**ABSTRACT**

Whey butter is the result of the rational use of the whey component, which is cream whey. It is an alternative to milk cream butter. The aim of the presented study was to analyze the effect of storage conditions on water thermodynamics and cholesterol oxidation products as reliable markers of quality and safety. After 4 mo of storage, the water loss (at 3°C and 13°C) and water activity in whey butter (only at 13°C) were reduced. Three-factorial ANOVA showed that the value of water activity was independent of the type of butter in interaction with the storage temperature. The duration of the translational movement of water molecules from the inside of whey butter was definitely longer than in butter and shortened with storage time. This was in contrast to butter. For whey butter stored at 13°C, the kinetics of the movement of water molecules was at the highest speed. In the case of whey butter and butter, the higher storage temperature almost doubled the gloss. Increasing the temperature to 13°C resulted in different yellowness index, chroma, and browning index between whey butter and butter. There were no statistically significant differences in the percentage of fatty acids and triacylglycerols in whey butter and milk cream butter during storage. In whey butter, compared with butter, the cholesterol content was higher, but the amount of cholesterol oxidation products was smaller. However, in whey butter, these amounts increased significantly. The presence of epoxides and their transformation products (i.e., triol cholesterol) was found in storage whey butter.

**Key words:** whey butter, storage, water activity, oxysterol, triol cholesterol

**INTRODUCTION**

Cheese whey is a natural ingredient created during the production of cheese. The cheese industry annually produces ~145 Gkg of whey, more than half of which is reused by the food industry (Lavelli and Beccalli, 2022). Therefore, the global interest in cheese whey processing is still valid, mainly in the context of recycling processes (Donzella et al., 2022; Lavelli and Beccalli, 2022). Targeted processing is primarily related to the rational use of all whey ingredients of various technological and nutritional importance, which is fully consistent with the Sustainable Development Goals adopted by all United Nations Member States in 2015 (United Nations, 2015). The food created using whey ingredients has been given new physical features, such as ice cream (Kamińska-Dwórzniak et al., 2022), fermented milk (Encinas-Vazquez et al., 2023), chocolate (Jovanović et al., 2022), and bakery products (Dopazo et al., 2023; Iosca et al., 2023). This is a new attraction for consumers and is inscribed in their lifestyle. In addition, consumers continue to expect foods that maintain or improve health, leading to the development of the functional food sector with whey ingredients. Often such products (e.g., whey butter) represent a promising perspective for the dairy industry (Costa et al., 2022). Especially during production, environmentally and climate-friendly technological processes are used, which are economically justified and socially acceptable.

Among the main products of cheese whey fractionation, proteins play a primary role in food production. The structure, properties, and uses of whey proteins are well-known and documented (Yiğit et al., 2023). However, cream obtained from cheese whey and its potential further use (e.g., for the production of butter) have been the subject of interest in recent years (Kasapcopur et al., 2021; Brożek et al., 2022; Costa et al., 2022). According to Çetinkaya (2021), the production of butter with whey cream can be more economically viable than that of using butter with sweet cream. Therefore, whey cream can be used as an alternative raw material for the production of butter. However, despite the general similarity between milk cream and whey butter, there are differences in determining physical and organoleptic properties, and above all, storage stability. Storage stability is closely related to oxidative stability. Oxidation is a natural process of fat...
degradation, which is the main factor determining the durability of butter.

Whey butter-making involves centrifuging the whey to separate the cream. Then, the whey cream is processed and whipped in the same way as in the conventional milk cream process from milk cream (Nadeem et al., 2015; Kasapcopur et al., 2021). Costa et al. (2022) showed that the approximate yield of whey butter was 5 kg of butter/1,000 L of whey. The physicochemical composition of whey butter has no important differences compared with milk butter (Nadeem et al., 2015). The differences between whey butter and milk butter are mainly related to the fatty acid profile (Nadeem et al., 2015), texture (Jinjarak et al., 2006), and sensory properties (Brożek et al., 2022). Jinjarak et al. (2006) showed the potential applications of whey butter, especially in products that have a more intense sour odor and taste, would not be adversely affected. A higher content of UFA and higher content of linolenic fatty acid in whey butter compared with milk cream butter causes a higher nutritional quality and higher healthiness (Costa et al., 2022). However, due to the higher content of UFA in whey butter and the related autoxidation process, the shelf life of whey butter is limited (Nadeem et al., 2015).

Unfortunately, the lack of data regarding the oxidative stability of whey butter is due to the lack of specific studies and development of technologies on milk processing. This is important because of the potential benefits provided by whey cream, such as biologically active compounds that originate from the milk fat globule membrane. We considered the question, will the popular trend of using whey cream to formulate foods with a longer shelf life, such as butter, lead to an increased supply of oxysterols? In this manuscript, for the first time, we tried to tackle this question. We designed an experiment to determine the role of factors (time and temperature) in inducing changes in the quality of stored whey butter in terms of variation in the main components of butter: lipid oxidation and water thermodynamics, especially the translational movement of water molecules. Our results provide knowledge on this topic, mainly oxysterols as reliable markers of quality and safety. Additionally, they can lead to the development of a whey butter storage protocol based on real information about its degree of cholesterol oxidation.

