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INFLUENCE OF STORAGE CONDITIONS ON CHANGES IN THE FAT FRACTION OF UHT MILK

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This study determined the influence of storage conditions (3 and 6 months at temperatures of 4°C and 23°C) on the changes in the fat fraction of UHT milk with 3.2% fat content produced under industrial conditions by two methods of sterilization (indirect and direct). Lipolytic activity, the total content of free fatty acids (FFA), the contents of volatile and non-volatile free fatty acids, the content of peroxides, and acid value were determined as well as a TBA test was conducted. An increase of lipolytic activity in the milk, dependent on temperature and time of storage, was found to occur during UHT milk storage and in consequence a growth of the FFA content in the product was observed. A greater dynamics of FFA content changes was also noticed in milk samples stored at 23°C rather than 4°C. In addition, oxidizing changes in UHT milk fat were found and their intensity was higher in the milk stored at room temperature as compared to the milk kept in cold storage.

INTRODUCTION

As a multi-component composition, milk undergoes important changes during heating. The range of these changes depends on the amount of thermal loading of milk (and many other dairy products), which affects the quality as well as the storage stability of these products. The influence of high temperatures on milk components is particularly significant in the process of UHT milk production. In this milk, the changes induced by heating can continue further in different directions and with different intensity during storing. This is important for a relatively long (2–4 months) period of storage. The influence of UHT milk production methods (indirect and direct heating) on the changes in the fat phase was presented in our previous publication [Panfil-Kuncewicz *et al.*, 2004].

The objective of the present study was to determine the influence of storage conditions on the changes in the UHT milk fat fraction produced by indirect and direct sterilization.

MATERIAL AND METHODS

UHT milk samples of a declared fat content of 3.2% produced under industrial conditions by two methods of sterilization: direct and indirect, taken straight from a producer, were evaluated. UHT milk products sterilized by the indirect method were homogenized at 63°C and 15 MPa, and then sterilized in a plate exchanger at 137–139°C for 8 sec and packed aseptically in Tetra Pack-type cartons of 1 L capacity. UHT milk products manufactured by the direct method were homogenized at 60°C and 20 MPa, sterilized at 140°C for 4 sec by introducing steam into the milk, and packed aseptically in Pure Pack-type cartons of 1 L capacity. Fresh milks stored for 3 and 6 months at the cold store temperature of 4°C and room temperature of 23°C were tested. In total, 12 samples of UHT milk produced by the indirect method were analysed from 12 products obtained from different parts of the bulk milk along with 12 UHT milk samples produced by the direct method from 12 products obtained from different parts of the bulk milk. The analysed samples of the UHT milk came from the summer period of production.

The content of free fatty acids (FFA) was determined in fresh milk and stored milk using the Dole extracting-titratable method in Deeth and Fitz-Gerald modification [Deeth *et al.*, 1975] and the contents of volatile [Ross *et al.*, 1963] and non-volatile free fatty acids (FFA) were established by the gas chromatography method [Kuzdzal-Savoie & Kuzdzal, 1967]. The principle of the free fatty acid determination method depends on the extraction of FFA from a milk sample by a mixture of 2-propanol: petroleum ether: 2 mol/L H_2SO_4 (40:10:1) and titration of the determined amount of the ethereal layer by a methanolic solution 0.005 mol/L KOH in the presence of α-naphthophthalein. Determining free volatile and non-volatile fatty acids involved extracting them from a milk sample acidified to pH 2 with sulfuric acid with the addition of a particular amount of the known concentration of C9 acid solution (inner standard) with petroleum ether and saponifying them by titration 0.25 mol/L NaOH in the presence of phenolphtalein. The obtained soaps were dried in a boiling water bath and weighted amounts were prepared from them to determine the content of volatile and non-volatile fatty

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acids. In the process of determining non-volatile fatty acids from a weighted amount of the previously obtained soaps, the soaps were released by adding H_2SO_4 and extracted by hexane. The hexane extract of FFA was transmitted to a vial, 8 mL of 3%-methanolic solution of H_2SO_4 was added, the phial was sealed and methylated by heating in a boiling water bath for one hour. The hexanoic solution of methyl esters of FFA (1 mL) was injected into the chromatographic column. Chromatographic separations were carried out using a HP 6890 (Hewlett-Packard Co., USA) gas chromatograph.

