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Ozonation of the pasteurized skim milk to extend the shelf life: evaluation of the chemical and microbial properties

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ABSTRACT

Today, the desire to use ozone as a strong antimicrobial compound, cost-effective, and eco-friendly innovation, has increased to increase the shelf life of food products. This study was conducted in order to evaluate the chemical and microbial properties of thermally pasteurized skim milk treated with pressurized ozone injection at concentrations of zero, 1.5, 5, and 10 ppm. The chemical, microbial and sensory characteristics of the treated milk samples were evaluated on days 0, 4, 7 and 15 of storage in the refrigerator. The total viable count (TVC) in the treated samples with ozone compared to the control had a noticeable decrease in all storage days. The highest antimicrobial effect was observed on the 15th day of storage with 10 ppm ozone and the lowest effect was observed on the 4th day with 1.5 ppm ozone. However, there was no significant difference in pH (6.8-6.88) and acidity (0.16-0.18) between treated and control samples. Although the peroxide number (PV) in the treated samples and the control samples was higher than the standard, but its reduction was evident in the treated samples compared to the control samples. Also, by comparing the sensory characteristics among the samples, no significant differences were observed. In general, treatments with 5 and 10 ppm ozone had the best efficiency in increasing the shelf life of pasteurized milk. According to the results, ozone can be used as an auxiliary method to increase the effectiveness of thermal processes in pasteurization and increase the shelf life of milk.

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1. Introduction

Milk is a nutritious food subject to microbial and chemical spoilage. The initial quality of raw milk is effective in its shelf life, but in order to increase its shelf life, several methods are used, especially thermal processes [1]. Thermal processes are traditionally the most common safe method for controlling microorganisms. However, the nutritional value and sensory characteristics of the product can be affected [2].

Pasteurized milk in cold conditions has a short shelf life due to the survival and activity of thermodiuretic bacteria, so the dairy industry uses more heat to produce a product with a longer shelf life (ESL).[1]) use. Due to the harmful effects of thermal processes on the micronutrient composition of milk, consumers prefer alternative or combined methods with thermal processes to increase the shelf life of milk, without causing adverse changes in the organoleptic properties and maintaining the health of milk [3]. Therefore, research on the addition of chemical compounds or the use of physical (non-chemical) methods along with thermal processes to increase the shelf life of dairy products is expanding [1].

Processing with ozone gas is one of the methods suggested to increase the shelf life of food with the least adverse effect. The use of ozone as a strong oxidizer in the food industry has many advantages, including increasing the level of hygiene in production centers, the absence of residues in the product, controlling effective organisms during the shelf life of food, and preserving the aroma of the product [4]. The antimicrobial effects of ozone on a wide range of microorganisms have been confirmed in various studies [2]. Therefore, the use of ozone in liquid or gaseous phases can improve the microbial quality of food without causing adverse changes in chemical, physical and nutritional properties [5]. Adding ozone before heat application can reduce the enzymes produced by organisms and increase the stability of milk proteins during its storage period [6].

The use of ozone in the industry is limited in the duration of its application and its concentration in addition to its corrosive properties on metal equipment. In some studies, the treatment of food with ozone has caused changes in the sensory characteristics of food and oxidation of lipids [4]. Regarding the possible changes made in milk and the amount of ozone efficiency in increasing the shelf life of pasteurized milk, no special research and studies have been done [1]. Therefore, the current research can give the consumers of dairy products the assurance that using ozone treatment, as a cost-effective and environmentally friendly method, along with thermal processes, can lead to increasing the shelf life of milk without causing adverse sensory and chemical changes. be pasteurized Therefore, the present study was carried out with the aim of evaluating the effect of effective concentrations of ozone gas along with the pasteurization process on the chemical, microbial and sensory characteristics of milk.

2- Materials and work methods

Pasteurized milk was obtained from Pegah milk factory in Hamedan. Ozone gas was provided by an ozone machine (Aqua Life Care, Iran) with a production rate of 5 grams of ozone gas per hour. In this study, microbial culture medium and chemicals from Merck (Germany) were used.

[1]- Extended Shelf life

1.2. Test plan and preparation of treatments

Skimmed milk with 1.2% fat, acidity of 15 (6.6 pH) and freezing point of 520 at 78 degrees Celsius for 15 seconds (by HTST method[2]) was pasteurized. Ozone treatments including concentrations of zero, 1.5, 5 and 10 ppm were separately injected into the cooled pasteurized milk in the tank of the filling machine with the help of a gas guiding pipe in the milk storage tank (simultaneously with the homogenizer activity) and in one kilo bags. It was packed. Samples without ozone injection were considered as control group. The useful concentrations of ozone were calculated based on the power of the device and the time to reach these concentrations. Chemical and microbial evaluations of the samples (each treatment includes 12 one-kilogram packages of milk) were done in three replications on days 0, 4, 7, and 15 of storage at 4 degrees Celsius.

2.2. Microbial analysis

To count the total microbial population (TVC[3]) in the samples, successive dilutions were prepared using sterile peptone water solution (0.1%) and using the mixed culture method.[4] in PCA culture medium[5] were cultured sterile. Then the cultured agar plates were placed in a greenhouse at 37°C for 48 hours. Total microbial population based on logarithmic CFU[6] It was reported in every milliliter of sample [7].

