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The effect of convective heating and microwave heating on antioxidant enzymes in pooled mature human milk

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ABSTRACT

The effects of convective and microwave heating at constant temperature (62.5, 66 and 70 °C) on the activity of antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx) in pooled mature human milk were compared. Activities of the enzymes were determined using spectrophotometric kits. Activity of GPx decreased significantly in the first stage of heating when milk samples were warmed to pasteurisation temperature. CAT was the most thermolabile enzyme but microwave heating induced a smaller decrease in CAT activity than convective heating. SOD was most resistant to thermal pasteurisation, regardless of the heating method. SOD and GPx activity temporary increased during microwaves heating. Considering shorter pasteurisation period and lower demand for energy, it can be concluded that microwaves pasteurisation enjoys special merits. However, still there is no clear answer as to whether the microwave field itself can affect the antioxidant enzymes of human milk.

1. Introduction

According to recommendations of the World Health Organisation (WHO), infants should be exclusively breastfed in the first six months of life to promote optimal growth, development and health. Infants that cannot be fed their mother's breast milk should be administered milk from a human milk bank (HMB). The main beneficiaries of a HMB are preterm or sick children. Human milk components have confirmed therapeutic properties and should be incorporated into nutritional treatments for infants and young children (Boyd, Quigley, & Brocklehurst, 2007). Human milk is particularly important for premature infants with incredibly low (ILBW) and extremely low (ELBW) birth weights. The immune system of newborns is not fully developed. Premature and sick babies are more susceptible to infections and the adverse effects of harmful factors, including reactive oxygen species (ROS).

ROS are produced by living organisms during normal cellular metabolism. Under physiological conditions, low concentrations of ROS play an important role by regulating the transduction of cell signals, but at high concentrations, they exert harmful effects by deactivating important cellular molecules (Valko et al., 2007). Overproduction of ROS and a deficiency of enzymatic and non-enzymatic antioxidants in biological systems contribute to oxidative stress. Oxidative stress is a disturbance in the prooxidant/antioxidant balance in favour of the prooxidant. Premature infants are especially susceptible to oxidative stress. Oxidative stress seems to be a contributing factor to the pathogenesis of many neonatal diseases, such as respiratory distress syndrome, bronchopulmonary dysplasia, necrotising enterocolitis, renal failure and retinopathy (Davis, 2002; Okur et al., 1995; Saugstad, 2001; Schaller, 2005; Weinberger, Laskin, Heck, & Laskin, 2002).

The antioxidant system prevents or inhibits changes caused by ROS. It involves ROS-degrading enzymes as well as low-molecular-weight compounds that are less specific than enzymes. Low molecular weight compounds degrade free radicals which are not neutralised by enzymes (Valko et al., 2007). The most important antioxidant components of human milk include enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants such as vitamins C and E, β-carotene, uric acid, glutathione and coenzymes Q (L'Abbe & Friel, 2000; Silvestre et al., 2008a; Yuksel, Yigit, Cinar, Atmaca, & Onaran, 2015).

Milk from HMB is usually subjected to low-temperature long-time pasteurisation (LTLT) at 62.5 °C for 30 min which effectively eliminates vegetative microbial pathogens. However, pasteurisation significantly decreases the concentrations of nutrients and bioactive components in milk, including antioxidant enzymes (Henderson, Fay, & Hamosh, 1998; Landers & Updegrove, 2010;

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Silvestre et al., 2008a; Tully, Jones, & Tully, 2001). Therefore, new preservation methods are being developed to guarantee the microbiological safety of human milk without compromising its nutritional and biological value. Pasteurisation using microwave radiation is one such alternative treatment.

Microwave radiation has insufficient energy to break chemical bonds, and it is a non-ionising form of radiation. Microwaves generate heat due to molecular friction of polar compounds that attempt to align themselves under in the oscillating electrical field (water dipoles and ions), which produces friction with other food components (Ahmed & Ramaswamy, 2007). During microwave treatment, the preset temperature is achieved at a much faster rate throughout the entire sample than during convective heating (Zhu & Chen, 2014). Microwave heating is an efficient and economical processing method. Many authors have demonstrated that microwave treatment is highly effective in eliminating various microorganisms. Microwave heating has numerous applications in the food industry, including blanching, drying, cooking, pasteurisation and sterilisation (Sumnu & Sahin, 2005).

