

New high-fat dairy products with colour attractants

¹Pylypenko, L., ¹Sevastyanova, O., ^{1*}Makovska, T. and ²Oliinyk, L.

¹Odessa National Academy of Food Technologies, Kanatnaya Str., 112, Odessa, 65039, Ukraine

²Poltava University of Economics and Trade, Koval Str., 3, Poltava, 36000, Ukraine

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Abstract

In the present work, the nutritional value of high-fat dairy product - melted butter, which was produced in Ukraine, was characterised. It was shown that, along with the traditional fatty acid compositions, this product had an insufficient concentration of biologically active substances that contributes to storage stability, expands organoleptic characteristics, and prevents main spoilage processes such as rancidity and tallowiness. The appropriate biologically active colouring attractants for improvement of organoleptic and biological properties of melted butter were observed. The formulations of new melted butter-based products were tested by using two individual carotenoids (carotene and xanthophyll); namely chlorophyll and chlorophyll-carotenoid complex additives in a concentration of 3 - 7%. The antioxidant activity of the samples, which was determined volumetrically by the intensity of initiated oxidation of isopropyl benzene, made it possible to establish an increase (4.40 - 6.52 times the period of induction) of oxidation for melted butter variants, with colorants. The study of organoleptic, physico-chemical, and microbiological indicators confirmed the biological stability and safety of the developed high-fat dairy products, thus fulfilling the body requirement for carotenoids, magnesium, and several other biologically active compounds. According to the results, the guaranteed shelf life of the developed melted butter types with colouring attractants of carotenoid and chlorophyll, which met the safety requirements, could be prolonged by 31 - 48%. In conclusion, the present work improved the technical and economic indicators, and confirmed the feasibility of their production.

Keywords

melted butter,
chlorophyll-carotenoid
attractants,
nutritional value,
stability

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Introduction

Nutrition is one of the most important factors which determines the health status of a population. Fats are an important component of daily human ration, and involved in various food technology areas (O'Brien, 2003). Modern biotechnological methods aim to enrich fats with biologically active valuable substances and to increase their shelf life by preventing unwanted changes in their content (Golubeva *et al.*, 2014).

The colour of food products, including fats, is an important attractant which determines the sensory characteristics. In EU countries, among the well-known synthetic food colouring additives, 23 are banned due to being unsafe. An increase in the consumption of synthetic food additives may lead to food poisoning, contribute to an increase of incidence in the population, and changes in the gene pool (Pylypenko *et al.*, 2009; Mysakov, 2016). The creation and widespread use of this new assortment of healthy food products, based on traditional raw food materials which are rich in biologically valuable substances and indispensable nutrition components, are currently

being explored by the food industries (ROSPATENT, 2010; Shilman *et al.*, 2016). Some of the industrialised countries has shown prospects to produce dairy products which do not contain ballast substances. In particular, the melted butter has an advantage over butter and cream, as it has higher storage stability, occupies less storage space, and has lower cost of transportation (Golubeva *et al.*, 2013; 2014).

Melted butter is widely used in confectionery and other industries, household, as well as cosmetology. Analysis of literature data on the biochemical composition of melted butter shows that the practical absence of mineral elements and insufficient content of several vitamin-active compounds (O'Brien, 2003; Paronyan, 2006; ROSPATENT, 2010). This indicates the feasibility of its enrichment with biologically active stabilisers, as well as colour and composition improvers (ALTERNATIVE, 2004; Pylypenko *et al.*, 2007). In this regard, improving the assortment of fats with positive properties such as increased sensory and biological values, improved antioxidant and organoleptic properties, with prolonged shelf life is an actual scientific and practical task (Shilman *et al.*,

*Corresponding author.
Email: makov-t@ukr.net

2016). The traditional type of butter produced in Ukraine contains up to 17.8 - 27.8% non-fat phase, which causes its biological and chemical instability during storage. Therefore, a special place is occupied by the types of melted butter produced, according to technological schemes (Sheifel, 2003; Vyshemirskij, 2010).

