RESEARCH ARTICLE

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Effect of Ozone Treatment Exposure Time on Oxidative Stability of Cream Milk

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Milk cream is a fluid milk product comparatively rich in fat, in the form of an emulsion of fat-in-skimmed milk, obtained by physical separation from milk, and widely used in the food industry. This study aims to evaluate the consequences of ozone treatment exposure time on lipid oxidation of milk cream, using peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) methods. Ozonation process significantly affects PV and TBARS value ($P < 0.05$), in particular, the lipid oxidation increases with the ozone exposure time. The color characteristics (L^*, a^*, and b^* values) of milk cream samples are also affected by ozone treatment ($P < 0.05$); the decrease of color parameters can be related to carotenoids degradation due to ozone treatment. ***Practical applications:*** **Although ozone treatment is an extremely useful method to reduce the microbiological load of foods, the duration of ozone treatment is a key factor to determine the capacity of the ozonation process to balance microbial and chemical quality of foods.**

1. Introduction

The ozonation is an eco-friendly green technology;[1,2] in fact, the ozone, approved by the Food and Drug Administation[3] as a direct food additive,[4] does not leave a chemical residue on either food or food contact surfaces because it quickly auto decomposes to non-toxic products, thus reducing environmental impacts and business costs.[5–7] Furthermore, the current need to formulate new sanitation procedures in the food industry in order to reduce the risks of contamination, and in particular by COVID 19, has led to a greater interest in the use of ozone as studies reported that it is also able to inactivate Coronaviruses,[8–10] and in particular COVID 19.[11] In the dairy industry, ozone treatment includes sanitation of: milk processing equipment; fluid and powdered milk;[12] cheese, and ripening and storage rooms;[13] and dairy wastewater.[14] However, it is known that ozone is very unstable both in the gas phase and in solution, as it decomposes into hydroxyl radicals, hydroperoxide, and superoxide which can react with food macromolecules, especially unsaturated organic compounds, such as fatty acids (UFA), influencing their oxidative stability.[15,16] Lipid oxidation not only reduces the nutritional value and sensory characteristics of the products but causes the generation of compounds that implicate various human diseases, including atherosclerosis, cancer, inflammation, and ageing processes, among others.[17] Ozone reacts readily with olefins giving origin to Criegee zwitterion which combines with the resultant aldehyde to form an "ozonide".[18] The Criegee zwitterion can also react with active hydrogen compounds such as alcohols or acids to yield the hydroperoxides which might decompose by the homolytic scission and to initiate free radical peroxidation of lipids.[18] Ozonides on the other hand reach the oxidation state of ketones or aldehydes,[13] contributing to off-flavors in foods. Alonso et al.[19] reported that the effectiveness of ozone was influenced by the treatment conditions such as temperature, pH, and humidity as well as by the chemical composition of the used matrix. Therefore, the study of oxidative effects of ozone on milk and dairy products is a key factor to determine the capacity of ozone treatment of balancing microbial and chemical quality of products. Despite this, few studies in the literature report the effect of ozone treatment on the oxidative stability of milk and dairy products;[13,20,21] moreover, none of these have evaluated the ozone effect on lipid oxidation of milk cream. Milk cream is a fluid milk product comparatively rich in fat, in the form of an emulsion of fat-in-skimmed milk, obtained by physical separation from milk, widely used in the food industry as ingredient in many foods, including whipped cream, ice cream, many sauces, soups, stews, puddings, custard bases, and cakes. Therefore, the aim of this study was an evaluation of the evolution of some parameters of milk cream in relation to ozone treatment exposure time. (**Table 1**)

2. Results and Discussion

In this study, the primary oxidation products of milk cream samples at different exposure time of ozone was evaluated by PV

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Table 1. Chemical composition of the milk cream.

Parameter	Mean	SD
Fat [%]	55.47	2.83
Proteins [%]	2.68	0.32
Lactose [%]	3.42	0.43
Ashes [%]	0.57	0.08
Moisture [%]	38.19	3.28
pH	5.91	0.53
Density [kg L^{-1}]	1.110	0.07

Table 2. Peroxide and thiobarbituric acid reactive substances (TBARS) values of milk cream samples at different ozone exposure times.

