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FATTY ACID PROFILE OF RAW MATERIALS AND CHOCOLATE MILK MASS DEPENDING ON TEMPERATURE AND TIME OF MIXING

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Summary. The effect of time and temperature of mixing on the fatty acids profile of chocolate milk mass was analyzed. An analysis of fatty acid composition was performed using chromatographic methods in roasted and unroasted cocoa beans and liquor, milk powder, and prepared chocolate milk mass (CM). Both cylinder-dried and spray-dried milk powders had very similar fatty acid profiles. The effect of mixing parameters on the fatty acids composition was shown, but it was ambiguous. The greatest differences were found after 85 and 120 minutes of basketing at temperatures of 70 and 80°C, especially in unsaturated acids.

Key words: cocoa beans, chocolate, cocoa mass, milk powder, fatty acids

INTRUDUCTION

Chocolates are the most preferred product among confectionery assortments. Although dark chocolates are more valuable, milk ones are consumed more often. Milk chocolate is a complex rheological system having solid particles (cocoa, milk powder and sugar) dispersed in cocoa butter (fat phase) [Glicerina et al. 2015]. Cocoa fat is a precious product due to its physical, organoleptic, and chemical properties. The dominant fatty acids in cocoa fat are palmitic (25.0–33.7%), stearic (33.7–40.2%), and linoleic (26.3–35.0%)

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acids [Jahurul et al. 2013]. The fatty acids profiles and their arrangement in triacylglycerols (TAG) influence the high stability of cocoa fat and products derived from it, as well as the physical and sensory properties of these products. Moreover, the location of unsaturated oleic acids between saturated acids provides a natural protective barrier that results in the preservation of their bonds even after technological processing and during storage [Torres-Moreno et al. 2015].

The use of milk with the correct fat content is important for obtaining the right properties of milk chocolate. The most valuable fatty acids in milk are long-chain fatty acids and butyric acids [Lipiński et al. 2012]. The chocolate production process is multistep. The chocolate production process consists of fermenting, drying, roasting, grinding cocoa beans, mixing all ingredients (cocoa liquor, sugar, cocoa butter, emulsifiers, aroma, and dairy ingredients if needed), conching and tempering. Roasting is carried out at temperatures ranging from 120–160ºC and its purpose is to reduce the amount of water, get rid of undesirable volatile compounds, develop precursors of color and flavor, and loosen the structure enabling the separation of the husk from the kernel. During this stage, some bioactive compounds are lost, e.g., polyphenols. To meet the expectations of consumers who pay attention to the quality and composition of the products in terms of nutritional value and the content of bioactive compounds, the technology for the production of "raw chocolate" was developed [Urbańska et al. 2019]. Cocoa beans intended for the production of raw chocolates are not subjected to a roasting process, but to many hours of stirring at temperature not exceeding 55°C, which enables the preservation of its valuable components, including antioxidants [Żyżelewicz et al. 2018]. Conching is carried out to obtain the appropriate viscosity, remove moisture, and impart the desired color [Barišić et al. 2019], as well as to evaporate volatile acid, including acetic acids, alcohols (mainly linalool and 2-phenylethanol), and other volatile compounds (ketones, aldehydes) [Domínguez-Pérez et al. 2020]. Tempering is a process of stirring the cocoa mass for a few hours combined with lowering and increasing the tempera-ture. The aim of this process is to obtain a stable form of crystalline fat (V), so that the chocolate is stable and does not dissolve in the fingers [Urbańska et al. 2019].

The aim of the study was to determine the effect of the properties of raw materials and mixing parameters on fatty acid profile of chocolate milk mass from unroasted cocoa beans.

MATERIAL AND METHODS

The experimental material (Tables 1, 2) consisted of: 2 types of cocoa beans (roasted and unroasted *Forastero,* obtained from producers), 5 cocoa liquors (obtained from producers), 5 milk powders (dried by spray or cylinder method) and chocolate milk mass prepared from these raw materials. The water content is essential indicator in the production of chocolate. Therefore, MLK5 milk with the lowest water content (2.44%) was selected for further research. For the preparation of chocolate milk mass, the cocoa liquor obtained from unroasted cocoa beans was selected due to the low water content (2.25%) (Table 1).

