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The effect of UV-C irradiation on lipids and selected biologically active compounds in human milk

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a b s t r a c t

The effect of UV-C irradiation of human milk on lipid oxidation, content of antioxidants (vitamin C and catalase, CAT) and bactericidal compounds (lysozyme), as well as the total antioxidant capacity (TAC), of the breast milk was investigated. In parallel, the extent of inactivation of some bacteria was also determined. UV-C at doses from 85 to 740 J L^{-1} caused total inactivation of *Escherichia coli and Staph-ylococcus aureus,* but bacteria of the genus *Enterococcus* were reduced only partially. There was a sig-nificant increase in content of primary (lipid peroxides, LP) and secondary (thiobarbituric acid reactive substances, TBARS) oxidation products of lipids (by 33% and 36%, respectively) but decreased vitamin C and lysozyme content (by 35% and 41%). UV-C had no effect on the value of the TAC and caused a smaller decrease in CAT activity (by 14%) than conventional pasteurisation (by 60%).

1. Introduction

The quantitative and qualitative composition of human milk fully meets the nutritional requirements of newborns. According to the World Health Organisation, exclusive breastfeeding for 6 months is the optimal way of feeding infants (WHO, 2011). The unique properties of human milk can be attributed to the presence of biologically active components that are vital for the healthy growth and development of infants and that presently cannot be recreated in milk formulas due to technological limitations. Those components include substances with antimicrobial, anti-inflammatory, antioxidant and immunomodulatory properties as well as hormones and enzymes (Gibbins, Wong, Unger, & O'Connor, 2013).

Antimicrobial and anti-inflammatory compounds, including lysozyme, lactoferrin and sIgA, are present in high concentrations in colostrum. These play a protective role in young children whose defence mechanisms against pathogens have not yet fully developed (Levy, 1998; Lonnerdal, 2003). Human milk contains also antioxidants, including vitamins A and C, catalase (CAT) and glutathione peroxidase (GPx). Both full-term and pre-term infants are exposed to strong oxidative stress in initial stages of life, which is why those protective compounds have to be supplied in the required amounts with mother's milk (Canfield, Clandinin, & Davies, 2003; Friel, Friesen, Harding, & Roberts, 2004).

Infants that cannot be nursed may be fed milk from a human milk bank (HMB), if it is possible. The beneficiaries of HMB are mainly children in neo-natal intensive care units. High-risk infants, including sick and premature babies, are fed HMB milk to prevent many diseases such as necrotising enterocolitis, retinopathy of prematurity and alveolar capillary dysplasia (Quigley, Henderson, & Anthony, 2007; Schanler, Lau, & Hurst, 2005). The immune system of premature infants is not fully developed; therefore, HMB milk has to be characterised by high microbiological quality. Milk deposited in HMB is subjected to Holder pasteurisation, also known as low-temperature-low-time (LTLT) treatment, to ensure its microbiological safety. During the process, milk is heated to 62.5 °C for 30 min, which eliminates pathogens but also partially or completely degrades nutrients and biologically active compounds (Moltó-Pouigmarti, Castellote, Permanger, Franch, & López-Sabater, 2011; Siegel, 1993; Silvestre, Miranda, Almansa, Jareno, & Romero, 2008; Thomas & Holt, 1978; Tully, Joned, & Tully, 2001). For this reason, there is a demand for alternative methods that would guarantee milk's microbiological safety while retaining its high biological value. One such method may involve UV-C irradiation.

Ultraviolet (UV) light has a wavelength of 100–400 nm. The electromagnetic spectrum of UV radiation can be divided into three

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ranges based on their photochemical properties and biological effects: UV-A (315–400 nm), UV-B (280–315) and UV-C (200–280) (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Ultraviolet irradiation is a non-thermal method for extending the shelf life of fresh foods (Koutchma, 2009). The UV-C spectrum, in particular the wavelength range 250–270 nm, is most widely used in the food processing industry and medicine.

UV-C radiation exerts bactericidal effects because it is absorbed by bacterial DNA and cytoplasmic proteins. A quantum of light absorbed by DNA leads to cross-linking between the bases of adjacent pyrimidine dimers (thymine and cytosine) in the same DNA strand (Miller, Jeffrey, Mitchell, & Elasri, 1999). The degree of cross-linking is proportional to the amount of UV radiation, and the modified bases lose the ability to form hydrogen bonds with purine bases in the opposite DNA strand. Those processes disrupt DNA transcription and replication, which, in combination with photochemical damage to cytoplasmic proteins, leads to the loss of microbial cell functions and ultimately to cell death (Miller et al., 1999; Rongies et al., 2011; Sastry, Datta, & Worrobo, 2000).