MATERIALS AND METHODS

Sample Whey Butter and Milk Cream Butter

Whey Butter and Milk Cream Butter. The following were commercially available: whey butter, Masło Serwatkowe z Michowa (SM Michowianka, Michów, Poland), and milk cream butter, Masło Ekstra z Michowa (SM Michowianka, Michów, Poland). The storage conditions specified on the packaging were as follows: temperature below 10°C and must be protected against light. Unit package was a 200-g cube (100 × 75 × 30 mm). The samples were stored in the original aluminum-paper-polyethylene laminate packaging.

Experimental Design. Samples of whey butter (sample code WB) and milk cream butter (sample code B) were purchased monthly from February to June (n = 5). Each month, 3 packages of each type of butter were purchased. Samples were stored for 4 mo at 3 ± 0.5°C (samples WB/C and B/C), and at 13 ± 0.5°C (samples WB/R and B/R). These conditions were similar to industrial storage and are often used by the consumer at home. According to Méndez-Cid et al. (2017), butter storage temperature (4°C or 12°C) is more important than storage time (9 mo) in relation to loss of fatty acids, and that losses are almost negligible during storage at low temperatures. The same authors showed that fat oxidation is enhanced by higher storage temperatures and the addition of salt. However, fat oxidation when storing butter at low temperatures was less intense.

The butter was protected from light and monitored during storage (TES-1335, TES Electrical Electronic Corp., Taipei, Taiwan). All samples were analyzed in triplicate. Samples for color and gloss analysis were taken from the center at the intersection of the diagonals of the outer layer. Preliminary research showed no differences between the outer layer and the central layer of butter and whey butter. In the remaining determinations and measurements of the experiment, samples were taken from the outer and inner layers after cutting the cube in half.

Standards and Reagents. All chemicals were of analytical grade. 19-Hydroxycholesterol (95% purity) and oxycholesterol standards were purchased from Steraloids Inc. (Newport, RI). 5-α-Cholesterol (95% purity), pyridine (min. 99.8%), and N,N-bis(trimethylsilyl) trifluoroacetamide (C₅H₁₂F₃NOSi₂) with 1% trimethylchlorosilane (purity min. 99%) were from Sigma-Aldrich (Darmstadt, Germany). Sodium methylate 30% solution in methanol (purity min. 99%), methanol (min. 99.8%), hexane (min. 99.5%), tert-butyl methyl ether (min. 99.5%), chloroform (min. 99.5%), acetone (min. 99.5%), acetonitril (min. 99%), and dichloromethane (min. 99%) were obtained from Merck (Darmstadt, Germany). The solid-phase extraction column SEP-PAK NH2 (aminopropyl sorbent) 300 mg was obtained from Waters Corporation (Milford, MA). The 37-component FAME mix standard was from Supelco (Bellefonte, PA).
Compositional and Water Thermodynamics

Basic Composition. Fat and moisture in butter were determined according to AOAC International standard methods described by Kasapcopur et al. (2021). Water content was determined using a rapid moisture analyzer MA 50.X2.A (Radwag, Radom, Poland).

Water Activity and Water Loss. Water activity was measured using an AquaLab Series 4TE instrument (Decagon Devices Inc., Pullman, WA). Water loss was determined according to the method described by Jones and Martini (2022) using Titania IM190 (Imperia, Italy).

Water Transport. An AWC-11 water activity meter (Cobrabid, Poznań, Poland) equipped with a Rotronic probe was used to assess water transport. During 780 min of analysis, instantaneous water activity values were recorded every 10 min, and the plotted curve was divided into 3 areas. The first area was the constancy of the meter (water moved between the sample and the sensor in the chamber), the second area was related to the translational movement of water molecules inside the sample (beginning with an increase in the speed of water activity, and ending with reaching the equilibrium water activity; i.e., differences in water activities were less than 0.001), and the third area was related to surface processes (i.e., evacuation of water outside the sample, water activity = constant).

Color, Gloss, and Texture Measurement. The CIELAB lightness (L*) and coordinates a* (red-green color) and b* (blue-yellow color) were measured using an X-Rite SP-60 camera (X-rite, Grandville, MI), a D65 light source, and a 10° observation angle. Based on the obtained measurements, the yellowness index (Chudy et al., 2021), chroma (Cais-Sokolińska and Walkowiak-Tomczak, 2021), and browning index (Queiroz et al., 2021) were calculated.

