

# The Influence of Storage Conditions on the Color Profile of Sweetened Condensed Whole Milk

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## ABSTRACT

**Introduction:** The expansion of logistical routes for exported canned dairy products to the Arctic zone and regions with hot climates underscores the need for research aimed at reducing transportation costs and preserving the quality of dairy preserves under extreme temperature conditions. In this regard, product color is an important organoleptic indicator that shapes initial perceptions of quality parameters and is considered one of the markers of spoilage mechanisms in sweetened condensed whole milk (SCWM). Currently, standardized methods for assessing organoleptic indicators, particularly color, are qualitative and subjective, which do not reliably measure the degree of color variation in identical food products. Therefore, digitizing the color indicator of SCWM and correlating it with changes in the food matrix under extreme temperature exposure is a relevant and timely research direction.

**Purpose:** To study the impact of simulated transport conditions within an extreme temperature range from 50°C to -50°C, and subsequent storage at 5°C, as well as the effectiveness of homogenization on the color of SCWM and associated physicochemical indicators to expand acceptable storage and transport conditions for the product.

**Materials and Methods:** The study object was SCWM from batches with varying homogenization efficiency, stored under different temperature conditions. Changes in sample color were recorded through photo documentation. The content of free amino acids was determined by capillary electrophoresis. Color difference, whiteness index, and color saturation were calculated. Active and titratable acidity were measured using potentiometric and titrimetric methods, respectively. The protein profile was determined by electrophoresis in polyacrylamide gel.

**Results:** It was found that a single-stage heating to 50°C and storage at this temperature for 7 and 14 days caused the formation of high-protein aggregates, changes in free amino acid content, pH, and product darkening. Multistage heating and freezing cycles to 50°C and -50°C, as well as single-stage freezing to -50°C, did not critically affect the color of SCWM. The effectiveness of homogenization was found to influence SCWM's susceptibility to darkening. Acidity analysis results showed that a high rate of pH change in the product correlated with the formation of a darker color during prolonged storage.

**Conclusion:** The obtained data contributed to the scientific basis for developing new standards documentation for SCWM intended for transport to the Far North and hot climate regions, as it was shown that multistage temperature changes do not alter product quality.

**Keywords:** sweetened condensed whole milk; color profile; Maillard reaction; darkening; CIELAB color space

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# Влияние условий хранения на цветовой профиль цельного сгущенного молока с сахаром

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## АННОТАЦИЯ

**Введение:** Расширение логистических путей экспортируемой молочно-консервной продукции в Арктическую зону и районы с жарким климатом актуализирует исследования, направленные на снижение транспортных затрат и сохранение качества молочных консервов в условиях воздействия экстремальных температурных факторов. В данном аспекте цвет продукта является важным органолептическим показателем, формирующим первичное восприятие параметров качества и рассматривается в качестве одного из маркеров активации механизмов порчи цельного сгущенного молока с сахаром (ЦСМС). На сегодняшний день стандартизованные методики оценки органолептических показателей и, в частности, цвета являются качественными и субъективными, что не позволяет достоверно оценить степень цветового различия идентичных по составу пищевых продуктов. В связи с этим оцифровка показателя цвета ЦСМС и сопоставление с изменениями, происходящими в пищевой матрице при воздействии экстремальных температур, является актуальным и своевременным направлением исследований.

**Цель:** Изучить влияние смоделированных условий транспортирования в диапазоне экстремальных температур от 50°C до минус 50°C и последующего хранения при 5°C, а также эффективности гомогенизации на изменение цвета ЦСМС и ассоциированных с этим процессом физико-химических показателей для расширения допустимых режимов хранения и транспортирования продукции.

**Материалы и методы:** Объект исследований – ЦСМС от партий с различной эффективностью гомогенизации, подвергнутое хранению при различных температурных условиях. Изменение цвета образцов регистрировали фотофиксацией. Содержание свободных аминокислот определяли методом капиллярного электрофореза. Определение цветового различия, индекса белизны и насыщенности определяли расчётным способом. Определение активной и титруемой кислотности проводили потенциометрическим и титриметрическим методом соответственно. Белковый профиль определяли с помощью электрофореза в полиакриламидном геле.

**Результаты:** Установлено, что одноступенчатое нагревание до 50°C и хранение при этой температуре в течение 7 и 14 суток вызывает образование высокобелковых агрегатов, изменение содержания свободных аминокислот, pH и потемнение продукта. Выявлено, что многоступенчатые циклы нагревания и замораживания до 50°C и минус 50°C соответственно, как и одноступенчатое замораживание до минус 50°C не оказывают критического влияния на цвет ЦСМС. Обнаружено влияние эффективности гомогенизации на потенциал ЦСМС к потемнению. Результаты анализа кислотности показали, что высокая скорость изменения pH в продукте коррелировала с формированием более темного цвета в продукте в процессе длительного хранения.

**Выводы:** Полученные данные стали частью научного обоснования разработки новой документации в области стандартизации на ЦСМС, предназначенное для транспортирования в районы Крайнего Севера и регионы с жарким климатом, так как позволили доказать, что многоступенчатый режим изменения температур не вызывает изменения качества продукта.

**Ключевые слова:** цельное сгущенное молоко с сахаром; цветовой профиль; реакция Майяра; потемнение; цветовое пространство CIELAB

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## INTRODUCTION

Condensed whole milk (CWM) is one of the most common types of canned milk due to its ease of transportation, long shelf life, and high nutritional and energy values (Efimova et al., 2022; Ryabova et al., 2022). It is not only popular among individual consumers but is also used by different sectors of the food industry (Petrov et al., 2017; Ryabova et al., 2022). Condensed milk is included in the Russian food reserve and supplied to the Russian military units (Usov et al., 2016).

CWM needs to be transported in specialized isothermal equipment to maintain the storage conditions established by State Standard 31688–2012, namely the maximum temperature of 25°C and the minimum temperature of –30°C (Ryabova et al., 2023). However, the cost of cold chain logistics is growing in the current geopolitical situation with changes in logistics routes (Bartsaev, 2023; Gavrilov et al., 2024), affecting the distribution of canned dairy products. Therefore, we need to explore changes in the quality of CWM in a wide temperature range in order to justify new storage and/or transportation conditions for this product. This will improve the economic efficiency of dairy canning companies, increase the exports of Russian produce, and provide the regions where dairy farming is limited with affordable dairy products. This area of research is also consistent with the Russian Doctrine of Food Security approved by the Presidential Decree No. 20 of January 21, 2020, which prioritizes affordable and safe high-quality food products.

