/pr9071146.

Rtibi K., Jabri M.A., Selmi S. Souli A., Sebai H., El-Benna J., Amri M., Marzouki L. (2015), Gastroprotective effect of carob (*Ceratonia siliqua* L.) against ethanol-induced oxidative stress in rat, *BMC Complementary Medicine and Therapies*, 15, 292, DOI: 10.1186/s12906-015-0819-9.

Selvasekaran P., Chidambaram R. (2021), Advances in formulation for the production of low-fat, fat-free, low-sugar, and sugar-free chocolates: An overview of the past decade, *Trends in Food Science & Technology*, 113, pp. 315–334, DOI: 10.1016/j.tifs.2021.05.008.

Souza L. B. A., Pinto R. A., Nascimento L. G. L., Stephani R., Carvalho A. F., Perrone Í. T. (2021), Low-sugar strawberry yogurt: Hedonic thresholds and expectations, *Journal of Sensory Studies*, 36(3), DOI: 10.1111/joss.12643.

Stabnikova O., Marinin A., Stabnikov (2021), Main trends in application of novel natural additives for food production, *Ukrainian Food Journal*, 10(3), pp. 524–551, DOI: 10.24263/2304-974X-2021-10-3-8.

Stavrou I.J., Christou A., Kapnissi-Christodoulou C.P. (2018), Polyphenols in carobs: A review on their composition, antioxidant capacity and cytotoxic effects, and health impact, *Food Chemistry*, 269, 355-374, DOI: 10.1016/j.foodchem.2018.06.152.

Turhan I., Tetik N., Aksu M., Karhan M., Certel M. (2006), Liqiuid-solid extraction of soluble solids and total phenolic compounds of carob bean (*Ceratonia siliqua* L.), *Journal of Food Process Engineering*, 29(5), pp. 498–507, DOI: 10.1111/j.1745-4530.2006.00078.x.

Urbańska B., Kowalska J. (2019), Comparison of the total polyphenol content and antioxidant activity of chocolate obtained from roasted and unroasted cocoa beans

from different regions of the world, *Antioxidants*, 8(8), p. 283, DOI: 10.3390/antiox8080283.

Vitali Čepo D., Mornar A., Nigović B., Kremer D., Radanović D., Vedrina Dragojević I. (2014), Optimization of roasting conditions as an useful approach for increasing antioxidant activity of carob powder, *LWT – Food Science and Technology*, 58(2), pp. 578–586, DOI: 10.1016/j.lwt.2014.04.004.

Wahl D. R., Villinger K., König L. M., Ziesemer K., Schupp H. T., Renner B. (2017), Healthy food choices are happy food choices: Evidence from a real life sample using smartphone based assessments, *Scientific Reports*, 7(1), p. 17069, DOI: 10.1038/s41598-017-17262-9.

Quality indicators of multicomponent dairy-vegetable concentrates

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Abstract

Introduction. Effective milk processing with optimal use of its components, search for new sources of protein, and partial replacement of proteins of animal origin with vegetable ones could be considered as the ways to solve the problem of protein deficiency.

Materials and methods. The dairy-vegetable concentrate was obtained from the dairy-vegetable mixture by the thermal acid precipitation of proteins. The yield (mass) was determined by the weighing method. The mass fraction of moisture was determined by the thermogravimetric method. Determination of water activity of model samples of concentrates was carried out on a hygrometer.

Results and discussion. The results of determining the yield of concentrates, their physico-chemical and sensory indicators confirmed the possibility of partial replacement of skimmed milk with whey-plant suspension with *Arachis hypogaea* fruit at the ratio of 7:3 in mixtures for thermal acid precipitation of proteins. Dairy-vegetable concentrates had a more developed spatial configuration compared to control. It is the "framework" of the samples obtained with the maximum content of vegetable coagulant that determines the greater rigidity of the structure, and therefore provides the best structural and mechanical characteristics and, accordingly, the moisture-retaining capacity. According to the indicator of water activity (Aw), which shows the influence of enzymatic, microbiological and other processes on the intensity of reactions, the predicted rational storage conditions for milk-plant concentrates are a temperature of 4±2 °C and a duration of 72 hours. It was determined that the evaporation of the main part of moisture from the protein-vegetable concentrate was slower by 3±0.5 min compared to the milk-protein concentrate, due to the presence of *Arachis hypogaea* proteins and carbohydrates that bind free water.

Conclusions. The feasibility of using the fruits of *Arachis hypogaea* in mixtures for thermal acid precipitation of proteins to obtain milk-plant concentrates with appropriate functional properties and increased biological value due to a more balanced amino acid composition has been proven.

Introduction

Dairy-vegetable products are widely developed and introduced into the human diet (Onopriichuk et al., 2023). The composition of essential nutrients in such products is optimized, which is ensured by the selection and combination of proteins of animal and vegetable origins (Liem et al., 2019; Savarino et al., 2021). The active use of Non-Dairy Milk is associated both with the individual intolerance of lactose and/or milk casein in a large number of consumers, as well as with the popularization of vegetarianism and the physiological benefits of vegetable protein consumption, especially in herodietic nutrition (Makinen et al., 2016; Sethi et al., 2016). There are known production of plant-based milk alternatives manufactured from nuts, legumes and cereal crops, which have a pleasant taste and smell and are also comparable by the sensory properties with traditional dairy raw materials (Cichońska et al., 2021; Rybak, 2016). As a result of precipitation of plant milk proteins and/or its mixtures with cow milk, concentrates are obtained, among which soy ones are especially common (Zheng et al., 2020). The above raw materials are used as an alternative to animal milk. And this, to a certain extent, serves to solve the problem of a general protein deficiency in the diet of the population.

The fruits of *Arachis hypogaea*, commonly known as peanuts, which have a high nutritional value due to the presence of a significant amount of easily digestible proteins and fats, could serve a suitable raw material for the production of vegetable milk. The fat (about 50%) of peanuts contains about 20% saturated and 80% unsaturated fatty acids, of which oleic and linoleic acids occupy the largest share (Ciftci et al., 2022). The protein composition of *Arachis hypogaea* fruit kernels is represented by such proteins as albumins, globulins, and glutelins. The high biological value of peanut proteins is due to their content of 8 essential amino acids and 10 replaceable amino acids, which brings it close to animal proteins by composition. It is known that the *Arachis hypogaea* fruits is rich by such amino acids as arginine, glycine, leucine, alanine and methionine, but others are present in small amounts (Cichońska et al., 2021; Rybak, 2016). The vitamin composition of Arachis hypogaea fruits is characterized by a high content of vitamins, mg/100g: E, 6.93; B1, 0.438; B2, 0.098; B3, 13.5; B5, 1.4; B6, 0.256, and B9, 145 (Settaluri et al., 2012). One of the functional components of peanuts is resveratrol, which has antioxidant, anticarcinogenic, and anti-inflammatory properties (Salesa et al., 2014). The polyphenolic composition of *Arachis hypogaea* fruits is mainly represented by n-coumaric and ferulic acids, esterified derivatives of n-coumaric and hydroxybenzoic acids (Dubinina et al., 2017). The above-mentioned polyphenolic compounds have antioxidant properties (Pavlyuk et al., 2019). Therefore, *Arachis hypogaea* fruits have a high content of biologically active substances, which makes it possible to recommend it for use in the technology of multicomponent milk plant concentrates. Products with a multicomponent composition are good for health and have a lower calorie content compared to traditional ones (Bocker et al., 2022). In addition to direct consumption, plant milk is also used as a basis for the preparation of traditional dairy products such as cream, yogurt, cheeses and ice cream (Gomes et al., 2021; Montemurro et al., 2021).

The aim of the present research was to study the quality indicators of multicomponent concentrates obtained by thermal acid precipitation of proteins from the dairy-vegetable mixture of the skimmed milk with the fruits of *Arachis hypogaea*.

Materials and methods

Preparation of dairy-vegetable concentrates

Skimmed milk with a mass fraction of solids 11.5%, protein 3.6%, active acidity 6.5, which was produced by separating and removing the fat from whole milk, was chosen as the dairy base.

Dairy-vegetable suspension was added to skimmed milk in amounts of 10, 20, 30, and 40%, which corresponds to the ratio of components in milk-vegetable mixtures as 9:1, 4:1, 7:3, 3:2. They had an active acidity of 6.7±0.1 pH units, with the size of the dispersed phase ranging from 10 to 60 μm. To obtain the milk-plant suspension of *Arachis hypogaea* fruits, they were pre-soaked and left to swell for 8-10 hours, washed 2-3 times, poured with low-fat milk whey in a ratio of 5:1 to the fruits, after which the resulting mixture was ground to a homogeneous state for 5-7 minutes in a disperser with a speed of 1000 s^{-1}. The resulting dairy-vegetable mixture was filtered and added to the prepared skimmed milk.

The dairy-vegetable concentrate was obtained from the dairy-vegetable mixture by the method of thermal acid precipitation of proteins. The acidity and the amount of coagulant were selected in such a way as to reach the pH of the mixture in the range of 5.3–5.8, which corresponds to the isoelectric point of casein and the main fractions of plant proteins. The coagulation process was carried out at a temperature of 92±2 °C by addition of the dairy-vegetable mixture to whey, the acidity of which was 150±10 °T in a ratio of 1:10 ÷ 5:10 and holding for 4±1 min. Under these conditions, the pH of the mixture reaches the isoelectric point of the proteins, the salt bonds of its particles are destroyed, and a concentrate is formed, which is then subjected to self-pressing for (30±2) min. A milk-protein concentrate without the addition of a dairy-vegetable mixture was used as control.

Methods

Order of the research. During the experiments, standard and well-known methods to determine quality indicators were used.

The yield (mass) was determined by the weighing method (in grams): the sample was weighed after self-pressing the concentrate obtained from 1 dm^3 of the dairy-vegetable mixture.

The water-retaining capacity (WRC) of multicomponent dairy-vegetable and milk-protein concentrates was determined by method based on the determination of the water amount that is released from the product by light pressing, which is absorbed by filter paper.

The mass fraction of moisture was determined by the thermogravimetric method on the ADGS 50 series electronic laboratory scale-hygrometer.

Determination of moisture. Determination of moisture was carried out by the thermogravimetric method on a laboratory electronic hygrometer ADGS 50 manufactured by the company "AXIS" (Poland). The method consists in determining the mass of the studied sample before and after drying it by heating to a temperature not higher than 160 °C. The hygrometer scales are equipped with general-purpose laboratory weights of accuracy class 3 with a built-in drying device and have the following characteristics: the largest/smallest weighing limit 50/0.02 g; discreteness of reading mass values is 0.5 mg; limit of permissible

error of mass determination is 0.5 mg; the limit of the permissible error of moisture determination is 0.3%.

Evaporation of moisture from the sample during heating leads to a decrease in its mass, which makes it possible to calculate the moisture content in the sample, which was before the sample drying process, based on the mass measurement data. Determination of moisture in the sample can be carried out with the same accuracy at significantly different values of the drying temperature of the sample (the difference will be only at the time of conducting the study). The principle of moisture determination consists in automatic mass measurement before, during and after drying of samples. Moisture is calculated automatically by the hygrometer according to the formula (Grek et al., 2017). Automatic termination of the drying procedure occurs if the difference between several consecutive measurements of the mass of the sample does not exceed 20 mg.

Photomicrographs of multicomponent dairy-vegetable and milk-protein concentrates were obtained using a fluorescence and phase-contrast microscope XSP-139A-TP (Manufactured by Shanghai Sanshen Medical Instrument Co., LTD, China) with a Canon-66 digital camera (at a magnification of x 600). Structural and mechanical characteristics were studied after long-term pressing of concentrates and maximum extraction of whey.

Determination of water activity of model samples of concentrates was carried out on a Rotronic hygrometer of the Hygro Palm AW modification (Switzerland). Measurement range: water activity 0–1 aw (0–100 %), temperature from minus 10 to plus 60 °C. Limits of absolute error±1% (± 0.008 aw),±0.1 °C. The range of application of the electronic unit is 0–100 %, minus 20 to plus 60 °C. The hygrometer consists of a manual unit with a display, control keys and a water activity measuring probe. The analysed sample is taken into a container and placed in the measuring chamber. A water activity probe is installed from above. The electrodes provide a signal based on the relative humidity in the closed chamber, which is converted by software. The measurement cycle lasts 3–5 minutes at room temperature, after which the water activity and temperature values are shown on the screen.

Statistical analysis

Data were expressed as means±standard deviations for triplicate determination. Differences were considered to be significant at validity of α=0.95.

Results and discussion

Determination of the optimal ratio of skimmed milk and dairy-vegetable suspension in mixtures for precipitation

The maximum possible level of replacement of skimmed milk with dairy-vegetable suspension in mixtures for precipitation was determined, taking into account the yield, physico-chemical and sensory indicators of multicomponent dairy-vegetable concentrates. Model samples were prepared from skimmed milk and dairy-vegetable suspension in the ratio of components 9:1, 4:1, 7:3, 3:2, the active acidity of them was pH 6.7±0.1. The choice of the optimal ratio was based on the preservation of normative physico-chemical indicators characteristic of milk-protein concentrates, which can be the basis for the production of various types of cheese products. In addition, the intensity of precipitation of proteins of

dairy-vegetable mixtures and the duration of high-temperature processing, which depends on the yield of concentrates, were taken into account (Kamal et al., 2021).

The change in yield and mass fraction of moisture of milk-plant concentrates depending on the ratio of components in mixtures with *Arachis hypogaea* fruits is shown in Figure 1.

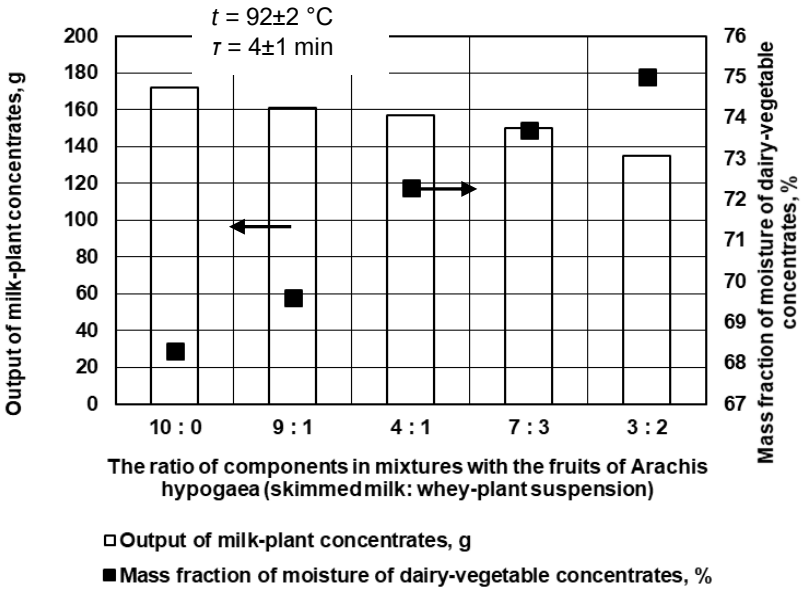


Figure 1. Yield and mass fraction of moisture of milk-plant concentrates depending on the ratio of components in mixtures with *Arachis hypogaea* fruits

The obtained concentrates are polydisperse colloidal systems, in which the dispersion medium is whey, and the dispersed phase is proteins and fats of plant and animal origin. The stability of protein globules is due to the conformation of the particles, the determined charge and the presence of a hydrated shell (Ifeanyi et al., 2021). For the separation of milk and vegetable proteins, it is necessary to break the balance of at least two factors of stability, which occurs during thermal denaturation (Dumpler et al., 2020). This process is accompanied by a change in the configuration, hydration and aggregate state of the particles, which become less stable (Huppertz et al., 2016). In addition, the degree of thermal denaturation of protein globules in dairy-vegetable mixtures depends on the temperature and duration of heating (Čurlej et al., 2022). The use of the proposed heat and time regimes makes it possible to carry out simultaneous coagulation of both milk proteins – caseins, albumins, globulins, and vegetable proteins – glutelins. The main part of the fat contained in the fruits of *Arachis hypogaea* is concentrated together with the proteins during precipitation, the rest is lost in the whey. Because of heating dairy-vegetable mixtures to a temperature of 92±2 °C in the presence of a coagulant (acidic whey), proteins co-precipitate in a single protein complex, which also includes a fat component.

The results of the research (Figure 1) showed a tendency to decrease the yield and increase the mass fraction of moisture of the obtained dairy-plant concentrates with an increase in the amount of introduction of the dairy-vegetable suspension from the fruits of *Arachis hypogaea*. Under the same conditions of the thermal acid precipitation process with a change in the ratio in mixtures from 9:1, 4:1, 7:3 to 3:2, a decrease in the yield of dairy-

vegetable concentrates by 4.16–10.11% compared to milk-protein concentrate is observed obtained exclusively from dairy raw materials. This effect is associated with a decrease in the total mass fraction of protein in mixtures in which a part of skimmed cow milk is replaced by whey and vegetable components of *Arachis hypogaea* fruits.

It was shown that the peculiarities of the composition of dairy-vegetable mixtures affect the process of dehydration of concentrates. The indicators of the mass fraction of moisture in the concentrates obtained under the same pressing conditions increased from 68.25 to 73.36%, depending on the ratio of components in the mixtures (Fig. 1). In particular, dairy-vegetable concentrates, which were obtained from skimmed milk and dairy-vegetable suspension in a ratio of 9:1, had a creamier consistency compared to the control sample. With a change in the ratio in the mixture to 3:2, concentrates with a delicate pasty consistency were obtained, while the process of whey separation (self-movement) was prolonged.

The change in the moisture-retaining capacity of multicomponent dairy-vegetable concentrates depending on the ratio in mixtures of skimmed milk and dairy-vegetable suspension with *Arachis hypogaea* fruits was studied, which shows the effect on the structure and quality indicators of the finished product (Figure 2).

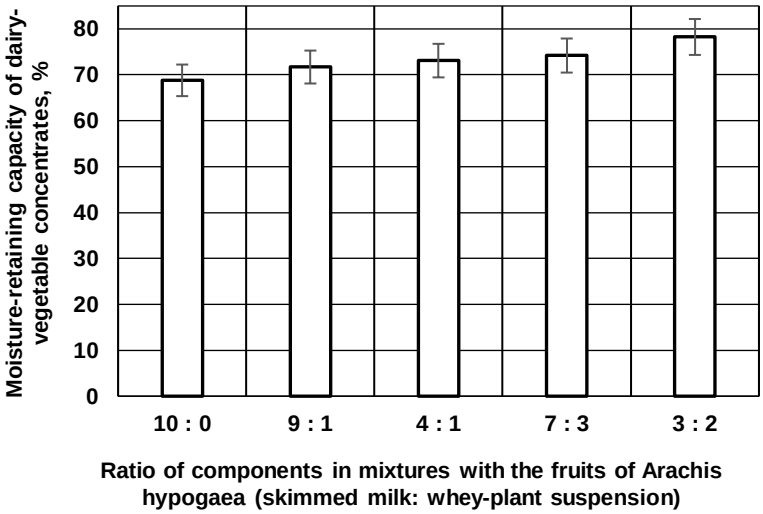


Figure 2. Changes in the moisture-retaining capacity of milk-vegetable concentrates depending on the ratio in mixtures of skimmed milk and dairy-vegetable suspension with *Arachis hypogaea* fruits

The results of research (Figures 2, 3) indicate an increase in the strength of multicomponent dairy-vegetable concentrates with an increase in the amount of dairy-vegetable suspension with the fruits of *Arachis hypogaea*. The moisture-retaining capacity of the model samples of the concentrates increased with the compaction of their structure. Thus, when the ratio of components in the mixtures was 3:2, the indicator of moisture retention was the highest and was 78.25±0.1%, which indicates an active decrease in the surface charge of micelles, which is a factor of colloidal destabilization of the protein phase of the mixtures (Anema et al., 2013).

According to the results of the quality indicators and yield of multicomponent milk-vegetable concentrates, the optimal ratio in mixtures of skimmed milk and dairy-vegetable suspension with *Arachis hypogaea* fruits was determined at the level of 7:3. Such concentrates retain a milky taste and aroma with a light peanut flavour, a uniform paste-like consistency. At a ratio of skimmed milk to dairy-vegetable suspension 3:2, a decrease in the yield of the concentrate by 22.62% was observed, with a too pronounced taste and aroma of peanuts.

Figure 3. Visualization of milk-vegetable concentrate obtained from a mixture with a ratio of skimmed milk and dairy-vegetable suspension with *Arachis hypogaea* fruits – 7:3

Polycomponent microstructure of dairy-vegetable concentrates

For the production of multicomponent dairy-vegetable concentrates of appropriate quality, it is necessary to take into account the changes that occur with the protein component in the mixtures during thermal acid deposition (Oliveira et al., 2022). One of the main parameters of such concentrates is the consistency of the product (Janahar et al., 2022). The structure of the concentrates was recorded microscopically using a fluorescent and phase-contrast XSP-139A-TP microscope with a Canon-66 digital camera (at a magnification of x 600).

In order to determine the features of the structure of the dairy-vegetable concentrate obtained from a mixture with the optimal ratio of skimmed milk and whey-plant suspension with the fruits of *Arachis hypogaea* – 7:3, comparative studies of the microstructure of the control and experimental samples were conducted (Tables 1, 2).

Table 1

Parametric characteristics of the concentrates microstructure, μm

Type of concentrate	Areas with compacted protein structure	Fat splash	Microvoids
Milk-protein (control)	60–380	–	35–120
Dairy-vegetable	50–520	7.5–11.5	35–90

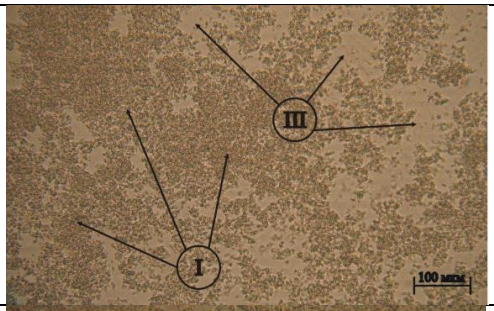
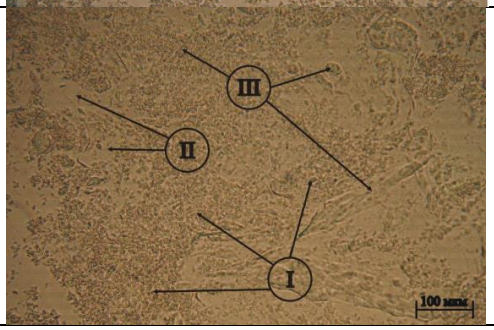
The microstructure of the dairy-vegetable concentrate consists of areas with a compacted protein structure with interspersed fat and microvoids of irregular shape and has differences compared to the milk-protein (control) (Tables 1, 2).

The microstructures of dairy-vegetable and milk-protein concentrates, having much in common, differ in the number of fat inclusions distributed in the protein phase in the form of large and small particles. However, there is a uniform distribution of fat with high dispersion – 5–7 microns.

It is obvious (Table 2) that the microstructure of the dairy-vegetable concentrate had different features from the control.

Table 2

Characteristics of the concentrates' microstructure

Type of concentrate	Photomicrographs of concentrates	Characteristics of the microstructure
Milk-protein (control)		Homogeneous, consisting of coarsely dispersed and finely dispersed protein aggregates (I), between which microvoids of irregular shape are located (III)
Dairy-vegetable		Solid, consisting of finely dispersed protein aggregates (I), between which there are single inclusions of vegetable fat with an average size of 10 μm (II), single inclusions of fibers of vegetable raw materials with a length of up to 60 μm (III)

The microstructure of the milk-vegetable concentrate had different features from control (Table 2). They have a pronounced homogeneous consistency. Their microstructure is uniform and includes evenly distributed micropores of minimal size with fat inclusions. In the micrographs of concentrates obtained from a mixture of skim milk and dairy-vegetable suspension with *Arachis hypogaea* fruits – 7:3, the protein structure consists of particles more densely connected to each other than in the control sample. The structure of the concentrate has a more developed spatial configuration – a "framework" characteristic of tightly dispersed systems, which prevents the free mutual movement of its links (Oliveira et al., 2022). Greater stiffness of the structure provides the best structural and mechanical characteristics and moisture retention capacity for the experimental sample. In samples of dairy-vegetable concentrate, such a component as fat is distributed evenly in the form of small inclusions. The dispersion of fat components in model samples is determined by the structure of the protein concentrate.

State of moisture and water activity in multicomponent dairy-vegetable concentrates

In order to determine the qualitative and quantitative state of moisture in the multicomponent milk-vegetable concentrate in comparison with control, the dynamics of water evaporation from model samples was determined on electronic scales-hygrometers of the company "AXIS", presented in Figure 3.

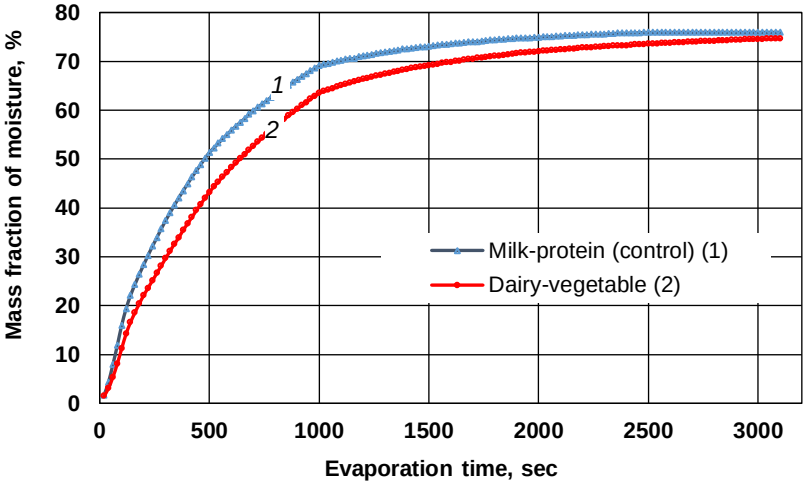


Figure 3. Dynamics of moisture evaporation from concentrates: milk-protein (control) and milk-vegetable with a ratio of components in the mixture of 7:3

According to the measurement results, the extraction of the main part of moisture (free) from the sample of milk-protein concentrate (control) occurred more intensively by 15.8±0.1% compared to the dairy-vegetable concentrate. During thermoacidic coagulation, irreversible reactions of deposition of protein substances occur with the loss of native properties, which is accompanied by the unfolding of polypeptide chains of protein molecules (Verruck et al., 2019). Because of such chain transformations and destruction of tertiary and secondary structures, hydrophobic groups appear on the surface of protein molecules. At the same time, protein substances lose their solubility, aggregate and precipitate (Li et al., 2021; Osman et al., 2020). Probably, the interaction of skimmed milk and whey proteins with the components of *Arachis hypogaea* fruits leads to the formation of additional complexes that differ in the presence of strong bonds between them. From a practical point of view, the use of a whey-plant suspension with the fruits of *Arachis hypogaea* in the composition of mixtures for thermoacidic coagulation of proteins will provide a higher amount of bound moisture.

The study of water activity of multicomponent dairy-vegetable concentrates was carried out according to the methodology that has already been used for milk-protein concentrates and is sufficiently informative (Sukmanov and Sklyarenko, 2012). Technological and consumer properties, the shelf life of such products are largely determined by the properties of the water contained in them. The level of water activity records the impact of the intensity of reactions, such as lipid oxidation, the activity of enzymatic, microbiological and other processes. With the help of the water activity indicator, a relationship is found between the presence of water available for microorganisms in the product and the probability of vital

activity of certain types of microflora (Allen, 2018). Thus, by controlling the "water activity" (Aw) indicator, it is possible to predict optimal conditions and storage capacity. According to the level of "water activity", products with high (Aw = 1.0–0.9), intermediate (Aw = 0.9–0.6) and low moisture content (Aw = 0.6–0.0). The value of "water activity" in multicomponent milk plant concentrates is presented in Table 3.

Table 3

Water activity (A_W) in multicomponent dairy-vegetable concentrates (n = 3, p≥0.05)

Type of concentrate	Ratio of components in mixtures		Water activity A_W
	Skimmed milk	Whey-plant suspension with *Arachis hypogaea* fruits	
Milk-protein (control)	10	–	0.955
Dairy-vegetable	9	1	0.958
	4	1	0.963
	7	3	0.972
	3	2	0.974

The studied dairy-vegetable concentrates belong to high-moisture foods (A_w = 1.0–0.9). Most of the moisture is in a free, unbound state, which enables the development of biochemical and oxidative processes (Grek et al., 2019). Such concentrates have a rather limited shelf life under appropriate temperature conditions (Trmcic et al., 2016). The difference in values of water activity between control and test samples is not significant, which gives reason not to specify the conditions of storage of these concentrates, but to apply the parameters accepted in production conditions: temperature 4±2 °C and duration 72 hours.

Conclusions

1. The feasibility of using the fruits of *Arachis hypogaea* in mixtures for thermal acidic precipitation of proteins to obtain milk-plant concentrates with appropriate functional properties and increased biological value due to a more balanced amino acid composition has been proven. The results of determining the yield of concentrates, their physico-chemical and organoleptic indicators confirmed the possibility of partial replacement of skimmed milk with whey-plant suspension with *Arachis hypogaea* fruits at the ratio 7:3 in mixtures for thermal acidic precipitation of proteins.
2. The microstructure of samples of concentrates obtained from a mixture of skimmed milk and whey-plant suspension with *Arachis hypogaea* fruits with the ration 7:3 was studied. It was found that dairy-vegetable concentrates had a more developed spatial configuration compared to control. It is the "framework" of the samples obtained with the maximum content of vegetable coagulant that determines the greater rigidity of the structure, and therefore provides the best structural and mechanical characteristics and, accordingly, the moisture-retaining capacity.
3. According to the indicator of water activity (Aw from 0.974 to 0.955), rational conditions and the ability to store milk-plant concentrates are predicted: temperature 4±2 °C and duration 72 hours. It was determined that the better release of the main part of moisture from the protein-vegetable concentrate was slower by 3±0.5 min compared to the milk-protein concentrate, due to the content of *Arachis hypogaea* proteins and carbohydrates that bind free water.

References

Abdualrahman A. (2013), Chemical, in-vitro protein digestibility, minerals and amino acids composition of edible peanut seeds (*Arachis hypogaea* L.), *Science International*, 1(6), pp. 199–202.

Allen L. (2018), Quality control: Water activity considerations for beyond-use dates, *International Journal of Pharmaceutical Compounding*, 22, pp. 288-293.

Anema S., De Kruif C. G. (2013), Protein composition of different sized casein micelles in milk after the binding of lactoferrin or lysozyme, *Journal of Agricultural and Food Chemistry*, 61, DOI: 10.1021/jf401270h.

Bocker R., Silva E.K. (2022), Innovative technologies for manufacturing plant-based non-dairy alternative milk and their impact on nutritional, sensory and safety aspects, *Future Foods*, 5, DOI: 10.1016/j.fufo.2021.100098.

Ciftci S., Suna G. (2022), Functional components of peanuts (*Arachis hypogaea* L.) and health benefits: A review, *Future Foods*, 5, pp. 100140, DOI:10.1016/j.fufo.2022.100140.

Čurlej J., Zajác P., Čapla J., Golian J., Benešová L., Partika A., Fehér A., Jakabová S. (2022), The effect of heat treatment on cow's milk protein profiles. *Foods (Basel, Switzerland)*, 11(7), pp.1023, DOI: 10.3390/foods11071023.

Dubinina A. A., Lenert S. O., Khomenko O. O., Cherevychna N. I. (2017), Study of the chemical composition and the quality assessment of peanut varieties adapted for cultivation in Ukraine, KhDUHT, Kharkiv.

Dumpler J., Huppertz T., Kulozik U. (2020), Invited review: Heat stability of milk and concentrated milk: Past, present, and future research objectives, *Journal of Dairy Science*, 103, 12, pp. 10986-11007, DOI.org/10.3168/jds.2020-18605.

Gomes I., Venâncio A., Lima J., Freitas-Silva O. (2021), Fruit-based non-dairy beverage: A new approach for probiotics, *Advances in Biological Chemistry*, 11, pp. 302-330, DOI: 10.4236/abc.2021.116021.

Grek O., Onopriichuk O., Tymchuk A., Chubenko L. (2017), Determination of moisture connection forms of protein-herbal clots, *Ukrainian Food Journal*, 6(3), pp. 534-542, DOI:10.24263/2304-974X-2017-6-3-13.

Grek O., Tymchuk A., Tsygankov S., Onopriichuk O., Savchenko O., Ochkolyas O. (2019), Research of recipe components influence on the properties of dairy-protein mashes for semi-finished products, *Eastern-European Journal of Enterprise Technologies*, 6(11 (102), pp. 41–48, DOI: 10.15587/1729-4061.2019.183549.

Huppertz T. (2016), Heat stability of milk. *Advanced Dairy Chemistry*, pp. 179-196, DOI: 10.1007/978-1-4939-2800-2_7.

Janahar J. J., Balasubramaniam V. M., Jimenez-Flores R., Campanella O. H., García-Cano I., Chen D. (2022), Pressure, shear, thermal, and interaction effects on quality attributes of pea–dairy protein colloidal dispersions, *Food Hydrocolloids*, 107811, DOI: 10.1016/j.foodhyd.2022.107811.

Kamal H., Le C. F., Salter A. M., Ali A. (2021), Extraction of protein from food waste: An overview of current status and opportunities, *Comprehensive Reviews in Food Science and Food Safety*, 20, pp. 2455–2475, DOI: 10.1111/1541-4337.12739.

Li H., Zhao T., Yu J. (2021), Effect of heat treatment on the property, structure, and aggregation of skim milk proteins, *Frontiers in Nutrition*, 8, pp. 714869, DOI: 10.3389/fnut.2021.714869.

Liem D. G., Russell C. G. (2019), The influence of taste liking on the consumption of nutrient rich and nutrient poor foods, *Frontiers in Nutrition*, 6, pp. 174-188, DOI: 10.3389/fnut.2019.00174.

Makinen O. E., Wanhalinna V., Zannini E., Arendt E. K. (2016), Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products, *Critical Reviews in Food Science and Nutrition*, 56(3), pp. 339–349, DOI:10.1080/10408398.2012.761950.

Montemurro M., Pontonio E., Coda R., Rizzello C. G. (2021), Plant-based alternatives to yogurt: State-of-the-art and perspectives of new biotechnological challenges, *Foods*, 10, pp. 316, DOI: 10.3390/foods10020316.