The objective of this study was to compare the effects of convective and microwave heating (at parameters that guarantee the microbiological safety of the processed product) on the activity of antioxidant enzymes in pooled mature human milk.

All experimental procedures have been approved by the Local Ethics Committee of the Medical University of Gdansk. The subjects gave their informed consent before the start of any procedure.

2. Materials and methods

2.1. Collection of samples

Samples of mature human milk were collected from ten healthy and non-smoking women who had full-term pregnancies without complications. All newborns were in good health (Apgar score ≥ 9 in the first minute of life), and their body weights were within the norm (3100–3800 g). Milk was pumped by the mothers at home with an electric breast pump (Symphony, Medela, Poland) with observance of general hygiene standards. The samples were collected from the mothers within 24 h and stored in a refrigerator (4 °C). In a laboratory, 2 mL specimens were collected from each sample to determine the individual levels of the tested enzymes in every woman. The remaining milk was immediately pooled and divided into smaller samples of 50 mL. Raw milk samples were frozen and stored at −80 °C, for no longer than one month. Directly before processing, milk was thawed and heated to 22 °C in an incubator (Binder, Warsaw, Poland).

2.2. Convective heating

Thawed milk samples (50 mL) were transferred to beakers and heated to the appropriate temperature in a water bath. The temperature at the central point of the beaker was controlled with a thermometer. The heating process was carried out with two variants - with and without stirring. The preset temperature was achieved within 19 ± 1 min in samples that were not stirred, which is equivalent to the time of LTLT pasteurisation in medical pasteurisers at HMB. In stirred samples, the above period was shortened to 13 ± 1 min (Fig. 1). Milk was heated to 62.5, 66 and 70 °C, and each temperature was maintained for up to 30 min. Samples for analysis were collected when the appropriate temperature had been reached (time 0) and then every 10 min. The processed milk was cooled immediately to 20 °C by immersion in an ice/water bath. Cooling time was approximately 7 min in both variants (Fig. 2). All pasteurisation treatments were performed in four replicates.

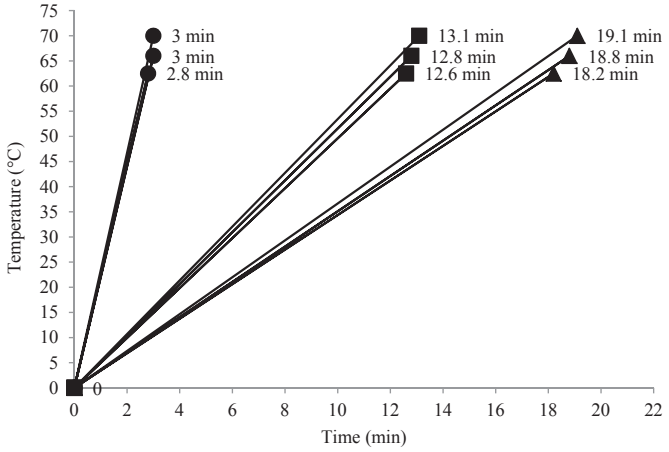

Fig. 1. The average time to reach the preset temperature (time 0) of human milk samples: microwave heating (●), convective heating with stirring (■) and convective heating without stirring (▲). Target temperatures were: 62.5 °C, 66 °C and 70 °C.

2.3. Microwave heating

Milk samples were subjected to microwave heating in a prototype device where their temperature was kept constant for a preset time (2450 MHz, 800 W, Enbio Technology, Gdańsk, Poland). Milk samples of 50 mL were transferred to a beaker and placed in the microwave pasteuriser (Fig. 3). Silicon pipes were submerged in the milk. Milk was pumped through a temperature sensor, and it was simultaneously stirred. The temperature sensor measured the temperature and controlled a magnetron that was turned on and off in a sequence of several seconds, depending on the recorded milk temperature.

The preset temperature was reached within approximately 3 min (Fig. 1). High temperature generation in a very short time is characteristic of microwave application in the food industry. Milk samples were heated to 62.5, 66 and 70 °C. Samples for analysis were collected at time 0 and after 1, 3, 5, 10 min of heating. After treatment, the samples were immediately cooled to 20 °C in a cooling exchanger with the use of tap water. Cooling time was approximately 2 min (Fig. 2). All treatments were performed in four replicates.

