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A Review on Influence of Ozone and Pasteurization on physico-chemical properties, Microbiology, and stability of milk

Shima Esamaldin Hamodah[1*], Robi Andoyo[2], EfriMardawati[2*] Ibrahim.A.I[1*]
[1] *Department of Food Science & Technology, Faculty of Agriculture, Omdurman Islamic University, P.O.BOX, www.oiu.edu.sd, Khartoum, Sudan.*
[2] *Faculty of Agro-Industrial Technology, University of Padjadjaran, UNPAD, Jl.Bandung-Sumedang Km 21, Jatinangor, Indonesia*
Corresponding Author: Shima Esamaldin Hamodah

Abstract: *In this study, attempt was made to determine if pasteurization and ozone treatments can effect on physicochemical properties, microbiology, and stability of milk.Ozone applications in foods, milk and dairy products are among the modern methods used to obtain high-quality safe food free from microbes. Pasteurization is one of the treatments used to reduce microbial load and prolong the shelf life of the raw milk. The pasteurization has been positive effect on reduce the microbial load in milk, but it is a Despite the effectiveness of pasteurization, it results to lose of some vitamins, calcium, significant impact on whey protein with a change in some nutritional and sensory properties that includes color, taste, and flavor of milk. Ozonation treatment is capable to reduce microbial load in fresh milk, however, it is not effect in levels of oxidation in lipids and without significant changes milk ph. In addition, ozone does not leave chemical residue on the contact surfaces for food or food because it automatically transferred to non-toxic products, reducing both environmental impacts and company costs.*

Keywords*: Ozone, Pasteurization, Physicochemical properties, Microbiology, Stability, Milk*

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I. Introduction

Milk is food in human diet; it is a good source of proteins, important vitamins and essential minerals has made it become part of recommended food habits (Pereira, 2014), (Contreras et al. 2015).Milk is a complex food from a molecular composition perspective, which is important part of human's diet, because it has high nutritional value (Mcmahon, 2013), (Raikos, 2010), (Šestan, 2016). The milk contains four essential components based on quantity, namely water, fat, protein and lactose. While the secondary constituents are minerals, enzymes and vitamins. Cow milk is less rich in fat, protein and lactose content. But the mineral content is similar(Guetouache et al., 2014). Milk has been contains several groups of nutrients, which are proteins, and energy components, carbohydrate, lipids, and dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium (Bylund, 1995). According to Indonesian national standard (SNI) in 2011 set the microbial contamination in fresh milk at a maximum limit Enterobacteriaceae consisting $1x10^3$CFU/ml and Staphylococcus aureus of $1x10^2$ CFU/mL. The total plate count (TPC) has a maximum $1x10^6$ CFU/Ml. However, the standards by SNI of 7388:2009 Salmonella sp. must be negative/25ml on fresh milk, pasteurization milk and UHT milk (Raikos, 2010).

The characteristics of microbial populations in raw milk at the time of processing has a significant influence on shelf life, organoleptic quality, spoilage and yields of the raw milk, processed milk as well as on the other dairy products (Samarzijaet al., 2010). Heat treatment of milk is an essential step in the production of milk adopted by the dairy industry. The thermal processing of milk aims to prolong the shelf life and improve the quality of this complex biological fluid by reducing the microbial load, thus, reducing the risk of food poisoning. In other cases, where milk is use as a food ingredient in milk-based products, heat treatment is use to improve the sensory properties of these dairy compounds by manipulating the function of milk proteins (varga, 2016), (Raikos, 2010).

Pasteurization is one of the treatments used to reduce microbial load and prolong the shelf life of the raw milk. Despite the effectiveness of pasteurization, it results to lose of some vitamins with a change in some nutritional and sensory properties that includes color, taste and flavor of milk. Although pasteurization is effective in obtaining safe milk for human consumption, heating has a significant impact on the nutritional value and sensory properties of milk. (Cavalcante, et al., 2013).

Ozone is new alternative technologies in food applications. However, increased global production, distribution and consumer awareness on health benefits, risks associated with consumption foods, thus, make the use ozone technology as a prominent and with novel regulatory action. Which is effective alternative method and offers unique advantages for foods with minimal effects on physicochemical properties(Jegadeeshwa, 2017).Ozone (O_3) is a safe gas that decomposes to $Co_2$and has certified by the US department of Agriculture and US regulations allows it to be use as safe additive in food (Kim et al., 1999), (O'Donnell et al., 2012). Ozone applications in foods, milk and dairy products are among the modern methods used to obtain high-quality safe food free from microbes. Ozone is a powerful disinfectant that reduces microbial load and eliminates pathogenic and non- pathogenic microbes such as viruses, fungi and protozoa (Khudir and mahmood, 2017). Ozone is preferred among other methods because of the ability to destroy all forms of microorganisms at relatively low concentrations (Greeneet al., 2012), (varga, 2016).