Lipids also undergo photo-oxidation. Light radiation, in particular UV light, transforms triplet oxygen into highly reactive singlet oxygen $^1O_2$, which significantly accelerates the oxidation of polyunsaturated fatty acids (PUFAs). Photosensibilised oxidation leads to the generation of hydrogen peroxides and secondary oxidation products that differ significantly in molecular weight, beginning from low-molecular-weight compounds such as esters, ethers and aldehydes, to oxidised triacylglycerols (TAG) and polymers (Frankel, 2012). The resulting volatile compounds can significantly influence the sensory properties of food products (Engin & Yuceer, 2012; Matak et al., 2007). Selected lipid peroxidation products exert harmful effects by modifying the properties of cell membranes and contributing to organ dysfunction (Gutteridge & Hallwell, 1990; Szymańska, Bruchajzer, & Rotnowski, 1996).

In the food processing industry, UV-C radiation is used to inactivate bacteria on the surface of equipment and products such as meat and fruit, and to disinfect water (Guerrero-Beltran & Barbosa-Canovas, 2004). Pasteurisation with UV light has several limitations. Penetration depth of UV radiation decreases with an increase in the absorption coefficient of the irradiated material (Guerrero-Beltran & Barbosa-Cánova, 2004). Dense and non-transparent liquid foods, such as milk and fruit juice, have to be irradiated in a thin layer or stirred vigorously around the area of contact with the radiation source (Christen, Lai, Hartmann, & Geddes, 2013a; Christen, Lai, Hartmann, Hartmann, & Geddes, 2013b; Guerrero-Beltran & Barbosa-Canovas, 2004).

There is a general scarcity of published information about the effect of UV-C irradiation on the components of human milk. Christen et al. (2013a) exposed human milk to UV-C irradiation and found that the radiation dose that induced a 5-log reduction in bacterial counts (five species of bacteria) did not induce significant changes in the activity of bile salt-stimulated lipase (BSSL), alkaline phosphatase (ALP) or the content of fatty acids. The applied treatment reduced the content of IgA, lactoferrin and lysozyme, but the noted decrease was less profound than that induced by conventional Holder pasteurisation (Christen et al., 2013b).

Research has demonstrated that human milk pasteurisation under exposure to UV-C radiation could offer an effective alternative to Holder pasteurisation. It should be noted, however, that UV light leads to the photo-oxidation of food components, in particular lipids. The aim of this study was to determine the effect of UV-C irradiation, in doses that eliminate bacteria to the extent accomplished by Holder pasteurisation, on the generation of primary and secondary products of lipid oxidation in human milk. The influence of UV-C irradiation on biologically active components (lysozyme), antioxidants (catalase, vitamin C) and the total antioxidant capacity (TAC) of human milk was also evaluated.

All experimental procedures were 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. Materials

Mature milk was obtained from five healthy mothers, residents of Gdańsk, who gave birth to full-term babies at the Department of Obstetrics of the Clinical Hospital in Gdańsk. Perinatal complications were not noted. All newborns were in good health, and their body weights were within the norm (3100–3800 g). Milk was pumped by the mothers with an electric breast pump (Symphony, Media) with observance of general hygiene standards. Milk was collected from the mothers within 24 h after pumping, and was stored at 4 °C. Milk samples were pooled and divided into smaller portions. The samples were freeze-stored at a temperature of −80 °C until analysis, but not longer than for 3 weeks.

The analysed microorganisms, *Staphylococcus aureus* (PCM 2054), *Escherichia coli* (K-12), *Enterococcus faecalis* (PCM 896) and *Enterococcus faecium* (ATCC 6057), were supplied by the Polish Microbial Collection of the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław. Cultures of the above microorganisms in the stationary phase of the bacterial lifecycle were obtained by incubation at 37 °C for 24 h in tryptic soy broth (TBS) (Merck, Poland).

2.2. Holder pasteurisation

Freeze-stored (−80 °C) human milk was thawed at 37 °C. Glass beakers containing 100 mL of human milk each were placed in a water bath with a magnetic stirrer and was heated at 62.5 °C for 30 min. The time to heating of milk from an initial temperature 18 °C–62.5 °C was approximately 20 min. After pasteurisation, samples were quickly cooled to 20 °C.

2.3. Preparation of samples for microbiological analysis

Milk after pasteurisation, free from any virulent microorganism, was inoculated with the analysed microorganisms to obtain a final concentration of 10^5 cfu mL^{-1}.

2.4. Exposure of milk samples to UV-C irradiation

A milk sample of 120 mL with a temperature of around 18 °C was transferred to a 250 mL beaker. A UV-C lamp (Aquael Steriliser UV AS-3W, Poland) was placed in the centre of the beaker. During irradiation, the sample was stirred with a magnetic stirrer (MR Hei-Standard, Heidolph, Germany) at a speed of 500 rpm. Samples of 4 mL each were collected for analyses after 5, 10, 20, 30 and 40 min of irradiation.

