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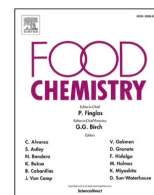

Effect of microwave and convection heating on selected nutrients of human milk

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ABSTRACT

The aim of this study was to determine the influence of the microwave heating method (MWH) on selected determinants of the nutritional value of human milk (HM) and compare to the effect exerted by the standard convection heating (CH) method, including holder pasteurization (HoP). It was showed that using MWH under conditions assumed to ensure microbiological safety, changes in the level of the nutrients were not observed. In these conditions concentration of fatty acids (FAs), lipid peroxides (LP) and α-lactalbumin (α-La) were on a similar level as in raw milk, and furosine was not formed. MWH treatment of HM led to slight increase in the malonaldehyde (MDA) and protein carbonyls (PC) content. However, MDA content in HM as a result of MWH was lower than after application CH.

1. Introduction

Human Milk Banks (HMB) are institutions that collect, pasteurize and properly store donors' milk and subsequently transfer to neonatal units where babies who were born prematurely or with low or extremely low birth weight are fed. Donors' milk is not only a part of newborns diet because it not only provides necessary nutrients, but is also considered as a factor determining health in adulthood (Gartner et al., 2005). Although HM microbiota is an important component for the proper development of the immune resistance and infant's intestinal microbiota, due to sensitivity of HMB beneficiaries, the donors' milk is pasteurized at 62.5 °C for 30 min (LTLT – low temperature long time pasteurization or holder pasteurization – HoP). Such conditions ensure microbiological safety, but lead to a significant reduction of nutrients and biologically active components such as immunoglobulins, enzymes, cytokines and vitamins (Peila et al., 2016). Therefore, research on the possibility of implementing other methods of HM preservation into the HMB environment is currently conducted by many research teams (Escuder-Vieco et al., 2018; Wesołowska et al., 2018). Flash-heating to pasteurization of HM is already being successfully used in the HMB at King Edward Hospital in Pietermaritzburg ("Program for Appropriate Technology in Health" handout: https://path.azureedge.net/media/documents/MCHN_5_milk_banking_models.pdf).

In our previous studies, we showed that the degree of inactivation of the HM microbiota depends on the initial level of contamination. In milk where the initial number of vegetative microbiota does not exceed 10^5 colony forming units (cfu) per mL (according to European Milk Bank Association maximal recommended level of cfu/mL of milk), complete inactivation can be achieved at the temperature of 62.5 and 66 °C after 5 and 3 min, respectively, of using microwave heating (MWH) (Malinowska-Pańczyk et al., 2019). In these experiments, a prototype pasteurizer (Enbio Technology Co.) was used, which allowed heating of milk in strictly programmed conditions (time and temperature), and its rapid cooling after the process.

The aim of the studies was to determine the influence of MWH on selected nutrients of HM: lipids and proteins, and compare to the effect exerted by the standard convection method commonly used in HMB.

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Table 1
Demographic and obstetrical characteristics of study participants.

Parameter	Value*
No. of subjects	25
Maternal age (years)	27.6 ± 4.8 (19–37)
Parity (n)	1.4 ± 0.71 (1–4)
Maternal weight at delivery (kg)	77.0 ± 9.48 (59–94)
Gestational age (weeks)	39.9 ± 1.42 (37–42)
Birth weight (g)	3 453 ± 422 (2750–4515)
Apgar score at 1st min (points)	9.8 ± 0.37 (9–10)
Infant gender	female 14, male 11
Lactation time (day)	29.0 ± 2.39 (22–31)

*Values presented as arithmetic means ±(SD) and ranges (in parenthesis).

2. Materials and methods

2.1. Milk preparation

Mature milk was collected between days 21 and 30 after birth from 25 healthy mothers who gave birth on the scheduled date and without complications at the Department of Obstetrics of the Clinical Hospital in Gdańsk (Table 1). All newborns were in good health (Apgar score ≥ 9 in the first minute of life), and their body weights were within the norm (2750 – 4515 g). HM was sampled by the mothers at home with an electric breast pump (Symphony, Medela Polska Sp. z o.o., Warsaw, Poland) into sterile containers designed for food contact, according to standard hygiene requirements. The samples were collected by the mothers within 24 h and stored in a refrigerator at around 5 °C. Milk samples from different mothers were delivered to the laboratory at the same time; they were immediately pooled and divided into smaller samples. Milk was frozen and stored at −80 °C until analysis, but not longer than three weeks.

All of the experimental procedures were approved by the Local Ethics Committee of the Medical University of Gdansk. The patients gave written consent to participate in the study.

2.2. Convection heating

Milk samples (50 mL) were transferred to screw-cap laboratory bottles DURAN® and heated to the appropriate temperature in a water bath. The temperature at the central point of the laboratory bottles was controlled with a digital laboratory thermometer. The temperature 62.5 °C was achieved within 18.1 ± 0.8 min, which is equivalent to the time of achieved preset temperature during HoP in medical pasteurizers used in HMB. Heating the milk samples to the temperature of 66 and 70 °C was 18.3 ± 1.0 and 18.8 ± 1.2 min. The milk samples were held at each temperature for 30 min. Samples for analysis were taken after reaching the appropriate temperature (time 0), and then every 10 min. The processed milk was immediately cooled to 20 °C by immersion in an ice/water bath. All treatments were performed in three replications.

2.3. Microwave heating

MWH was achieved by subjecting milk to microwave field as described previously in Patent No. PL235534-B1 (2020). HM were heated in a microwave prototype device where the temperature was kept constant for a limited time (2450 MHz, 800 W, Enbio Technology, Poland). Milk samples (50 mL) were transferred to a screw-cap laboratory bottles DURAN® and placed in a microwave pasteurizer. During the process, the milk was stirred by pumping in a closed circuit by using silicon pipes. The milk was flowing through a temperature sensor, which turned on and off of the magnetron in depending on the recorded milk temperature (Martysiak-Żurowska, Puta & Kiełbratowska, 2019). The temperature 62.5, 66 and 70 °C were reached within about 2.6 ± 0.3, 2.9 ± 0.5 and 3.3 ± 0.5 min, respectively. Milk samples were heated at each temperature for 1, 3, 5 and 10 min. Previous studies have shown that the complete pasteurization effect can be achieved by using MWH at 62.5 or 66 °C for 5 and 3 min, respectively and guarantee the microbiological safety of the processed milk (Malinowska-Pańczyk et al., 2019). All treatments were replicated three times.

2.4. Determination of essential macronutrients

MIRIS Human Milk Analyzer (HMA) (Miris AB, Uppsala, Sweden) based on semi-solid mid-infrared (MIR) transmission spectroscopy, was used to analyze macronutrients: total fat (g/100 mL), total protein (g/100 mL), nutritional protein (g/100 mL), total sugar (g/100 mL) and energy content (kJ/100 mL) in milk samples. A daily calibration check was performed prior to analysis using the calibration solution provided by the supplier. Total protein, refers to content based on the total amount of nitrogen in a sample; non-protein nitrogen compounds are also included in this value. True protein is corrected for non-protein nitrogen compounds and represents only the content of actual protein. Each sample before analysis was heated to 40 °C in a thermostatic bath and then homogenized using MIRIS Sonicator. Repeatability for instrument was defined based on information published in the Miris HMA Human Milk Analyzer User Manual: fat, crude protein, true protein ≤ 0.05 g/100 mL; carbohydrate ≤ 0.08 g/100 mL (Available from: https://www.mirissolutions.com/support/user-manuals). Precision of the determination of crude protein, true protein, fat and carbohydrate for the using Miris HMA reached 10.51, 9.56, 8.31 and 3.49%, respectively. Each sample was analyzed in triplicate.