Triacylglycerols Analysis. Triacylglycerols were separated according to equivalent carbon numbers (ECN). Samples were dissolved in dichloromethane and analyzed using a HPLC Agilent 1260 Infinity II equipped with an evaporative light scattering detector (ELSD: Agilent, Santa Clara, CA), and InfinityLab Poroshell 120 EC-C18 (100 × 4.6 mm, 2.7 µm) column (Agilent). The column was thermostated at 30°C. The mobile phases consisted of A (acetonitrile) and B (dichloromethane) and were programmed as follows: 0

Butter Fat Composition

Fat Extraction. Approximately 5 g of each butter sample was flooded with Folch’s mixture (chloroform: methanol, 2:1, vol/vol; Folch et al., 1957). Then the samples were stirred using a magnetic stirrer for 2 h (Raczyk et al., 2022). Distilled water was added to each sample. The aqueous layer was separated using a separatory funnel, and the organic layer was evaporated under a stream of nitrogen.

Fatty Acid Composition. The analysis of fatty acids was carried out using a gas chromatograph with a flame ionization detector, after derivatization of fatty acids into FAME (Belt et al., 2023). Approximately 0.01 g of fat was weighed into a test tube, and 1 mL of hexane, and 1 mL of 0.4 M sodium methoxide were added. After 15 min, the methylation reaction was quenched via the addition of 5 mL of distilled water. After the layers were separated, the upper layer was transferred to a chromatography vial. The fatty acid methyl esters were analyzed using a GC-FID Trace 1300 (ThermoScientific, Waltham, MA), with a SP-2560 capillary column (100 m × 0.25 mm × 0.2 µm; Supelco, Bellefonte, PA). The temperature of the injection port and detector was set at 240°C. The carrier gas was hydrogen, with a flow rate of 1.5 mL/min, and the analysis was performed in split mode. The initial oven temperature was set at 160°C, held for 1 min, and increased to 220°C at a rate of 6°C/min. The temperature was maintained at 220°C for 17 min. One microliter of the sample was used for the analysis. The obtained retention times were compared with those of standard 37 Component FAME Mix (Supelco, Bellefonte, PA).

Triacylglycerols Analysis. Triacylglycerols were separated according to equivalent carbon numbers (ECN). Samples were dissolved in dichloromethane and analyzed using a HPLC Agilent 1260 Infinity II equipped with an evaporative light scattering detector (ELSD: Agilent, Santa Clara, CA), and InfinityLab Poroshell 120 EC-C18 (100 × 4.6 mm, 2.7 µm) column (Agilent). The column was thermostated at 30°C. The mobile phases consisted of A (acetonitrile) and B (dichloromethane) and were programmed as follows: 0
min 80% A and 20% B; within 30 min, phase proportion changed to 55% A and 45% B; changing the phase composition to initial parameters by 80 min and maintained for 10 min. The mobile phase flow was 0.5 mL/min, and the injection volume was 1 µL. The ELSD parameters were as follows: evaporator and nebulizer temperature was 30°C, gas flow rate was 1.6 standard liters per minute, and photomultiplier tube gain was 1.0 (Rudzińska et al., 2022).

### Statistical Analyses

The data were analyzed using the Statistica software, version 13.3.0 (TIBCO Software Inc., Palo Alto, CA). The influence of the composition and storage time on the samples was evaluated by one-way ANOVA followed by Tukey’s honestly significant difference post hoc tests for multiple comparisons. Effective hypotheses were decomposed using ANOVA for multifactor systems, where the qualitative factor was the type of butter, storage time, and temperature, and the examined parameters were dependent variables.

### RESULTS AND DISCUSSION

#### Composition, Water Activity, and Mobility in Whey Butter and Milk Cream Butter

The fat and water content of whey butter and milk cream butter complied with the formal requirements of many countries (Codex Alimentarius Commission, 2011; Lee et al., 2018). However, after 4 mo of storage at 13°C, the total fat content of both types of butter increased ($P < 0.05$, Table 1). These changes were not observed after storage at 3°C ($P > 0.05$). After storage, whey butter showed a lower value of water loss compared with before storage samples (2.18%). The higher the storage temperature, the lower the whey butter water loss (3°C water loss was 2.05%, and at 13°C water loss was 1.92%). After the same storage time, the water loss of milk cream butter did not change ($P > 0.05$). Storing whey butter at 13°C, compared with 3°C, decreased the water activity value from 0.9563 to 0.9022 (i.e., by 5.7%, $P < 0.05$). Water activity of milk cream butter stored under the same conditions decreased by 3.6% ($P < 0.05$). However, 3-factorial ANOVA showed that the value of water activity was independent of the type of butter in interaction with the storage temperature ($P = 0.463$, Supplemental Table S1, https://data.mendeley.com/datasets/mmgz3cxbsd/1/files/9a1fd16a-db64-4645-a324-2452919542b4). A significant relationship was observed between the type of butter in interaction with storage and storage temperature due to the water loss ($P = 0$ and $P = 0.001$, respectively). Hence, the effect of butter type on the water loss value depended on the storage temperature used.