Table 1. List of raw materials: symbols, origin, harvest/milking date, heat treatment, dry matter and protein content

Tabela 1. Wykaz surowców: symbole, pochodzenie, termin zbioru/udoju, obróbka cieplna, zawartość suchej masy i białka

Raw material Surowiec	Sample's symbol Symbole próbek	Country of origin Kraj pochodzenia	Date of harvest Milking Termin zbioru/udoju	Characteristics of the samples Charakterystyka próbek	Dry matter content Zawartość suchej substancji [%]	Protein content Zawartość białka [%]
cocoa beans	Z1	Ghana	2018	roasted	98.10	bo/nm
cocoa beans	Z2	Ivory Coast	2018	roasted	97.50	bo/nm
cocoa beans	Z3	Peru	2018	unroasted, organic farming	93.90	bo/nm
cocoa liquor	MZG1	Ghana	IV 2018	from roasted cocoa beans	98.96	14.25
cocoa liquor	MZG2	Ghana	2017	from roasted coca beans	96.23	14.05
cocoa liquor	MZG3	Ghana	V 2017	from roasted cocoa beans	96.85	15.19
cocoa liquor	MZG4	Ivory Coast	2018	from roasted coca beans	96.92	14.67
cocoa liquor	MZG5	Peru	2018	from unroasted cocoa beans	97.75	13.62
milk preparation	MLK1	France	IV 2018	cylindrically dried	96.55	18.90
milk preparation	MLK2	France	IV 2018	cylindrically dried	97.26	13.19
milk powder	MLK3	Poland	IV 2018	spray dried	95.17	26.43
milk powder	MLK4	Poland	V 2018	spray dried	96.24	27.02
milk powder	MLK5	Poland	V 2018	spray dried	97.56	26.76

Chocolate milk mass (CM) were prepared in a Thermomix machine (Vorwek, Germany, Wuppertal). The mixing used was suitable for the conching process in industrial conditions. Due to the lack of the conche, the Thermomix was used to evaluate the parameters of the mixing stage, referring to the tests carried out by Aidoo et al. 2014. The mass contained: cocoa liquor MZG% (16.2%), cocoa fat (12.3%), sugar (50%), milk powder MLK5 (18%), whey (3.2%), lecithin (0.3%). Fat and cocoa liquors were liquefied at 60°C for 2–3 min and 50°C for 5 min. The liquefied liquor, 10% fat, sugar, milk powder, and whey were dosed and mixed for 10 min at 40°C. The rest of the fat and lecithin were then added. The ingredients were conched at four temperature, 50, 60, 70, and 80°C for 35–120 min, taking a sample for analysis every 10 min (Table 2).

Table 2. Mixing parameters and coding of sample chocolate milk mass (CM)

Tabela 2. Parametry mieszania oraz kodowanie próbek czekoladowych mas mlecznych (CM)

Process time Czas procesu [min]	Process temperature – Temperatura procesu			
	50°C	60°C	70°C	80°C
	MC1	MC2	MC3	MC4
35	MC11	MC21	MC31	MC41
45	MC12	MC22	MC32	MC42
55	MC13	MC23	MC33	MC43
65	MC14	MC24	MC34	MC44
75	MC15	MC25	MC35	MC45
85	MC16	MC26	MC36	MC46
95	MC17	MC27	MC37	MC47
105	MC18	MC28	MC38	MC48
120	MC19	MC29	MC39	MC49

Determination of dry matter content [PN-84/A-88027]

Determination of the dry matter content was carried out using drying to constant weight at 105°C in a WAMED SUP-65 WG laboratory oven (Warsaw, Poland). Samples of cocoa liquors and chocolate milk mass were dried with sand to increase the evaporation surface.