2.5. Determination of fatty acid composition

Lipids were extracted from HM samples by the Röse-Gottlieb method (EN:ISO 1211:2011). Fatty acid methyl ester (FAME) profiles were determined according to the European Standard (EN:ISO 5509:2000). FAMEs were separated by high-performance gas chromatography (HP-GC) based on the length of the hydrocarbon chain and the degree of FAME unsaturation. Analyses were carried out using a Hewlett-Packard GC system (Hewlett-Packard, Palo Alto, CA, USA) with a split/splitless injector, Rtx 2330 column (100 m × 0.25 mm, Restek, Bellefonte, PA, USA) and a flame ionization detector (FID). FID and injector temperature were maintained at 250 °C. The initial column oven temperature was 155 °C for 30 min, after which it was gradually increased to 210 °C at the rate of 1.5 °C/min and held at this temperature until the end of the analysis. Qualitative and quantitative analyses of FAs in the evaluated samples were performed with FAME standards (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) (Martysiak-Żurowska et al., 2011). The results were expressed as the percentage of the individual FAs in total FAs.

2.6. Determination of lipid peroxides (LP)

LP in milk was determined by the method described by Turoli Testolin Zanini & Bellu (2004). LP were recovered by liquid–liquid extraction of milk sample with chloroform/methanol (2:1, vol:vol). The organic layer was evaporated under nitrogen and the sample was dissolved in 1.8 mL of acetic acid/chloroform solution (3:2, vol:vol). Next 120 µl saturated solution of potassium iodide was added and the mixture was incubated at room temperature for 15 min. Then, 1.8 mL of 60 mM cadmium acetate was added to stop the reaction. After centrifugation at 10 °C, 3 000 × *g* for 5 min, the aqueous upper layer absorption was read at 353 nm. The content of LP in µM was calculated based on the value of ε for I_3^- which is set at 2.19×10^4 L/M × cm at 353 nm (Balla et al. 1991).

2.7. Determination of malondialdehyde (MDA)

A quantitative analysis of the MDA as MDA:2TBA complex was carried out by HPLC. The analytical procedure with some alterations was based on methods described by Fenaille et al. (2001). A 1 mL

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portion of HM was collected into centrifuge tubes. The reaction mixture (1 mL) containing equal volumes of 15% trichloroacetic acid (TCA), 0.25 M HCl and 0.375% thiobarbituric acid (TBA) as well as 0.1 mL of a 0.4% solution of butylated hydroxytoluene (BHT) in 96% ethanol were added. The mixture was homogenized and centrifuged at 6 000 × *g* for 5 min. The upper phase was transferred to twist test tubes. The precipitate left was again homogenized with 1 mL of the reaction mixture and centrifuged. The combined upper phases were heated for 30 min at temperature of 95 °C under nitrogen atmosphere. Then, the reaction was stopped through rapid chilling of the test tube in ice bath. The mixture was made up to 4 mL with distilled water.

RP-HPLC conditions: PerkinElmer Series 2000 chromatograph, chromatographic column Hypersil® BDS C18 (250 × 4.6 mm, d_p 5 μm) (Agilent Technologies), column temperature 20 °C, detection at 534 nm, flow rate 0.7 mL/min, injection volume 10 μl. The mobile phase was 5 mM phosphate buffer (pH 7.0):ACN (85:15, vol:vol). In these parameters, the retention time of MDA:2TBA complex was 8.76 min. Standard MDA solution was obtained by acid hydrolysis of 1,1,3,3–tetramethoxypropane (TMP, Sigma–Aldrich, Merck KGaA, Darmstadt, Germany) in 0.01 M hydrochloric acid. Using standard solutions of MDA a series of dilutions in the range of from 0.97 to 306.28 μg MDA/L was made. Method detection limit (MDL) of MDA with the use of described technique was 3.93 μg MDA/L. Recovery of MDA from the spiked milk sample reached 98.7%. The results were expressed in μg MDA/100 mL of the sample.

2.8. Determination of furosine (FUR) content

FUR content was determined by Ion-Pair Reversed Phase High-Performance Liquid Chromatography with UV detection. The analytical procedure was performed on the basis of methods used by Martysiak-Żurowska & Stołyhwo (2007).

Acid hydrolysis: One and a half millilitres of HM sample was put in a hermetically closed vial with 8 mL of 8 M HCl. Samples were blown out for 1 min by a stream of nitrogen, closed and heated for 23 h at 110 °C. After that time, the hydrolysates were cooled, filtrated and filled up with water to 8 mL. Directly before HPLC analysis 1 mL of hydrolysate was neutralized with 0.4 NaOH to pH 7 and diluted with distilled water to 3 mL. Three mL were withdrawn from the resulting solution and passed through a SPE C-18 column (Bakerbond), and then the FUR adsorbed on the bed was eluted with 2 mL of water.

RP-HPLC conditions: PerkinElmer Series 2000 chromatograph, Zorbax SB-C18 (250 × 4.6, I.D., d_p 5 μm) chromatographic column (Merck Darmstadt, Germany), column temperature 25 °C, detection at 280 nm, flow rate 0.8 mL/min, injection volume 20 μl. As mobile phase solution of 5.5 mM sodium heptanesulfonate with 15% acetonitrile and 0.2% formic acid in ultrapure water was use. In these parameters, the analyte retention time was 9.5 min. Using a standard of FUR (PolyPeptide Group, Limhamn, Sweden) a series of dilutions in the range of 0.03155 to 0.5027 μg FUR/mL was made. MDL of FUR for used methodology accounted 0.26 mg/100 g of proteins. The average recovery of FUR equal 74.25% was determined by the using spiked samples. The results were expressed in mg FUR/100 g of protein.

2.9. Determination of protein carbonyls

The methodology of determination of the level of oxidized proteins on the basis of the number of carbonyl groups using the Levine spectrophotometric method was adapted from Scheidegger, Pecora, Radici, & Kivatinitz (2010). Two hundred microliters of HM were incubated with 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl (1 mL volume), for 30 min at room temperature. A control sample was simultaneously prepared by adding 1 mL of 2 M HCl instead of 2,4-DNPH in 2 M HCl. Milk proteins were precipitated with 10% (wt/vol) TCA (final concentration) and recovered by centrifugation at 7 500 × g for 5 min. The supernatant was removed and protein pellets were washed 3 times with 1 mL of ethanol/ethyl acetate (1:1; vol:vol) to remove free DNPH reagent. Finally, protein re-dissolved in 1 mL of 6 M guanidine hydrochloride, pH 2.3. Protein carbonyls were determined by UV spectrophotometry at 360 nm against control sample using an extinction coefficient of 2.2×10^4 M/cm (Scheidegger et al. 2010), with a Jenway 6305 UV–Vis spectrophotometer (Jenway, Bibby Scientific, Staffordshire, UK). Results were expressed as nanomoles of carbonyl per milligram of protein.