There are very few studies on the impact of higher or lower storage temperatures on the physicochemical, microbiological, and sensory properties of CWM. For example, Guryeva et al. (2019) reported that storing condensed milk at 45°C for three months affected the product's taste and aroma, as well as titratable acidity and viscosity. In addition, the authors found a 28 % increase in oleic acid and a decrease in the saturation index of fatty acids from 1.96 to 1.79–1.82, which might indicate oxidation and rancidity processes. Ryabova et al. (2023; 2022) analyzed the impact of negative temperature fluctuations (up to –95°C) on the quality of condensed milk and its model analog systems with different concentrations of sugar and milk. They also studied phase transitions by differential scanning calorimetry. As a result, the authors selected three temperature programs that differed in heating/cooling cycles. They found that a 20°C

product exposed to a temperature cycle of (–95°C)→(–35°C)→(–75°C)→(+30°C) had a cryoscopic temperature of  $-32.2 \pm 0.2^\circ\text{C}$ , a glass transition temperature of  $-47.3^\circ\text{C}$ , and a melting enthalpy of 20.5 J/g, with 6.1 % of frozen moisture. Although the quality of condensed milk involves a whole range of properties and indicators (Turovskaya et al., 2018), its spoilage is primarily indicated by changes in its taste, aroma, consistency, and color.

The color of condensed milk can change at elevated temperatures as a result of protein-carbohydrate interaction and the formation of flavor-aromatic colored compounds, which causes darkening (Maillard reaction) (Van den Oever et al., 2021; Xiang et al., 2021). In addition to storage and transportation conditions, process factors can also affect the rate of color change in the product. For example, a change in the active acidity stimulates the Maillard reaction and accelerates the process of melanoidin formation. Homogenization also affects the rate and degree of color change in the milk matrix (Shao et al., 2023; Tribst et al., 2020). Shao et al. (2023) reported an inversely proportional relationship between the efficiency of homogenization and changes in the color characteristics of pasteurized milk, which was due to the effect that the emulsion particle size had on the product's ability to reflect light. However, there is a lack of generally accepted quantitative methods for sensory evaluation of food products. Qualitative methods, which are most commonly used for this purpose, cannot reliably assess the degree of color difference in identical products. Alternatively, a system based on a Lab color space is a quantitative tool that can digitize the color indicators of a product, thereby increasing the accuracy of research (Al-Hilphy et al., 2022). Since such techniques have not been applied to condensed milk yet, we sought to analyze the effects of process factors (homogenization mode) in addition to post-process factors (an extended range of storage temperatures) on the quality of the product.

In this study, we aimed to determine changes in the color (primary indicator of spoilage) of condensed whole milk under simulated transportation (from 50°C to –50°C) and subsequent storage (5°C) conditions. We also studied the effect of homogenization efficiency on the product's color, as well as other indicators of spoilage such as changes in acidity, free amino acids, and the protein profile.

## MATERIALS AND METHODS

### Materials

Samples of condensed whole milk were provided by Promkonservy Dairy Canning Company (Russia). They came from two batches differing in homogenization efficiency (Batch I and Batch II) and were stored at different temperatures according to the experimental plan. Different homogenization efficiency was achieved by using two homogenization modes at the first stage of production (7–10 MPa and 15 MPa), while the same pressure of 3 MPa was applied at the second stage. Table 1 presents the average physicochemical indicators of condensed whole milk produced for this study.

### Study Design

Our study consisted of two consecutive stages.

At the first stage, we assessed changes in the properties of condensed whole milk (CWM) after its exposure to varying extreme storage temperatures (from  $-50^{\circ}\text{C}$  to  $50^{\circ}\text{C}$ ). The samples were tested under five storage conditions: K, A, B, C, and D (Table 2).

The samples were taken for analysis at the control points (main and additional) according to the schedule (Figure 1) after a specified thermal treatment (temperature, duration). The samples were letter- and number-coded. The letters

Table 1

Average Physicochemical Indicators of Freshly Produced Condensed Whole Milk

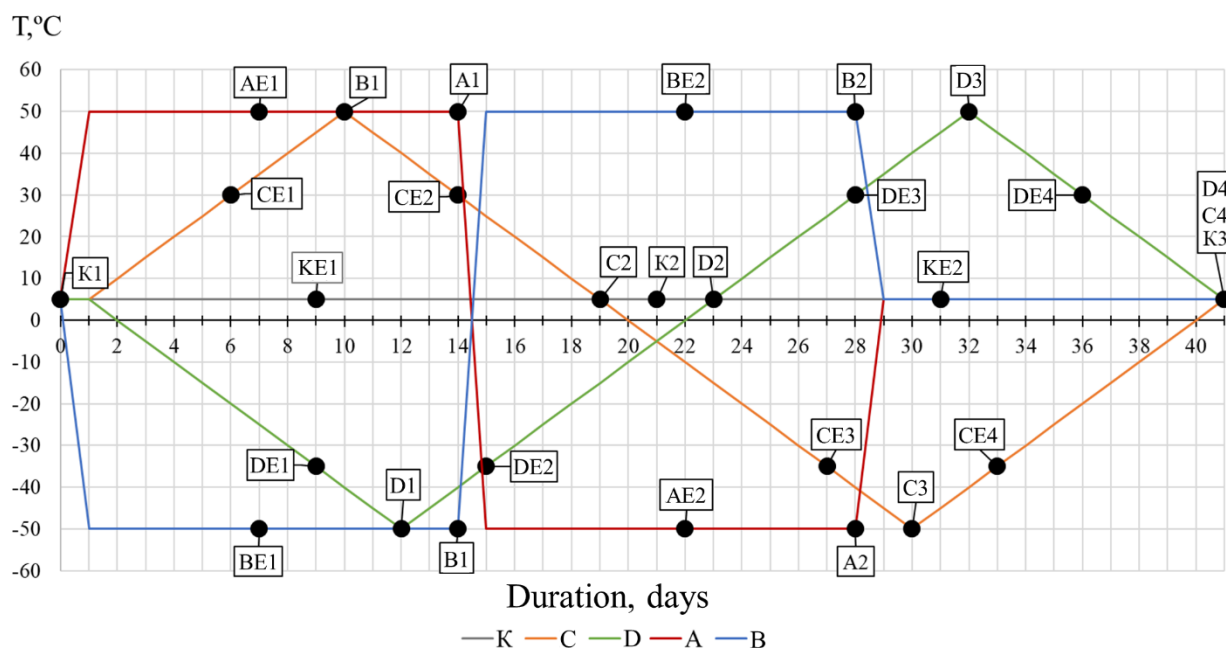
Indicator	Value	
	Batch I	Batch II
Moisture, %	25.8±0.6	26.0±0.4
Sucrose, %	45.3±0.2	45.0±0.1
Fat, %	8.7±0.2	8.8±0.2
Protein, %	7.6±0.1	8.2±0.1
Acidity, °T	35±1	41±2
Viscosity, Pa·s	7.6±0.7	8.4±0.2
Size of milk sugar crystals, µm	3.5±0.3	3.5±0.3
Homogenization efficiency		
Degree of homogenization, %	23	46
Average size of fat globules, µm	3.4±0.3	2.3±0.1