Oliveira I. C., de Paula Ferreira I. E., Casanova F., Cavallieri A. L. F., Lima Nascimento L. G., de Carvalho A. F., Nogueira Silva N. F. (2022), Colloidal and acid gelling properties of mixed milk and pea protein suspensions, *Foods*, 11, pp. 1383, DOI: 10.3390/foods11101383.

Osman M., Abdalla M., Mohammed H., Salih A. (2020), Effect of heat treatment of milk on the physicochemical, microbiological and sensory characteristics of white cheese (*Gibna bayda*). *GSC Advanced Research and Reviews*, 3, pp. 20-28, DOI: 10.30574/gscarr.2020.3.3.0044.

Pavlyuk R., Pogarskaya V., Balaba K., Kravchuk T., Pogarskiy A. (2019), Of sour-milk healthy beverages with the use of natural herbal, *Food Science and Technology*, 13(4), DOI:10.15673/fst.v13i4.1566.

Rybak O. (2016), Milk fat in structure formation of dairy products: a review, *Ukrainian Food Journal*, 5(3), pp. 499-514, DOI:10.24263/2304-974X-2016-5-3-9.

Salesa J. M., Resurreccion A. V. A. (2014), Resveratrol in peanuts, *Food Science and Nutrition*, 54(6), pp. 734–770, DOI: 10.4236/fns.2012.312215.

Savarino G., Corsello A., Corsello G. (2021), Macronutrient balance and micronutrient amounts through growth and development, *Italian Journal of Pediatrics*, 47**,** pp. 109-121, DOI: 10.1186/s13052-021-01061-0.

Savchuk Yu., Usatiuk S. (2017), Methods of receiving of proteinageous products from plant raw materials, *Podilian Bulletin: Agriculture, Engineering, Economics*, 26, pp. 64-71.

Sethi S., Tyagi S. K., Anurag R. K. (2016), Plant-based milk alternatives an emerging segment of functional beverages: a review, *Journal of Food Science and Technology*, 53(9), pp. 3408–3423, DOI:10.1007/s13197-016-2328-3.

Settaluri V. S., Kandala C. V. K., Puppala N., Sundaram J. (2012), Peanuts and their nutritional aspects − A review, *Food and Nutrition Sciences*, 3, pp. 1644–1650, DOI: 10.4236/fns.2012.312215.

Sukmanov V. A., Sklyarenko E. V. (2012), Study of water activity in fresh soft cheeses, *Scientific works of Odessa National Academy of Food Technology*, 41(1), pp. 18-22. (In Ukrainian).

Trmcic A., Ralyea R., Meunier-Goddik L., Donnelly C., Glass K., D'Amico D., Meredith E., Kehler M., Tranchina N., McCue C., Wiedmann M. (2016), Consensus categorization of cheese based on water activity and pH—A rational approach to systemizing cheese diversity, *Journal of Dairy Science*, Doi: 100. 10.3168/jds.2016-11621.

Verruck S., Sartor S., Marenda F. B., Barros F., Silva E., Canella C., Prudencio M. H. (2019), Influence of heat treatment and microfiltration on the milk proteins properties, *Advances in Food Technology and Nutritional Sciences*, 5(2), pp. 54-66, DOI: 10.17140/AFTNSOJ-5-157.

Zheng L., Regenstein J.M., Teng F., Li Y. (2020), Tofu products: A review of their raw materials, processing conditions, and packaging, *Comprehensive Reviews in Food Science and Food Safety*, 19, pp. 3683–3714, DOI: 10.1111/1541-4337.12640.

Comparison of characteristics of sweet cherry varieties grown in Georgia and their changes during the storage

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Abstract

Introduction. The aim of this research was to study the changes in the quality of sweet cherries grown in Georgia during their storage.

Materials and methods. Characteristics of sweet cherry varieties such as weight loss, titratable acidity, and content of total soluble solids, total phenolic compounds and anthocyanins, as well as the antioxidant activity were monitored at the beginning and after 21 and 42 days of storage in the fridge chamber at the temperature of 0–1 ºC and the relative humidity of 90–95%.

Results and discussion. Three commercially produced varieties of sweet cherry, particularly, Cordia, Regina and Sweetheart, were chosen for study. Cherry variety Cordia contained the highest amount of total phenolic content (TPC), 213.95mg100g^{-1}, and anthocyanins, 145.7mg100g^{-1}, compared to the other varieties, meanwhile variety Sweetheart contained the lowest amount of TPC, 116.0mg100g^{-1}, and anthocyanins, 29.75mg100g^{-1}. A positive correlation between the phenolic content and antioxidant activity for studied varieties was observed. The best correlation was found for Regina cherry (R^2=0.98), meanwhile for Cordia and Sweetheart it was 0.88 and 0.83, respectively. The physico-chemical properties of sweet cherries changed during the storage: content of total soluble solids, titratable acidity, total phenolic content, anthocyanins content, and antioxidant activity decreased gradually.

The total decrease in fruit mass after 42 days was measured together with the microbiological losses. Sweetheart has the highest weight loss compared to the others.

Based on the three-year data, total weight loss in Cordia was 10.13% at the end of the storage period (42 days) at temperature 0–1 ºC and relative humidity 90–95%. This value was 13.51% for Regina and 14.49% for Sweetheart variety.

Conclusion. The studied three sweet cherry varieties are close by their chemical composition. However, it can be concluded that Cordia is the best in terms of the content of valuable substances and storage stability.

Introduction

Sweet cherries (*Prunus avium* L.) are one of the most popular seasonal fruit, which is highly appreciated by consumers for its taste, attractive appearance and nutritional quality (Antognoni et al., 2022; Faniadis at al., 2010; Gabriele et al., 2013; Pirog et al., 2022; Usenik et al., 2005; Wani et al., 2002). According to FAO the world's total sweet cherry production was estimated as 2,609,550 metric tons, and in Georgia sweet cherry production is increased by almost 31% in 2016-2020 (FAO, 2020).

Attractive colour, sweetness, sourness, firmness, high content of antioxidants and nutrients are the main characteristics for cherry quality (Esti et al., 2002; Gabriele et al., 2013; Nunes et al., 2021; Usenik et al., 2005). The health benefits of cherry intake are linked mainly to strong antioxidant activities (Nunes et al., 2020; Yoo et al., 2010), supporting the potential preventive health benefits in relation to several chronic diseases including cardiovascular disease, diabetes, and arthritis (Ferretti et al., 2010; Jacob et al., 2003; Mamani-Matsuda et al., 2006; Mc Cune et al., 2011; Wani et al., 2002). Sweet cherries are also rich in phenolic acids such as hydroxycinnamic acid derivatives (neochlorogenic acid, p-coumaroylquinic acid and chlorogenic acid) (Liu et al., 2011; Usenik et al., 2010). These components are important for their potential contribution to the colour of the cherry fruits through co-pigmentation with anthocyanins (Mazza & Brouillard, 1990; Mozetic et al., 2002). Moreover, strong correlations were found between the phenolic content and antioxidant activity of fruits (Serra et al., 2011; Tobar et al., 2019; Usenik et al., 2008).

Commercial cultivation and storage of sweet cherries is generally difficult and expensive. High levels of care in transportation and storage should be taken to achieve premium quality fresh fruit for serving in the market (Looney & Jackson, 2011). Cherry is highly perishable with a limited shelf life of 7–10 days (Wani et al., 2002). Low temperature is the basic parameter for storing; it keeps the fruit firm longer and reduces the color loss (Shick and Toivonen, 2002). Reported as recommended conditions to store cherries is the temperature range 0 to 2 °C and the relative humidity of 90 to 95% (Crisosto et al., 1993; Looney et al., 1996; Suran et al., 2019). The narrow harvest season together with sweet cherry soft texture limits availability of this fruit in the market over a longer period (Yoo et al., 2010), and in conventional storage conditions, the shelf life of cherries is very short.

The aim of the present research was to characterize the chemical compositions of sweet cherries grown in Georgia and their changes during sweet cherry storage.

Materials and methods

Chemicals

Ascorbic acid, sodium hydroxide, Folin-Ciocalteu reagent, TPTZ-2,4,6-Tris (2-pyridyl)-s-triazine, sodium carbonate, ethyl acetate, ferric (III) chloride and ethanol were purchased from Sigma-Aldrich (Steinheim, Germany), (Sigma-Aldrich, Switzerland), hydrochloric acid was provided by Merck (Darmstadt, Germany). All other reagents were commercially available at the local market and were of analytical grades.

Sweet cherry samples

Three commercially produced sweet cherry (*Prunus avium* L.), Cordia, Regina and Sweetheart, grown in the village of Jighaura (Farming of Scientific Research Center of

Agriculture), west Georgia, municipality of Mtskheta (WGS84 41° 55′ 25″ N, 44° 46′ 35″ E 41.923611, 44.776389) were selected for study. The sweet cherry samples for the experiment were harvested at the end of June and in early July. The test samples were kept in the fridge chamber at the temperature of 0–1 °C and the relative humidity of 90–95 %. Initially, during and at the end of storage the physico-chemical and antioxidant properties of sweet cherry fruit of each variety were examined and compared.

Determination of quality parameters

Quality parameters such as weight loss, total soluble solids (TSS), titratable acidity (TA), total phenolic content (TPC) and anthocyanin content (AC), as well as the antioxidant capability were monitored at the beginning, after 21 and 42 days of storage at 0–1 °C. The samples were prepared for TPC and antioxidant analysis: about 40 g of cherry fruits were squashed and weighed (5g), 70% ethanol was added to the sample. The extract was left at room temperature for 30 min and then filtered. The extracts were stored in the refrigerator at 5 °C.

To determine weight loss fruit, 15 pieces of fruit were taken in triplicate and weighted. The loss of weight was expressed as the percent from the original weight.

To measure the microbiological loss, 10 kg of cherry samples were tested in triplicates. Microbial spoilage was monitored at intervals of 5 days. Microbiological changes were observed after 21 and 42 days of storage. Samples were weighted then, and the weight loss percentage was determined compared to the original weight.

Content of total soluble solids was determined using a digital refractometer (WAY, 2S, China) according to ºBrix reading.

Titratable acidity was determined by titration with 0.1 N NaOH to a pink color using 1% phenolphthalein as indicator and expressed as g 100 g $^{-1}$ malic acid (Morris et al., 1985).

The total phenolic compound content was determined using the Folin–Ciocalteu method Bond et al., 2003). 1.0 mL of extract was diluted with 10 mL distilled water, mixed thoroughly with 1.0 mL of Folin-Ciocalteau reagent for 8 min, followed by the addition of 4 mL of 7.5% ($w \cdot v^{-1}$) sodium carbonate. The samples and standards (dilute gallic acid standard working solutions: 10-50 μg/mL) were allowed to stand at room temperature for 60 minutes in the dark, and absorbance was measured spectrophotometrically (UV/Vis spectrophotometer, A&E Lab Co LTD, UK) at 765 nm with a 10 mm path length cell, and the total phenolic compound content was calculated as mg of gallic acid equivalents per 100 gram of fresh fruit.

Determination of total anthocyanins was conducted by the pH differential method (Giusti et al., 2001). Samples were diluted 1:150 in pH 1.0 and pH 4.5 buffers, and the absorbance was measured at 520 nm and 700 nm in a UV-visible spectrophotometer (A & E Lab Co LTD, UK), based on a cyanidin 3-glucoside molar extinction coefficient of 26,900 ΔE/mol and a molecular weight of 449.2 g mol^{-1}. The resulting values were expressed in terms of mg of anthocyanin per 100 g of fresh fruit.

The ferric reducing ability of plasma (FRAP) assay was carried out as previously described by (Benzie and Strain, 1996). The experiment was carried out at 37 °C and pH 3.6 with a blank sample in parallel. In the FRAP assay, the reductants ("antioxidants") in the sample reduce the Fe(III)/tripyridyltriazine complex to the blue ferrous form, with an increase in absorbance at 593 nm. The results were expressed as mg ascorbic acid equivalents (AAE) per 100 gram fresh fruit (mg AAE100 g^{-1} FW) (Dziadek et al., 2019).

Statistical Analysis

The data represents the mean of three replicates±standard deviation (SD). Data were subjected to the t-test. All calculations were performed with Microsoft Excel (Version 4, statistical functions, Microsoft Corp., Redmond, WA, USA).

Results and discussion

The mean three-year indicators show that Cordia has a high content of dry soluble solids (20.50%) and Sweetheart has the lower (18.25%) followed with Regina (17.10%). Cordia has the highest titratable acidity (0.50%), Sweetheart is the second (0.46%) followed by Regina (0.40%) (Table 1).

Table 1

Chemical characteristics of fresh cherry fruit

Varieties	TSS, %	TA, %	TPC, $mg100g^{-1}$	AC , $mg100g^{-1}$	AAE, $mg\ 100g^{-1}$
Cordia	20.50±1.36	0.50±0.10	213.95±6.44	145.70±4.35	288.33±7.78
Regina	17.10±1.02	0.46±0.15	122.69±5.11	51.52±2.87	127.13±5.62
Sweetheart	18.35±1.58	0.40±0.10	116.00±5.02	29.75±2.10	93.33± 4.51

The data represents the mean of three replicates±SD

The total polyphenols were analyzed, and Cordia was found to have 213.95 $mg100g^{-1}$, while Sweetheart has only half of this amount and Regina has 122.69 $mg100g^{-1}$. Cordia has high content of anthocyanins and high level of antioxidant activity: 145.7 $mg100g^{-1}$ and 288.33 AAE $mg100g^{-1}$, respectively, and for Sweetheart these values are much lower: 29.75 $mg100g^{-1}$ and 93.33 AAE $mg100g^{-1}$, respectively. Thus, Cordia is a cultivar with the highest content of useful substances while Sweetheart has the lowest.

The changes in the chemical characteristics during the storage were also studied. The research showed that studied cultivars differed in terms of change of their chemical composition during the storage (Figures 1–5).

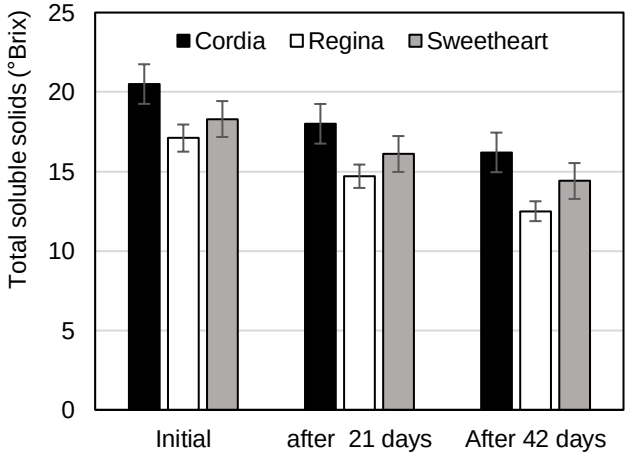

Figure 1. Change of total soluble solids (%) in cherry varieties during the storage.

The content of total soluble solids gradually decreased for all three varieties during the storage. However, the level of this decrease was different for each variety. For example, after 42 days of storage, the dry soluble solids in Cordia decreased from origin 20.5 to 16.2%, and the percentage of decrease was 20.9%. Whereas the same values for Regina and Sweetheart were 26.9 and 21.3 %, respectively.

The change in titratable acidity in the studied varieties also occurred in different ways (Figure 2).

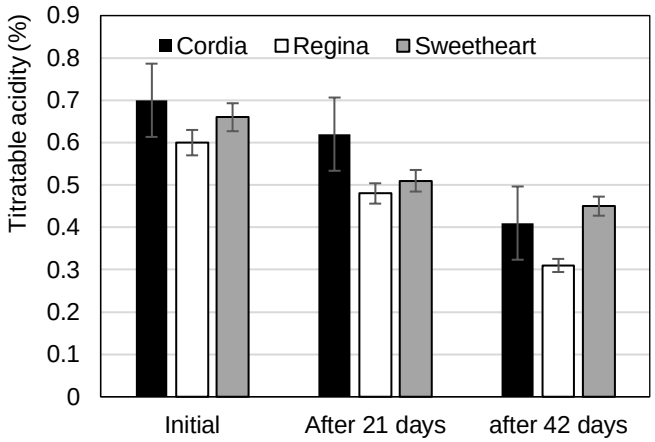

Figure 2. Change of titratable acidity (%) in cherry varieties during storage.

The reduction of sweet cherry titratable acidity during storage has been shown (Horak et al., 2016). In our case, at the end of storage period titratable acidity was reduced by 41.4% in Cordia, by 31.8% in Sweetheart and by 48.3% in Regina.

The analyzed cultivars differ in terms of decrease in total polyphenols. The Figure shows that at the end of the storage period the total polyphenols decrease by 52.6%, while in Regina this indicator is 46.7% and in Sweetheart it is 45.8%. The data illustrates that in terms of the decrease in total polyphenol there is only a little difference between the cultivars (Figure 3).

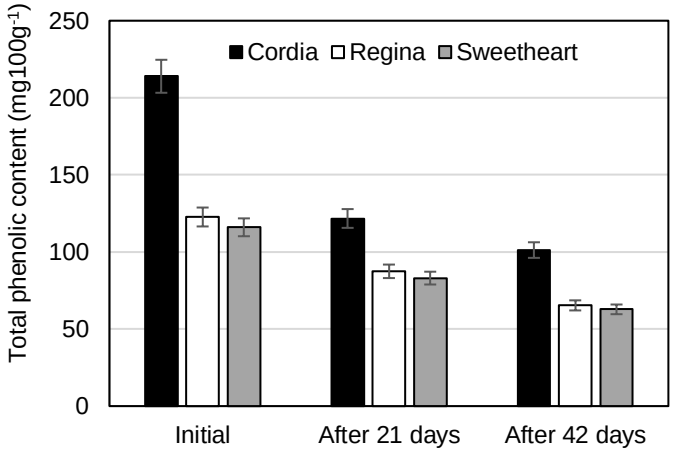

Figure 3. Change of total phenolic content (mg100g^{-1}) in cherry varieties during storage.

The analysis of the change in the anthocyanins content showed its decrease during the storage. The change was most noticeable for Sweetheart (decrease by 36.13%). The least decrease occurred in Regina. For Cordia the decrease in anthocyanins amounts to 22.92% after 42 days of storage was detected (Figure 4).

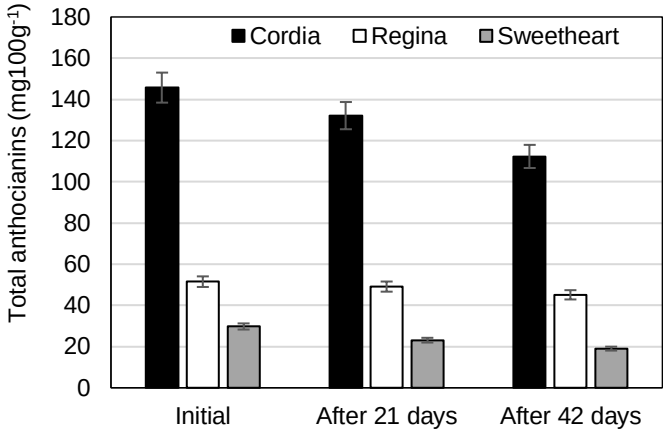

Figure 4. Change of anthocyanins content (mg100g^{-1}) in cherry varieties during storage.

The change in antioxidant activity showed that it decreased in all three varieties. The higher decrease, 36.4%, was observed in Cordia, the lowest, 14.9%, in Sweetheart, and in Regina it was 31.8% (Figure 5).

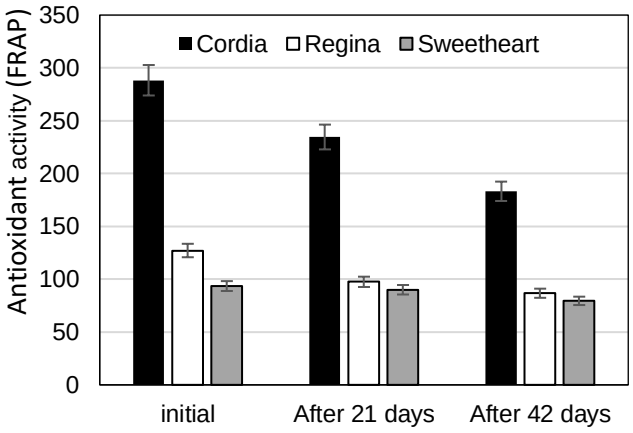

Figure 5. Change of an (FRAP) in cherry varieties during storage.

A positive correlation between the phenolic concentration and antioxidant activity of sweet cherry fruit has been shown by Tobar et al. (2019). For studied cherry varieties correlation of total phenolic content and antioxidant activities was different for studied cherry varieties. Thus, high correlation (R^2=0.98) was found for Regina cherry, meanwhile it was lower for the other varieties: in the case of Cordia R^2=0.88 and Sweetheart R^2=0.83 (Figure 6).

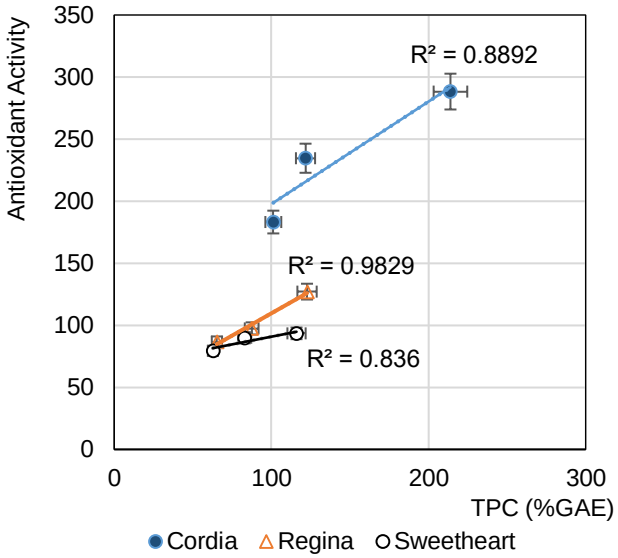

Figure 6. Correlation between total phenolic content and antioxidant activity for studied cherry fruits varieties

The cherries of the selected varieties were stored at 0–1 °C temperature and 90–95 % relative humidity. A total weight losses in fruit after 42 days was measured together with the microbiological postharvest losses. Microbiological postharvest loss is one of the major types of postharvest loss and it refers to losses caused by microorganisms like moulds, yeasts and bacteria (Bist and Bist, 2021). Based on the three-year data, the natural weight loss in Cordia was 5.83%, microbiological loss was 4.30%, and the total loss at the end of the storage period (42 days) was 10.13% (Table. 2).

Table 2

Natural, microbiological and total weight losses on different stages of cherry fruit storage

Varieties	Natural weight losses, %	Microbiological losses %	Total weight loss, %
21 days of storage			
Cordia	3.80±0.10	2.50±0.05	6.30±0.20
Regina	4.92±0.12	2.84±0.06	7.76±0.14
Sweetheart	6.12±0.17	4.38±0.01	10.50±0.23
42 days of storage			
Cordia	5.83±0.22	4.30±0.10	10.13±0.13
Regina	6.30±0.11	7.21±0.31	13.51±0.72
Sweetheart	8.23±0.78	6.26±0.50	14.49±0.36

The data represents the mean of three replicates±SD

The natural weight loss for Regina was 6.30%, microbiological losses was 2.84%, and the total loss was 13.51%. Sweetheart had the highest natural weight loss, 8.23%, compared to the others. Its microbiological loss was 6.26% and the total loss is 14.49%.

Conclusions

Comparison of three cherry varieties grown in Georgia showed that:

1. Cherry variety Cordia contained the highest amount of total phenolic compounds and anthocyanins compared to the other varieties. This variety had also a higher antioxidant activity. Cordia variety can be stored under refrigerated conditions – 0–1 ºC and 90–95% relative humidity for 42 days with 10.13% total weight loss.
2. Regina variety had a lower content of total phenols and anthocyanins and a lower antioxidant activity compared to Cordia. Cherries of this variety can be stored at 0–1ºC and 90–95% relative humidity for 42 days with 13.5% total loss. Among the studied varieties, Sweetheart had the lowest total phenolic and anthocyanin contents and antioxidant activity. While storing sweet cherries of this variety under the refrigerated conditions for 42 days, total losses was 14.49 %.
3. Based on the obtained data, it can be concluded that among studied varieties Cordia was the best in terms of storage stability and content of valuable substances.

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References

Antognoni F., Potente G., Mandrioli R., Angeloni C., Freschi M., Malaguti M., Hrelia S., Lugli S., Gennari F., Muzzi E., Tartarini S. (2020), Fruit quality characterization of new sweet cherry cultivars as a good source of bioactive phenolic compounds with antioxidant and neuroprotective potential, *Antioxidants (Basel)*, 9(8), p. 677, DOI: 10.3390/antiox9080677.

Benzie I., Strain, J. (1996), The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, *Analytical Biochemistry*, 239(1), pp. 70–76, DOI: 10.1006/abio.1996.0292.

Bist N., Bist P. (2021), Role of microorganisms in post-harvest loss of agricultural products: A review, *Sustainability in Food and Agriculture (SFNA)*, 2(1), pp. 1–4, DOI: 10.26480/sfna.01.2021.01.04.

Bond T., Lewia J., Davis A., Davis A. (2003), *Methods of polyphenols analysis*, The Royal Society of Chemistry, London.

Chiabrando V., Garavaglia L., Giacalone G. (2019), The postharvest quality of fresh sweet cherries and strawberries with an active packaging system, *Foods*, 8(8), pp. 335, DOI: 10.3390/foods8080335

Crisosto C., Garner D., Doyle J., Day, K. R. (1993), Relationship between fruit respiration, bruising susceptibility, and temperature in sweet cherries, *Horticulture Science*, 28(2), pp. 132–135, DOI: 10.21273/HORTSCI.28.2.132.

Dziadek K., Kopec A., Tabaszewska M. (2019), Potential of sweet cherry (*Prunus avium* L.) by-products: bioactive compounds and antioxidant activity of leaves and petioles, *European Food Research and Technology*, 245, pp. 763–772, DOI: 10.1007/s00217-018-3198-x.

Esti M., Cinquanta L., Sinesio F., Moneta E., Mateo, M. (2002), Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage, *Food Chemistry*, 76, pp. 399–405, DOI: 10.1016/S0308-8146(01)00231-X.

Faniadis D., Drogoudi P., Vasilakakis M. (2010), Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (Prunus *avium* L.), *Scientia Horticulturae*, 125, pp. 301–304, DOI: 10.1016/j.scienta.2010.04.013.

FAO (2020), Cherry production, *Statistical Database-Agriculture*, Food and Agricultural Organisation (FAO).

Ferretti G., Bacchetti T., Belleggia A., Neri D. (2010), Cherry antioxidants: From farm to table, *Molecules*, 15, pp. 6993–7005, DOI: 10.3390/molecules15106993.

Gabriele B., Continella A., Gentile A., Amenta M., Fabroni S., Rapisarda P. (2013), Fruit quality and bioactive compunds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy, *Food Chemistry*, 140, pp. 630–638, DOI: 10.1016/j.foodchem.2012.11.024.

Giusti M., Wrolstad R. (2001), *Current protocols in food analytical chemistry*, Hoboken: John Wiley & Sons, US.

Hancock J. (2008), *Temperate Fruit Crop Breeding*, Springer Science Business Media, USA.

Horak M., Golias J., Hic P., Nemcova A., Kulichova J. The effect of post-harvest treatment on the quality of sweet cherries during storage, *Potravinarstvo® Scientific Journal for Food Industry*, 10(1), pp. 570–577, DOI: 10.5219/667.

Jacob R., Spinozzi G., Simon V., Kelley D., Prior R., Hess-Pierce, B. (2003), Consumption of cherries lowers plasma urate in healthy women, *Journal of Nutrition*, 133(6), pp. 1826–1829, DOI: 10.1093/jn/133.6.1826.

Liu Y., Liu X., Zhong F., Tian R., Zhang K., Zhang X., Li T. (2011), Comparative study of phenolic compounds and antioxidant activity in different species of cherries, *Journal of Food Science*, 76(4), pp. 633–638, DOI: 10.1111/j.1750-3841.2011.02150.x.

Looney N., Webster A. (1996), *Cherries: crop physiology, production and uses*, Wallingford, Oxon, UK.

Looney N., Jackson M., Bunker M., Thiele G. (2011), *Temperate and subtropical fruit production*, Cambridge University Press, UK.

Mamani-Matsuda M., Kauss T., Al-Kharrat A., Rambert J., Fawaz F., Thiolat D. (2006), Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decrease macrophage inflammatory mediators, *Biochemical Pharmacology*, 72, pp. 1304–1310, DOI: 10.1016/j.bcp.2006.08.001.

Mazza G., Brouillard R. (1990), The mechanism of co-pigmentation of anthocyanins in aqueous solutions, *Phytochemistry*, 29(4), pp. 1097–1102, DOI: 10.1016/0031-9422(90)85411-8.

McCune L., Kubota C., Stendell-Hollis N., Thomson C. (2011), Cherry and health: A review, *Critical Reviews in Food Science and Nutrition*, 51(1), pp. 1–12, DOI: 10.1080/10408390903001719.

Morris, J., Sistrunk W., Sims C., Main G., Wehunt E. (1985), Effects of cultivar, postharvest storage, preprocessing dip treatments and style of pack on the processing quality of strawberries, *Journal of the American Society for Horticultural Science*, 110(2), pp. 172–177, DOI: 10.21273/JASHS.110.2.172.

Mozetič B., Trebše P., Simčič M., Hribar J. (2004), Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunus avium* L.), *LWT – Food Science and Technology*, 37(1), pp. 123–128, DOI: 10.1016/S0023-6438(03)00143-9.

Nunes A.R., Gonçalves A.C., Falcão A., Alves G., Silva L.R. (2021), *Prunus avium* L. (sweet cherry) by-products: A source of phenolic compounds with antioxidant and anti-hyperglycemic properties—A review, Applied Sciences, 11(18), 8516, DOI: 10.3390/app11188516.

Pirog T., Stabnikov V., Antoniuk S. (2022), Application of surface-active substances produced by Rhodococcus erythropolis IMB Ac-5017 for post-harvest treatment of sweet cherry, *Ukrainian Food Journal*, 11(1), pp. 164–174, DOI: 10.24263/2304-974X-2022-11-1-15.

Pirog T., Stabnikov V., Stabnikova O. (2022), Bacterial microbial surface- active substances in food- processing industry, In: Paredes-Lopez O., Stabnikov V., Shevchenko O., Ivanov V., eds., Bioenhancement and Fortification of Foods for a Healthy Diet, CRC Press, Boca Raton, London, pp. 271–294, DOI: 10.1201/9781003225287-18.

Serra A., Matias A., Almeida P., Bronze M., Alves P., Sousa C., Duarte M. (2011), Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 2. Evaluation of SCF extracts as promising natural chemotherapeutical agents, *The Journal of Supercritical Fluids*, 55(3), pp. 1007–1013, DOI: 10.1016/j.supflu.2010.06.006.

Serra A., Duarte R., Bronze M., Duarte C. (2011), Identification of bioactive response in traditional cherries from Portugal, *Food Chemistry*, 125(2), pp. 318–325, DOI: 10.1016/j.foodchem.2010.07.088.

Shick J., Toivonen P. (2002), Reflective traps at harvest reduce stem browning and improve fruit quality of cherries during subsequent storage, *Postharvest Biology and Technology*, 25, pp. 117–121.

Suran P., Zeleny L., Skrivanova A. (2019), Impact of storage technologies on length of storage period and fruit quality of sweet cherries, *Acta Horticulturae*, 1256, pp. 615–622, DOI: 10.17660/ActaHortic.2019.1256.88.

Tobar D., Ponder A., Hallmann E., Glowacka A., Rozpara E. (2019), The profile and content of polyphenols and carotenoids in local and commercial sweet cherry fruits (*Prunus avium* L.) and their antioxidant activity *in vitro*, *Antioxidants (Basel)*, 8(11), pp. 534, DOI: 10.3390/antiox8110534.

Usenik V., Fajt N., Mikulic-Petkovsek M., Slatnar A., Stampar F., Veberic R. (2010), Sweet cherry pomological and biochemical characteristics influenced by rootstock, *Journal of Agricultural and Food Chemistry*, 58(8), pp. 4928–4933, DOI: 10.1021/jf903755b.

Usenik V., Fabčič J., Štampar F. (2008), Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.), *Food Chemistry*, 107(1), pp. 185–192. DOI: 10.1016/j.foodchem.2007.08.004, DOI: 10.1021/jf903755b.

Usenik V., Kastelec D., Stampar F. (2005), Physico-chemical changes of sweet cherry fruits related to application of gibberellic acid, *Food Chemistry*, 90(4), pp. 663–671, DOI: 10.1016/j.foodchem.2004.04.027.

Wani A., Singh P., Gul K., Wani M., Langowski H. (2014), Sweet cherry (*Prunus avium*): Critical factors affecting the composition and shelf life, 1(1), *Food Packaging and Shelf Life*, pp. 86–89, DOI: 10.1016/j.fpsl.2014.01.005.

Yoo K., Al-Farsi M., Lee H., Yoon H., Lee C. (2010), Antiproliferative effects of cherry juice and wine in Chinese hamster lung fibroblast cells and their phenolic constituents and antioxidant activities, *Food Chemistry*, 123(3), pp. 734–740, DOI: 10.1016/j.foodchem.2010.05.043.

Influence of the amino acid and fractional composition of dry milk of mammals on the digestibility of dry milk mixtures

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Abstract

Introduction. The aim of the study was to determine the influence of the amino acid and fractional composition of dry milk of mammals on the digestibility of dry milk mixtures.

Materials and methods. Use of goat, mare, sheep dry milk and dry milk mixtures for baby food were studied. Amino acid composition was determined by ion exchange chromatography; fractional composition by electrophoresis; digestibility of dry milk mixtures was studied *in vitro*.

Results and discussion. The limiting amino acid of dry cow and sheep's milk proteins is cystine, the amino acid scores of which are 46.0% and 47.0%, respectively. The limiting amino acid of mare and goat milk proteins is tyrosine, the amino acid scores of which are 52.0% and 57.0%, respectively. Mare milk protein belongs to the albumin type as well as protein of human milk. Mare and sheep's milks is known to contain various protective proteins including immunoglobulin and lactoferrin. Mare and sheep's milks contain large amount of immunoglobulin A close to human milk, namely, 515% and 6.92%, respectively. The ratio of casein to whey proteins in mare milk is 1:1, in cow's milk is 4:1, and in goat and sheep's milks is 3:2. Differences in composition are due to different genotypes of mammals and protein losses during drying process. The highest degree of digestibility in human milk and the mixture based on mare's milk 'Ligans' is 717 mg and 780 mg of amino acids per 100 g of product, respectively. The lowest degree of digestibility of proteins is in the mixture based on cow's milk 'Malyutka hypoallergenic'. A low degree of digestibility can be caused by the reaction between amino acids during drying, and the formation of casein complexes with denatured whey proteins.