Chromatographic separation conditions: Detector – FID. Capillary column 30 m, internal diameter – 0.32 mm, liquid phase – Supelcowax 10, film thickness – 0.25 μm. Temperature: 250°C for detector, 225°C for injector, for column 60°C (1 min) – 180°C, Δt=8°C/min. Carrier gas – helium, flow rate – 1 mL/min. Split 100:1.

The content of volatile fatty acids was determined after the release from the previously obtained soaps using formic acid in the presence of a defined amount of hexane to dissolve volatile fatty acids. The hexanoic solution of volatile fatty acids was injected (1 mL) into the chromatographic column. Chromatographic separations were carried out using a HP 6890 (Hewlett-Packard Co., USA) gas chromatograph.

Chromatographic separations conditions: Detector – FID. Capillary column 30 m, internal diameter – 0.32 mm, liquid phase – HP-Innowax, film thickness – 0.25 μm. Temperatures: 280°C for detector, 250°C for injector, 60°C – for column – isothermal cycle – 140°C. Carrier gas – helium, flow rate – 1 mL/min. Split – 50:1.

The content of free fatty acids was calculated on the basis of the comparison of the peak size of a determined fatty acid in a tested sample to the peak size of the added internal standard (C9) taking into account correction coefficients for the determined acids.

In the fat extracted from milk by the Rose-Gotlieb method [Budsławski, 1973], the peroxide content [International Standard FIL-IDF 74:1974], acid value of fats [Krełowska-Kułas, 1993] and lipolytic activity were determined by the pH static titratable method and expressed in lipolytic activity units (LAU). LAU – lipolytic activity of lipases expressed as the number of μmoles of fatty acids released by the lipases contained in 1 mL of a sample in 1 min [Jacobsen *et al.*, 1989]. A TBA test was carried out as well (values expressed in units of absorbance – A) [Krełowska-Kułas, 1993]. Determinations were conducted twice. The obtained results were assumed as averages for 12 samples.

The statistical analysis of the results obtained was carried out using two-factor variance analysis in the Excel 2000 calculating spread sheet (Microsoft Office 2000 Professional), assuming the significance level of $\alpha = 0.05$. The Duncan test was applied to compare the averages [Gawęcki & Wagner, 1984].

RESULTS AND DISCUSSION

The fat fraction of UHT milk is considered to be one of the sources of undesirable changes, including organoleptic features, of the product. As a result of milk fat hydrolysis and oxidation, numerous compounds are created resulting in the decrease of the organoleptic features of the product. Thermostable bacterial lipases are the primary cause of fat hydrolysis and their presence in UHT milk depends on the microbiological quality of the raw milk as well as on the milk heat treatment [Choi & Jeon, 1993; Hittu-Matta & Punj, 1999; Rye & Schraft, 1999; Chen *et al.*, 2003].

Walstra and Jenness [1984] found that milk native lipase was inactivated by heating at 98°C for 1 sec, whereas inactivation of bacterial lipases (*Pseudomonas fluorescens*) occured after 20 min of heating at 138°C. Griffiths *et al.* [1981] observed that lipases of several different species of psychrotrophic bacteria still preserved about 30% of their initial activity in milk heated at 140°C for 5 sec. The research of Andersson *et al.* [1981] showed that the lipases of microbiological origin (*Pseudomonas fluorescens*) preserved 50% of their initial activity after heating milk at 138°C for 3 sec. These lipases were able to continue lipolysis in UHT milk during storage.

In the tested UHT milk samples we found that, in both methods of sterilization, the inactivation of lipases was not complete. The average lipolytic activity of tested fresh UHT milk produced by the indirect method was 0.15±0.044 LAU (Lipolytic Activity Unit), while in the case of the direct method it was on average 0.12±0.079 LAU. Changes of the lipolytic activity in the tested stored milk samples were not unique. The course of the curves in Figure 1 shows that in milk stored at 4°C for 3 months, produced by the indirect method, the lipolytic activity increased on average by 25% and reached 0.19±0.022 LAU. In milk samples sterilized by the direct method, stored under the same conditions, the average value of lipolytic activity was near the value obtained for samples directly after production (Figure 1). After six months of storage at 4°C, a decrease of the lipolytic activity in milk samples from all products was noted. Milk produced by the direct method showed an even lower