2.4. Determination of the activity of antioxidant enzymes

Enzyme activity in the human milk samples was determined using spectrophotometric kits (Cayman Chemical Company, Ann Arbor, MI, USA): superoxide dismutase (SOD) assay kit No. 706002, catalase (CAT) assay kit No. 707002 and glutathione peroxidase

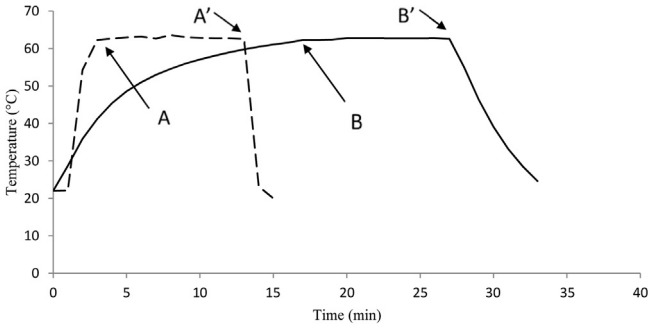

Fig. 2. Temperature curve of human milk pasteurization to 62.5 °C for 10 min: microwave heating (- - -) and convective heating (—). A).Time of reaching the preset temperature in microwave heating, B) in convective heating. A') the beginning of sample cooling after microwave heating, B') after convective heating.

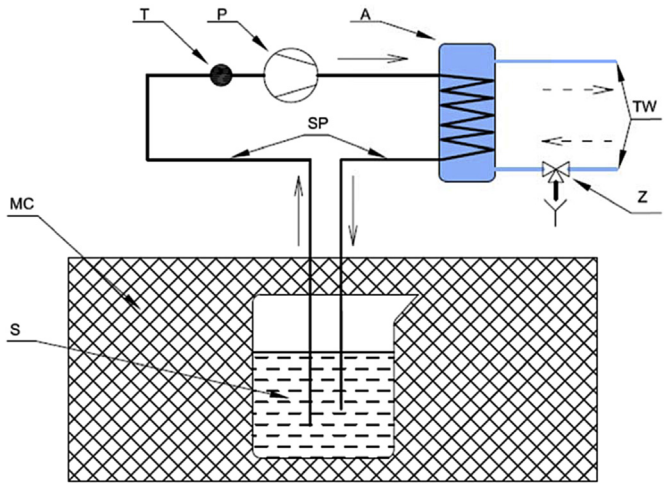

Fig. 3. Diagram of a microwave pasteuriser (Enbio Technology, Gdańsk, Poland): SP, silicone pipe; T, temperature sensor; P, pump; A, cooling exchanger; Z, three-way valve; S, sample; MC, microwave chamber; TW, tap water; —, sample flow; ----, tap water flow.

(GPx) assay kit No. 703102, according to the manufacturer's instructions. Enzyme activity was analysed in milk samples immediately after processing. All analytical determinations were carried out in duplicate.

2.5. Statistical analysis

Mean values and standard deviation of the mean were determined. Data were processed statistically in the GraphPad Prism 7.01 system (GraphPad Company, San Diego, CA, USA). The differences between control and processed (heated) samples were evaluated by one-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test. The results were regarded as significant at $p \leq 0.05$.

3. Results

3.1. Enzyme activity in raw human milk

The activity of the analysed antioxidant enzymes in human milk samples differed between women. The activity of SOD ranged from 0.69 to 1.66 U mL^{-1}, CAT from 14.57 to 27.06 nmol min^{-1} mL, and GPx from 9.94 to 15.80 nmol min^{-1} mL. Enzyme activities in pooled laboratory samples (raw milk, control) were determined to be: SOD, 1.27 ± 0.44 U mL^{-1}; CAT, 19.15 ± 1.24 nmol min^{-1} mL; GPx, 10.18 ± 0.86 nmol min^{-1} mL.

3.2. The influence of convective heating

In the present study, human milk samples subjected to LTLT pasteurisation with and without stirring at a temperature of 62.5 and 66 °C were not characterised by significant differences in SOD activity. A significant decrease in SOD activity (around 25% relative to raw milk) was noted only in the human milk sample processed by convective heating at 70 °C for the longest period of 30 min without stirring (Table 1).