Conclusions. The amino acid and fractional composition of proteins of dry mare milk is close to composition of human milk proteins, so the digestibility of products based on mare milk is similar to that of human milk.

Introduction

Artificial feeding has a negative impact on the infant's health. 72% of formula-fed infants suffer from dysbacteriosis, and the number of children suffering from an allergy to cow's milk proteins is 2–7.5% (Mousan et al., 2016). Meanwhile, for artificial feeding of children, mixtures based on cow's milk are used. Allergic reactions can be caused by differences in the protein composition of human and cow's milk.

Mass fraction of casein is the largest in the protein composition of cow's milk (about 80%), and in human milk, which belongs to the albumin type, whey proteins predominate (about 65%). At the same time, there are differences in the fractional composition of casein – α-casein predominates in cow's milk, and β-casein is the main protein in human milk. The main serum protein of cow's milk is β-lactoglobulin, and α-lactalbumin and immunoglobulins are main serum proteins in human milk. Casein and β-lactoglobulin are the main allergens of milk, while immunoglobulins have protective function in the child's body (Zeng et al., 2019). Human milk proteins contain a larger number of essential amino acids compared to cow's milk proteins. In addition, the amino acids of human milk are in the most favorable ratio for children (Barreto et al., 2019).

There are three classes of immunoglobulins (A, G and M) in cow's milk, and four (A, E, G, M) in human milk. Human milk contains large amount of lactoferrin, so iron absorption for newborns during breast feeding is 80%, while iron absorption is only 20% during artificial feeding (Avershyna, 2012). The amount of iron ions in the child's body is extremely important, since iron is involved in many metabolic processes (El Amrousy et al., 2022). Lactoferrin acts as a factor that limits the amount of iron available to microorganisms in the body. Binding excess iron ions and other metals, lactoferrin deprives pathogenic flora of vital trace elements (Griffin, 2020; Lonnerdal et al., 2021).

The source of proteins for feeding babies is the milk of domestic animals, especially goat, mare, sheep, the use of which is not widespread in the production of baby food products. Therefore, the study of the amino acid and fractional composition of goat, mare, and sheep's milk proteins, as well as their influence on the digestibility of baby food products based on them, is an important task. A study of the amino acid composition of native mare's milk was conducted, but the question of the amino acid composition of dry mare's and sheep's milk remains insufficiently studied (Mazhitova et al., 2015). The results of the study of the fractional composition of proteins of native sheep's and mare's milk are also given, but the issue of the fractional composition of proteins of mare's and sheep's milk powder needs to be studied more carefully (Barreto et al., 2019; Navarro et al., 2018).

The analysis of literature data showed that the protein composition of dry sheep, goat and mare's milk, as well as its effect on digestion under *in vitro* conditions, wasn't sufficiently studied. The aim of the study is to determine the influence of the protein composition of animal milk powder on the ability of food products based on it to be digested *in vitro*.

Materials and methods

Materials

Whole cow's milk, whole goat's milk, whole mare's milk, whole sheep's milk were used for laboratory research. Milk was obtained from farms in Donetsk, Khmelnytskyi, Chernivtsi and Zakarpattia regions in Ukraine. Research was conducted in different periods of animal lactation. Human milk samples were collected during the lactation period of 1–8 weeks at Children's Clinical Hospital No. 8, Kyiv, Ukraine.

Dry milk mixtures based on sheep's "Agnus" and mare's milk "Ligans" were used to investigate digestion. The mixtures are developed at the Department of bakery and confectionery goods technology of the National University of Food Technologies (Kyiv, Ukraine). The recipes of the mixtures are presented in Table 1.

Table 1

Recipes of dry milk mixtures for children from birth to 6 months

Raw material, %	Mixture 'Ligans'	Mixture 'Agnus'	Mixture 'Malyutka hypoallergenic'
Dry demineralized whey	–	–	48.20
Cow's milk	–	–	26.40
Sheep powder milk	–	56.00	–
Milk powder mare	84.65	–	–
Mixture of vegetable oils	–	–	13.30
Soybean oil	–	4.00	–
Sunflower oil	7.00	2.00	–
Olive oil	8.00	–	–
Lactose	–	36.30	3.00
Lactulose	–	1.00	–
Fat-soluble vitamins, mg/100 g	0.03	0.03	–
Water-soluble vitamins, mg / 100 g	0.23	0.31	–
Minerals:	0.21	0.56	–
Sodium citrate salts, mg / 100 g	–	4.63	–
Citrate potassium salts, mg / 100 g	–	13.88	–
Taurine	0.03	0.03	–
Inositol	0.11	0.11	–
Total:	100.00	100.00	

Methods

Drying milk

Semi-industrial spray dryer "Nyro-Atomizer" (Denmark) with a working volume of the chamber of 0.9 m^3 and a productivity of evaporated moisture up to 5.0 kg/h was used for milk drying. Drying agent speed was 0.5 m/s and a drying agent relative humidity was 25%. The size of the droplets of the sprayed product was 40-50 μm, the mass fraction of dry substances in the product was 40-43%. The temperature of the drying agent for goat milk was 160–170 °C, for mare's milk was 150–160 °C, for sheep's milk was 170–180 °C.

Amino acid composition

The amino acid composition of milk proteins was determined by ion exchange chromatography using an automatic T 339 amino acid analyser (Carta et al., 2010). The elution of amino acids from the column was conducted, in turn, by Li-citrate buffers from pH 2.75±0.01; pH 2.95±0.01; pH 3.2±0.02; pH 3.8±0.02; pH 5.0±0.2. Amino acids were detected at a wavelength of 560 nm by rectification with a ninhydrin solution on a photometer. The results of detection were registered by a variplotter in the form of the peaks of absorption of light of ninhydrin positive substances in an eluent that number the direct ratio concentrations

of this substance in solution. The correlation of the solution of ninhydrin reagent and eluents was 12; the temperature of thermostatic T1 = 38.5 ºC; T2 = 65 ºC. The prototype was diluted in Li-citrate buffer by pH 2.2±0.02 and inflicted on an ion exchange column. The quantitative estimation of chromatograms of the pre-production model settled in relation to the Bio-Rad standard mixture of amino acids. The mass of every amino acid, expressed as g per 100 g protein (Ai), in the investigated solution was calculated by the following formula:

$$Ai = \frac{M_i \cdot S_i}{S_i^3}$$

where M_i is molecular mass of each amino acid; S_i is an area of peak of each amino acid on an aminogram from the investigated solution; S_i^3 is an area of peak of each amino acid on an aminogram from the solution of the standard mixture of amino acids which accords to one micromole (Litvynchuk et al., 2022).

Fractional composition of milk proteins

Determination of the fractional composition of milk proteins was carried out by the electrophoresis method. Electrophoresis was performed on a Hoefer Mighty Small apparatus (Amersham Biosciences, USA) at an amperage of 19 mA for concentrating and 35 mA for separating gels (Massouras et al., 2017, Yukalo et al., 2019).

Processing of the obtained data by the method of disk electrophoresis of electrophoregrams was carried out using the ImageMaster TotalLab v.2.01 program (Amersham Biosciences) (Massouras et al., 2017, Yukalo et al., 2019).

Protein digestibility

Protein digestibility was determined under *in vitro* conditions. Sequential enzymatic hydrolysis of samples of dry milk mixtures was carried out with the complex of proteinases pepsin-chymotrypsin. Digestion efficiency was estimated by accumulation of the products of hydrolysis (Tagliazucchi et al., 2018).

Statistical analysis

The statistical processing of the results was performed by sequential regression analysis using the Microsoft Excel XP and Origin Pro8 software calculating correlation coefficients (Hinkle et al., 2003).

Results and discussions

Amino acid composition of dry milk samples

The results of the study of the amino acid composition of samples of dry milk are presented in the Table 2.

Dry cow's milk and human milk were selected as control samples. The obtained results showed significant differences in the protein composition of the studied animal milk and its difference from human milk. This is explained by the fact that milk differs in the content of total protein and in the content of individual fractions. The milk of mammals is intended to ensure the rapid growth and strengthening of the skeleton of their offspring.

Table 2

Amino acid composition of dry animal milk

Amino acid	Cow's milk		Mare's milk		Goat's milk		Sheep's milk		Breast milk	
	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %
Mass fraction of protein, %	23.0		16.1		29.6		25.2		0.8–1.5	
Lysine	7.80	153	6.67	131	7.29	143	8.4	153	7.40	155
Histidine	2.50	86	2.23	76	2.59	89	2.11	73	2.20	96
Arginine	3.50	80	4.33	96	2.67	61	2.54	58	6.70	128
Aspartic acid	5.10	–	5.32	–	7.42	–	8.67	–	9.90	–
Threonine	4.30	123	3.89	111	4.88	140	4.10	117	4.80	138
Serine	4.90	–	8.17	–	5.24	–	5.37	–	5.50	–
Glutamic acid	20.6	–	19.31	–	20.40	–	22.30	–	16.4	–
Proline	7.4	–	9.73	–	14.43	–	7.72	–	6.40	–
Glycine	–	–	1.71	–	2.12	–	2.61	–	–	–
Alanine	2.40	–	3.31	–	2.98	–	5.40	–	2.96	–
Cystine	1.20	46	1.39	64	1.74	67	1.23	47	2.96	100
Valine	6.60	138	4.60	96	5.05	105	4.79	100	5.20	116
Methionine	2.50	96	3.09	119	2.10	81	2.82	108	2.50	120
Isoleucine	5.50	131	3.80	91	4.18	100	3.59	86	6.30	160
Leucine	9.60	137	14.39	206	8.45	121	9.08	130	17.00	220
Tyrosine	4.30	59	3.75	52	4.18	57	4.45	61	5.50	107
Phenylalanine	5.2	71	4.03	55	4.25	58	4.82	66	6.30	120

The limiting amino acid in cow's and sheep's milk is cystine, the amino acid ratio of which is 46 and 47%, respectively. In goat's and mare's milk cystine content is also quite low – 67% and 64%, respectively, though it is not the limiting amino acid. The limiting amino acid for dry mare's and goat's milk is tyrosine, the content of which is also insufficient in cow's and sheep's milk due to the low amino acid score. Lysine, threonine, valine and leucine are essential amino acids, and they are contained in the proteins of dry milk of animals in sufficient amounts, which even exceed their amount in an ideal protein. The histidine content is low in the protein composition of all types of milk.

An irreplaceable amino acid for the child's body is also arginine, the content of which in mare's milk corresponds to its content in ideal protein. Egg protein is considered to be ideal protein due to the fact that amino acid score of all its essential amino acids is more than 100% (Toghyani et. al., 2020). The amino acid score of arginine in cow's milk is 80%, which is 14% less than in mare's milk. Arginine amino acid scores of goat and sheep's milk are 61% and 58%, respectively, which are 19 and 22% lower compared to cow's milk.

The authors (Hodgkinson et al., 2018) claim that native mare's milk contains all 9 essential amino acids in the levels of need established by the World Health Organization. The content of the amino acid methionine is sufficient in the proteins of mare's and sheep's milk, the scores of which are 119% and 108%, respectively. The content of methionine in cow's milk approaches its content in ideal protein. Cow's and sheep's milk protein contain 29–35% less phenylalanine than the ideal protein, amino acid score is 71 and 66%, respectively.

Fractional composition of proteins of dry milk samples

The fractional composition of milk, as well as the amino acid composition, is also determined for different types of milk. The fractional composition of proteins is presented on the electrophoregram (Figure 1) and in quantitative form in the Table 3.

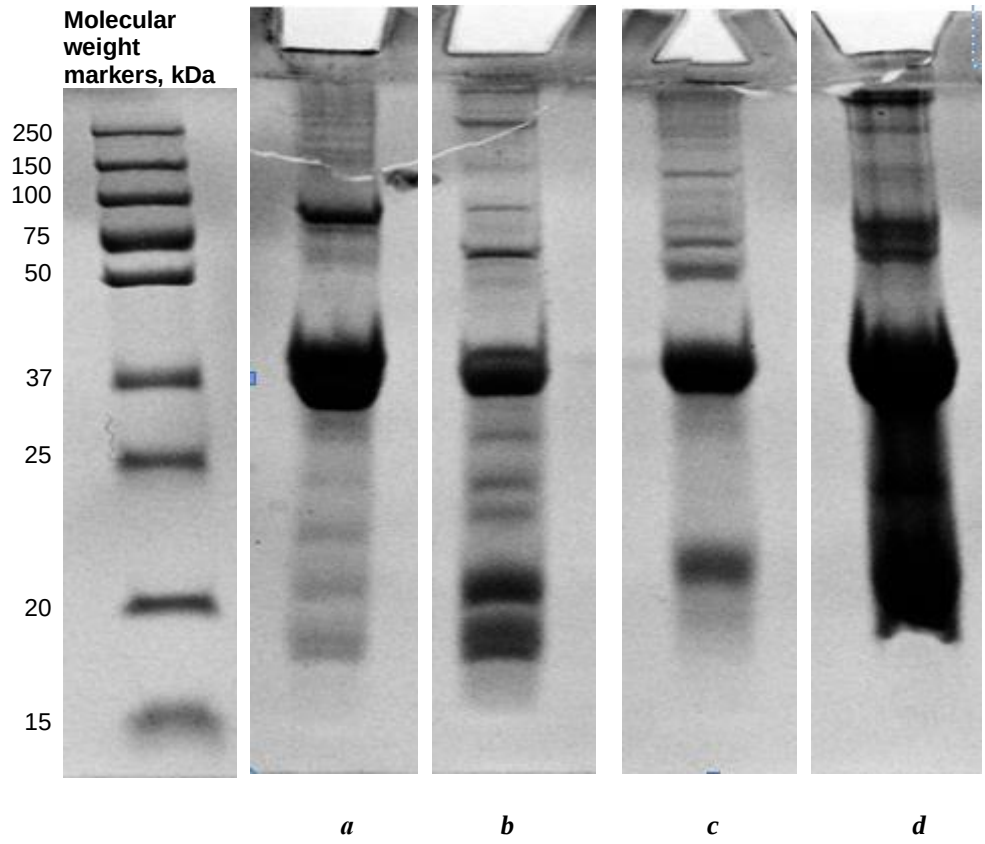

Figure 1. Electrophorogram of dry milk:
***a* – cow's milk; *b* – goat's milk; *c* – sheep's milk;**
***d* – mare's milk**
(molecular weight markers: 250, 150, 100, 75, 50, 37, 25, 20, 15 kDa)

The molecular weight of the proteins of the detected fractions ranges from 10 to 250 kDa. The molecular mass of 19-24 kDa is typical for caseins, 18 kDa for lactoglobulin, 14 kDa for lactalbumin, 69 kDa for albumin, and 150–1000 kDa for immunoglobulins. Cow's milk proteins have the lowest content of immunoglobulins among all samples. Mare's and goat's milk contain them 12 and 3 times more. Sheep's milk contains 10 times more immunoglobulins than cow's milk. Immunoglobulin M is contained in small amounts in cow's milk, and is absent in goat's milk. The content of immunoglobulin M in mare's and

sheep's milk is 20 times higher than in human milk. The smallest amount of lactoferrin is in cow's milk. Other types of milk contain 3-7 times more lactoferrin.

In the casein fraction of human milk, β-casein predominates (Zeng et al., 2019). The same is observed in mare's, goat's and sheep's milk. In cow's milk casein composition, α-casein predominates. The obtained data are similar to the other studies of the fractional composition of native milks. Minor differences are observed, which are explained by the fact that the chemical composition of milk depends on the genetic characteristics and breed of the mammal, geographical and climatic conditions, nutrition, lactation period, season, and ecological condition of the area (Kala et al., 2019, Markevich-Kenshycka et al., 2013).

Table 3

Fractional composition of proteins of dry milk

Milk proteins	Cow's milk (control)	Mare's milk	Goat's milk	Sheep's milk	Human milk
Casein, %	83.0±2.2	45.0±1.2	70.0±1.9	65.0±1.7	24.0±0.8
α-casein	53.56±1.4	7.5±0.3	23.24±0.7	20.4±0.7	not found
β-casein	26.68±1.0	24.48±1.0	33.94±1.1	35.94±1.1	23.0±0.7
κ-casein	0.66±0.01	2.56±0.2	2.76±0.3	3.46±0.3	0.96±0.01
γ-casein	2.46±0.2	8.1±0.3	9.18±0.4	2.17±0.2	not found
Whey proteins, %	17.00±0.7	55.00±1.4	30.0±1.1	35.0±1.1	76.0±1.9
β-lactoglobulin	10.35±0.4	20.61±0.7	0.99±0.01	7.2±0.4	not found
α-lactalbumin	2.33±0.2	7.76±0.3	12.88±0.4	4.89±0.3	35.0±1.1
Albumin	1.7±0.1	6.72±0.3	4.9±0.3	4.19±0.3	6.1±0.3
Immunoglobulins:					
Ig G	1.2±0.1	13.3±0.5	4.72±0.3	7.95±0.4	0.65±0.01
Ig A	0.30±0.01	5.15±0.4	0.26±0.01	6.92±0.3	4.8±0.3
Ig M	0.0001±0.00001	0.02±0.001	–	0.02±0.001	0.001± 0.00001
Ig E	0.01±0.001	0.02±0.001	–	0.02±0.001	0.30±0.01
Total:	1.51±0.1	18.49±0.7	4.98±0.3	14.91±0.5	5.75±0.3
Lactoferrin	0.68±0.01	1.82±0.01	4.98±0.3	2.9±0.2	14.5±0.5

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

The predominant whey protein in goat's milk, as well as in human milk, is α-lactalbumin, and in mare's, sheep's and cow's milk it is β-lactoglobulin, which causes food allergies. It was established that immunoglobulin A in mare's and sheep's milk is 17–23 times more than in cow's milk.

β-lactoglobulin was poorly absorbed under all conditions of gastric digestion. Caseins responded differently to pH change than whey proteins, with lower casein digestion at pH 3.0 than at pH 5.0. Goat milk caseins are generally absorbed more efficiently compared to cow's milk caseins, and the peptide profiles of goat milk differ from cow's milk (Hodgkinson et al., 2018). The drying mode has a significant effect on the amino acid composition of dry milk proteins. Heating milk proteins can lead to the formation of cross-links between different amino acids within the protein (Lieshout et al., 2015).

The formation of lysinoalanine in α-lactalbumin lead to a decrease in protein digestibility (Lieshout et al., 2020). Cross-linking of amino acids may or may not affect protein digestibility, thus demonstrating the need and relevance of the conducted research.

The ratio of casein to whey proteins in mare's milk is 1:1. In cow's milk, the casein to whey protein ratio is 4:1. In goat's and sheep's milk, the ratio between the fractions is 3:2. Thus, it can be concluded that mare's milk belongs to milk of the albumin group, as well as human milk. So, the composition of mare's milk contains significant amounts of finely dispersed proteins (albumins), the composition of cow's, goat's and sheep's milk has large amounts of caseins.

Protein digestibility of milk mixtures

At high temperatures of milk drying casein interacts with denatured whey proteins, which can cause reduced hydrolysis of caseins. It is also indicated that at high temperatures, cross-linking of amino acids with the formation of lysinoalanine is observed in milk. Cross-linking could affect protein digestibility by altering enzyme availability (Lieshout et al., 2020, Roy et al., 2021).

Milk mixtures based on mare ("Ligans") and sheep ("Agnus") milk were studied for the ability to digest protein substances *in vitro*. Human milk and "Malyutka hypoallergenic" (Khorolsky milk-canned plant for children's products, Ukraine) mixtures were selected as controls.

The results of the study of the digestion of protein substances are presented in the Figure 2.

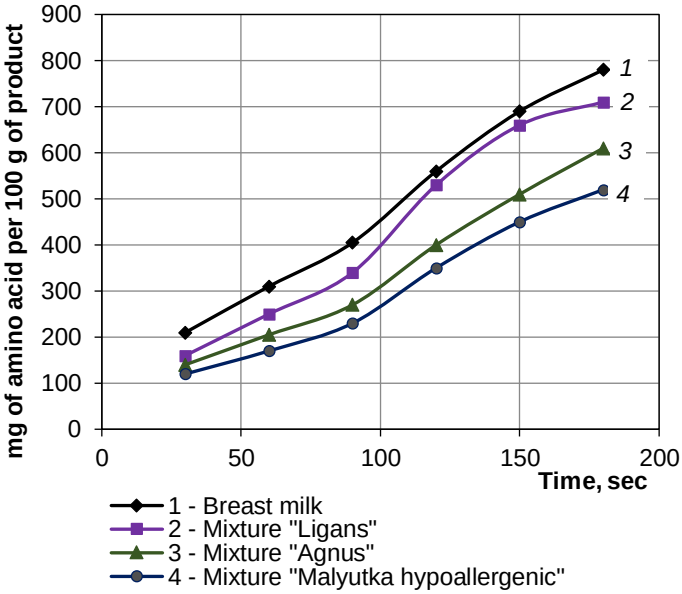

Figure 2. Digestibility of protein substances of the studied products
* Data are given as an average result of triplicate trials.

Process of digestion of protein substances in the developed products and in human milk proceeds with higher intensity (Figure 2) than in the mixture "Malyutka hypoallergenic"

(Khorol milk canned plant for children's products – the only Ukrainian manufacturer of dry milk mixtures). The degree of digestibility of proteins by pepsin and renin and the dynamics of this process had their own characteristics for different samples: the highest degree of digestibility was observed in the sample of human milk and mixture "Ligans" and was 717 mg and 780 mg of AA per 100 g of product, respectively. The lowest digestibility of proteins was found in the "Malyutka hypoallergenic" mixture compared to other samples. Accumulation of amino acids in this mixture is 33% less, compared to human milk. There is a peak value of amino acids during pepsin and renin hydrolysis at 90 min of the experiment in all samples, except for "Malyutka hypoallergenic" mixture. The digestion process of the "Ligans" mixture containing milk powder mare is similar to the intensity of digestion of human milk. At the end of hydrolysis with trypsin, 8% less amino acids were accumulated in the "Ligans" mixture than in human milk. This is explained by the immediate action of the enzyme on the protein molecules, which are immediately subjected to fermentation and splitting since proteins of the albumin group are more susceptible to the action of proteases (Hodgkinson et al., 2018). "Agnus" mixture containing sheep powder milk is characterized by slower protein hydrolysis compared to human milk and "Ligans" mixture (16% less than "Ligans" and 28% less than human milk).

The explanation for this is the higher content of casein in milk, as well as the processes which change the features of coagulation of milk by citric acid salts (Roy et al., 2021). The higher the amount of casein and calcium salts in milk, the faster the clotting speed and the stronger the protein clot. In case of the addition of citric acid potassium and sodium salts in milk, the coagulation of its proteins by rennet enzymes slows down (Hodgkinson et al., 2018). Sodium and calcium citric acid salts interact with free calcium ions, as a result of which calcium in the form of poorly soluble calcium citric acid is removed from the sphere of action, but does not precipitate. All these processes inhibit the rapid breakdown of proteins, the cleavage occurs more smoothly and for a longer time (Roy et al., 2021). The researchers compared the in vitro digestibility, selected biological activities, and digested products of proteins from skimmed cow's, camel's, goat's, and sheep's milk. It was established that goat's milk had the highest digestibility. According to our results the digestibility of products based on mare and sheep's milk is similar to the digestibility of human milk and "Malyutka hypoallergenic" mixture.

Conclusions

1. The amino acid composition of dry animal milk was determined and a comparative analysis was carried out with the amino acid composition of human milk proteins. It was shown that the limiting amino acid of cow's milk powder (46.0%) and sheep's milk powder (47.0%) is cystine. The limiting amino acid of dry mare milk (52.0%) and dry goat milk (57.0%) is tyrosine.
2. The fractional composition of proteins of different types of dry milk was studied. It was found that only mare milk belongs to the albumin type, as well as human milk. Mare and sheep's milk contain the largest amount of milk protective factors (immunoglobulins and lactoferrin), which makes it possible to consider these types of milk as hypoallergenic. It was found that mare and sheep's milk contain the amount of immunoglobulin A close to human milk (5.15 and 6.92%, respectively),
3. It was established that the digestibility of products based on mare and sheep's milk is similar to the digestibility of human milk and "Malyutka hypoallergenic" mixture.

Accumulation of amino acids in mixtures based on mare and sheep's milk occurs more intensively than in the mixture "Malyutka hypoallergenic".

References

Avershyna A.S. (2012), Obgruntunannia parametrav fermentatsii molochnoi osnovy u biotehnologii napoiu kyslomolochnogo dlia dytiachogo kharchuvannia «Biolakt», *Kharchova Nauka i Tehnologiia*, 2, pp. 32–36.

Barreto L., Marcell I. (2019), Equine milk and its potential use in the human diet, *Food Science and Technology*, 39, pp. 1–7.

Carta G., Jungbauer A. (2010), *Protein chromatorgraphy: Process development and scale Up*, Wiley VSH, 346 p.

El Amrousy D., El-Afify D., Elsawy A., Elsheikh M., Donia A., Nassar M. (2022). Lactoferrin for iron-deficiency anemia in children with inflammatory bowel disease: a clinical trial, *Pediatric Research*, 92, pp. 762-766, DOI: 10.1038/s41390-022-02136-2

Griffin I. (2020), The Effects of Different Forms of Lactoferrin on Iron Absorption, *Journal of Nutrition*, 150(12), pp. 3053–3054, DOI: 10.1093/jn/nxaa314

Hinkle D.E., Wiersma W., Jurs S.G. (2003), *Applied statistics for the behavioral sciences*, Boston, Mass: Houghton Mifflin, DOI: 10.1016/B978-0-12-824135-6.00030-1

Hodgkinson A., Wallace O., Boggs I., Broadhurst M., Prosser C. (2018), Gastric digestion of cow and goat milk: Impact of infant and young child in vitro digestion conditions, *Food Chemistry*, 245, pp. 275–281, DOI: 10.1016/j.foodchem.2017.10.028

Kala R., Samkova E., Hanus O., Pecova L., Sekmokas K., Riaukiene D. (2019). Milk protein analysis: An overview of the methods – development and application, *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(1), pp. 345–359, DOI: 10.11118/actaun201967010345

Lieshout G., Lambers T., Hettinga M. (2020), How processing may affect milk protein digestion and overall physiological outcomes: A systematic review, *Critical Reviews in Food Science and Nutrition*, 60(14), pp. 2422–2445, DOI: 10.1080/10408398.2019.1646703

Litvynchuk S., Galenko O., Cavicchi A., Ceccanti C., Mignani C., Guidi L., Shevchenko A. (2022), Conformational changes in the structure of dough and bread enriched with pumpkin seed flour, *Plants*, 11, 2762, DOI: 10.3390/plants11202762

Lonnerdal B., Du X., Jiang R. (2021). Biological activities of commercial bovine lactoferrin sources, *Biochemistry and Cell Biology*, 99(1), pp. 35-46, DOI: 10.1139/bcb-2020-0182

Markevich–Kenshycka M., Vujtovskii J. (2013), Chemical composition and whey protein fraction of late lactation mares' milk, *International Dairy Journal*, 31, pp. 62–64, DOI: 10.1016/j.idairyj.2013.02.006

Massouras T, Triantaphyllopoulos K., Theodossiou I. (2017), Chemical composition, protein fraction and fatty acid profile of donkey milk during lactation, *International Dairy Journal*, 75, pp. 83–90, DOI: 10.1016/j.idairyj.2017.06.007

Mazhitova A., Kulmyrzaev A. (2015), Amino acid and fatty acid profile of the mare's milk produced on suusamyr pastures of the Kyrgyz Republic during lactation period, *Procedia – Social and Behavioral Sciences*, 195, pp. 2683–2688, DOI: 10.1016/j.sbspro.2015.06.479

Mousan G., Kamat D. (2016), Cow's milk protein allergy, *Clinical Pediatrics*, 55, pp. 1054–1063, DOI: 10.1177/000992281666451

Navarro F., Galan–Malo P. (2018), Lactoferrin and IgG levels in ovine milk throughout lactation: Correlation with milk quality parameters, *Small Ruminant Research*, 168, pp. 12–18, DOI: 10.1016/j.smallrumres.2018.09.002

Roy D., Ye A., Moughan P., Singh H. (2021), Structural changes in cow, goat, and sheep skim milk during dynamic in vitro gastric digestion, *Journal of Dairy Science*, 104(2), pp. 1394–1411, DOI: 10.3168/jds.2020-18779

Tagliazucchi D., Martini S., Shamsia S., Helal A., Conte A. (2018), Biological activities and peptidomic profile of in vitro–digested cow, camel, goat and sheep's milk, *International Dairy Journal*, 81, pp. 19–27, DOI: 10.1016/j.idairyj.2018.01.014

Toghyani M., McQuade L.R., Mclnerney B.V., Moss A.F., Selle P.H., Liu S.Y. (2020), Initial assestment of protein and amino acid digestive dynamics in protein-rich feegstuffs for broiler chickens, *PLos ONE*, 15(9), e0239156, DOI: 10.1371/journal.phone.0239156.

Yukalo V., Datsyshyn K., Storozh L. (2019), Electrophoretic system for express analysis of whey protein fractions, *Eastern-European Journal of Enterprise Technologies*, 2(98), pp. 37–44.

Zeng Y., Zhang J. (2019), Assessment of cow's milk–related symptom scores in early identification of cow's milk protein allergy in Chinese infants, *BMC Pediatrics*, 191, pp. 1–7.

Influence of chemical structure of alcohols on extraction and stability of anthocyanins

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Abstract

Introduction. The aim of this research was to evaluate the efficiency of the anthocyanin extraction from grape skins with different alcohols under different conditions such as temperature and pH.

Materials and methods. Grape pomace of red grapes of the Vitis Vierul variety, which are obtained after the production of wine by white way and are considered as winemaking waste, were studied. The mass concentration of the anthocyanins was measured by pH differential spectrophotometry and expressed in mg of equivalent cyanidin-3-glycoside per gram of dry matter. The rate constant and half-life of the anthocyanin degradation were calculated for a temperature of 60 ºC.

Results and discussion. The effect of pH and the chemical structure of the molecule of alcohol extractant on the efficiency of anthocyanin extraction and its spectral characteristics was studied. The number of hydroxyl groups in the alcohol used as extractant as well as the length of the hydrocarbon moiety had a key role in the process efficacy. In particular, for monohydric alcohols, the efficacy follows the order: 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol. On the other side, increasing the number of OH groups in the line ethanol > ethane-1,2-diol > propane-1,2,3-triol does not enhance the extraction performance. The spectral characteristics of extractions obtained with ethanol and polyhydric alcohols are similar, the absorption maximum is unclear and is in the range of 530–560 nm. When the length of the carbon chain of alcohol increases, the electronic absorption spectra are characterized by different intensity and a wide, indistinct absorption maximum. The thermal stability of anthocyanins in the extracts was determined by the rate constant of the anthocyanin degradation reaction and the half-life. Decreasing the pH of the extract leads to an increase in the thermal stability of anthocyanins. Ethanol is the best extractant in terms of the technological and economic efficiency of natural pigment extraction and for its application in the food industry. Anthocyanin half-life in ethanol is about 10 hours at 60 °C that indicates its suitability to be used in industrial processing.

Conclusions. The results obtained make it possible to evaluate the efficiency of the extraction of anthocyanins with alcohols from grape skins and their thermal stability.

Introduction

Food production and processing generate high amounts of waste and by-products containing valuable compounds and biological active substances, and can be turned into useful products (Sirohi et al., 2020). As example, there is a growing scientific interest towards raw materials with organic dye compounds, which have not only the ability to provide a stable color, but also antioxidant properties (Qin et al., 2021). Natural flavonoid pigments, particular anthocyanins, are related to such substances, although anthocyanins are easily degraded being effected of the environment factors (Enaru et al., 2021; Delgado-Vargas et al., 2000). To preserve their properties, modern methods of extraction with supercritical carbon dioxide and subcritical water could be used (Essien et al., 2020). However, as these methods require special equipment, improvements in the extraction of anthocyanins from plant raw materials, as well as their application in various industries (food, chemical, pharmaceutical, cosmetic) are still open issues.

From a chemical point of view, anthocyanin dyes are mono- or diglycosides, the aglycone in which are anthocyanidins – phenolic derivatives of 2-phenylchromene in the form of benzpyrylium salts, such as pelargonidine, cyanidin, delphinidin. The main aglycones also include their methyl ethers: syringidine (malvidin), peonidine, hirsutidine. The properties of dyes are exhibited by both aglycones and the corresponding glycosides (Loarce et al., 2021). A mixture of different anthocyanins gives fruits, berries and flower petals of various colors from red to blue (Konieczynski et al., 2021). The chemical structure of anthocyanins causes easy solubility in water and polar solvents, especially in acidic media.

It is known that anthocyanins are present in solutions in several tautomeric forms, depending on a number of factors: the acidity of the medium, the polarity of the solvent and its ability to form hydrogen bonds. In general, depending on the pH value, anthocyanin dyes exist in the form of a flavylium cation, a carbinol base, a chalcone or a quinoid form. In an acidic environment, anthocyanins are mainly in the form of a bright red cation of flavylium. As the pH shifts to an alkaline medium, the color turns purple as the quinoid form appears. The formation of colorless structures in the form of a carbinol base and a chalcone is also possible (Etxabide et al., 2021; Manzoor et al., 2021). In addition, they are sensitive to the conditions of technological processing and storage, namely, they may lose or change color under the action of high temperatures, enzymes, the presence of oxygen, and heavy metal ions (Loarce et al., 2021).

Promising raw materials for the production of anthocyanin extracts are winemaking waste from dark grapes. The amount of dyes in the extract depends on the grape variety and the method of wine production. Red grape pomace, which remain as waste in the production of wine by the white method, has a higher content of dyes. When the grapes ripen, the number of anthocyanins is constantly increasing. The content of anthocyanins in the skin can be from 3 to 6% of the dry weight of the skin during the ripening of the grapes, and from 0 to 500 mg/dm^3 in the pulp. The composition of anthocyanins depends on the grape variety and place of growth (Perestrelo et al., 2020).

The most widespread as extractants of anthocyanins from plant raw materials are water, solutions of ethyl alcohol, and alcohol-glycerol mixture (Kurambhatti et al., 2020; Popović et al., 2020). Typically the extraction is carried out with aqueous or aqueous-alcoholic solutions, acidified with mineral or organic acids, using operations of infusion, pressing, filtration and concentration in vacuum at a temperature 60°C. The disadvantages of these methods include the difficulty of evaporating the extract containing pectin, tannins and mineral or organic acids. In fact, during evaporation, the degradation of dyes occurs due to hydrolysis and polycondensation of anthocyanins with the formation of insoluble products.