2.10. Determination of α-lactalbumin (α-La) content

α-La content of HM was determined according to the method of Santos & Ferreira (2007). Milk samples were centrifuged (4 °C, 12,000 × g, 15 min) and the clear supernatant was collected, and vial contents were diluted 1:5 (vol:vol) with ultra-pure water. The samples were analyzed by High-Performance Liquid Chromatography in the Perkin Elmer series 200 chromatograph with a UV–VIS detector. Separation was performed on the Kinetex C18 chromatographic column (4.0 × 150 mm, 5 μm, 100A, Phenomenex) with a guard column SB-C18 (4.6 × 12 mm). The analysis was carried out in linear gradient elution mode from 65% A:35% B (0 min) to 50% A:50% B (20 min), where eluent A was 0.1% trifluoroacetic acid in distilled water, and eluent B was 0.1% solution of trifluoroacetic acid in acetonitrile. Volume flow was 1 mL/min. Recovery of α-La was calculated by comparing the results for the directly injected α-La standards (0.1, 0.6 and 1.0 mg/mL) with the concentration of α-La in the spiked samples at 0.1, 0.6 and 1 mg/mL. α-La recovery was determined at 98.42%. The α-La content of milk was calculated with a calibration curve where peak area was plotted against α-La concentration in standard solutions (α–Lactalbumin from HM, Sigma-Aldrich, St. Louis, MO, USA).

2.11. SDS-PAGE electrophoresis

Samples of HM (10 μl) were dissolved in 10 μl of 0.125 M Tris-HCl buffer (pH = 6.8) containing 4% SDS, 20% (by volume) glycerol, 0.2 M dithiothreitol (DTT), 0.02% bromophenol blue (reducing conditions) or containing 20% (by volume) glycerol and 0.02% bromophenol blue (non-reducing conditions). Mixtures were heated at 95 °C for 5 min and subsequently centrifuged at 1000 × g for 2 min. Ten microliters of samples were deposited on the 5% stacking gel. Separation was performed on 15% gel. Molecular weight standard from 250 to 10 kDa (HyperPage II Prestained Protein Marker – Bioline no. BIO-33067) was used as a reference. Samples were separated on the Vertical Electrophoresis System (Bio-Rad). Fixation of the proteins was by 12% trichloroacetic acid for 1 h and staining with 0.125% Coomassie brilliant blue R-250 and then destained in a solution containing 50% methanol and 10% acetic acid.

2.12. Statistical analysis

The statistical analysis was conducted in SigmaPlot 11.0 (Systat Software, Inc., CA). The results were presented as mean values and standard deviations (mean ± SD). The normality of all variables was evaluated with the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) and Tukey's HSD test was used to determine if treatment time in a given temperature and way of temperature generation (MWH, CH) had the significant influence on the content or activity of the compounds in processed HM. Differences between means were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Macronutrients contents

In the tested samples of pooled raw HM, the total protein content was 1.6 g/100 mL, carbohydrates 7.25 g/100 mL, fat 3.58 g/100 mL, water

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Table 2
Concentration of macronutrient and energy content in raw HM (control) and pasteurized HM by convection and microwaves heating under different conditions (temperature and time).

Heating time [min]	Content of						Heating time [min]	Content of					
	Crude protein (g/100 mL)	True protein (g/100 mL)	Carbohydrates (g/100 mL)	Total fa(g/100 mL)	Energy value (kJ/100 mL)	Water (g/100 mL)		Crude protein, (g/100 mL)	True protein (g/100 mL)	Carbohydrates (g/100 mL)	Total fat (g/100 mL)	Energy value (kcal/100 mL)	Water (g/100 mL)
	after convection heating at						after microwaves heating at						
	62.5 °C												
Raw milk	1.30 ± 0.02^{a}	1.60 ± 0.04^{a}	7.25 ± 0.05^{a}	3.58 ± 0.08^{a}	293.08 ± 3.43^{a}	87.33 ± 0.14^{a}	Raw milk	1.30 ± 0.04 ^{a}	1.60 ± 0.05^{a}	7.25 ± 0,05^{a}	3.58 ± 0.08^{a}	291.82 ± 3.43^{a}	87.33 ± 0.14^{a}
RST*	1.33 ± 0.05^{a}	1.63 ± 0.05^{a}	7.28 ± 0.04^{a}	3.77 ± 0.10^{b}	297.26 ± 2.13^{a}	87.17 ± 0.08^{a}	RST*	1.25 ± 0.05^{a}	1.45 ± 0.05^{b}	7.18 ± 0.04^{a}	3.62 ± 0.16^{a}	290.98 ± 3.52^{a}	87.15 ± 0.16^{a}
10	1.37 ± 0.05^{a}	1.70 ± 0.04^{a}	7.30 ± 0.04^{a}	3.82 ± 0.04^{b}	302.29 ± 1.72^{b}	86.97 ± 0.05^{a}	1	1.27 ± 0.08^{a}	1.62 ± 0.10^{a}	7.35 ± 0.05^{b}	3.70 ± 0.15^{a}	293.91 ± 5.57^{a}	87.20 ± 0.15^{a}
20	1.32 ± 0.04^{a}	1.65 ± 0.05^{a}	7.25 ± 0.05^{a}	3.82 ± 0.04^{b}	299.36 ± 2.30^{a}	87.13 ± 0.06^{a}	3	1.28 ± 0.10^{a}	1.62 ± 0.10^{a}	7.35 ± 0.05^{b}	3.75 ± 0.14 ^{a}	297.26 ± 6.49^{a}	87.12 ± 0.20^{a}
30	1.27 ± 0.08^{a}	1.60 ± 0.11^{a}	7.32 ± 0.10^{a}	3.77 ± 0.15^{b}	298.10 ± 5.57^{a}	87.12 ± 0.08^{a}	5	1.30 ± 0.11^{a}	1.60 ± 0.11^{a}	7.35 ± 0.05^{b}	3.75 ± 0.16 ^{a}	295.17 ± 6.87^{a}	87.07 ± 0.16^{a}
							10	1.29 ± 0.10^{a}	1.61 ± 0.09^{a}	7.33 ± 0.04^{b}	3.71 ± 0.12 ^{a}	294.3 ± 1.42^{a}	87.10 ± 0.11^{a}
	66 °C												
Raw milk	1.30 ± 0.02^{a}	1.60 ± 0.04^{a}	7.25 ± 0.05^{a}	3.58 ± 0.08^{a}	293.08 ± 3.43^{a}	87.33 ± 0.14	Raw milk	1.30 ± 0.04^{a,b}	1.60 ± 0.05^{a}	7.25 ± 0.05^{a}	3.58 ± 0.08^{a}	291.82 ± 3.43^{a}	87.33 ± 0.14^{a}
RST*	1.32 ± 0.05^{a}	1.61 ± 0.05^{a}	7.29 ± 0.04^{a}	3.78 ± 0.10^{b}	297.26 ± 2.13^{a}	87.15 ± 0.18^{a}	RST*	1.27 ± 0.05^{a}	1.55 ± 0.05^{a}	7.26 ± 0.04^{a}	3.71 ± 0.16^{a,b}	295.17 ± 3.52^{a}	87.05 ± 0.10^{a}
10	1.27 ± 0.08^{a}	1.60 ± 0.11^{a}	7.35 ± 0.10^{a}	3.72 ± 0.13^{b}	297.26 ± 2.13^{a}	86.96 ± 0.06^{b}	1	1.35 ± 0.05^{b}	1.70 ± 0.04^{b}	7.23 ± 0.05^{a}	3.85 ± 0.05^{b}	300.61 ± 3,14^{a}	86.98 ± 0.12^{a}
20	1.30 ± 0.11^{a}	1.60 ± 0.11^{a}	7.30 ± 0.05^{a}	3.68 ± 0.13^{a,b}	302.29 ± 1.72^{b}	86.98 ± 0.08^{a}	3	1.38 ± 0.04^{b}	1.71 ± 0.04^{b}	7.30 ± 0.04^{a}	3.87 ± 0.05^{b}	298.10 ± 4,10^{a}	87.03 ± 0.10^{a}
30	1.28 ± 0.10^{a}	1.60 ± 0.11^{a}	7.33 ± 0.05^{a}	3.72 ± 0.13^{b}	296.01 ± 6.32^{a}	87.01 ± 0.09^{a}	5	1.33 ± 0.05^{a,b}	1.68 ± 0.04^{a,b}	7.30 ± 0.04^{a}	3.78 ± 0.04^{b}	300.17 ± 2.18^{a}	87.06 ± 0.13^{a}
							10	1.35 ± 0.05^{a}	1.70 ± 0.04^{b}	7.29 ± 0.04^{a}	3.82 ± 0.04^{b}	298.10 ± 2.09^{a}	86.96 ± 0.09^{a}

*RST - Reaching the set temperature. Each value is expressed as mean ± SD of three samples per triplicate.
^{a,b,c} – Means value within a column with different superscripts are significantly different at a given temperatures ($P < 0.05$).