A method to produce food colouring from plant materials was developed as a high priority work (UKRPATENT, 2000). According to Pylypenko *et al.* (2009), there is an information on the advisability of using colouring in a number of long-term storage products with a significant concentration of the water phase, but they were not used for fat product - melted butter. Therefore, the present work was aimed to develop new type of safe and biologically stable products with long-term storage life, increased nutritional value, and improved functional properties; high-fat dairy products with colour attractants. The purpose was to expand the assortment and substantiate the formulations of melted butter, enriched with safe biologically active plant components of various chemical nature, from raw materials of the Ukrainian region. Specifically, the present work (a) characterised the nutritional value of a high-fat dairy product - melted butter, which was produced in Ukraine; (b) monitored appropriate biologically active colouring attractants to improve the organoleptic and biological properties of the melted butter; and (c) explored the set of properties and recommended formulations of new products based on melted butter.

Materials and methods

Regional raw cow butter with fat content of 82.5% was used as a feedstock, produced at the Ternopil Dairy Plant under TM "Molokiya". Samples of the melted butter were obtained from sweet cream butter by heating the fat phase (DSTU, 2005; ROSPATENT, 2010; Karpenya *et al.*, 2018;).

For biologically active colouring additives, three different types of natural food colours were used, namely carotene, xanthophyll, and chlorophyll; and chlorophyll-carotenoid complex colourings from wastes of canning and essential oil production was received by the developed technology (Oleynik, 2000; UKRPATENT, 2000). The advantage of developed technology for the production of carotene, xanthophyll, and chlorophyll colourings was the identity of a number of initial and final stages of production which makes it possible to produce them using just one equipment, thereby, increasing the profitability of production.

The main indicators of the chemical

composition of the samples were as follows: humidity, mass fraction of fats, proteins, carbohydrates, concentration of minerals, vitamins, acid number, peroxide number, and acidity; and these were determined by classical biochemical methods (Ermakov *et al.*, 1987; Arutyunyan *et al.*, 2004; Krainyuk, 2009).

The preparation of samples for determining fatty acid composition is based on the methylation of hexane extracts of triglyceride samples. Fatty acid compositions were determined by gas chromatography (Clarus 500, Perkin Elmer), flame ionisation detector, quartz capillary column (Supelco SP-2380; 30 m length, 0.32 mm diameter); and an active layer thickness of 0.20 μm . The carrier gas was helium, temperature of the injector was 250°C, temperature of the detector was 250°C, temperature of the thermostat was from 50 to 250°C for 25 min, and the volume of the injected sample was $1 \times 10^{-3} \text{ cm}^3$. Chromatograms were recorded and processed using the Total Chrom by Perkin Elmer (ISO, 2000; 2010).

The amount of liposoluble pigments was also studied in the composition of general lipids (Ermakov *et al.*, 1987).

An aliquot of the extract intended for the study of carotenoids was fractionated into carotenes and xanthophylls on sucrose columns under dim light, with an antioxidant added to the extracts. After evaporation on a rotary evaporator (IR-15, Russia), a thin-layer chromatography (TLC) and L5/40 silica gel plates with gypsum (Czech Republic) was performed. For the separation of carotenoids (oxygen-free derivatives), a solvent petroleum ether-acetone (98:2) or hexane-acetone (96:4) was used.

Individual xanthophylls were obtained by TLC on cellulose (Nagel & Co, Germany) using the petroleum ether:acetone:propanol system (90:10:0.25). Spots of the separated pigments were removed from the plates, eluted, and identified based on chromatographic mobility and spectral characteristics; and the amount of each pigment was determined spectrophotometrically based on individual extinction coefficients (Kudritskaya, 1990).

Carotenoids were also identified by comparing the chromatographic mobility of the test samples with carotenoid preparations (Fluka, Switzerland), and the maximum absorption of pigments were calculated in the region of 200 - 700 nm.

An aliquot of the extract after preliminary purification from the accompanying impurities were obtained by column chromatography on silica gel L100/160 (Scientific Production Company SIBEKOSERVICE, Russia), and was used for the separation and quantification of chlorophylls. The

separation of chlorophylls and their structural analogues, as well as the quantitative determination of their individual species and forms, were carried out according to Pylypenko (1994) and Oleynik (2000).