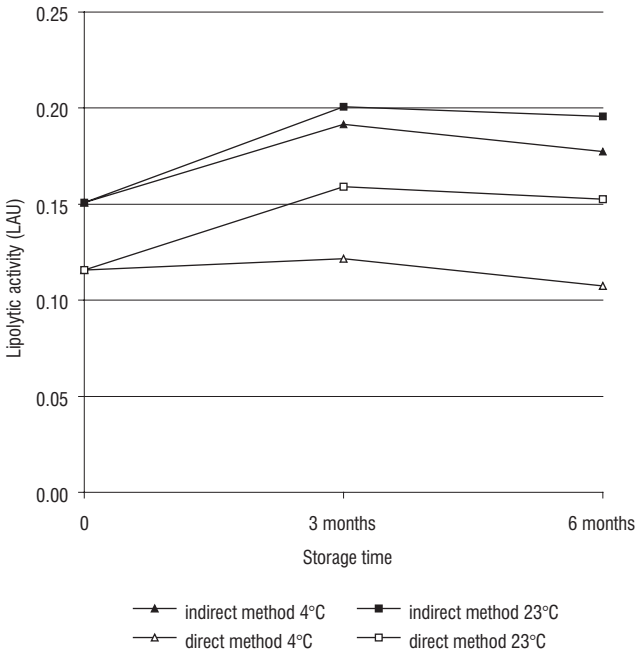

FIGURE 1. Changes of lipolytic activity during UHT milk storage.

lipolytic activity than it did directly after production. The decrease of the lipolytic activity during milk storage can be connected with their inactivation by bacterial proteinases in UHT milk. Kelly and Foley [1997], as well as Sajko and Cichosz [1998] associate it with the activity occurring in milk plasmin. The storage of samples at 23°C for 3 months caused a significant increase in the lipolytic activity both in the milk produced by the indirect method and the direct method (Figure 1). Further storage did not alter the activity significantly (Figure 1). Choi and Jeon [1993] claim that low initial lipolytic activity directly after the UHT process can be connected with the loss of a part of the lipases active centres, whereas the increase in the lipolytic activity during milk storage is presumably connected with partial reproduction of these centres. The configuration of curves (Figure 1) shows that the course of changes of the lipolytic activity in the tested samples of the stored UHT milk was similar.

Changes in the lipolytic activity in UHT milk resulted in changes in the free fatty acids (FFA) content in milk. A comparison of the milks sterilized by the indirect and direct methods indicated that the milk sterilized by the direct method showed a lower content of free fatty acids than the milk sterilized by the indirect method. The average amount of FFA in fresh milk produced by the indirect and direct methods was 0.688±0.162 μEq/mL and 0.540±0.146 μEq/mL, respectively. It can be assumed that a relatively high content of FFA in fresh UHT milk was a result of induced lipolysis caused by the influence of physical factors on milk during pumping, separating and homogenization [Kiełczewska & Kruk, 1997; Staniewski, 1998]. Sensitivity increase in the fat phase under the influence of the above mentioned factors, as well as of heating, encouraged hydrolytic changes in fat conveyed by the thermostable lipases remaining in milk. The lower increase of the FFA content in the milk sterilized by the direct method rather than by the indirect one can be explained by removing some volatile FFA in a vacuum chamber during sterilization by this method.

Milk storage both at room temperature and in cool room conditions caused an increase in the free fatty acids content. After 3 months of storage at 4°C, an average amount of those acids in the milk produced by the indirect method increased up to 0.971±0.148 μEq/mL and up to 1.349±0.252 μEq/mL in the milk stored at 23°C. The free fatty acids content of the milk produced by the direct method after 3 months, was 0.812±0.126 μEq/mL and 1.220±0.287 μEq/mL in the milk stored at 4°C and 23°C, respectively (Figure 2).

Storing tested milk samples for another 3 months caused a further increase in the FFA content depending on the sterilization method and storage temperature. After 6 months of storage, the average amount of FFA in the milk produced by the indirect method was 1.894±0.394 μEq/mL (4°C) and 2.624±0.608 μEq/mL (23°C), whereas in the milk produced by the direct method it was 1.284±0.163 and 2.020±0.313 μEq/mL, respectively. At room temperature, the increase in the FFA contents in milk sterilized by the direct and indirect methods was nearly quadruple as compared to their contents in fresh milk (Figure 2). A significant increase in the FFA content during storage, particularly at the higher temperature, was observed by Schmidt and Renner [1978]. This concerned mainly short-chain fatty acids, which were responsible for the rancid smell and the taste of the milk [Kinsella, 1969; Kwak *et al.*, 1989]. However, Schmidt and Renner state that despite a relatively large increase in the fatty acids level in the stored UHT milk, it did not exceed threshold values perceived organoleptically. In domestic research, Usarek *et al.* [1997] observed a systematic growth of free fatty acids during the storing of milk, and the growth was only slightly dependent on temperature.