87.33 g/100 mL and calorific value 293,08 kJ/100 mL (Table 2). There was no significant effect of CH at 62.5 and 66 °C on the content of total protein, nutritional protein, carbohydrates, water and energy value of HM. Similarly, García-Lara et al. (2013) and Espinosa-Martos et al. (2013) found that HoP did not affect the protein content and carbohydrates in HM. A statistically significant higher fat content, by 6.7% in relation to the fat content in raw milk, was found in milk samples heated (CH) at 62.5 °C for 10 and 20 min (Table 2). A higher level of fat was also found in milk heated at 66 °C, although the increase in fat content was not higher than 5.6%. García-Lara et al. (2013) found a slight, 3.5% decrease in fat content in milk after HoP.

In the case of MWH, the fat content was stable in milk samples subjected to heating at 62.5 °C, while an increase in the fat content was found in milk heated at 66 °C. After 1 and 3 min of MWH, the fat content of the milk samples was higher than that of the raw milk, by about 7.5 and 8%, respectively. No statistically significant changes were found in the concentration of the nutrients in comparison with raw milk in the samples of HM heated by MWH in conditions ensuring the total pasteurization effect (62.5 °C, 5 min). Also, the use of higher MWH temperatures did not affect the basic nutrients and content of water in HM. Data on the influence of MW heating on the nutrients of HM are fragmentary. Tacken et al. (2009) did not find differences in these components in raw and MWH milk at temperature 35–40 °C for 15–30 s.

The two-way ANOVA analysis results showed that influence of time and temperature of HM heating on the nutrients is independent of the way of generating the high temperature (MWH or CH). The observed changes in fat content in samples subjected to heating probably result from a change in the form in which fat is present in milk and the measurement technique used in the study. The fat in HM occurs in the form of globules with an average diameter of 4 μm, surrounded by a triple phospholipid-protein membrane, the so-called milk fat globule membrane (MFGM) (Lopez & Ménard, 2011). Treatment of HM by pasteurization, freezing, spray drying and lyophilization affects the structure of fat components, partially disrupting the MFGM, leading to a significant decrease in the diameter of the fat globules (Cavazos-Garduño et al., 2016). HMA™ based on semi-solid mid-infrared transmission spectroscopy was used to analyze content of macronutrients. The wave ranges used in the device are specific for groups: carbonyl (5.7 μm) for fat, amide groups (6.5 μm) for protein and hydroxyl groups (9.6 μm) for carbohydrate. Rémillard, Robin, Martel, & Paquin (1993) showed a

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Table 3
Content of selected fatty acids FA (% of total FA) in raw HM (control) and HM pasteurized by CH and MWH under different conditions (temperature and time).

Fatty acid		Content (% of total FA) in HM after CH for				Content (% of total FA) in HM after MWH for			
	Raw milk	RST*	10 min	20 min	30 min	1 min	3 min	5 min	10 min
		62.5 °C							
SFA	39.3 ± 1.55	38.8 ± 1.50	38.8 ± 1.53	38.9 ± 1.49	38.7 ± 1.49	38.8 ± 1.50	38.9 ± 1.55	38.9 ± 1.49	38.7 ± 1.59
MUFA	45.8 ± 1.26	46.1 ± 1.23	46.1 ± 1.21	45.8 ± 1.20	45.7 ± 1.42	45.9 ± 1.36	45.9 ± 1.15	45.9 ± 1.22	46.0 ± 1.32
PUFA	14.7 ± 0.53	14.8 ± 0.56	14.8 ± 0.67	14.9 ± 0.66	14.8 ± 0.55	15.1 ± 0.62	14.9 ± 0.66	14.9 ± 0.65	15.0 ± 0.59
PUFA (n-6)	11.9 ± 0.46	12.1 ± 0.45	12.1 ± 0.38	12.1 ± 0.35	12.1 ± 0.35	12.2 ± 0.44	12.1 ± 0.38	12.1 ± 0.35	12.1 ± 0.35
PUFA (n-3)	2.0 ± 0.08	2.0 ± 0.07	2.0 ± 0.07	1.9 ± 0.08	1.9 ± 0.08	2.0 ± 0.08	2.0 ± 0.07	2.0 ± 0.09	2.0 ± 0.09
		66 °C							
SFA	39.3 ± 1.55	40.9 ± 1.53	39.9 ± 1.50	40.2 ± 1.38	40.8 ± 1.44	40.6 ± 1.49	39.9 ± 1.56	40.9 ± 1.51	40.9 ± 1.58
MUFA	45.8 ± 1.26	46.8 ± 1.33	44.9 ± 1.21	45.6 ± 1.25	44.9 ± 1.33	44.9 ± 1.37	45.5 ± 1.18	44.8 ± 1.31	44.0 ± 1.27
PUFA	14.7 ± 0.53	14.2 ± 0.55	14.3 ± 0.66	14.7 ± 0.59	14.3 ± 0.58	14.3 ± 0.63	14.4 ± 0.71	14.1 ± 0.61	14.0 ± 0.60
PUFA (n-6)	11.9 ± 0.46	11.5 ± 0.47	11.9 ± 0.38	11.6 ± 0.40	11.5 ± 0.39	11.6 ± 0.45	11.7 ± 0.41	11.4 ± 0.37	11.4 ± 0.36
PUFA (n-3)	2.0 ± 0.08	1.9 ± 0.07	1.9 ± 0.06	2.0 ± 0.09	1.9 ± 0.08	1.9 ± 0.08	1.9 ± 0.08	1.9 ± 0.10	1.9 ± 0.10
		70 °C							
SFA	39.3 ± 1.55	40.6 ± 1.48	39.5 ± 1.53	40.2 ± 1.51	40.4 ± 1.49	40.0 ± 1.46	40.2 ± 1.53	39.6 ± 1.48	40.0 ± 1.56
MUFA	45.8 ± 1.26	44.8 ± 1.27	45.3 ± 1.26	44.8 ± 1.30	45.0 ± 1.29	45.5 ± 1.36	45.3 ± 1.18	45.5 ± 1.23	44.6 ± 1.32
PUFA	14.7 ± 0.53	14.3 ± 0.55	14.4 ± 0.69	14.1 ± 0.61	14.3 ± 0.63	14.3 ± 0.63	14.4 ± 0.65	14.7 ± 0.67	14.1 ± 0.56
PUFA (n-6)	11.9 ± 0.46	11.6 ± 0.43	11.7 ± 0.41	11.4 ± 0.35	11.6 ± 0.37	11.6 ± 0.45	11.6 ± 0.39	11.9 ± 0.41	11.5 ± 0.37
PUFA (n-3)	2.0 ± 0.08	2.0 ± 0.06	2.0 ± 0.08	1.9 ± 0.09	1.9 ± 0.10	2.0 ± 0.08	1.9 ± 0.06	2.0 ± 0.08	1.9 ± 0.08

Means from triplicate experiments ± SD; *RST - Reaching the set temperature

Table 4
The concentration of lipid oxidation products: LP (μM) and MDA (μg/100 mL); PC (nM/mg protein) and α-La (mg/mL) in raw (control) and pasteurized HM by CH and MWH under different conditions (temperature and time).