Table 2

Storage Conditions for Condensed Whole Milk

Storage parameters	Storage options				
	K	A	B	C	D
Temperature	const, $t_{\text{air}} = 5^{\circ}\text{C}$	Changes in the range from $-50^{\circ}\text{C}$ to $50^{\circ}\text{C}$			
Mode of temperature change	—	Single-stage mode		Multi-stage mode	
		$t_{\text{air}} = \text{const}$ ; at the initial point, the ambient air temperature is maximum ( $50^{\circ}\text{C}$ ) or minimum ( $-50^{\circ}\text{C}$ ), depending on the type of thermal exposure		$t_{\text{air}} = t_p + 5^{\circ}\text{C}$ ; the ambient air temperature changes by $5^{\circ}\text{C}/\text{day}$ until the maximum ( $50^{\circ}\text{C}$ ) or minimum ( $-50^{\circ}\text{C}$ ) temperature is reached	
Cyclicity	—	Two-cycle structure			
Type of cycles (1 — direct; 2 — reverse)	—	1. Heating to $50^{\circ}\text{C}$ 2. Freezing to $-50^{\circ}$	1. Freezing to $-50^{\circ}\text{C}$ 2. Heating to $50^{\circ}\text{C}$	1. Heating to $50^{\circ}\text{C}$ 2. Freezing to $-50^{\circ}\text{C}$	1. Freezing to $-50^{\circ}\text{C}$ 2. Heating to $50^{\circ}\text{C}$
Note. $t_{\text{air}}$ — ambient air temperature, $t_p$ — product temperature.					

Figure 1

**Storage Timeline for Condensed Whole Milk Samples**

K, A, B, C, and D designated storage conditions, while the numbers indicated the sequence of control points. Additional control points were marked with the letter E. The batches were numbered as I or II.

The second stage involved storing all the samples at  $5 \pm 2^\circ\text{C}$  for 12 months and studying changes in their properties at monthly control points.

## Equipment and Methods

### Photographic Recording of Color Change

The color change was recorded via photography using a Samsung Galaxy Z Flip4 smartphone (Samsung, Suwon, South Korea) with a 12 MP camera, as well as a light-tight imaging station of the View gel documentation system (Helikon, Russia). The samples were placed at an equal distance from the sides of the station. Photographs were taken with a flash to ensure uniformity of measurements in terms of luminous flux.

### Determination of Free Amino Acids

Contents of free amino acids were determined by capillary electrophoresis using the KAPEL system (Lumex, St. Petersburg, Russia) without hydrolysis. Phosphate with the addition of beta-cyclodextrin was used as a background electrolyte. Free amino acids were separated at 25 kV,

$30^\circ\text{C}$ , and 254 nm. The content of free tryptophan was determined directly, while the contents of other amino acids were measured through their phenylthiocarbamyl derivatives.

### Determination of Color Difference, Whiteness, and Saturation

The color difference, whiteness, and saturation values were calculated using the CIE Lab data obtained via photography and analysis with the ColorMeter software (White Marten GmbH, Baden-Württemberg, Germany). The color difference ( $\Delta E$ ) was calculated as:

$$\Delta E = \sqrt{(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2}, \quad (1)$$

where  $L_f$  is the value of  $L$  for the final sample,  $L_i$  is the value of  $L$  for the initial sample,  $a_f$  is the value of  $a$  for the final sample,  $a_i$  is the value of  $a$  for the initial sample,  $b_f$  is the value of  $b$  for the final sample,  $b_i$  is the value of  $b$  for the initial sample.

The whiteness index ( $WI$ ) was calculated as:

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}, \quad (2)$$

where  $L$ ,  $a$ ,  $b$  are the corresponding values according to the Lab system.

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The color saturation (*Ch*) was calculated as:

$$Ch = ((a)^2 + (b)^2)^{0.5} \tag{3}$$

where *a*, *b* are the corresponding values according to the Lab system.

Determination of Acidity and pH

Titrateable acidity was determined by the titrimetric method in accordance with State Standard 30305.3–95, while pH was determined by the potentiometric method in accordance with State Standard 32892–2014.

Electrophoretic Separation of Proteins

The protein composition was determined by disk electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate using the Laemmli method in a Mini-PROTEAN® Tetra Cell vertical chamber (Bio-Rad, California, USA).

Data Processing

One-way analysis of variance (ANOVA) and Tukey’s post-hoc test were performed to analyze experimental data using the

RStudio software package (Posit Software, Massachusetts, USA). All the parameters were analyzed in 3–5 replicates. Tukey’s test was used for multiple comparisons.

RESULTS AND DISCUSSION

We investigated the effect of storage conditions on the color of condensed whole milk (CWM) and related indicators (acidity, free amino acids, and protein profile). CWM was studied in two stages: at extreme temperatures and during prolonged storage. The results are presented accordingly.

Storage of Condensed Whole Milk at Extreme Temperatures

Color Change

First, a color palette was formed for the CWM samples using photography, which changed by the end of storage at extreme temperatures (Figures 2–4).

The degree of color change depended on storage conditions and CWM homogenization efficiency. A single-

Figure 2  
Color Change in Samples A and B

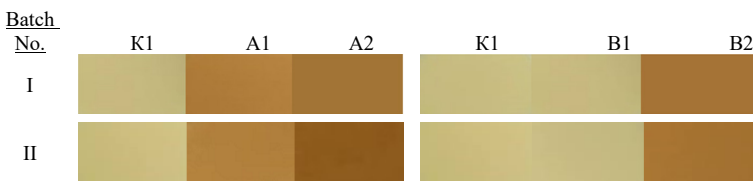


Figure 3  
Color Change in Sample C

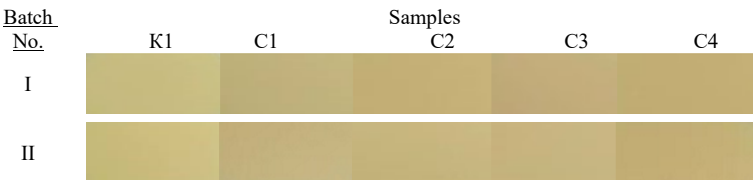
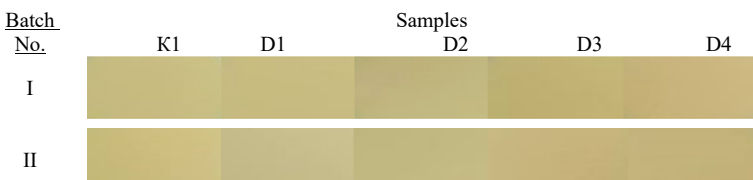


