RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 40 Issue 2: 147-151 (June 2021)

Effect of High Temperature, Short Time (HTST) Pasteurization on Milk Quality Intended for Consumption

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10.18805/ajdfr.DR-210

ABSTRACT

Background: In the dairy industry, hygienic quality and safety of milk and dairy products are very important for human health.

Methods: The samples taken at the inlet and outlet of the HTST pasteurization process were subjected to physicochemical, bacteriological and sensory analysis.

Result: showed that pasteurization has not only been beneficial, as in the case of the hygienic quality of raw milk, where the number of the Total Mesophilic Aerobic Flora dropped by 94% after heat treatment, to reach an average of 5.62×10^4 CFU ml^{-1} of the thermo-resistant bacteria. Heat treatment resulted in a decrease of protein and lactose values for both types of milk. In fact, this loss is more marked in raw milk than in reconstituted milk (4% against 1.8%, respectively, $p<0.05$). Wetting was more marked after pasteurization for raw milk (0.102°C) compared to reconstituted milk (0.014°C). Sensory profiles were modified in both milk types, with a significant decrease in color, viscosity and flavor descriptors and a significant increase in the intensity of smell and taste ($p<0.05$).

Key words: Bacteriological, Milk, Pasteurization, Physicochemical, Sensory evaluation.

INTRODUCTION

Milk and dairy products are an important source of nutrients. They are considered as the main food products in the human diet (Leksir *et al*., 2019). Milk can be consumed without heat treatment (raw milk), most popular in recent years, in a context of organic, natural and eco-friendly consumption, with a possible potential positive impact on human health (Claeys *et al*., 2013), or after heat treatment (pasteurized milk) to ensure microbiological safety for human consumption and to extend its shelf life (Tadjine *et al*., 2020).

Currently and despite the publication of several scientific reviews, which have concluded that there was no reliable scientific evidence to support suggested health benefits (Macdonald *et al.,* 2011), several organizations across the world continue to promote the production and consumption of unpasteurized milk, "nature's perfect food" (Natural Milk.org, 2003) (Raw-Milk-Facts.com, 2009), which still fuels the current debate around the impacts of milk heat treatment on human health (Claeys *et al.,* 2013; Verraes *et al*., 2014).

However, pasteurization can provoke slowly damage components that could be beneficial to consumers; availability of the nutritional relevant vitamins in milk, particularly vitamin B2 and vitamin B12 can be slowly affected. Small losses have been reported for B6 and the fat-soluble vitamins A, D and E, even during conventional sterilization of milk (Schaafsma, 1998). From epidemiological studies, populations with a similar genetic background shown that children growing up on a farm have a lower risk of developing asthma and allergies due to the consumption of raw unpasteurized milk (Waser *et al*., 2007). Therefore, this study aimed to assess the effect of heat treatment using high-temperature short-time (HTST) on the physicochemical and microbial properties and sensory characteristics of both raw and reconstituted milk to provide a better understanding of the changes that occur in the milk quality.

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How to cite this article: Bousbia, A., Gueroui, Y., Boudalia, S., Benada, M. and Chemmam, M. (2021). Effect of High Temperature, Short Time (HTST) Pasteurization on Milk Quality Intended for Consumption. Asian Journal of Dairy and Food Research. 40(2): 147-151. DOI: 10.18805/ajdfr.DR-210.

Submitted: 23-11-2020 **Accepted:** 18-02-2021 **Online:** 30-03-2021

MATERIALS AND METHODS

Type of milk and sampling

The study was conducted from early October 2014 to late march 2015. Random sampling was carried out at four dairy plants located in the Guelma area (North-East of Algeria) on whole raw milk (WRM) and on partially reconstituted skimmed milk (PRSM) prepared from two types of anhydrous powder, with two fat content measurements; 26% and 0% (w/w); to obtain milk with a fat content ranging between 1.5% and 1.8% (w/w). The control group (raw milk and reconstituted milk) without heat treatment was taken inlet the HTST process. However, heat-treated group (raw milk pasteurized at 85°C during 30 seconds and reconstituted milk pasteurized at 72°C during 15 seconds) was taken at the outlet HTST process, after cooling. Samples were collected in glass bottles which previously autoclaved at a temperature of 120°C during 20 min. As well, the milk has been taken in respect of the Good Laboratory Practices (GLP) and the aseptic rules. The bottles were immediately transported for cooling at 4°C to the university laboratory (Université 8 Mai 1945 Guelma, Algeria).

Physicochemical analysis

Physicochemical parameters were measured using the milk analyzer "Lactoscan" (Milkotronic Ltd, Nova Zagora, Bulgaria) according to the manufacturer's instructions. The analysis was based on the principle of fourier transform infrared spectroscopy, which combines the recording of infrared spectra and the data processing with high accuracy and stability. Acidity was titrated with a sodium hydroxide solution (N/9) and the results were expressed in Dornic degrees.