Heating time (min)	Content of				Heating time (min)	Content of			
	LP(μM)	MDA(μg/100 mL)	PC(nM/mg protein)	α–La(mg/mL)		LP(μM)	MDA(μg/100 mL)	PC(nM/mg protein)	α–La(mg/mL)
	after convection heating at					after microwaves heating at			
	62.5 °C								
Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.99 ± 0.11^{a}	Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.68 ± 0.52^{a}
RST*	23.3 ± 2.81^{a}	$12.6 \pm 1.12^{a*}$	1.26 ± 0.16^{a}	2.91 ± 0.11^{a}	RST*	23.9 ± 5.04^{a}	$10.7 \pm 0.31^{a*}$	1.19 ± 0.14^{a}	2.61 ± 0.54^{a}
10	21.7 ± 2.90^{b}	$12.2 \pm 1.21^{a*}$	$1.13 \pm 0.14^{a*}$	$2.86 \pm 0.10^{a,b}$	1	21.0 ± 3.27^{a}	$10.8 \pm 0.79^{a*}$	$1.68 \pm 0.19^{a*}$	2.51 ± 0.47^{b}
20	20.7 ± 2.55^{b}	13.1 ± 1.37^{a}	1.64 ± 0.23^{a}	2.81 ± 0.12^{b}	3	23.6 ± 5.12^{a}	12.7 ± 1.07^{a}	1.85 ± 0.19^{a}	2.46 ± 0.55^{b}
30	21.2 ± 1.11^{b}	13.1 ± 1.05^{a}	1.72 ± 0.12^{a}	$2.84 \pm 0.02^{a,b}$	5	25.2 ± 4.62^{a}	11.8 ± 0.91^{a}	2.02 ± 0.12^{b}	$2.5 \pm 0{,}48^{b}$
					10	25.5 ± 5.43^{a}	12.7 ± 0.89^{a}	2.06 ± 0.15^{b}	2.21 ± 0.07^{c}
	66 °C								
Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.73 ± 0.26^{a}	Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.68 ± 0.52^{a}
RST*	23.6 ± 1.50^{a}	$12.6 \pm 1.71^{a*}$	1.37 ± 0.15^{a}	2.50 ± 0.13^{b}	RST*	25.8 ± 5.12^{a}	11.3 ± 0.95^{a}	1.55 ± 0.08^{a}	$2.63 \pm 0.13^{a,b}$
10	23.4 ± 2.61^{a}	13.2 ± 0.89^{b}	$2.39 \pm 0.14^{b*}$	2.13 ± 0.27^{c}	1	22.6 ± 2.52^{a}	12.1 ± 0.83^{a}	$1.78 \pm 0.05^{a,b*}$	$2.55 \pm 0.33^{a,b}$
20	$23.9 \pm 2.29^{a*}$	$14.8 \pm 0.69^{b*}$	2.25 ± 0.25^{b}	2.07 ± 0.07^{c}	3	$25.5 \pm 3.49^{a*}$	$13.7 \pm 1.18^{b*}$	2.12 ± 0.07^{b}	2.32 ± 0.14^{b}
30	22.4 ± 2.12^{a}	14.7 ± 1.49^{b}	2.37 ± 0.21^{b}	2.04 ± 0.02^{c}	5	24.2 ± 3.14^{a}	14.3 ± 0.63^{b}	2.37 ± 0.13^{b}	2.54 ± 0.20^{b}
					10	20.3 ± 2.11^{b}	12.7 ± 0.89^{a}	2.61 ± 0.09^{c}	2.14 ± 0.15^{c}
	70 °C								
Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.73 ± 0.26^{a}	Raw milk	24.1 ± 2.74^{a}	11.7 ± 0.34^{a}	1.35 ± 0.07^{a}	2.72 ± 0.46^{a}
RST*	23.7 ± 2.90^{a}	$12.4 \pm 1.09^{a*}$	$2.09 \pm 0.19^{b*}$	2.03 ± 0.02^{b}	RST*	22.7 ± 3.46^{a}	$10.2 \pm 1.26^{a*}$	$1.56 \pm 0.07^{a*}$	2.41 ± 0.39^{b}
10	21.8 ± 3.48^{b}	$13.6 \pm 0.90^{b*}$	$3.45 \pm 0.26^{c*}$	$1.84 \pm 0.11^{b*}$	1	22.7 ± 2.77^{a}	$10.4 \pm 1.06^{a*}$	$2.07 \pm 0.14^{b*}$	$2.21 \pm 0.24^{c*}$
20	$23.9 \pm 3.56^{a*}$	$13.3 \pm 1.04^{b*}$	$3.73 \pm 0.28^{c*}$	$1.74 \pm 0.18^{b*}$	3	$27.6 \pm 2.95^{b*}$	$11.7 \pm 0.97^{a*}$	$1.97 \pm 0.13^{b*}$	$2.26 \pm 0.49^{c*}$
30	22.5 ± 2.58^{a}	14.5 ± 1.63^{b}	$4.33 \pm 0.23^{c*}$	$1.66 \pm 0.22^{b*}$	5	24.7 ± 3.28^{a}	14.3 ± 0.82^{b}	$2.14 \pm 0.11^{b*}$	$2.22 \pm 0.58^{c*}$
					10	22.3 ± 4.46^{a}	16.3 ± 1.69^{b}	2.95 ± 0.14^{c}	1.82 ± 0.02^{c}

*RST - Reaching the set temperature. Each value is expressed as mean ± SD of three samples per triplicate.
[a,b,c] - Means value within a column with different superscripts are significantly different at a given temperatures ($P < 0.05$).
*- in the rows indicates significant treatment effect at the same temperature and in the analogues heating time ($P < 0.05$).

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significant influence of the size of the fat globules on the measurement accuracy of the fat content in milk using the infrared analysis method.

Giuffrida et al. (2019) compared results obtained using HMA system and reference methods for determination of fat, protein, and lactose in HM (Röse-Gottlieb extraction, Kjeldahl method and high-performance anion exchange chromatography with pulsed amperometric detection, respectively). Significant differences were found between the HMA and reference method for the determination of fat content. However, the difference in total fat was lower than 12%, which was within the variability reported by the HMA supplier. The authors demonstrate that the MIRIS HMA is suitable for the quantification of total fat with regard to trueness and precision (Giuffrida et al., 2019). Thus, it can be assumed that the differences found in our research (maximum 8%) in the total fat content in heat treated milk do not constitute a significant difference.