Butter is a milk fat product and water-in-oil emulsion (Seo, 2023), in which water droplets are dispersed in a partially crystallized and continuous fat phase, in which the fat globules have a size of 3.41 µm (Dias et al., 2022), although Walter et al. (2020) indicated that the fat globules in the milk of Holstein-Friesian cows ranges from 2.5 to 5.7 µm. Butter grain size plays an important role, as smaller grains create more surface area and consequently retain more water (Rønholt et al., 2013). According to Rønholt et al. (2012), water droplets are dispersed in the continuous fat phase of butter and form a network together with the fat globules. The internal water is that which has been incorporated into the butter grains during churning. The amount of water in the internal aqueous phase is determined by the amount of crystallized fat in the fat globules. The increased water droplet size is related to the lower hardness of the butter, whereas the low water content increases the stability of the fatty network and increases the stability of larger water droplets (Jones et al., 2023). Bocharova-Leskina and Verbytskyi (2019) showed that the degree of dispersion and distribution of moisture correlates with the physicochemical properties and storage temperature of butters. The authors also indicated that the degree of dispersion and distribution of moisture is one of the indicators to predict the shelf life of butter as a mathematical modeling tool.

### Table 1. Fat and water content and thermodynamics in whey butter and milk cream butter after storage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WB</th>
<th>WB/C</th>
<th>WB/R</th>
<th>B</th>
<th>B/C</th>
<th>B/R</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content (%)</td>
<td>82.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.006</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>15.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.019</td>
</tr>
<tr>
<td>Water loss (%)</td>
<td>2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.004</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.9563&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9536&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9022&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9307&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9551&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8971&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>-<sup>b</sup>Means within a row with different superscripts differ ($P < 0.05$).
According to Brighenti et al. (2021) whey cream is a fat source, which has smaller fat droplets and slightly different chemical composition than sweet cream. In whey cream there is a large proportion of nonspherical fat, resulting from the destabilization of fat globules that occurs during cheese production, pumping or centrifugal separation of the whey. Whey cream contains fewer proteins from skim milk, and milk fat globule membrane can be recovered from the aqueous phase due to the destabilization of fat globules (Fox and McSweeney, 2006). According to Nadeem et al. (2015) there are no differences between the milk and whey butters in terms of the free fatty acid, moisture, fat, solid and protein contents. However, Kasapcopur et al. (2021) report that the differences may result from the type of cheese production (rennet- or acid-coagulated), and biochemical and chemical changes that occur in the period from the production of whey to its processing into butter.

Molecular Dynamics of Water and Water Transport

Analysis of the course of water activity until the equilibrium water activity could be determined, which revealed the area dominated by the translational movement of water molecules from the inside of the sample (Figure 1). The duration of water movement in whey butter was longer than in butter, and when the storage time was shortened (mostly at 13°C, Δτ from 320 to 150 min). This was in contrast to butter. In WB/R, the kinetics of the movement of water molecules was at the highest speed (Vm = 3.85 min × 10^{-3}, Table 2). For provenance in B/R it was Vm = 0.75 min × 10^{-3}. This proved the reverse mechanism of water movement differentiating whey butter from milk cream butter. The average values of water activity in whey butter were higher than in milk cream butter (P < 0.05), and the time to reach the final water activity in the area of translational movement was more diverse in whey butter (from 180 to 370 min) than in milk cream butter (from 300 to 340 min).

Probably the degree of water dispersion in fat largely determines water activity. The smaller the water droplet, the higher the vapor pressure above its surface and the higher the water activity. However, the emulsion components are exposed to hydrolytic and oxidative changes during storage, which also affects the translational movement of water. In the literature, the translational movement of water in a water-in-oil emul-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Translational movement of water in whey butter and milk cream butter. WB = unstored whey butter, control; WB/C = whey butter stored for 4 months in the cold at 3°C, in the darkroom; WB/R = whey butter stored for 4 months at 13°C, in the darkroom; B = unstored milk cream butter, control; B/C = milk cream butter stored for 4 months in the cold at 3°C, in the darkroom; B/R = milk cream butter stored for 4 months at 13°C, in the darkroom; awm = mean water activity translation area; Δτ = duration of translational water movement in minutes.
It is necessary to determine the influence of kneading time, the correlation between kneading time and the air content and the degree of wetting during storage, and the transformation of the mobile fraction into the immobilized one during storage.