II. Benefit used ozone and pasteurization in milk processing

Ozonation Applications in food processing have legally approved, although to Varying degrees in most Developed Countries (Tiwari and rice, 2012). Ozone treatments are environmentally friendly and cost-effective food processing technology. They have successfully used to remove milk residues and bacteria formed from microfilm and milk processing, including liquid milk, powdered milk and cheese. Ozone has shown to prevent mold growth on cheese and disrupting portable molds in the air in cheese maturation and storage facilities. Ozone treatment also founded to be a promising way to reduce the concentration of pollutants in wastewater from dairy products (Vargav and Oszigeti, 2016).Although it is a highly effective disinfectant, ozone does not leave chemical residue on the contact surfaces for food or food because it automaticallytransferred to non-toxic products, reducing both environmental impacts and company costs (Patil and Bourke, 2012).Thephysical properties and generation of ozone showed on table 1.The factors affecting on ozone efficiency that which ozone efficiency in food processing applications is affected by a range of variables, including processing temperature, PH, relative humidity and amount of ozone-depleting compounds, because these factors have a different effect on solubility and ozone stability (Cullen and Norton).).Ozonation has been capable to reduced aflatoxin about 50% after milk treat by ozone to 5 minute (Mohammadet al., 2017).The Physical properties and generation of ozone showed on Table1.

Table1. Physical properties and generation of ozone

Parameter	Value
Molecular weight	48
Density (Kg/m3)	2.14
Boiling point (ºC)	-111.9
Melting point (ºC)	-192.6
Critical temperature (ºC)	-12.1
Critical pressure (atm)	54.6
Oxidation potential (V)	-2.07
Solubility in water at o (ºC) (L/L)	0.640
Solubility in water at 15 (ºC) (L/L)	0.456
Solubility in water at 40 (ºC) (L/L)	0.112
Solubility in water at 60 (ºC) (L/L)	0.000

Source: (Vargav and Oszigeti, 2016).

III. Effect of ozone and pasteurizationon milkproperties

Fluid milk is treat by traditional methods such as thermal processing for to be safe for human consumption. Heating treat, moreover, may negative effect on nutritional value and sensory characteristics of milk (Cavalcante et al., 2013).Thermal processing is oldest methods applied to reduce microbiology load in raw milk, which is consideration it very effective and simple. However, it has affect on sensory and nutritional values in product (Pedraset al., 2012).

3.1. Effect of ozone on physicochemical properties and microbiology of milk

The efficacy of an ozonation process usually based on the effects of both direct and indirect reaction mechanisms. The effectiveness of ozone against microorganisms present in food systems depends on several factors including the amount of ozone applied, the residual ozone in the medium and various environmental factors such as medium PH, temperature, relative humidity, additives and the amount of organic matter surrounding the cells (O' Donnell et al., 2012).(Table2) The use of ozone to milk treatments can be reducing of the microbiology count in raw milk, and improving the milk quality and increasing its shelf life(Cavalcantet al., 2013).

Table2. Decimal reductions in microbial counts in milk after ozonation

Microorganisms	Without Tween	control with Tween	ozonated sample during 5min	ozonated sample during10min	ozonated sample during15min
TMA	-	0.40 ±0.60	0.70 ±0.61	0.58 ±0.0	0.60 ±0.04
Enterobacteriaceae	-	0.02 ±0.03	0.13 ±0.12	0.58 ±0.11	0.96 ±0.14
Psychrotrophic	-	0.00 ±0.05	0.08 ±0.07	0.08 ±0.05	0.13 ±0.06
Staphylococcus sp	-	0.06 ±0.05	0.16 ±0.18	0.52 ±0.14	1.02 ±0.09
Yeasts and Molds	-	0.02 ±0.04	0.07 ±0.06	0.25 ±0.08	0.48 ±0.09
Salmonella sp.	n.f.*	n.f.	n.f.	n.f.	n.f.