2.5. Microbiological analysis

Pasteurised, UV-C treated and untreated milk samples were serially diluted with saline peptone water. The pour plate method was applied for enumeration of bacteria with the use of tryptic soy agar (TSA) (Merck, Poland). Plates were incubated for 48 h at 37 °C.

2.6. Lipid peroxidation assay

Lipid peroxidation in milk was determined by the method described by Turoli, Testolin, Zanini, and Bellu (2004). Lipids were extracted from milk with organic solvents, peroxides were reacted with potassium iodide, and the released iodine was measured in a spectrophotometer at 353 nm wavelength. The concentration of lipid peroxides (LP) [μM] was calculated based on a coefficient, ε, for J^{3-} at 2.19×10^4 M^{-1} cm^{-1} (Aust, 1985).

2.7. Determination of the content of thiobarbituric acid reactive substances

The oxidation of PUFAs in human milk fat was determined by measuring the content of thiobarbituric acid reactive substances (TBARS), the secondary products of lipid peroxidation, in milk samples (Turoli et al., 2004). The results were expressed as malondialdehyde (MDA) based on the standard curve of the relationship between absorbance and MDA concentrations in reference solutions.

2.8. Determination of total antioxidant capacity

The total antioxidant capacity (TAC) of human milk was determined with the use of the ABTS reagent (Martysiak-Żurowska & Wenta, 2012). The results were expressed as Trolox equivalent antioxidant capacity (TEAC) based on the standard curve illustrating the relationship between absorbance and Trolox concentrations in reference solutions. TAC expressed as Trolox (TEAC) results from the activity and quantity of all hydrophilic and lipophilic antioxidants in the analysed sample, including vitamins, non-biological systems that scavenge free radicals and their mutual interactions.

2.9. Determination of vitamin C content

The vitamin C content of milk was determined by reversed-phase high-performance liquid chromatography with UV detection (RP-HPLC/UV) according to the method described by Romeu-Nadal, Castellote, and Lopez-Sabater (2008). Vitamin C occurs in the form of ascorbic acid (AsA) and dehydroascorbic acid (DHsA) and, to determine total vitamin C content, DHsA was reduced to AsA with DL-dithiothreitol (DTT). Ascorbic acid was stabilised with 0.56% metaphosphoric acid, and the transparency of the resulting solutions was achieved by adding 5% trichloroacetic acid (TCA). The content of vitamin C (AsA) in milk samples (mg 100 mL^{-1}) was calculated based on calibration curves illustrating the relationship between peak areas and AsA concentrations in reference solutions. Analytical recovery of vitamin C was taken into account in the final calculations.

2.10. Determination of catalase activity

Catalase (CAT) activity was determined with the use of the Catalase Assay Kit (Cayman Chemicals, Ellsworth Rd, USA). The method is based on the reaction of catalase with methanol in the presence of hydrogen peroxide at the optimal concentration. The concentration of the produced formaldehyde was measured spectrophotometrically after staining with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald).

2.11. Determination of lysozyme concentration

The concentrations of lysozyme in samples of untreated human milk and milk irradiated with UV-C were determined by the sandwich ELISA assay with the use of the Lysozyme ELISA Kit (Immundiagnostik AG, Bensheim, Germany).

2.12. Statistical analysis

The results were expressed as mean values with standard deviation. Data were processed statistically in the Statistica 12.0 program. The significance of differences between analyte concentrations in samples of untreated milk and milk exposed to various doses of UV-C radiation was determined by one-way ANOVA and Tukey's post-hoc test. The results were regarded as being statistically significant at $P \leq 0.05$.

3. Results

3.1. UV-C irradiation

The UV-C radiation dose absorbed by the sample was calculated based on the volume of the irradiated sample (L), actual output of the UV-C lamp (W) and time of exposure (s) (Shama, 2014). Radiation intensity at a wavelength of 254 nm, measured at the point of contact with the sample with the Photo-Radio meter HD2302.0 with a UV-C probe (Delta OHM, Italy), was 9.1 W m^{-2}. The absorption coefficient of milk (300 cm^{-1}) was used in the calculations (Shama, 2014). The absorbed dose was determined based on the area of contact between the radiation source and vigorously stirred milk (Fig. 1).

The UV-C radiation dose supplied during 5, 10, 20, 30 and 40 min of exposure was determined at 85, 172.9, 355.1, 544 and 740.1 J L^{-1} of milk, respectively. Milk temperature was controlled during the process. The average temperature of milk samples under exposure to the maximum UV-C dose was 27.1 ± 1.1 °C (Fig. 2). The above results suggest that all changes induced by UV-C in milk are related to the radiation dose rather than thermal processing.