During convective heating, CAT activity decreased even in the warming up stage, before the preset temperature had been reached. The decrease in CAT activity was exacerbated with an increase in pasteurisation temperature. CAT activity decreased by around 66% in the process of heating milk to 70 °C without stirring. Under pasteurisation conditions identical to those applied in HMB (62.5 °C, 30 min, no stirring), CAT activity also decreased significantly from 19.2 to 8.2 nmol min^{-1} mL, i.e., by nearly 60%. Extent of decrease was also influenced by the pasteurisation variant. In samples pasteurised at 66 and 70 °C, the decrease in CAT activity was significantly less in stirred samples compared with non-stirred samples. In samples exposed to the longest heating time (30 min) at 70 °C, the activity of CAT decreased by 75% in stirred samples and by 87% in non-stirred samples.

At temperatures higher than 62.5 °C GPx was more stable than CAT in response to convective heating. The highest significant changes in GPx activity were observed in the first stage of heating during which the samples were warmed up to the preset temperature. In stirred samples, the activity of GPx decreased by around 30% by the time the temperature of milk samples had reached 62.5 and 66 °C, and by 40% by the time sample temperature had reached 70 °C. Convective heating without stirring at a temperature of 62.5 and 66 °C led to a significantly greater decrease in GPx activity which was estimated at 42%. In both stirred and non-stirred samples, pasteurisation for 30 min at all temperatures decreased GPx activity by 50–55%.

Table 1
The activity of antioxidant enzymes in raw human milk (control) and human milk pasteurised by conventional heating under different conditions (temperature and time).[a]

Heating time (min)	SOD (U mL^{-1} ± SD)		CAT (nmol min^{-1} mL ± SD)		GPx (nmol min^{-1} mL ± SD)	
	With stirring	Without stirring	With stirring	Without stirring	With stirring	Without stirring
62.5 °C						
Raw milk	1.27 ± 0.44	1.27 ± 0.44	19.15 ± 1.24^{abcd}	19.15 ± 1.24^{abcd}	10.18 ± 1.86^{abcd}	10.18 ± 1.86^{abcd}
0	1.29 ± 0.08	1.29 ± 0.23	14.33 ± 2.00^{abcd}	14.78 ± 2.58^{acd}	7.23 ± 0.14^{a}	5.79 ± 0.68^{a}
10	1.20 ± 0.10	1.33 ± 0.20	11.05 ± 1.93^{ab}	11.95 ± 2.24^{bd}	5.62 ± 0.98^{b}	5.19 ± 0.55^{b}
20	1.30 ± 0.05	1.20 ± 0.17	10.43 ± 1.76^{ac}	10.45 ± 1.74^{ac}	5.31 ± 1.08^{c}	5.18 ± 0.99^{c}
30	1.21 ± 0.11	1.20 ± 0.21	9.47 ± 0.74^{ad}	8.20 ± 1.78^{abd}	5.26 ± 0.39^{d}	4.97 ± 0.55^{d}
66 °C						
Raw milk	1.27 ± 0.44	1.27 ± 0.44	19.15 ± 1.24^{abcd}	19.15 ± 1.24^{abcd}	10.18 ± 1.86^{abcd}	10.18 ± 1.86^{abcd}
0	1.39^{a} ± 0.11	1.52 ± 0.22	13.78 ± 1.70^{abcd}	7.71 ± 1.35^{acd}	7.22 ± 0.86^{ad}	6.00 ± 0.87^{a}
10	1.09^{a} ± 0.06	1.33 ± 0.25	10.91 ± 1.48^{ab}	6.24 ± 1.55^{bcd}	6.90 ± 0.30^{bd}	5.71 ± 0.64^{b}
20	1.18 ± 0.15	1.32 ± 0.11	9.45 ± 1.77^{ac}	3.82 ± 0.68^{abc}	5.19 ± 0.89^{c}	5.68 ± 1.26^{c}
30	1.11 ± 0.14	1.13 ± 0.07	9.23 ± 1.84^{ad}	3.47 ± 1.44^{acd}	5.09 ± 0.55^{abd}	5.52 ± 0.26^{d}
70 °C						
Raw milk	1.27 ± 0.44	1.27 ± 0.44	19.15 ± 1.24^{abcd}	19.15 ± 1.24^{abcd}	10.18 ± 1.86^{abcd}	10.18 ± 1.86^{abcd}
0	1.40 ± 0.11	1.46^{a} ± 0.09	7.97 ± 1.32^{abcd}	6.55 ± 1.48^{abcd}	6.23 ± 1.04^{a}	5.66 ± 0.83^{a}
10	1.25 ± 0.23	1.29 ± 0.18	4.06 ± 0.57^{ab}	3.15 ± 1.20^{ab}	6.27 ± 0.77^{b}	5.81 ± 0.39^{b}
20	1.11 ± 0.24	1.18 ± 0.19	4.95 ± 0.63^{ac}	2.91 ± 1.04^{ac}	6.45 ± 1.53^{c}	6.13 ± 0.12^{cd}
30	1.10 ± 0.11	0.95^{a} ± 0.21	4.90 ± 0.94^{ad}	2.51 ± 0.96^{ad}	4.58 ± 0.61^{d}	4.47 ± 0.48^{cd}

