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RESEARCH OF MILK FAT OXIDATION PROCESSES DURING STORAGE OF BUTTER PASTES

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ABSTRACT

Basic quality indicators studied: acidity, peroxide, anisidine value and integrated value of complete fat oxidation. Butter paste was selected as a reference, consisting of butter, skim milk powder and fat-soluble emulsifiers. Peroxide value during storage at the temperature of (4 ±2 °C) for the first 4 days did not exceed 5.0 1/2 O mmol.kg^{-1}, on the 15th day fat peroxide value of butter paste with milk-vegetable protein exceeded permissible limits that is indicative of milk fat contamination. At the temperature of (-3 ±1 °C) butter paste fat couldn't be qualified as fresh when storing during 15 days, peroxide value exceeds permissible limits on the 25th day of storage. Rising of the peroxide value above 5 1/2 O mmol.kg^{-1} was detected on the 25th day of storage, exceeding of threshold value was on the 45th day. It was established that rate of oxidation processes in butter pastes with vegetable protein is the highest among all studied samples in each particular control and observation point. It was determined that the rate of secondary lipid oxidation depends on the storage temperature and is observed when storing butter paste samples at a temperature of (-3 ±1 °C) on the 10th day, (-24 ±2 °C) – on the 30th day of storage. Acid value did not exceed recommended limits (2.5 °K) and was on average – 2.3 °K when storing butter paste during 10 days at a temperature of (4 ±2 °C); 2.1 °K during 20 days at the temperature of (-3 ±1 °C), 2.4 °K during 40 days at the temperature of (-24 ±2 °C). In view of obtained results of fat phase stability evaluation of studied butter pastes, the following storage maximum time is recommended: at the temperature of (4 ±2 °C) – 7 days, at the temperature of (-3 ±1 °C) – 15 days, at the temperature of (-26 ±2 °C) – 30 days.

Keywords: butter paste; milk protein; vegetable protein; milk; fat; oxidation

INTRODUCTION

Relatively high calorific value of butter often serves as limitation when adding to food ration of a modern human and compels scientists and producers of dairy products to develop technologies similar to butter with lower fat content. Fat exclusion from the ration for a long term is inadvisable and dangerous in as much as lipids have exceptional value in human physiology. Lipids are source of energy and fulfil a range of functions in the body: heat insulation, protection of internal injury, exercise anatomic and tectonic function as part of cell membrane, arranging order of metabolites flow in the body **(Kubicová, Predanocyová and Kádeková, 2019)**. Biological value of fats is determined first of all by availability of polyunsaturated fatty acids – linoleic, linolenoic and arachidonic acids. These fatty acids are not synthesized by human body, if they are insufficient in food products lipid exchange processes fail in the body. Linoleic and linolenoic acids are contained primarily in vegetable fats, arachidonic – in animal fats **(Zahorui, Mazur and Kalinina, 2019; Bozhko et al., 2017)**. Milk fat is unique as it contains all three aforesaid fatty acids at once. Besides, fat-soluble vitamins, in particular vitamin A and β-carotene are also assigned to milk lipids. Therefore, development of new butter paste types which are low-caloric substitute of butter is topical trend of researches.

Since butter pastes are defined by high moisture contents, skim milk powder, milk or vegetable proteins, protein polysaccharide complexes etc. are used for their structure stabilization **(Kochubei-Lytvynenko et al., 2018a; Ondrušíková et al., 2019; Kumbár et al., 2017)**. Free moisture is suitable environment for biochemical transformations, that is why during production and storage of butter pastes there is a danger of biochemical transformations of milk fat and other components, primarily proteins and carbohydrates limiting storage life of such products **(Golian et al., 2018; Frolova et al., 2019)**.

One of the main processes occurring during storage of butter pastes and causing product spoilage is milk fat oxidation. Fatty acid content of milk fat exercises significant influence on preservation of butter pastes

during storage **(Dyman and Zahoruy, 2008; Bubelová et al., 2017)**. First of all polyunsaturated fatty acids are prone to oxidation under influence of atmospheric oxygen, causing accumulation of peroxide and carbonyl compounds. Therefore, the course of oxidation changes in developed butter pastes were studied when controlling peroxide and anisidine values. Oxidation stability of fat as part of a product also depends on production conditions, heat and light impact, concentration and type of oxygen, presence of free fatty acids, mono- and diglycerides, metals of variable valency, peroxides, thermal oxidized compounds, pigments and antioxidants. These factors mutually influence on the oxidation process and it is practically impossible to determine their individual effect, however at initial oxidation stages chemical and organoleptic indicators of fat have little or no change.