Bacteriological analysis

Milk samples were cultured using standard microbiological methods according to the International Dairy Federation guidelines (IDF, 2013). Analyses were carried out for two types of bacteria, total mesophilic aerobic flora (TMAF) and (THF). Briefly, TMAF was enumerated on plate count agar (PCA) and incubated in aerobic at 30°C for 72 h. Similarly, thermophilic Flora was determined using Plate Count Agar medium, previously melted at 45°C, followed by incubation 55°C for 24 h (Ronimus *et al*., 2006). The total number of bacteria was expressed as colony-forming units per ml of milk (CFU ml^{-1}).

Sensory analysis

The panelists were recruited on the basis of their degree of taste preference for dairy products and their availability. Participants were instructed to rinse their mouth with tap water (20°C) before they began testing and between samples. Milk samples were evaluated for their color, viscosity, odor, taste and flavor using 9 categories hedonic scale method.

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software for Windows (version 20.0) (Armonk, New York, USA). Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as a percentage. For each milk type (raw milk and reconstituted milk), physicochemical, bacteriological and sensory analysis were performed separately. For the comparison of the means, the Student's t-test for two paired samples was used when the normality of the distributions was respected. The Spearman rank test was used to search correlations between the different floras.

RESULTS AND DISCUSSION

Effect of heat-treatment on milk physical qualities

The mean freezing points of raw milk increased significantly ($p<0.05$) from -0.520°C before pasteurization to reach an average of -0.426°C after pasteurization (Table 1). According to Zee *et al*. (1982), the increase in freezing point is probably due to water content caused by technological imperfections in the milk production. The wetting was observed much more after heat treatment, where the increase in water content is mainly due to pasteurization (Zagorska and Ciprovica, 2013). The same trend was noted for density, where the values are slightly lower than those of raw milk before pasteurization ($p<0.05$), with an average decrease of - 0.14%. The same result was observed for reconstituted milk where the mean density of the samples dropped by - 0.06% ($p<0.05$) (Table 1). The main causes of milk wetting are related to the construction defects of the heat treatment machines, thus when cleaning the different parts of the machines condensed and residual water can passes into the milk (Zee *et al*. 1982). Regarding pH values, no significant difference was recorded for both milk types (raw and reconstituted) before and after pasteurization. However, the acidity was significantly increased ($p<0.05$) in raw milk. This increase in Dornic acidity can be attributed to the lactose transformation into lactic acid and the precipitation of calcium phosphate (Walstra *et al.* 2005).

Effect of heat-treatment on milk chemical qualities

In raw milk, fat contents were decreased slightly by 0.46 g kg^{-1} after pasteurization to 34.56 ± 2.31 g kg^{-1}, ($p>0.05$). For reconstituted milk, pasteurization does not affect the fat content (Fig 1A).

Tallini (2015) were recorded any change in fat levels or fatty acid profile after pasteurization and Ultra-High-

Table 1: Physical quality before and after pasteurization.

Milk type	WRM		PRSM	
Treatment	Before HT	After HT (85°C/30 s)	Before HT	After HT (72°C/15 s)
n	784	784	784	784
FP (°C)	- 0.528	- 0.426*	- 0.432	- 0.418#
Density (mg cm^{-3})	1028.57	1027.05*	1028.16	1027.46#
pH	6.60	6.61	6.41	6.42
Dornic Acidity (°D)	16.07	17.88*	16.42	16.40

n: number of analyzed samples; **s:** second; **FP:** Freezing point; **°D:** Dornic degrees; **WRM:** Whole Raw Milk; **PRSM:** Partially Reconstituted Skimmed Milk; **HT:** Heat treatment; *and # Indicate values significantly different from within the same group before and after heat treatment (Student t-test, $p<0.05$).

Temperature (UHT) treatments on milk fat content. However, heat treatment was decreased protein content in significant manner in both milk types (raw milk and reconstituted milk, Fig 1B) ($p<0.05$). From literature, milk proteins can undergo a structural change known as denaturation which causes the deployment of proteins and the exposure of hydrophobic groups depending mainly on the temperature and treatment duration (Raikos, 2010). Pasteurization process (70 to 75°C for 15 seconds) causes only minimal denaturation in protein structure without any precipitation (Winarso and Foekh, 2011). Results showed that the lactose content decreased significantly after pasteurization in both milk types (raw and reconstituted milk, Fig 1C). It can be attributed to the wetting level recorded after heat-treatment and possibly to the degradation of Amadori compounds as a result of Maillard's reaction (Claeys *et al*., 2013). In both milk types, mineral content was not affected by heat-treatment (Fig 1D), which is in accordance with data from the literature (Claeys *et al*. 2013). The defatted dry extract is represented by the protein, mineral and the lactose contents. For both milk types, the defatted dry extract content after pasteurization was decreased ($p<0.05$) (Fig 1E). In the same way, total dry extract content was significantly decreased for both milk types (Fig 1F). Here, we show that pasteurization can affect some milk properties and these results are in accordance with data from the literature (Boudalia *et al*., 2016; Bousbia *et al*., 2018).