In addition, the use of aqueous extracts of anthocyanins is not suitable in the manufacture of some perfumes and cosmetics based on hydrophobic compositions. The use of alcohols of different chemical structure will increase the thermal stability of the extracts due to obtaining anthocyanins without carbohydrates and resistance to microorganisms.

The aim of this work was to determine the effect of the number of hydroxyl groups and the carbon chain of the aliphatic alcohols of the extractants on the efficiency of anthocyanin extraction from red grape pomace, optical characteristics and stability of anthocyanins in the obtained extracts. The proposed protocols can open new routes for a by-products disposal by the recovery and recycling of valuable substances.

Material and methods

Materials

Vitis Vierul grapes were obtained from local farms in Ukraine. Grape pomace obtained after two days of fermentation in the production of wine according to the "white method", squeezing and freezing was used as raw material.

Extraction of anthocyanins

Extraction of anthocyanins was performed in conical flasks with reflux in a water bath with a temperature of 60±2°C under stirring. The extraction process was performed at module 10 for 20 minutes with the following extractants: methanol, ethanol, propan-2-ol, butan-1-ol, 2-methylpropan-1-ol, ethane-1,2-diol (ethylene glycol), propane-1,2,3-triol (glycerol). The extracts were cooled, filtered through a blue ribbon filter and adjusted to the initial level with fresh extractant solution (Pérez et al., 2021).

The efficiency of the extraction process was determined by the amount of extracted anthocyanins at the pH of the obtained extracts (5.2–6.7 depending on the alcohol-extractant) and with acidification of the extracts with hydrochloric acid to pH 2.9, because at this pH anthocyanins are almost 100% flavylium cation form.

Determination of the concentration of anthocyanins

pH-differential spectrophotometry was used to determine the concentration of anthocyanins. For this purpose, 2.5 cm^3 aliquots of the filtrate were placed into two volumetric flasks with a capacity of 50 cm^3 and filled up with a buffer solutions of pH=1 (0.025 M potassium chloride) and pH=4.5 (0.4 M sodium acetate), respectively. After stirring, the optical density of each solution was measured at wavelengths of 510 and 700 nm on a spectrophotometer Spekol-11 (Germany). Measurements of the optical density at 700 nm were performed to establish the amount of light absorption by impurities.

The concentration of anthocyanins in grape pomace was calculated as equivalent of cyanidin-3-glucoside in mass per gram of dry matter (AC, mg CG/g DM) according to (Wrolstad et al., 2001):

$$AC = (\Delta A \cdot M \cdot V \cdot F \cdot 10^3)/(m \cdot \varepsilon \cdot l) \quad (1)$$

where, ΔA – the difference absorption solutions at different wavelengths 510 and 700 nm and the corresponding pH values, $\Delta A=(A_{510} - A_{700})_{pH1}-(A_{510} - A_{700})_{pH4,5}$; M – molar mass of cyanidin-3-glucoside; V – volume of prepared solution, l; F – dilution factor; m – mass of absolutely dry sample, g; ε – molar extinction coefficient cyanidin-3-glucoside, $l \cdot mol^{-1} \cdot cm^{-1}$; l – optical path length of the cuvette, cm.

Determination of the thermal stability of anthocyanins

To determine the thermal stability of anthocyanins, the extracts were kept in a closed flask at 60 °C for 4 hours. Determination of anthocyanin content was performed every 60 minutes. To study the kinetics of the process of anthocyanin decomposition under the influence of temperature, the rate constant of anthocyanin degradation reaction (k, h^{-1}) and half-life ($t_{1/2}$, h) were calculated. The first-order reaction was used as a kinetic model of anthocyanin degradation (Mulyawantia et al., 2018). The reaction rate constant was calculated by the formula:

$$k = -\ln (c/c_0)/t \quad (2)$$

c – current concentration of anthocyanins in solution, mg/l;
c_0 – initial concentration of anthocyanins in solution, mg/l;
t – time, hours.

The half-life was obtained as:

$$t_{0,5} = \ln 2/k \quad (3)$$

Results and discussion

Influence of the number of hydroxyl groups in an aliphatic alcohol molecule on the efficiency of extraction

At the first stage, the goal was to investigate the influence of the number of hydroxyl groups in the aliphatic alcohol molecule on the extraction efficiency. Mono-, di- and trihydric alcohols were chosen as extractants. The process of extraction of anthocyanins was carried out at different pH, which was created by the introduction of hydrochloric acid. The results of the experiments are given in the Table 1.

Table 1

Effect of pH and the number of hydroxyl groups in the extractant molecule on the number of extracted anthocyanins

Parameter	Extractant					
	ethanol		ethane-1,2-diol		propane-1,2,3-triol	
pH	5.4	2.9	5.6	2.9	5.9	2.9
AC, mg CG/g DM	7.71		5.78		4.49	

The efficiency of the extraction follows the order propane-1,2,3-triol < ethane-1,2-diol < ethanol (Table 1). The worst results in the extraction of anthocyanins were obtained for extractants with the largest number of carbon atoms and hydroxyl groups in the structure. The amount of extracted anthocyanins by two-stage extraction for each alcohol is: propane-1,2,3-triol (glycerol) 4.49 mg/g; ethane-1,2-diol (ethylene glycol) 5.78 mg/g; while with ethanol it increases up to 7.71 mg/g. The latter value indicates that ethanol and ethylene glycol compared to glycerol have 1.7 and 1.3 times higher efficacy, respectively, due to the different ability to form hydrogen bonds, as well as the intermolecular interaction between solvent and anthocyanins.

The spectral characteristics of the extractions obtained with ethanol and polyhydric alcohols are presented in Figure 1.

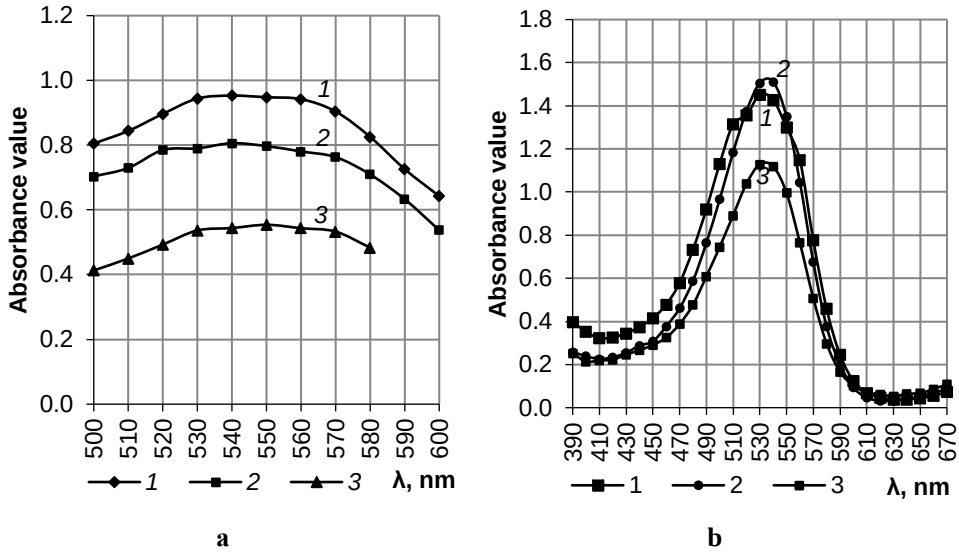

Figure 1. Absorption spectra of extracts from grape skins at:
a – pH 5.2 – 6.7; b – pH 2.9;
1 – ethanol; 2 – ethane-1,2-diol; 3 – propane-1,2,3-triol.

When comparing the spectral characteristics of the extractions obtained with ethanol and polyhydric alcohols, it should be noted that they are similar, the absorption maximum is not clear and is in the wide range of 530–560 nm, indicating a not bright red color with blue hues (Figure 1a). Provided that the introduction of hydrochloric acid is the cleavage of monosaccharides from the anthocyanin molecule to obtain the flavylium cation, the extracts were adjusted to pH 2.9 to obtain a more stable form of the natural pigment, namely – anthocyanidin. From Figure 1b it would be seen that the typical absorption maximum at 530–540 nm becomes clearer and more pronounced, and the value of the optical density increases by 1.5–1.8 times compared to the initial pH. The color of the solutions becomes bright red, which indicates the transition of the dye into the form of the flavylium cation. The results shown in Figure 1, b, show the advantages of using ethane-1,2-diol and ethanol in comparison with propane-1,2,3-triol in 1.4 times. Moreover, the use of ethane-1,2-diol is more effective than ethanol and allows you to obtain extracts with a clear maximum, which indicates the absence of a shade in the color.

Influence of the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins

It is known that anthocyanins are thermolabile compounds and degraded during heat treatment (Bakowska-Barczak, 2005). Temperature can have a negative effect not only on the color of dyes, but also changes their antioxidant properties (Martinsen et al., 2022). The exact mechanism of thermal degradation of anthocyanins is still unclear. However, it is known the formation of chalcones in the first stage of the process, the loss of glycosidic fragments and the formation of adiketone before the formation of final products, including coumarin derivatives, benzoic acid derivatives and threehydrobenzaldehyde (Reyes et al., 2007; Zhao et al., 2013). Therefore, it is important to investigate the ability of anthocyanins

to degrade when the extracts are kept at the temperature of their concentration with a decrease in pressure.

When studying the effect of the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins, it was found that the thermal stability increases as follows ethane-1,2-diol < propane-1,2,3-triol < ethanol (Figure 2, Table 2).

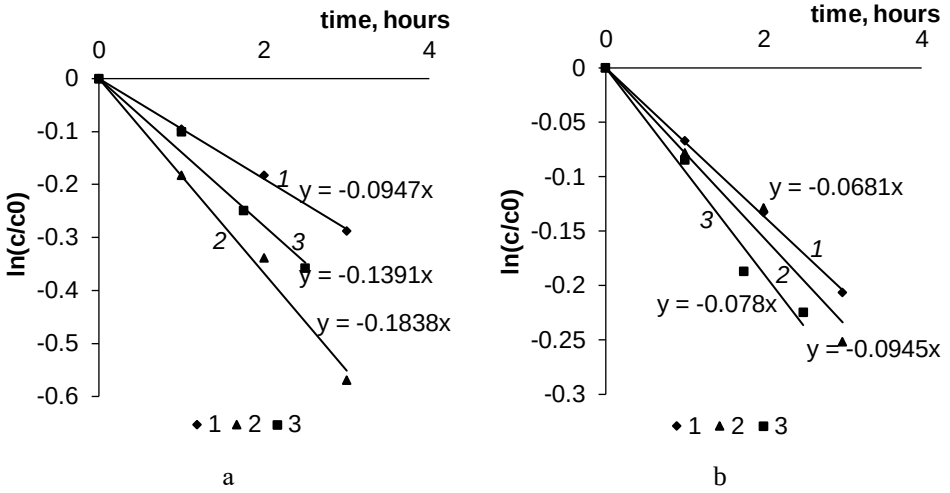

Figure 2. Kinetic curves of destruction of anthocyanins in extractants at:
a – initial pH of solvent; b – pH = 2.9: 1 – ethanol; 2 – ethane-1,2-diol; 3 – propane-1,2,3-triol.

Table 2

The effect of pH and the number of hydroxyl groups in the extractant molecule on the thermal stability of anthocyanins

Parameter	Extractant					
	ethanol		ethane-1,2-diol		propane-1,2,3-triol	
pH	5.4	2.9	5.6	2.9	5.9	2.9
k, h^{-1}	0.0947	0.0681	0.1838	0.0779	0.1391	0.0945
$t_{1/2}$, h	7.32	10.18	3.77	8.89	4.98	7.33

The results of the reaction rate constant of the decomposition of anthocyanins are given in Table 2 and they evidence the stability of anthocyanins in the form of the flavylium cation (pH 2.9). The decrease in the decomposition reaction constant of anthocyanins when moving to a more acidic environment was for: ethanol by 1.4 times, ethane-1,2-diol by 2.4 times, propane-1,2,3-triol by 1.5 times, with better stability of anthocyanins observed in ethanol at both pH values. It can be assumed that with an increase in the number of hydroxyl groups in an alcohol molecule, the ability to form intermolecular hydrogen bonds also increases, which negatively affects the stabilization of anthocyanins. The use of ethanol at pH 2.9 is 12% more effective than ethane-1,2-diol, and 23% more effective with propane-1,2,3-triol.

Influence of the length and structure of the carbon chain of monatomic alcohols on the amount of extracted anthocyanins

Further studies were aimed at determining the influence of the length and structure of the carbon chain of monohydric alcohols on the number of extracted anthocyanins – Table 3.

The largest amount of extracted anthocyanins was obtained when low molecular weight alcohols were used as extractants, which are able to penetrate better into the cells of grape pomace. Among alcohols of normal structure, butanol extracts 1.5–2 times less anthocyanins. Alcohols of the isostructure extract a smaller amount of anthocyanins due to the spatial structure of their molecule. Methanol and ethanol extract 2.4 and 3.0 times more anthocyanins compared to propan-2-ol. Ethanol extracts 2 times more anthocyanins than 2-methylpropan-1-ol.

Table 3

Influence of pH and structure of carbon chain of monohydric alcohols on the amount of extracted anthocyanins

Parameter	Extractant									
	methanol		ethanol		propan-2-ol		butan-1-ol		2-methylpropan-1-ol	
pH	6.7	2.9	5.4	2.9	5.75	2.9	5.6	2.9	5.2	2.9
AC, mg CG/g DM	6.34		7.71		2.60		4.04		3.94	

It was found that the efficiency of extractants increases as 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol (Figure 3b). When comparing the spectral characteristics of alcohol extracts with the linear structure of the carbon chain, we observe an increase in the number of anthocyanins in solution with a reduction in the carbon chain of the extractant: methanol – ca. 3 times, ethanol – 2.4 times compared to butan-1-ol. Extraction of anthocyanins by methanol occurs not only in cationic but also in quinoid form, as evidenced by the spectral characteristics of the extracts, which have a wide spectrum of absorption (Figure 3, a) and the highest maximum at pH 2.9 (Figure 3b). Alcohols of isostructure under these conditions showed better results than alcohols with the appropriate number of carbon atoms of normal structure. The absorption intensity of extracts with 2-methylpropan-1-ol is 2 times more than butan-1-ol.

Studies of the spectral characteristics of the obtained extracts (Figure 3a, b) showed that with increasing the length of the carbon skeleton of alcohol, the absorption spectra are characterized by different intensities, the absorption maximum is in the range of 520–560 nm.

The lack of a clear maximum in the extracts can be explained by the fact that the extraction of anthocyanins occurs not only in the form of the flavylium cation, but also in the form of other structures.

When obtaining a stable form – flavylium cation, there is (Figure 3b) an increase in the absorption intensity for methanol at 540 nm by three times and a shift of the maximum by 10 – 15 nm towards long waves for alcohols of the isostructure and butan-1-ol. From Figure 3b, we can see the advantages of using methanol (2.7 times) and ethanol (2.3 times) in comparison with the alcohols of the isostructure. Thus, the extraction of anthocyanins depends on the polarity of the solvent and is associated with better diffusion properties of alcohols with lower molecular weight across plant cell membranes.

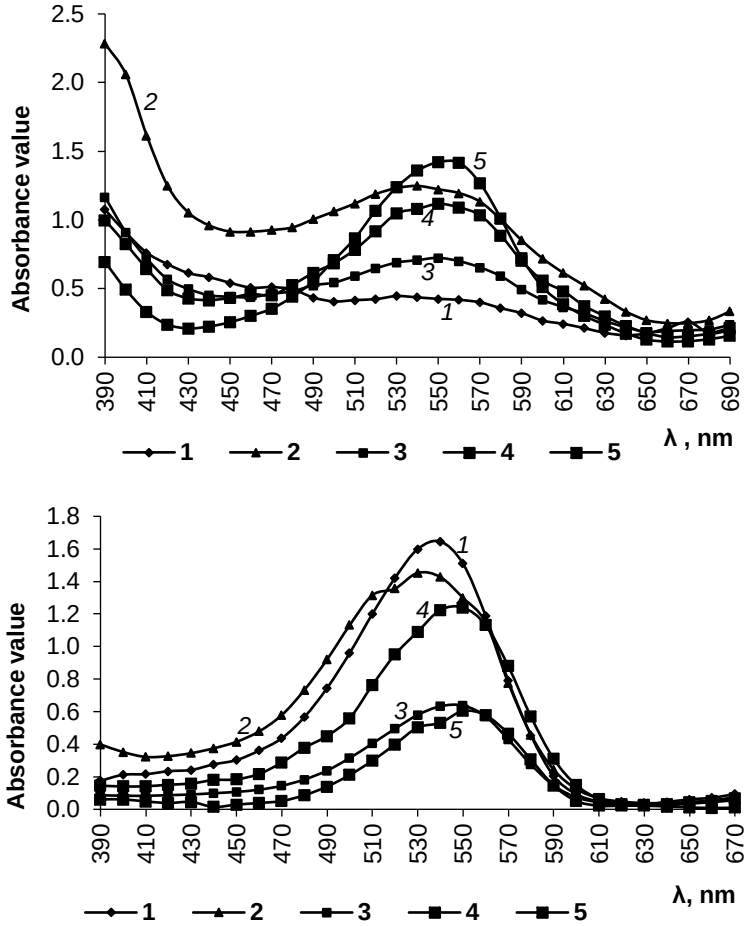

Figure 3. The spectrum of absorption of the extract from the skins of grapes at:
a – pH 5.2 – 6.7; b – pH 2.9;
1 – methanol; 2 – ethanol; 3 – propan-2-ol;
4 – butan-1-ol; 5 – 2-methylpropan-1-ol.

Influence of the length and structure of the carbon chain of alcohols on the thermal stability of anthocyanins

Determination of the influence of the structure of the carbon chain of monohydric alcohols on the thermal stability of anthocyanins is presented in Figure 4 and in Table 4.

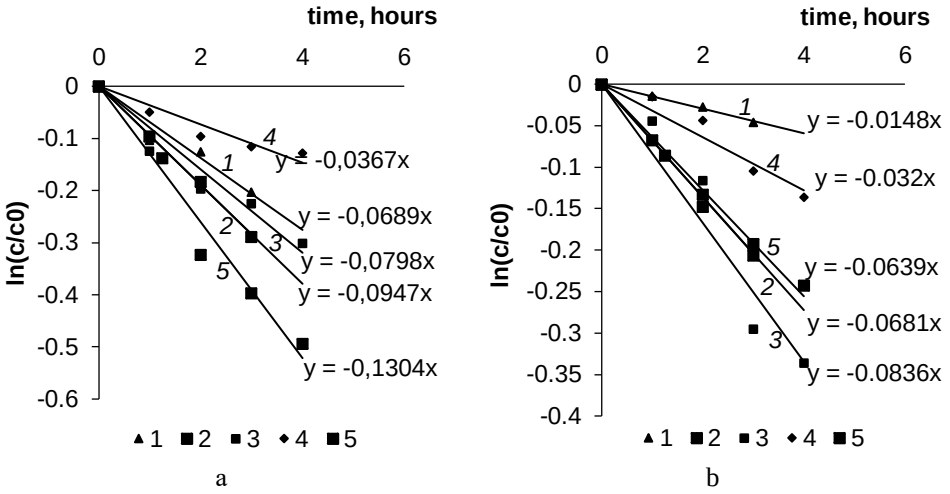

Figure 4. Kinetic curves of destruction of anthocyanins in extractants at:
a – initial pH of solvent; b – pH = 2.9: 1 – methanol; 2 – ethanol;
3 – propan-2-ol; 4 – butan-1-ol; 5 – 2-methylpropan-1-ol.

Table 4

Influence of pH and structure of carbon chain of monohydric alcohols on the amount of extracted anthocyanins

Parameter	Extractant									
	methanol		ethanol		propan-2-ol		butan-1-ol		2-methylpropan-1-ol	
pH	6.7	2.9	5.4	2.9	5.8	2.9	5.6	2.9	5.2	2.9
k, h^{-1}	0.069	0.015	0.095	0.068	0.084	0.080	0.037	0.032	0.130	0.064
$t_{1/2}$, h	10.06	46.83	7.32	10.18	8.29	8.68	18.89	21.66	5.32	10.85

It was found that the thermal stability increases in the series of 2-methylpropan-1-ol < ethanol < propan-2-ol < methanol < butan-1-ol (Table 4). The half-life of anthocyanins in butan-1-ol is the longest and is 18.89 hours, which is an order of magnitude higher than other tested extractants.

The stability of anthocyanins in the form of the flavylium cation (pH 2.9) is higher for all extractants: for propan-2-ol by 1.05 times, for ethanol by 1.4 times, for 2-methylpropan-1-ol by 2 times, for butan-1-ol by 1.15 times, for methanol by 4.7 times. The best thermal stability indicators were obtained for anthocyanins in methanol. Compared with the neutral medium, the stability in acidified methanol is 4.7 times higher, and the half-life is 46.83 hours. The second most effective storage of anthocyanins is butano-1-ol, in which the half-life of the dye is 21.66 hours. As evidenced in Table 4, the efficiency of extraction of

anthocyanins by ethanol is the highest 7.71 mg/g, but the half-life is less compared to methanol 4.6 times, and butan-1-ol by a factor 2. The use of alcohols of isostructure does not provide advantages when storing natural dyes.

Conclusions

1. The influence of hydrogen index value and structure of extractant alcohol molecule on anthocyanin extraction from Vitis Vierul red grape pomace was investigated and anthocyanin stability in extracts was determined by calculating the anthocyanid degradation reaction rate constant and half-life.
2. An increase in the efficiency of the use of extractants depending on the number of hydroxyl groups as: propane-1,2,3-triol < ethane-1,2-diol < ethanol and depending on the structure of the carbon fragment of monohydric alcohols as: 2-methylpropan-1-ol < butan-1-ol < propan-2-ol < ethanol < methanol.
3. It was found that the stability of anthocyanins in the form of cation flavylium (at pH 2.9) is higher in the corresponding extracts compared to the stability of the extracts at pH 5.2–6.7. The best extractant in terms of the efficiency of extraction of natural pigment and use in the food industry is ethanol. The kinetic curves of anthocyanin degradation under the influence of temperature were constructed and the half-life was calculated, which is 10.18 hours in ethanol at a temperature of 60°C. The obtained results allow to evaluate the efficiency of extractants in the extraction of anthocyanins from grape pomace and their thermal stability.

References

Bakowska-Barczak A. (2005), Acylated anthocyanins as stable, natural food colorants – a review, *Polish Journal of Food and Nutrition Sciences*, 14/55(2), pp. 107–116.

Enaru B., Drețcanu G., Pop T.D., Stănilă A., Diaconeasa Z. (2021), Anthocyanins: Factors affecting their stability and degradation, *Antioxidants (Basel)*, 10(12), 1967, DOI: 10.3390/antiox10121967.

Essien S. O., Young B., Baroutian S. (2021), Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials, *Trends in Food Science & Technology*, 97, pp. 156–169, DOI: 10.1016/j.tifs.2020.01.014.

Etxabide A., Kilmartin P. A., Maté J. I. (2021), Color stability and pH-indicator ability of curcumin, anthocyanin and betanin containing colorants under different storage conditions for intelligent packaging development, *Food Control*, 121, 107645, DOI: 10.1016/j.foodcont.2020.107645.

Delgado-Vargas F., Jiménez A.R., Paredes-López O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains--characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, 40(3), pp. 173-289. DOI: 10.1080/10408690091189257.

Konieczynski P., Lysiuk R., Wesolowski M. (2021), Total flavonoid and ionic element content in 32 medicinal plants collected from natural habitats in Northern Ukraine, *Journal of Herbal Medicine*, 100492, DOI: 10.1016/j.hermed.2021.100492

Kurambhatti Ch., Kumar D., Rausch K. D., Tumbleson M. E., Singh V. (2020), Improving technical and economic feasibility of water based anthocyanin recovery from purple

corn using staged extraction approach, *Industrial Crops and Products*, 158, 112976, DOI: 10.1016/j.indcrop.2020.112976.

Loarce L., Oliver-Simancas R., Marchante L., Díaz-Maroto M. C., Alañón M. E. (2021) Modifiers based on natural deep eutectic mixtures to enhance anthocyanins isolation from grape pomace by pressurized hot water extraction, *LWT*, 149, 111889, DOI: 10.1016/j.lwt.2021.111889.

Manzoor M., Singh J., Gani A., Noor N. (2021), Valorization of natural colors as health-promoting bioactive compounds: Phytochemical profile, extraction techniques, and pharmacological perspectives, *Food Chemistry*, 362(15), 130141, DOI: 10.1016/j.foodchem.2021.130141.

Martinsen B. K., Aaby K., Skrede G. (2022), Effect of temperature on stability of anthocyanins, ascorbic acid and color in strawberry and raspberry jams, *Food Chemistry*, 316, 126297, DOI: 10.1016/j.foodchem.2020.126297

Mulyawantia I., Budijantob S., Yasni S. (2018), Stability of anthocyanin during processing, storage and simulated digestion of purple sweet potato pasta, *Indonesian Journal of Agricultural Science*, 19(1), pp. 1–8, DOI: 10.21082/ijas.v19n1.2018.p1-8.

Perestrelo R., Bordiga M., Locatelli M., Silva C., Câmara J. S. (2020), Polyphenols, biogenic amines and amino acids patterns in Verdelho wines according to vintage Part I: Vintage and ripeness effects, *Microchemical Journal*, 153, 104383, DOI: 10.1016/j.microc.2019.104383.

Pérez B., Endara A., Garrido J., Ramírez Cárdenas, L. de los Ángeles (2021), Extraction of anthocyanins from Mortiño (Vaccinium floribundum) and determination of their antioxidant capacity, *Revista Facultad Nacional de Agronomía Medellín*, 74(1), pp. 9453–9460, DOI: 10.15446/rfnam.v74n1.89089.

Popović B. M., Blagojević B., Latković D., Četojević-Simin D., Kucharska A. Z., Parisi F., Lazzara G. (2021), A one step enhanced extraction and encapsulation system of cornelian cherry (*Cornus mas* L.) polyphenols and iridoids with β-cyclodextrin, *LWT*, 141, 110884, DOI: 10.1016/j.lwt.2021.110884.

Qin D., Xi J., (2021), Flash extraction: An ultra-rapid technique for acquiring bioactive compounds from plant materials, *Trends in Food Science & Technology*, 112, pp. 581–591, DOI: 10.1016/j.tifs.2021.04.025.

Reyes L. F., Cisneros-Zevallos L. (2007), Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (*Solanum tuberosum* L.), *Food Chemistry*, 100, pp. 885–894, DOI: 10.1016/j.foodchem.2005.11.002.

Sirohi R., Tarafdar A., Singh Sh., Negi T., Gaur V. K., Gnansounou E., Bharathiraja B. (2020), Green processing and biotechnological potential of grape pomace: Current trends and opportunities for sustainable biorefinery, *Bioresource Technology*, 314, 123771, DOI: 10.1016/j.biortech.2020.123771.

Wrolstad R. E., Giusti M. M. (2001), Characterization and measurement of anthocyanins by UV-visible spectroscopy, *Current Protocols in Food Analytical Chemistry*, DOI: 10.1002/0471142913.faf0102s00.

Zhao M., Luo Y., Li Y., Liu X., Wu J., Liao X., Chen F. (2013), The identification of degradation products and degradation pathway of malvidin-3-glucoside and malvidin-3,5-diglucoside under microwave treatment, *Food Chemistry*, 141(3), pp. 3260–3267, DOI: 10.1016/j.foodchem.2013.05.147.

Destruction of biofilms by surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in the presence of competitive microorganisms

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Abstract

Introduction. The aim of this study was to investigate the role of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in media with glycerol in the presence of biological inductors in destruction of biofilms.

Materials and methods. Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in a mineral medium using refined glycerol or crude glycerol, the waste of biodiesel production, as carbon sources. Biological inductors were introduced as live or inactivated cells of *Bacillus subtilis* BT-2, as well as the supernatant after strain BT-2 cultivation. Surfactants were extracted from the supernatant of the culture liquid with a modified mixture of Folch (chloroform and methanol, 2:1). The degree of biofilm destruction in the presence of surfactants was determined by spectrophotometric method.

Results and discussion. Regardless of the substrate used, the introduction of both live and inactivated cells of *B. subtilis* BT-2 into medium used for cultivation of *A. calcoaceticus* IMV B-7241 was accompanied by the synthesis of surfactants, the degree of biofilm destruction of which was higher than those obtained in the medium without an inductor. The degree of destruction of bacterial and yeast biofilms achieved by the action of *A. calcoaceticus* IMV B-7241 surfactants obtained on refined glycerol in the presence of inductor cells was 36.5–85% and was 1.5-3 times higher compared to using surfactants synthesized in medium without inductors. Note that, surfactants synthesized in the presence of biological inductors destroyed biofilms of the test cultures at fairly low (7.5–960 μg/ml) concentrations. Similar results were observed for the usage of surfactants obtained on the waste of biodiesel production. Therefore, introduction of live cells of *B. subtilis* BT-2 into the medium with the crude glycerol was accompanied by synthesis of surfactants, which at concentration 1.8-960 μg/ml caused destruction of *B. subtilis* BT-2, *Proteus vulgaris* PA-12 and *Enterobacter cloacae* C-8 biofilms at 30.1–80.7% and was higher than using similar surfactant concentrations obtained during cultivation without inductors (24.1–75%). The destruction of biofilms of *Staphylococcus aureus* BMS-1, *Candida albicans* D-6 and *Candida tropicalis* PE-2 under the action of surfactants (1.8-960 μg/ml) synthesized on crude glycerol in the presence of both live or inactivated cells of *B. subtilis* BT-2 was 1.5–8 times higher than surfactants synthesized in medium without inductor.

Conclusion. The possibility to regulate the ability to destroy bacterial and yeast biofilms of surfactants synthesized by *A. calcoaceticus* IMV B-7241 by introducing into the medium competitive bacteria *B. subtilis* BT-2 was found.

Introduction

Biofilm formation significantly contributes to microbial survival in hostile environments and it is currently considered a key virulence factor for pathogens responsible for serious chronic infections (Huigens et al., 2019). More than 90% of the studied species of bacteria are able to form biofilms. Biofilm formation was detected in more than 80% of chronic diseases of microbial etiology. Thus, about 60% of all hospital-acquired infections are caused by microorganisms located in biofilms (Parrino et al., 2019). Bacteria capable of forming biofilms are considered a major cause of chronic and acute bacterial infections. For several decades, the bacteria that cause widespread or severe infections have acquired resistance to every new antibiotic that comes on the market (D'Cunha et al., 2018).

Alternative methods for biofilm prevention and/or eradication are urgently required to modify the traditional treatments. The ability of several novel natural antimicrobial compounds (probiotics, bacteriophages, enzymes) to efficiently control biofilm formation has been identified (Algburi et al., 2017). New potential biocides (microbial surfactants, peptides) are actively investigated (Lin et al., 2021).

There is an increased need for recycling of waste products from food, wood industry and agriculture in recent years. For example, crude glycerol which is a side product of biodiesel production because the problem today is the need to dispose of large amounts of it. In recent years, there have been developments to utilize glycerol and convert it to usable biomass (Chmielarz et al., 2021). It was also tested as an additive with other waste substrates to make valuable products (Poladyan et al., 2020).

Increasing the efficiency of microbial surfactant technologies is one of the ways to use cheap industrial waste as a substrate, crude glycerol as waste of biodiesel production, in particular (Salazar-Bryam et al., 2017). The most effective way to dispose of such waste is to use them as substrates in biotechnological processes to obtain practically valuable products (Diamantopoulou et al., 2020).

In addition, there are more papers in the literature devoted to the cultivation of microorganisms in the the presence of biological inductors, the presence of which enhances the biological activity of final metabolites (peptides, bacteriocins, surfactants) and the synthesis of new metabolites with biological activity (Kumar et al., 2021).

It was previously found that *Acinetobacter calcoaceticus* IMV B-7241 synthesizes a complex of surfactants on a wide range of carbon substrates, including glycerol of different degrees of purification (Pirog et al., 2018). A study of the biological activity of surfactants synthesized crude glycerol showed that such surfactants were less effective biofilm destructors compared to those synthesized on purified glycerol.

Previous studies (Pirog et al., 2021) showed the possibility of regulating the antimicrobial activity of the surfactants *A. calcoaceticus* IMV B-7241 by adding into the cultivation medium of cells of competitive bacteria *B. subtilis* BT-2. It is important that the antimicrobial activity of surfactants synthesized on crude glycerol was significantly increased under such cultivation conditions.

Since one of the mechanisms for biofilm destruction under the influence of microbial surfactants is their antimicrobial activity (Sharma et al., 2019), it was suggested that cultivation the producer with competitive bacteria *B. subtilis* BT-2 to allow increase not only antimicrobial activity of surfactants, but also their ability to destroy biofilms.

In connection with the above, the aim of the work is to investigate the role in biofilm destruction of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of biological inductors in medium with glycerol of different degrees of purification.

Materials and methods

Object of research

The main object of research was strain *Acinetobacter calcoaceticus* K-4, registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the number IMV B-7241.

Bacterial strains (*Bacillus subtilis* BT-2, *Staphylococcus aureus* BMS-1, *Proteus vulgaris* PA-12, *Enterobacter cloacae* C-8) and yeast (*Candida* D-6, *Candida tropicalis* PE-2) from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used as test cultures in determining the ability of surfactants to destroy bacterial and yeast biofilms.

Medium composition and conditions of cultivation

Strain *A. calcoaceticus* IMV B-7241 was grown in the liquid mineral medium (g/l): $(NH_2)_2CO$ – 0.35; $MgSO_4 \cdot 7H_2O$ – 0.1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; pH 6.8–7.0. Yeast autolysate – 0.5% (v/v) and microelement solution – 0.1% (v/v) were additionally added into the medium. The micronutrient solution contained (g/100 ml): $ZnSO_4 \cdot 7H_2O$ – 1.1; $MnSO_4 \cdot H_2O$ – 0.6; $FeSO_4 \cdot 7H_2O$ – 0.1; $CuSO_4 \cdot 5H_2O$ – 0.004; $CoSO_4 \cdot 7H_2O$ – 0.03; H_3BO3 – 0.006; KI – 0.0001; EDTA (Trilon B) – 0.5.