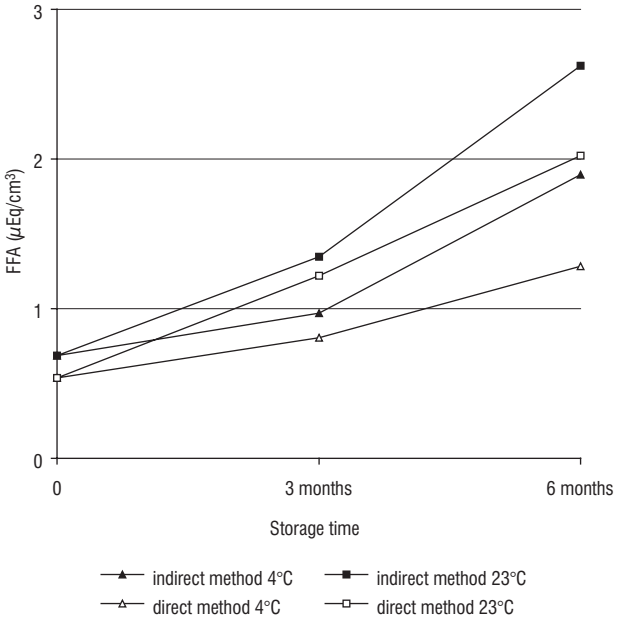

FIGURE 2. Changes of free fatty acids (FFA) content during UHT milk storage.

The majority of authors maintain that the basic reason for the growth of the FFA content during storage of sterilized milk can be found in the low microbiological quality of raw milk and its contamination with psychrotrophic microflora. Mottar [1981] confirms that this relationship does not concern the overall number of psychrotrophs in raw milk, but only lipolytic psychrotrophic bacteria.

Choi's *et al.* [1994] studies concerning isolation and characteristics of lipases from fat globule membranes and UHT milk serum showed the appearance of several active variations of this enzyme, characterised by a different ability to release fatty acids from milk triacylglycerols. They also found that these reactions proceeded with greater intensity at a temperature of 35°C than at 4°C. Results of the studies by Collin *et al.* [1993] also prove that an increase in the free fatty acids content of UHT milk depends on the temperature of storage. The authors, testing milk stored at 20°C, 30°C and 40°C, proved that after 4 months of storage the increase in the free fatty acids content was respectively: 20%, 50% and 80%. As many other authors, they confirm the relation between the change of the FFA content during storage and the quantity of psychrotrophic bacteria in raw milk. Milk produced from the raw material with the largest number of psychrotrophic bacteria was found to contain the highest amount of FFA during storage, although it was not a linear dependency.

Schmidt and Renner [1978] found that the growth of the total content of FFA from 20 to 25% can be noted

organoleptically. However, the majority of researchers maintain that UHT milk sensory properties are influenced by the FFA quality composition, particularly the participation of short-chain fatty acids, rather than by the FFA content. The susceptibility of particular fatty acids to hydrolysis is different and depends, among other factors, on the length of the carbon chain of a fatty acid and the position of its esterification in acylglycerol. Choi and Jeon [1993] observed that medium-chain fatty acids (C10, C12, C14) were released from acylglycerols to the greatest extent. After 12 weeks of UHT milk storage at room temperature, the content of these acids increased by 45–60%. The rate of release of short-chain (C4, C6) and long-chain (C16, C18) fatty acids was significantly weaker, and the growth of the content of these acids under these conditions was from 9% to 26%. Higher storage temperatures accelerated the rate of release in both aforementioned groups of fatty acids in UHT milk.