[a] Heating time of zero is the moment of reaching the preset pasteurisation temperature; identical letters in columns indicate significant differences in enzyme activity at different temperatures ($P < 0.05$).

Table 2
The activity of antioxidant enzymes in raw human milk (control) and human milk pasteurized by microwave heating under different conditions (temperature and time).[a]

Heating time (min)	SOD (U mL^{-1} ± SD)	CAT (nmol min^{-1} mL ± SD)	GPx (nmol min^{-1} mL ± SD)
62.5 °C			
Raw milk	1.27 ± 0.44	19.15 ± 1.24^{abcd}	10.18 ± 0.86^{acd}
0	1.24 ± 0.09	15.05 ± 1.01	8.51 ± 0.56^{a}
1	1.40 ± 0.18^{a}	12.58 ± 1.57^{a}	6.35 ± 0.67^{ad}
3	1.25 ± 0.09	12.77 ± 1.55^{b}	9.01 ± 1.14^{abcd}
5	1.14 ± 0.07	12.31 ± 1.78^{c}	7.26 ± 0.98^{ab}
10	1.00 ± 0.14^{a}	11.76 ± 1.33^{d}	5.65 ± 0.61^{ac}
66 °C			
Raw milk	1.27 ± 0.44	19.15 ± 1.24^{abcd}	10.18 ± 0.86^{abcd}
0	1.32 ± 0.16	12.95 ± 1.32	8.85 ± 0.69^{a}
1	1.53 ± 0.19^{ab}	11.16 ± 2.26^{a}	6.39 ± 1.01^{ae}
3	1.09 ± 0.06^{a}	11.71 ± 2.01^{b}	7.35 ± 0.49^{b}
5	1.13 ± 0.03	10.37 ± 2.02^{c}	5.86 ± 1.16^{ce}
10	1.01 ± 0.13^{b}	9.88 ± 1.31^{d}	6.07 ± 1.12^{d}
70 °C			
Raw milk	1.27 ± 0.44^{a}	19.15 ± 1.24^{abcde}	10.18 ± 0.86^{abcd}
0	1.29 ± 0.17^{a}	12.25 ± 0.86^{a}	8.89 ± 0.83^{a}
1	1.71 ± 0.07^{abcd}	11.87 ± 1.73^{b}	4.76 ± 0.74^{ae}
3	1.08 ± 0.18^{b}	9.19 ± 1.51^{c}	7.07 ± 1.88^{b}
5	1.24 ± 0.05^{c}	10.15 ± 2.06^{d}	5.20 ± 1.23^{ce}
10	1.02 ± 0.13^{d}	9.82 ± 0.84^{e}	4.77 ± 0.96^{d}

[a] Heating time of zero is the moment of reaching the preset pasteurisation temperature; identical letters in columns indicate significant differences in enzyme activity at different temperatures ($P < 0.05$).

3.3. The influence of microwave heating at constant temperature

The microwave pasteuriser device ensured even mixing of the sample and even temperature distribution in the liquid during microwave heating. Microwave heating at 62.5, 66 and 70 °C for 1 min led to a significant increase in SOD activity in breast milk samples, which was determined to be 10%, 21% and 34%, respectively (Table 2). After the longest exposure to microwave heating (10 min) at all analysed temperatures, SOD activity was equivalent to about 80% compared with unheated milk.