**Interference of Storage Conditions on Color and Gloss of Whey Butter and Milk Cream Butter**

Samples of WB and B did not differ in measured color and gloss parameters ($P > 0.05$, Table 3). Four mo of storage at 3°C resulted in differences in lightness and gloss between the 2 types of butter. As determined above, increasing the temperature to 13°C promoted differences in yellowness index, chroma, and browning index between whey butter and milk cream butter ($P < 0.05$). In the case of whey butter and butter, the higher storage temperature almost doubled the gloss. Interestingly, the yellowness index of butter stored at 13°C increased from 43.10 to 54.00 ($P < 0.05$). This tendency was not demonstrated for whey butter.

Color analysis of dairy products, especially during storage, is an important indicator of quality (Milovanovic et al., 2020). Kasapcopur et al. (2021) compared...
whey butter with milk butter, where milk butter was visibly yellower and duller. The same authors also reported that the color of butter was mainly related to the content of carotenoids, the size and distribution of fat globules, water droplets, and carotenoid oxidation. Brožek et al. (2022) showed that the decrease in parameter L* from 60 d storage was attributed to the production process (effects of washing butter) and oxidation of the color pigments of butter. Color analysis is also important in the selection of packaging materials for butter. According to Asdagh and Pirsa (2020), butter packed in active packaging (such as composite film) was characterized by the smallest color change during storage.

After breaking down the variability into additive factors such as the type of butter, storage, and storage temperature, ANOVA allowed for the determination of whether a given factor plays a significant role in shaping the experimental results (Supplemental Table S2, https://data.mendeley.com/datasets/mmgz3cxbsd/1/files/289af7d2-11c2-49e7-ba14-f1bb7c81f80c). The results of the 3-factorial ANOVA confirmed that the type of butter and its storage differed only in lightness (P = 0.004 and P = 0.033, respectively). In the case of color and chroma indexes, neither the type of butter nor storage and temperature had a significant individual effect. However, these parameters were influenced by the interaction of the type of butter and its storage. It was found that the storage temperature as an individual factor differentiated the gloss value of the butter (P = 0). The interactions that occurred between the 3 factors also highlighted the influence of the type of butter on gloss depending on the storage temperature used (P = 0.003). Thus, the earlier observation regarding the differences in gloss between the samples was confirmed. Post hoc analysis with the application of Tukey’s test revealed a very reduced gloss associated with elevated whey butter storage temperature (Table 3). Therefore, gloss and previously described water loss were the 2 most significant determinants of whey butter quality changes during storage. Visible changes on the surface of the sample, such as changes in gloss, could be associated with a quick entry into the area of water evacuation from the whey butter surface, as indicated in Table 2.

Gloss or shine is the visual aspect of the surface light reflections (Lavrentev et al., 2021). Baygut et al. (2023) reported that gloss measurement may be important in the visual assessment of the product by the consumer. The same observations were described by Rafiq and Rafiq (2019), who claimed that the use of whey-based formulations positively affected the visual properties (dry as glossy) of coated products. Truong et al. (2018) reported that gloss in butter might depend on air content. Jinjarak et al. (2006) compared whey butter with other butters (cultured and sweet cream) showed that cultured butter and butter from sweet cream were shinier than whey.

The conducted analysis (Table 3 and Supplemental Table S2) proved the need to specify a different storage protocol for whey butter than milk cream butter due to changes in color and gloss indices. This was important because the storage of whey butter and milk cream butter often takes place under the same time-temperature conditions prevailing in warehouses and during distribution. In the opinion of the consumer, the right appearance, including the color, reflects the quality of the butter. Changes in color indices, and especially in gloss, stem from changes occurring in and between butter components, related to water mobility and lipid changes. Hence, a 3-way (analytical, thermodynamic, and structural) approach to the water in butter and understanding the direction of lipid transformations, especially the formation of oxysterols, is necessary.

**Textural Properties of Whey Butter and Milk Cream Butter**

The penetration test showed an important effect of storing samples on their firmness (Table 4). The force determining the firmness of whey butter and milk cream butter was significantly lower after 4 mo than before storage, which indicated that the sample was softer. However, only in the case of whey butter did the temperature have a significant impact. The higher the temperature, the lower the firmness (P < 0.05). In addition, work of penetration decreased from 11,268.9 to 10,761.3 g·s (i.e., by 4.5%; P < 0.05), which also proved significant softening of the sample. However, the negative region of the graph, produced on the probe’s return, which defined the resistance to probe withdrawal, remained unchanged, and proved no changes in the adhesiveness of the samples as a result of storage (P > 0.05). Although, when comparing unstored samples, whey butter showed greater adhesion to the probe than butter.