The conducted determination of the selected contents, characteristic of milk fat fatty acids released into the lipolysis process in UHT milk samples produced by the indirect method and stored for 3 months at 4°C proved the highest average content increase (in proportion to fresh milk) of the following acids: butyric (C4) of 73.8%, caproic (C6) of 63.6%, capric (C10) of 21.8%, palmitic (C16) of 25.7%, oleic (C18:1) of 31.5%, and linoleic (C18:2) of 36%. The results of the analyses showed that an increase in the storage temperature up to 23°C caused a significant growth in FFA content, particularly the short-chain type: butyric acid (C4) – 150% on average, caproic (C6) – 152.5% on average, palmitic (C16) – 70% on average, oleic (C18:1) – 70.5% on average, and linoleic (C18:2) – 86.8% (Figuress 3 and 4).

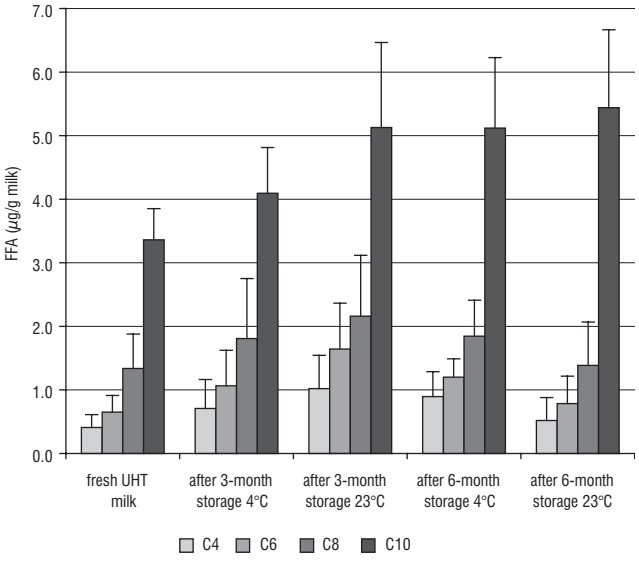

FIGURE 3. Free short-chain fatty acids content in stored UHT milk produced by the indirect method.

In milk samples stored for 6 months at 4°C, the composition and content of free fatty acids did not undergo any significant changes in comparison to their content in the milk samples stored at the same temperature for 3 months. In UHT milk samples stored for 6 months at 23°C, there was a slight decrease in the content of acids C4, C6 and C8 and a slight increase in the content of acid C10, as well as of the long-chain acids in comparison to their content in UHT milk samples stored at the same temperature for 3 months (Figures 3 and 4).

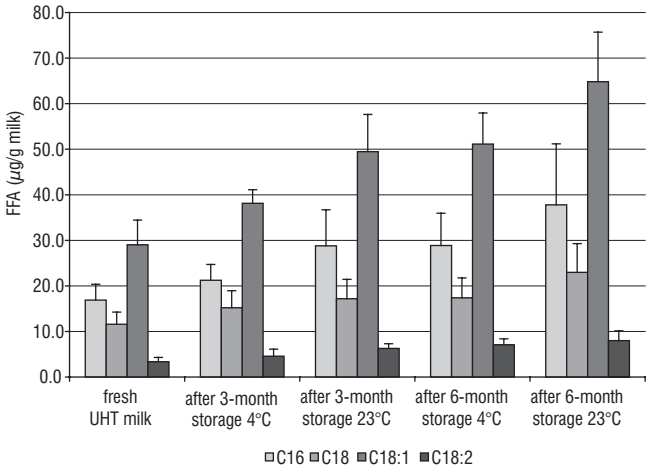

FIGURE 4. Free long-chain fatty acids content in stored UHT milk produced by the indirect method.

In the case of UHT milk produced by the direct method, after the first period of storage at 4°C, a slight decrease was noted in the short-chain FFA content in proportion to their content in fresh milk samples (Figure 5). Similar tendencies were observed in milk samples stored at room temperature (Figure 5). At the same time, a growth of long-chain FFA was observed, except for the contents of acid C16, both in the milk stored at 4°C and 23°C. This increase was higher in the samples stored at the higher temperature (Figure 6). After 6 months of storage, the milk produced by the direct method was further observed. There was a slight decrease in the short-chain FFA content and an increase in the long--chain FFA content, except for acid C16 (Figure 6). Higher rates of change in the acid contents were observed in UHT milk stored at 23°C (Figure 6). The range of content changes in the above mentioned acids in the results of lipolysis in the tested milk samples can be explained by differences in the degree of preserving the native lypolitic activity of milk as well as lipases of bacterial origin after heating