Source: (Cavalcantet al., 2013)

Ozonation treatment is capable to reduce microbial load in fresh milk, however, it is not effect in levels of oxidation in lipids and without significant changes milk pH (Mohammadet al., 2017). Ozonation up to 15 minutes did not change the milk physicochemical properties and milk centesimal composition (Cavalcantet al., 2013).Ozone is a powerful antimicrobial agent in food applications. Its effect in inhibiting microorganisms is rapid by interacting with the enzymes inside the cell, nuclear material and its cellular envelope components, spore coats, or viral caps(Jegadeeshwa, 2017), (Khadret al., 2001). Table3 showed the physicochemical properties of milk before and after ozonation**,** and Figure1 showed the Disruption of Salmonella enteritidis outer membrane.

Table3. Physicochemical properties of milk before and after ozonation

Parameters	without Tween®	control with Tween®	ozonated sample during 5min	ozonated sample during10min	ozonated sample during15min
Protein (%)	3.37±0.02	3.39±0.03	3.39±0.03	3.38±0.04	3.35±0.04
Fat (%)	3.67±0.14	3.64±0.10	3.64±0.10	3.66±0.13	3.62±0.06
Density (gmL^{-1})	1.0329±0.00	1.0329±0.00	1.0324±0.00	1.0321±0.00	1.0329±0.00
NFDE (%)	8.69±0.0	8.70±0.0	8.70±0.0	8.77±0.0	8.72±0.05
Acidity (^oD)	15.87±0.76	16.10±0.80	16.10±0.80	15.90±0.74	15.8±0.71

Source: (Cavalcantet al., 2013)

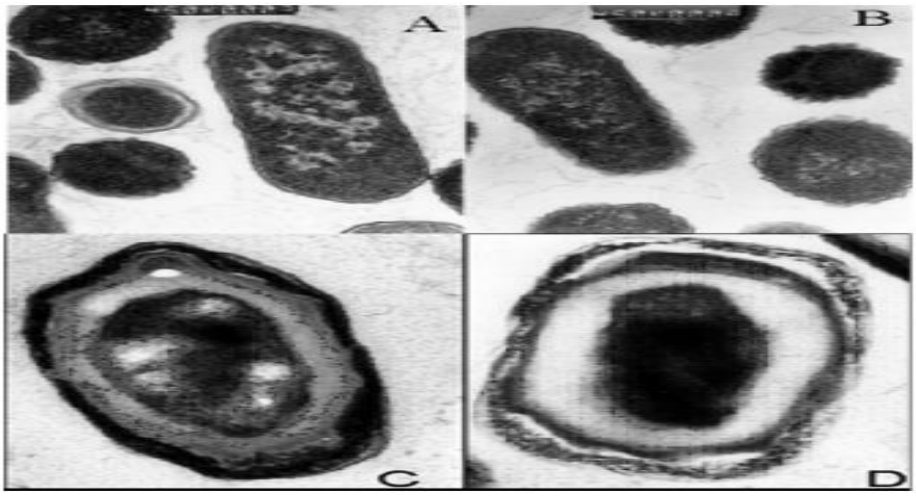

Fig1. Disruption of Salmonella enteritidis outer membrane and the outer coat of Bacillus subtilis spores after treatment with sublethal levels of ozone as seen by the transmission electron microscope. (A and B) S. enteritidis; (C and D) B. subtilis, before and after ozone treatment, respectively.

Source: (Khadret al., 2001)**.**

3.2. Effect of pasteurization on physicochemical properties and microbiology of milk

Pasteurization and ultra-high temperature processing does not effect on composition and fatty acids profile in raw milk (Pestanaetal., 2015).Pasteurization has been a positive effect on microbial contents in milk, which is reducing the total count bacteria, Coliform bacteria count and pathogens (El- Zubeir et al., 2007).Pasteurization has capable to extended shelf life of milk and reducing microbial loaded in it, and it does not effect in values of chemical contents (AbdElrahmanet al., 2013).Pasteurizing milk by thermo-ultrasonicated can be decrease nutritional values of milk such as protein content and decrease the Ph, density of milk. In other side, it increase the butter fat and Titratable acidity in milk (Aguirrre et al., 2009). The proximate analysis of raw milk, heat-pasteurized milk and thermos-sonicated pasteurized milkshowed in Table4, and physicochemical

characteristics of raw milk, heat-pasteurized milk and thermos-sonicated pasteurized milk showed on Tabe5. Heat treatment of milk leads to more processes, such as reduction the amount of water that makes milk durable. In addition, heat treatment of milk up to 80ºC to 15sec leads to loss of calcium between 20-40% (Šestan, 2016).The concentration of Ca2+ ions before and after thermal treatment showed in Figure2.