Determination of protein content of the test material by Kjeldahl method

The principle of the method was to digest the samples in concentrated sulfuric acid (VI) in the presence of a catalyst (selenium-copper mixture) in a Buchi 426 Dugestion Unit (Germany, Burladingen). The protein nitrogen was converted to ammonium ion, which after alkalization was distilled as ammonia and bound in excess boric acid in a Buchi B-316 distillation unit. The ammonia solution was determined by potentiometric titration with 0.1 N hydrochloric acid standard solution. The nitrogen content of the sample was calculated knowing that 1 cm^3 of 0.1 N hydrochloric acid solution corresponds to 0.0014 g of nitrogen.

Determination of fatty acids composition [PN-EN ISO 5509:2001]

The analysis was performed using a gas chromatograph coupled to a Shimadzu QP2010 mass spectrometer (Shimadzu Corporation, Japan, Kyoto). A 30 m × 0.25 mm × 0.25 µm bpx70 column was used (SGE Analytical Science, (Ringwood, Australia, Victoria). The initial column temperature was set at 50°C with an increment of 3°C/min until it reached 220°C. The samples were injected in divider mode at a split of 250 : 1. Helium was used as the carrier gas with a flow rate of 0.75 ml/min. The temperature of the dispenser was 250°C and at the line connecting the gas chromatograph to the mass spectrometer 220°C. Other MS operating parameters: ion source temperature 200°C, quadrupole filter sweep range 50–400 m/z, with ionization energy of 70 eV. The fatty acids were identified by comparing the retention time of the acids with the retention time of available standards and data contained in library databases (labraries – NIST 47, NIST 147, and Wiley 175).

Statistical analysis

All determinations were carried out in three replicates. Microsoft Excel 2013 for Windows 10 was used to calculate the average values of the obtained results and to make graphs. Statistical analysis of the obtained results, correlation, and significance of differences using Tukey's test was conducted with the use of Statistica 13.0**.**

RESULTS AND DISCUSSION

Fatty acid profiles in milk powder

Fat in milk powder constitutes about 26% of its total weight. It consists of about 98% TAG, about 2% phosphoglycerides, and trace amounts of diacylglycerides and sterols [Safaei et al. 2020]. It contains many valuable acids in its composition, including conjugated linoleic acids (CLA) (C18:2), which has been attributed to health-promoting effects [Cichosz and Czeczot 2012]. Milk fat is known to inhibit fat efflorescence. It slows the crystallization rate of cocoa butter.

Milk samples were characterized by different content of lauric acid C12:0 (Table 3). MLK4 milk contained 29–38% more of this acid than the other milk samples. The

Table 3. Fatty acid content of the tested samples of milk [%]

Tabela 3. Zawartość kwasów tłuszczowych w badanych próbkach mleka [%]