3.2. The effect of UV-C on bacteria

Bacteria of the genus *Enterococcus* were relatively resistant to the applied doses of UV-C radiation, and the degree of inactivation varied between the bacterial species. *E. faecium* was more

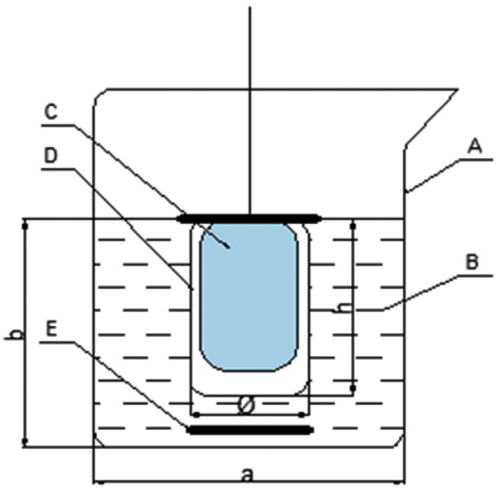

Fig. 1. Diagram of the apparatus for irradiating human milk with UV-C. A, beaker; B, liquid (milk); C, filament; D, external quartz shield of filament (h = 37 mm, ϕ = 27 mm); E, magnetic stirrer. Dimensions: a, width of beaker; b, height of the liquid column.

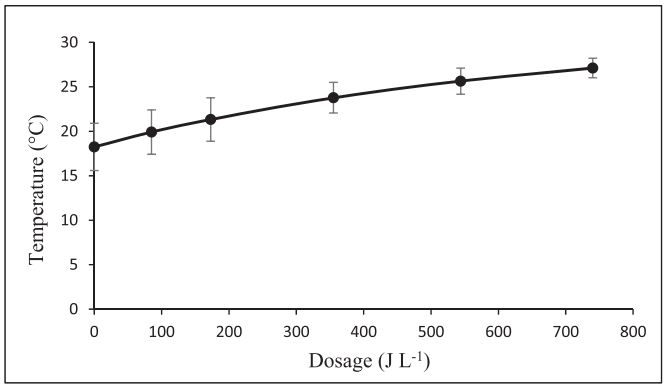

Fig. 2. The average increase in milk temperature during exposure to UV-C radiation. Error bars represent standard deviation of data from ten replicates.

resistant to radiation than *E. faecalis*. The maximum radiation dose (740 J L^{-1}) decreased the number of bacteria by 3.95 log cycles (Fig. 3C), whereas numbers of *E. faecalis* decreased by 2.9 log cycles relative to the initial population size of approximately 10^5 cfu mL^{-1} (Fig. 3D). *S. aureus* and *E. coli* were more sensitive to UV-C treatment, and they were not detected in 1 mL samples after exposure to 400 and 700 J L^{-1}, respectively (Fig. 3A and B).

These results are similar to those obtained by Holder pasteurisation (62.5 °C, 30 min), after which *E. coli* and *S. aureus* were also inactivated and bacteria of the genus *Enterococcus* were reduced only partially. Heat treatment reduced the number of *E. faecalis* and *E. faecium* by 3.92 log and 4.28 log cycles, respectively (Table 1).

Table 1
The effect of Holder pasteurisation on bacterial survival in human milk.[a]

Strain	Bacterial counts (log cfu mL^{-1})	
	Raw milk	Pasteurised milk
Staphylococcus aureus (PCM 2054)	5.20	Not detected
Escherichia coli (K-12)	5.08	Not detected
Enterococcus faecalis (PCM 896)	4.95	1.03
Enterococcus faecium (ATCC 6057)	4.90	0.62

[a] Bacterial counts were performed on untreated (raw) and pasteurised (62.5 °C for 30 min) milk samples.

3.3. The effect of UV-C on the biochemical composition of milk

The analysed milk was characterised by a relatively high content of antioxidants, vitamin C (34 mg L^{-1}) and catalase (18 nmol min^{-1} mL), which corresponded to TEAC values of raw human milk at 23 mg TE 100 mL^{-1}. Lysozyme content was estimated to be 153 μg mL^{-1}. Untreated milk contained the primary and secondary products of lipid peroxidation: lipid peroxides at a concentration of about 10 nM mL^{-1} and TBARS at approximately 37 μg MDA 100 mL^{-1} of milk.

3.3.1. Lipid peroxidation products

The content of lipid peroxides (LP) expressed in μM L^{-1} changed during UV-C irradiation. Treatment at 173 J L^{-1} increased the concentrations of primary products of lipid peroxidation by approximately 35% relative to the initial value. Continued exposure to UV-C irradiation led to a gradual decrease in lipid peroxide levels, to approximately 12 μM (Table 2).