Jinjarak et al. (2006) showed that whey butter was less hard and more spreadable than butter from sweet cream. Furthermore, the decrease in hardness and increase in spreadability was associated with an increase in unsaturated fat. This was also confirmed by Aly (2009), where whey butter showed lower values of hardness and cohesiveness and higher values of adhesiveness, and the obtained texture results were correlated with the fatty acids pattern of gas chromatograph.
Fatty Acids Profile in Whey Butter and Milk Cream Butter

The fatty acid profile is the most commonly used method for fat analysis. The results of fatty acids composition in WB and B are presented in Table 5. The results were similar to other analyses of fatty acids in butter produced in winter (Staniewski et al., 2021) and in whey butter (Kasapcopur et al., 2021). This is especially important when examining the nutritional value of dairy fat. An important feature of such fat is the proportion of PUFA and the level of short-chain fatty acids (SCFA) and dienes of CLA. According to the fatty acids composition, we calculated the percentage of SFA, MUFA, and PUFA. The share of individual groups of fatty acids affects human health. The high content of SFA adversely affects the cardiovascular system (Briggs et al., 2017).

Table 6 compared the fatty acid groups in WB and B, where no statistically significant differences in all 3 groups (SFA, MUFA, PUFA) in WB and B were observed. Both WB and B were dominated by SFA (71–72%), follow the MUFA (25–26%), and PUFA (2%). Greater differences between the groups of fatty acids in B and WB were observed by Brożek et al. (2022).

There were no statistically significant differences in the percentage of fatty acids in whey butter and milk cream butter during storage (Table 6). Therefore, the percentage of individual fatty acids was not a good indicator of changes occurring during storage of whey butter and milk cream butter. The SCFA ranged from C2:0 to C6:0 are useful components in the human diet because of appetite regulation and energy homeostasis (Byrne et al., 2015). They have beneficial effects from weight loss to normalization of insulin levels and amelioration of hepatic steatosis (Ilyés et al., 2022). Short-chain fatty acids have been reported to exert anticancer (Balkwill and Mantovani, 2012) and anti-inflammatory effects (Nogal et al., 2021). Short-chain fatty acids can also be a regulatory factor in lipid metabolism (He et al., 2020) and they can enhance fatty acid oxidation and production of heat, block fatty acid synthesis, and reduce storage of fat in the body (Kimura et al., 2020). Khatibjoo et al. (2018) showed that the combination of SCFA and medium-chain fatty acids (MCFA) can decrease the serum cholesterol concentration of broilers. In addition, MCFA are a good source of energy because they are easily absorbed and quickly transferred to the mitochondria (Yuan et al., 2022). The MCFA also inhibit bacterial toxin production and the expression of other virulence factors by interfering with signal transduction (Mathis et al., 2005; Kipper et al., 2022). The effect of these acids is both bactericidal and bacteriostatic depending on the concentration, synergism among them, and target bacterial strain (Batovska et al., 2009; Deschepper et al., 2003). The MCFA have been linked to the treatment of both aging and neurodegenerative disease via their effects on metabolism (Dunn et al., 2023).

The share of SCFA were similar, and share 5% in WB and 4% in B. The MCFA in both butters amounted to ~8%, and long-chain fatty acid content was 88% and 86%, respectively. The CLA are positional and geometric isomers of octadienoic acid (C18:2). The highest concentration of these acids are found in dairy products (Rodriguez-Alcalá and Fontecha, 2007). The analyzed milk cream butter showed 0.6% of CLA, whereas in...

Table 4. Texture of whey butter and milk cream butter after storage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WB</th>
<th>WB/C</th>
<th>WB/R</th>
<th>B</th>
<th>B/C</th>
<th>B/R</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmness (g)</td>
<td>9.671.8e</td>
<td>7.898.2b</td>
<td>6.777.2a</td>
<td>6.777.6d</td>
<td>5.778.0e</td>
<td>5.278.7c</td>
<td>8.624.0</td>
</tr>
<tr>
<td>Work of penetration (g·s)</td>
<td>11.268.9b</td>
<td>10.061.2ab</td>
<td>10.761.3a</td>
<td>10.604.8a</td>
<td>10.418.4b</td>
<td>10.291.9a</td>
<td>2.803.0</td>
</tr>
<tr>
<td>Resistance to probe withdrawal (g·s)</td>
<td>−1.215.3b</td>
<td>−1.237.4b</td>
<td>−1.151.7b</td>
<td>−1.007.7b</td>
<td>−863.6b</td>
<td>−719.5ab</td>
<td>50.906.0</td>
</tr>
</tbody>
</table>

a–eMeans within a row with different superscripts differ (P < 0.05).

1WB = unstored whey butter, control; WB/C = whey butter stored for 4 mo in the cold at 3°C, in the darkroom; WB/R = whey butter stored for 4 mo at 13°C, in the darkroom; B = unstored milk cream butter, control; B/C = milk cream butter stored for 4 mo in the cold at 3°C, in the darkroom; B/R = milk cream butter stored for 4 mo at 13°C, in the darkroom; MSE = mean square error of intergroup variability (n = 5).