3.2. Chemical analysis

1.3.2. acidity

To measure acidity, 10 ml of human milk sample with a volume of 100 ml was homogenized with 0.5 ml of phenolphthalein reagent. Then, titration was done with 0.1 normal sodium hydroxide solution. Total acidity was calculated using the following equation:

Acidity: N×0.009×100/V

N: Amount of ml of 0.1 normal soda consumed

V: Sample volume

2.3.2. pH measurement

After making sure that the pH meter was calibrated, the samples were poured separately into a beaker with a volume of 100 ml and the electrode of the pH meter was completely inserted into the sample. The temperature of the evaluated samples was set at 20 degrees Celsius. After 45 seconds of contact between the electrode and the sample, the pH of the samples was recorded.

3.3.2. Evaluation of lipid oxidation

In order to extract fat, 10 ml of each sample was mixed well with 25 ml of dichloromethane-methanol solution (with a ratio of 2:1) and centrifuged for 20 minutes (1800 g). After separating the milk fat from the upper phase of the solution, the remaining solvent was removed under vacuum in the evaporator. Evaluation of fat oxidation (PV) in samples using AOCS method[7] And it was done based on the amount of hydroperoxide in the samples [8].

4.3.2. Fatty acid profile measurement

Evaluation of the type and amount of fatty acids in the fat of the treatments was done by gas chromatography (Shimadzu Corp, SGE-BPX70, Japan). One microliter of extracted pure fat of

[2]- High Temperatue Short Time

[3]- Total Viable Count

[4]- Pour plate culture

[5]- Plate count agar

[6]- Colony Forming Unit

[7]6-American Oil Chemists' Society

each sample was injected into the machine. In this study, a column with an inner diameter of 0.25 mm and a column length of 60 meters was used. At the beginning, the temperature of the column is 150 degrees Celsius and kept at this temperature for 1 minute. Then gradually increase the temperature to 240 degrees and give time for 10 minutes to achieve the maximum release of fatty acids. Nitrogen gas with a flow of 1.1 ml/min was used as carrier gas [9].

5.3.2. The main components of milk

In order to measure the amount of protein, fat, lactose and water, Milkscan device (Funck Gerber - Germany) was used. In order to analyze these values approximately, the electrode of the device was inserted into each sample separately [10].

6.3.2. Evaluation of sensory properties

The evaluated sensory characteristics included the appearance, texture, taste and overall acceptance of the sample, which was done in the form of a five-point hedonic test by 20 people who were present in the Pegah factory production line. These people were male and between the ages of 25 and 45.

4.2. statistic analysis

This research was conducted based on a completely randomized design in 3 replications. The tested treatments included pasteurized milk without additives as a control group and ozonized pasteurized milk with concentrations of 1.5, 5, and 10 ppm, which were stored in the cold conditions of the refrigerator for four time periods of 0, 4, 7, and 15 days, microbial characteristics, were evaluated chemically and organoleptically. The difference in the results between different treatments was determined based on a completely randomized factorial statistical design using ANOVA variance analysis at a 95% confidence level. Comparison of average data was done based on Duncan's test using IBM SPSS 26 software.

3. Results and Discussion

1.3. Microbial analysis

Chart 1 shows the results of microbial evaluation of the effect of different concentrations of ozone on the total count of microorganisms in pasteurized milk during storage at 4 degrees Celsius. The growth trend of microorganisms in the control sample from day 0 to day 7 of storage was stable and relatively decreasing, while it showed an increasing trend from day 7 to day 15. Similar to the control group, the microbial population in the samples treated with ozone had a downward trend until the 7th day, and then the population increase was maintained until the 15th day. However, the ozone treatments significantly compared to the control group ($P<0.05$) were associated with a decrease in the microbial population. In such a way that ozone treatments with concentrations of 5 and 10 ppm led to a decrease of more than 0.5 logarithmic cycle of the microbial population of milk in all storage days. This effect in the ozone treatment with a concentration of 1.5 ppm on the 15th day of storage is significantly compared to the control group ($P<0.05$) was evident. The concentrations of 5 ppm and 10 ppm of ozone did not have a significant difference in the level of antimicrobial effectiveness.$P>0.05$) and the effectiveness of both concentrations was greater than ozone 1.5 ppm treatment. The highest and lowest antimicrobial effect of ozone was on the 15th day of storage with a concentration of 10 ppm and on the 4th day of storage with a concentration of 1.5

ppm.Although the increase in the microbial population in the ozone treated samples after the 7th day of storage was less than the control group, but factors such as the gradual decrease in the half-life of ozone in the treated samples and the slow growth of heat-resistant bacteria after milk pasteurization can lead to an increase in the total population of microorganisms in these treatments. The limit of aerobic microbial population in pasteurized milk is 10 CFU/ml^{4}7.5 × is determined[11]. Based on the results obtained from the total count test of aerobic microorganisms in the studied treatments, all the samples were within the microbial standard range of pasteurized milk during storage for 15 days. Studies on the use of ozone to control microorganisms in milk and dairy products have shown that with increasing ozone concentration and contact time in these products, the antimicrobial effect increases [6]. In this regard, Genecya et al. (2020) reported that ozone treatment for 11 minutes can reduce the population of microorganisms in pasteurized colostrum by 97.2 logarithmic cycles [12].

In another study, the microbial evaluation of dry milk powder treated with different concentrations of ozone over successive periods of time showed that its antimicrobial effectiveness increased with the increase in exposure time to ozone. According to studies, the mechanism of this action can be caused by the gradual oxidation of microorganisms [13]. Despite the destruction of all pathogens and the reduction of spoilage bacteria during the milk pasteurization process, a large number of spores and thermodiuretic vegetative cells remain in the pasteurized milk, which limits the shelf life of this product in the refrigerator.become Therefore, applying more heat or using additional processes such as ozonation treatment can be effective in reducing spoilage microorganisms and increasing the shelf life of this product [14].