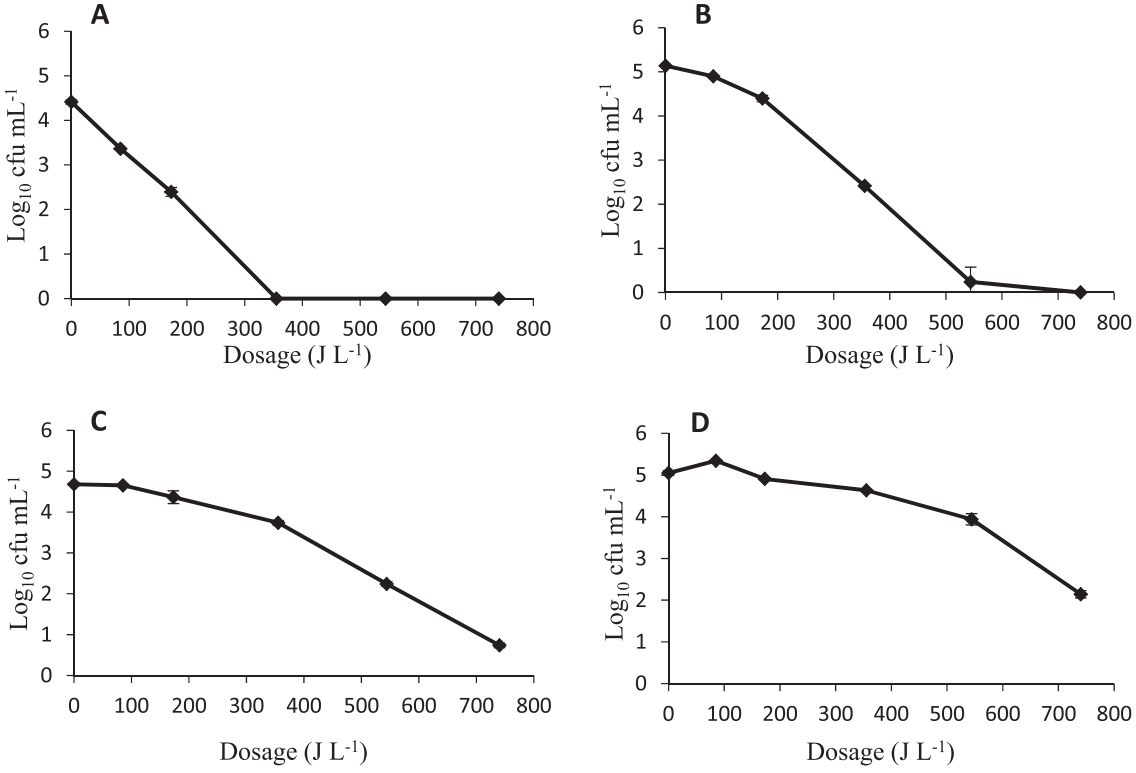

Fig. 3. The effect of UV-C irradiation on the survival of bacteria: A, *Staphylococcus aureus* (PCM 2054); B, *Escherichia coli* (K-12); C, *Enterococcus faecium* (ATCC 6057); D, *Enterococcus faecalis* (PCM 896), suspended in human milk (mean value ± SD).

Table 2
Concentrations of selected compounds in untreated and UV-C irradiated human milk.[a]

UV-C dose (J L^{-1})	LP (nM mL^{-1}) n = 12	TBARS (μg MDA 100 mL^{-1}) n = 23	Vitamin C (mg L^{-1}) n = 20	CAT (nmol min^{-1} mL) n = 12	TAC (mg TE 100 mL^{-1}) n = 18	Lysozyme (μg mL^{-1}) n = 12
0 (raw milk)	10.8 ± 1.25^{a,b}	37.1 ± 4.60^{a}	34.5 ± 3.67^{a}	18.4 ± 1.17	23.1 ± 2.81	152.9 ± 14.89^{a,b}
85	10.7 ± 1.53^{a,c} (99.1)	38.1 ± 4.98^{a} (102.7)	32.7 ± 3.39^{a} (94.8)	19.0 ± 2.92 (96.8)	24.0 ± 2.38 (103.9)	161.7 ± 24.02^{a} (105.8)
173	14.5 ± 1.95^{d} (134.3)	40.5 ± 5.39^{a,b} (109.2)	29.4 ± 4.88^{b} (85.2)	16.5 ± 2.06 (111.5)	24.5 ± 2.86 (106.1)	140.0 ± 25.16^{b} (91.6)
355	13.7 ± 2.73^{d,e} (126.9)	41.7 ± 7.75^{b} (112.4)	26.6 ± 4.52^{b,c} (77.1)	16.1 ± 3.60 (114.3)	24.6 ± 3.56 (106.5)	107.4 ± 31.53^{c} (70.2)
544	12.0 ± 2.22^{b,c,e} (111.1)	46.5 ± 6.81^{c} (125.3)	25.4 ± 3.90^{c} (73.6)	15.7 ± 1.04 (85.3)	23.6 ± 3.36 (102.2)	93.2 ± 19.16^{c} (61.0)
740	12.1 ± 2.12^{b,c,e} (112.0)	50.2 ± 6.56^{d} (135.3)	22.3 ± 3.69^{d} (64.6)	16.1 ± 3.23 (114.3)	22.9 ± 4.01 (100.9)	90.3 ± 16.07^{c} (59.1)