3.2. Fatty acid composition

The presence of more than 50 FAs, including cis- and *trans*-monoenic isomers of 14:1, 15:1 16:1 and 17:1 FAs, was determined in the lipids extracted from the pooled milk sample (Table 3). The predominant FAs in HM fat were: oleic acid C18:1 9c (38.8%), palmitic acid C16:0 (22.4%), linoleic acid (LA) C18:2n–6 (10.9%), stearic acid C18:0 (6.4%), myristic acid C14:0 (ok. 4.3%) and lauric acid C12:0 (3.7%). The above FAs accounted for nearly 87% of the total fatty acids in HM lipids. The remaining 13% was composed of very important for infants LC-PUFAs such as arachidonic acid (AA) 20:4n-6, eicosapentaenoic acid (EPA) 20:5n-3, decosahexaenoic acid (DHA) 22:6n-3, alpha-linolenic acid (ALA) C18:3 n-3 and bioactive conjugated linoleic acids (CLAs). The obtained composition and content of KT is characteristic of the milk of women from Gdańsk and the surrounding area (Martysiak-Żurowska et al., 2011). Saturated fatty acids (SUFAs) accounted for about 39% of the total FAs content, monounsaturated fatty acids (MUFAs) about 46% and polyunsaturated fatty acids (PUFAs) about 15%. In PUFAs group, the FAs n-6 families were found at the level of about 12% of the total FAs content, the n-3 families - about 2% (Table 3)

The obtained results showed no significant influence of any of the tested thermal preservation methods on the composition and content of HM fat FAs. The literature data also indicate no effect of the HoP on the FA profile of HM fat (Delgado et al., 2014). Only Ewaschuk et al. (2011) reported slight changes in the relative composition of medium-chain fatty acids (MCFAs) in HM after HoP.

According to our best knowledge, these are the first results concerning the influence of MWH on the FA composition of HM fat. Ovensen, Jakobsen, Leth, & Reinholdt (1996) have studied the effect of MWH on chosen PUFAs (linoleic acid and linolenic acid) in HM and showed that content of FAs was stable over the tested temperature range of 37–77 °C (the authors did not report the duration of heat treatment). Therefore, our studies provide evidence that MWH of HM at 62.5, 66 and 70 °C by 10 min do not change its FAs concentration.

3.3. Primary and secondary lipid oxidation products

In the pooled sample of HM, a low LP content at the level of 24 μM, was found. During CH of HM at all temperatures the LP level fluctuated slightly with a decreasing tendency along with extension of time processing (Table 4). LP concentration was lower by about 7 and 6%, after 30 min of heating at 66 and 70 °C, respectively, compared to the LP content in raw milk. However, these changes were no statistically significant. Decrease in LP value, for about 13%, was found in milk heated at 62.5 °C for 20 and 30 min. No differences were found in treatment effect on LP value in heating HM. There were statistically significant differences between MWH in 5 min and CH in 20 min of heating at 66 and 70 °C ($P = 0.041$ and 0.018, respectively) but the difference in the mean values only in these conditions was not great enough to exclude the possibility of a random error. Human milk contains a number of antioxidant components, including enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Our previous paper showed that pasteurization of human milk decreases but does not completely deactivate the antioxidant enzymes. Especially SOD was resistant to thermal pasteurization, regardless of the heating method (Martysiak-Żurowska et al. 2019). Thus, it can be assumed that the relatively high activity of enzymes, which constitute the first line of defense against reactive oxygen species (ROS), and the lack of oxygen access during heating prevented the formation of new LPs.

Decrease in LPs content in pasteurized milk may indicate the breakdown of unstable primary lipid oxidation products or their transformation into secondary oxidation products. To verify this, the effect of heating on the concentration of malondialdehyde (MDA) in milk was determined. MDA is one of the secondary products of lipid oxidation, detrimental to health, formed as a result of the oxidation process of fatty acids containing at least three double bonds. Measurement of MDA content is a generally accepted method for determining the degree of fat degradation in food (Fenaille et al., 2001).

The concentration of MDA in milk increased after 20 min of CH at all temperatures. Heat treatment for 30 min at 62.5 and 70 °C caused about 10 and 25%, respectively, increased of MDA content in HM (Table 4). However, changes at 62.5 °C were not significant statistically. The lack of an increase in the concentration of LP in HM with a simultaneous increase in the level of MDA indicates the formation of secondary products from LP present in milk rather, than a progressive degradation of milk lipids. It may also be confirmed by the lack of changes in PUFAs in CH milk (Table 3). According to literature data HoP did not affect the oxidative status of HM as measured by MDA content (Silvestre et al., 2008).

MWH of milk at 62.5 °C for up to 10 min and at 66 °C for up to 5 min, did not change in the levels of lipid oxidation markers. It can therefore be concluded that the heating of HM under such conditions did not cause oxidative changes in HM lipids. After 10 min of treatment at 66 °C, a decrease in LP content for about 15% compared to the concentration in raw milk was found. This correlated with the temporal increase in milk MDA levels after 5 min of heating. The process carried out at 70 °C for 3 min generated an increase in LP level by 15% compared to the control sample, which indicates a probable acceleration of the lipid oxidation reaction under these conditions. As a result, in the milk heated at 70 °C for 5 min, a clear increase in MDA content was found, by about 22% (Table 4). Statistical differences in the effect of the generating method of HM heating on the MDA level were shown in the initial heating periods: RST (time to reaching the set temperature) and during first 10 (CH) and 1 min (MWH) heating at 62.5; 66 and 70 °C. Despite the faster temperature rise when using MWH, the use of CH generated a higher increase in MDA content in milk.

The ingestion of oxidized lipids with milk may be a significant problem for newborns oxidative balance because their antioxidant defense mechanisms are still poorly developed and may be overcome by an excessive presence of ROS. These latter are involved in serious diseases in premature infants, such as necrotizing enterocolitis, chronic lung disease, retinopathy of prematurity and intraventricular haemorrhage (Turoli et al., 2004). It should be noted, however, that despite the increase in MDA concentration in HM treated by MWH at highest temperature for the longest time (70 °C, 10 min), the level of this analyte, 16.3 μg/100 mL, in milk still corresponded to the physiological concentration of MDA in HM. Earlier studies showed that milk from Polish women donors contained MDA in the range from 9.20 to 27.10 μg/100 mL (Martysiak-Żurowska & Stołyhwo, 2006). For comparison, in milk of Spanish women donors more considerable dispersion of MDA content has been reported - to range from 2.16 to 182 μg (Miranda et al., 2004).

3.4. The content of furosine

One of the most significant changes caused by high temperature in food products are the reactions of nonenzymatic browning (Maillard reactions), which consists in a reaction between a carbonyl group of

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reducing sugars and a free amino group of amino acids, peptides and proteins (Van Boekel, 1998). Maillard reactions have a negative influence on the nutritional and health value of food because lead to loss and degradation of essential amino acids: lysine, arginine, methionine, tryptophan and histidine. Products of the Maillard reactions limit the digestibility of proteins by blocking the availability of a peptide bond for trypsin and carboxypeptidase, and they are inhibitors of digestive enzymes (proteases and disaccharidases) (Van Boekel, 1998). On the other hand, some authors observed antioxidant activity of Maillard reactions products in a system containing of milk proteins and saccharides (Oh et al., 2016). Furosine is a routinely used indicator of the thermal degradation of dairy products in the early stage of the Maillard reactions (Espinosa-Martos et al., 2013). Raw HM can contain certain amounts of furosine, from 1.3 to 2.5 mg/100 g protein (Escuder-Vieco et al., 2018) and from 3.50 to 11.60 mg/100 g protein in unpasteurized colostrum (Espinosa-Martos et al., 2013).

In the pooled sample of raw HM, the content of furosine was below the quantification limit of the analytical method used, i.e. below 0.26 mg/100 g of protein. MWH and CH of HM, did not initiate the Maillard reactions except samples subjected to heating at 70 °C for 10 (MWH) and 30 min (CH) where a small content of furosine was found (data not showed). The samples contained 0.86 mg/100 g protein and 1.17 mg/100 g protein, respectively. Escuder-Vieco et al. (2018) showed, that the content of furosine in HM after HoP (4.7 mg/100 g protein) was more than twice as high as in raw HM (2.1 mg/100 g protein). However, the level of furosine in pasteurized HM was still lower than those reported for infant formulas (IFs). Sabater et al. (2018) determined the concentration of furosine in IFs, ranging from 94 to 1226 mg/100 g of protein.