As carbon sources used (%, v/v): refined glycerol – 3, crude glycerol – 5. Concentrations of glycerol of different quality are equimolar on carbon.

Culture in the exponential phase was used as an inoculum, grown in a medium of the above composition with 0.5 % of the corresponding substrate. The inoculum with the number of bacteria 10^4–10^5 cells/ml was added in an amount of 10% of the medium volume.

The bacterial strain *Bacillus subtilis* BT-2 was used as a biological inductor and were introduced as live, inactivated cells, as well as the supernatant after BT-2 strain cultivation. At the beginning of the cultivation process, inductor inoculums were added to *A. calcoaceticus* IMV B-7241 culture. *Bacillus subtilis* BT-2, grown on meat-peptone agar for 24 h, was suspended in 100 ml of sterile tap water and 2.5 ml of suspension per 100 ml of surfactant-producing culture medium was added. Inactivated cells (heat treated at 131 °C for 1 hour) were added, 10 ml per 100 ml of culture medium. Cell-free supernatant was added, 2.5 ml per 100 ml of culture medium.

Cultivation of *A. calcoaceticus* IMB B-7241 in the presence of supernatant, live and inactivated *B. subtilis* BT-2 cells and without inductors was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30 °C for 7 days.

Determination of extracellular surfactant concentration

The amount of extracellular surfactants was determined using our modified Bligh and Dyer method. The surfactants extraction was with a mixture of chloroform and methanol (2:1) from the supernatant of the culture liquid. Then, the culture liquid was centrifuged at 5000 g for 20 min to obtain the cell-free supernatant.

As *A. calcoaceticus* IMB B-7241 synthesizes a complex of non-polar and polar lipids, and the well-known Bligh and Dyer method used for surfactant isolation allows the separation of mainly non-polar lipids, we modified the classical solvent system (Folch mixture) by adding to it 1 M HCl (chloroform – methanol – water = 4:3:2). Such a system allows for maximum separation of both non-polar and polar lipids.

In a 100 ml cylindrical separation glass-stoppered funnel, 25 ml of supernatant was placed, 1 M HCl solution was added until the pH value is reached 4.0–4.5 (about 5 ml), the funnel was covered with a stopper and shaken for 3 min, then 15 ml of chloroform and methanol mixture (2:1) was added and shaken (lipid extraction) for 5 min. The mixture obtained after extraction was left in a separating funnel to separate the phases, after which the lower fraction was drained (organic extract 1) and the aqueous phase was re-extracted. During the second extraction, 1 M HCl solution was added to the aqueous phase to reach a pH 4.0–4.5 (about 5 ml), 15 ml chloroform-methanol mixture (2:1) and the lipids were extracted for 5 min. At the third stage, 25 ml of chloroform-methanol mixture (2:1) was added to the aqueous phase and extracted as described above to obtain organic extract 3. Extracts from 1 to 3 were combined and evaporated on IP-1M2 rotary evaporator at 50 °C and absolute pressure of 0.4 atm to constant weight.

Obtaining surfactant preparations

A. calcoaceticus IMB B-7241 surfactant solutions of various concentrations were used in the research. The dry surfactant residue was dissolved in sterile phosphate buffer (0.1 M, pH 7.0) to the original volume (25 ml) and further diluted with this buffer to the required concentration. The surfactant solutions were sterilized in an autoclave at 112 °C for 30 min.

Study of the degree of the biofilm destruction under the action of surfactants

The effect of surfactants on biofilm degradation was carried out as described in (Allegrone et al., 2021). For obtaining the biofilm formation, 180 µl of meat-peptone broth (MPB) or liquid wort and 20 µl of one-day test culture suspension were added to immunoassay microplates. Then, it was incubated for 24 h at optimal temperature for the test culture, followed by draining the culture liquid, added 180 µl of fresh MPB (liquid wort) and 20 µl of test culture suspension and further incubated for 24 h. This 48-hour cultivation is decent for the formation of a biofilm in the microplate wells. After 48 h, the culture liquid was drained and 200 µl of surfactant preparations (0.005–1.28 mg/ml) were added to the microplate wells (with the test culture biofilm formed on them previously). Sterile tap water (200 µl) was added instead of surfactant preparations to control variants (wells). Wells were washed three times with 200 µl of distilled water after 24 h of exposure and the number of adherent cells was determined spectrophotometrically. The degree of biofilm destruction (%) was determined as the difference between cells adhesion in untreated and surfactant-treated wells of the immunoassay plate.

Statistical analysis

All experiments were performed in 3 replicates, the number of parallel determinations in the experiments was 3–5.

Results and discussion

The functionality of various types of biosurfactants as antibiofilm agents is mainly determined by their types: glycolipids, rhamnolipids, sophorolipids, lipopeptides. Mechanisms of bacterial biofilm degradation differ depending on the type of surfactant (Paraszkiewicz et al., 2021). It is reported that most microbial surfactants can increase cell

surface hydrophobicity and destabilise lipid structure (Ohadi et al., 2020), as one of the mechanisms of biofilm destruction. According to other data, these changes increase the permeability of cell membranes and reduce microbial adhesion to different surfaces (Bionda et al., 2016). According to other studies, microbial surfactants inhibit the expression of bacterial genes involved in biofilm formation (Allegrone et al., 2021). In addition, a paper (Yan et al., 2019) reported a mechanism of anti-biofilm activity against *S. aureus* CMCC 26003 of microbial surfactants synthesized by lactic acid bacteria. Surfactants were found to affect the expression of biofilm-associated genes by interfering with the release of signaling molecules.

Effect of biological inductors in a medium with crude glycerol on the ability of surfactants synthesized by *A. calcoaceticus* IMV B-7241 to destroy biofilms

The degradation of biofilms under the influence of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with crude glycerol in the presence of live *B. subtilis* BT-2 cells is shown (Table 1).

Table 1

Destruction of biofilms under the action of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with crude glycerol in the presence of live *B. subtilis* BT-2 cells

Test culture	Presence of inductor in the medium	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)				
		60	120	240	480	960
Bacillus subtilis BT-2	−	50	51	51	52	55
	+	55	55	55	61	65
Enterobacter cloacae C-8	−	65	70	70	71	71
	+	60	70	70	81	81
Proteus vulgaris PA-12	−	52	58	61	66	70
	+	76	77	77	77	77
Candida tropicalis PE-2	−	40	45	52	57	58
	+	53	54	57	62	63
Candida albicans D-6	−	26	30	31	35	38
	+	39	42	48	51	52

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

An increase in the degree of biofilm destruction of bacterial and yeast test cultures was achieved by the action of surfactants synthesized in the presence of live inducer cells in the medium with crude glycerol. The use of inactivated cells or supernatant as inductor was accompanied by the synthesis of surfactants under the influence of which biofilm degradation was the same as preparations synthesized in medium without inductors (Tables 2 and 3). Using surfactant concentrations below 60 μg/ml obtained during cultivation of *A. calcoaceticus* IMV B-7241 on crude glycerol in the presence of live *B. subtilis* BT-2 cells, the destruction of both bacterial and yeast biofilms was not different from that in the action of preparations synthesized without inductors.

A significant difference (9–10 %) in the degradation of *B. subtilis* BT-2 and *E. cloacae* C-8 biofilms in the presence of surfactants synthesized with and without an inductor was observed only when surfactants with the highest concentrations studied (480–960 μg/mL) were used. At the same time, the degree of destruction of *P. vulgaris* PA-12 biofilm by surfactants synthesized by strain IMV B-7241 in medium with inductor was 76–77 %, regardless of surfactant concentration, and was 7–24 % higher than those obtained without inductor. The highest difference (19–24 %) in the degradation of *P. vulgaris* PA-12 biofilm under the influence of surfactants synthesized with and without inductor was observed when using low concentrations of preparations (60–120 μg/ml).

The degree of destruction of yeast biofilms under the action of surfactants synthesized in the presence of *B. subtilis* BT-2 cells was 5–17% higher than that under the influence of preparations obtained without an inductor. In the case of surfactants obtained under cultivation of IMV B-7241 strain with the inductor, the destruction of *C. tropicalis* PE-2 biofilm was rather high, 53–63% in the whole range of surfactant concentrations tested. The degree of destruction of *C. albicans* D-6 biofilm exceeded 50% only under the influence of high concentrations (480–960 μg/ml) of the preparations obtained in the presence of the inductor.

In contrast to other bacterial biofilms (see Table 1), an increasing *S. aureus* BMS-1 biofilm destruction was observed when surfactants synthesized in the presence of all inductors (supernatant, live and inactivated *B. subtilis* BT-2 cells) were used: the degree of biofilm destruction was 10–19% higher than under the action of preparations obtained during *A. calcoaceticus* IMB B-7241 cultivation in medium without inductors (Figure 1).

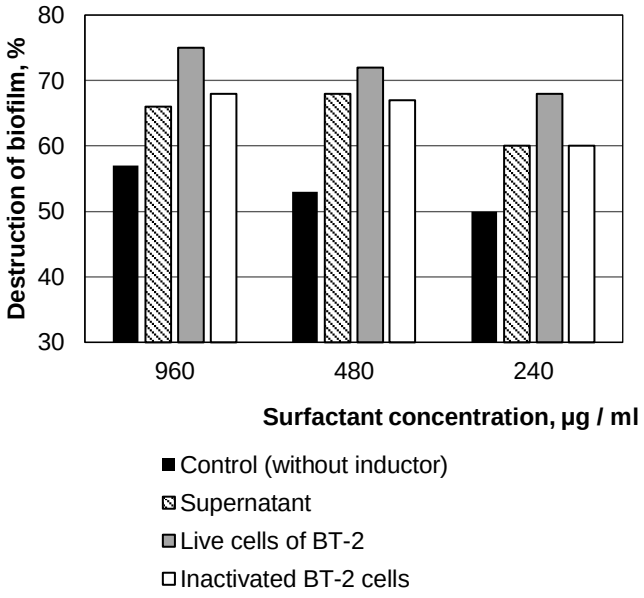

Figure 1. Effect of biological inductors in *A. calcoaceticus* IMV B-7241 in a medium with the waste of biodiesel production on the ability of synthesized surfactants to destroy *Staphylococcus aureus* BMS-1 biofilm

Under the action of lower concentrations of surfactants (7.5–120 μg/ml) synthesized on crude glycerol in the presence of supernatant, live and inactivated *B. subtilis* BT-2 cells, the destruction of *S. aureus* BMS-1 biofilm was at the same level as that caused by surfactants obtained in medium without inductors.

Destruction of biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 on purified glycerol in the presence of biological inductors

The data presented in Table 2 show that in contrast to the cultivation of *A. calcoaceticus* IMV B-7241 on crude glycerol, the growth of surfactant producer on purified glycerol in the presence of both live and inactivated inducer cells as well as supernatant showed the synthesis of surfactants under the influence of which the destruction of bacterial biofilms was on average 10–20% higher compared to the action of preparations synthesized without inductor.

Table 2

Effect of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with purified glycerol in the presence of *B. subtilis* BT-2 cells on bacterial biofilms destruction

Test culture	Biological inductor	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)					
		240	120	60	30	15	7,5
Bacillus subtilis BT-2	Control	55	54	43	40	38	35
	Supernatant	54	52	41	40	36	36
	Live cells	64	60	58	50	50	48
	Inactivated cells	59	58	50	45	43	40
Staphylococcus aureus BMS-1	Control	38	33	31	30	30	28
	Supernatant	56	52	48	40	34	33
	Live cells	65	65	50	46	40	36
	Inactivated cells	55	53	48	40	35	33
Enterobacter cloacae C-8	Control	55	55	50	40	35	30
	Supernatant	60	60	55	55	45	40
	Live cells	80	75	73	70	70	65
	Inactivated cells	76	68	65	60	55	51
Proteus vulgaris PA-12	Control	38	38	38	36	32	29
	Supernatant	47	45	45	40	40	36
	Live cells	58	55	55	50	43	42
	Inactivated cells	58	52	50	48	40	40

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

The second fundamental difference between surfactants synthesized on purified glycerol in the presence of inductors and those obtained under similar conditions of strain cultivation on crude glycerol is a lower effective concentration ensuring maximum (over 50%) degradation of bacterial biofilms (30–240 and 60–960 μg/ml respectively (Tables 1 and 2). The most effective inductor under *A. calcoaceticus* IMV B-7241 cultivation on purified glycerol was *B. subtilis* BT-2 live cells: the surfactants synthesized in their presence were characterized by a higher ability to destroy most bacterial biofilms than those obtained using inactivated cells or cell-free supernatant. Data about the destruction of yeast biofilms by surfactants synthesized in the presence of inductors are shown in Table 3.

Table 3

Destruction of yeast biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with purified glycerol in the presence of live and inactivated *B. subtilis* BT-2 cells

Test culture	Biological inductor	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)					
		240	**120**	**60**	**30**	**15**	**7,5**
Candida tropicalis PE-2	Control (without inductor)	43	42	40	39	33	30
	Live cells of *Bacillus subtilis* BT-2	57	57	56	54	53	42
	Inactivated *Bacillus subtilis* BT-2 cells	51	50	45	45	43	43
Candida albicans D-6	Control (without inductor)	41	41	41	36	28	23
	Live cells of *Bacillus subtilis* BT-2	64	60	55	46	46	45
	Inactivated *Bacillus subtilis* BT-2 cells	48	48	42	40	34	30

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

Increased destruction of yeast biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 on purified glycerol in the presence of inductors was observed only when live and inactivated *B. subtilis* BT-2 cells, except supernatant were used as inductors, and besides live cells were been more effective compared to inactivated cells.

The degree of destruction of yeast biofilms by surfactants produced by IMV B-7241 strain in medium with live cells of inductor was 6–16% and 10–23% higher compared to those established for surfactants synthesized with *B. subtilis* BT-2 inactivated cells and without inductor, respectively.

Both bacterial biofilm destruction (see Table 2) and destruction of *C. tropicalis* PE-2 and *C. albicans* D-6 biofilms were at the maximum level (40–64%) when surfactants produced in the presence of an inductor were used at 30–240 μg/ml concentrations. Similar degree destruction of yeast biofilms under the influence of surfactants synthesized in the medium with crude glycerol and inductors was achieved at a higher surfactant concentration (60–960 μg/ml) (Table 1).

Note that, there is limited data in the literature on the ability of surfactants synthesized in the presence of biological inducers (live or inactivated cells, or supernatant) to destroy microbial biofilms.

The work (Kimelman and Shemesh, 2019) showed that in the presence of supernatant after co-cultivation of *B. subtilis* with *Lactobacillus plantarum* the degree of *S. aureus* biofilm destruction reached up to 61%, whereas supernatant after growing *L. plantarum* monoculture inhibited the formation of biofilm by only 40% after 24 h. The authors found that the increasing in biofilm destruction by the supernatant after co-cultivation of the two strains was due to the synthesis of antimicrobial lipopeptides by *Bacillus subtilis* under these conditions.

It was found by other researchers (Hamza et al., 2018) that the supernatant after co-cultivation of *Staphylococcus lentus* SZ2 and *Vibrio harveyi* MTCC 7771 was able to inhibit the biofilm of *V. harveyi* MTCC 7771 pathogen. In addition, the degradation of the *V. harveyi* biofilm after 24 h treatment with the supernatant obtained after *Staphylococcus lentus* SZ2

monoculture cultivation was 40%, while under the action of the supernatant after the strains co-cultivation reached 79%.

In research (Mohamed et al., 2020), two bacterial strains of *Micromonospora* sp. UR56 and *Actinokineospora* sp. EG49 produced new metabolites that were not typical for monocultures. The induced metabolites were phenazine derivatives and *they* showed the ability to destroy biofilms of *B. subtilis*, *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Phenazine at a concentration of 10 μl destroyed 24-hour biofilms of *E. coli* by an average of 54%, *S. aureus* by 50%, *B. subtilis* by 18% and *P. aeruginosa* by 42%.

Previously (Pirog et al., 2020a), it waas found that regardless of the time of introduction of competitive bacteria (*E. coli* IEM-1 and *B. subtilis* BT-2) into medium of *Nocardia vaccinii* IMV B-7405 cultivation and their physiological state (live, inactivated cells) the synthesis of surfactants was observed, after treatment with which the degree of *B. subtilis* BT-2, *S. aureus* BMS-1, *Pseudomonas* sp. MI-2 biofilm destructions were 10–35% higher compared to those established for surfactants obtained in medium without competitive microorganisms.

Our other studies (Pirog et al., 2020b) it was shown that the degree of destruction of bacterial (*B. subtilis* BT-2, *S. aureus* BMS-1, *Pseudomonas* sp. MI-2) and yeast (*C. albicans* D-6 and *Candida utilis* BBC-65) biofilms under the influence of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 in the presence of live *E. coli* IEM-1 and *B. subtilis* BT-2 cells, reached up to 40-94 % and was higher compared to those surfactants synthesized by IMV Ac-5017 strain in a medium without inductors (32–65%).

To compare the degree of biofilm destruction by our and other well-known microbial surfactants (lipopeptides, rhamnolipids, sophorolipids) synthesized on glycerol, the literature data on the ability of such microbial surfactants to destroy microbial biofilms was analysed.

It is shown that under action of *B. subtilis* VS16 lipopeptides at fairly high concentrations (3000–5000 μg/ml) destruction of *S. aureus* ATCC 29523 biofilms was 67.4%, *E. coli* MTCC 65 – 63.9%, *S. typhimurium* ATCC 19430 – 61.1% (Giri et al., 2019). In addition, in the work (Sen et al., 2020) the authors showed that the destruction of *Trichophyton rubrum* MTCC 8477 and *Trichophyton mentagrophytes* NCCPF 800049 biofilms after treatment with rhamnolipids synthesized by *P. aeruginosa* SS14 reached 80-85% at the surfactant concentration of 2000 and 250 μg/ml, respectively.

In research (Borah et al., 2019) was found that rhamnolipids synthesized by *P. aeruginosa* SS14 on waste of alcohol production reached the highest (90–95%) degree of *C. tropicalis* MTCC 1000 destruction at concentrations of 500–1000 μg/ml, respectively.

The results of our studies showed that the introduction of inductors in *A. calcoaceticus* IMB B-7241 cultivation medium allowed to obtain surfactants that effectively destroy bacterial and yeast biofilms at much lower (several orders of magnitude) concentrations (7.5-480 μg / ml), than described in the literature.

Note that at present we could not find information in the available literature on the increased efficiency of yeast biofilm destruction (including *Candida* yeast) in the presence of microbial metabolites synthesized during co-cultivation of microorganisms.

Conclusion

Consequently, as a result of this work, the possibility of regulating the biological activity of *A. calcoaceticus* IMV B-7241 surfactants has been established by introducing into the cultivation medium the competitive bacteria *B. subtilis* BT-2 cells, which are inductors of synthesis of surfactant with a higher ability to destroy both bacterial and yeast biofilms.

References

Algburi A., Comito N., Kashtanov D., Dicks L., Chikindas M.L. (2017), Control of biofilm formation: antibiotics and beyond, *Applied and Environmental Microbiology Journal*, 83(3), DOI: 10.1128/AEM.02508-16.

Allegrone G., Ceresa C., Rinaldi M., Fracchia L. (2021), Diverse effects of natural and synthetic surfactants on the inhibition of *Staphylococcus aureus* biofilm, *Pharmaceutics*, 13(8), pp. 1172, DOI: 10.3390/pharmaceutics13081172.

Bionda N., Fleeman R.M., Fuente-Núñez C., Rodriguez M.C., Reffuveille F., Shaw L.N., Pastar I., Davis S.C., Hancock R., Cudic P. (2016), Identification of novel cyclic lipopeptides from a positional scanning combinatorial library with enhanced antibacterial and antibiofilm activities, *European Journal of Medicinal Chemistry*, 108, pp. 354–363, DOI: 10.1016/j.ejmech.2015.11.032.

Borah S.N., Sen S., Goswami L., Bora A., Pakshirajan K., Deka S. (2019), Rice based distillers dried grains with solubles as a low cost substrate for the production of a novel rhamnolipid biosurfactant having anti-biofilm activity against *Candida tropicalis*, *Colloids and surfaces. B, Biointerfaces*, 182, pp. 110358, DOI: 10.1016/j.colsurfb.2019.110358.

Chmielarz M., Blomqvist J., Sampels S., Sandgren M., Passoth V. (2021), Microbial lipid production from crude glycerol and hemicellulosic hydrolysate with oleaginous yeasts. *Biotechnology for Biofuels*, 14(1), pp. 65, DOI: 10.1186/s13068-021-01916-y.

Diamantopoulou P., Filippousi R., Antoniou D., Varfi E., Xenopoulos E., Sarris D., Papanikolaou S. (2020), Production of added-value microbial metabolites during growth of yeast strains on media composed of biodiesel-derived crude glycerol and glycerol/xylose blends, *FEMS Microbiology Letters*, 367(10), DOI: 10.1093/femsle/fnaa063.

D'Cunha N.M., Peterson G.M., Baby K.E., Thomas J. (2018), Impetigo: a need for new therapies in a world of increasing antimicrobial resistance, *Journal of Clinical Pharmacy and Therapeutics*, 43(1), pp. 150–153, DOI: 10.1111/jcpt.12639.

Giri S.S., Ryu E., Sukumaran V., Chang Park S. (2019), Antioxidant, antibacterial, and antiadhesive activities of biosurfactants isolated from Bacillus strains, *Microbial Pathogenesis*, 132, pp. 66−72, DOI: 10.1016/j.micpath.2019.04.035.

Hamza F., Kumar A.R., Zinjarde S. (2018), Coculture induced improved production of biosurfactant by *Staphylococcus lentus* SZ2: Role in protecting *Artemia salina* against *Vibrio harveyi*, *Enzyme and Microbial Technology*, 114, pp. 33–39, DOI: 10.1016/j.enzmictec.2018.03.008.

Huigens R.W., Abouelhassan Y., Yang H. (2019), Phenazine antibiotic-inspired discovery of bacterial biofilm-eradicating agents, *Chembiochem: a European journal of chemical biology*, 20(23), pp. 2885–2902, DOI: 10.1002/cbic.201900116.

Kimelman H., Shemesh M. (2019), Probiotic bifunctionality of *Bacillus subtilis*—rescuing lactic acid bacteria from desiccation and antagonizing pathogenic *Staphylococcus aureus*, *Microorganisms*, 7, 407, DOI: 10.3390/microorganisms7100407.

Kumar A., Singh S.K., Kant C., Verma H., Kumar D., Singh P.P., Modi A., Droby S., Kesawat M. S., Alavilli H., Bhatia S.K., Saratale G.D., Saratale R.G., Chung S.M., Kumar M. (2021), Microbial biosurfactant: a new frontier for sustainable agriculture and pharmaceutical industries, *Antioxidants (Basel, Switzerland)*, 10(9), pp. 1472, DOI: 10.3390/antiox10091472.

Lin Z., Yuan T., Zhou L., Chen, S., Qu X., Lu P., Feng Q. (2021), Impact factors of the accumulation, migration and spread of antibiotic resistance in the environment, *Environmental Geochemistry and Health*, 43(5), pp. 1741−1758, DOI: 10.1007/s10653-020-00759-0.

Mohamed S.H., Hossam M., Mohammed R., Mohamed M.F., Ahmed S.M., Ahmed A.H., Sameh F.A., Mostafa E.R., Hani A.A., Ramadan A.U. (2020), Induction of antibacterial metabolites

by co-cultivation of two red-sea-sponge-associated *Actinomycetes micromonospora* sp. UR56 and *Actinokinespora* sp. EG49, *Marine Drugs*, 18(5), pp. 243, DOI: 10.3390/md18050243.

Ohadi M., Forootanfar H., Dehghannoudeh G., Eslaminejad T., Ameri A., Shakibaie M., Adeli-Sardou M. (2020), Antimicrobial, anti-biofilm, and anti-proliferative activities of lipopeptide biosurfactant produced by *Acinetobacter junii* B6, *Microbial Pathogenesis*, 138, DOI: 10.1016/j.micpath.2019.103806.

Paraszkiewicz K., Moryl M., Płaza G., Bhagat D., Satpute S., Bernat P. (2021), Surfactants of microbial origin as antibiofilm agents, *International Journal of Environmental Health Research*, 31(4), pp. 401–420, DOI: 10.1080/09603123.2019.1664729

Parrino B., Schillaci D., Carnevale I., Giovannetti E., Diana P., Cirrincione G., Cascioferro S. (2019), Synthetic small molecules as anti-biofilm agents in the struggle against antibiotic resistance, *European Journal of Medicinal Chemistry*, 161, pp. 154–178, DOI: 10.1016/j.ejmech.2018.10.036.

Pirog T.P., Lutsai D.A., Shevchuk T.A., Iutynska G.O., Elperin I.V. (2018), Antimicrobial and anti-adhesive activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 on technical glycerol, *Mikrobiologichny Zhurnal*, 80(2), pp. 14−27, DOI: 10.15407/microbiolj80.02.014.

Pirog T.P., Skrotska O.I., Shevchuk T.A. (2020a), Influence of biological inducers on antimicrobial, antiadhesive activity and biofilm destruction by *Nocardia vaccinii* IMV V-7405 surfactants, *Mikrobiologichny Zhurnal*, 82(3), pp. 35-44, DOI: https://doi.org/10.15407/microbiolj82.03.035.

Pirog T., Kluchka L., Skrotska O., Stabnikov V. (2020b), The effect of co-cultivation of *Rhodococcus erythropolis* with other bacterial strains on biological activity of synthesized surface-active substances, *Enzyme and Microbial Technology*, 142, 109677, DOI: 10.1016/j.enzmictec.2020.109677.

Pirog T., Ivanov M., Yarova H. (2021), Antimicrobial activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants, synthesized in the presence of biological inductors, *Scientific Works of NUFT***,** 27(4), pp. 43-52.

Poladyan A., Blbulyan S., Semashko T., Dziameshka V., Zhukouskaya L., Trchounian A. (2020), Application of organic waste glycerol to produce crude extracts of bacterial cells and microbial hydrogenase-the anode enzymes of bio-electrochemical systems, *FEMS Microbiology Letters*, 367(7), DOI: 10.1093/femsle/fnaa056.

Salazar-Bryam A.M., Lovaglio R.B., Contiero J. (2017), Biodiesel byproduct bioconversion to rhamnolipids: Upstream aspects, *Heliyon*, 3(6), DOI: 10.1016/j.heliyon.2017.e00337.

Sen S., Borah S.N., Bora A., Deka S. (2020), Rhamnolipid exhibits anti-biofilm activity against the dermatophytic fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes*, *Biotechnology Reports*, 27, DOI: 10.1016/j.btre.2020.e00516.

Sharma D., Misba L., Khan A.U. (2019), Antibiotics versus biofilm: an emerging battleground in microbial communities, *Antimicrobial Resistance and Infection Control*, 8**(**76), DOI: 10.1186/s13756-019-0533-3.

Yan X., Gu S., Cui X., Shi Y., Wen S., Chen H., Ge J. (2019), Antimicrobial, anti-adhesive and anti-biofilm potential of biosurfactants isolated from *Pediococcus acidilactici* and *Lactobacillus plantarum* against *Staphylococcus aureus* CMCC26003, *Microbial pathogenesis*, 127, pp. 12–20, DOI: 10.1016/j.micpath.2018.11.039.

Increase the ecological safety of the soil biogrouting using plant urease

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Abstract

Introduction. Biocement is a new building material based on the use of bacterial urease. The application of plant urease is the way for a wide and environmentally friendly application of this biotechnology for soil biogrouting.

Materials and methods. Urease activity of germinated soy seeds was determined by changing the electrical conductivity of the urea solution, μS/cm, due to its hydrolysis under the action of the urease enzyme. Calcium concentration was determined by titration with ethylenediaminetetraacetate using Eriochrome black T indicator. Assessment of sand biocementation was provided by the change of its water permeability.

Results and discussion. The disadvantages of the biocementation process are the potential biohazard from the used urease-producing bacteria and the unpredictable effect of introducing a significant amount of live bacterial biomass into the environment in the case of soil biogrouting. A possible replacement for bacterial urease may be plant-derived urease. Screening of seeds of agricultural crops grown in Ukraine showed that soybean seeds can be used as a source of urease for biocementation in the form of a crude aqueous extract from the crushed mass of the seeds themselves or germinated within 24-48 hours.

The urease activity of the homogenized mass of soy seeds was higher than the activity of pure extracts, and the specific activity – activity per unit of plant material – of both the homogenized mass and the extract was higher when using seeds that were germinated for 24–48 hours and specific activity of seeds germinated for 96 hours decreased.

The use of a crude extract from soybean seeds showed its effectiveness for the precipitation of calcium carbonate from a solution of calcium chloride and urea. Application of plant urease for sand biocementation made it possible to reduce water permeability by 600 times and obtain values of water seepage $1 \cdot 10^{-6}$ m/s that allowed the use of plant urease instead of urease-producing bacteria for soil biogrouting.

Conclusions. The possibility to replace in biocementation bacterial urease with plant-derived urease, particularly extract from soybean seeds, was shown. Plant urease effectively precipitated calcium carbonate from a mixture of solutions of calcium chloride and urea, and its use in the biocementation of sand reduced its water permeability to values corresponding to the seepage of sand biocemented with urease-producing bacteria.

Introduction

A new area of biotechnology – the use of microorganisms for the needs of construction has been successfully developing in the world over the past 15 years. A special place among new building materials is occupied by the production of biomement and biogrouts based on the application of urease-producing bacteria, which, in the presence of urea and calcium ions in an alkaline medium, catalyze the process of the so-called microbial induced calcium carbonate precipitation (MICP) and form insoluble calcite crystals (Ivanov and Stabnikov, 2017; Rosquoet et al., 2002).

Bacteria-induced mineral precipitation is a well known phenomena in Nature, which plays a significant role in Earth's mineral deposits (Hoffmann et al., 2021). The formation of calcium carbonate $CaCO_3$ is one of the most researched and studied biomineralization processes (Bang et al., 2010; Fernandez et al., 2018; Ronholm et al., 2014). Biocement is considered an ecological material inspired by nature itself (Achal, 2015).

An interesting example of natural biocementation in Ukraine can be a rock in the village of Podkamen, Brody district, Lviv region, Ukraine. This is a huge rock 16 m high and about 11 million years old (Figure 1a). In places where the outer hard crust is broken, clean sand is visible. An example of the use of MICP, the transformation of 1 m^3 of sand into stone using urease-producing bacteria, is shown in Figure 1b (Ivanov and Stabnikov, 2017).

a ***b***

Figure 1. Object of natural biocementation: the rock in the village of Pidkamin, Ukraine (*a*) and the result of artificial biocementation using MICP of sand (*b*)

To obtain biocement, most researchers use bacterial biomass with urease activity, since the use of the urease enzyme is expensive. Urease-producing bacteria *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) that is the most commonly used for biocementation (Bang et al., 2010; Keykha et al., 2018; Mortensen and DeJong, 2011; Whiffin et al., 2007) are belonging to the RG1 group of microorganisms (biologically safe) according to the European Union Directive (Directive 2000/54/EC). At the same time, there are many opportunistic and pathogenic species, halotolerant and alkaliphilic, with significant urease activity, which are considered in some studies as possible biological agents for biocementation (Dosier, 2014; Han et al., 2013; Maheswaran et al., 2014; Varalakshmi, 2014; Zaghloul et al., 2021). Some authors have isolated active urease-producing bacteria for further use in biocementation processes from activated sludge of sewage treatment plants (Al-Thawadi, 2012; Varalakshmi and Devi, 2014; Xu et al., 2017), while the risk of isolation of opportunistic pathogens is high. The use of biosafe and relatively cheap sources of urease is indicated as one of the essential requirements for the widespread practical use of soil biogrouting, when a large number of living cells of urease-producing bacteria are introduced into the environment (Ivanov et al., 2019).

A possible replacement for bacterial urease could be an environmentally friendly plant-derived urease (Dilruksh and Kawasaki, 2016; Ivanov et al., 2019). The main role of urease in plants is to allow the organism to use urea as a source of nitrogen. Several families of common plants are very rich in urease, including certain varieties of beans, melons and pumpkins, and even the pine family (Das et al., 2002). It is known to use Jack beans (*Canavalia ensiformis* and *Canavalia adans*) for the industrial production of plant urease (Kakimoto et al., 1992). There are studies where plant leaves, rather than seeds, are studied as a source material with urease activity, for example, mulberry leaves (*Morus alba*) (Hirayama et al., 2000), wheat and soybean leaves (Hogan et al., 1983).

The authors usually propose the use of local plant materials to obtain extracts with urease activity for their further application as a source of urease in biocementation. For example, crude extracts from watermelon (*Citrullus lanatus*) seeds, which are considered food waste (Al Imran et al., 2021; Dilruksh et al., 2018), from crushed outer leaves of cabbage and soy pulp (Baiq et al., 2020), and jack beans (*Canavalia gladiata*) (Tirkolaei et al., 2020) for biocementation.

The aim of the present study was to increase the ecological safety of the process of soil biograiting using urease of agricultural plants.

Materials and methods

Plant seeds

To determine the possibility of using the seeds of agricultural crops as a source of urease, the seeds of green lentils, peas, white beans, black beans, soybeans, pumpkins, watermelons and melons were germinated by water-air method. Seeds were soaked for 48 hours: the period of exposure to water was 6 hours, followed by 6 hours of exposure to air), and then self-germination was carried out for 96 hours with periodical spraying with water to prevent the seeds from drying out (Stabnikova et al., 2021; 2023).

Sand

For biocementation, river sand sifted through a metal sieve with holes (diameter of 0.5 mm) was used. Particle size distribution and mean size were measured using a Bettersizer S3 Plus particle size analyser (Bettersize Instruments, Dandong, China). For each sample, 3 measurements were done to determine the average particle size. The maximum size of 10% (D10), 50% (D50) and 90% (D90) of all particles was also determined.

Determination of urease activity

Urease activity was determined using a TDS-3 portable conductometer: the amount of released ammonium was determined according to the calibration graph by changing the electrical conductivity of the solution, μS/cm, due to hydrolysis of urea under the action of the urease enzyme. The molar concentration of NH_4^+ (Y) correlated linearly ($R^2 = 0.999$) with the change in the electrical conductivity of the solution (ΔX) in μS/cm over 5 minutes. Urease activity (UA) was defined as the amount of ammonium formed in 1M urea solution per minute.

To determine the urease activity of plant raw materials, seeds together with sprouts or sprouts separately were ground in a mortar, 0.5 g of grounded material were weighed, and mixed with 10 ml of distilled water in a glass beaker (the concentration of the obtained extract was 50 g/l) and urease activity of this extract was determined.