	MLK1	MLK2	MLK3	MLK4	MLK5
C 6:0	1.39 ^{c,d,e,f}	1.21 ^{c,d}	1.37 ^{a,b}	1.59 ^{a,b}	0.83 ^{a,b}
C 8:0	1.00 ^{a,b,c,d,e}	0.89 ^{b,c}	1.01 ^{a,b}	1.21 ^{a,b}	0.57 ^{a}
C 10:0	1.88 ^{f}	1.70 ^{e}	2.205 ^{a,b}	2.87 ^{c}	2.21 ^{d,e}
C 12:0	2.99 ^{g}	2.72 ^{f}	2.975 ^{a,b}	4.36 ^{d}	3.09 ^{e}
C 14:0	12.23 ^{h}	11.57 ^{g}	12.18 ^{c}	16.32 ^{f}	11.60 ^{f}
C 14:1	0.81 ^{a,b,c,d}	0.78 ^{a,b,c}	0.815 ^{a,b}	1.17 ^{a,b}	0.72 ^{a}
C 15:0	1.00 ^{a,b,c,d,e}	0.96 ^{b,c}	1.065 ^{a,b}	1.51 ^{a,b}	1.23 ^{a,b,c}
C 16:0	36.48 ^{j}	36.25 ^{j}	38.07 ^{e}	20.23 ^{g}	36.04 ^{h}
C 16:1	1.55 ^{d,e,f}	1.49 ^{d,e}	1.6 ^{a,b}	2.18 ^{b,c}	1.71 ^{b,c,d}
C 17:0	0.52 ^{a,b}	0.55 ^{a,b}	0.56 ^{a,b}	0.70 ^{a}	0.74 ^{a}
C 18:0	12.17 ^{h}	12.89 ^{h}	11.26 ^{c}	14.17 ^{e}	11.61 ^{f}
C 18:1	24.09 ^{i}	25.07 ^{i}	23.29 ^{d}	29.40 ^{h}	25.49 ^{g}
C 18:1 trans	0.96 ^{b,c,d,e,f}	1.13 ^{c,d,e}	1.15 ^{a,b}	1.25 ^{a}	1.27 ^{a,b,c}
C 18:2	1.62 ^{e,f}	1.69 ^{e}	1.55 ^{a,b}	2.26 ^{b,c}	1.98 ^{c,d}
C 18:3	0.35 ^{a}	0.35 ^{a}	0.31 ^{a}	0.43 ^{a}	0.38 ^{a}
C 20:0	0.58 ^{a,b,c}	0.60 ^{a,b}	0.625 ^{a}	0.63 ^{a}	0.64 ^{a}

a–j – homogeneous group at α = 0.05, code designation as in in Table 1

a–j – grupy homogeniczne przy, α = 0,05, oznaczenia kodów jak w Table 1

concentration of palmitic acid (C16:0) in MLK4 was significantly lower (about 20.2%) than the other samples (36.1–38.1%). The composition of milk depends on the milking season and the type of feed given to the animals [Lipiński et al. 2012]. Milk obtained in the summer (May– September) has a C16:0 palmitic acid content of 25%, while in the autumn and winter period it is above 34%. The increase concentation palmitic acid is also accompanied by an increase of concentration myristicacid. A similar relationship was observed in the present study. The concentration of myristic acid was significantly higher (16.3%) in MLK4 than in the other samples (11.6–12.2%). As shown by Felkner-Poźniakowska et al. [2012], the concentration of oleic acid increases in summer milk, while it decreases in the autumn and winter months. In the present study, the amount of oleic acid in the MLK4 sample was about 29.4%, while in other samples 15–20 IU less concentration of this acid was found. It may indicate that it is milk from the winter period.

In the study by Felkner-Poźniakowska et al. [2012], the fat of the winter period milk contained more saturated fatty acids, which was mainly influenced by the higher content of palmitic (C16:0), myristic (C14:0) and lauric (C12:0) acids. Milk from the summer period had a higher content of trans isomers of C18:1 and C18:2 acids (4.0 and 3.4%, respectively). In the present study, MLK4 milk from the summer period contained a total 3.5% of C18:1 and C18:2 trans fatty acids, while the milk from the winter period 2.6–3.3% Milk and milk products contain 3–7% of natural trans fatty acids [Kowalska and Cichosz 2013].

Fatty acid profiles of the fats obtained from roasted and unroasted cocoa beans

Cocoa fat properties and fatty acid composition depend on the origin of the bean, cultivar, growing season, and cultivation method [Sirbu et al. 2018]. Soft cocoa fat has a higher content of 1-palmitoyl-2-3-dioleoyl-glycerol (POO) and 1-stearoyl-2-3-diole-