Exposure time	Peroxide value[meq O_2 kg^{-1} fat]		TBARS value[mg MDA1 kg^{-1}]	
	Mean	SD	Mean	SD
Control	0.288[a)]	0.015	1.416[a)]	0.021
10 min	1.387[b)]	0.037	1.490[a)]	0.080
20 min	1.735[c)]	0.024	1.983[b)]	0.090
30 min	1.957[d)]	0.026	2.061[b)]	0.030
40 min	2.437[e)]	0.037	2.643[c)]	0.032
50 min	3.997[f)]	0.039	4.630[d)]	0.036
60 min	4.086[f)]	0.108	7.587[e)]	0.012

[a–f)] Means within a column with different superscripts differ ($P < 0.05$). [1]MDA: malondialdehyde

(**Table 2**). This simple and rapid method can be used to evaluate fat stability, thus monitoring the overall ozonation process.[22,23] Hydroperoxides are formed during the reaction propagation phase; these compounds have no odour or taste and therefore do not affect the sensory profile of food products. Before ozone treatment, the PV of milk cream was in line with what was detected by other authors in milk fat.[24,25] The O_3^- exposure time (0, 10, 20, 30, 40, 50, and 60 min) significantly influenced the PV of milk cream ($P < 0.05$), which ranged from 0.288 meqO_2 kg^{-1} fat in control milk cream to 4.086 meqO_2 kg^{-1} fat at the end of O_3^- exposure time ($P < 0.05$), highlighting that different oxidation products were generated during ozone treatment. The findings of this study are in line with[19] (2017) who found a significant PV increase in fresh milk after 10 min of ozone treatment (80 mg O_3 min^{-1}). Fuhrmann et al.[26] also detected significant oxidative process on egg surface already after low gaseous flow ozone treatment (10 and 25 mL L^{-1} per 60 min). On the contrary, Segat et al.[21] found no significant difference in PV in high moisture mozzarella cheese produced using ozone (30 mg m^{-1} for a time maximum of 120 min) in several phases of the production chain. In this study, already after 10 min of ozone exposure, the PV of milk cream increased by about five times than control, in addition, the PV increased significantly ($P < 0.05$) as the ozone exposure time increased, in agreement with what was reported by Sadowska et al.[27] in both olive and soybean oil. This is due to the action of the third oxygen atom of the ozone molecule, which not being stably bonded, it can easily detach, generating a highly reactive oxidizing system that affects the structural integrity of other molecules. However, no significant difference in PV was detected after 50 min of ozone exposure; this could be due to high hydroperoxides' decomposition into oxidation secondary products (such as aldehydes and ketones) after 50 min of ozone treatment. In fact, the PUFA oxidation generates a fatty acid radical that rapidly adds oxygen forming peroxyl radicals, which can oxidize further PUFA, producing hydroperoxides that can break down to yet more radical species and to a wide range of compounds, in particular aldehydes.[28] Several studies reported the ozone ability to alter UFA and form reactive secondary products, especially acrolein,[29] which can inhibit the nucleic acid synthesis, and interact with the amino group of enzymes, proteins, and DNA causing cellular damage. For this reason, on milk cream samples at different O_3^- exposure time, the TBARS assay was carried out (Table 2). MDA is considered a mark of lipid oxidation because it is the most important aldehydes produced during the secondary lipid oxidation of PUFA. As expected, ozone treatment significantly increased the MDA amount in milk cream; in particular, TBARS value ranged from 1.416 mg MDA kg^{-1} in control to 7.587 mg MDA kg^{-1} at the end of O_3^- exposure time ($P < 0.05$). However, no significant difference was detected after 10 min of ozone exposure; this is due to the initial oxidation of UFA that led to the formation of main hydroperoxides. After 20, 30, and 40 min of O_3^- exposure, the TBARS value increased by 1.40, 1.45 and 1.87 times, respectively, compared to control ($P < 0.05$); while, after 50 min of ozone treatment, the increase was marked compared to the control (3.27 times; $P < 0.05$) until reaching, at the end of the exposure time (60 min), a value 5.37 times greater than the control ($P < 0.05$). To date, there are no research data related to the effect of ozone treatment on milk cream, therefore, results of the current study can only be compared with the results of ozone-treated on oxidation of milk and other dairy products. In agreement with our results, Ipsen[30] detected significant lipid oxidation in whole milk powder after ozone treatment, while, Torlak and Sert[20] highlighted a negative but insignificant impact of ozone treatment (5.3 mg L^{-1} for 120 min) on whole milk powder. Furthermore, Sert and Mercan[31] detected that ozone water treatment during the churning process significantly reduced the oxidative stability of butter. On the contrary, several studies reported the ability of ozone to extend the shelf life of fluid milk without causing a significant oxidation in product.[32] Sert[33] also found no significant difference in TBARS value in high moisture mozzarella cheese produced using ozone (30 mg m^{-3} for a time maximum of 120 min) in several phases of the production chain.