Determination of calcium concentration

The amount of calcium carbonate was measured by filtration and drying at 60°C.

Calcium concentration was determined by the standard APHA 2340C method with ethylenediaminetetraacetate (EDTA) titration (APHA, 1999). A liquid sample, 50 cm^3, was placed in a conical flask, 1 cm^3 of buffer solution to maintain pH 10.0 and a few drops of Eriochrome black T indicator were added. The sample was titrated with 0.01 M EDTA solution until the colour changed from purple to blue.

Determination of water permeability of biocemented sand

To determine the water permeability of the treated sand samples, 0.1–0.2 dm^3 of tap water was supplied by gravity from 1 dm^3 of a container with water at a practically constant hydraulic pressure of 0.5 m of water. This measurement was close to ASTM D2434-68 (2006) "Standard Test Method for Permeability of Granular Soils". The hydraulic permeability of sand, P, in a sand core was calculated according to equation:

$$P = \frac{V}{t} \cdot A, \text{ m/s},$$

where – V is the volume of water supplied to a sand column; t is the time for which water passes through the sand; A is the cross-sectional area of the column.

Statistical analysis

The experiments were carried out in triplicates. Statistical processing of the experimental results was carried out using special computer programs for personal computers. Data are presented as arithmetic mean standard deviation.

Results and discussion

Selection of a plant whose seeds can be used as a safe source of urease for biocementation

Seeds of agricultural crops were selected for the study. The urease activity was measured in water extracts of germinated seeds with sprouts, as well as in sprouts separated from seeds. The urease activity of some plant materials is shown in Table 1.

Table 1

Urease activity of germinated seeds and sprouts of agricultural plants

Plant	Urease activity, mM hydrolyzed urea/min/g	
	Germinated seeds	Sprouts
Green lentils	0.29	0.31
Pumpkin	0.23	N/D*
White beans	0.26	024
Black beans	0.29	0.20
Soya	0.84	0.55
Watermelon	0.54	0.43
Melon	0.56	0.39

* N/D –not determine.

The urease activity of the germinated seeds with sprouts was a little bit higher than that of the sprouts only. According to the level of urease synthesis, the germinated seeds of the studied plants were arranged as follows: seeds of soybean, melon, watermelon, green lentil, black bean, white bean and pumpkin. Germinated soybean seeds showed higher urease activity. The urease activity of germinated seeds of melon, green lentil, black bean, white bean and pumpkin was 66, 64, 35, 35, 31 and 27% of the urease activity of germinated soybean seeds, respectively. Compared with the literature data on the urease activity of plants, urease activity of soybean seeds can be respected as relatively high. For example, the urease activity of the original extract from the leaves of mulberry (*Morus alba* L.), which was used to obtain purified plant urease, was 0.064 mM/min (Hirayama et al., 2000), and the activity of the pumpkin seed extract used in the work (Al Imran et al., 2021) was 0.01 mM/min. Soybean seeds were chosen as the most promising plant source of urease.

Choice of the form, which will be advisable to use plant urease for biocementation

Urease activity of germinated soybean seeds, 0.84 mM hydrolyzed urea/min, is lower than the activity of urease-producing bacteria commonly used in biocementation processes, but it is sufficient to use for soil biogrouting, since it has been shown that surface treatment of contaminated soil with small doses of biocement is effective in controlling its wind and water erosions and significantly reduces the release of dust, as well as bacterial and chemical pollutants into the environment (Hao et al., 2021; Ivanov and Stabnikov, 2020; Namdar-Khojasteh et al., 2022; Stabnikov et al., 2013).

To characterize the accumulation of urease in plant biomass, soybeans were germinated and samples of seeds were taken at 24, 72, and 96 hours. The value of urease activity was determined both in the homogenized mass of crushed seeds together with sprouts, and in the aqueous extract, which was obtained after removal of the suspended plant mass by centrifugation. For the preparation of extracts, different amounts of the homogenizing mass of seeds with sprouts were used, namely in proportions, g of mass to 10 ml of distilled water: 1 (10%); 2 (20%) and 4 (40%). The results on the urease activity of the homogenized mass of germinated soybean seeds and the extract obtained after removal of the plant mass by centrifugation are shown in Figure 2.

Urease activities of the homogenizing mass of seeds with sprouts were higher than the activities of water extracts and the activities of both were higher at the beginning of seeds germination. So, there was no significant change in urease activity during germination for 24–72 hours, but it decreased by 30–80% at 96 hours of germination. During the germination of peas (*Cicer arietinum* L.), another pattern was found: urease activity increased in germinated seeds and was maximum in the extract at 192 hours of germination, 0.03 mM/ml, and then decreased (Pervin et al., 2009).

To select a method for preparing plant materials to be used as a source of urease, the specific urease activity of the homogenizing mass of soybean seeds with sprouts and water extracts obtained by removing the suspended plant mass were compared. The seeds were germinated for 3 days, a homogenized mass was prepared from seeds and sprouts at 24, 48 and 96 hours of germination by treatment in a blender for 2 minutes to destroy plant cells and tissues, and then mixed thoroughly. Mixtures with different content of homogenized mass were prepared (10, 20 and 40% w/v), and to make extracts plant biomass was separated by centrifugation, and specific urease activities were determined in homogenized mass and extracts. Specific urease activity remained almost constant during 24–72 hours of germination and decreased by 96 hours. According to the data obtained, the homogenized mass of germinated seeds had a higher urease activity than the extracts, but the introduction of a large mass of plant material during biocementation can interfere with the process, so the possibility of using a homogenized mass, hereinafter a crude extract, had to be checked experimentally (Table 2).

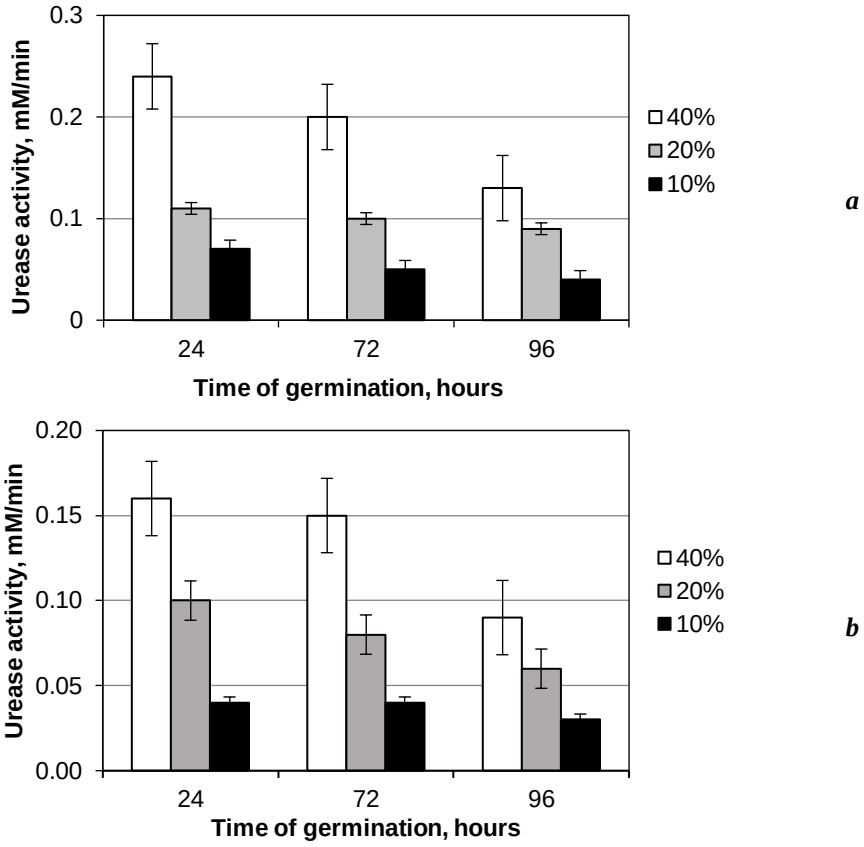

Figure 2. Urease activity of the homogenized mass with different amounts of crushed seeds with sprouts (10, 20, and 40%) (a) and the extracts obtained after removal of the plant mass by centrifugation (b).

Table 2

Specific urease activity of homogenized mass of germinated soybean seeds and extracts depending on germination time

Homogenized mass, %	Specific urease activity, mM/min·g at time of generation, h		
	24	72	96
homogenized mass (crude extract)			
40	0.6±0.05	0.5±0.03	0.3±0.02
20	0.6±0.03	0.5±0.02	0.5±0.02
10	0.7±0.05	0.5±0.05	0.4±0.03
extract			
40	0.4±0.02	0.4±0.02	0.2±0.01
20	0.5±0.05	0.4±0.04	0.3±0.01
10	0.4±0.01	0.4±0.04	0.3±0.02

Using crude extracts from soybean seeds as a source of urease for the precipitation of calcium carbonate

To confirm the possibility of using urease of soybean seed for biocementation, precipitation of $CaCO_3$ was done from a solution of chemical compounds used for microbially initiated precipitation of calcium carbonate, namely, a mixture of equimolar solutions of $CaCl_2$ and urea, with the addition of crude extracts from soybean seeds or germinated seeds. 20 ml of a mixture of equimolar solutions of $CaCl_2$ and urea with different molar concentrations were added to the propylene tubes. Prepared 10% crude extracts of soy seeds and germinated soy seeds without centrifugation, were added in the amount of 5 ml per tube and incubated at 30 °C for 48 hours. Part of the tubes were placed on a laboratory shaker at 150 rpm and stirred for 4 h every day. Precipitation of $CaCO_3$ was determined by titration with the indicator Eriochrome black (Tables 3 and 4).

Table 3

Calcium precipitation using soy seed crude extracts (no agitation)

$CaCl_2$: urea	Initial concentration of Ca^{2+}, g/l	Precipitated Ca^{2+} using crude extract of			
		Seeds		Germinated seeds	
		g/l	%	g/l	%
0.3 M:0.3 M	12	11.87	98.92	11.78	98.17
0.5 M:0.5 M	20	16.04	80.20	17.10	85.5
0.7 M:0.7 M	28	17.35	61.96	19.23	68.68
1.0 M:1.0 M	40	21.18	52.95	22.32	55.80
1.5 M:1.5 M	60	23.47	39.12	24.79	41.32

Table 4

Calcium precipitation using soy seed crude extracts (with agitation)

$CaCl_2$: urea	Initial concentration of Ca^{2+}, g/l	Precipitated Ca^{2+} using crude extract of			
		Seeds		Germinated seeds	
		g/l	%	g/l	%
0.3 M:0.3 M	12	10.63	88.58	11.92	99.30
0.5 M:0.5 M	20	10.35	51.75	17.67	88.35
0.7 M:0.7 M	28	13.69	48.89	29.19	72.11
1.0 M:1.0 M	40	22.12	55.30	23.73	59.33
1.5 M:1.5 M	60	25.81	41.68	25.21	42.02

The concentration of dissolved calcium in the liquid under precipitate was determined by titration with the indicator Eriochrome black. The amount of precipitated calcium was determined from the difference between the initial content of calcium and that remaining in the solution, and the percentage of precipitation was calculated. As can be seen, the precipitation of calcium occurred better with the use of crude extracts from germinated seeds and with periodic mixing of the solutions, but this difference was insignificant and amounted to no more than a few percent. The amount of precipitated calcium increased with an increase in its initial value, but the percentage of removal, on the contrary, decreased. Therefore, when

carrying out soil biogrouting or biocementation, an equimolar ratio of calcium chloride and urea above 1.5 should not be used, and application of seeds without germination as a source of urease resulted in formation of calcium carbonate.

Biocementation of sand using plant urease

Screened river sand was used for biocementation. Particle size distribution and mean size were measured using a Bettersizer S3 Plus particle size analyser (Bettersize Instruments, Dandong, China) (Figure 3, Table 5).

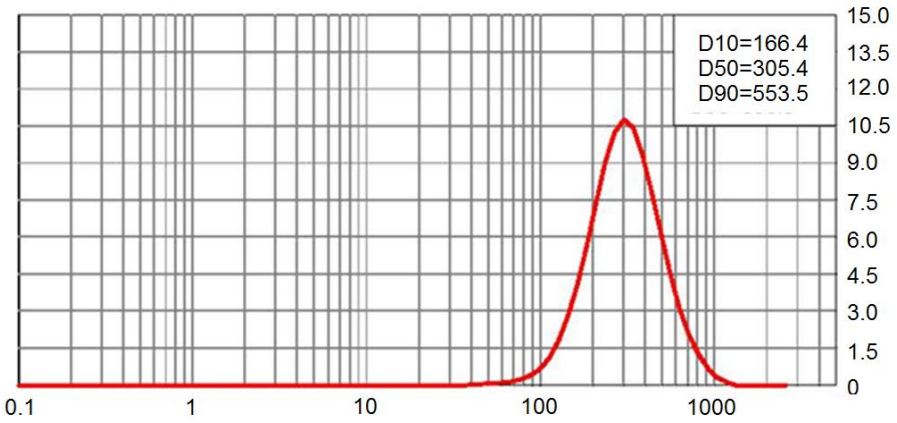

Figure 3. Particle size distribution of screened sand

Table 5

Particle size distribution of screened sand

d, µm	0-200	200-300	300-400	400-500	500-600	600-700	700-800	800-900	900-1000	1000-1500
%	18.12	30.31	23.74	13.47	7.22	3.44	1.86	0.99	0.50	0.35

Thus, the main sand fractions contained particles ranging in size from 200 to 300 µm (30.31%) and in size from 300.0 to 400 µm (23.74). D_{10}=166.4 µm; D_{50}=305.4 µm; D_{90}=553.5 µm, i.e. 10% of the sand had particles with diameter less than 166.4 µm, 50% less than 330 µm and 90% less than 1600 µm. Based on the grain size of the particle, sand is classified as fine sand (75 to 425 µm), medium sand (425 to 2000 µm), and coarse sand (2000 to 4750 µm). That is, the sand that was used for biocementation was fine. For comparison, biocementation studies typically use: ASTM (American Society for Assaying and Materials) sorted sand with an average grain size of 400 µm; standard round sand (Societe Nouvelle du Littoral, France) with an average grain size of 420 µm; standard Ottawa sand with an average size of 300 µm with a particle size variation from 150 to 1180 µm.

Biocementation of screened sand was carried out in syringes (3 cm diameter and 10 cm in length). Sand was placed into each syringe in an amount of 60 g. Suspension of homogenized mass of seeds or germinated seeds (seeds with sprouts) were used as a source of urease. The efficiency of biocementation was evaluated by the change in the permeability of the treated sand. The zero point was the rate of water passage in the sand before the start of biocementation.

Caputo (2004) defines soil permeability as the amount of water passing through the pores between particles at different speeds over a certain period of time. Estimating the rate of water passage is relevant because the water content of any soil zone is related to the relationship between soil tension and pressure, which depends on the increased amount of water infiltrating. The coefficient of water permeability (the amount of passing water) is usually advisable to use for porous media. There were 8 cycles of biocementation, and they consisted of the following steps: slow feed of the crude extract, 10 ml; exposure for 2 hours; supply of solutions of calcium chloride and urea with different ratio $CaCl_2$: urea, namely: 0.5 M : 0.5 M; 0.7 M: 0.7 M; 1.5 M: 1.0 M. After each biocementation, the sand was allowed to stand for 24 hours for calcium carbonate crystals to form, and the water permeability of the sand was determined. The results are presented in Figure 4.

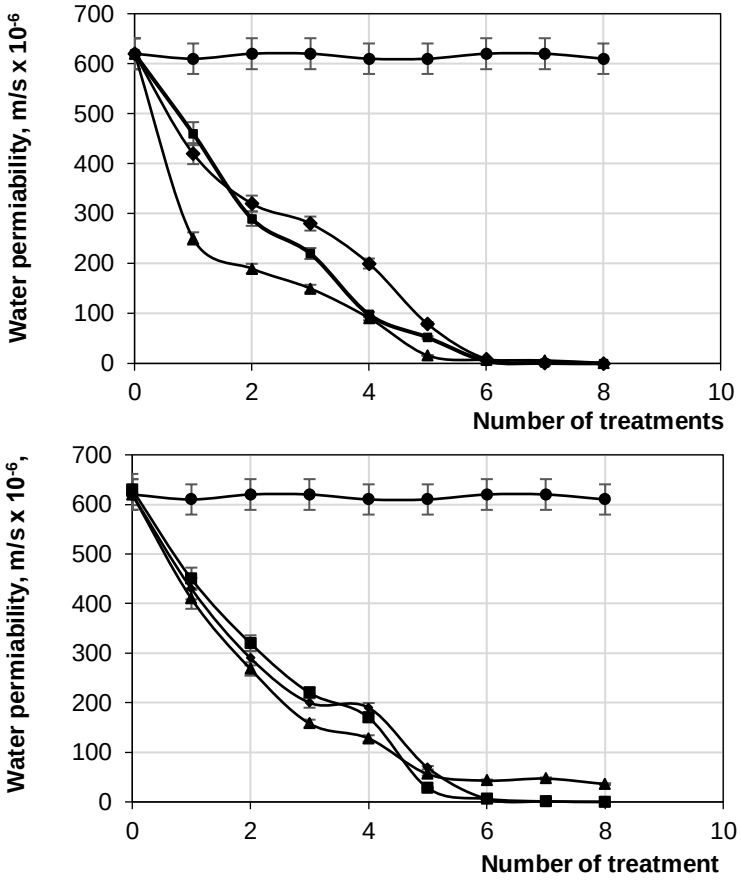

Figure 4. Changes in the water permeability of biocemented sand when using an crude extract from germinated seeds (a) and seeds (b):

● control;
▲- sand, which was treated with a 0.5 M solution of urea and calcium chloride;
■ – sand that was treated with a 1.0 M solution of urea and calcium chloride;
◆ – sand, which was treated with a 1.5 M solution of urea and a 1 M solution of calcium chloride.

In general, during the biocementation of sand using plant urease, the water permeability decreased from $6 \cdot 10^{-4}$ m/s to $1 \cdot 10^{-6}$ m/s, that is, it decreased by 600 times. The values obtained correspond to the seepage rates of sand biocemented with conventionally used urease-producing bacteria (Chu et al., 2013; Ivanov and Stabnikov, 2017), and seepage rates from actual aquaculture ponds (Teichert-Coddington et al., 1988; Weisburd and Laws, 1990). Thus, due to the action of plant urease, because of the formation of calcium carbonate $CaCO_3$, the pores in loose sand are filled, the sand particles are bound, the water permeability is reduced and, as a result, their strength is increased. Similar results were obtained in the work of Japanese scientists (Al Imran et al., 2021), who used crushed and mixed watermelon seeds (both dry and germinated) as a source of the urease enzyme for sand biocementation at neutral pH ~7 and temperature 30 °C. Biocementation assessed with decrease of water permeability was going faster when solutions with low concentration of calcium chloride and urea were used and the use of 1M solutions should be considered optimal, which provided a lower final water permeability of the sand samples. Photos of biocemented sand are shown in Figure 5.

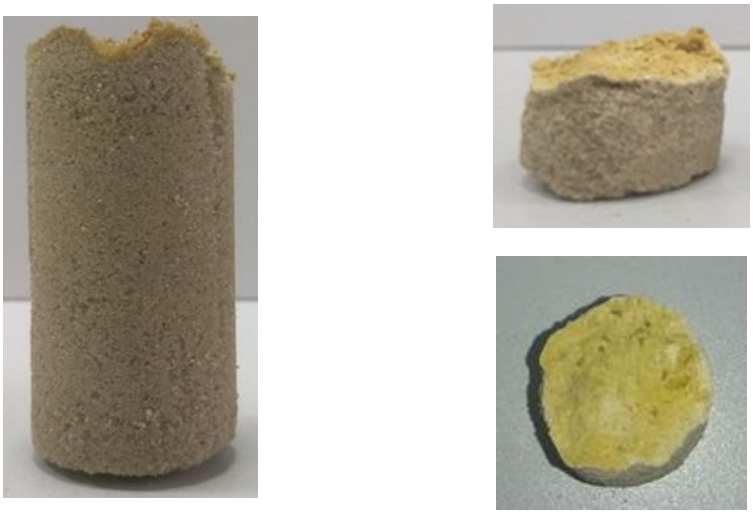

Figure 5. Sand after biocementation using soybean seed urease

The advantages of this method of biocementation are evident: (a) there is no need to grow a microbial producer of urease, that is, the technology is greatly simplified; (b) the crude extract can be prepared just prior to its use; (c) the cost of the biocementation process is significantly reduced, since there is no need for a nutrient medium for growing bacteria, electricity consumption for aeration in the growing process, and the use of highly qualified personnel to obtain microbial biomass with urease activity; (d) one of the important problems of biocementation is completely solved – cells of the microbial urease producer do not enter the environment, and an aqueous solution of homogenized plant biomass does not need additional determinations of its biosafety. This biocementation method can be used as an environmentally friendly and sustainable method for solving numerous geotechnical problems, such as soil stabilization, elimination of the consequences of soil liquefaction during earthquakes, soil protection from erosion, the atmosphere from dust, and chemical pollutants.

Conclusions

1. Screening of seeds of agricultural crops grown in Ukraine showed that soybeans can be used as a source of urease for biocementation in the form of a crude aqueous extract from the seed mass itself or germinated within 24–48 hours.
2. The use of a crude soybean seed extract has been shown to be effective in precipitating calcium carbonate from a mixture of calcium chloride and urea solutions.
3. The use of a crude soybean extract in the biocementation of sand made it possible to reduce its water permeability and obtain values corresponding to the seepage rates of sand biocemented with traditionally used urease-producing bacteria.

References

Achal V., Mukherjee A., Kumari D., Zhang Q. (2015), Biomineralization for sustainable construction – A review of processes and applications, *Earth-Science Reviews*, 148, pp. 1-17, DOI: 10.1016/j.earscirev.2015.05.008

Al Imran M., Nakashima K., Evelpidou N., Kawasaki S. (2021), Improvement ofusing crude extract urease from watermelon seeds for biocementation technology, *Geomate Journal*, 20(78), pp. 142–147. https://geomatejournal.com/geomate/article/view/197

Al-Thawadi S. (2012), Calcium carbonate crystals formation by ureolytic bacteria isolated from Australian soil and sludge, *Journal of Advanced Science and Engineering Research*, 2(1), pp. 12–26. https://www.academia.edu/8004358/ Calcium_ Carbonate_Crystals_Formation_by_Ureolytic_Bacteria_Isolated_from_Australian_So il_and_Sludge

APHA. Standard Methods for the examination of Water and Wastewater, 20th ed., American Public Health Association: Washington, DC, 1999.

Baiq H.S., Yasuhara H., Kinoshita N., Putra H., Johan E. (2020), Examination of calcite precipitation using plant derived urease enzyme for soil improvement, *International Journal of GEOMATE*, 19(72), 231–237. https://geomatejournal.com/geomate/article/view/1

Bang S.S., Lippert J.J., Yerra U., Mulukutla S., Ramakrishnan, V. (2010), Microbial calcite, a bio-based smart nanomaterial in concrete remediation, *International Journal of Smart and Nano Materials*, 1(1), 28–39, DOI: 10.1080/19475411003593451

Chu J., Ivanov V., Stabnikov V., Li B. (2013), Microbial method for construction of aquaculture pond in sand, Géotechnique, 63(10), pp. 871 – 875. https://doi.org/10.1680/geot.SIP13.P.007

Das, N., Kayastha, A.M., Srivastava, P.K. (2002). Purification and characterization of urease from dehusked pigeonpea (Cajanus cajan L) seeds. Phytochemistry, 61(5), 513-521, DOI: 10.1016/s0031-9422(02)00270-4

Dilrukshi R.A.N., Kawasaki, S. (2016), Effective use of plant-derived urease in the field of geoenvironmental/geotechnical engineering, *Journal of Civil and Environmental Engineering*, 6, p. 207, DOI: 10.4172/2165-784X.1000207

Dilrukshi R.A.N., Nakashima K., Kawasaki, S. (2018), Soil improvement using plant-derived urease-induced calcium carbonate precipitation, *Soils and Foundations*, 58(4), pp. 894-910, DOI: 10.1016/j.sandf.2018.04.003

Directive 2000/54/EC (2000), *Directive 2000/54/EC of The European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to*

exposure to biological agents at work, Available at: https://www.legislation.gov.uk/eudr/2000/54/data.pdf

Dosier G.K. (2014), Methods for making construction material using enzyme producing bacteria, US8728365B2. https://patents.google.com/patent/US8728365B2/en

Fernández M.S., Montt B., Ortiz L., Neira-Carrillo A., Arias J.L. (2018), Effect of carbonic anhydrase immobilized on eggshell membranes on calcium carbonate crystallization in vitro, In Endo, K., Kogure, T., Nagasawa, H. (Eds.), Biomineralization. Springer, Singapore, DOI: 10.1007/978-981-13-1002-7_4

Han J., Lian B., Ling H. (2013), Induction of calcium carbonate by *Bacillus cereus*, *Geomicrobiology Journal*, 30(8), pp. 682-689, DOI: 10.1080/01490451.2012.758194

Hao M., Gao Y., He J., Yongshuai Q., Lei, H. (2021), Microbially induced carbonate precipitation for wind erosion control of desert soil: Field-scale tests, *Geoderma*, 383, p. 114723, DOI: 10.1016/J.GEODERMA.2020.114723

Hirayama C., Sugimura M., Saito H., Nakamura, M. (2000), Purification and properties of urease from the leaf of mulberry, *Morus alba*, *Phytochemistry*, 53(3), pp. 325-330, DOI: 10.1016/s0031-9422(99)00521-x

Hoffman P.F., Halverson G.P., Schrag D.P., Higgins J.A., Domack E.W., Macdonald F.A. et al. (2021), Snowballs in Africa: sectioning a long-lived neoproterozoic carbonate platform and its bathyal foreslope (NW Namibia), *Earth-Science Reviews*, 219, p. 103616, DOI: 10.1016/j.earscirev.2021.103616

Hogan M.E., Swift I.E., Done, J. (1983), Urease assay and ammonia release from leaf tissues, *Phytochemistry*, 22(13), pp. 663–667, DOI: 10.1016/S0031-9422(00)86958-7

Ivanov V., Stabnikov V. (2017), *Construction Biotechnology: Biogeochemistry, Microbiology and Biotechnology of Construction Materials and Processes*, Green Energy and Technology, Springer, DOI: 10.1007/978-981-10-1445-1

Ivanov V., Stabnikov V. (2020), Microbially-mediated decontamination of CBRN agents on land and infrastructure using biocementation. In: Sidorenko A., Hahn H. eds., *Functional Nanostructures and Sensors for CBRN Defence and Environmental Safety and Security*. *NATO Science for Peace and Security Series C: Environmental Security*, Springer, Dordrecht, Netherlands, pp. 233-244, DOI: 10.1007/978-94-024-1909-2

Ivanov V., Stabnikov V., Stabnikova O., Kawasaki S. (2019), Environmental safety and biosafety in construction biotechnology, *World Journal of Microbiology and Biotechnology*, 35, p. 26, DOI: 10.1007/s11274-019-586 2598-9

Kakimoto S., Sumino Y., Suzuki T. (1992), Acid urease and production thereof. , Patent US5093255A. https://patentimages.storage. googleapis.com/5f/ef/04/787f5cfd420497/US5093255 pdf

Keykha,, H.A., Asadi A., Huat B.B.K, Kawasaki, S. (2019), Microbial induced calcite precipitation by *Sporosarcina pasteurii* and *Sporosarcina aquimarina*, *Environmental Geotechnics*, 6(8), pp. 562-566, DOI: 10.1680/jenge.16.00009

Maheswaran S., Dasuru S.S., Murthy A.R.C., Bhuvaneshwari B., Kumar V.R., Palani G.S., Iyer N.R., Krishnamoorthy S., Sandhya S. (2014), Strength improvement studies using new type wild strain *Bacillus cereus* on cement mortar, *Current Science*, 106(1), pp. 50-57. http://www.jstor.org/stable/24099862

Mortensen B.M., Haber M.J., DeJong J.T., Caslake L.F., Nelson D.C. (2011), Effects of environmental factors on microbial induced calcium carbonate precipitation, *Journal of Applied Microbiology*, 111(2), pp. 338-349, DOI: 10.1111/j.1365-2672.2011.05065.x

Namdar-Khojasteh D., Bazgir M., Babaheidari S.A.H., Asumadu-Sakyi A.B. (2022), Application of biocementation technique using *Bacillus sphaericus* for stabilization of

soil surface and dust storm control, *Journal of Arid Land*, 14, pp. 537–549, DOI: 10.1007/s40333-022-0017-9

Pervin M., Jahan M.G., A.Y.K.Md., M.R., Sana N.K., Rahman M.H., Shaha, R.K. (2013), Effects of some environmental variables on urease in germinating chickpea (*Cicer arietinum* L.) seed, *Journal of Stress Physiology & Biochemistry*, 9, pp. 345-356.

Ronholm J., Schumann D., Sapers H.M., Izawa M., Applin D., Berg B., Mann P., Vali H., Flemming R.L., Cloutis E.A., Whyte, L.G. (2014), A mineralogical characterization of biogenic calcium carbonates precipitated by heterotrophic bacteria isolated from cryophilic Polar Regions, *Geobiology*, 12(6), pp. 542-556, DOI: 10.1111/gbi.12102

Rosquoet F., Alexis A., Khelidj A., Phelipot-Mardelé A. (2003), Experimental study of cement grout: Rheological behavior and sedimentation. *Cement and Concrete Research*, 33(5), pp. 713-722, DOI: 10.1016/S0008-8846(02)01036-0

Stabnikov V., Chu J., Myo A.N., Ivanov V. (2013). Immobilization of sand dust and associated pollutants using bioaggregation, *Water, Air, & Soil Pollution*, 224(9), pp. 1631–1700, DOI: 10.1007/s11270-013-1631-0

Stabnikova O., Antonuk M., Stabnikov V., Arsen'eva L. (2019), Ukrainian dietary bread with selenium-enriched malt, *Plant Foods for Human Nutrition*, 74(2), pp. 157–163, DOI: 10.1007/s11130-019-00731-z

Stabnikova O., Stabnikov V., Antoniuk M., Arsenieva L., Ivanov V. (2022), Bakery products enriched with organoselenium compounds. In Paredes-Lopez O., Stabnikov V., Shevchenko O., Ivanov V. eds., *Bioenhancement and Fortification of Foods for a Healthy Diet*, CRC Press, Boca Raton, London, pp. 89 – 111. ndhttps://doi.org/10.1201/9781003225287

Teichert-Coddington D.R., Stone N., Phelps R.P. (1988), Hydrology of fish culture pos in Gualaca, Panama, *Aquacultural Engineering*, 7(5), pp. 309–320, DOI: 10.1016/0144-8609(88)90013-1

Tirkolaei H.K., Javadi N., Krishnan V., Hamdan N., Kavazanjian E. (2020), Crude urease extract for biocementation, *Journal of Materials in Civil Engineering*, 32(12), Article number 04020374. https://doi.org/10.1061/(ASCE)MT.1943-5533.0003466

Varalakshmi V., Devi A. (2014), Isolation and characterization of urease utilizing bacteria to produce biocement, *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8, pp. 52-57, DOI: 10.9790/2402-084252

Weisburd R.S.J., Laws E.A. (1990), Free water productivity measurements in leaky mariculture ponds. *Aquacultural Engineering*, 9(6), pp. 377–403, DOI: 10.1016/0144-8609(90)90028-X

Whiffin V.S., van Paassen L.A., Harkes M.P. (2007), Microbial carbonate precipitation as a soil improvement technique, *Geomicrobiology Journal*, 24 (5), pp. 417–423, DOI: 10.1080/01490450701436505

Xu G., Li D., Jiao B., Li D., Yin Y., Lun L., Zhao Z., Li S. (2017), Biomineralization of a calcifying ureolytic bacterium *Microbacterium* sp. GM-1. *Electronic Journal of Biotechnology*, 25, pp. 21–27, DOI: 10.1016/j.ejbt.2016.10.008

Zaghloul E.H., Ibrahim H.A.H., El-Badan D.E. (2021), Production of biocement with marine bacteria *Staphylococcus epidermidis* EDH to enhance clay water retention capacity. *The Egyptian Journal of Aquatic Research*, 47(1), pp. 53-59, DOI: 10.1016/j.ejar.2020.08.005

Impact of the crisis caused by the coronavirus on Hungarian consumer behavior related to food purchases

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Abstract

Introduction. Due to the worldwide coronavirus epidemic, models of consumer behaviour related to the purchase of food need to be revised.

Materials and methods. An empirical study was conducted with a questionnaire survey. The research took place in Hungary in the summer of 2020, involving 724 consumers.

Results and discussion. A high percentage of consumers in Hungary reacted to the coronavirus crisis by panic buying. The consumption of healthier foods has spread in connection with the coronavirus crisis, and that the demand of Hungarian consumers for dairy products has also increased. Furthermore, there are differences between the products consumed by panic buyers and normal shoppers. The results showed those who were not afraid of the coronavirus paid less attention to their eating habits (Pearson's correlation: -0.119, sign.:0.01). Some 59.9% of respondents said that it was worth storing large quantities of food because of the coronavirus epidemic. Females and males did not differ on this issue (Chi-square 0.160, df: 2, sign.:0.923, $p>0.05$). Correlation tests showed that for dairy purchases, butter, cheese, fruit yoghurt and sour cream were significantly correlated with each other. By age, respondents aged 40–60 bought the most dairy products, while consumers aged 30 bought the least.

Conclusion. The coronavirus pandemic has seriously affected not only the global economy, but also the daily life of the world's population. Negative consequences were also reflected in the attitude to the purchase of food and there were significant changes in the composition of consumed products.

Introduction

Throughout history, infectious diseases have always been present in people's lives. The coronavirus epidemic is certainly not the first epidemic from the past period. At the same time, it cannot be denied that this virus has had the biggest impact on the global economy in the last twenty years. The coronavirus was declared a global epidemic by the World Health Organization on March 11, 2020.

The coronavirus caused serious economic damage worldwide, affecting not only national economies, but also people's everyday lives through massive job losses (Grueso-Hinestroza et al., 2022; Mostenska et al., 2022). Several economic sectors, such as tourism, hospitality, and retail were extremely negatively affected by the crisis (Nayak et al., 2020). The epidemic has had different economic impacts in each individual country, the main reason for this being that the public health and government measures taken to contain the Covid-19 epidemic have varied widely (Post et al., 2021; Ram et al., 2021).