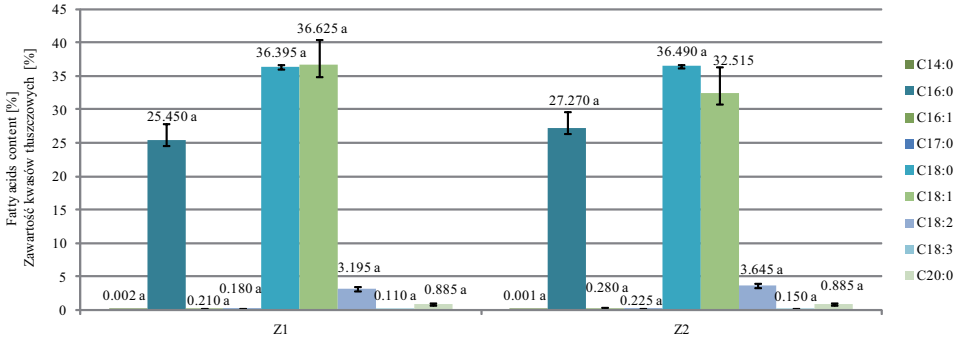

Fig. 1. Average percentage of fatty acids in the fat of roasted (Z1) and unroasted (Z2) cocoa beans; a, b, c – homogeneous group at α = 0.05, code designation as in Table 1

Rys. 1. Średni procentowy udział kwasów tłuszczowych w tłuszczu ziaren kakaowych prażonych (Z1) i nieprażonch (Z2); a, b, c – grupy homogeniczne przy α = 0,05, oznaczenia kodów jak w Tabeli 1

oyl-glycerol (SOO), while harder cocoa fat has an increased content of saturated fatty acids [Sirbu et al. 2018].

The fatty acids with the highest percentage in both roasted Z1 and unroasted Z2 beans (Fig. 1) were: palmitic C16:0 (Z1 – 25.45%, Z2 – 27.27%), stearic C18:0 (Z1 – 35.24%, Z2 – 36.49%) and oleic C18:1 (Z1 – 35.62%, Z2 – 32.51%) acids. In the analyzed beans was also determined 2.5% of polyunsaturated linoleic acid. Comparable results were obtained by Torres-Moreno et al. [2015] and Grassia et al. [2019].

Fatty acid profiles of the fats obtained from cocoa liquors

Cocoa liquor contains about 55% fat. Melo et al. [2020] showed the influence of the cocoa variety used in chocolate processing on the fatty acids profile, which was also confirmed in their study by Torres-Moreno et al. [2015]. In addition, Mustiga et al. [2019] showed the effect of genotype on the chemical composition of the beans, including the fatty acid profile. The results obtained for cocoa liquors obtained from beans cultivated in Ghana (MZG1, MZG2, and MZG3) are the confirmation of the literature data (Table 4). MZG1 was obtained from beans grown one year later than the other beans . This cocoa liquor was characterized by the lowest content of palmitic and linoleic acids, and the highest content of stearic acid.

Table 4. Content of fatty acids [%] in the the fat of the analysed cocoa liquors

Tabela 4. Zawartość kwasów tłuszczowych [%] w tłuszczu analizowanych miazg kakaowych

	MZG1	MZG2	MZG3	MZG4	MZG5
C14:0	0.04	0.07	0.09	0.09	1.53
C15:0	0.01	0.02	0.02	0.03	0.01
C16:0	24.36	27.91	25.18	24.50	29.56
C16:1	0.14	0.19	0.21	0.19	0.29
C17:0	0.15	0.18	0.16	0.17	0.13
C18:0	37.85	29.57	36.95	37.50	34.02
C18:1	34.29	37.77	33.66	33.67	30.98
C18:2	2.32	2.61	2.74	2.27	1.95
C18:3	0.06	0.10	0.11	0.10	0.18
C20:0	0.72	0.99	1.02	0.94	0.91
C22:0	0.03	0.09	0.13	0.10	0.10

MZG1–4 cocoa liquor based on roasted beans, MZG5 cocoa liquor based on unroasted beans

MZG1–4 miazgi kakaowe otrzymane z prażonych ziaren kakaowych, MZG5 miazgi kakaowe otrzymane z nieprażonych ziaren kakaowych

Statistical inference of three dominant fatty acids was carried out in unroasted cocoa beans and liquor obtained from it (Fig. 2). No influence of the crushing and grinding process on the content of analyzed acids was demonstrated.