Fat oxidation process is free radical chain reaction, comprising initiation, branching and termination stage and which takes place involving molecular oxygen **(Piven, 2007)**:

Chain initiation	$H \rightarrow R\cdot + H\cdot$
Chain branching	$R\cdot + 3O_2 \rightarrow ROO\cdot$
	$ROO\cdot + RH \rightarrow ROOH + R\cdot$
Chain termination	$ROO\cdot + R\cdot \rightarrow ROOR$
	$R\cdot + R\cdot \rightarrow RR$
	(R – lipid radical)

Depending on storage conditions and initial qualities of product the oxidation may take place under different mechanisms. Products of described reactions are first and foremost hydroperoxides, from which aldehydes, ketones, short-chain hydrocarbons, spirits and ethers are formed by autoxidation mechanism. Hydroperoxides formed in the reaction with oxygen can be determined by peroxide value, products of their further transformation – by anisidine value **(Tsisaryk, Musiy and Shereshkov, 2016)**.

Lipids oxidation is followed by deep breakdown of triacyl glycerides with formation of peroxide compounds, aldehydes, ketones etc. At this time foreign flavours and odours appear in the product. Oxidation may be nonenzymic and enzymic. Nonenzymic oxidation is followed by peroxides accumulation at the beginning, thereafter aldehydes, ketones, oxyacids and other compounds, which add foreign flavours and odours to dairy products. This process takes place as a result of fat interaction with molecular oxygen. At first free milk butter not protected by membrane is exposed to oxidation, out of fatty acids – unsaturated ones. Oxidation process is controlled by peroxide value **(Javůrková et al., 2016; Korablova, 2011)**.

This value is very sensitive and is a reference for beginning and rate of fat oxidation. Fresh fat is free from peroxides. At initial oxidation stages for some time chemical and organoleptic indicators of fat are almost unchanged. During the storage fat begins spoiling. It can be detected by increase in peroxide value and change of organoleptic properties of fat **(Decker, 2002; Li et al., 2018; Saláková et al., 2019)**. End products of milk fat splitting are free fatty acids **(Martin-Polvillo, Marquez-Rui and Dobarganes, 2004)**, accumulation of which result in bad taste and odour of the product and acidity level increase. In addition to, accumulation of organic acids may be a result of deep breakdown of proteins and carbohydrate components, in particular lactose.

Thus, the study of the milk fat oxidation processes of butter pastes during storage at different temperatures will allow specifying conditions and storage life of products and ensuring product safety for a consumer.

Scientific hypothesis

Fat splitting process in butter pastes, stabilized by protein polysaccharide complexes, has the same tendency, but slightly slower as compared to butter paste, which contains skim milk powder. Process deceleration can be explained by reduction of free moisture content through using more active structure-forming agents and by synergism of their interaction.

MATERIAL AND METHODOLOGY

Material

Authors developed formulations of butter pastes, stabilized by protein polysaccharide complex and justified technological modes of their production. **(Kochubei-Lytvynenko et al., 2018a; Kochubei-Lytvynenko et. al., 2018b)**. Figure 1 represents the photo of a butter paste stabilized with protein-polysaccharide complex.

Figure 1 Butter paste stabilized with protein-polysaccharide mixture.

In order to ensure stable properties of butter pastes and prevention of their spoilage during storage, changes in quality during storage by acidity, peroxide (PV) and anisidine (AV) values were studied. Product samples were stored in an unsealed package at three technological modes: at a temperature of (4 ±2 °C) during 15 days, (-3 ±1 °C) during 25 days and at a temperature of (-26 ±2 °C) during 45 days at relative air humidity of not less than 85% without sunlight. Acidity was determined directly in collected sample, oxidation indicators – at fat extraction by digestion method after each 5 days of storage (3 days at a temperature of (4 ±2 °C). Butter paste was selected as a reference, consisting of butter, skim milk powder and fat-soluble emulsifiers.