Antioxidant activity was determined by volumetric method, where the total concentration of antioxidants was evaluated by the period of isopropylbenzene oxidation induction (Pylypenko and Pylypenko, 2014). The testing was carried out at the initiation rate $W_i = 6.8 \times 10^{-5} \text{ mol/dm}^3$, and the initiator used was azoisobutyronitrile. The concentration of antioxidants was determined using Eq. 1:

$$[\text{In H}] = t \cdot W_i \quad (\text{Eq. 1})$$

where, $[\text{In H}]$ = the concentration of the inhibitor obtained experimentally, and t = the duration of the induction period, s.

Microbiological parameters were determined by classical as well as accelerated PCR methods using microbiological media Compact Dry (Nissui Pharmaceutical Co. Ltd., Japan) (Kapreliants *et al.*, 2016; Pylypenko *et al.*, 2017; Berhilevych *et al.*, 2019; Danylova *et al.*, 2019).

Statistical processing of the results obtained during the experiment was carried out according to Shilov (2015).

Results and discussion

Cow butter is a valuable high-fat food product, which contains saturated and unsaturated fatty acids with high biological and physiological activity. As noted, the common in the schemes for producing melted butter is the separation of the fat fraction during heat treatment. The fatty acid composition of the butter produced in Ukraine and its general chemical composition can be seen in Tables 1 and 2.

Based on the results obtained, it should be noted that melted butter contains essential fatty acids that are necessary for the synthesis of biological cell membranes. It was found that the recommended daily intake of melted butter does not provide the body with some biologically valuable nutrients, as well as substances that stabilise the quality of production during storage. Previous studies (Pylypenko, 1994; Oleynik, 2000; Pylypenko *et al.*, 2009) showed that biologically active substances of secondary raw materials was not only a valuable and safe food colourings which possess antimutagenic properties, but also gives the product colour attractiveness, antimicrobial, antioxidant, and other positive characteristics. In this regard, melted butter samples were prepared with a concentration of three individual and carotenoid-chlorophyll complex additives, oriented to the body's needs for these biologically

Table 1. The chemical composition of melted butter ($n = 3$; $p \leq 0.05$).

Indicator	Value	Indicator	Value
Fat (%)	99.0	Polyunsaturated fatty acid (%)	2.38
Saturated fatty acid (%)	57.03	C18:2 Linoleic	1.73
C4:0 Butyric	0.94	C18:3 Linolenic	0.58
C6:0 Caproic	1.76	C20:4 Arachidonic	0.07
C8:0 Caprylic	1.39	Vitamin (10^{-3} %)	
C10:0 Capric	2.98	Vitamin A ₁ (retinol)	0.57
C12:0 Lauric	1.75	β-carotene	0.2
C12:0 Isolauroic	0.94	Vitamin E (α-tocopherol)	1.5
C14:0 Myristic	8.96	Mineral (10^{-3} %)	24.7
C15:0 Pentadecanoic	-	Potassium	5.0
C16:0 Palmitic	26.37	Calcium	6.0
C16:0 iso-14-methylpentadecane	1.30	Sodium	4.0
C18:0 Stearic	9.41	Phosphorus	9.5
C20:0 Arachidic	1.23	Iron	0.2
Monosaturated fatty acids (%)	28.24	Magnesium	0
C14:1 Myristoleic	1.95		
C16:1 Palmitoleic	2.38		
C18:1 Oleic	23.06		
C20:1 Paullinic	0.85		

Table 2. Characterisation of cow butter samples with carotene colour attractants ($n = 3$; $p \leq 0.05$).