There are no data in the literature on the effect of MWH on the content of furosine in HM. Such data can be found for microwave heat-treated bovine milk. Concentration of furosine in bovine milk, heated at 100 °C for 30 min, increased to about 177 mg/100 g protein (6.2 mg/100 mL) from<5 mg/100 g protein in raw milk. Higher temperatures enhanced formation this compound. Finally, the content of furosine in milk treated at 120 °C for 30 min was at the level of about 343 mg/100 g protein (12.0 mg/100 mL) (Villamiel et al., 1996).

3.5. Protein carbonyls

The content of PC groups is an indicator of protein oxidation in biological matrices. The content of PC in raw HM was 1.35 nM/mg protein (Table 4). CH of HM at the temperature of 62.5 °C had no significant effect on the level of carbonyl groups in the samples. The increase in the heating temperature to 66 °C caused the growth of oxidized forms of proteins after 10 min of heating. At the temperature of 70 °C, a significant increase in the PC concentration, by about 50%, was found in the milk samples when the set temperature was obtained. After 30 min of heating at 66 and 70 °C, the concentration of PC was 75 and 220%, respectively, higher than in raw milk (Table 4).

MWH also generates an increase in the concentration of oxidized forms of proteins. Formation of PC increased as a function of heating time for all temperatures, but was lower than in CH. The significant,

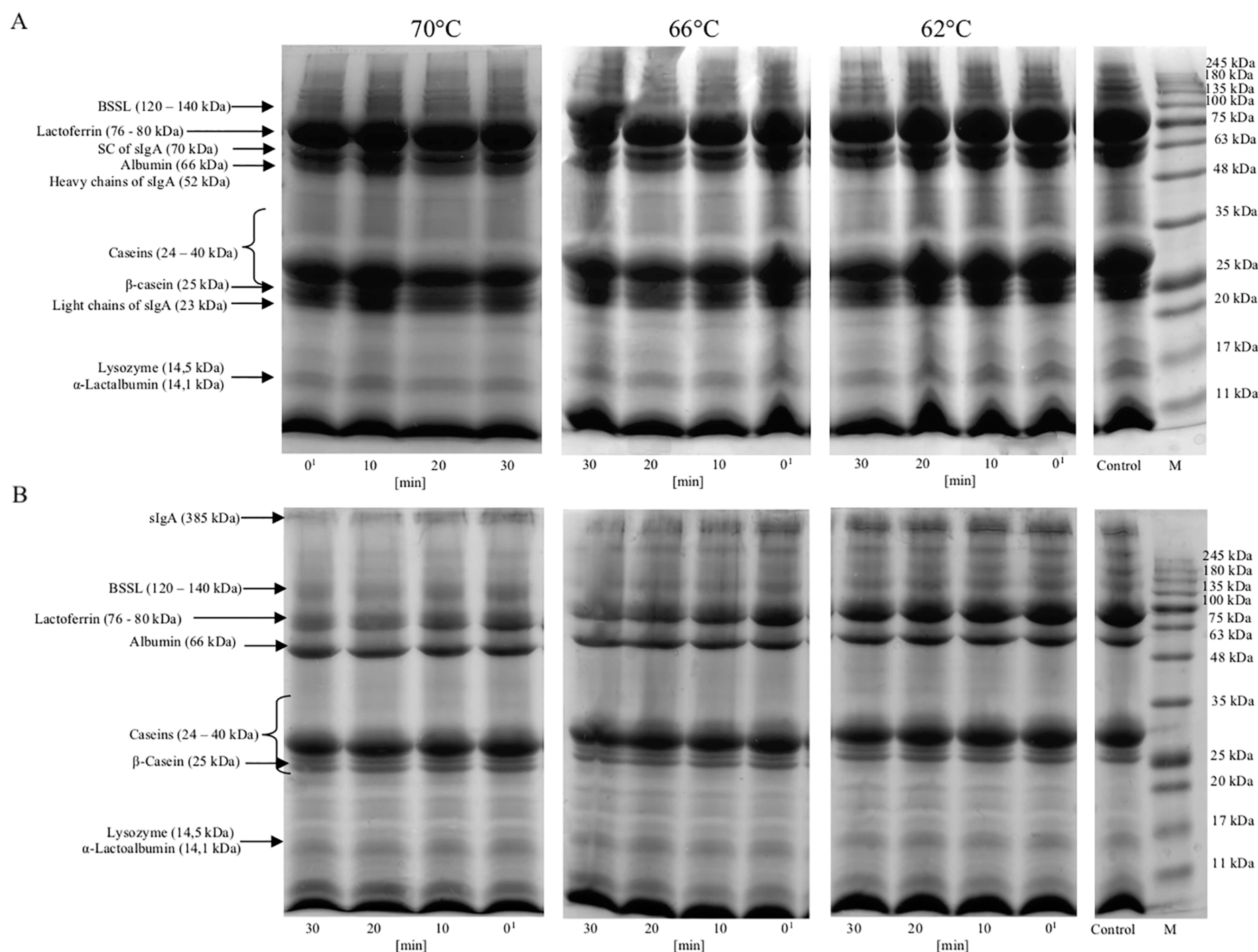

Fig. 1. SDS-PAGE total HM protein profile after CH A) with DTT, B) without DTT – control: raw milk, M - marker.

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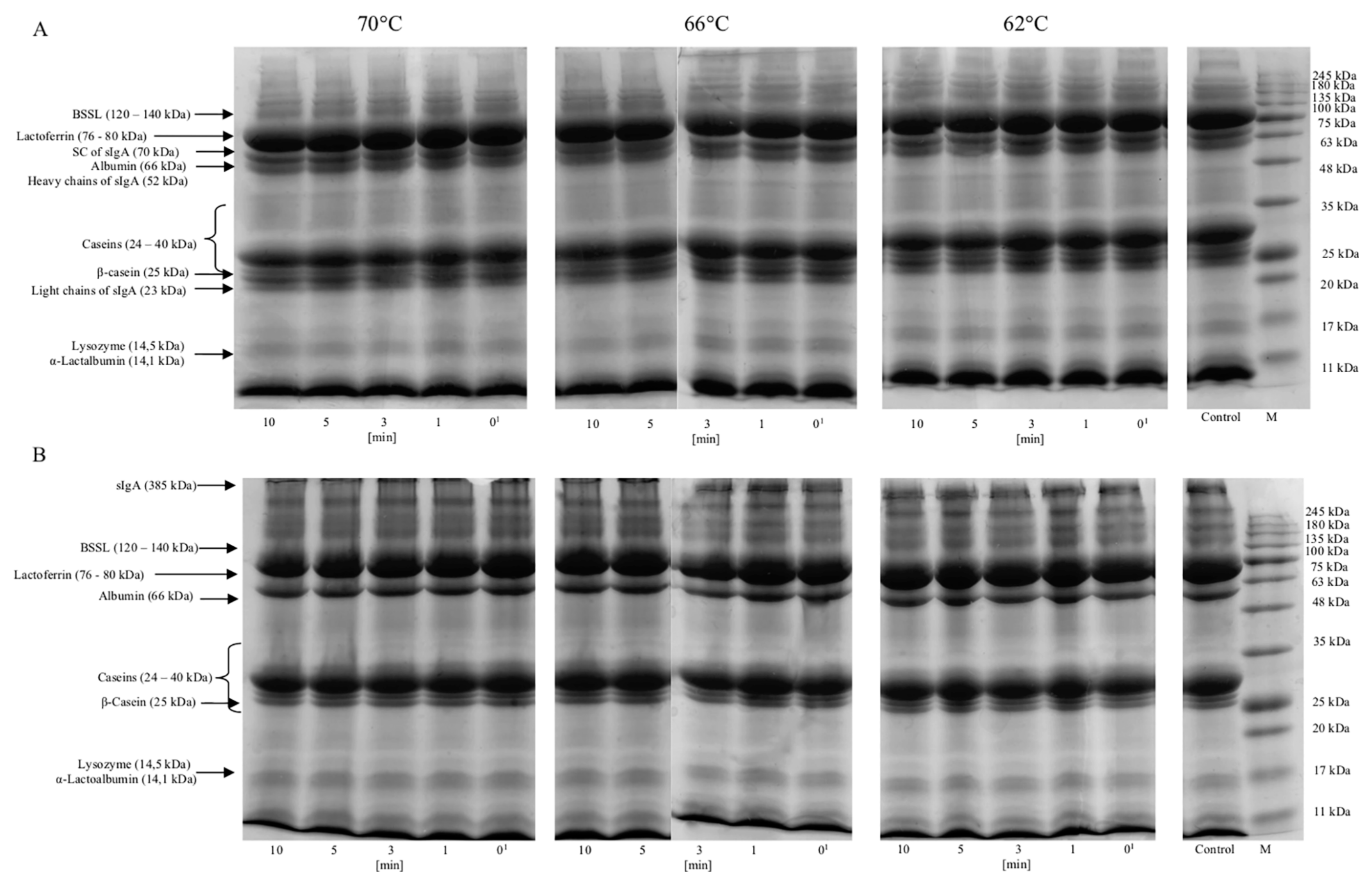