[a] Values are the mean ± standard deviation with changes in the value of selected compounds expressed (in brackets) as percentage relative to the value of the raw milk (100%); means in the same columns followed by different superscript letters denote significant differences in the evaluated parameter ($P < 0.05$). Abbreviations are: LP, lipid peroxides; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; CAT, catalase; TAC, total antioxidant capacity; TE, Trolox equivalents.

The TBARS content of irradiated milk samples continued to increase proportionally to the absorbed UV-C dose to a final level of 50 μg MDA 100 mL^{-1} (an increase of approximately 36%).

This study demonstrated that pasteurisation induces an estimated 17% decrease in LP and a minor, statistically non-significant increase in TBARS levels (by 8.6% relative to the initial value) (Table 3).

3.3.2. Antioxidant compounds

UV-C irradiation of human milk led to a statistically significant decrease in vitamin C concentration (around 15%) under exposure to a radiation dose of 173 J L^{-1}. The highest dose of radiation (740 J L^{-1}), induced a nearly 35% decrease in vitamin C levels in comparison with untreated milk (Table 2). Holder pasteurisation (62.5 °C, 30 min) lowers the vitamin C content of human milk by about 40% (Table 3).

In this study, UV-C radiation doses higher than 85 J L^{-1} decreased CAT activity by nearly 14%, but the noted change was not statistically significant (Table 2). The influence of Holder pasteurisation on CAT levels in human milk has not been investigated to date. Our results indicate that CAT activity decreases by around 57% in milk heated to 62.5 °C for 30 min. These findings suggest that CAT is more susceptible to degradation caused by thermal processing than by UV-C irradiation.

A minor and statistically non-significant increase in TAC values was observed when milk was exposed to UV-C doses of 85–355 J L^{-1}, whereas, at the highest radiation dose of 740 J L^{-1}, TAC reached the level noted in untreated milk (around 23 mg TEAC 100 mL^{-1}). No changes in TAC values were noted in samples subjected to Holder pasteurisation.

3.3.3. Lysozyme

The UV-C radiation dose of 85 J L^{-1} did not affect lysozyme concentrations in the analysed milk samples. A gradual increase in UV-C dose decreased lysozyme levels and, after treatment at 740 J L^{-1}, the lysozyme content of milk was decreased by around 40% relative to the initial value. In a study by Christen et al. (2013b), the lysozyme content of milk decreased by 9%, 16% and 35% after exposure to UV-C for 8.3, 14.8 and 26.5 min, respectively. In milk subjected to Holder pasteurisation, lysozyme concentrations decreased by approximately 47% relative to the initial value.

3.4. Correlations between changes in the content of lipid peroxides and bioactive substances

The content and activity of the analysed milk components and lipid peroxidation products were correlated with the applied doses of UV-C radiation. Significant changes in the content of the evaluated compounds were not observed under exposure to 85 J L^{-1} of UV-C radiation. The threshold dose was 173 J L^{-1} (Table 2), and this led to a decrease in the levels of vitamin C and CAT, compounds which scavenge free radicals and neutralise oxidation products. Photo-oxidation induced by UV-C radiation significantly increased peroxide concentrations and led to a gradual increase in TBARS values. The processes observed in human milk exposed to UV-C doses of 173–544 J L^{-1} suggest a correlation between LP degradation, the formation of TBARS (which rely on free radicals and peroxides as reaction substrates) and the degradation of lysozyme and vitamin C. Catalase activity and TAC levels in the above UV-C range remained constant. UV-C doses higher than 544 J L^{-1} led to a further increase in TBARS (by another 10%) and a decrease in vitamin C concentrations.

4. Discussion

S. aureus and *Enterococcus* are considered the natural microflora in human milk (Fernandez et al., 2013). A criterion for acceptability of milk in HMB is a correspondingly low level of coagulase-negative Staphylococci (>10^4–10^5 cfu mL^{-1}; Arslanoglu et al., 2010). Among the microorganisms that can affect the microbiological quality of breast milk are coliforms; significant numbers of *E. coli* in milk suggests a general lack of cleanliness in handling and improper storage.