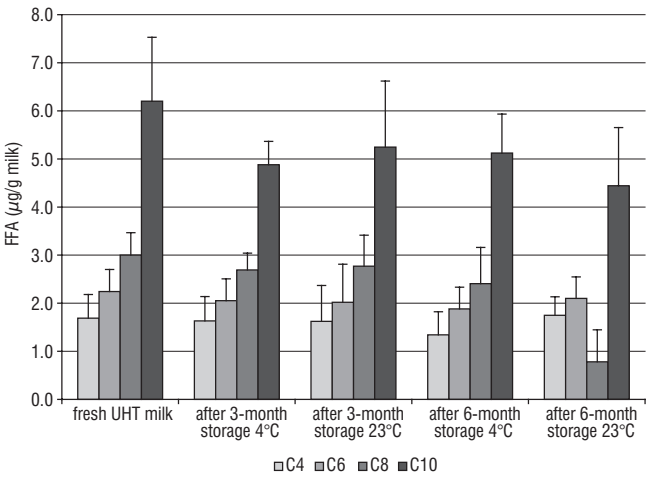

FIGURE 5. Free short-chain fatty acids content in stored UHT milk produced by the direct method.

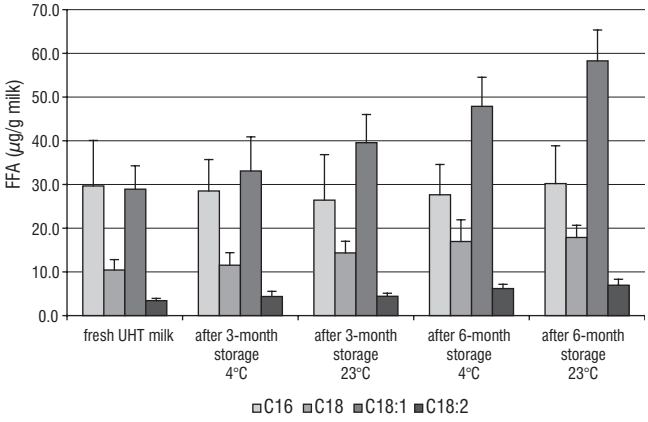

FIGURE 6. Free long-chain fatty acids content in stored UHT milk produced by the direct method.

procedures. It may result from (despite applying the same parameters of heating for each product) the different quality of the raw milk designed for processing, particularly during the period of storage from the moment of obtaining the raw milk till its processing.

The growth of free fatty acids was connected with the growth of fat acidity. The fat acidity value of fresh UHT milk produced by the indirect method ranged from 0.68 to 1.20 and its average value was 0.88. After storing the milk for 3 months at 4°C, an increase in fat acidity value was noted, on average *ca*. 30% (from 0.88 to 1.15), whereas at 23°C the average was 49% (from 0.88 to 1.31) (Figure 7). After 6 months, the fat acidity value in UHT milk increased up to 100% (Figure 7) at both storage temperatures. Increases of fat acidity in milk samples produced by the indirect method were lower as compared to the milk produced by the indirect method. An average increase in the fat acidity value in the milk produced by the direct method after 3 months of storage at 4°C was 23% (from 0.60 to 0.74), whereas at 23°C it was about 43% (from 0.60 to 0.86). After 6 months of storage, acidity values increased respectively to 1.15 at 4°C and to 1.37 at 23°C (Figure 7). The noted tendencies of change in the acidity value were convergent with the previously presented results of the analyses of the total FFA content. Choi and Jeon [1993] found a growth in milk fat acidity value stored at 23°C for 3 months from 0.62 to 0.77 in the case of milk produced by the direct method and from 1.39 to 1.50 in the case of milk produced by the indirect method. Duncan and Christen [1991] showed that the acidity value depended mainly on medium-chain and long-chain free fatty acids, which due to their higher hydrophobicity, remain in milk fat. To a lesser degree this value is affected by short-chain fatty acids, which have a significant influence on the organoleoptic properties of the product. That is why acidity value does not seem to be a reliable index of hydrolytic changes in milk fat.