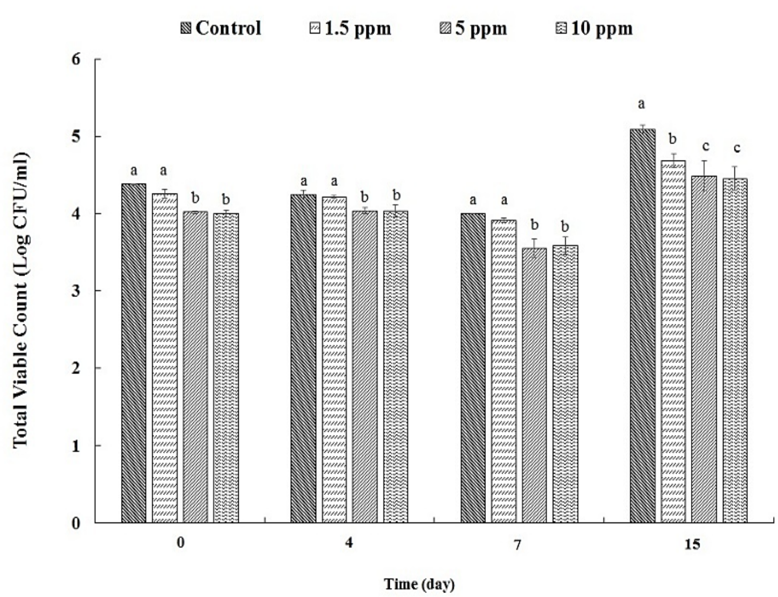

Figure 1 Evaluation of the effect of ozone on Total Viable Count in the treated skim milk samples stored at 4°C

2-3- Chemical analysis

1-2-3- Assessment of acidity and pH of samples

The results of evaluating pH changes in control and ozonized treatments during storage at 4 degrees Celsius are shown in Table 1. In general, the results showed that there were no noticeable changes in the pH of the samples.The highest pH was related to 10 ppm ozone treatment on day 15 and the lowest value was 5 ppm treatment on day 0.As shown in Table 1 The acidity of the control sample was 1.6 on day 0, and the acidity of this sample was 1.8 on day 15, which corresponds to the increase in microbial growth. The numerical value of acidity in the control sample decreased on day 4 and increased on days 7 and 15. The acidity of ozonated samples with a concentration of 1.5 ppm on day 0 reached about 1.6 and on day 15 it reached 1.8. While the use of ozone concentrations of 5 ppm and 10 ppm did not cause any change in the samples during days 0 and 15. Finally, the highest amount of acidity on day 7 was related to the injection of 10 ppm ozone and on day 15 in the control sample and the concentration of 1.5 ppm ozone, and the lowest value was related to the concentration of 10 ppm ozone on day zero.

Measuring acidity and pH are important criteria to be considered in evaluating the shelf life of food. The change of these factors in food during storage can be due to the changes caused by enzyme activity and the growth of microorganisms. According to the standards of the World Health Organization, the pH value of quality milk under heating conditions at a temperature of 100 to 117 degrees Celsius should be around 6.6, which in the present study, all the samples were in this range [15]. According to the research of Lu et al. (2013), there is a significant relationship between the increase of free fatty acids in milk and the decrease of casein percentage with the decrease of pH. Therefore, the pH level can indicate the progress of spoilage, and when the flavor of milk changes due to the decrease in pH, it indicates the oxidation level of free fatty acids and caseins [16]. Genecya et al. (2020) reported that there was no significant change in the pH of control, whole milk, and unpasteurized milk samples [12]. Also, in another study, by injecting ozone into fresh cow's milk at 0.5, 1, 5 and 10 minutes, it was observed that the pH of the samples did not change significantly [17]. This report was consistent with the results of our study.

The acidity of fresh milk is around 0.14-0.16 according to the amount of lactic acid or 14-16 according to Dernik degree. According to the World Health Organization, the level of acidity in quality milk is 17% based on the amount of lactic acid [15]. Among the reasons for high acidity in pasteurized milk, we can point out the non-observance of the cold chain and high ambient temperature. Also, the insufficient heat of pasteurization and the remaining microbial contamination and their related enzymes can increase the acidity in pasteurized milk [18]. Based on the observations of Lu et al. (2013), when spoilage occurs, the acidity of milk increases. Therefore, one of the determining factors in milk quality is acidity. The natural acidity of milk is between 0.16 and 0.18 [16]. By comparing the results of the control sample and the ozone-infused sample with a concentration of 1.5 ppm on day zero, it can be concluded that there is a significant relationship between ozone injection and acidity values. As the microbial population was high at first and ozone did not cause a shock in the microbial population, as a result the acidity of these samples is high. But when a higher concentration of ozone was used, the microbial growth decreased more, as a result of increasing acidity, it was controlled. It was also significant by row comparison of the data in the amount of acidity with the passage of time.

Table 1 the changes of pH and acidity of the treated skim milk samples during storage at 4 ˚C.