The UV-C doses applied in this study exerted similar effects on *E. coli* and *S. aureus* to those noted for Holder pasteurisation. The above microorganisms were not detected in 1 mL milk samples exposed to UV-C doses of 544 J L^{-1} and 355 J L^{-1}, respectively. Similarly to Holder pasteurisation, the tested UV-C doses failed to inactivate *E. faecium* and *E. faecalis* bacteria (McAuley, Gobius, Britz, & Craven, 2012). The analysed pathogens are opportunistic microorganisms, and they can pose a health threat when present in high concentrations in milk, in particular to pre-term infants (Becker et al., 2004; Kluytmans, van Belkum, & Verbrugh, 1997;

Table 3
Concentrations of selected compounds in unheated (raw) and pasteurised (62.5 °C for 30 min) human milk.[a]

Treatment	LP (nM mL^{-1}) n = 12	TBARS (μg MDA 100 mL^{-1}) n = 12	Vitamin C (mg L^{-1}) n = 11	CAT (nmol min^{-1} mL) n = 8	TAC (mg TE 100 mL^{-1}) n = 20	Lysozyme (μg mL^{-1}) n = 6
Raw milk	26.7 ± 1.74^{a}	35.8 ± 7.78	53.1 ± 5.29^{a}	17.0 ± 1.56^{a}	40.5 ± 5.44	140.8 ± 9.46^{a}
Holder pasteurisation	20.4 ± 1.33^{b}	38.9 ± 7.19	32.7 ± 4.42^{b}	7.3 ± 0.96^{b}	38.8 ± 4.52	76.6 ± 13.70^{b}

[a] Values are means ± standard deviation; means in a column with a different superscript letters are significantly different in the evaluated parameter ($P < 0.05$). Abbreviations are: LP, lipid peroxides; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; CAT, catalase; TAC, total antioxidant capacity; TE, Trolox equivalents.

Murray et al., 2003). There is a general absence of published data relating to the effect of UV-C radiation on *Enterococcus* bacteria in mammalian milk. Crook, Rossitto, Parko, Koutchma, and Cullor (2015) demonstrated that radiation doses of 1500–2000 J L^{-1} reduced *S. aureus* and *E. coli* counts by only 5 log cycles relative to an initial population of 10^7 cfu mL^{-1}.

The exposure of human milk to a UV-C dose of 740 J L^{-1} decreased vitamin C content, whereas the TAC of milk was not significantly affected. The effect of irradiation on the vitamin C content and the TAC values of milk were similar to the values noted after Holder pasteurisation. Vitamin C is the most abundant vitamin in human milk and, due to its high sensitivity to external factors, including UV radiation, vitamin C is one of the key indicators of changes in the properties of foods during processing and storage. The influence of UV-C irradiation on the vitamin C content of human milk has not been investigated to date. A study of bovine and caprine milk revealed significant loss of vitamins, including vitamin C, under exposure to UV-C radiation (Guneser & Karagul Yuceer, 2012). In the cited study, UV-C doses of 12.6–88.2 J mL^{-1} decreased vitamin C levels by 45–100%. By comparison, Holder pasteurisation (62.5 °C, 30 min) lowers the vitamin C content of human milk by 20% (Moltó-Pouigmarti et al., 2011) to around 36% (Van Zoeren-Grobben, Schrijver, Van den Berg, & Berger, 1987).

The observed drop in lysozyme content under exposure to the UV-C dose of 740 J L^{-1} was similar to the noted after Holder pasteurisation. Catalase was more resistant to UV-C radiation than to prolonged heating at a temperature of 62.5 °C. This enzyme was less degraded by UV-C radiation than by Holder pasteurisation.

The results of this study clearly indicate that UV-C radiation generates the primary and secondary products of lipid peroxidation. A significant increase in the peroxide content of human milk (by around 35% relative to the initial value) was noted already after treatment at a UV-C dose of 173 J L^{-1}.

Singlet oxygen produced under exposure to UV-C is approximately 1000 times more reactive than triplet oxygen (Frankel, 2012). Singlet oxygen reacts directly with double bonds within the fatty acid chains to form hydrogen peroxide, and it initiates the oxidation of PUFAs. In successive stages of oxidation, hydrogen peroxides are transformed into secondary oxidation products, TBARS. There are no published data relating to the direct effect of UV-C radiation on the TBARS content of human milk. Matak et al. (2007) exposed caprine milk to a UV-C radiation dose of 15.6 mJ cm^{-2} and observed a nearly 90% increase in TBARS from 31 to 59 μg 100 mL^{-1}. Bandla, Choudhary, Watson, and Haddock (2012) also reported a significant increase in TBARS values (by around 85% relative to the initial value) in bovine milk exposed to a UV-C radiation dose of 16.8 mJ cm^{-2}. Holder pasteurisation of human milk does not significantly affect the oxidative status of milk (Elisia & Kitts, 2010).