Fatty acid profiles of the fat obtained from chocolate milk mass

The dominant fatty acids in cocoa butter are palmitic, stearic, and linoleic acids. Their total content in cocoa butters obtained from analyzed chocolate mass ranged from 89.95 to 95.39% (Fig. 3a). An important acid in cocoa butter is also C18:2 linoleic acid, present

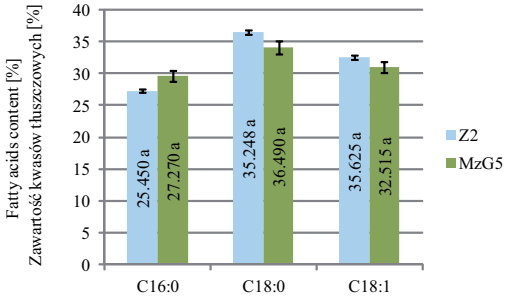

Fig. 2. Influence of roasting on the content of dominant fatty acids in the fats obtained from unroasted cocoa beans and MZG5 liquor

Rys. 2. Wpływ prażenia na zawartość dominujących kwasów tłuszczowych w tłuszczach otrzymanych z nieprażonych ziaren kakaowych i miazgi MZG5

in the concentration approx. 3% in the analysed cocoa butters. Due to its polyunsaturated bonds, this acid is important from a nutritional point of view.

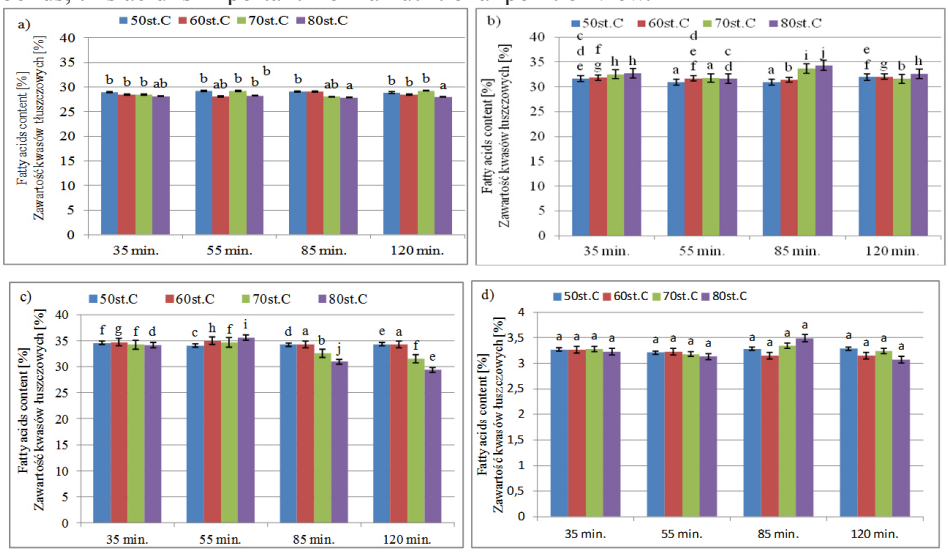

Fig. 3. Effect of temperature and time mixing on the content of dominant fat acids: a) C16:0, b) C18:0, c) C18: 1, d) C18:2, in the tested cocoa butters obtained from chocolate milk mass; a, b, c – homogeneous groups at α = 0.05

Rys. 3. Wpływ temperatury i czasu mixing na zawartość dominujących kwasów tłuszczowych: a) C16:0, b) C18:0, c) C18:1, d) C18:2 w badanym tłuszczu kakaowym uzyskanym z mlecznych mas czekoladowych; a, b, c – grupy homogeniczne przy α = 0,05

Statistical analysis of the effect of mixing temperature and time as a result of the interaction of both factors on palmitic acid C16:0 content (in 15.9 to 30.2% range) showed 3 homogeneous groups (*p*-value 0.0472). However, there was a significant effect of temperature (*p*-value 0.0187) but no influence of mixing time (*p*-value 0.0657) on the analyzed acid. Palmitic acid, like other saturated acids, is stable and shows no significant

changes under the influence of temperature. The content of palmitic acid could change as a result of changes taking place, for example, in C16:1 – palmitoleic acid. This acid was determined in the analyzed mass at the level of 0.2–0.25% of cocoa butters, which was an irrelevant amount for the palmitic acid content.

