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The Effect of Treatment with Ozone on Buffalo Milk and the Yoghurt made from it

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A B S T R A C T

The present study aimed at identifying the effect of Ozone on the chemical and physical properties of buffalo milk including the fat, protein, solid materials, lactose, salts, specific weight and pH. the results showed that treating the buffalo milk led to slight changes in the rate of fat content, solid materials, lactose, salts, specific weight and an increase in the viscosity of the milk. when some properties of milk samples were studied, it was observed that there was a slight decrease in the viscosity of the yoghurt samples made from buffalo milk and a decrease in whey osmosis with a rise in pH. after conducting the sensed assessment, the samples of yoghurt had the highest value of appearance and color when the treatment period was (60) minutes, while the treatment of (40) minutes gave the highest value in terms of the flavor and no change was observed in the property of the form for all the periods of exposure to the ozone.

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Introduction

The ozone (O_3) is a blue gas with a sharp smell and it is unstable with a high oxidation potential that might reach (- 2.07 volts) and this makes it have antimicrobial properties. Ozone was discovered in 1839 by Sch Obein and it is considered as the second more powerful oxidizing agent after the fluorine [1] and it doesn't leave any harmful chemical residuals. In 1997, The United States Food and Drug Administration (US-FDA) included the ozone in the list of the materials that are known as (Generally Recognized As Safe GRAS) and officially permitted its use for direct contact with the food in 2001. Ozone has been used for sterilization as a gas or a liquid [2]. On the other hand, [3] mentioned that ozone is characterized with antibacterial properties and its ability to fully dissolve with producing any wastes or poisonous products. One of the processes of milk treatment that can cause damage to milk is heating to high temperature degrees. The treatment with ozone has an effect on the functional properties of the milk like the emulsification and solubility and the properties of foam formation to isolate the whey protein (WPI) [4].[5] mentioned that the decomposition of ozone increases with the increase of pH and this leads to producing the hydroxyl roots (*OH) as the ideal milk fat comprises 10% of unsaturated multi fatty acids, 8% of saturated mulit fatty acids and 82% of the mono unsaturated fatty acids. The decomposition of fats generates fatty acids, especially short-chain fatty acids that contribute to the emergence of a strange flavor [6]. The treatment with the ozone results in a change in the milk color [4] [7]. The reason behind the change of milk color after it is treated with ozone due to the reaction of ozone with the double bonds of β-carotene molecules as they represent strong defensive locations for reaction with the ozone and this leads to the decrease in the beta carotene content due to this reaction [8].

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Materials and methods

Determining the fat, non-fatty solid materials, lactose, protein, salts and pH:

Buffalo milk samples used in yogurt manufacturing were obtained from Badush/Mosul area. The basic components of the buffalo milk were determined, which include: the percentages of fat, total solid materials, lactose, protein, salts and pH using the Lactoscan (Milk) device, Bulgarian origin model SP-CB011092.

Determining the specific weight

The specific weight of buffalo milk was determined according to the method mentioned by [9]using the Pycnometer with a volume of 25 ml at 20 C° as shown in the following equation:

$$\text{Specific weight} = \frac{\textit{weight of a certain volume of the sample}}{\textit{weight of the same volume of water}}$$

Determining the viscosity in milk and yoghurt

The apparent viscosity of the samples of milk and yoghurt was determined in 20 C° using the device HAAKE Viscotester 3R Version, German origin according to the method mentioned by [10] plus conducting some modification as the axial spindle R1 with a volume of 50 ml of the sample was used and it was left to rotate inside the sample (speed of 150 for (60) seconds and then the reading was taken and the unit for measurement was d pa.s.

Determining the flavor compounds and pH

Fat extraction

The method described by [11] was used to extract the fat from the milk sample as the unit Majonnier was used for this purpose. Ten (10) grams of milk was taken and put in a separation funnel (volume 500 ml) and then (0.5) g of table salt was added and mixed thoroughly and then 1.5 ml of ammonium hydroxide (95%) was added continuously and mixed for (30) seconds. After that, (10) ml of ethanol (95%) was added and (25) ml of ether Ethyl and (25) ml of petroleum ether (boiling point 35-60C°) were added with continuous shaking to the content of the funnel. After that the funnel was left stable for (30) minutes to separate the upper layer of fat with the organic solvents that were withdrawn from the separation funnel, evaporating them and keeping then in class container until decomposition is performed.