Treatments		Storage days			
		0	4	7	15
Control	pH	6.81 ± 0.03	6.82 ± 0.01	6.86 ± 0.00	6.86 ± 0.01
	Acidity	1.5 ± 0.02	1.5 ± 0.01	1.6 ± 0.01	1.8 ± 0.00
1.5 ppm	pH	6.82 ± 0.01	6.81 ± 0.01	6.86 ± 0.00	6.87 ± 0.00
	Acidity	1.6 ± 0.02	1.5 ± 0.05	1.7 ± 0.00	1.7 ± 0.00
5 ppm	pH	6.81 ± 0.02	6.82 ± 0.00	6.86 ± 0.00	6.87 ± 0.00
	Acidity	1.5 ± 0.01	1.6 ± 0.05	1.6 ± 0.00	1.6 ± 0.00
10 ppm	pH	6.80 ± 0.01	6.81 ± 0.01	6.86 ± 0.00	6.88 ± 0.00
	Acidity	1.5 ± 0.11	1.5 ± 0.00	1.7 ± 0.00	1.6 ± 0.05

2-2-3- Evaluation of peroxide number in samples

The results of the evaluation of the amount of peroxide production in the control and ozonized samples during 15 days of storage are shown in graph No. 2. By comparing the data in a row, it was observed that the peroxide value increased significantly in all the control and ozonated samples (in all concentrations) with the passage of time. Also, there was a significant relationship between the concentration of ozone used and the reduction of peroxide number. Thus, with the increase of ozone concentration, the peroxide value decreased more than the control sample. On the 15th day of storage, the concentration of 1.5 ppm of ozone was significantly associated with a greater increase in the peroxide number than the control sample, and in the concentrations of 5 ppm and 10 ppm, with the same values, the peroxide number was higher than the control sample and lower than the concentration of 1.5 ppm. .

Fat oxidation is one of the most important limiting factors in the shelf life of dairy products as well as reducing their quality, nutritional value and bad taste. Among the most important factors influencing the rate of oxidation are temperature, time, pH, fatty acid, antioxidant concentration and the presence of oxygen [19]. On average, the composition of fatty acids in milk fat is 70% saturated, 25% monounsaturated and 5% polyunsaturated fatty acids. also Bacterial lipases and natural lipases in milk can hydrolyze triglycerides and produce free fatty acids and glycerol [20]. The highest amount of peroxide value (PV) in the sample treated with ozone with a concentration of 1.5 ppm was on day 15 and the lowest value was for the sample treated with ozone with a concentration of 10 ppm on day 4. The standard of peroxide value in Iran is less than 5, which was out of standard in all the control and ozonized samples, which could be due to excessive mixing of milk in the production line [11].

Mohammadi et al. (2017) reported that ozone contact with tire milkIt did not have any effect on the peroxide number for 5 minutes, but finally it reached its highest level for 10 minutes [17]. According to these results, it can be concluded that with the increase of dissolved ozone in milk over time, the oxidation process occurs. In a research conducted by Segat et al. (2014), by injecting ozone up to a maximum of 30 mg/L into a sample of mozzarella cheese with high moisture, this sample underwent slight oxidation compared to the sample without ozone [21]. Khanashyam et al. (2021) showed that by injecting ozone into nonfat dry milk, whole milk powder, and cheese, the oxidative stability of these products did not change significantly compared to before ozone injection [22]. As a result, it can be hoped that the peroxide number in the ozonized samples is within the standard range and does not differ significantly from the control sample.

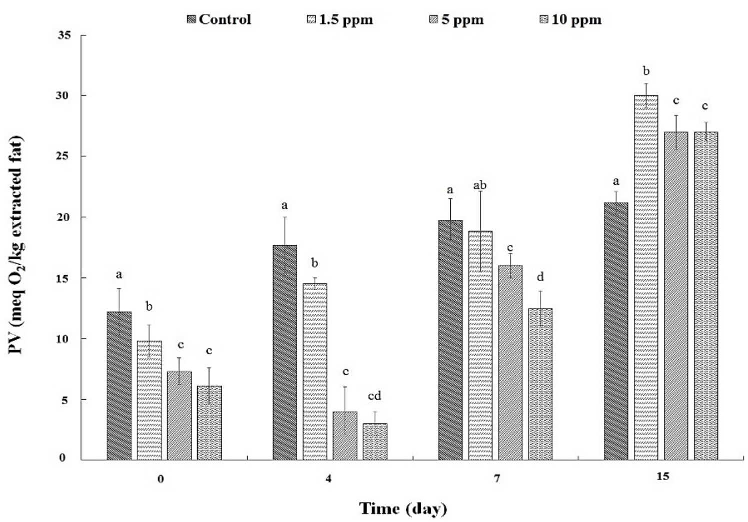

Figure 2 The effect of ozone on the peroxide value of the treated skim milk samples stored at 4°C

3-2-3- Fatty acid profile analysis

The results of gas chromatography analysis of fats extracted from untreated and treated colostrum milk samples with different concentrations of ozone are shown in Table No. 2. According to the obtained data, the highest percentage of C4 was related to the sample treated with ozone with a concentration of 10 ppm on day 4 and the control sample on day 7, and the lowest percentage was related to the sample treated with ozone with a concentration of 10 ppm on day zero. By comparing the available data, no significant relationship was observed between the control and ozonized samples over time.The highest percentage of C6 related to all ozonized samples was on day 4, the control sample was on days 7 and 15, and the lowest percentage was related to the ozonized sample with a concentration of 10 ppm on day zero. By comparing the data, there was no significant relationship between fatty acid levels with the passage of time.Also, the highest percentage of C8 corresponds to the concentration of 1.5 ppm on day 4 and the lowest percentage corresponds to the concentration of 10 ppm on day zero. The highest percentage of C10 corresponds to the ozone concentration of 1.5 ppm on day 4 and the lowest percentage corresponds to the concentration of 5 ppm on day 4.