Figure 4  
Color Change in Sample D





stage heating to 50 °C caused the samples A1, A2, C1, and C2 to turn brown or dark brown (Figure 2). A darker shade of brown was found in the sample II-A2 (compared to I-A2) after successive cycles of single-stage heating to 50 °C and freezing to -50 °C, with a storage period of 14 days. This finding is consistent with the data reported by Tribst *et al.* (2020), who investigated the effect of different physical processes (mixing, dispersion, and homogenization at 3.5 MPa and 50 MPa) on the color of sheep milk (fresh, stored at -18 °C for a month, and thawed at 7 °C). The researchers found that higher homogenization pressure increased the color difference in both fresh and frozen/thawed milk samples.

Chen *et al.* (2021) investigated the effect of thawing methods (air thawing at 20 °C, water thawing at 20 °C, microwave thawing, and ultrasound thawing) on the properties of frozen concentrated milk. They found the greatest color difference in the concentrated milk thawed in air at the lowest speed. The authors emphasized that despite the statistical significance of their results, the samples thawed by different methods had a similar color profile. We observed the same effect for CWM: the samples B1 and D2 did not differ significantly in color, although they were thawed at different rates (Figure 2.4). This was probably due to only a slight change in protein particles or

fat globules which determine the color of milk by reflecting light. In addition, Chen *et al.* (2021) reported pH values in the range from 6.56 to 6.59, indicating that the thawing methods did not affect the rate of physicochemical processes. This finding is consistent with our results for the samples D2 and B1, which had the same acidity values (Table 3).

Chemical reactions in milk obey the Arrhenius law, which states that an increase in temperature leads to an increase in reaction rate constants (Halabi *et al.*, 2020). However, the degree of thermally induced changes also depends on the rate of temperature change (Sahu & Kumar Mallikarjunan, 2016; Anema, 2020). This explains why a single-stage heating of the samples A1 and B2 to 50 °C (storage up to 14 days) led to critical changes in acidity (an increase in titratable acidity by  $14 \div 23$  °T and a decrease in pH by  $0.21 \div 0.53$  compared to K), indicating physicochemical processes in the system. Such changes did not occur in the samples C and D, showing that multi-stage heating and freezing, as well as their successive cycles, can preserve the quality of condensed whole milk.

According to Anema (2020), when milk temperature is gradually increased to 70 °C, most of the denatured  $\beta$ -LG and  $\alpha$ -LA bind to  $\kappa$ -casein, presumably through disulfide

Table 3

**Acidity Changes in Condensed Whole Milk After Primary Thermal Exposure Cycles**

Sample	Titratable acidity, °T		Active acidity, pH	
	I	II	I	II
	K			
K	35±1 <sup>b</sup>	41±2 <sup>c</sup>	6.70±0.07 <sup>a</sup>	6.36±0.03 <sup>ab</sup>
K3	34±1 <sup>b</sup>	40±3 <sup>c</sup>	6.63±0.02 <sup>ab</sup>	6.40±0.10 <sup>a</sup>
	A			
A1	56±3 <sup>a</sup>	55±2 <sup>b</sup>	6.17±0.06 <sup>c</sup>	6.15±0.10 <sup>b</sup>
A2	56±2 <sup>a</sup>	69±2 <sup>a</sup>	6.19±0.02 <sup>c</sup>	5.79±0.06 <sup>c</sup>
	B			
B1	36±1 <sup>b</sup>	41±1 <sup>c</sup>	6.59±0.02 <sup>ab</sup>	6.38±0.14 <sup>a</sup>
B2	57±3 <sup>a</sup>	64±2 <sup>a</sup>	6.10±0.09 <sup>c</sup>	5.93±0.09 <sup>c</sup>
	C			
C2	36±1 <sup>b</sup>	42±2 <sup>c</sup>	6.58±0.02 <sup>ab</sup>	6.36±0.01 <sup>ab</sup>
C4	36±1 <sup>b</sup>	41±2 <sup>c</sup>	6.56±0.02 <sup>b</sup>	6.35±0.01 <sup>ab</sup>
	D			
D2	36±3 <sup>b</sup>	44±1 <sup>c</sup>	6.54±0.02 <sup>b</sup>	6.38±0.01 <sup>a</sup>
D4	34±3 <sup>b</sup>	42±1 <sup>c</sup>	6.58±0.02 <sup>ab</sup>	6.36±0.06 <sup>ab</sup>

Note. Significant differences ( $P < 0.05$ ) are indicated by lowercase letters a-c.