Table 5. Percentages of fatty acid composition in whey butter and milk cream butter

<table>
<thead>
<tr>
<th>Sample1</th>
<th>4:0</th>
<th>6:0</th>
<th>8:0</th>
<th>10:0</th>
<th>12:0</th>
<th>14:0</th>
<th>14:1</th>
<th>15:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1t</th>
<th>18:1c</th>
<th>18:2</th>
<th>18:2 CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB (%)</td>
<td>3.1b</td>
<td>1.9a</td>
<td>1.3a</td>
<td>3.1b</td>
<td>3.8b</td>
<td>12.6d</td>
<td>1.2a</td>
<td>1.2a</td>
<td>35.9c</td>
<td>1.7a</td>
<td>9.3c</td>
<td>1.6c</td>
<td>21.4c</td>
<td>1.5c</td>
<td>0.4c</td>
</tr>
<tr>
<td>B (%)</td>
<td>2.3c</td>
<td>1.7a</td>
<td>1.2a</td>
<td>2.9b</td>
<td>3.6c</td>
<td>12.7c</td>
<td>1.3c</td>
<td>1.1a</td>
<td>36.0c</td>
<td>1.6c</td>
<td>10.2c</td>
<td>1.5c</td>
<td>21.9c</td>
<td>1.5c</td>
<td>0.6b</td>
</tr>
</tbody>
</table>

a,bMeans within a column for the same parameter with different superscripts differ (P < 0.05).

1WB = unstored whey butter, control; B = unstored milk cream butter, control.
whey butter it was 0.4%. These fatty acids play a role in atherogenesis (Benito et al., 2001), and their properties in type-2 diabetes have been investigated (Zhang et al., 2017).

### Triacylglycerol Composition

An equally popular fat analysis method is triacylglycerol analysis, which has increased in popularity in recent years. It is used to detect adulteration in various fats, such as cocoa butter, milk fat, or olive oil (Rom-baut et al., 2009). Table 7 shows the percentage of each ECN group present in whey butter and milk cream butter fresh and after storage. The largest percentage was ECN 50 (21%–23%) and 38 (18%–19%), and the smallest group was 32 (0.5%–0.6%) and 34 (1%), which included triacylglycerols that contained UFA. There were no statistically significant changes in the percentage share of individual groups. Park et al. (2014) suggested the potential of triacylglycerols ECN 36, 38, 40, 50, 52, and 54 as biomarkers in dairy products. Our results showed that C54 was not detected, and no differences between whey butter and milk cream butter were observed. Also, during storage, differences in triacylglycerol composition were not found. As in the case of the percentage composition of fatty acids, triacylglycerols were not sufficient indicators to differentiate whey butter and milk cream butter and to identify changes during their storage.

### Oxysterols as Reliable Markers of Quality and Safety

Table 8 presents the content of cholesterol and COP in the analyzed whey butter and milk cream butter fresh and after storage. In milk cream butter, the amount of cholesterol was statistically significantly lower than in whey butter (1.12 g/kg fat and 1.24 g/kg fat, respectively). Due to butter being an animal product, cholesterol is an indispensable element. This is an important compound because it is a precursor to bile acids and steroid hormones (Kulig et al., 2016). The problem is the changes that occur during the storage of cholesterol. In the presence of oxygen, cholesterol is transformed into COP, which can increase the risk of cardiovascular diseases. In addition, COP can also have cytotoxic and mutagenic effects (Ubhayasekera et al., 2010). More and more publications show the impact of, among other factors, temperature and storage time of products containing cholesterol or phytosterols on the formation of COP or phytosterol oxidation products (Conchillo et al., 2005).