Besides hydrolytic changes in UHT milk fat, oxidizing changes induced by the presence of oxygen and free fatty acids resulting from lipolysis could occur. The presence of fat oxidizing products, *i.e.* peroxides, aldehydes and low-molecular ketones seems important. The peroxide values for fresh UHT milk fat produced by the indirect method accounted for 0.417±0.173 mgO_2/kg of fat and for the milk produced by the direct method – for 0.160±0.076 mgO_2/kg on average. Regardless of storage conditions and the method of production, an increase in the amount of peroxides in UHT milk after 3 months of storage was observed. In milk produced by the indirect method, the average peroxide values were 0.467±0.086 (4°C) and 0.541±0.114 (23°C) mgO_2/kg fat respectively, whereas after 6 months they were 0.573±0.129 and 0.785±0.225 mgO_2/kg fat, respectively. In milk produced by the direct method and stored for 3 months, the peroxide values were as follows: 0.180±0.069 mgO_2/kg fat (4°C) and 0.217±0.069 mgO_2/kg fat (23°C), and after 6 months: 0.235±0.101 mgO_2/kg fat and 0.270±0.080 mgO_2/kg fat, respectively. The configuration of curves in Figure 8 shows that oxidizing changes were more advanced and proceeded more intensively during the storage of the UHT milk produced by the indirect method than the direct one, particularly at room temperature. Statistical analysis showed significant differences in peroxide values between the milks produced by the indirect and direct methods, fresh

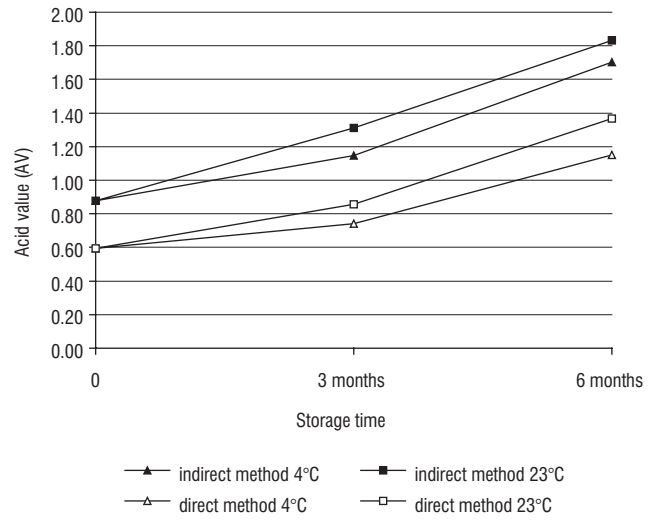

FIGURE 7. Changes of acid value of UHT milk fat during storage.

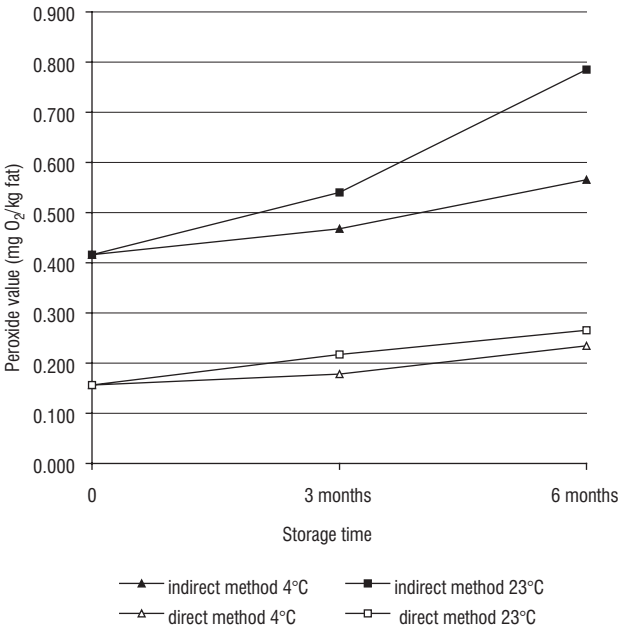

FIGURE 8. Changes of peroxide value of UHT milk fat during storage.

and stored for 3 months at both temperature ranges. After 6 months of storage, significant differences concerned only the products stored at room temperature.