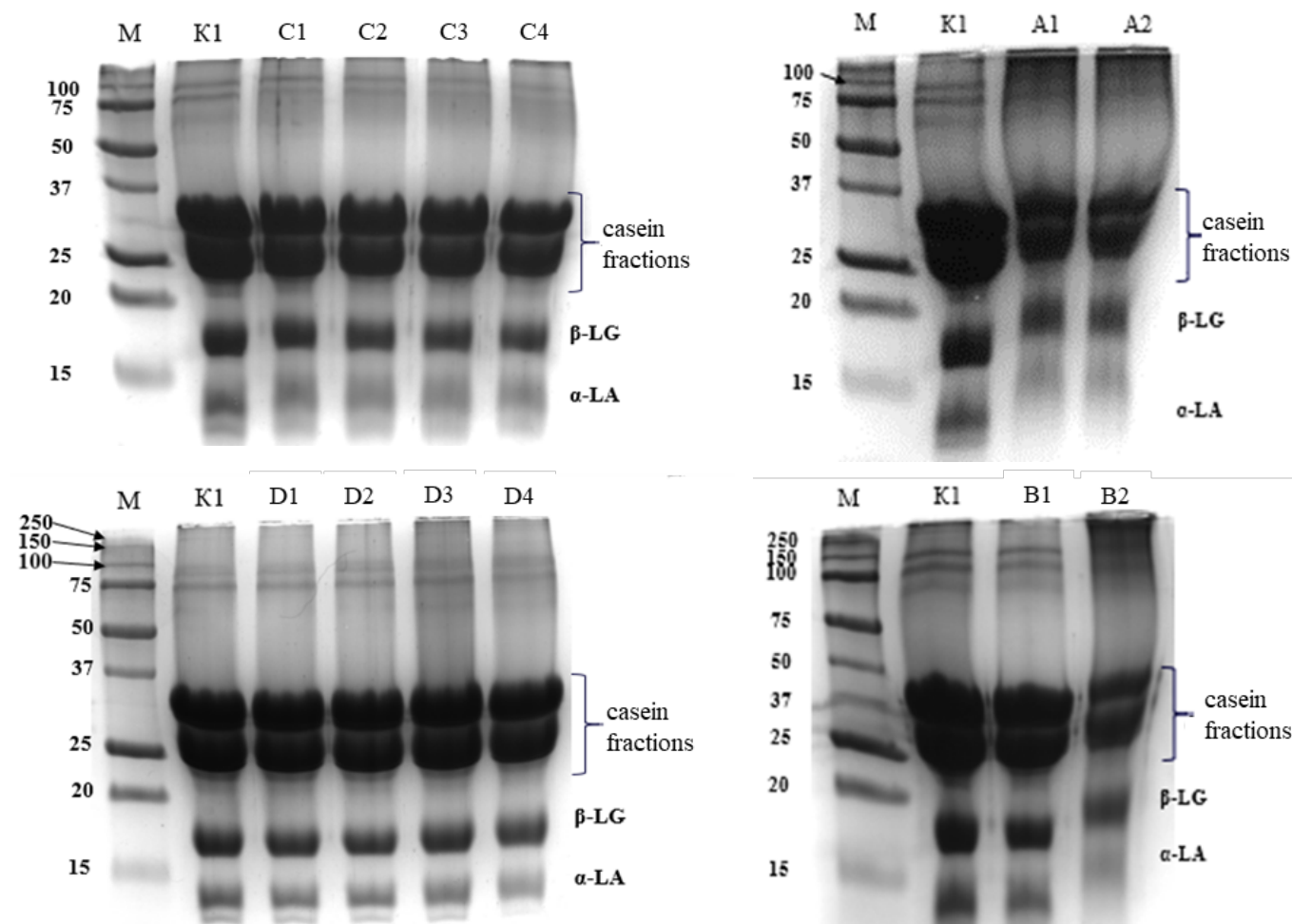
bonds on the surface of micelles. Rapid heating, however, causes only half of denatured whey proteins to form complexes with casein, the other half remaining in the dissolved phase or aggregating with each other. In our study, electrophoretic analysis revealed a decrease in the content of whey proteins ( $\beta$ -LG,  $\alpha$ -LA) and casein after a single-step heating and freezing, as well as the formation of high-molecular aggregates (Figure 5). These results were consistent with those reported by Meyer et al. (2011), who heated milk to 120 °C for 60 minutes. The authors also found carboxymethyllysine, a product of the protein glycation reaction, in the high-molecular fraction. In another study, Jongberg et al. (2012), who investigated  $\beta$ -LG glycation in a dry milk model system, found that holding the sample at 60 °C for 60 minutes can shift the  $\beta$ -LG band towards a higher molecular weight on the electrophoregram. Similar results were reported by Liu et al. (2012) and observed in

our study for the samples A1, A2, and B2 after a single-stage heating and a storage for 14 days (Figure 5).

Liu et al. (2012) noted that the formation of high-molecular glycoproteins depended on the storage conditions (temperature, pH, holding time) of the milk system and its composition, especially carbohydrates. The authors reported that the binding of  $\beta$ -LG with glucose increased the proportion of tetramers and octamers, while  $\beta$ -LG modified with lactose increased the proportion of octamers. This might explain the intense bands that we observed in the high-molecular-weight zone of the samples A1, A2, and B2. Condensed whole milk is rich in sucrose, with glucose being one of its monomers, and contains about 12.5% lactose. High-molecular-weight aggregates in the milk system can be associated with both glycation products (changing the color in the samples A1,

Figure 5

## Electrophoregrams of Condensed Whole Milk from Batch I





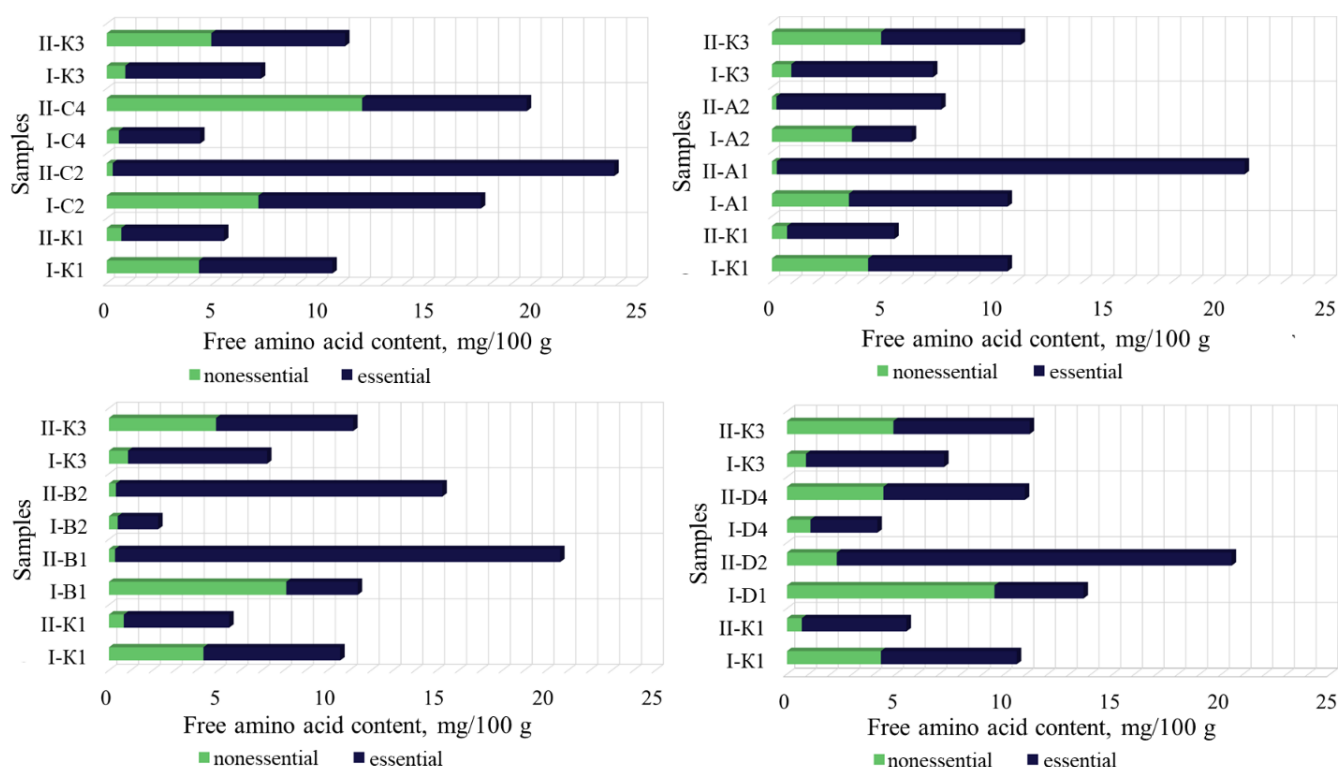
A2, and B2) and protein associates, which are not separated due to charge loss. Sharma et al. (2021), who compared different methods for electrophoretic separation of milk proteins, found that electrophoresis under denaturing conditions with dodecyl sulfate caused casein aggregates to accumulate in the high-molecular-weight zone, being unable to migrate into the gel. Similar observations were reported in other studies (Gazi et al., 2022; Jean et al., 2006; Considine et al., 2007). In addition, Considine et al. (2007) noted that heat treatment can expose previously hidden hydrophobic groups of amino acids, which can lead to protein aggregation or cause free amino acids to form. Fox et al. (2015) reported the exposure of amino acids and their activation due to the denaturation of proteins in the fat globule membranes. These amino acids can then react to form flavoring substances or glycation products. In our study, we found changes in the contents of free amino acids in the condensed whole milk samples exposed to extreme temperatures, namely nonessential amino acids (mainly glutamine and glutamic acid) in the samples of Batch I and essential amino acids (mainly methionine) in the samples of Batch II (Figure 6).