According to the obtained results, we saw an increasing trend in all short chain fatty acids with ozone injection in all concentrations on day 4 and a decreasing trend on days 0, 7 and 15 compared to the control sample. In the present study, the highest percentage of C18:0, C16 was related to the concentration of 5 ppm and the lowest percentage was related to the concentration of 10 ppm and 15 ppm on day zero. In connection with fatty acid C18:1,The highest percentage was related to 5 ppm concentration on day 4 and the lowest percentage was related to 5 ppm concentration on day 7 and 10 ppm concentration on day 4. According to the obtained data, ozone with a concentration of 10 ppm led to an increase in this fatty acid from day 4 to 15.

in between Palmitic acid has the highest amount of saturated fatty acids in milk, so it is expected that the highest amount is related to this fatty acid in GC analysis. Also, among unsaturated fatty acids, oleic acid has the highest amount.The general pattern among long chain fatty acids C16:0 and C18:0 is almost similar and ozone injection in all concentrations has increased these fatty acids. Among fatty acids, ozone has a high affinity with oleic acid (C18:2n-6) and this reason makes this fatty acid act as a natural antioxidant and reduce the effect of ozone on other fatty acids. Also, ozone had a significant effect on linoleic acid, which is a polyunsaturated fatty acid, compared to palmitic acid, which is a saturated fatty acid.[23]. In a study by Jiang et al., the composition of fatty acids was investigated after storage at 25, 30 and 40 degrees Celsius. The results indicated that during storage at all three temperatures, saturated fatty acids and monounsaturated fatty acids did not undergo any changes. But polyunsaturated fatty acids decrease at temperatures of 30 and 40 degrees Celsius.P<0.05). The cause of this phenomenon can be the high level of reaction of double bonds with free radicals and the degree of occurrence of oxidation [19]. One of the most important factors of milk fat hydrolysis and the subsequent release of fatty acids is excessive stimulation of milk under conditions such as rough handling of milk and late cooling of raw milk, which increases the lipolytic activity of fat-decomposing enzymes (lipase). Lipase enzymes that are naturally present in milk are removed by thermal processes, but lipolytic enzymes of bacterial origin are resistant to heat and remain in milk and cause the oxidation of milk fat during storage [20].

Table 2 GC analysis of the treated skim milk samples stored at 4°C.

Time storage (day)	Treatments	Fatty acid									
		C4	C6	C8	Q10	C12	C14:0	C14:1	C15:0	C15:1	C16:0
0	Control	6644/1	9763/0	7249/0	9108/1	5898/2	0166/10	1483/1	5600/0	1632/1	2105/35
	1.5 ppm	9873/0	8492/0	6917/0	9021/5	6269/2	3892/10	1747/1	2680/1	2946/0	6857/36
	5 ppm	1338/1	0420/1	7961/0	0405/2	6691/2	0001/50	1451/1	5554/0	1414/1	4622/34
	10 ppm	5350/0	6134/0	6012/0	5098/1	1401/2	4533/8	9532/0	4770/5	9943/0	4283/31
4	Control	6621/0	7833/0	6603/0	8862/1	6339/2	3969/10	1689/1	2088/1	2980/5	4143/36
	1.5 ppm	4299/1	4138/1	0753/1	5497/2	0514/3	8587/10	2580/1	6147/0	2033/1	8730/35
	5 ppm	--	--	--	8550/0	6480/1	4602/8	8997/0	5637/0	1081/0	5320/37
	10 ppm	8001/5	3826/1	9483/0	4342/2	1535/3	7379/11	3414/1	6390/0	3611/5	3435/38
7	Control	7076/1	4061/5	9769/0	4027/2	9722/2	6166/10	2081/1	5718/0	1402/1	2040/35
	1.5 ppm	3363/1	2219/1	9166/0	4115/2	0987/3	3144/11	3051/5	6134/0	2124/1	4558/36

	5 ppm	9047/0	8154/0	6501/0	8582/1	6970/2	0808/11	2767/1	6404/0	3183/0	0048/40
	10 ppm	4376/1	1016/1	7818/0	0547/2	7312/2	5241/10	2369/1	5918/0	2262/1	3075/36
15	Control	8064/1	3402/1	9080/5	2745/2	9364/2	0928/11	2841/1	6137/0	2926/1	0437/37
	1.5 ppm	4261/5	1121/5	8546/0	5894/2	4581/2	8574/11	4041/1	6082/0	3957/1	0582/36
	5 ppm	7812/0	8032/0	6742/0	9238/1	6776/2	6562/10	2263/1	5968/0	2558/1	5400/37
	10 ppm	2381/5	0582/1	7682/0	9528/1	8594/2	7421/10	2481/5	5869/0	1258/1	5432/35