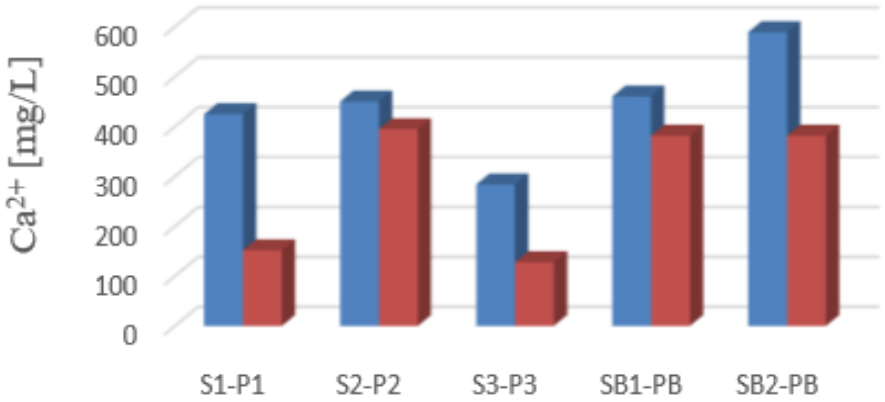

Fig2. The concentration of Ca2+ ions before and after thermal treatment

Source: (Šestan, 2016).

Table4. Proximal analysis of raw milk, heat-pasteurized milk and thermos-sonicated pasteurized milk

Milk sample	Protein Content (%)	Butter fat Content (%)	Added water to milk (%)	Solid nonfat (%)
Raw	3.28 ± 0.04	4.04 ± 0.05	0.00 ± 0.00	9.15 ± 0.04
Thermal treatment	3.55 ± 0.01	4.22 ± 0.02	0.00 ± 0.00	9.50 ± 0.03
30%	3.02 ± 0.03	4.21 ± 0.04	4.35 ± 0.92	8.08 ± 0.09
60%	3.04 ± 0.03	4.28 ± 0.05	3.88 ± 0.91	8.13 ± 0.08
90%	3.03 ± 0.04	4.29 ± 0.05	4.28 ± 1.12	8.36 ± 0.43
100%	3.00 ± 0.01	4.24 ± 0.02	4.92 ± 0.3	8.03 ± 0.04

Source: (Aguirrre et al., 2009).

Table5. Physicochemical characteristics of raw milk, heat-pasteurized milk and thermos-sonicated pasteurized milk

Milk sample	PH	Acidity	Density (g/cm3)	Freezing point (C) $\times 10^{-2}$
Raw	6.80 ± 0.04	0.109 ± 0.012	1. 0303 ± 0.0014	-51.60±0.031
Thermal treatment	6.67± 0.06	0.126 ± 0.008	1. 0317 ± 0.0010	-61.73 ±0.015
30%	6.64± 0.02	0.142 ± 0.014	1. 0260 ± 0.0032	-53.20±0.053
60%	6.04 ± 0.05	0.141 ± 0.009	1.0261 ±0.0029	-53.47 ±0.049
90%	6.03 ± 0.02	0.136 ± 0.012	1.0259 ± 1.0038	-53.20±0.061
100%	6.00 ± 0.01	0.141 ± 0.009	1.0257±0. 0015	-52.83 ± 0.021

Source: (Aguirrre et al., 2009).

IV. Effect of ozone and pasteurization on stability of milk

Extended lifespan products are common on the market today due increased consumer and retail demand for longer-life products, which are usually sterilization by ultra-high temperature treatment (UHT) such as protein-rich milk drinks. Theevolution from health trends that are often related to diet. These products often come with instability because of their complexity, which during storage leads to loss of product quality (Datta and Deeth, 2001).In order to improve the structural stability of dairy products, stabilizers such as carrageenan is added. However, there are some problems we face while developing a recipe that needs to be taken into consideration, including environmental conditions such as pH, temperature and storage time. In addition, you must monitor the amount of the installer to optimize the product stability (Tziboula& Horne, 2000). The pH has been of primary importance for the stability of product, where a higher pH seemed to result in less stable products (Pulkkinen, 2014). Milk pasteurization by ultrasound technology capable to produce a sate and stable product from the microbial for 16 days at 4ºC, despite effect on the apparent slight decrease in protein content, but it improved in other characteristics, such as better availability of butter fat content, and better color, showed in Figure4 (Aguirrre et al., 2009).The measured pH in the different products during twelve weeks of storage showed that in figure 3, and Figure4.