Indicator	Butter	Melted butter with the addition of carotene attractant in quantity (%)			
		Melted butter without additive	3.0	5.0	7.0
Organoleptic indicator					
Taste and smell	Brisk smack of butter and pasteurisation, without extraneous smacks and odours. From light yellow, homogeneous throughout the mass.	Brisk smack of butter and pasteurisation, without extraneous smacks and odours. Pale yellow, homogeneous throughout the mass.	Brisk smack of butter and pasteurisation, without extraneous smacks and odours. Yellow-orange, homogeneous throughout the mass.	Brisk smack of butter and pasteurisation, without extraneous smacks and odours. Dark orange, homogeneous throughout the mass.	Brisk smack of butter and pasteurisation, without extraneous smacks and odours. Solid, homogeneous at $12 \pm 2^\circ\text{C}$; for the product in molten form: transparent, without sediment.
Colour	Homogeneous, elastic, dense surface, shiny, dry sectional view.	Solid, homogeneous at $12 \pm 2^\circ\text{C}$; for the product in molten form: transparent, without sediment.	Solid, homogeneous at $12 \pm 2^\circ\text{C}$; for the product in molten form: transparent, without sediment.	Solid, homogeneous at $12 \pm 2^\circ\text{C}$; for the product in molten form: transparent, without sediment.	Solid, homogeneous at $12 \pm 2^\circ\text{C}$; for the product in molten form: transparent, without sediment.
Consistency					
Physical-chemical characteristic					
Humidity (%)	11.5	0.89	0.87	0.86	0.88
Lipid (%)	81.9	99.0	99.1	99.1	99.0
Protein (%)	2.4	-	-	-	-
Carbohydrate (%)	4.1	-	-	-	-
Carotenes (10^{-3} %)	0.2	0.2	15.1	25.0	34.8
β -carotene	0.15	0.13	12.8	21.5	30.0
Including ($\cdot 10^{-3}$%)					

α -carotene	0.05	-	1.1	1.8	2.5
γ -carotene	-	-	0.5	0.7	0.9
δ -carotene	-	-	0.2	0.3	0.5
Lycopene	-	-	0.3	0.5	0.6
Xanthophyll	-	-	0.1	0.2	0.3
Chlorophyll	-	-	0.1	0.2	0.3
Stability and security indicator					
Oxidation induction period (s)	65	72	320	445	478
Concentration of antioxidants (InH) (mol/kg)	-	0.49×10^{-5}	2.18×10^{-5}	3.01×10^{-5}	3.26×10^{-5}
Peroxide number (mg O ₂ /g)	0.117	0.114	0.110	0.100	0.094
Acid number (mg KOH/g)	1.15	1.10	0.98	0.94	0.93
QMAFAnM (CFU/g)	2.4×10^3	9.6×10^2	9.4×10^2	8.7×10^2	8.9×10^2
Moulds (CFU/g)	5	25	22	20	20
Coliform bacteria, including shigatoxigenic <i>Escherichia coli</i> (STEC) (CFU/g)	N/D	N/D	N/D	N/D	N/D
<i>Salmonella</i>	N/D	N/D.	N/D	N/D	N/D
<i>Bacillus cereus</i> (CFU/g)	7	N/D	N/D	N/D	N/D
<i>Clostridium perfringens</i> (CFU/g)	-	N/D	N/D	N/D	N/D
<i>Staphylococcus aureus</i> (CFU/g)	N/D	N/D	N/D	N/D	N/D
<i>Listeria monocytogenes</i> (in 25 g)	N/D	N/D	N/D	N/D	N/D

active substances. Table 2 shows a set of indicators characterising the samples of different types of cow butter with carotene colour attractants.

As shown in Table 2, supplementation of carotene additives not only improved the organoleptic characteristics of the melted butter samples, but also enriched them with provitamins A, thus increasing their stability and safety. A wide range of established microbiological indicators also confirmed the safety and potential biological stability of the samples during storage. It is advisable to introduce 5% carotene attractant into the butter, according to the results given in Table 2. This approach was later used in the development of formulations of melted butter with other colour additives.

The composition of xanthophyllic additives is represented by the following colour ingredients: lutein, violaxanthin, neoxanthin, zeaxanthin, β -cryptoxanthin, and lutein-5,6-epoxide, as well as a small amount of chlorophylls and mineral elements mainly magnesium and calcium. The xanthophyll colouring additive turned the colour of melted butter samples into yellow. Meanwhile the intensity of the colour depended on the amount of the additive.