Time storage (day)	**Treatments**	**Fatty acid**									
		C16:1	**C17:0**	**C17:1**	**C18:0**	**C18:1c**	**C18:1t**	**C18:2c**	**C20:0**	**C20:1**	**C22:0**
0	Control	98/1	6907/0	6060/0	2515/9	7362/24	7668/0	7259/4	6062/0	3720/0	2919/0
	1.5 ppm	0851/2	6898/0	6460/5	5777/9	4278/24	7674/0	6337/3	6067/0	4021/0	2937/0
	5 ppm	9401/5	6267/0	5818/0	7878/8	8668/23	0819/1	7212/6	7550/5	3700/5	2813/0
	10 ppm	6743/1	5550/5	4992/0	3574/8	2817/24	0546/1	5043/14	3668/1	--	--
4	Control	0445/2	6698/0	6236/0	4421/9	3546/24	7687/0	6395/4	6667/0	3954/0	2820/0
	1.5 ppm	0868/2	6512/0	5999/0	6249/8	3023/23	0214/1	2531/3	5301/0	3813/0	2213/0
	5 ppm	0208/2	6919/0	7421/0	4193/11	9869/28	2750/1	3329/3	5910/5	4737/0	3992/0
	10 ppm	2062/2	6987/0	6302/0	9042/8	9294/20	0088/0-	3586/2	5092/0	3976/0	2322/0
7	Control	9690/1	5998/0	5326/0	2798/8	8403/23	0377/0-	5021/5	5100/0	3517/0	2458/0
	1.5 ppm	9674/2	6670/0	6503/0	4045/8	1030/24	9207/0	3144/2	4806/0	3873/0	2182/0
	5 ppm	2962/2	7571/0	7088/0	3101/50	5776/20	0033/0	7740/2	5904/0	4583/0	2771/0
	10 ppm	7476/2	6849/0	6770/0	0931/9	0289/24	9884/0	4909/2	5230/5	4097/0	1091/0
15	Control	1190/2	6647/0	6077/0	5735/8	8496/22	9979/0	5094/2	5099/0	3580/0	2174/0
	1.5 ppm	4825/3	6769/0	5919/0	2028/7	8780/23	3208/1	0125/2	4200/5	3852/0	2642/0
	5 ppm	1441/2	6989/0	6890/5	5177/9	8016/24	2444/0	5664/2	5353/0	4103/0	2571/0
	10 ppm	2468/3	6980/5	7032/0	2938/9	6852/23	2580/1	5611/2	5010/0	4190/0	2298/0

4-2-3- Analysis of the main components of milk

The results obtained regarding the effect of ozone on the measurement of the main components of colostrum milk during the storage period at 4 degrees Celsius are shown in graph No. 3. On day 0, the ozonated sample with a concentration of 5 ppm had more fat than the control sample. On the 4th day, with the increase in ozone concentration, the amount of fat in the sample increased and on the 7th day, all the samples treated with ozone in all concentrations had an increase in fat compared to the control sample. On the 15th day, the amount of fat in the sample treated with ozone with a concentration of 1.5 ppm significantly decreased compared to the control sample, and at higher concentrations of ozone, the amount of fat was almost the same and lower than the control sample. The highest amount of fat was on day 4 with a concentration of 10 ppm and the lowest amount of fat was on day 15 with an ozone concentration of 1.5 ppm. The highest amount of protein was related to the concentration of 5 ppm on day 4 and the lowest amount was related to the concentration of 1.5 ppm on day 7 (Chart 3). The highest amount of lactose was on day 4 in the control and ozonated sample with a concentration of 5 ppm and the lowest amount was in the ozonized sample with a concentration of 1.5 ppm on day 7.. The highest amount of minerals was related to the ozonized sample with concentrations of 1.5 ppm and 10 ppm on day 4 and the lowest was related to the ozonized sample with concentration of 5 ppm on day 15 (Chart 3).Milk fat has a more complex composition than the fat of other foods and is composed of various types of fatty acids. The most important fatty acids in milk are C14:0, C16:0, C18:0, and C18:1.[16]. In general, according to the obtained data, no significant relationship between ozone concentration and sampling days was observed.

The results showed that on the 7th day of the evaluation of the samples, the acidity increased and the lactose decreased, which indicates the beginning of the fermentation process. During this process, lactic acid bacteria use the available protein, so we should have a decrease in protein, which is completely consistent with our data [24]. Also, no significant correlation was observed between the data related to ozone concentration.

By comparing the data on the days of sampling, it was observed that the amount of lactose decreased significantly in the control and ozonized samples during the 7th and 15th days with the increase in microbial growth. Also, there was an increase in acidity on the 7th and 15th day, which indicated an increase in microbial growth. The trends of these data are completely consistent. In general, the only factor that causes a decrease in the amount of lactose is fermentation, and fermentation increases acidity because lactose is converted into lactic acid in this process [24]. By comparing column data, no significant relationship was observed between ozone injection in different concentrations and control samples. On the other hand, the amounts of minerals in control and ozonated samples decrease with time. By observing the data related to ozone concentration, there was no significant relationship between the ozone-treated samples in all concentrations and the control.According to the research done bySuprapto et al. found that the injection of ozone into fresh milk for a maximum period of 30 minutes and a concentration of 21.06 mg/liter had no significant effect on the density, protein and fat of milk and on the general physicochemical properties.[23].