Unlike SOD, CAT in human milk is highly sensitive to high temperature during microwave heating. In milk samples microwaved at a temperature of 62.5, 66 and 70 °C for 1 min, CAT activity decreased by 34%, 42% and 38%, respectively. Prolonged heating at each of the above temperatures did not induce further significant changes in CAT activity.

The most significant decrease in GPx activity was observed after 1 min of microwave heating. The activity of GPx decreased by around 38% at 62.5 and 66 °C and by around 53% at 70 °C. Interestingly, in the third minute of microwave heating a temporary increase in GPX activity was observed at all three temperatures. In samples pasteurised for 10 min, GPx activity decreased by 44%, 40% and 54%, respectively.

4. Discussion

Activity of the enzymes analysed in this study corresponded to the values reported in the literature (Gutiérrez-Repiso et al., 2014; Yuksel et al., 2015). Significant differences in enzyme activity in milk samples collected from different women can be attributed to individual variations and, in case of SOD and CAT, stage of lactation (Yuksel et al., 2015). Activity of GPx remains stable throughout the entire breastfeeding period (L'Abbe & Friel, 2000).

The pasteurisation temperature for pasteurisers use on human donor milk cannot be programmed. Pasteurisation temperature is determined by the capacity of the pasteuriser and the initial temperature of the processed milk sample. In the applied procedure, the time required to reach the initial temperature was equivalent to that of HMB pasteurisation. In HMB, milk is subjected to LTLT at 62.5 °C. Milk samples are exposed to convective heating in a water bath for 30 min without stirring. The influence of LTLT on antioxidant enzymes has been poorly described in the literature (Marinković et al., 2016; Silvestre et al., 2008a). The effect of convective heating under different conditions (time and temperature) with and without stirring during pasteurisation has not been researched to date. It should also be noted that the standard conditions of LTLT have been set for cows' milk. The aim of pasteurisation is to eliminate vegetative microbial pathogens, rather than to preserve the highest biological quality of milk. LTLT can be optimised by shortening heating time at higher temperature or by stirring the sample to ensure uniform distribution of temperature throughout the heated milk.

For all heat treatments using in this study significant differences in the degradation of GPx were not observed among samples that were pasteurised with and without stirring. The most significant decrease in GPx activity was observed in the first stage of processing, during which milk was brought to pasteurisation temperature. Further convective heating had a less detrimental effect on GPx activity. Sample stirring inhibited enzyme inactivation only in the first stage of pasteurisation (less than 10 min) conducted at 62.5 °C. Thirty minutes of pasteurisation at 62.5 °C decreased GPx activity by approximately 53%. Similar results were reported by Marinković et al. (2016) and Silvestre et al. (2008a). According to the cited authors, LTLT significantly decreased GPx activity by 63% and 54%, respectively.

Applied convection heating variants (with and without stirring) did not influence the extent of change in the activity of SOD and GPx during treatment. Sample stirring did not influence CAT degradation at 62.5 °C, but it significantly inhibited CAT inactivation when milk samples were subjected to convective heating at higher temperatures. Degradation of CAT was decreased by at least 23% in milk samples pasteurised at 66 °C with stirring. In human milk, CAT is highly sensitive to elevated temperature. Conventional pasteurisation conducted under conditions identical to those used in HMB decreased CAT activity by 57%. In milk samples pasteurised at 70 °C with stirring, CAT activity decreased by 60% already in the first stage of heating. The enzyme can be used as a marker of changes induced by high temperature in convective heating. The observed changes in CAT activity indicate that human milk pasteurisation by convective heating should not be carried out without

stirring at temperatures higher than 66 °C. SOD in human milk is highly resistant to high temperatures. In the analysed group of antioxidant enzymes, SOD was characterised by the greatest stability during convective heating with and without stirring and during microwave heating.

Convective heating at a temperature of 62.5 °C for 30 min did not induce significant changes in SOD activity. At higher processing temperature of 66 and 70 °C, SOD activity decreased by around 13% after 30 and 20 min, respectively, but the observed changes were not statistically significant. A significant decrease in SOD activity was noted only in the human milk sample processed by convective heating at 70 °C for 30 min without stirring (25%) and by controlled microwave heating at the same temperature after 10 min (20%).