The first waves of the epidemic affected both population centres and economic actors most unexpectedly (Ruiz Estrada, 2020). In addition to the sectors listed above, the epidemic also had an extremely negative impact on agriculture and the food industry due to illness and the loss of employees, government restrictions, changes in customer markets, and reductions in logistics services (Csiszárik-Kocsir et al., 2021a; 2021b; Gyenge et al., 2021; Khodakivska et al., 2020; Kitukutha et al, 2021; Reményik et al., 2020; Sokil et al., 2021).

The increase in demand and the appearance of food shortages have launched an inflationary process in certain sectors affected by the epidemic, including the food industry (Ghosh, 2021), while in the case of other – primarily convenience and luxury products – a price drop was experienced due to the sudden decline in demand (Akter, 2020; Victor et al., 2021). In China, where the epidemic began, the food problem was even more pronounced due to its large population (Lin and Huang, 2021; Marinova et al., 2022; Yang et al., 2022). Measures introduced to deal with the coronavirus crisis, such as working from home and social distancing, also changed people's food purchasing habits (Baarsma and Groenewegen, 2021; Güngördü Belbağ, 2021;Kolte et al., 2022; Maryati, 2020; Naz et al. 2020; Naz et al., 2022).

Panic buying began in stores around the world (David et al., 2021), and this panic buying fever appeared not only for hygienic products and medicines but also for food, which resulted in periodic shortages of certain food products (Bozsik et al., 2022; Chang and Mayerhoefer, 2020; Garai-Fodor and Csiszárik-Kocsir, 2022; Lehberger et al., 2021; Yu et al., 2020; Alshaabani et al., 2021). In addition to panic buying, another significant change in consumer behaviour was that fear of the coronavirus led consumers to online shopping, thereby increasing the number and frequency of online food purchases (Alaimo et al., 2020; Lu et al., 2021). Szymkowiak et al. (2020) examined shopping habits during the coronavirus epidemic. During their research, they distinguished eight factors that appeared as consumers' risk-reduction expectations during the pandemic. Such factors included, among others, the reduction of shopping opportunities, the emphasis on the importance of social distancing while shopping, the issue of food safety, the quality of product packaging, and the emphasis on personal safety. Nowadays, after the "fifth wave", we can no longer detect the panic purchases experienced in the first wave, but it is clear that online orders have stabilized at a high level and many people continue to buy less in traditional ways (Gu et al., 2021; Semerádová and Weinlich, 2022). According to Google Trends (2020) data, the proportion of online purchases has also increased significantly in Hungary (Figure 1).

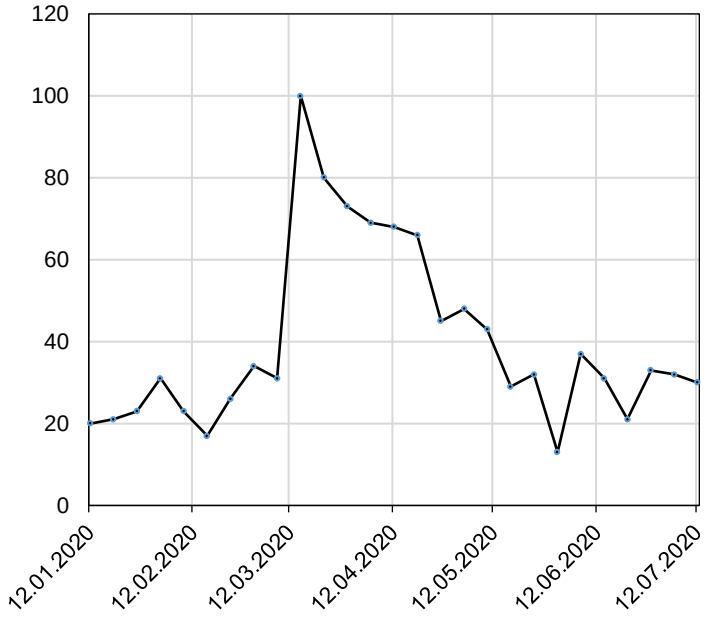


Figure 1. Percentage of searches for the keyword "Online shopping" in the first half of 2020 (percentage)
(Google search for 'online shopping' in the first 6 months of 2020)

All of this is well supported by the fact that 9% of the population in Hungary tried online grocery shopping for the first time in their lives. In addition, behavioural habits related to food safety have also changed, as for example, it is now less common to check baked goods by touch (Kasza et al., 2020). Even before the epidemic, supermarkets tried home delivery of food, but there was not much demand at the time. Soós (2020) in his research highlighted that 63% of online grocery shoppers liked "this method", but would return to traditional shopping as soon as possible. 11% of the participants in the mentioned research liked it very much, while 21% did not like the possibility of online shopping.

Taking into account the trends presented above and in international articles, the first hypothesis of the current research is as follows:

Hypothesis H1: The coronavirus crisis affected the shopping habits of Hungarian consumers, and this demonstrated differences in gender, age, and education.

Consumers are increasingly aware of values such as health, quality of life, food safety, and digital security (De and Giri, 2020; Ivanov et al., 2021; Megits et al., 2020; Brumă et al., 2021). Several international studies (Hall et al., 2021; Marty et al., 2021) also examined food-purchasing habits during the coronavirus. The researchers showed that the demand for dairy products, cereals, vegetables and fruits increased the most, which mainly benefited local producers (Cappelli and Cini, 2020; Palau-Saumell et al., 2021; Máté et al., 2022). In their research, Laguna et al. (2020) showed that the most frequently purchased products in Spain included pasta, vegetables, oil seeds, cheese, and chocolate.

On the other hand, the demand for foods considered unhealthy and fattening, such as desserts, decreased. In their publication, Xu et al. (2022) pointed out that the health awareness

of consumers increased during the coronavirus crisis, and the consumption of healthier products, including dairy products, increased significantly. According to the results of their research, 79% of consumers were willing to buy more dairy products after the outbreak of the epidemic, so that the purchase rate of dairy products increased by an average of 17.49% compared to the period prior to the outbreak of the epidemic.

This tendency is also confirmed by the 2022 study by Hambardzumyan and Gevorgyan, who found that the demand for dairy products among consumers in Armenia was significantly increased, especially in the first month after the declaration of the state of emergency.

Based on the above, the second hypothesis of research is the following:

Hypothesis H2: At the beginning of the outbreak of the coronavirus crisis, Hungarian consumers bought dairy products regardless of whether they were perishable goods or not.

The 2019 coronavirus epidemic affected not only our health, but also our shopping and food consumption habits. The current study was carried out in the initial phase of the emergency, so our goal was to demonstrate how and to what extent the sudden, high-impact changes affected the food consumption behaviour of Hungarian consumers. The research clearly illustrates the "circumstances" that characterized food consumption and shopping habits both in Hungary and globally in 2020.

Materials and methods

The research started in March 2020 and ended in June 2020. Consumers were contacted with the help of interviewers, who questioned research participants in shopping centres (Tesco, Metro, etc.), in smaller grocery stores (e.g. Coop), and in other larger stores that sell food (e.g. Lidl, Aldi). The empirical study was based on a questionnaire survey. The starting point for compiling the sample taken from the basic population was the most recent 2011 census data of the Central Statistical Office. The sample size, the subgroups formed on the basis of the various background variables, were the size of the statistical regulations, this number of elements is common in both international and domestic public opinion research.

When compiling the sample taken from the basic population, the goal was to achieve an accurate representation, so the authors implemented the following sampling procedure:

Hungary was divided into regions.

- Based on the population data of each region, it was determined how many of the 724 consumers should participate in the research from each region.
- The researchers selected one county per region, and then based on KSH (Central Statistical Office of Hungary) guidance, they determined the number of people living in the county, cities and villages with a smaller population, and their proportion.
- The population of the test settlements over the age of 16 was further grouped according to gender, age and education based on the KSH data, and the quota of persons to be interviewed was compiled on this basis. With the developed sampling procedure, the composition of the sample population in terms of gender, age, education and region is the same as that of the base population.
- Finally, the quota system of each region was established, and the questionnaire was divided accordingly.

Taking advantage of the possibility of random selection, the so-called random walking method was used, the important feature of which is that each person has the same chance of being included in the sample, while still striving for randomness. Thanks to the sampling procedure, 724 consumers were included in the analysis, and all of the questionnaires could be evaluated. The data of the questionnaire was analysed with the SPSS 28 program. The methods of analysis were frequency, mean, standard deviation, ANOVA.

The following Table 1 presents the demographic data of the consumers interviewed during the research.

Table 1

Demographic characteristics of the consumers (%)

Variables	Sample Size	%
By gender		
Male	335	46.30
Female	389	53.70
By age		
Between 15–29	161	22.20
Between 30–39	149	20.60
Between 40–59	236	32.60
60 and over	178	24.60
By education		
Elementary	427	59.00
High school	212	29.30
University	85	11.70
By region		
Central Hungary	185	25.60
Central Transdanubia	81	11.20
Western Transdanubia	90	12.40
Southern Transdanubia	84	11.60
Northern Hungary	93	12.80
Northern Great Plain	95	13.10
Southern Great Plains	96	13.30
By settlement type		
Capital city	250	34.50
City	242	33.40
Village	232	32.00

20.3% of the females in the sample are under 30 years old, 19.0% are in their 30's, 35.0% are over 40 years old, while every fourth respondent is over 60 years old. 24.5% of males are between 15 and 29 years old, 22.4% are between 30 and 39 years old, and 29.9% are older than 40 but younger than 60, while 23.3% of male respondents are older than 60 years. 55.8% of females have an elementary school education. This ratio is 62.7% for male, while 32.6% of female, and 25% of males have a high school degree. 11.6% of females and 11.9% of males have university diplomas. The questionnaire also asked about the average

monthly net income per person. This value was below HUF 20,000 in about 4.3% of the respondents, 39.9% of them were above HUF 20,000, but no more than HUF 40,000. For 33.6% of them, it was above HUF 40,000, but not more than HUF 60,000, while in the case of 16.9% it is an amount over HUF 60,000. The majority of people under the age of 20 (26.6%) live with income between HUF 30,000 and 40,000, while the largest proportion (20%) of people between the ages of 30 and 40 earn a similar amount. Regarding the place of residence and the average monthly income per capita, about 7% of the people living in the villages lived on amounts below HUF 20,000 per person, this ratio was only 2.6% in the case of cities.

Results and discussion

Respondents' opinions about the coronavirus situation and shopping habits

First the respondents had to decide how much they agreed with the given statement. They had to rate the definitions on a five-point Likert scale (1 – totally do not agree, 5 – totally agree). The responses to the statements are presented in Table 2:

Table 2

Respondents' opinions about the coronavirus situation and shopping habits (mean, standard deviation)

Claims	N		Mean	Std. Deviation
	Valid	Missing		
I am not afraid of the coronavirus	712	12	1.9846	1.56474
I like ready-to-cook products	712	12	2.8975	1.24807
Epidemiological regulations must be observed	715	9	3.5483	1.42854
What they think of me is important	713	11	3.2903	1.20095
I pay attention to my eating habits	718	6	3.5362	1.07498
My friends often ask me for advice	713	11	3.2202	1.13451
I believe in family traditions	714	10	3.8852	1.08966
I often worry about the future	713	11	3.6830	1.19360
If prices go up, I will buy less, but I will not go for the cheaper brands	717	7	2.9442	1.17098
I am one of the first to try new products	712	12	1.9228	1.10666
Comfort is important to me	710	14	3.8056	1.15826
I mostly buy well-known products	715	9	3.3441	1.15674
I usually pay attention to prices	719	5	3.8804	1.06718
I plan every purchase carefully	713	11	3.1683	1.26525

It is clear from the data that at the time of the investigation there was not yet a complete fear of the epidemic in Hungary. People knew that there would be rules to follow regarding the coronavirus, such as the wearing of masks, shopping schedules determined by age groups, digital education, and home office employment. At the same time, the majority were worried about the future, and this is not necessarily just due to Covid-19. The respondents consciously paid attention to prices, planned their purchases, and preferred well-known products, but it

was important for them that the product purchase was also a positive and comfortable experience. They pay attention to quality, which means that the rise in prices does not necessarily mean that the respondents will buy products of a lesser quality.

It was shown that those who were not afraid of the coronavirus paid less attention to their eating habits (Pearson's correlation: -.119 sign.:0.01). Those who believe that the epidemiological rules should be followed preferred ready-made meals less (Pearson's correlation: -.126 sign.: 0.00), paid attention to their eating habits (Pearson's correlation: .141 sign.: 0.00), they often worried about the future (Pearson's correlation: .183 sign.: 0.00), paid attention to prices (Pearson's correlation: .174 sign.:0.00), and planned purchases (Pearson's correlation: .137 sign.: 0.00). For those who are worried about the future, it was important what they thought about them (Pearson's correlation: .137 sign.: 0.00), and they mostly bought well-known brands (Pearson's correlation: .125 sign.: 0.

The authors used a non-parametric test (Mann-Whitney and Kruskal-Wallis) to analyse whether differences could be identified in the given sample based on gender, age, and education. In Table 3, the researchers highlighted the cases where any significant differences could be identified, as well as of which sample group the given statement is most typical.

The data in Table 3 clearly shows that for almost every statement a significant difference could be identified either according to gender, age, or educational level. According to gender, women were more afraid of the coronavirus than men were, they believed that the rules would have to be followed more stringently, they looked at prices and they planned their purchases more carefully. The younger age group was the least afraid of the coronavirus, while those over 60 felt greater uncertainty, so they rather measured their purchases and paid attention to prices. According to the educational level, those with a higher educational level believed that it was possible to protect oneself consciously by following the rules against the coronavirus, while those with an elementary educational level believed in well-known products and conscious price monitoring.

The questionnaire also asked whether it is worth storing large amounts of food due to the coronavirus epidemic. About 59.9% of respondents said the answer was yes, while 37.9% said no, and 2.2% could not answer the question. Females and males did not differ on this issue (Chi-square .160 df: 2 sign.: .923 $p>0.05$). 60% of females and males believed that food should be stored now. Based on educational level, 64.7% of those with a higher educational level, 71.6% of those with a secondary educational level, and 53.2% of those with an elementary educational level considered purchasing larger amounts of food. In these cases, the Chi-square test showed a significant difference (Chi-square .21.119 df: 4 sign.: .0.000 $p<0.05$). The tests did not show any differences in this question either based on where the respondents live or on the basis of their income.

Based on the results reported above the first hypothesis is accepted, according to which the coronavirus crisis had an impact on the shopping habits of Hungarian consumers, and this showed differences in gender, age, and education based on the examined sample.

Another focus of the research was the consumption of dairy products. First, the respondents had to answer what their habits are when purchasing dairy products. They had to answer on a five-point Likert scale (1 – not characteristic of me at all, 5 – completely characteristic of me). The average and standard deviation of the responses to the statements are summarized in Table 4.

Table 3

Respondents' opinions about the coronavirus situation and shopping habits in the light of gender, age, education

Claims	No	Age	Educational level
I am not afraid of the coronavirus	Mann-W.:57762. sign.:.019 Male	Kruskal-W:28.959 sign.:0.00 15–29-year-olds	
I like kitchen-ready, convenient products		Kruskal-W:14.225 sign.:0.00 15–29-year-olds	
Epidemiological regulations must be observed	Mann-W.:40955. sign.:.000 Female		Kruskal-W:7.194 sign.:0.027 University
What they think of me is important		Kruskal-W: 7.895 sign.: 0.048 40–59-year-olds	Kruskal-W:6.920 sign.:0.031 University
I pay attention to my eating habits	Mann-W.:52571. sign.:.000 Female	Kruskal-W:21.662 sign.:0.00 Over 60 years	
My friends often ask me for advice		Kruskal-W: 9.997 sign.: 0.019 15–29-year-olds	Kruskal-W:10.267 sign.:0.006 University
I believe in family traditions	Mann-W.:57305. sign.:.021 Female	Kruskal-W:33.026 sign.:0.00 Over 61 years	Kruskal-W:14.396 sign.:0.00 High school
I often worry about the future			Kruskal-W:6.781 sign.:0.034 High school
If prices go up, I will buy less, but I will not go for cheaper brands	Mann-W.:56662. sign.:.006 Female		Kruskal-W:10.008 sign.:0.007 University
I am one of the first to try new products		Kruskal-W:43.037 sign.:0.00 15–29-year-olds	Kruskal-W:11.421sign.:0.003 University
Comfort is important to me			Kruskal-W:10.868sign.:0.004 Elementary
I mostly buy well-known products		Kruskal-W:23.272 sign.:0.00 Over 60 years	Kruskal-W:7.901sign.:0.019 Elementary
I usually pay attention to prices	Mann-W.:55112 sign.:.000 Female	Kruskal-W:51.454 sign.:0.00 Over 60 years	Kruskal-W:16.044 sign.:0.001 Elementary
I plan every purchase carefully	Mann-W.:54570.5 sign.:.001 Female	Kruskal-W:76.271 sign.:0.00 Over 60 years	

Respondent statements regarding the consumption of dairy products

Table 4

Respondent statements regarding the consumption of dairy products (mean, standard deviation)?

	Mean	Standard deviation
I would not buy more dairy products even if they were cheaper	2.7143	1.60518
I prefer to buy lower priced products	3.2881	1.18028
Good quality dairy products are easily affordable	2.0505	1.14912
Dairy products are not very expensive compared to other foods	2.5676	1.10790
I am willing to pay more for better quality	3.2700	1.15194
I regularly consume functional food	3.7188	1.31244
Homemade products are expensive compared to other foods	3.2697	1.37502
Organic foods are healthier	4.0328	1.22983
I would rather buy functional food (e.g. probiotic yogurt, bread enriched with vitamins)	4.0942	1.14129
I consider dairy products healthy	4.4803	0.87127
Butter is healthier than margarine products	3.2645	1.37235
Food of local/regional origin gives me confidence	3.6667	1.46244
I am afraid of unknown diseases	3.1918	1.45248
I like convenience products (e.g. frozen Camembert cheese)	2.7806	1.04275
I buy branded foods	2.5221	1.12435
Consideration of advertising	2.0648	1.10456
I prefer to buy organic food/food from controlled farms	2.8200	1.64666

From the data in Table 4, it can be seen that the respondents consider foods made from milk to be healthy. Functional products are preferred, and organic products are also considered healthy. Dairy products are popular and prices influence the amount consumed.

For the purpose of further analysis, the given statements were compressed into factors by the authors. One variable was not suitable for factor formation: "I like convenience products (e.g. frozen Camembert cheese)", this statement was left out of the factor formation. Based on the KMO and Bartlett test, KMO: .628. Chi square: 956.755 df: 120 sign: .001. The factors were created with Varimax rotation, and the explained fraction was: 62.198%. 7 factors were created, whose names and component matrix with Cronbach Alpha values are presented in Table 5.

Table 5 shows that the following factors were formed: the health factor, the brand, functional foods, pricing, the dairy product is cheap and good, the organic choice, local trust. In the following, the authors examined whether differences could be identified according to gender, age, education, and place of residence.

Based on the examination by gender, significant differences could be identified for the following factors: health factor (F:5.018 sig.: .025 $p<0.05$), dairy products are cheap and good (F:10.243 sig.: .001 $p<0.05$). In both cases, female consumers were more strongly sensitive than male consumers were.

Table 5

Rotated component matrix

		Component						
		1	2	3	4	5	6	7
Health factor	H1 I consider dairy products healthy	0.801						
	H2 Organic foods are healthier	0.778						
	H3 I would rather buy functional food	0.718						
Cronbach's Alpha		0.672						
Brand	B1 Consideration of advertising		0.831					
	B2 I buy branded food		0.636					
Cronbach's Alpha			0.555					
Function Foodstuffs	F1 I regularly consume functional food			0.774				
	F2 Homemade products are expensive compared to other			0.700				
Cronbach's Alpha				0.524				
Pricing	P1 I prefer to buy the lower priced product				-0.740			
	P2 I am willing to pay more for better quality				0.636			
Cronbach's Alpha					0.597			
Dairy is cheap and good	D1 I would not buy more dairy products even if they were cheaper					0.711		
	D2 Dairy products are the other foods. They are not very expensive in comparison					0.598		
	D3 Among dairy products, good quality ones are easily affordable					0.547		
Cronbach's Alpha						0.331		
Boy selection	BIO1 I am afraid of unknown diseases						0.718	
	BIO2 I prefer to buy organic food/food from controlled farms						0.724	
Cronbach's Alpha							0.170	
Local trust	T1 Food of local/regional origin instils confidence in me							0.680
	T2 Butter is healthier than margarine products							0.691
Cronbach's Alpha								0.175

Based on age, the health factor (F:5.302 sig.: .001 p<0.05), functional foods (F:4.771 sig.: .003 p<0.05), and pricing (F:5.704 sig.: .001 p <0.05) there was a significant difference. The health aspect is the most important for the over 60 years old respondents, functional foods for the 40-50 years old, and pricing for the over 60 years old consumers.

Based on educational level, there was a significant difference in pricing (F:12.889 sig.: .001 p<0.05) and based on the perception of the product (F:5.726 sig.: .003 p<0.05). In both cases, the question was cardinal for those with university educational level.

Based on place of residence, the organic choice was significantly different (F: 5.126 sig.: .006 p<0.05) and cities were the strongest in this respect.

After that, the authors examined how the intention to accumulate due to Covid-19 is related to the given factors. ANOVA tests did not confirm the correlations.

Purchases of dairy products during the period

Part of the research was also how much the respondents bought on a given weekend and by what percentage on average this purchase exceeded a normal weekend purchase. The data in Table 6 shows that Hungarian consumers typically bought one and a half times more of the types of dairy products surveyed.

Table 6

By what percentage did purchases change on average during the first wave of the coronavirus epidemic (mean)?

Products	N	Mean
Butter	724	72.410
Sour cream	724	74.558
Cheeses (e.g. Trappist, Pannonian)	724	74.572
Fruit yogurts	724	76.107
Liquid milk	724	116,826

The correlation studies proved that dairy products are significantly related to each other (butter, cheese, fruit yogurt, sour cream). The authors looked at how purchases developed according to gender, age, education, and place of residence. Men bought more butter, yogurt, sour cream and cheese than women. By age, the 40-60-year-old respondents accumulated dairy products the most, while the 30-year-old consumers the least. City residents bought more of all five dairy products than rural residents. Based on educational level, those with elementary school educational level and those with university degree bought more dairy products.

It was also examined which foods are stored differently by those respondents who think that there is no need to stock up now, and those who decide to stock foods during the epidemic. Those who did not stock up on dairy products bought significantly less milk and butter. Overall, it can be concluded that the onset of the coronavirus had an impact on the procurement of dairy products, so the authors also accept their second hypothesis.

Conclusion

1. The coronavirus pandemic seriously affected not only economic factors, but also the everyday lives of consumers. The negative consequences also had an impact on consumer attitudes, consumer food purchasing habits, and significant changes took place in the composition of the products consumed. Even today, during crises, many people try to prepare for unexpected events by panic buying (Sim et al., 2020, Chua et al., 2021). Fear, panic, and uncertainty are direct human responses to crises, which depend on many individual and socio-economic factors (Shadiqi et al., 2020, Arafat et al., 2020). Panic buying is generally seen as a psychological reaction to stress, fear, and uncertainty (Sikos et al., 2021). According to Wang and Hao (2020), food stockpiling, as one example of this behaviour, represents an attempt to reduce the negative consequences caused by the crisis situation and therefore represents a coping mechanism for individuals (Janssen et al., 2021, Gunter, 2022). The current research also demonstrated that, like consumers living in other countries, Hungarian consumers reacted to the shock caused by the coronavirus crisis and the resulting fear by panic buying, and in this way, they now try to prepare for an uncertain future.
2. Since the epidemic caused by the coronavirus primarily affected people's health, consumers have become more aware of which foods they buy (Chowdhury and Nandi, 2021, Selvi, 2021). Health awareness and quality food of organic origin became the focus of consumers' purchases (Nandini, 2021). According to the data received on some of the products, Hungarian consumers, for example, typically bought one and a half times more dairy products at the outbreak of the coronavirus crisis than before. Men bought more butter, yogurt, sour cream and cheese than women did. According to age, people between 40 and 60 years of age stocked up on dairy products, while respondents in their 30's purchased larger quantities the least.
3. The results of current research also highlighted which foods the respondents differ in cumulating. Customers who did not panic bought significantly less milk and butter, so they were not fans of storing. The surveyed Hungarian consumers consider foods made from milk to be healthy and prefer buying functional products when shopping. Since this study was conducted, there has been a russian invasion of Ukraine, and now Hungarian clients now have to face new challenges. On the one hand, the drastic increase in food and energy prices, and on the other hand, in the case of certain foods, the quantitative restrictions imposed on one-time purchases. In addition, of course, we cannot forget that Covid-19 is still among us.

References

Akter S. (2020), The impact of COVID-19 related "stay-at-home" restrictions on food prices in Europe: findings from a preliminary analysis, *Food Security*, 12(4), pp. 719–725, DOI: 10.1007/s12571-020-01082-3.

Alaimo L.S., Fiore M., Galati A. (2020), How the covid-19 pandemic is changing online food shopping human behaviour in Italy, *Sustainability*, 12, 9594, DOI: 10.3390/su12229594.

Alshaabani, A., Naz, F., Magda, R., Ildikó, R. (2021). Impact of Perceived Organizational Support on OCB in the Time of COVID-19 Pandemic in Hungary: Employee Engagement and Affective Commitment as Mediators. *Sustainability*, 13(14), DOI: 10.3390/su13147800

Arafat S. M. Y., Kar S. K., Marthoenis M., Sharma P., Hoque Apu E., Kabir R. (2020), Psychological underpinning of panic buying during pandemic (COVID-19), *Psychiatry Research*, 289, 113061, DOI: 10.1016/j.psychres.2020.113061.

Baarsma B., Groenewegen J. (2021), COVID-19 and the demand for online grocery shopping: Empirical evidence from the Netherlands, *De Economist*, 169(4), pp. 407–421, DOI: 10.1007/s10645-021-09389-y.

Bozsik N., Cubillos T. JP., Stalbek B., Vasa L., Magda R. (2022), Food security management in developing countries: Influence of economic factors on their food availability and access, *Plos One*, 17(7), e0271696, DOI: 10.1371/journal.pone.0271696.

Brumă I.S., Vasiliu C.D., Rodino S., Butu M., Tanasă L., Dobos S., Butu A., Coca O., Stefan G. (2021), The behavior of dairy consumers in short food supply chains during COVID-19 pandemic in Suceava area, Romania, *Sustainability*, 13, 3072, DOI: 10.3390/su13063072.

Cappelli A., Cini E. (2020), Will the COVID-19 pandemic make us reconsider the relevance of short food supply chains and local productions? *Trends in Food, Science & Technology*, 99, pp. 566–567, DOI: 10.1016/j.tifs.2020.03.041.

Chang H., Meyerhoefer C. D. (2020), COVID-19 and the demand for online food shopping services: Empirical evidence from Taiwan, *American Journal of Agricultural Economics*, 103(2), pp. 448–465, DOI: 10.1111/ajae.12170002/cb.1853, DOI: 10.1002/cb.1853.

Chowdhury T., Nandi S. (2021), Food safety, hygiene, and awareness during combating of COVID-19. *Environmental and Health Management of Novel Coronavirus Disease (COVID-19)*, pp. 305–24, DOI: 10.1016/B978-0-323-85780-2.00002-0.

Chua G., Yuen K. F., Wang X., Wong Y. D. (2021), The determinants of panic buying during COVID-19, *International Journal of Environmental Research and Public Health*, 18(6), 3247, DOI: 10.3390/ijerph18063247.

Csiszárk-Kocsir Á., Garai-Fodor M., Varga J. (2021a), what has become important during the pandemic? – reassessing preferences and purchasing habits as an aftermath of the coronavirus epidemic through the eyes of different generations, *Acta Polytechnica Hungarica*, 18(11), pp. 49–74, DOI: 10.12700/APH.18.11.2021.11.4.

Csiszárk-Kocsir Á., Varga J., Garai-Fodor M. (2021b), A múlt és a jelen pénzügyi válságainak ismerete a pénzügyi oktatás függvényében, *Pénzügyi Szemle/Public Finance Quarterly*, 66(2), pp. 215–234, DOI: 10.35551/PSZ_2021_2_3.

David J., Visvalingam S., Norberg M. M. (2021), Why did all the toilet paper disappear? Distinguishing between panic buying and hoarding during COVID-19, *Psychiatry Research*, 303, 114062, DOI: 10.1016/j.psychres.2021.114062.

De, M., Giri, B. C. (2020), Optimal decisions on pricing and greening policies of multiple manufacturers under governmental regulations on greening and carbon emission. *Decision Making: Applications in Management and Engineering*, *3*(1), pp. 43–59, DOI: 10.31181/dmame2003034d.

Garai-Fodor M., Csiszárik-Kocsir, Á. (2022), The validity of value-based consumer behavioral models in the financial consciousness of the Z generation, *On-Line Journal Modelling the New Europe*, 27, pp. 107–131, DOI: 10.24193/OJMNE.2018.27.05.

Garai-Fodor M., Popovics A., Csiszárik-Kocsir Á. (2022), The perception of Hungarian food by consumer segments according to food purchasing preferences based on primary research results, *Plos One*, 17(8), pp. 1–19, DOI: 10.1371/journal.pone.0273023.

Ghosh S. (2021), COVID-19, Stock market, exchange rate, oil prices, unemployment, inflation, geopolitical risk nexus, the case of the BRICS nations, *Advances in Finance, Accounting, and Economics*, pp. 85–105, DOI: 10.4018/978-1-7998-8314-2.ch005.

Google Trends (2020), *'Online vásárlás' kulcsszóra történő keresések aránya az elmúlt 12 hónapban*, Available at: https://trends.google.com/trends/explore?geo=HU&q=online20vC3A1sC3A1rlC3A1s.

Grueso-Hinestroza M. P., Sanchez-Riofrio A., López-Santamaría M., Antón C. (2022), What we know about workplace responses during the COVID-19 pandemic, *Advances in Human Resources Management and Organizational Development*, pp. 35–45, DOI: 10.4018/978-1-7998-8827-7.ch003.

Gu S., Ślusarczyk B., Hajizada S., Kovalyova I., Sakhbieva A. (2021), Impact of the COVID-19 pandemic on online consumer purchasing behavior, *Journal of Theoretical and Applied Electronic Commerce Research*, 16, pp. 2263–2281, DOI: 10.3390/jtaer16060125.

Güngördü Belbağ A. (2021), Impacts of Covid-19 pandemic on consumer behavior in Turkey: A qualitative study, *Journal of Consumer Affairs*, 56(1), pp. 339–358., DOI: 10.1111/joca.12423.

Gunter B. (2022), Lockdown and Panic Buying Behaviour, *Psychological Impact of Behaviour Restrictions During the Pandemic*, pp. 98–114., DOI: 10.4324/9781003274377-6.

Gyenge B., Máté Z., Vida I., Bilan Y. Vasa L. (2021), A New strategic marketing management model for the specificities of e-commerce in the supply chain, *Journal of Theoretical and Applied Electronic Commerce Research*, 16(4), pp. 1136–1149, DOI: 10.3390/jtaer16040064.

Hall C.M., Fieger P., Prayag G., Dyason D. (2021), Panic buying and consumption displacement during COVID-19: Evidence from New Zealand, *Economies*, 9, pp. 46, DOI: 10.3390/economies9020046.

Hambardzumyan G., Gevorgyan S. (2022), The impact of COVID-19 on the small and medium dairy farms and comparative analysis of customers' behavior in Armenia, *Future Foods*, 5, 100110, DOI: 10.1016/j.fufo.2021.100110.

Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7–36, DOI: 10.24263/2304-974X-2021-10-1-3.

Janssen M., Chang B. P. I., Hristov H., Pravst I., Profeta A., Millard J. (2021), Changes in food consumption during the COVID-19 pandemic: Analysis of consumer survey data from the first lockdown period in Denmark, Germany, and Slovenia, *Frontiers in Nutrition*, 8, DOI: 10.3389/fnut.2021.635859.

Kasza G., Dorkó A., Pósa E., Szakos D. (2020), *Élelmiszerbiztonság és tartalékolás a háztartásokban*, V. NÉBIH Kerekasztal.

Khodakivska, O, Pugachov M., Patyka N., Shpykuliak O., Mohylnyi O., Tulush L. (2020), Impact of COVID-19 on the agrarian sector and country's food security, *Ekonomika APK*, 314(12), pp. 6–21., DOI: 10.32317/2221-1055.202012006.

Kitukutha,M. N., Vasa L., Oláh J. (2021), The impact of COVID-19 on the economy and sustainable e-commerce, *Forum Scientiae Oeconomia*, 9(2), pp. 47–72. DOI: 10.23762/fSo_Vol9_no2_3.

Kolte A., Mahajan Y., Vasa L. (2022), Balanced diet and daily calorie consumption: Consumer attitude during the COVID-19 pandemic from an emerging economy, *PLoS One*, 17(8), e0270843.

Laguna L., Fiszman S., Puerta P., Chaya C., Tárrega A. (2020), The impact of COVID-19 lockdown on food priorities. Results from a preliminary study using social media and an online survey with Spanish consumers, *Food Quality and Preference*, 86(12), pp. 1–9., DOI: 10.1016/j.foodqual.2020.104028.

Lehberger M., Kleih A.K., Sparke K. (2021), Panic buying in times of coronavirus (COVID-19): Extending the theory of planned behavior to understand the stockpiling of nonperishable food in Germany, *Appetite*, 161, 105118, DOI: 10.1016/j.appet.2021.105118.

Lin W., Huang J. (2021), Impacts of agricultural incentive policies on land rental prices: New evidence from China, *Food Policy*, 104, 102125, doi:10.1016/j.foodpol.2021.102125.

Lu,M., Wang R., Li P. (2021), Comparative analysis of online fresh food shopping behavior during normal and COVID-19 crisis periods, *British Food Journal*, 124(3), pp. 968–986., DOI: 10.1108/bfj-09-2020-0849.

Marinova D., Bogueva D., Wu Y., Guo X. (2022), China and changing food trends: A sustainability transition perspective, *Ukrainian Food Journal*, 11(1), pp. 126–147, DOI: 10.24263/2304-974X-2022-11-1-13.

Marty L., de Lauzon-Guillain B., Labesse M., Nicklaus S. (2021), Food choice motives and the nutritional quality of diet during the COVID-19 lockdown in France, *Appetite*, 157, 105005, DOI: 10.1016/j.appet.2020.105005.

Maryati T. (2020), Consumer behavior changes post pandemic Covid-19, *International Journal of Halal Research*, 2(2), pp. 84–89, DOI: 10.18517/ijhr.2.2.84-89.2020.