Figure 6 shows a trend towards an increase in free amino acids in the samples A1, B1, C2, and D2 (Batches I and II) and a trend towards a decrease in free amino acids in the samples A2, B2, C4, and D4 (Batches I and II). Bottiroli et al. (2021), who studied changes in free amino acid contents in lactose-free milk exposed to ultra-high heat treatment during storage at different temperatures (4 °C, 20 °C, 30 °C, and 40 °C), found the highest contents of glutamic and aspartic acids, as well as aliphatic amino acids. The greatest increase in amino acids was observed in the milk stored at  $\geq 30^{\circ}\text{C}$ , especially the content of glutamic acid. This correlates with our data for Batch I samples of condensed whole milk. Bottiroli et al. (2021) associated this effect with the specificity of enzymes with proteolytic activity in the milk system.

Meltretter et al. (2008) reported the formation of carboxymethyllysine and methionine sulfoxide, as well as the cyclization of the N-terminal glutamic acid, as the main heat-induced changes in the milk system associated with amino acids. Jansson et al. (2020) noted that thermal denaturation of  $\beta$ -LG led to the release of sulfur-containing amino acids, including methionine. According to Augustin

Figure 6

## Changes in the Free Amino Acid Content in Condensed Whole Milk



et al. (2007), the higher the homogenization pressure, the lower the thermal stability of the milk system after processing. In our study, this might explain the increased content of free amino acids in Batch II samples with higher homogenization efficiency as mainly due to methionine released as a result of  $\beta$ -LG denaturation. García-Risco et al. (2002) also described the dependence of whey protein denaturation on homogenization. The authors found that in whole milk homogenized at 20 MPa, the enzymatic degradation of  $\alpha$ S1-CN and  $\beta$ -CN was 75.5 % lower. Caseins are rich in glutamic acid and glutamine. Their enzymatic degradation might explain the changes in the contents of glutamic acid and glutamine in Batch I samples of condensed milk with lower homogenization efficiency. Another possible explanation for these changes is the Maillard reaction and the formation of primary unstable reaction products, which could increase the concentration of amino acids during decomposition (Adrian, 2019).

#### Prolonged storage of condensed whole milk

Figure 7 shows changes in the color characteristics of condensed whole milk (CWM) samples during long-term storage.

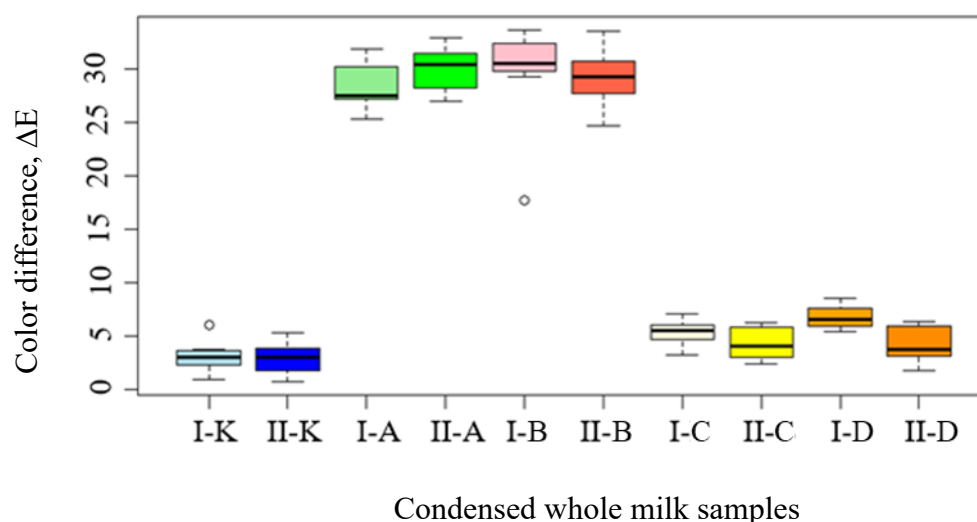
As can be seen in Figure 7, there were no significant differences between the I-K and II-K samples. The I-C and I-D samples had a smaller variation of data and a higher concentration of color difference values above or in the upper quartiles of the data sets for the II-C and II-D samples. This indicates a color stability of I-C and I-D, but, at the same

time, their greater potential for darkening in comparison with II-C and II-D. A similar effect was observed by Shao et al. (2023), who studied the effect of homogenization pressure on changes in the color characteristics of pasteurized milk during storage. They found the smallest change in color difference after storage for 1 and 7 days in the samples homogenized at the highest pressure of 30 MPa. The authors established that the size of the emulsion particles affected the reflectivity of light and, accordingly, the color of milk.