Determining pH

pH value was determined according to the method mentioned by [11] by melting (5) grams of fat that was extracted with the solvent composed of acetic acid and chloroform (2:3) of mixing and (0.5) ml of it was added with the saturated potassium iodide solution and then the mix was kept in a dark place for (5) minutes. After that, (30) ml of sterilized water was added and the mix was bureted with the sodium thiosulfate solution (0.1N) with continuous shaking until the yellow color disappears. Then (0.5) ml of starch solution (0.5%) is added with shaking and bureting with sodium thiophosfate solution until the blue color disappears. Also, the control sample (Blank) was prepared using the sterilized water instead of oil, according to the following equation:

$$\textbf{PV (milliequivalent/kg of oil)} = \frac{\textit{sodium thiosulphate} - \textit{sodium thiosulphate for the blank } x\ \mathrm{N}x1000}{\textbf{milliequivalent/kg of oil}}$$

manufacturing the yoghurt from buffalo milk treated with ozone

the raw buffalo materials was received and filtered and then a sample of the milk was taken before treating it with the ozone and this sample was considered as the control sample. The milk was treated with the ozone and several samples were taken from it to be exposed for periods of (20), (40)

and (60) minutes. The traditional primer of the yoghurt (3%) was added to the samples and then they were packaged in plastic containers and incubated in a temperature of (42 ± 2 C°) for (3-4) hours and then they were cooled and kept in the fridge (4 C°). after that, the chemical and physical tests were conducted in addition to the sensed evaluation (color, flavor, form and scent).

Physical tests

Determining the spontaneous whey osmosis

The whey osmosis was determined according to the method described by [12] by putting the cup of yoghurt in a tilted position (angle 45°) in a temperature of (5 C°) for one hour and then the whey osmosed from the surface using the injector. Them the cup was weighed again after one hour. This process was performed for (10) seconds to avoid extra whey osmosis.

Determining the pH

The pH was determined using the pH meter provided by EUTECH (Singaporean made) from the buffalo milk and that was done by immersing the electrode inside the yoghurt sample directly and the reading was recorded.

Sensible evaluation

The yoghurt was evaluated sensibly by the teaching staff members and higher studies students at the department of foods sciences according to the following table:

Table 1. Questionnaire form of the sensed evaluation of the yoghurt manufactured from the buffalo milk treated with the ozone

Property	Score	Yoghurt made from milk untreated with ozone (control)	Yoghurt made from milk untreated with ozone for (20) minutes	Yoghurt made from milk untreated with ozone for (40) minutes	Yoghurt made from milk untreated with ozone for (60) minutes
Appearance	25				
Flavor	25				
Color	25				
Form	25				

Results and discussion

1- The effect of treating the milk with ozone components of the buffalo milk

From table (1), it is evident that there was a slight decrease in the fat content as the value was (7.11%) in the control treatment and the value was (6.60%) in the treatment with ozone for (60) minutes. This slight change might not affect greatly the microbial risks. Nevertheless, fats in milk could protect some organisms from the effect of ozone. According to a study conducted by [13], the existence of organic materials like fats might decrease the effectiveness of ozone in terms of inhibiting the bacteria growth. It was also, noticed that the percentage of non-fatty solid materials for the control sample was (10.50%) and it rose to be (10.94%) when treating the milk with the ozone for (60) minutes. These changes could affect the environment of organisms' growth in the milk. on the other hand, [14] indicated that the non-fatty components in milk, such as protein and saccharides can affect the growth and survival of the epidemic organisms. As for the lactose, it was observed that there was a continuous decrease in the content of lactose fro, (5.80%) to (3.86%) with the longer period of treatment with the ozone. This decrease in lactose might affect the growth of some organisms that depend on the lactose as a source of energy. According to the study conducted by [15], lactose is considered a main source of energy for many organisms in milk and the decrease in its level might affect the growth of those organisms and the decrease in the level of salts from (0.90%) to (0.69%) at the treatment for (60) minutes, might in turn, decrease the osmotic pressure of the milk and thus affect the survival of the organisms. Moreover, in a study conducted by [16], it was concluded that the ionic balance in milk can affect the growth and survival of epidemic organisms. In terms of the protein content, it was observed that there was a slight decrease in the protein content from (4.18%) to (4.11%) at the treatment with ozone for (60) minutes. Proteins in the milk can be a good source of nutrition for the organisms, but they are, also, contain some antibacterial compounds. [17], in the study they conducted, that some proteins in the milk like the lactoferine and the lysozyme have antibacterial characteristics and it was observed that the changes in the specific weight of the milk is due to the exposure to ozone and those changes were very slight. This result is in conformity with several previous studies that found that treating with the ozone didn't affect significantly the physical properties of the milk. The changes in the specific weight of the milk might be correlated with the changes in the milk structure such as the protein and fat content that could be affected by the exposure to the ozone.