Fig. 2. SDS-PAGE total HM protein profile after MWH A) with DTT, B) without DTT – control: raw milk, M - marker.

50% growth in the PC content was observed in HM heated for 5 min at 62.5 °C. In milk heated by 10 min at 70 °C, the content of PC was 118% higher than in raw milk. The two-way ANOVA analysis results show that increase in PC levels depends on temperature and method of treatment (MWH or CH). The highest level of PC in HM was found after CH at 70 °C. During heating at 70 °C a statistically significant interaction between time and the way of treatment was detected ($P =< 0.001$).

As with MDA, PC consumption by children, especially preterm infants, which have an immature antioxidant defense system and are frequently exposed to oxidative stress caused by infection, mechanical ventilation or intravenous nutrition (Inayat et al., 2015) should be kept to a minimum. It is worth noted, however, that despite the fact that such a significant increase in the level of PC was found in HM subjected to heating, these concentrations are still at a lower level than in infant formula. Maximal concentration of PC within these latter may be about 9 nM/mg protein (about 14 μM/100 mL) (Pozzo et al., 2019). The high concentration of oxidative compounds, such as PC, in infant formula can deriving from extensive heat treatments, that are applied during their manufacturing processes.

3.6. *The content of α-La*

The protein content of HM is approximately 1 g/100 mL, of which 25–30% is α-La (Ballard & Morrow, 2013). It plays an important role in nutrition and is part of the antimicrobial complex, the so-called HAMLET (human α-La made lethal to tumor cells), which *in vitro* sensitized some pathogens, like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* (Marks et al., 2012). It was shown that HoP did not cause significant changes in α-La concentration (Table 4). No changes in α-La content after holder and vat (63 °C for 30 min) pasteurization was also observed by (Meredith-Dennis et al., 2018). According to McGuffey (2004) denaturation of this protein starts at relatively high temperatures, about 64 °C, and total destruction take place at 96 °C for 30 min. We observed that CH temperature 66 °C by 10 min caused a decrease in the content of α-La by about 20%. The extension of the heating time did not lead to any further changes in α-La concentration. However, when temperature of process was reaching to 70 °C, the amount of α-La decreased by about 25%, and after 30 min of treatment at this temperature by about 40% compared to the initial value. Loss of 75% of this protein in HM, was demonstrated by Meredith-Dennis et al. (2018) after sterilization at 121 °C for 5 min.

MWH of HM at 62.5 and 66 °C for 5 min did not change significantly the content of α-La, but 10 min treatment caused 16 and 18% denaturation of this protein, respectively. At higher temperature, during reaching the 70 °C about 10% of α-La denaturation took place, and after 10 min of heating 32% of losses of content this protein was observed. (Table 4). Significant treatment effect on α-La degradation was detected only in the case of heating at 70 °C. Villamiel et al. (1996) also showed that microwave and convection treatments of cow's milk at 70 °C for 10, 20, 30 min resulted in the same level of denaturation of α-La.

3.7. *SDS-PAGE analysis of milk proteins*

For the evaluation of changes occurring in milk proteins after heating, electrophoretic analysis was used under reducing (in the presence of DTT) and non-reducing conditions (without DTT). It was found that, depending on the conditions of electrophoretic separation, the profiles of milk proteins differ. In both cases, the presence of such proteins as: bile salt activated lipase (120–140 kDa), lactoferrin (76–80 kDa), β-casein (25 kDa), lysozyme and α-La (14.5 kDa and 14.1 kDa respectively) were found (Kunz & Lönnerdal, 1989). On the other hand, in protein profiles without DTT, an intense band with a mass above 245 kDa is visible, demonstrating the presence of protein complexes in milk, which did not migrate to the separating gel. It is sIgA with a molecular

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weight of 385 kDa (Garcia-Pardo et al., 1981). sIgA is composed of IgA monomers consisting of polypeptide chains: H - heavy and L-light linked by disulfide bonds, chain linking monomers IgA - J and secretory component - SC. The J polypeptide chain has eight cysteine residues, six of which form disulphide bridges between them, and the other two, Cys14 and Cys68, form covalent links to Cys471 in IgA monomers. During electrophoresis in the presence of DTT, disulfide bonds in sIgA are broken, which is reflected in electrophoresis in the form of additional bands: 70 kDa - SC, 52 kDa - heavy chains H and 23 kDa - light chains L. The same electrophoresis separation under reducing and non-reducing conditions was obtained by Garcia-Pardo et al. (1981) and Kunz & Lönnerdal (1989). The electrophoretic profiles of raw milk proteins as well as those subjected to MWH and CH do not differ from each other (Figs. 1 and 2), which indicates that there are no degradation changes in the proteins as well as no formation of covalent bonds. Mayayo et al. (2014) demonstrated that 30 min heating at 65 °C and 10 min at 90 °C caused evident reduction in the intensity of the bands corresponding to the LF monomer. These discrepancies may result from a different heating temperature.

4. Conclusions

The nutrients and FAs content in HM does not change after MWH and the level lipid oxidation markers LP only slightly fluctuate in the conditions ensuring microbiological safety. MDA content also did not change after pasteurization at 62 °C for 5 min but at 66 °C for 3 min slightly increases by 16%. Both, CH and MWH do not lead to the formation of furosine. The presence of this compound in the milk samples was found, only after applying the highest temperature and the longest heating time (70 °C, 30 min). CH and MWH at 62.5 and 66 °C for 5 min do not cause denaturation of α-La, but in these conditions an increase in the degree of protein oxidation was noted. However, the level of PC was higher after CH than MWH. Our research shows that the use of MWH under controlled conditions may be a promising method of pasteurizing HM, which will preserve its valuable nutrients.

CRediT authorship contribution statement

Dorota Martysiak-Żurowska: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Edyta Malinowska-Pańczyk:** Conceptualization, Methodology, Validation, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Małgorzata Orzołek:** Methodology, Investigation, Data curation. **Barbara Kusznierewicz:** Methodology, Validation. **Bogumiła Kiełbratowska:** Resources, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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