Máté, D., Oláh, J., Erdei, E., Estiyanti, N. M., Bács, Z., Kovács, S. (2022), The impacts of sustainable industrial revolution (IR) on the profitability of Hungarian food companies, *Frontiers in Sustainable Food Systems*, 6, 1034010, pp. 1–14, DOI: 0.3389/fsufs.2022.1034010

Megits, N., Neskorodieva, I., & Schuster, J. (2020), Impact assessment of the COVID-19 on trade between Eastern Europe and China, *Journal of Eastern European and Central Asian Research (JEECAR)*, 7(3), pp. 385–399, DOI:10.15549/jeecar.v7i3.579

Mostenska, T. L., Mostenska, T. G., Yurii, E., Lakner, Z. Vasa, L. (2022). Economic affordability of food as a component of the economic security of Ukraine, *PLoS ONE*, 17(3), e0263358.

Nandini V. D. (2021), Awareness of Healthy Eating and Implementation of Immune Boosting Superfoods in the Diet during COVID 19, *Asian Pacific Journal of Health Sciences*, 8(3), pp. 150–154, DOI: 10.21276/apjhs.2021.8.3.26.

Nayak P., Mishra V., Singh M., Tambuwala M. M. (2020), Impacts and consequences of COVID-19 epidemic on global economy, *Coronaviruses*, 01, DOI: 10.2174/2666796701999200905094151

Naz, F., Kumar, A., Upadhyay, A., Chokshi, H., Trinkūnas, V., Magda, R. (2022). Property Management Enabled by Artificial Inteligence Post COVID-19: An Exploratory Review and Future Propositions. *International Journal of Strategic Property Management*, 26(2), pp. 156–171. http://doi.org/10.3846/ijspm.2022.16923

Naz, F., Oláh, J., Vasile, D., Magda, R. (2020), Green purchase behavior of university students in Hungary: An empirical study. *Sustainability*, 12(23), 10077, pp. 1–21.DOI: 10.3390/su122310077

Palau-Saumell R., Matute J., Derqui B,. Meyer, J. H. (2021), The impact of the perceived risk of COVID-19 on consumers' attitude and behavior toward locally produced food, *British Food Journal*, 123(13), pp. 281–301, DOI: 10.1108/BFJ-04-2021-0380.

Post R. A. J., Regis M., Zhan Z., van den Heuvel E. R. (2021), How did governmental interventions affect the spread of COVID-19 in European countries?, *BMC Public Health*, 21(1), DOI: 10.1186/s12889-021-10257-2.

Ram S. K., Sornette D. (2021), Impact of Governmental interventions on epidemic progression and workplace activity during the COVID-19 outbreak, *Scientific Reports*, 11(1), DOI: 10.1038/s41598-021-01276-5.

Remenyik B., Horváth D. Vasa L. (2020), Relationships between cycle theories, sustainable tourism, and the effects of the COVID-19 in Hungary, *Economic Annals XXI*, 185(9–10), pp. 79–90.

Ruiz Estrada M. A. (2020), Economic waves: The effect of the Wuhan COVID-19 on the World Economy (2019–2020), *SSRN Electronic Journal*, DOI: 10.2139/ssrn.3545758.

Selvi K. S. (2021), Health status and nutritional awareness of college students during COVID 19 lockdown, *Issues and Development in Health Research*, 8, pp. 23–31., DOI: 10.9734/bpi/idhr/v8/15065d.

Semerádová T., Weinlich P. (2022), Changes in Grocery Shopping Patterns Due to COVID-19, *Advances in Electronic Commerce*, pp. 23–48, DOI: 10.4018/978-1-7998-8294-7.ch002.

Shadiqi M. A., Hariati R., Hasan K. F. A., I'anah N., Al Istiqomah W. (2020), Panic buying pada pandemi COVID-19: Telaah literatur dari perspektif psikologi, *Jurnal Psikologi Sosial*, 19(2), pp. 131–141, DOI: 10.7454/jps.2021.15.

Sikos T., Papp V., Kovács A. (2021), Panic buying in Hungary during Covid-19 pandemic, *Theory, Methodology, Practice*, 17(1), pp. 56–65, DOI: 10.18096/tmp.2021.02.06.

Sim K., Chua H. C., Vieta E., Fernandez G. (2020), The anatomy of panic buying related to the current COVID-19 pandemic, *Psychiatry Research*, 288, 113015., DOI: 10.1016/j.psychres.2020.113015.

Sokil O., Zvezdov D., Vasa L. (2021), The Perspective of European globalization and capitalization of agriculture in the post-covid period, *Oblik i Finansy / Accounting and Finance*, 3(93), pp. 90-96., DOI: 10.33146/2307-9878-2021-3(93)-90-96.

Soós G. (2020), Az élelmiszer-fogyasztói szokások változása a COVID-19 vírus megjelenéséhez kapcsolódóan Magyarországon, ELTE, EKE, *Oeconomia*, 54(3), pp. 15–27., DOI: 10.15170/MM.2020.54.03.026

Szymkowiak A., Gaczek P., Jeganathan K., Kulawik P. (2020), The impact of emotions on shopping behavior during epidemic. What a business can do to protect customers, *Journal of Consumer Behaviour*, 10.1.

Victor V., Karakunnel J.J., Loganathan S., Meyer D.F. (2021), From a recession to the COVID-19 pandemic: Inflation-unemployment comparison between the UK and India, *Economies*, 9, pp. 73, DOI: 10.3390/economies9020073.

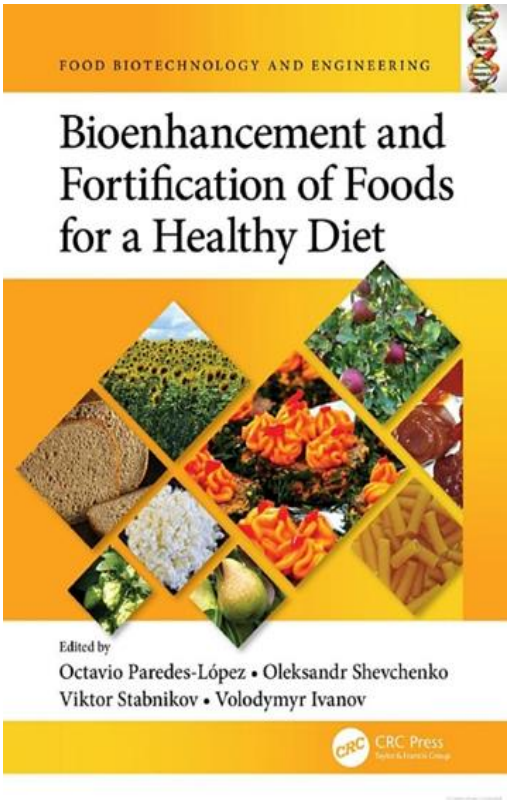
Wang H.H., Hao N. (2020), Panic buying? Food hoarding during the pandemic period with city lockdown, *Journal of Integrative Agriculture*, 19(12), pp. 2916–2925., DOI: 10.1016/S2095-3119(20)63448-7.

Xu J., Wang J., Li C. (2022), Impact of consumer health awareness on dairy product purchase behavior during the COVID-19 pandemic, *Sustainability*, 14, pp. 314, DOI: 10.3390/su14010314.

Yang B., Asche F., Li T. (2022), Consumer behavior and food prices during the COVID-19 pandemic: Evidence from Chinese cities, *Economic Inquiry*, DOI: 10.1111/ecin.13067

Yu X., Liu C., Wang H., Feil, J.H. (2020), The impact of COVID-19 on food prices in China: evidence of four major food products from Beijing, Shandong and Hubei Provinces, *China Agricultural Economic Review*, 12(3), pp. 445–458.

"Bioenhancement and Fortification of Foods for a Healthy Diet": International publication of Ukrainian scientists research studies



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The authoritative international publishing house of scientific literature CRC Press Taylor&Francis group published the book "**Bioenhancement and Fortification of Foods for a Healthy Diet**" under the leadership of the famous biochemist and nutritionist Octavio Paredes-López.

The book is dedicated to the memory of the leading scientist in the field of biotechnology, Professor Volodymyr Ivanov.

The book is a comprehensive collection of food science reviews and covers the technological and nutritional aspects of the use and processing of cereals, dairy products, vegetables and fruits. Developments in the field of food technology are demonstrated in the book and an overview of current knowledge in the field of biotechnological processing and bioimprovement of food products is provided.

Five parts of the Book are devoted to bread and confectionery products, technologies for improving the quality of grain and dairy products, food additives and new technological processes.

The book helps scientists and engineers to contribute to the development of high-quality food products, and is undoubtedly useful for university teachers, graduate students, researchers and professionals in the field of food technology.

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Анотації

Харчові технології

Моделювання зарум'янення хлібних виробів під час випікання: огляд

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Вступ. Розглянуто результати наукових досліджень щодо наявності в хлібних виробах неферментативних сполук потемніння, механізму та факторів, що впливають на їх утворення, а також прогнозування і контролю розвитку зарум'янення хлібних виробів за допомогою математичного моделювання.

Матеріали і методи. Аналітичні дослідження механізму зарум'янення на поверхні хлібних виробів, прогнозування і контроль розвитку зарум'янення в хлібних виробах за допомогою математичного моделювання на основі вже наявних наукових статей.

Результати і обговорення. Утворення кольору в хлібних виробах під час фази випікання відоме як зарум'янення. Коричневий колір на поверхні хлібних виробів забезпечують меланоїдин (нерозчинний коричневий пігмент) і карамель, які є продуктами неферментативних реакцій зарум'янення (реакцій Маяра і карамелізації). Ці реакції також можуть утворювати небажані продукти з потенційно мутагенним ефектом (акриламід, гідроксиметилфурфурол і фурфурол), що призводить до втрати поживної цінності продукту. Зміна кольору поверхні виробу вважається істотним параметром для визначення закінчення процесу випікання хлібних виробів. Необхідно докласти зусиль для розробки швидкого, недорогого, автоматизованого, розумного і об'єктивного методу відстеження зміни кольору під час випікання. Розроблення математичної моделі зарум'янення є важливим для прогнозування і контролю цього явища під час випікання як функції робочих умов і рецептури продукту. Кінетичні моделі для зміни кольору хлібних виробів поділяються на дві групи. Перша група складається з кінетичних моделей зміни кольору, де незалежною змінною є час. До цієї групи входять кінетичні моделі реакцій нульового, першого та другого порядку та експоненціальна емпірична модель. Друга група складається з кінетичних моделей зміни кольору, де незалежною змінною є втрата маси.

Висновок. Зарум'янення впливає на загальну якість хлібних виробів і призводить до змін органолептичних і поживних властивостей (зменшення біодоступності білків і амінокислот, утворення акриламіду, гідроксиметилфурфуролу, речовин з антиоксидантною активністю), тому це питання викликає великий інтерес серед технологів харчових продуктів.

Ключові слова: *випікання, хліб, зарум'янення, колір, карамелізація, реакція Маяра, кінетика.*

Кондитерський соус з порошком ріжкового дерева (*Ceratonia siliqua*)

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Вступ. Розглянуто виробництво кондитерського ріжкового соусу без додавання цукру, визначено його функціональні та фізико-хімічні властивості.

Матеріали і методи. Для обґрунтування використання ріжкового дерева в рецептурі какао-солодкого кондитерського соусу введено порошок стручків і бобів ріжкового дерева. Функціональні та фізико-хімічні властивості отриманого соусу визначено за реологічними показниками, хімічним складом, органолептичним аналізом, антиоксидантною активністю та вмістом загальних фенолів.

Результати і обговорення. Включення морфологічних частин ріжкового дерева (бобів або м'якоті стручків) у рецепт кондитерського соусу для заміни цукру та какао знизило його енергетичну цінність на 60% порівняно з оригінальним рецептом (з какао та цукром). Додавання порошку стручків ріжкового дерева до складу кондитерського соусу збільшувало вміст Ca та Fe у 2,9 та 5,1 раза відповідно .

Зважаючи на 1,1-дифеніл-2-пікрилгідразилу (DPPH) антиоксидантну активність, біологічна цінність соусу з порошком стручків ріжкового дерева підвищилися до 95,97% порівняно з 60% у контролі. Вміст загального фенолу зріс до 29,12 мг еквівалента галової кислоти (GAE) на г порівняно з 5,11 GAE/г для контрольного зразка. Додавання порошку стручка ріжкового дерева в рецептури позитивно впливає на реологічні властивості соусів, що призводить до підвищення їх в'язкості, а також стійкості завдяки збільшенню напруження швидкості зсуву.

Органолептична оцінка соусів, приготованих з додаванням порошку стручків або порошку бобів ріжкового дерева, показала, що всі соуси мали тонку та однорідну консистенцію, приємні смакові та запахові характеристики доданих інгредієнтів: кондитерський соус із порошком стручків ріжкового дерева мав специфічний запах і смак темного шоколаду, а кондитерський соус із порошком бобів ріжкового дерева – відтінок карамельного смаку.

Висновки. Додавання порошку стручків або бобів ріжкового дерева до кондитерських соусів для заміни какао та цукру покращило якість і біологічну цінність соусу за рахунок підвищення вмісту мінеральних речовин, антиоксидантної активності, загального вмісту фенолів. Водночас енергетична цінність продукту зменшилася.

Ключові слова: *ріжкові плоди, функціональний, кондитерський соус, антиоксидант, фенол, ожиріння.*

Показники якості полікомпонентних молочно-рослинних концентратів

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Вступ. Шляхами вирішення проблеми білкової недостатності можуть бути ефективна переробка молока з раціональним використанням його компонентів, пошук нових джерел протеїну, часткова заміна білків тваринного походження на рослинні.

Матеріали і методи. Молочно-рослинний концентрат отримували з молочно-рослинної суміші методом термокислотного осадження білків. Вихід (масу) визначали ваговим методом – зважували зразок після самопресування концентрату, що отримували з 1 дм^3 молочно-рослинної суміші. Визначення активності води модельних проб концентратів проводили на гігрометрі.

Результати і обговорення. Результати визначень виходу концентратів, їх фізико-хімічних та органолептичних показників підтвердили можливість часткової заміни знежиреного молока на сироватково-рослинну суспензію з плодами арахісу *Arachis hypogaea* на рівні 7:3 в сумішах для термокислотного осадження білків. Молочно-рослинні концентрати мали більш розвинену просторову конфігурацію порівняно з контролем. Саме «каркас» зразків, отриманих з максимальним вмістом рослинного коагулянту, обумовлює більшу жорсткість структури і тому забезпечує найкращі структурно-механічні характеристики та, відповідно, вологоутримувальну здатність. За показником активності води (A_w від 0,974 до 0,955), що відображає вплив ферментативних, мікробіологічних та інших процесів інтенсивності реакцій, спрогнозовані раціональні умови зберігання молочно-рослинних концентрів – температура 4±2 °С і тривалість 72 год. Визначено, що випаровування основної частини вологи з білково-рослинного концентрату відбувалося повільніше на 3±0,5 хв порівняно з молочно-білковим завдяки вмісту білків і вуглеводів *Arachis hypogaea*, які зв'язують вільну вологу.

Висновки. Доведена доцільність використання плодів *Arachis hypogaea* у сумішах для термокислотного осадження білків з отриманням молочно-рослинних концентратів з відповідними функціональними властивостями, підвищеною біологічною цінністю за рахунок більш збалансованого амінокислотного складу.

Ключові слова: *молоко, арахіс, Arachis hypogaea, коагуляційний концентрат.*

Порівняльна характеристика змін під час зберігання сортів черешні, вирощених у Грузії

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Вступ. Метою дослідження було вивчення зміни якісних показників черешні, вирощеної в Грузії, під час її зберігання.

Матеріали і методи. Контролювали такі показники сортів черешні, як втрата маси, титрована кислотність і вміст загальної розчинної речовини, загальної кількості фенольних сполук і антоціанів, а також антиоксидантну активність на початку, через 21 і 42 доби зберігання в холодильній камері за температура 0–1 ºС і відносної вологості повітря 90–95%.

Результати і обговорення. Для дослідження обрано три комерційно вирощені сорти черешні, зокрема Кордіа, Регіна та Світхарт. Сорт черешні Кордія містив найбільшу кількість загального вмісту фенолів (ТРС) – 213,95 мг/100 $г^{-1}$ та антоціанів – 145,7 мг/100г, якщо порівняти з іншими сортами. Сорт Світхарт містив найменшу кількість ТРС – 116,0 мг/100 г, антоціанів – 29,75 мг на 100 г. Виявлено позитивну кореляцію між вмістом фенолів та антиоксидантною активністю для досліджуваних сортів. Найкраща кореляція виявлена для черешні Регіна (R^2=0,98), тоді як для сортів Кордія та Світхарт – 0,88 і 0,83 відповідно. У процесі зберігання змінювалися фізико-хімічні властивості черешні: поступово знижувався вміст загальної розчинної речовини, титрована кислотність, загальний вміст фенолів, вміст антоціанів і антиоксидантна активність.

Загальне зменшення маси плодів через 42 дні вимірювали разом із мікробіологічними втратами. У сорту Світхарт спостерігалася найбільша втрата ваги порівняно з іншими.

За трирічними даними загальна втрата маси черешні сорту Кордія склала 10,13% наприкінці періоду зберігання (42 дні) за температури 0–1 ºС і відносної вологості повітря 90–95%. Це значення становило 13,51% для сорту Регіна і 14,49% – для сорту Світхарт.

Висновок. Три досліджувані сорти черешні близькі за хімічним складом. Проте за вмістом цінних речовин і стійкістю до зберігання найкращі показники у сорту Кордія.

Ключові слова: *черешня, Prunus avium L., Кордіа, Регіна, Світхарт, антиоксидант, зберігання.*

Вплив амінокислотного та фракційного складу сухого молока на засвоюваність сухих молочних сумішей

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Вступ. Досліджено вплив амінокислотного і фракційного складу сухого молока ссавців на здатність сухих молочних сумішей до перетравлювання.

Матеріали і методи. Досліджували молоко сухе: козине, кобиляче, овече, сухі молочні суміші для дитячого харчування. Амінокислотний склад визначали методом іонообмінної хроматографії, фракційний склад – методом електрофорезу, здатність до перетравлювання – в умовах in vitro.

Результати і обговорення. Лімітуючою амінокислотою сухого коров'ячого та овечого молока є цистиїн, амінокислотний скор якого становить 46,0% та 47,0% відповідно. Лімітуючою амінокислотою кобилячого та козиного молока є тирозин, скор якого – 52,0% та 57,0% відповідно. Лише кобиляче молоко, як і жіноче, відноситься до альбумінового типу. Найбільшу кількість захисних факторів молока (імуноглобулінів і лактоферину) містить молоко кобиляче та овече. Кобиляче та овече молоко містить наближену кількість до жіночого молока імуноглобуліну А (5,15 % та 6,92 % відповідно). Співвідношення казеїну до сироваткових білків у кобилячому молоці становить 1:1, у коров'ячому молоці – 4:1, у козиному та овечому молоці – 3:2. Відмінності складу пояснюються різними генотипами ссавців і втратами білка при сушінні. Найвищий ступінь перетравлюваності у жіночого молока та суміші на основі кобилячого молока «Ligans» – 717 мг та 780 мг амінокислот на 100 г продукту відповідно. Найнижчий ступінь перетравності білків у суміші на основі коров'ячого молока «Malyutka hypoallergenic». Низький ступінь перетравності можуть зумовлювати реакції між амінокислотами, утворення комплексів казеїну з денатурованими сироватковими білками під час сушіння.

Висновок. Амінокислотний і фракційний склад білків сухого кобилячого молока наближений до жіночого, тому перетравність продуктів на його основі відбувається як і в жіночому молоці.

Ключові слова: *молоко овече, молоко кобиляче, білки, амінокислоти.*

Харчова хімія

Вплив хімічної будови спиртів на екстракцію і стабільність антоціанів

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Вступ. Мета цього дослідження – оцінювання ефективності екстракції антоціанів кількома спиртами зі шкірки винограду за різних умов, таких як температура та pH.

Матеріали і методи. Досліджено вичавки ягід червоного винограду сорту Vitis Vierul, які отримують після приготування вина за білим способом і вважають відходом від виноробства, а також вплив значення pH і структури молекули спиртового екстрагента на ефективність екстракції антоціанів та їх спектральні характеристики. Визначено стабільність цих природних пігментів в отриманих екстрактах. Масову концентрацію антоціанів від pH отримували за допомогою диференціальної спектрофотометрії pH та виражали у мг еквівалентного ціанідин-3-глікозиду на грам сухої речовини. Константу швидкості та період напіврозпаду реакції розпаду антоціанів розраховували при температурі 60°C.

Результати і обговорення. Кількість гідроксильних груп у спирті, що використовується як екстрагент, а також довжина вуглеводневого фрагмента відігравали ключову роль в ефективності процесу. Зокрема, для одноатомних спиртів ефективність відповідає такому порядку: 2-метилпропан-1-ол < бутан-1-ол <пропан-2-ол <етанол <метанол. З іншого боку, збільшення кількості груп OH в ряду етанол > етан-1,2-діол > пропан-1,2,3-тріол не покращує ефективність вилучення. Спектральні характеристики вилучень, одержаних за допомогою етанолу і багатоатомних спиртів, подібні, максимум поглинання нечіткий і знаходиться в межах 530 – 560 нм. При збільшенні довжини вуглецевого ланцюга спирту електронні спектри поглинання характеризуються різною інтенсивністю і широким, нечітким максимумом поглинання. Проведено визначення термостійкості антоціанів в екстрактах з визначенням константи швидкості реакції деградації антоціанів та часу напіврозпаду. Зниження pH екстракту призводить до збільшення термічної стабільності антоціанів. Етанол є найкращим екстрагентом з точки зору технологічної й економічної ефективності вилучення природного пігменту та його застосування в харчовій промисловості. Період напіввиведення антоціанів близько 10 год в етанолі при температурі 60°C свідчить про його придатність для фактичної промислової переробки.

Висновки. Отримані результати дають змогу оцінити ефективність вилучення антоціанів спиртами зі шкірки винограду та їх термічну стійкість.

Ключові слова: *антоціани, вилучення, деградація, стабільність, виноград.*

Біотехнологія, мікробіологія

Руйнування біоплівок під впливом поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241, синтезованих за наявності конкурентних мікроорганізмів

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Вступ. Метою статті було дослідження ролі в руйнуванні біоплівок поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241, синтезованих за наявності біологічних індукторів у середовищі з гліцерином різного ступеня очищення.

Матеріали і методи. Культивування *A. calcoaceticus* IMB B-7241 здійснювали в рідкому мінеральному середовищі з використанням як субстратів очищеного гліцерину та відходів виробництва біодизелю. Як біологічний індуктор (конкурентний мікроорганізм) використовували бактеріальний штам *Bacillus subtilis* БТ-2 у вигляді суспензії живих та інактивованих клітин, а також у вигляді супернатанту. Поверхнево-активні речовини екстрагували із супернатанту культуральної рідини модифікованою сумішшю Фолча. Ступінь руйнування біоплівки за наявності ПАР визначали спектрофотометричним методом.

Результати і обговорення. Встановлено, що незалежно від субстрату, що використовується, внесення у середовище культивування *A. calcoaceticus* IMB B-7241 як живих, так і інактивованих клітин *B. subtilis* БТ-2 супроводжувалося синтезом ПАР, ступінь руйнування біоплівок за дії яких був вищим, ніж у разі використання поверхнево-активних речовин, отриманих на середовищі без індуктора. Ступінь деструкції бактеріальних і дріжджових біоплівок під впливом ПАР, одержаних на очищеному гліцерині за наявності клітин індукторів, становив 36,5–85% і був у 1,5–3 рази вищим порівняно з використанням ПАР, синтезованих у середовищі без індуктора. Зазначимо, що ПАР, синтезовані за наявності біологічних індукторів, руйнували біоплівки досліджуваних тест-культур у досить низьких (7,5–960 мкг/мл) концентраціях. Подібні закономірності спостерігали і за використання ПАР, утворених на відходах виробництва біодизелю. Так, внесення в середовище з цим субстратом живих клітин *B. subtilis* БТ-2 супроводжувалося синтезом поверхнево-активних речовин, за дії яких у концентрації 1,8–960 мкг/мл ступінь руйнування біоплівок *B. subtilis* БТ-2, *Proteus vulgaris* ПА-12 та *Enterobacter cloacae* С-8 становив 30,1–80,7% і був вищим, ніж при використанні аналогічних концентрацій ПАР, одержаних під час культивування без індуктора (24,1–75%). Деструкція біоплівок *Staphylococcus aureus* БМС-1, *Candida albicans* Д-6 та *Candida tropicalis* РЕ-2 під впливом ПАР (1,8–960 мкг/мл), синтезованих на відходах виробництва біодизелю за наявності як живих, так і інактивованих клітин *B. subtilis* БТ-2, була у 1,5–8 разів вищою порівняно з такою за дії ПАР, синтезованих у середовищі без індуктора.

Висновки. Отже, в результаті проведеного дослідження доведено можливість регулювання біологічної активності поверхнево-активних речовин *A. calcoaceticus* IMB B-7241 шляхом внесення в середовище культивування клітин конкурентних бактерій *B. subtilis* БТ-2, які є індукторами синтезу ПАР з вищою здатністю до руйнування як бактеріальних, так і дріжджових біоплівок.

Ключові слова: *поверхнево-активні речовини, Acinetobacter calcoaceticus IMB B-7241, індуктор, біоплівка.*

Підвищення екологічної безпеки біозміцнення ґрунту при використанні рослинної уреази

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Вступ. Біоцемент – новий матеріал для будівництва, створений з використанням мікробної уреази. Використання уреази рослин – шлях до широкого та екологічно безпечного біозміцнення ґрунту.

Матеріали і методи. Уреазну активність пророщеного насіння визначали за зміною електропровідності розчину сечовини, μS/ см, внаслідок її гідролізу під дією ферменту уреази. Концентрацію кальцію визначали титруванням етилендіамінтетраацетатом з використанням індикатора Еріохром чорний Т. Оцінку ступеня біоцементації піску проводили за зміною його водопроникності.

Результати і обговорення. Недоліками процесу біоцементації є потенційна біологічна небезпека від запропонованих до використання уреазо-продукуючих бактерій і непередбачуваний ефект внесення в навколишнє середовище суттєвої кількості живої бактеріальної біомаси при біоукріпленні ґрунтів. Можливою заміною бактеріальної уреази може бути уреаза рослинного походження. Скринінг насіння сільськогосподарських культур показав, що насіння сої можливо використовувати як джерело уреази для біоцементації у вигляді неочищеного водного екстракту з подрібненої маси самого насіння або пророщеного протягом 24 – 48 годин. Уреазна активність гомогенізованої маси була вищою, ніж активність чистих екстрактів. Питома активність (активність на одиницю рослинної сировини) як гомогенізованої маси, так і в екстракті була вищою при застосування насіння, яке пророщувалося протягом 24 – 48 год, і знижувалася на 96 год пророщування.

Використання неочищеного екстракту з насіння сої підтвердило його ефективність для осадження карбонату кальцію з розчину хлориду кальцію та сечовини. Застосування рослинної уреази для біоцементації піску дало змогу знизити його водопроникність у 600 разів та отримати значення $1 \cdot 10^{-6}$ м/с, що підтверджує можливість використання рослинної уреази замість уреаза-продукуючих бактерій для біоцементації ґрунту.

Висновки. Доведено можливість заміни бактеріальної уреази уреазою рослинного походження, зокрема екстрактом з насіння сої. Рослинна уреаза ефективно осаджувала карбонат кальцію із суміші розчинів хлориду кальцію і сечовини, а її застосування при біоцементації піску знижувало його водопроникність до значень, що відповідають гідравлічній проникності піску біоцементованого з використанням уреаза-продукуючих бактерій.

Ключові слова: *рослинна уреаза, біоцементація, біоукріплення, ґрунт, екологічна безпека, водопроникність.*

Економіка і управління

Вплив кризи, спричиненої коронавірусом, на поведінку споживачів в Угорщині при купівлі харчових продуктів

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Вступ. Через світову епідемію коронавірусу моделі споживчої поведінки, пов'язані з купівлею харчових продуктів, потребують перегляду.

Матеріали і методи. Проведено емпіричне дослідження шляхом анкетування. Дослідження проводилося в Угорщині влітку 2020 року, в ньому взяли участь 724 споживачі.

Результати і обговорення. Високий відсоток споживачів в Угорщині відреагували на кризу через коронавірус панічними купівлями. Споживання більш здорової їжі поширилося у зв'язку з кризою, пов'язаною з коронавірусом. Також зріс попит угорських споживачів на молочні продукти. Крім того, існують відмінності між продуктами, які споживають покупці в паніці та звичайні покупці. Результати показали, що ті, хто не боявся коронавірусу, менше звертали уваги на свої харчові звички (кореляція Пірсона: -0,119, sign.:0,01). 59,9% респондентів заявили, що через епідемію коронавірусу варто зберігати велику кількість продуктів. Дані стосовно жінок і чоловіків не відрізнялися (Chi-square 0,160, df: 2, sign.:0,923, p>0,05). Кореляційні тести показали, що купівля молочних продуктів, масла, сиру, фруктового йогурту і сметани значно корелюють між собою. За віком найбільше молочних продуктів купували респонденти 40–60 років, найменше – 30 років.

Висновок. Пандемія коронавірусу серйозно вплинула не лише на світову економіку, а й на повсякденне життя населення планети. Негативні наслідки також відбилися на ставленні до придбання продуктів харчування, також відбулися значні зміни в складі споживаних продуктів.

Ключові слова: *їжа, покупка, коронавірус, Угорщина, паніка.*

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Divide your article into sections and into subsections if necessary. Any subsection should have a brief heading.

References

Please, check references carefully.

The list of references should include works that are cited in the text and that have been published or accepted for publication.

All references mentioned in the reference list are cited in the text, and vice versa.

Cite references in the text by name and year in parentheses. Some examples:

(Drobot, 2008); (Qi and Zhou, 2012); (Bolarinwa et al., 2019; Rabie et al., 2020; Sengev et al., 2013).

Reference list should be alphabetized by the last names of the first author of each work: for one author, by name of author, then chronologically; for two authors, by name of author, then name of coauthor, then chronologically; for more than two authors, by name of first author, then chronologically.

If available, please always include DOIs links in the reference list.

Reference style

Journal article

Please follow this style and order: author's surname, initial(s), year of publication (in brackets), paper title, *journal title (in italic)*, volume number (issue), first and last page numbers, e.g.:

Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies*, 2(2), pp. 104-108. DOI: 10.1016/j.jece.2020.103776

The names of all authors should be provided, but in case of long author lists the usage of “et al” will also be accepted. Journal names should not be abbreviated.

Book

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

Book chapter in an edited book

Coffin J.M. (1999), Molecular biology of HIV, In: Crandell K.A. ed., *The evolution of HIV*, Johns Hopkins Press, Baltimore, pp. 3-40.

Online document

Mendeley J.A., Thomson, M., Coyne R.P. (2017), *How and when to reference*, Available at: https://www.howandwhentoreference.com

Conference paper

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52-57.

Figures

All figures should be made in graphic editor using a font Arial.

The font size on the figures and the text of the article should be the same.

Black and white graphic with no shading should be used.

The figure elements (lines, grid, and text) should be presented in black (not gray) colour.

Figure parts should be denoted by lowercase letters (a, b, etc.).

All figures are to be numbered using Arabic numerals.

Figures should be cited in text in consecutive numerical order.

Place figure after its first mentioned in the text.

Figure captions begin with the term Figure in bold type, followed by the figure number, also in bold type.

Each figure should have a caption describing what the figure depicts in bold type.

Supply all figures and EXCEL format files with graphs additionally as separate files.

Photos are not advisable to be used.

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Number tables consecutively in accordance with their appearance in the text.

Place footnotes to tables below the table body and indicate them with superscript lowercase letters.

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Ensure that the data presented in tables do not duplicate results described elsewhere in the article.

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Submission

Email for all submissions and other inquiries:

ufj_nuft@meta.ua

Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **10 сторінок** формату А4 (без врахування анотацій і списку літератури)**.**

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.

Всі поля сторінки – по 2 см.

Структура статті:

1**.** УДК**.**
2. **Назва статті.**
3. Автори статті (ім’я та прізвище повністю, приклад: Денис Озерянко).
4. *Установа, в якій виконана робота*.
5. Анотація. **Обов’язкова** структура анотації:
   - Вступ (2–3 рядки).
   - Матеріали та методи (до 5 рядків)
   - Результати та обговорення (пів сторінки).
   - Висновки (2–3 рядки).
6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов’язкові розділи:
   - Вступ
   - Матеріали та методи
   - Результати та обговорення
   - Висновки
   - Література.

За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім’я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

У списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.

Оформлення цитат у тексті статті:

Кількість авторів статті	Приклад цитування у тексті
1 автор	(Arych, 2019)
2 і більше авторів	(Bazopol et al., 2021)

Приклад тексту із цитуванням: It is known (Bazopol et al., 2006; Kuievda, 2020), the product yield depends on temperature, but, there are some exceptions (Arych, 2019).

У цитуваннях необхідно вказувати одне джерело, звідки взято інформацію.
Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:

Автори А.А. (рік видання), Назва статті, *Назва журналу (курсивом)*, Том (номер), сторінки.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

1. Приклад:
   Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (Juglans regia L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108.

2. Посилання на книгу:

Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.

Приклади:

(2013), *Svitovi naukovometrychni bazy*, Available at: http://www.nas.gov.ua/publications/q_a /Pages/scopus.aspx

Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт.

Зручний сайт для транслітерації з української мови: http://translit.kh.ua/#lat/passport

Стаття надсилається за електронною адресою: ufj_nuft@meta.ua

УДК 663/664

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

Ukrainian Food Journal індексується наукометричними базами:

- Index Copernicus (2012)
- EBSCO (2013)
- Google Scholar (2013)
- UlrichsWeb (2013)
- CABI full text (2014)
- Online Library of University of Southern Denmark (2014)
- Directory of Open Access scholarly Resources (ROAD) (2014)
- European Reference Index for the Humanities and the Social Sciences (ERIH PLUS) (2014)
- Directory of Open Access Journals (DOAJ) (2015)
- InfoBase Index (2015)
- Chemical Abstracts Service Source Index (CASSI) (2016)
- FSTA (Food Science and Technology Abstracts) (2018)
- Web of Science (Emerging Sourses Citaton Index) (2018)
- Scopus (2022)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

Детальна інформація про Журнал, інструкції авторам, приклади оформлення статті та анотацій розміщені на сайті:

http://ufj.nuft.edu.ua

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