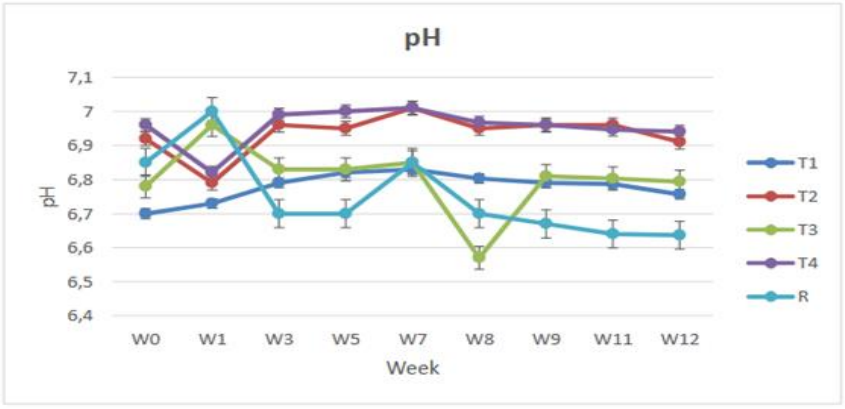

Fig3.The measured pH in the different products during twelve weeks of storage.
Source: (Pulkkinen, 2014).

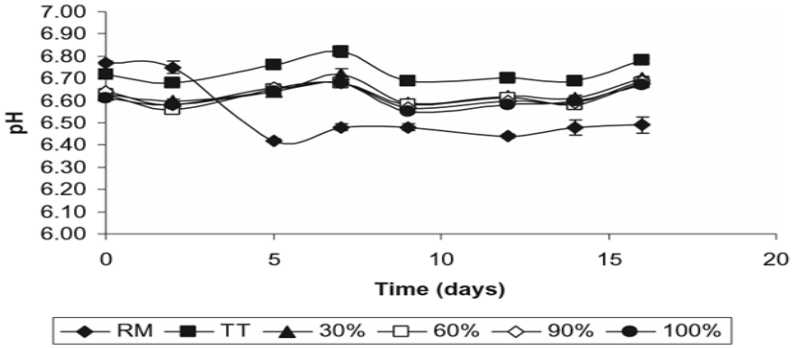

Fig4. The PH Behavior of Raw Milk (Rm), Heat-Pasteurized Milk (Tt) and Thermo-Sonicated Milk at Different Amplitude Intensities (30%, 60%, 90% and 100%) During Storage.
Source: (Aguirrre et al., 2009).

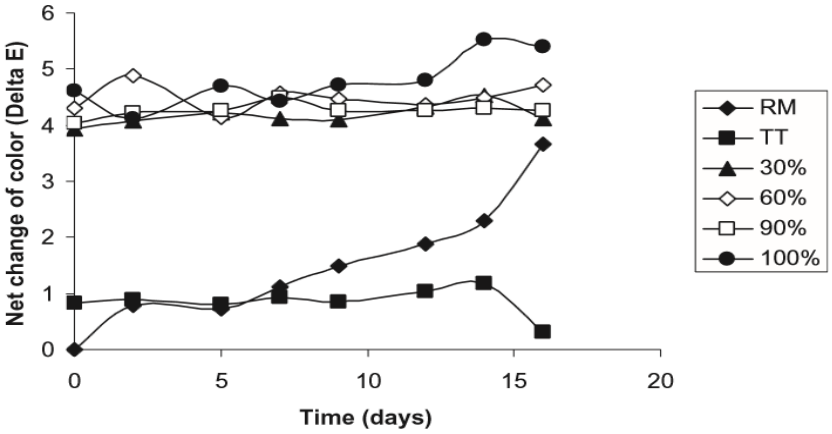

Fig5. Net change of color (Delta E) of raw milk (RM), heat-pasteurized milk (TT) and thermo-sonicated milk at Different ultrasound intensities (30%, 60%, 90%, and 100%) during storage using raw milk color at time zero as control.
Source: (Aguirrre et al., 2009).

V. Conclusion

Pasteurization has been positive effect on reduce the microbial load in milk. However,has significant impact on the nutritional value such as decrease protein content, lose vitamin and calcium? Also on some physicochemical characteristics and sensory properties of milk.Ozonation treatment has been capable to reduce microbial load in fresh milk, in addition, it is not effect in levels of oxidation in lipids and without significant changes milk ph. moreover, ozone does not leave chemical residue on the contact surfaces for food or food

because it automatically transferred to non-toxic products, reducing both environmental impacts and company costs.

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