Methods

Determination of fat peroxide value was conducted under State Standard of Ukraine DSTU 4570:2006 **(DSTU 4570, 2006)**. The method is based on the reaction of interaction of fat oxidation products (peroxides and hydroperoxides) with potassium iodide in acetic acid and chloroform solution and the subsequent quantitative determination of released iodine with sodium thiosulfate solution by the titrimetric method. For determination of peroxide number

such chemical reagent were used: distilled water, glacial acetic acid, chemically-pure (C.P.), chloroform (trichloromethane), aqueous solution of potassium iodide C.P. mass content 50 – 55% freshly prepared, solution of starch mass content 0.5%, aqueous solution of ($Na_2S_2O_3$. $5H_2O$) molarity 0.01 mol.L^{-1}, final of sodium sulphite with mass of substance in vacuum-sealed ampule , which equals 0.1 gram-equivalent. Peroxide value is a ration of substance amount in a sample related to active oxygen, which under reference conditions oxidize potassium iodide, to the weight of studied sample. The value characterizes the amount of primary products of fats oxidation – peroxide compounds (hydroperoxides, peroxides, dialkyl peroxides), which are able to separate iodine from aqueous solution of potassium iodide. It is expressed in millimole of active oxygen per sample kilogram. Peroxide value is an indicator of fats freshness grade. The method principle is based on the interaction reaction of fat oxidation products with potassium iodide in acetic acid and chloroform solution and further quantitative determination of discharged iodine by sodium thiosulfate solution using titrimetric method.

Peroxide value (PV) 1/2 O mmol.kg^{-1} is calculated by formula:

$$PV = (V - V_0)*c*1000/ m \quad (1)$$

Where: V, V_0 – volume of sodium thiosulfate solution in main and control study respectively, cm^3; C – concentration of sodium thiosulfate solution, mol.dm^{-3}; m – mass of studied sample, g; 1000 – ratio, which includes conversion of measurement result in mmol.kg^{-1}.

Peroxide value is expressed in 1/2 O millimole per kilogram, which corresponds to the amount of oxygen, used in the given oxidation-reduction reaction, in milliequivalents per kilogram.

Determination of anisidine value was conducted under DSTU 6885-2002 **(DSTU 6885, 2002)**. The principle of the method is in dissolving the test sample of the fat product in isooctane, interacting with p-anisidine, and measuring the optical density of the solution at a wavelength of 350 nm. For determination of anisidine value such chemical reagent were used: sodium sulfate (Na_2SO_4) C.P.; 2,2,4-trimethylpentane (isooctane), which has optical density ≤0.01 in relation to water within the wavelength 300-380 nm; 4-methoxyaniline (p-anisidine); glacial acetic acid C.P., with content of water ≤0.1%; sodium sulphite (Na_2SO_3) C.P., anhydrous; activated carbon clarify powdered; anhydrous calcium chloride ($CaCl_2$) C.P.; laboratory items: spectrometer UV-1800 SHIMADZU, range of values 190 – 1100 nm, pathlength of cuvette 10 mm, suitable for measurement at the wavelength 350 nm. Anisidine value (AV) shows concentration of aldehydes (primarily, 2-alcanal) in animal fats. This is a dimensionless value, which is numerically equal to centuplicated optical density fat solution in isooctane upon processing by anisidine reagent, measured on spectrophotometer with a wavelength of 350 nm. The method principle consists in preparation of the studied sample solution in isooctane, reaction with acetic solution of p-anisidine and measurement of optical density at 350 nm.

Anisidine value is calculated by formula:

$$AV = 100 \cdot Q \cdot V [1.2 (A_1 - A_2) - A_0] / m, \quad (2)$$

Where: Q – adjusted concentration of studied solution in g per cm^3 (Q = 0.01 g.cm^{-3}); V – dissolved sample volume (V = 25 mL), A_0, A_1, A_2 – optical density of nonreactive analytical, color and control solution respectively; m – sample mass, g.

Integrated value of full fat oxidation (IV) is calculated by formula:

$$IV = 2\times PV+AV \quad (3)$$

Fat acidity is expressed in degrees c (°K), it is a volume (cm^3) of sodium or potassium hydroxide solution of molar concentration 0.1 mol.dm^{-3}, spent for neutralization of free acids in 10 g of product.