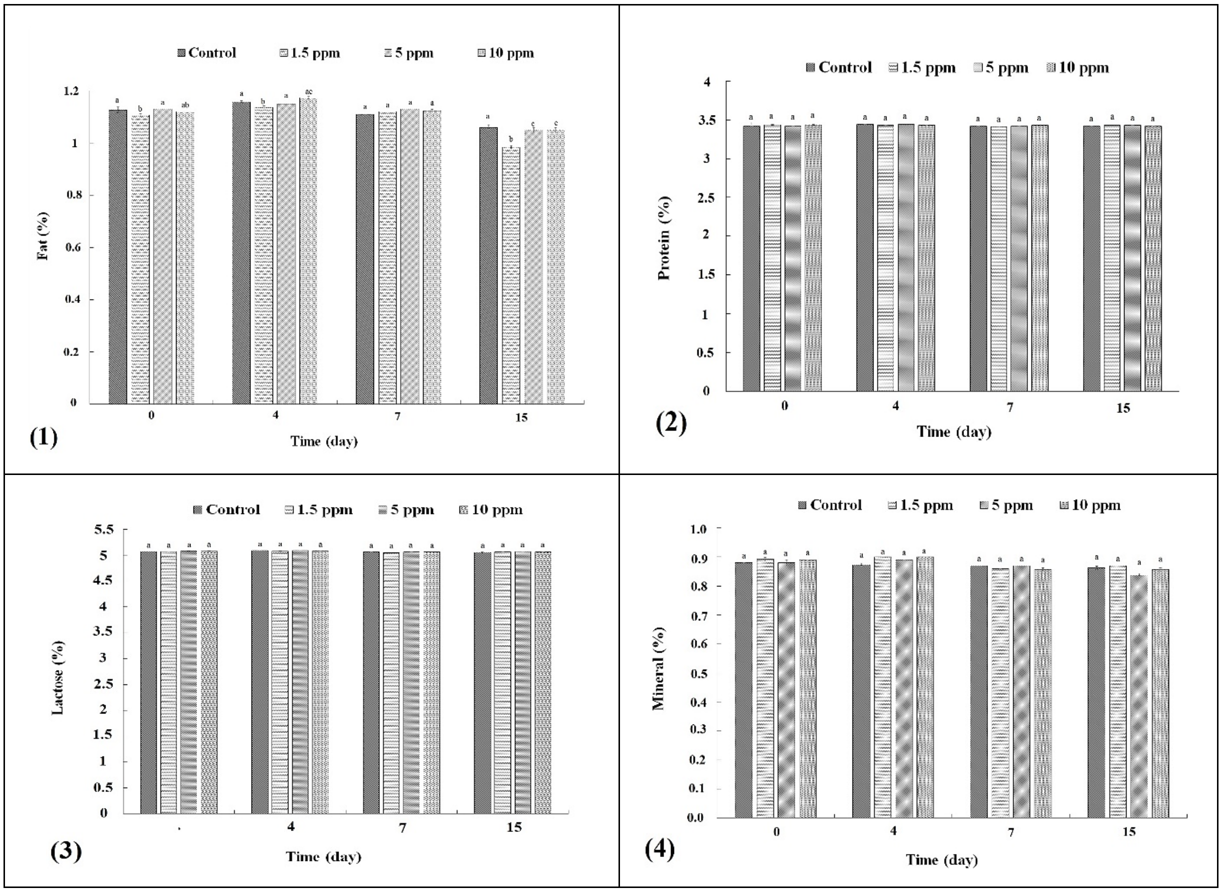

Figure 3 The effect of ozone on the main components of the treated skim milk samples stored at 4˚C. 1) Fat, 2) Protein, 3) Lactose, 4) Minerals.

5-2-3- Sensory characteristics of the samples

Sensory characteristics of pasteurized milk samples treated with ozone were performed based on the five-point hedonic method, and the highest grade was 5 and the lowest grade was 1. The results obtained regarding the effect of ozone on the sensory characteristics of the samples of wheeled shirps treated with ozone during the storage period at 4 degrees Celsius are given in chart number 4. By comparing the data between the treatments in the storage days and each treatment during the evaluation period, no significant relationship was observed.The highest score in terms of appearance on day zero concentrationppm1.5, on day 4, control and ozonated samples with concentrationppm5/1، On the 7th day, ozonated samples with concentrations ofppm 5/1ppm 5, and on day 15, control and ozonated samples with concentrationppm 1.5 had the highest score. The characteristics of taste on day 0 of ozonized samples in all concentrations had the same scores and a higher mean score than the control sample. On day 4, the highest score corresponds to ozonized milk with concentrationppmIt was 5. On day 7, the highest scores correspond to the ozonized sample with concentrationppm 10 and on the 15th day, the highest score related to the ozonated sample with concentrationppm It was 1.5. The highest odor index score on day zero is related to the ozonized sample with a concentration of 5 ppmppm 10, on day 4 the highest odor score related to the ozonized sample with concentrationppm1.5, on the 7th day, the highest score related to the ozonated sample with concentrationppm 5 and on the 15th day, the highest score related to the control sample and ozonated with concentrationppm It was 1.5. Finally, regarding the oral sensation on day zero of the ozonated sample with concentrations ofppm 5 andppm 10 highest, on day 4 ozone samples with concentrationppm1.5 is the highest, on day 7 of the ozonated sample with concentrationppm 5 highest and on day 15 of control and ozonized samples with concentrationppm 1.5 had the highest score.