It was noticed that there was a slight decrease in milk specific weight when the milk is exposed to ozone for (60) minutes and this could be due to the effect of ozone on the milk. In a study conducted by [18] it was found that the long period of milk exposure to the ozone could affect some components of the milk and this leads to slight changes in the specific weight. Although the changes in the specific weight are slight, but it could be important in some industrial applications as the specific weight of the milk affects the manufacturing processes like the sorting and drying and thus these changes, even if they were slight, are important in the process of manufacturing on a vast level. It is important to preserve the specific weight of the milk and keep it within the range for the purpose of quality assurance. According to Codex Alimentarius

Commission 2011, the specific weight of the milk should range between (1.028) and (10.034) at a temperature of 20 C˚. all the values recorded in this study are withing the range and this indicates that the treatment with the ozone had no negative effect on the quality of the milk in terms of the specific weight.

It is observed that the effect of ozone on the milk viscosity was limited, and this result is in agreement with the ones of study conducted by [13] , who found that the treatment with the ozone didn't have a significant effect of the physical properties of the milk. However, they noticed some slight changes in the chemical structure of the milk. Moreover, [19] observed that buffalo milk is characterized with higher viscosity compared to the cow milk and this is due to the high concentration of the solid materials, particularly the fats and proteins in the buffalo milk.

From table (1), it is noticed that there is a decrease in the buffalo milk viscosity when the milk is exposed to ozone for (40) minutes and this could be related to the structure of the milk of certain chemical reactions. According to [20] , the changes in milk viscosity might be correlated with the change in the size of casein molecules or the structure of the casein particles. Additionally, [21] mentioned that the milk viscosity affects the manufacturing processes like the evaporation, drying and the production of fermented dairy products, so it is important to understand these changes to Improve the quality of the final products.

Also, table (1) shows a gradual increase in pH with the increase of the exposure time and this increase indicates the occurrence of fats oxidation in the milk. According to the study conducted by [13], the ozone acts as a strong oxidant and this leads to the formation of peroxides in the fats. This explains the remarkable increase in pH with longer periods of exposure to the ozone. This result is consistent with the study conducted by [22] that concluded that increasing the time of exposure to ozone leads to the increase in fat oxidation in milk.

The increase in pH might have consequences on the quality of the milk and its apparent properties. According to the study performed by[23], the rise in pH could result in changes in the flavor and odor and might decrease the nutrition value of the milk. It is important to compare the values recorded with the internationally accepted limits of pH in milk as according to Codex Alimentarius Commission 2006, the maximum acceptable limit of pH in the milk is 0.8 milliequivalent/kg fat and it is observed that all the values recorded in the current study are higher than this limit, even for the samples that were not treated with the ozone, but the increase in pH indicate that it is necessary to balance between the benefits of sterilization and the consequences oxidation. In the study conducted by [24], it was proposed to use complementary techniques like the rapid cooling after treating the milk with the ozone to limit the consequences of oxidation and the results indicate that treating the milk with the ozone increase the fat oxidation in the buffalo milk.

Table 2. The effect of treatment with ozone on buffalo milk components

Component	Buffalo milk untreated with ozone (control)	Buffalo milk treated with ozone (20 minutes)	Buffalo milk treated with ozone (40 minutes)	Buffalo milk treated with ozone (60 minutes)
Fat %	7.11	6.90	6.80	6.60
SNF %	10.50	10.54	10.76	10.94
Lactose %	5.80	4.53	4.30	3.86
Salts %	0.90	0.79	0.73	0.69
Protein %	4.18	4.17	4.12	4.11
Specific weight	1.034	10.53	1.034	1.032
Viscosity (d pa.s)	4.0	4.0	3.0	4.0
Peroxide (milli equivalent/kg fat)	7.1	9.3	10.5	12.2

2- The effect of treatment with ozone on the properties of the yoghurt manufactured from the buffalo milk

From the results in table (2) concerning the treatment of buffalo milk with the ozone on the properties of the yoghurt made from it, it is clear that there was a slight change in its viscosity and a rise in the pH of the yoghurt with the increase of the period of exposure and the highest value was obtained for the exposure period (40) minutes as it was (4.7). Principally, pH value increase with the decrease of the active hydrogen ions (H+) and this can be attributed to the decomposition of some acidic compounds and the release of hydrogen ions and forming different compounds. The whey osmosis results showed an evident decrease in the quantity of whey osmosis from the yoghurt manufactured from the milk treated with the ozone depending on the periods of exposure and this decrease in the whey osmosis indicates the improvement in yoghurt in terms of preserving the water. Moreover, according to the study conducted by [25], treatment with the ozone can affect the microscopical structure of proteins in dairies and this leads to changes in the properties of preserving the water.