Among the individual pigments in the xanthophyll colouring additive, lutein dominated up to 57.2%, while chlorophyll α and β dominated in the chlorophyll dye (58.0 - 73.5%).

The compositions of chlorophyll colouring includes 12 individual pigments namely chlorophylls, pheophytins, chlorophyllides, ethylchlorophyllides, pheoforbides, and ethylpheoforbids in α and β forms. Chlorophyll colouring coloured the melted butter in a pleasant intense green colour. The depth and

brightness of the colour depended on the amount of the additive.

The supplementation of chlorophyll-carotenoid complex additive without dividing the pigments into groups also gave the melted butter samples a pleasant green colour, but less intense in comparison to the chlorophyll dye.

Experimental studies (Table 2) showed that should one want to achieve the improved organoleptic characteristics within the recommended level of biologically active compounds and manifestation of substantial stability and safety, it is sufficient to supplement 5% of carotene colour attractants. The same results were also observed with the supplementation of the following three colour additives (Table 3).

The results given in Table 3 shows the feasibility of bio-correction of melted butter with the developed colour attractants. The supplementation of chlorophyll and complex additives to melted butter allowed one to enrich the composition of melted butter with magnesium, which was originally absent in melted butter, as shown in Table 1.

The developed new formulations not only expanded the assortment line of melted butters, but also led to the stabilisation of their function, in particular, antioxidant properties and potential of prolongation of shelf life.

The result of storing samples of melted butter at 4 - 5°C in a hermetically sealed container confirmed the possibility of extending the shelf life of the samples by 34 - 48%, and enriching them with biologically active colour attractants without worsening the quality and safety indicators in comparison with the control sample. This is confirmed by the results of studying

Table 3. The influence of colour attractants for chlorophyll, xanthophyll, and complex nature additive on the quality indicators of melted butter samples ($n = 3$; $p \leq 0.05$).

Indicator	Melted butter		
	Chlorophyll	Xanthophyll	Chlorophyll-carotenoid complex
Oxidation induction period (s)	465	420	470
Concentration of antioxidants (In H) (mol/kg)	3.17×10^{-5}	2.86×10^{-5}	3.19×10^{-5}
Peroxide number (mg O ₂ /g)	0.100	0.115	0.097
Acid number (mg KOH/g)	1.12	1.15	1.00
QMAFAnM (CFU/g)	9.1×10^2	9.4×10^2	8.8×10^2
Moulds (CFU/g)	11	18	14
Coliform bacteria, including STEC (CFU/g)	N/D	N/D	N/D
<i>Salmonella</i>	N/D	N/D	N/D
<i>Bacillus cereus</i> (CFU/g)	N/D	N/D	N/D
<i>Clostridium perfringens</i> (CFU/g)	N/D	N/D	N/D
<i>Staphylococcus aureus</i> (CFU/g)	N/D	N/D	N/D
<i>Listeria monocytogenes</i> (in 25 g)	N/D	N/D	N/D

indicators of acid, peroxide numbers, and acidity (2.0 - 2.8 °K), which practically did not change throughout nine months, and did not exceed the permissible maximum.

Conclusion

A comprehensive analysis of the nutritional value of high-fat dairy product produced in Ukraine - melted butter, was characterised, and the need for bio-correction of its composition was shown. Experiments was carried out, and technologically feasible and biologically active colouring attractants were selected, taking into account the needs of the body; allowing for improvement of the organoleptic properties of melted butter by creating a colour line for the new products based on it. The complex properties of new products based on melted butter was also studied. It was found that 3 - 7% concentrations of additives increased the induction period of oxidation by 4.4 - 6.52 times, thus fulfilling the body requirement for carotenoids, and a number of mineral elements are recommended. The study of microbial contaminants and antioxidant properties of the samples showed the possibility of prolonging shelf life of melted butter with colour attractants by 31 - 48%, as well as obtaining a stable, safe, and biologically active high-fat dairy products. Thus, the assortment has been expanded and the formulations of melted butter types with colour attractants of various chemical nature from raw materials from the Ukrainian region have been substantiated.

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