Human milk is one of the most complex substances in nature. Its lipid content is estimated at 3.8%, and unsaturated fatty acids account for nearly 50% of the total fatty acid pool. Linoleic acid, C18:2, 9c12c, accounts for around 10% of total fatty acids, whereas long-chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid (AA) 20:4 (n-6), eicosapentaenoic acid (EPA) 20:5 (n-3) and docosahexaenoic acid (DHA) 22:6 (n-3), which are vital for the healthy growth of infants and small children, represent together 3–4% of total fatty acids in human milk (Koletzko, Rodriguez-Palmero, & Demmelmailr, 2001). The results of this study also demonstrated that photo-oxidation is responsible for the synthesis of secondary products of lipid peroxidation. After 20 min exposure to a UV-C dose of 355.1 J L^{-1}, the observed decrease in peroxide levels in milk samples corresponded with an increase in the concentrations of secondary products of lipid peroxidation expressed as TBARS (Fig. 3). UV-C radiation can also contribute to the formation of volatile compounds that significantly influence the sensory properties of milk (Engin & Yuceer, 2012; Matak et al., 2007). Volatile organic compounds (VOC) are produced mainly from oleic acid, C18:1 9c, under exposure to singlet oxygen. Matak et al. (2007) reported that a UV-C dose of 15.8 mJ cm^{-2} did not influence the fatty acid composition of caprine milk. The above authors also demonstrated that UV-C radiation induced the production of pentanal, hexanal and heptanal, compounds which lead to detectable changes in the sensory properties of milk. Caprine milk contains around 4% fat, and oleic acid accounts for around 18% of its fatty acids; human milk has a similar fat content, but it contains nearly twice the amount of oleic acid, at 34% on average. The above data suggest that the oxidation of oleic acid produces more volatile compounds in human milk than in caprine milk.

Ultraviolet radiation, in particular the UV-C spectrum, also contributes to the photo-oxidation of food proteins (Davies & Truscott, 2001; Frankel, 1984). Proteins can be oxidised when a quantum of light is directly absorbed by amino acids. Exposure to UV-C is particularly damaging for tryptophan, tyrosine, phenylalanine, histidine, cysteine and cystine. Ultraviolet radiation induces the production of reactive compounds, including free radicals (Davies & Truscott, 2001). Lysozyme molecules contain five of the above amino acids (Thompson, 1955), which, according to Christen et al., (2013b), makes lysozyme sensitive to UV-C-induced damage. The observed significant drop in the lysozyme content of irradiated milk could be attributed to the above process.

Human catalase also contains amino acids susceptible to photo-oxidation. Despite the above, UV-C irradiation did not significantly influence CAT activity in the evaluated milk samples, which can probably be related to the enzyme's specific structure. Human catalase is a homotetramer, and every subunit contains a hæme group with a central iron (Fe III) atom. Tyrosine residues react with arginine, histidine and asparagine residues to isolate hæme from the remaining parts of the enzyme in the active site (Putnam, Arvai, Bourne, & Tainer, 2000). The atoms surrounding iron in the hæme group undergo dynamic changes to ensure that the enzyme remains active, which probably also stabilises the enzyme's activity under exposure to UV-C radiation (Chelikani, Fita, & Loewen, 2004). Catalase was more resistant to UV-C radiation than to prolonged heating at a temperature of 62.5 °C.

The observed changes in the composition of human milk subjected to UV-C pasteurisation were not caused only by the direct effects of UV light or the formation of secondary oxidation products. The decrease in the concentrations of peroxides after 20 min of irradiation could also be attributed to their reaction with antioxidants present in human milk, such as glutathione peroxidase, vitamin E, glutathione and vitamin C. Those compounds inactivate highly reactive peroxides, hydrogen peroxides and their radicals (Saydama et al., 1997; Yu et al., 2005), thus limiting degradation processes. High concentrations of photoreactive products probably stimulate antioxidant activity in milk. The above hypothesis is substantiated by the relative stability of TAC values in the evaluated milk samples.

5. Conclusions

The exposure of human milk samples to UV-C doses of up to 740 J L^{-1} exerts similar effects on lysozyme and vitamin C concentrations and the level of bacterial inactivation to those observed in Holder pasteurisation. Unlike conventional pasteurisation, UV-C irradiation did not induce a significant decrease in catalase activity. It should be noted, however, that UV-C radiation generates hydrogen peroxides and the secondary products of lipid peroxidation, damages nucleic acids and amino acids, and induces free radical degradation of lipids and proteins in human milk. UV-C

irradiation influences mainly milk lipids and proteins. However, all unstable products of lipid and protein oxidation can interact with other components of the mixture, in particular in complex food matrices such as human milk. Those adverse effects should be studied in greater detail before UV-C irradiation is approved for pasteurising human milk, in particular milk fed to pre-term infants.

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