It is observed that increasing the period of exposure to ozone results in increasing the whey osmosis and this could be correlated with the effect of ozone on the milk proteins. Ozone can react with other proteins and this might lead to changes in their structures and eventually their ability to preserve the water. There was an acute decrease in whey osmosis for the treatment with ozone for (20) minutes compared to the control sample. This indicates that treatment with ozone, even for relatively short periods, can cause considerable changes in the properties of the yoghurt. The decrease in whey

osmosis might have a positive effect on the quality of the yoghurt. According to [26], the decrease in whey osmosis is correlated with the improvement in the form of the yoghurt and increasing its cohesion. Nevertheless, it is important to study these changes on the sensed properties of the final product. The effect of ozone on whey osmosis could be due to several mechanisms. In a study conducted by [27], it was proposed that ozone can affect the cazinean particles in milk and this results in changes in the properties of milk curdled and the formation of curd, which in turn, affect the potential of yoghurt in terms of preserving the water.

Table 3. The effect of treating the buffalo milk with ozone on the properties of the yoghurt made from it

Yoghurt properties	Control	20 minutes	40 minutes	60 minutes
Viscosity (d pa.s)	11	11	9	9
pH	4.2	4.4	4.7	4.5
Whey osmosis (ml/100g of yoghurt)	17.965	6.32	3.286	2.018

3- The sense evaluation of samples of yoghurt made from buffalo milk treated with ozone

Table (3) shows the results of sense evaluation of buffalo yoghurt made from buffalo milk treated with ozone. It was observed that there was a remarkable improvement in the shape (form) of the yoghurt manufactured from the milk treated with ozone (21-23 scores) compared to the yoghurt made from untreated milk (17 scores). This improvement was clearer with the increase of the period of exposure. The improvement in the form could be due to the effect of ozone on the molecules of fat and protein in the milk and this leads to more homogeneity and softer form. As for the flavor for the treated yoghurt, the score was (20-23 scores) for it and the yoghurt made from untreated milk the value was (19 scores). The best improvement was obtained for the exposure period (40 minutes) and these results are in consistency with the results of the study conducted by [28] that indicated that treating the milk with ozone can positively affect the flavor by means of removing some undesirable compounds and improving the sense properties of the milk and its products. As the color, it was noticed that there was a noticeable improvement in the degree of color for the yoghurt made from the buffalo milk treated with the ozone with a value of (22-23) compared to the yoghurt made from the untreated milk (17) and that was due to the effect of ozone in terms of the distribution of the fat molecules and its reaction with the natural dyes in the milk. As for the form, there was a remarkable improvement for the yoghurt made from milk treated with ozone (22 scores) compared to the control value, which was (18 scores) and this improvement might be due to the effect of ozone on the milk proteins and the distribution of fat molecules, which leads to more cohesive and softer form.

Table 4. The sensed evaluation of buffalo milk made buffalo milk treated with ozone

Treatment	Appearance	Flavor	Color	Form	Total
Buffalo yoghurt made from milk untreated with ozone	17	19	17	18	71
Buffalo yoghurt made from milk treated with ozone for (20) minutes.	21	20	22	22	85
Buffalo yoghurt made from milk treated with ozone for (40) minutes.	23	23	22	22	90
Buffalo yoghurt made from milk treated with ozone for (60) minutes.	23	22	23	22	90

Conclusions

Treating the buffalo milk with ozone changes the properties of the milk and cause changes, and these changes increase with the increase of exposure time. It was observed that the percentages of fat, protein and lactose decreased for the sample untreated with ozone and the pH value, on the contrary, increased with the increase of exposure time.

It was also, noticed that the yoghurt made from the milk treated with ozone was impacted in terms of the changes of the physical properties of the yoghurt as there was a decrease in viscosity, pH and a decrease in the whey osmosis with the longer times of exposure. It was observed also that treatment with ozone showed a remarkable in all the sensed properties of the buffalo yoghurt and that was manifested in the appearance, color and form with a slight improvement in the flavor. The treatment (40-60) minutes gave the best results in general. There results refer to that the treatment with ozone could be an effective method to improve some sensed properties of buffalo yoghurt.

From what has been mentioned above, it is evident that it is possible to use the ozone for milk treatment in order to decrease the damage in dairy products and to make the storage time longer and at the same time preserving the physical and chemical properties and sensed ones as well as the potential to

use ozone in treating various foods and dairy products with various concentration and exposure periods. It is recommended to study different types of ozonated milk with different concentrations and times in the manufacturing of yogurt or other types of dairy products.

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