The color characteristics (L^*, a^*, and b^* values) of milk cream samples were affected by ozone treatment ($P < 0.05$, **Table 3**). It is known that ozone treatment significantly affected color of milk and dairy products.[19–21,34–37,22–33] In this study L^* maintained a constant value (about 87.78) up to 40 min of treatment ($P > 0.05$), while a significant decrease was detected after 50 min of ozone exposure ($P < 0.05$), and at the end of ozone treatment L^* value was 85.76 ($P < 0.05$). The a^* and b^* value of ozonated milk cream samples also decreased during treatment; in particular, a^* value ranged from −2.68 in control milk cream to −3.57 at the end of O_3^- exposure time ($P < 0.05$), whereas b^* value ranged from 11.83 in control milk cream to 9.58 at end of O_3^- exposure time ($P < 0.05$). The trend of color parameters detected in this study is in agreement with what was detected by Sert[33] on ozone-treated

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Table 3. Colorimetric parameters (L^*, a^*, and b^* values) of milk cream samples at different ozone exposure times.

Exposure time	L^*		a^*		b^*	
	Mean	SD	Mean	SD	Mean	SD
Control	88.16[c)]	0.10	−2.68[d)]	0.22	11.83[e)]	0.55
10 min	87.80[c)]	0.28	−2.71[d)]	0.07	11.13[d)]	0.23
20 min	87.71[c)]	0.50	−2.92[c)]	0.06	11.12[d)]	0.24
30 min	87.70[c)]	0.19	−3.23[b)]	0.06	10.69[c)]	0.23
40 min	87.54[b,c)]	0.25	−3.40[a,b)]	0.03	10.56[c)]	0.26
50 min	86.95[b)]	0.70	−3.47[a)]	0.02	10.05[b)]	0.31
60 min	85.76[a)]	0.23	−3.57[a)]	0.13	9.58[a)]	0.16

[a–e)] Means within a column with different superscripts differ ($P < 0.05$).

milk cream. The variation of color parameters of milk cream during ozone treatment could be related to carotenoids degradation. In fact, it is well-known that carotenoids are associated with fat fraction and in milk cream, they are relevant in determining its color. Several studies confirmed that oxidation treatments can harmfully affect carotenoids.[38] Moreover, Benevideset al.[39] detected a reduction of β-carotene content after ozone treatment; in particular, these authors hypothesized that all double bonds of the carotenoid molecules are potential sites for ozone to react chemically, this leads to a wide range of oxidation products.[39,40] Total color difference (ΔE) indicates the magnitude of color difference between treated and control samples; it is considered a sensitive parameter for the measurement of color degradation in response to treatment on foods.[41] Overall, ΔE was analytically classified as very distinct ($\Delta E > 3$), distinct ($1.5 < \Delta E < 3$), and small difference ($1.5 < \Delta E$).[42] In this study, the high ΔE detected (3.41), highlighted the high color change undergone by milk cream samples following ozone treatment. Many authors reported that ozone treatment improved or did not alter the shelf life and the sensory quality in beef and eggs;[43,44] therefore, alterations in the sensory attributes were detected as a function of chemical composition of food, ozone dose, and treatment condition.[6]

3. Conclusions

Ozone treatment, which strong oxidizing agent, is an extremely used method to reduce the microbiological load for several applications in the food industry also directly on food.