In the case of the TBA test, its initial values in fresh UHT milk produced by the indirect method were slightly lower than in the milk produced by the direct one and reached 0.014 and 0.022, respectively. During storage, an increase in the TBA index value was noted. In milk produced by the indirect method, stored at 4°C after 3 months, the value was 0.029 and after 6 months the value reached the level of 0.051, whereas in the samples stored at 23°C these values were 0.046 and 0.063, respectively. In milk produced by the direct method, the values after 3 and 6 months of storage at 4°C were as follows: 0.031 and 0.053, whereas at 23°C 0.042 and 0.056, respectively. Despite a lower value in the TBA index in samples of fresh UHT milk, changes during storage were the highest in the milk sterilized by the indirect method and stored at 23°C. In the case of both methods, temperature and duration of storage in a similar way influenced the value changes in this test (Figure 9). Nevertheless, variation analysis did not show any significant differences ($\alpha = 0.05$) between the average values in the TBA test in relation to the method of UHT milk production.

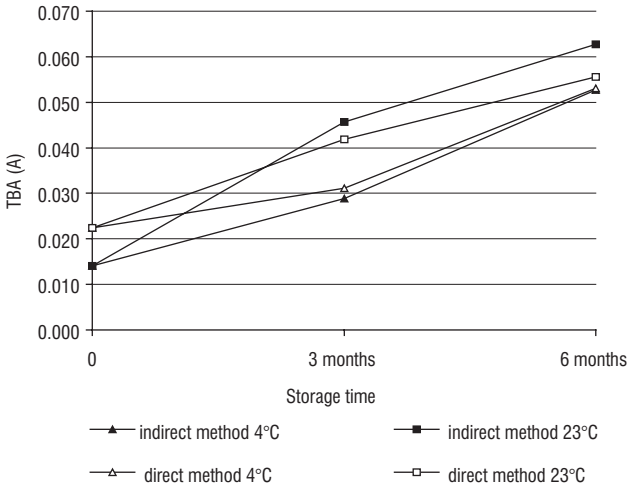

FIGURE 9. Changes of TBA index value during UHT milk storage.

Values in the index of fat oxidizing changes in UHT milk products did not exceed taste detection thresholds stated in Downey's studies [1968], *i.e.* 2.0 mgO_2/kg fat for the peroxide value and 0.080 in the case of the TBA index.

The results of the analyses showed that biochemical changes occurred in milk during its storage. These changes lower the product's quality and can lead to a decline in the commercial acceptability of sterilized UHT milk. It was found that the range and depth of changes in UHT milk fat fraction depended on temperature and period of storage. At room temperature, the intensity of changes was higher than at cool room temperature. If its good quality and commercial value are to be preserved, UHT milk must be stored under cool conditions.

Of significant importance to the fat phase durability is also the activity of lipases in fresh UHT milk, that depends on the psychrotrophic lipolytic microflora activity in raw milk and the type of thermal processing (direct or indirect sterilization).

CONCLUSIONS

1. An increase in the lipolytic activity in UHT milk depended on temperature and duration of storage.
2. The lipolytic activity increase caused an increase in the FFA content of stored UHT milk.
3. Milk samples stored at 23°C were characterised by higher dynamics of changes in the FFA content than those stored at 4°C. In the stored milk samples, a higher range of content changes were observed in short-chain fatty acids rather than in long-chain acids.
4. UHT milk fat was subject to oxidizing changes and the intensity of these changes was higher in milk stored at room temperatures as compared to the product stored in cool conditions.

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WPŁYW WARUNKÓW PRZECHOWYWANIA NA ZMIANY W TŁUSZCZOWEJ FRAKCJI MLEKA UHT

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W pracy określono wpływ warunków przechowywania (3 i 6 miesięcy w temp. 4 i 23°C) na zmiany w tłuszczowej frakcji mleka UHT o zawartości 3,2% tłuszczu wyprodukowanego w warunkach przemysłowych dwiema metodami sterylizacji: pośredniej i bezpośredniej. Oznaczano aktywność lipaz, ogólną zawartość wolnych kwasów tłuszczowych (WKT), zawartość WKT lotnych i nielotnych, zawartość nadtlenków, kwasowość tłuszczu oraz przeprowadzano test TBA. Stwierdzono, że podczas przechowywania mleka UHT nastąpił wzrost aktywności lipaz w tym mleku, zależny od temperatury i czasu przechowywania. Konsekwencją tego był wzrost WKT w produkcie. Zaobserwowano większy przyrost ilości WKT długołańcuchowych aniżeli kwasów o krótkich łańcuchach. Stwierdzono ponadto zmiany oksydacyjne w tłuszczu mleka UHT a ich natężenie było większe w mleku przechowywanym w temperaturze pokojowej w porównaniu z produktem przechowywanym w warunkach chłodniczych.