Statistical analysis

Obtained measurement results and graphical representation of experimental data were conducted using standard programs of statistical processing Microsoft Excel 2010. Precision of obtained results was ensured by three-, fivefold repetition of studies. The analysis of variance (ANOVA) of the obtained results was conducted; confidence range under t-criterion for parallel measurements with *p*-value less than 0.05 was determined. For building graphical dependencies and in the table the arithmetic results of parallel measurements with value of $p = 0.05$ are indicated.

RESULTS AND DISCUSSION

Since the peroxide value (PV) is regulated by state standards system for fat-containing products, there is a possibility to predict storage life of developed products under this value **(Coppin and Pike, 2001; Mehta et al., 2018)**. Generally accepted quality level of fat-containing products PV at the level up to 5.0 1/2 O mmol.kg^{-1}, when its value more than 10.0 1/2 O mmol.kg^{-1} the product loses its nutritional value **(Fatouh et al., 2005; Dyman and Zahoruy, 2008)**. Guaranteed storage life is established at the level of 50% of a term, during which the product does not lose its nutritional value, if expected storage life does not exceed 30 days and at the level of 70%, if expected storage life does not exceed 30 days **(Stele, 2006)**.

It was established that accumulation rate of peroxides during the storage of butter pastes directly depends on the storage temperature (Figure 2), caused by enzymic component of biochemical transformations of milk fat **(Jacobsen et al., 2008; Jones et al., 2005; Rodrigues and Gioielli, 2003)**. At temperature decrease the enzymes strength decreases and practically levels in condition of deep freezing (Figure 2a). It was determined that during storage at a temperature of (4 ±2 °C) (Figure 2a) within first 4 days, no major changes of peroxide value were observed, on further storage the accumulation rate of peroxides was rising ($p \leq 0.05$) significantly, on the 10^{th} day fat of butter pastes was defined by peroxide value higher than 5.0 1/2 O mmol.kg^{-1}, that was indicative of the beginning of spoilage processes, on the 15^{th} day of storage the peroxide value pf butter paste fat with milk and vegetable protein slightly exceeded permissible limits.