The initial amount of COP in fresh samples was lower than the amount of COP after storage (Table 8). The comparison of the initial amount of COP in whey butter and milk cream butter revealed that the lower cholesterol content in butter resulted in statistically significantly more COP in milk cream butter than in whey butter. Hence, cholesterol-related oxidative changes occurred faster in whey butter than in butter. To date, studies on the amount of cholesterol and COPs in whey butter have not been performed. To the best of our knowledge, this is the first such study. The comparison of the effect of the storage temperature of the samples (3°C and 13°C), showed that the storage of both whey butter and milk cream butter resulted in significantly more COP formation.
butter and milk cream butter at a higher temperature resulted in the formation of larger amounts of COPs. Storage at 3°C resulted in a slight increase (statistically significant) in the amount of COPs in whey butter and milk cream butter. At the same time, storage of whey butter and milk cream butter at 13°C promoted a significant increase in COPs (statistically significant). In whey butter, 7β-OHC, β-epoxyC, triolC, and 7-ketoC were formed during storage. In milk cream butter 7β-OHC and 7-ketoC were formed during storage. During the oxidative transformations of cholesterol, mainly changes related to 2 directions occurred: epoxidation and C-7 oxidation (Medina-Meza and Barnaba, 2013). In the C-7 oxidation pathway, 7α- and 7β-OHC were first formed, and 7-ketoC was formed during subsequent transformations. In samples WB/C and B/R, more 7-ketoC was formed than 7β-OHC, which meant a faster rate of formation of primary COP. In milk cream butter, there was no transformation toward the formation of epoxides. The presence of epoxides and their transformation products (i.e., triolC) was found in samples WB/C and WB/R. These transformations were initiated by the presence of water and acidic conditions (Cardenia et al., 2013). When whey butter was stored at 13°C, a rapid increase in the amount of β-epoxyC and triolC was observed. Among all COP determined, triolC is the most toxic compound, even at low concentrations (Cardenia et al., 2013). In a study by Hiesberger and Luf (2000), during the storage of butter, 7-ketoC, 7α-OHC, and 7β-OHC were detected. The amount of COP also increased during storage. Whey butter has more cholesterol than milk cream butter. The 4 mo storage procedure reduced the nutritional value of whey butter due to the presence of oxysterols. Hence, cholesterol-related oxidative changes occurred faster in whey butter than in butter. In milk cream butter, no transformation toward the formation of epoxides occurred. In whey butter, 7β-OHC, β-epoxyC, triolC, and 7-ketoC were formed during storage. We confirmed the role of 7β-OHC and 7-ketoC as reliable biomarkers of cholesterol oxidation during storage of whey butter. Qualitative tools to monitor cholesterol oxidation in whey butter were 7β-OHC, β-epoxyC, triolC, and 7-ketoC. For the first time, we reported the presence of high levels of COP in storage whey butter. After 4 mo at 3°C and 13°C, the presence of triol-C was detected.

Table 8. Levels of cholesterol and cholesterol oxidation products in whey butter and milk cream butter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WB</th>
<th>WB/C</th>
<th>WB/R</th>
<th>B</th>
<th>B/C</th>
<th>B/R</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (g/kg fat)</td>
<td>1.26b</td>
<td>1.29b</td>
<td>1.29b</td>
<td>1.12a</td>
<td>1.16a</td>
<td>1.14a</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol oxidation</td>
<td>7β-OHC</td>
<td>76.86e</td>
<td>30.75c</td>
<td>184.04f</td>
<td>61.89c</td>
<td>69.37d</td>
<td>53.24b</td>
</tr>
<tr>
<td>products (mg/kg fat)</td>
<td>β-epoxyC</td>
<td>ND1</td>
<td>14.94b</td>
<td>125.95b</td>
<td>ND1</td>
<td>ND1</td>
<td>ND1</td>
</tr>
<tr>
<td>triolC</td>
<td>ND2</td>
<td>17.84b</td>
<td>67.37f</td>
<td>102.09b</td>
<td>ND2</td>
<td>ND2</td>
<td>ND2</td>
</tr>
<tr>
<td>7-ketoC</td>
<td>17.50a</td>
<td>35.99b</td>
<td>147.44f</td>
<td>124.85d</td>
<td>129.98e</td>
<td>195.50c</td>
<td>0.017</td>
</tr>
<tr>
<td>Σ COP</td>
<td>94.36a</td>
<td>99.50b</td>
<td>479.44f</td>
<td>124.85d</td>
<td>129.98e</td>
<td>189.50c</td>
<td>0.017</td>
</tr>
</tbody>
</table>

a-fMeans within a row for the same parameter with different superscripts differ (P < 0.05).
1WB = unstored whey butter, control; WB/C = whey butter stored for 4 mo in the cold at 3°C, in the darkroom; WB/R = whey butter stored for 4 mo at 13°C, in the darkroom; B = unstored milk cream butter, control; B/C = milk cream butter stored for 4 mo in the cold at 3°C, in the darkroom; B/R = milk cream butter stored for 4 mo at 13°C, in the darkroom; MSE = mean square error of intergroup variability (n = 5).
27β-OHC = 7β-hydroxycholesterol; β-epoxyC = 5,6β-epoxycholesterol; triolC = cholestanetriol; 7-ketoC = 7-ketocholesterol; Σ COP = sum of cholesterol oxidation products.
3ND = not detected.

CONCLUSIONS

Water loss and gloss are the 2 most significant determinants of whey butter quality changes during storage. Whey butter has more cholesterol than milk cream butter. The 4 mo storage procedure reduced the nutritional value of whey butter due to the presence of oxysterols. Hence, cholesterol-related oxidative changes occurred faster in whey butter than in butter. In milk cream butter, no transformation toward the formation of epoxides occurred. In whey butter, 7β-OHC, β-epoxyC, triolC, and 7-ketoC were formed during storage. We confirmed the role of 7β-OHC and 7-ketoC as reliable biomarkers of cholesterol oxidation during storage of whey butter. Qualitative tools to monitor cholesterol oxidation in whey butter were 7β-OHC, β-epoxyC, triolC, and 7-ketoC. For the first time, we reported the presence of high levels of COP in storage whey butter. After 4 mo at 3°C and 13°C, the presence of triol-C was detected.

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