However, this preliminary investigation showed the reactivity of ozone with organic matter (lipids) containing in milk cream, and consequently the effects on the color of the sample's surface. Excessive exposure to ozone treatments caused lipid oxidation with the formation of metabolites that negatively affect health safety. The results of this study highlighted the importance to evaluate the effect of exposure time to ozone in food to predict the capacity of treatment of balancing microbial and chemical quality of products.

4. Experimental Section

Samples Collection and Ozonation: Cream (≈55% w/w fat; Table 1) was obtained from the higher-fat layer after separation for centrifugation of fresh skim milk. For ozonation treatments, samples were divided into portions (3 g) spread homogeneously as a thin layer (0.2 cm) on glass dishes (Ø 5 cm) and placed in a box handmade with a total volume of 2730 cm^3 (size: 21 × 13 × 10 cm). Ozone treatment was performed at room temperature (RT) for six exposure times (10, 20, 30, 40, 50, and 60 min) under the continuous stream. One milk cream sample not treated was used as a control. Ozone was produced by atmospheric air as a source of oxygen using a generator with an inlet flow of 300 mg O_3/h (Sander Ozone Generator 300, Erwin Sander Elektroapparatebau GmbH, Uetze-Eltze, Germany). Each treatment was carried out six times.

Determination of Lipid Oxidation: The determination of lipid oxidation was carried out on the control sample after the preparation of the milk cream and on the treated samples immediately at the end of the each ozonation time. Initial oxidation products were measured on lipid fraction by peroxide value (PV) method according to the ISO 3976:2006-IDF 74:2006 method.[34] The results were expressed as meq O_2 kg^{-1} fat. Rancidity effect of ozonation on milk cream was evaluated by thiobarbituric acid reactive substances (TBARS) assay as reported by Buege[35] with some modifications. Briefly, 50 mg of milk cream was homogenized with 1 mL of hexane:isopropanol (4:1) by a Polytron (PT-MR 2100, Kinematica AG, Littau, Luzern, Switzerland) at 13 500 rpm for 15 s. The homogenates were placed in an ultrasound water bath apparatus (Elma Transsonic 460/H, Singen, Germany) for 10 min at RT. An aliquot of each homogenate (145 µL) was transferred into a test tube, and 255 µL of distilled water and 600 µL of 2-thiobarbituric acid (TBA) solution (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 M HCl) were added. The mixture was kept at 90 °C for 30 min, followed by cooling in a refrigerator (4 °C) for 30 min. Subsequently, the mixture was centrifuged at 3600 × g for 15 min at RT, and the absorbance of the obtained supernatant was measured at 532 nm. TBARS value was calculated from the standard curve draw using 1,1,3,3-tetraethoxypropane (0.3–2 µg mL^{-1}), and the results were expressed as mg malondialdehyde (MDA) kg^{-1} sample. Each determination was made in triplicate.

Instrumental Color Measurement: Color (CIE L^*, a^*, b^*) was measured using a MINOLTA Chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ, USA) equipped with a D65 illuminant, the 10° Observer, and zero and white calibration. The measuring head has an 8 mm diameter measuring area. Before each measurement, the equipment was standardized against a white tile. On each sample was determined the following color coordinates: L^* (lightness), a^* (redness–greenness), and b^* (yellowness–blueness). The analysis was performed in quadruplicate. The total color difference (ΔE) was measured as the modulus of the distance vector between initial and final color values, using the following equation:[36]

$$\Delta E = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \quad (1)$$

Statistical Analysis: Data were analyzed according to the following linear model:[37]

$$y_{ij} = \mu + \alpha_i + \epsilon_{ij} \quad (2)$$

where y_{ij} is the observation; μ is the overall mean; α_i is the fixed effect of the i^{th} exposure time ($i = 1, 2,...7$); and ϵ_{ij} is the random error. Student's t-test was used for all variable comparisons and differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

All authors have contributed equally to this manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

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