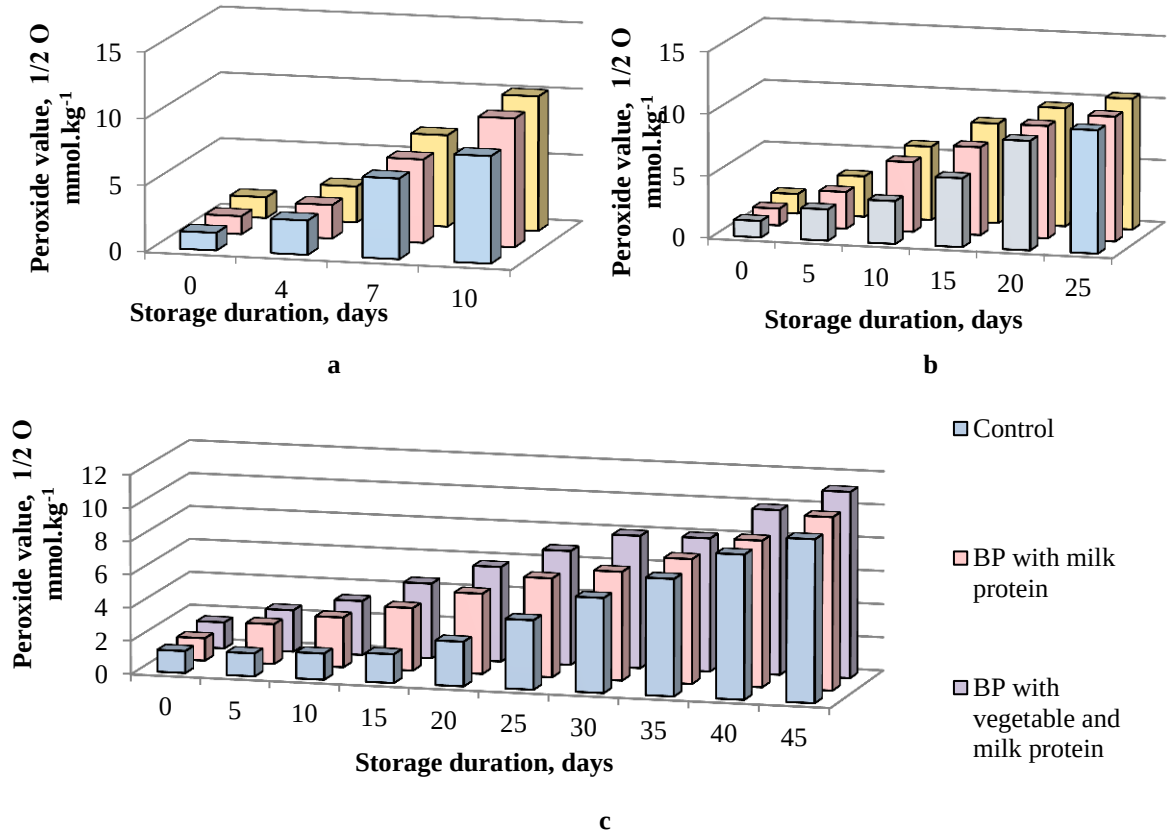

Figure 2 Dynamics of peroxide number of butter pastes at different temperature regimes: a – (4 ±2 °C); b – (-3 ±1 °C); c – (-24 ±2 °C); (n = 3 – 5, $p \leq 0.05$).

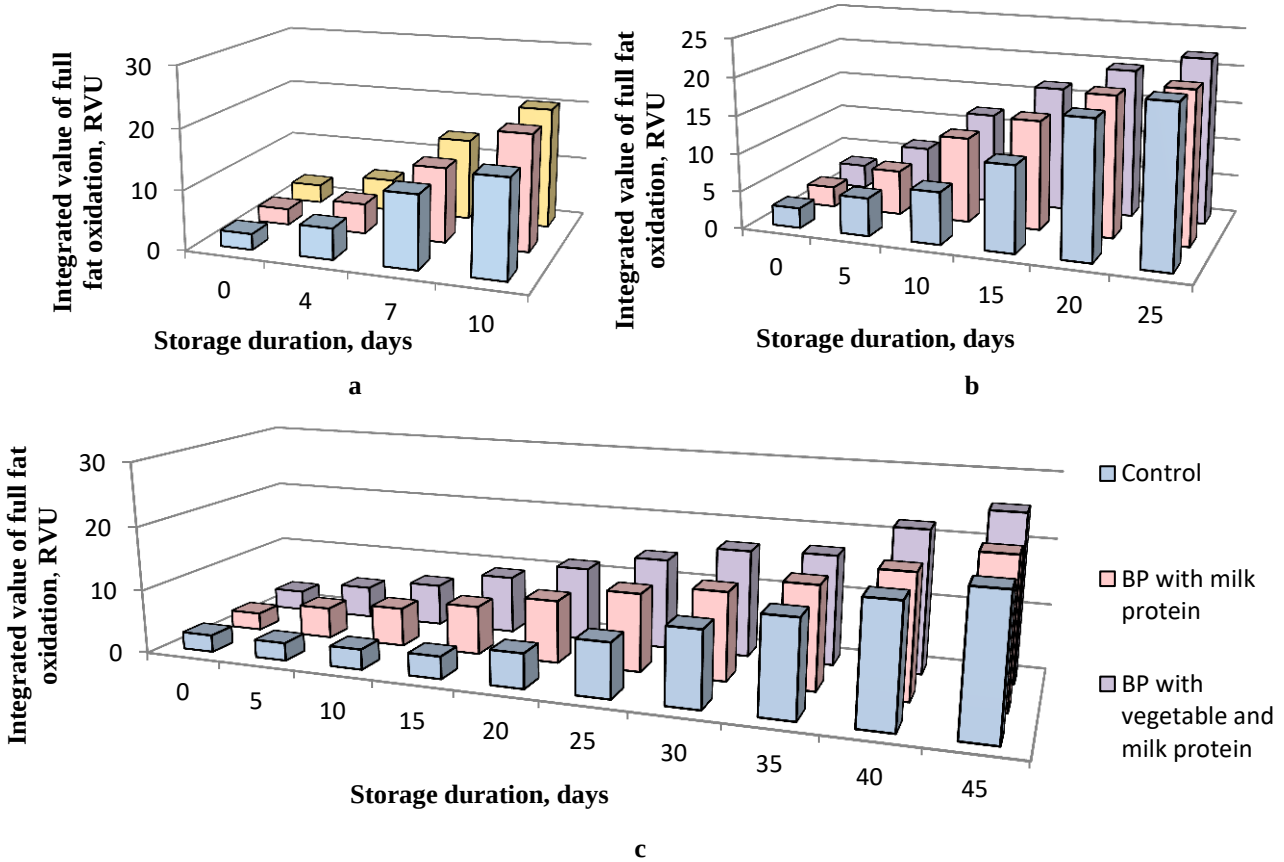

Figure 3 Dynamics of integral index of complete oxidation of butter pastes (relative value unit (RVU)) at different temperature regimes: a – (4 ±2 °C); b – (-3 ±1 °C); c – (-24 ±2 °C); (n = 3 – 5, $p \leq 0.05$).