The values of color difference in the A and B samples of Batches I and II ( $24 \div 34$ ) were significantly higher than those for the K, C, and D samples ( $1 \div 9$ ) throughout the entire storage period. This indicated the same trend we observed for the CWM samples stored under extreme temperatures. The heating rate and duration of storage at high temperature, which were the highest for the A and B samples, determined a high degree of change in the components of the milk system. This finding was consistent with some other studies (Oldfield et al., 2005; Manzo et al., 2015). The extreme values of color difference in the A and B samples revealed an insignificant effect of homogenization efficiency or the sequence of heating/freezing cycles throughout the entire 12-month experiment. Both storage options launched melanoidin formation, maintaining optimal conditions for this process for 14 days. Assumingly, at each storage stage, the A and B samples had early, intermediate, and final glycation products at different concentrations and with good chemical structures

Figure 7

#### Changes in Color Difference between Condensed Whole Milk Samples



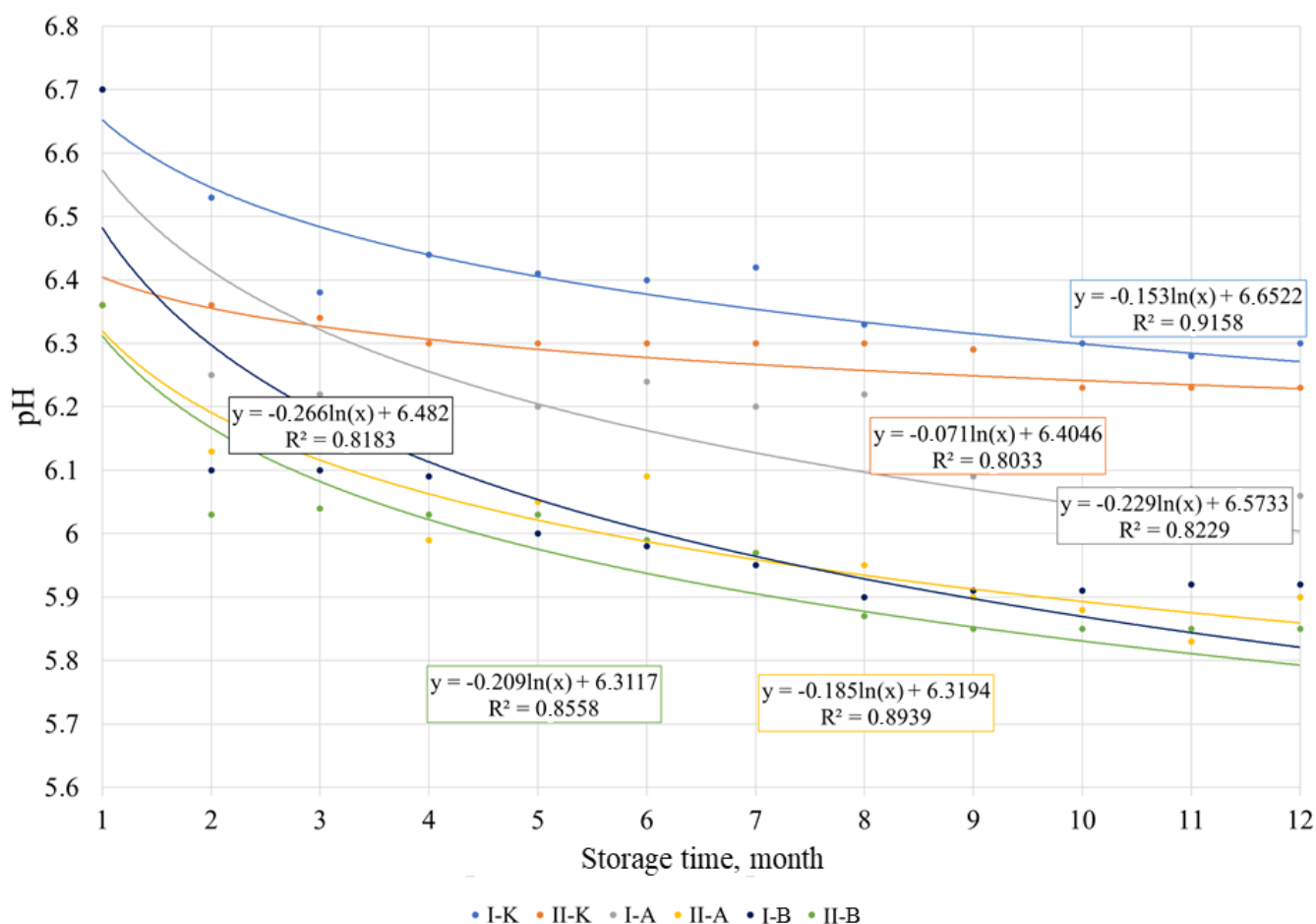
and properties. When the temperature was lowered to 5°C, these products could have both potential and limited capabilities for further transformation into brown pigments. This hypothesis is theoretically supported by Van Boekel (2001), who noted that each stage of the Maillard reaction has different sensitivity to temperature, and that it is the temperature that determines the predominant reaction pathway and the activity of the reactants. This may explain the insignificant influence of differences in storage conditions or homogenization modes on the color formation in condensed whole milk.

Although the storage options for the A and B samples eventually led to similar values of color difference (33–34), the I-B samples had the smallest data scatter located mainly in the upper quartiles of the samples I-A, II-A, and II-B. This indicates that lower homogenization efficiency and freezing before heating produce the greatest influence on the primary color change in condensed

whole milk. The stimulating effect of preliminary freezing of the I-B samples to -50°C before heating to 50°C on their darkening may be associated with a higher rate of pH change in I-B compared to I-A, II-A, and II-B (Figure 8). Liu et al. (2008), who studied the kinetics of color change in galactose and glycine model systems, noted a linear dependence of pH change on heating duration and a logarithmic dependence of pH on temperature. The authors emphasized that the initial pH value in the system significantly affected the course of the Maillard reaction. Similar observations were made by Van Boekel and Berg (2005) and Stojanovska et al. (2017). According to Liu et al. (2008), a lower pH value resulting from the interaction between sugars and amino groups during the Maillard reaction slowed down the darkening in a protein-carbohydrate system. Considering that the I-B samples had the highest rate of pH change, we can assume that glycation products formed at a high rate, too.

Figure 8

## pH Changes in Condensed Milk Samples A and B during Prolonged Storage



According to Pathania et al. (2019) and Alinovi et al. (2020), freezing/thawing processes can cause changes in the physicochemical properties and functionality of proteins, which can lead to their partial denaturation in the milk matrix. Partial denaturation, which involves structural change, can expose amino acids that were hidden from interaction before the heat treatment. Since the I-B samples had lower homogenization efficiency, their freezing/thawing might have caused great changes in their composition, speeding up the darkening of condensed whole milk and the related reactions.

The changes in the whiteness index and color saturation confirm our conclusions regarding the color difference (Figures 9, 10).