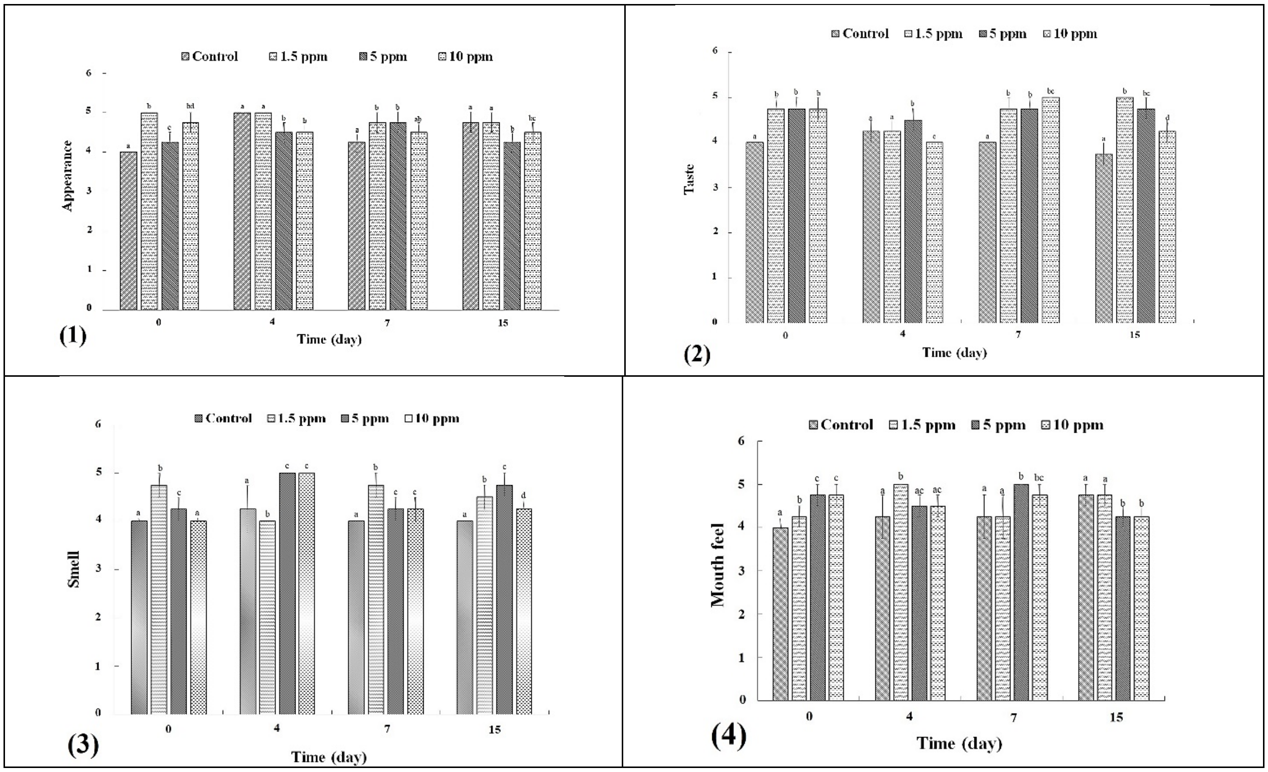

Figure 4 The effect of ozone on sensory characteristics of the treated skim milk stored at 4℃. 1) Appearance, 2) Taste, 3) Smell, 4) Mouthfeel.

4 - Conclusion

According to the obtained results, ozone injection led to a significant reduction of the total microbial population and the greatest effect of ozone was observed at a concentration of 10 ppm and on the 15th day. In examining acidity values, changes in ozone concentration had no significant effect on microbial growth, but acidity values increased on days 7 and 15. Adding ozone to milk in all concentrations and sampling days had no significant effect on milk pH and solute values. Evaluation of GC analysis showed that ozone injection in all concentrations did not cause significant changes in milk fatty acids. The acceptability of ozonized milk in all concentrations by the evaluators and the lack of distinguishing these samples from the control sample in sensory evaluation was significant. Therefore, ozone treatment can be used as an effective method to increase the residue of pasteurized milk.

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6- Resources

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تأثیر تیمار ازن بر افزایش ماندگاری شیر پس چرخ پاستوریزه: ارزیابی ویژگی های شیمیایی و میکروبی

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چکیده

امروزه تمایل برای استفاده از ازن به عنوان یک ترکیب ضدمیکروبی قوی مقرون به صرفه بودن و فناوری سازگار با محیط زیست، جهت افزایش ماندگاری محصولات غذایی افزایش یافته است. این مطالعه به منظور بررسی خصوصیات شیمیایی و میکروبی شیر بدون چربی پاستوریزه حرارتی تیمار شده با تزریق ازن تحت فشار در غلظت‌های صفر، ۱/۵، ۵ و ۱۰ ppm انجام شد. ویژگی‌های شیمیایی، میکروبی و حسی نمونه‌های شیر تیمار شده به همراه نمونه کنترل (۴ تیمار) در روزهای صفر، ۴، ۷ و ۱۵ نگهداری در یخچال، مورد ارزیابی قرار گرفت. جمعیت میکروبی کل در نمونه‌های تیمارشده با ازن نسبت به نمونه کنترل، در تمام روزهای نگهداری کاهش محسوسی داشت. بیشترین تاثیر ضد میکروبی ازن در روز ۱۵ نگهداری با غلظت ۱۰ ppm و کمترین تاثیر آن در روز ۴ با غلظت ۱/۵ ppm مشاهده گردید. با این حال، تفاوت معنی‌داری در میزان pH (۶/۸–۶/۸۸) و اسیدیته (۰/۱۶–۰/۱۸ درجه درنیک) بین نمونه‌های تیمار شده و کنترل وجود نداشت. اگر چه عدد پراکسید در نمونه‌های تیمار شده و نمونه‌های کنترل از حد استاندارد بالاتر بود ولیکن کاهش میزان آن در نمونه‌های تیمار شده نسبت به نمونه‌های کنترل مشهود بود. همچنین با مقایسه ویژگی‌های حسی در بین نمونه‌ها، هیچ گونه تفاوت معنی داری مشاهده نگردید. در مجموع تیمارهای ۵ و ۱۰ ppm ازن بهترین کارایی را در افزایش ماندگاری شیر پاستوریزه داشتند. با توجه به نتایج به دست آمده از این مطالعه، می توان از ازن به عنوان یک روش کمکی برای افزایش اثرگذاری فرآیندهای حرارتی در پاستوریزاسیون و افزایش ماندگاری شیر استفاده نمود.