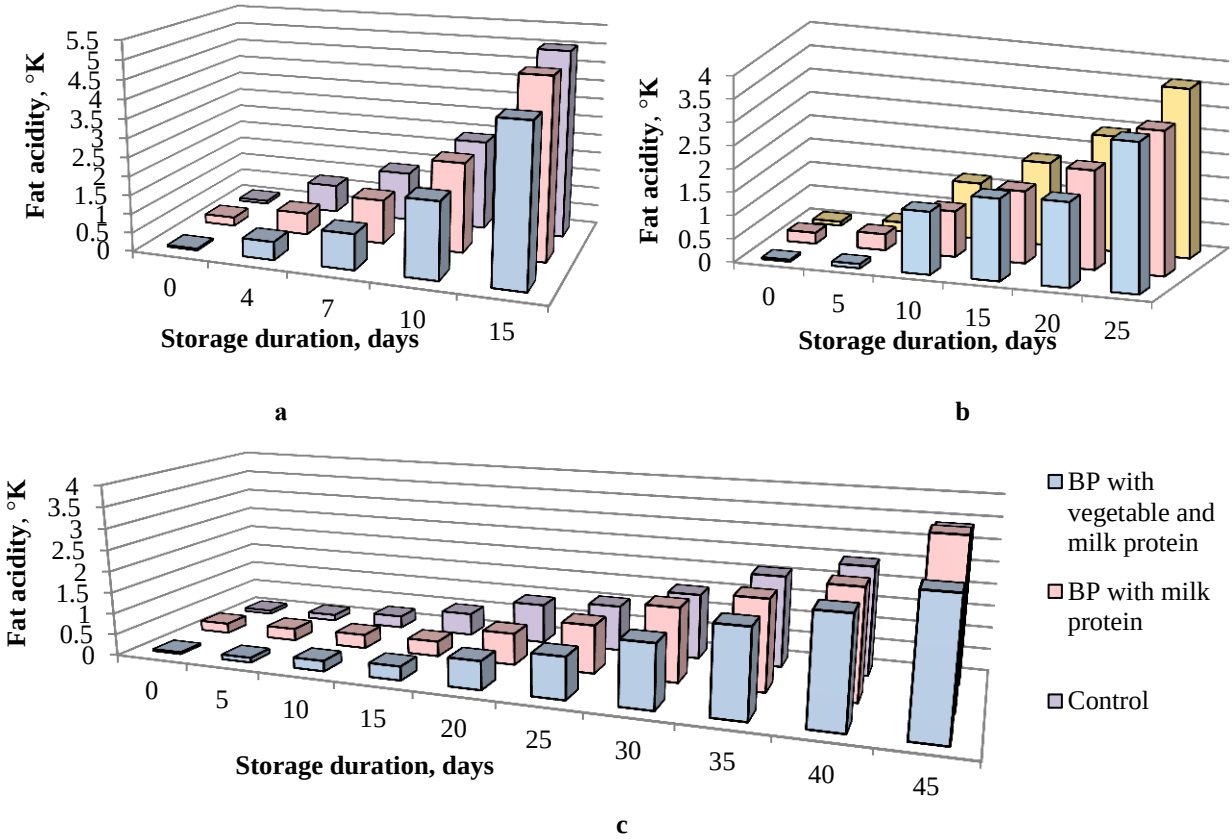

Figure 4 Acid dynamics of butter pastes at different temperature conditions: a – (4 ±2 °C); b – (-3 ±1 °C); c – (-24 ±2 °C); (n = 3 – 5, $p \leq 0.05$).

Based on obtained results we can conclude about the possibility of establishing guaranteed storage life – not more than 5 days. Accumulation rate of peroxides in butter pastes with vegetable protein is the highest among all studied samples in each particular control and observation point. The revealed trend can be explained by residual enzyme strength – lipooxidase, which is contained in vegetable isolate and catalyses reactions of lipid peroxide oxidation **(Lobanov and Shcherbin, 2003)**.