The interquartile ranges of whiteness indexes for the I-C and I-D samples ( $59 \div 60$  and  $58 \div 59$ , respectively) are within the median interquartile ranges for II-C and II-D or lower ( $\leq 59-60$ ), which indicates their higher potential for darkening (Figure 9). Among the A and B samples of Batches I and II, the samples I-A, II-A, and II-B had a median whiteness index of  $40 \div 41$ , while I-B had a lower median value of 38. Furthermore, the entire range of values for

Figure 9

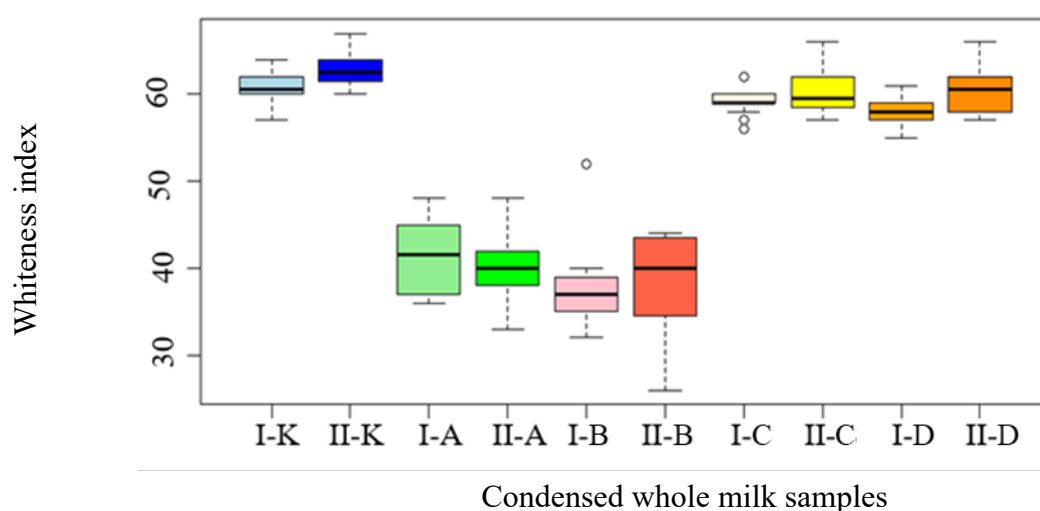
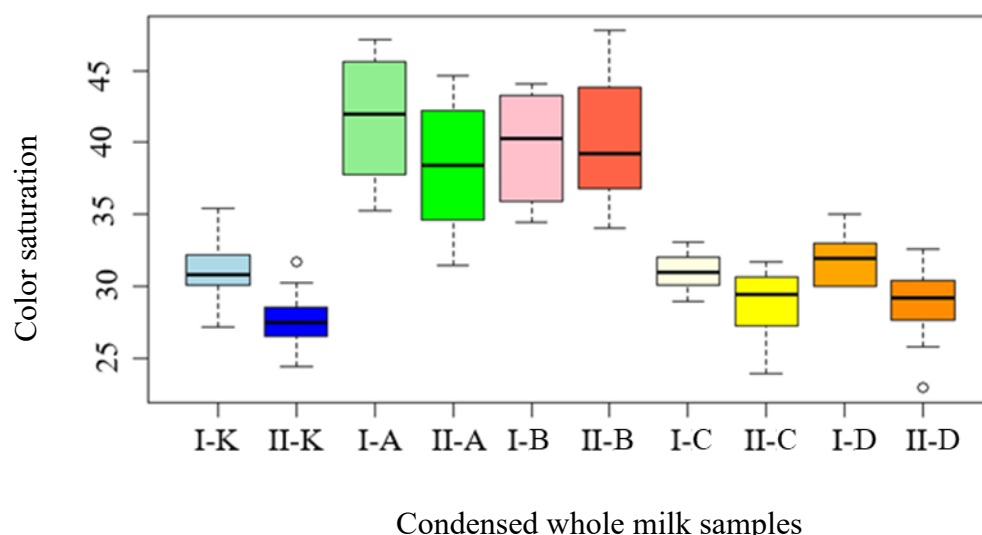
**Whiteness Index vs. Storage Conditions and Homogenization Efficiency**

Figure 10

**Color Saturation vs. Storage Conditions and Homogenization Efficiency**

I-B ( $32 \div 40$ ) was lower than the median whiteness index of II-B (40), which characterizes I-B as less white. The whiteness index values in the interquartile range for the II-K samples ( $61 \div 62$ ) were above the upper quartile for the I-K samples ( $60 \div 61$ ), indicating that the II-K samples were whiter throughout 12-month storage.

The I-K samples had higher color saturation than the II-K samples, while the I-C and I-D samples had higher color saturation than the II-C and II-D samples. This confirmed our general hypothesis that the efficiency of homogenization of condensed whole milk affects the potential of the milk system for darkening.

### CONCLUSION

We studied the effect of variable extreme storage conditions on the color and related properties of condensed whole milk with high and low homogenization efficiency. The study showed that a single-stage heating of condensed milk to 50°C and its storage at 50°C for 7 and 14 days caused irreversible changes in the milk matrix. These changes include the formation of high-protein aggregates, changes in the free amino acid content, pH, and the product's darkening. Conversely, we found that multi-stage heating and freezing cycles to 50°C and -50°C, respectively, or a single-stage freezing to -50°C, did not have a critical effect on the color of condensed whole milk.

Homogenization efficiency affected the darkening potential of condensed whole milk. The samples with lower homogenization efficiency had higher color difference values and a lower whiteness index during prolonged storage after exposure to extreme temperatures. According to acidity analysis, a high rate of pH change in the product correlated with a darker color developing during prolonged storage.

Our data can be used to substantiate the development of new standards for transporting condensed whole milk to the regions with extremely cold or hot climates. We studied condensed whole milk produced according to State Standard 31688–2012 and packed in tin cans No. 7 with a standard lid. Further research can focus on developing standardized methods for quantifying color difference based on the CIE Lab color space to digitize this color indicator and improve its control.

### CONTRIBUTIONS

**Ekaterina I. Bolshakova:** conducting research; methodology; draft preparation; manuscript writing and editing; visualization; data curation; project administration; formal analysis.

**Irina A. Barkovskaya:** conducting research; visualization.

**Alexander G. Kruchinin:** methodology; data verification; resources; manuscript writing and editing; visualization; research supervision; project administration; formal analysis.

**Svetlana N. Turovskaya:** resources; manuscript writing and editing.

**Elena E. Illarionova:** conducting research; resources.

**Elena S. Orlova:** conducting research.

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