Milk can also be processed by high temperature short time (HTST; 72 °C for 15 s) pasteurisation. This pasteurisation method retains the nutritional and sensory attributes of milk, but it leads to significant deactivation of milk enzymes (Hammershoj, Hougaard, Vestergaard, Poulsen, & Ipsen, 2010) and the loss of approximately half of its SOD activity (Donnelly, McLellan, Walker, & Robinson, 1989). Silvestre, Ruiz, Martinez-Costa, Plaza, and Lopez (2008b) demonstrated that HTST pasteurisation induces a significantly greater loss of the bactericidal capacity of human milk than low-temperature processing. In the HTST method, milk is pasteurised by a continuous system of plate heat exchangers, but this treatment is difficult to use to pasteurise small portions of milk in HMB. The HTST method also causes sedimentation on plate surfaces, which decreases heat transfer.

Microwave heating is more effective in retaining heat-sensitive nutrients and decreasing fouling than convective heating (Dehghan, Jamalian, Farahnaky, Mesbahi, & Moosavi-Nasab, 2012). Albert, Mándoki, Csapó-Kiss, and Csapó (2009) observed no significant differences in the amino acid composition, free amino acid content and biological value of milk processed by microwave heating and conventional heat treatment. Salamon et al. (2009) heated raw milk using microwave methods and did not report differences in the fatty acid content of heated milk and the control sample. Microwave heating at low temperatures (20 °C–71 °C) had no significant effect on the levels of total IgA and specific IgA to *Escherichia coli* serotypes 01 and 04 (Quan et al., 1992) or the content of fat and carotenoids in human milk (Tacken et al., 2009). The results of the present study also indicate that microwave heating retains heat-sensitive proteins in milk. Microwave heating had a less detrimental effect on CAT activity. In milk samples subjected to microwave heating at 70 °C for 10 min, CAT activity decreased by approximately 49%. Microwave heating had an equally unexpected effect on SOD and GPx activity. A transient increase in SOD and GPx activity was observed in human milk microwaved at a constant temperature; SOD activity increased after 1 min, and GPx activity – after 3 min of microwave heating.

The observed variations in SOD and GPx activity and the stability of CAT can probably be attributed to the release of enzymes from human milk cells or the specific effects of microwave heating.

Results of studies investigating the influence of microwave heating on enzymes are inconclusive. Recent research suggests that microwave heating has a more destructive effect on enzymes than conventional heating. The above can be attributed to the enhanced thermal effects of microwave heating on enzyme inactivation, which is not only related to temperature, as measured by ordinary means (Ahmed & Ramaswamy, 2007). However, electromagnetic field strength had no effect on the tertiary structure of trypsin. Simulations revealed that the electromagnetic field in a typical laboratory microwave reactor was 3–4 orders of magnitude too low to induce conformational changes in proteins or enzymes (Damm, Nusshold, Cantillo, & Kappe, 2012).

According to other authors, microwave irradiation could exert specific effects on the structural and functional properties of enzymes. Direct energy transfer between the electromagnetic field and polar protein domains could modify enzyme flexibility and, consequently, change enzymatic properties and increase the reactivity of the functional groups involved in an enzymatic reaction (Mazumder, Laskar, Prajapati, & Roy, 2004). Horikoshi, Nakamura, Kawaguchi, Kondo, and Serpone (2016) demonstrated that microwave radiation enhanced CAT activity, but only for a short time when heating time was less than 3 min.

The noted results could also be attributed to the release of enzymes from human milk cells that break down under the influence of thermal shock resulting from the rapid increase in the temperature of microwave-processed milk. Human milk contains two isoforms of SOD, copper and zinc superoxide dismutase (CuZnSOD), which is found mainly in the cytoplasm, and mitochondrial manganese superoxide dismutase (MnSOD) (Kasapović, Pejić, Mladenović, Radlović, & Pajović, 2005). GPx is a selenium-containing, cytosolic enzyme. CAT is ubiquitous in almost all mammalian tissues in both soluble and membrane-bound forms. It is located mainly in peroxisomes, where other enzymes of the oxidoreductase class, including L-amino acid oxidase and α-hydroxy acid oxidase, are also found. In mammalian peroxisomes, CAT may account for up to 16% of all proteins. A small amount of the enzyme was detected in the mitochondria and the endoplasmic reticulum (Ścibor & Czeczot, 2006).