Similar trend was observed when studying peroxide value history at a temperature of (-3 ±1 °C) (Figure 2b). It was determined that butter paste fat could not be qualified as fresh when storing during 15 days at a said temperature, peroxide value exceeds permissible limits and is on average 10.4 1/2 O mmol.kg^{-1}. Thus, the guaranteed storage life of butter pastes at a said temperature should be maximum 15 days.

Rising dynamics of the peroxide value during storage of butter pastes at a temperature of (-24 ±2 °C) (Figure 2c) was far slower, peroxide value above 5 1/2 O mmol.kg^{-1} was detected on the 25th day of storage, exceeding of threshold value was observed on the 45th day of storage, allowing to establish guaranteed storage life of butter pastes for not more than 30 days at a temperature of (-24 ±2 °C).

The anisidine value is used to control processes of secondary oxidation by content of malondialdehyde. Substances with aldehyde functional groups are formed as a result of oxygen effect on peroxides, consequently they appear only at deep oxidation stages. When storing butter pastes at a temperature of (4 ±2 °C) secondary oxidation products were not detected. Under such storage conditions intense primary milk fat splitting takes place, resulting in product spoilage earlier that secondary oxidation products accumulate in it.

Obtained results show that AV at subzero temperatures does not exceed 1 c.u. value during the whole storage duration (Table 1). Aldehydes accumulation begins during the second half of storage in all studied temperature conditions.

Rising dynamics of full oxidation of butter pastes, stabilized by protein polysaccharide complexes based on milk and vegetable protein, was faster as compared to control. It is explained by the presence of fat-soluble emulsifiers in the formulation of butter paste as a reference product **(Lucas et al., 2010)** (Figure 3) that have a protective effect on milk fat glycerides. Fat splitting process in butter pastes with protein polysaccharide complexes takes place with almost the same intensity (due to the influence of milk protein concentrate, **(Abdullah et al., 2018)**).

Fat breakdown with formation of fatty acids can assist in acceleration of oxidation process because free acids oxidize first. Total amount of acidic substances in the product is indirect indicator of hydrolytic fat decomposition during the product storage and is characterized by acid value **(Musiy and Tsisaryk, 2014)**. Besides, acidity of butter pastes is stipulated by the

presence of organic acids, formed during the breakdown process of plasma lactose, by acidic products of proteins breakdown etc. **(Hladyshev, 2012; Malfia et al., 2008)**. Results depicted on Figure 4 show that acid value of developed butter pastes is credibly lower than acidity of reference sample. Accumulation of organic acids takes place slower in butter pastes with protein polysaccharide complex under all studied temperature conditions regardless of freezing depth **(Ruttarattanamongkol, Afizah and Rizvi, 2015)**.

Thus, acid value did not exceed recommended limits (2.5 °K) and was on average – 2.3 °K when storing butter paste during 10 days at a temperature of (4 ±2 °C); 2.1 °K during 20 days at a temperature of (-3 ±1 °C), 2.4 °K during 40 days at a temperature of (-24 ±2 °C). Slightly lower acid values of milk fat of reference sample can be similarly explained by protective effect on lipid of formulation components – fat-soluble emulsifier **(Bodnarchuk, 2016)**.

CONCLUSION

It was established that storage of butter pastes at above-zero temperatures causes intensive milk fat splitting forming primary splitting products – peroxides that results in product spoilage. Research results of milk fat oxidation processes of new butter paste types stabilized by protein polysaccharide complexes and reference sample during storage at below zero temperatures showed common trends. At first accumulation of primary and secondary oxidation products takes place slower, upon which jumping increase at the expense of organic acids accumulation was observed that causes intensification of oxidation processes. Based on the obtained evaluation results of fat phase stability of studied butter pastes, the following storage life is recommended: at a temperature of (4 ±2 °C) during maximum 7 days, at a temperature of (-3 ±1 °C) during maximum 15 days, at a temperature of (-24 ±2 °C) during maximum 30 days. Research results and found regularities will be used for complex evaluation of quality and stability of new butter paste types, stabilized by protein polysaccharide complexes for the purpose of justification of their guaranteed storage life.

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