The content of stearic acid C18:0 significantly increased (in 30.9 to 37.3% range) with increasing temperature and mixing time (Fig. 3b), also as a result of the interaction of both factors, and a similar influence of this process was for C18:1 and C18:2. It was especially visible after 85 minutes of the process at 70 and 80°C. The explanation may be the changes that occurred in the C18:1 linoleic acid, especially after 85 and 120 minutes of mixing at the temperatures of 70 and 80°C (Fig. 3c). The configuration of this acid with one double bond is susceptible to high temperatures which breaks it down. Note that cocoa fat consists mainly of TAGs in which linoleic acid is between the saturated acids. This protects unsaturated bonds from the effects of temperature and external factors. The slight changes obtained in this study confirm these relationships. There was also no evidence of a significant effect of the mixing process parameters on the linoleic acid content. This acid, like oleic acid, is in the TAG configuration between saturated acids, which constitute a protective barrier for unsaturated bonds. The fatty acid profiles in cocoa products (liquor and mass) were similar to those obtained in cocoa beans, which is confirmed by the literature data [Torres-Moreno et. al. 2015, Zyżelewicz 2018, Grassia et al. 2019]. Moreover, Żyżelewicz et al. [2014] showed no significant influence of thermal processes on the fatty acids composition in cocoa butter, which is consistent with the results obtained in this study.

CONCLUSIONS

The roasting process did not significantly affect the fatty acid profile of cocoa beans and liquors. Small differences in the composition of fatty acids may result from the region of origin and date of cocoa bean harvest. For all analyzed samples, the most important and dominant fatty acids were palmitic acid C16:0, stearic acid C18:0 and oleic acid C18:1. The fatty acid composition of milk fat depended on the season of obtaining raw milk. Both rolled and spray-dried milk powders had very similar fatty acid profiles. The effect of mixing parameters on the composition of fatty acids was shown, but it was unequivocal. The greatest differences were found after 85 and 120 min of basketing at temperatures of 70 and 80°C. These differences were seen in the unsaturated fatty acids and stearic acid with the same number of carbon atoms as the unsaturated acids.

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PROFIL KWASOWO-TŁUSZCZOWY SUROWCÓW I MLECZNYCH MAS CZEKOLADOWYCH W ZALEŻNOŚCI OD TEMPERATURY I CZASU MIESZANIA

Streszczenie. Przeanalizowano wpływ zmiennych parametrów czasu i temperatury konszowania na profil kwasów tłuszczowych czekoladowych mas mlecznych. Przeprowadzono analizę składu kwasów tłuszczowych metodami chromatograficznymi w prażonych i nieprażonych ziarnach kakaowych i miazgach z nich otrzymanych, mleku w proszku oraz w przygotowanych czekoladowych masach mlecznych. Skład kwasów tłuszczowych tłuszczu mlecznego nie był zależny od metody uzyskania proszku mlecznego, a od pory roku pozyskania mleka surowego. Zarówno proszki mleczne suszone cylindrycznie, jak i suszone rozpyłowo charakteryzowały się bardzo zbliżonym profilem kwasów tłuszczowych. Profil kwasów tłuszczowych w konszowanych masach czekoladowych nie zależał od czasu i temperatury procesu, ale od właściwości surowców określonych przez region pochodzenie lub okres pozyskania.

Słowa kluczowe: ziarna kakaowe, miazga kakaowa, mleko w proszku, kwasy tłuszczowe