Li et al. (2015) demonstrated that viability of somatic cells in human milk decreased by 97% in samples heated at 60 °C for 30 min. One millilitre of human milk contains around 14,000 cells, including macrophages, neutrophils with a small percentage of lymphocytes, and mammary epithelial cells (Cregan et al., 2007). A rapid increase in the temperature of the entire sample (the final temperature was attained in around 3 min) leads to cell degradation. Thermal shock destabilises the cell membrane. The above suggests that the observed temporary increase in SOD and GPx activity was caused by the release of enzymes from human milk cells. However, prolonged heating at the above temperatures resulted in degradation of these proteins.

Enzymes are probably also released from human milk cells during LTLT pasteurisation. However, temperature increase is more gradual during convective heating (62.5 °C was attained in 12.6 min, process without stirring), therefore, it can be assumed that an increase in enzyme activity is levelled out by its inactivation under exposure to high temperature.

Time of exposure to the temperatures generated during conventional and microwave heating cannot be directly compared. Convective heat transfer is the transfer of heat from one place to another by the movement of fluids. Microwave energy is delivered through a molecular interaction with microwaves, molecular friction resulting from dipole rotation of polar solvents and conductive migration of dissolved ions (Ahmed & Ramaswamy, 2007). Microwave heating is more effective in the inactivation of microorganisms than convection heating using the same temperature and time of its interaction (Atmaca, Akdag, Dasdag, & Celik, 1996).

Our earlier research shows that application of microwave heating results in total inactivation of bacteria in the significantly shorter time than achieved during convectional heating, even in the case of heat-resistant enterococci. The pre-obtained results suggest that microwave heating at a constant temperature of 62.5 °C for 5 min achieves similar bactericidal effects as LTLT (62.5 °C, 30 min) (Malinowska-Pańczyk et al., 2019). In the present study, the activity of enzymes in milk samples pasteurised by microwave heating at the above temperature and time was comparable with or higher than in samples subjected to LTLT (90% versus 95%, 64% versus 43%, and 71% versus 49%, of initial activity for SOD, CAT and GPx, respectively).

5. Conclusions

The use of human milk in milk banks requires thermal processing to eliminate microbiological hazards. Human milk banks rely on LTLT which is not an ideal method. Pasteurisation guarantees high microbiological quality of milk, but it considerably inactivates many biologically active components. The influence of heating parameters and heat generation methods on these compounds, including antioxidant enzymes, should be taken into consideration when attempting to optimise the LTLT method. However, the tests performed in this study revealed that convective heating at temperatures higher than 66 °C causes significant changes in the activity of antioxidant enzymes in human milk when applied for longer than 20 min.

The results of this study revealed that microwave heating could be an alternative method of pasteurisation. This method supports the achievement of high temperature throughout the entire heated sample within a short period of time. However, the maintenance of constant temperature throughout the process poses a problem, and it can be achieved only in advanced microwave pasteurisers. Microwave heating is significantly affected by frequency, the sample's dielectric properties, initial temperature, moisture content, mass, geometry and location. These parameters and the initial temperature of the microwaved food product should be controlled or known, so that microwave power can be adjusted to obtain uniform final temperatures.

The quality of human milk after heat treatment has to be strictly controlled. The impact of microwaves on milk composition and the content of bioactive components in milk remains insufficiently investigated. During controlled microwave heating at constant temperature, human milk is exposed to high temperature for a significantly shorter period of time than during convective heating, which considerably inhibits the degradation of antioxidant enzymes. Microwave heating is an efficient and economical processing method. However, the reason for the temporary increase in enzyme activity during microwave heating has not been fully elucidated. It remains unknown whether this effect occurs due to a rapid increase in temperature within a short time and the release of enzymes from human milk cells or whether human milk enzymes are directly affected by the microwave field itself. The extent to which human milk cells are degraded under exposure to microwave heating has to be measured to clarify the above doubts.

To the best of the authors' knowledge, this is the first study evaluating the effects of microwave heating at constant temperature on antioxidant enzymes in human milk.

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