Effect of heat-treatment on the milk bacteriological qualities

Microbial populations found in raw and reconstituted milk before and after heat-treatment were shown in Table 2. A high level of total flora count (9.38×10^5 CFU ml^{-1}) detected in raw milk before treatment indicating a very poor quality compared to the accepted standards (10^5 CFU ml^{-1}) (OJAR, 1998). However, these data are consistent with the Benhalima *et al*. (2019). After pasteurization, thermofilic flora count of raw milk ranged between 2.4×10^4 to 29.4×10^4 CFU ml^{-1} with an average of $5.62 \times 10^4 \pm 13 \times 10^5$ CFU ml^{-1} considered as higher than the limit of 3×10^4 CFU ml^{-1} (OJAR, 1998). On the other hand, a positive correlation, between the total mesophilic flora and the thermophilic flora

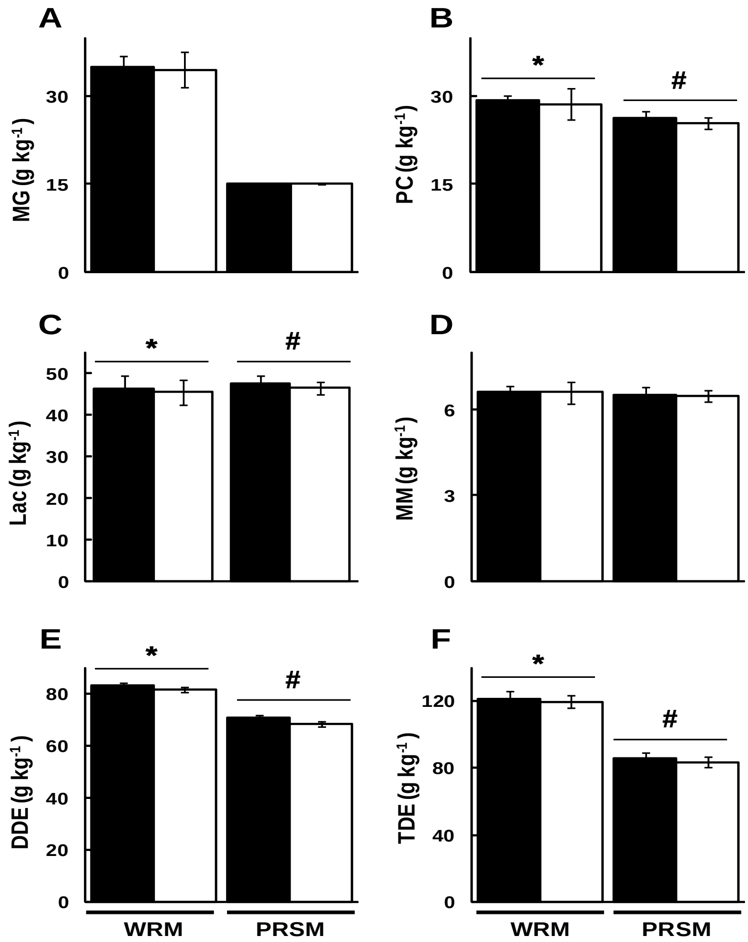

Fig 1: Pasteurization effects on chemical qualities: **A)** Fat Content (FC); **B)** Protein Content (PC); **C)** Lactose (Lac); **D)** Mineral Matter (MM); **E)** Defatted Dry Extract (DDE); **F)** Total Dry Extract (TDE). The results are presented in the form of mean ± SD. *, #Correspond to a significant difference in the same milk type (WRM and PRSM), n before pasteurization o after pasteurization ($p<0.05$).

was found (r = 0.65, $p<0.05$). Heat-treatment was as efficient (94%) in reducing the average count of common flora in raw milk. Nevertheless, the heat-treatment was not able to reduce microbial count to acceptable levels in all the samples, where 45% of raw pasteurized milk samples show a high concentration of thermophilic flora exceeding the standards (OJAR, 1998). The detection of the thermophilic flora of raw milk indicates not only the germ content of pasteurized milk, but also its shelf life when the milk is not contaminated after pasteurization (Mourgues *et al.,* 1983). Also, thermophilic flore is considered as spoilage microorganisms and hygiene indicators in processed milk because of their potential to form endospores (Burgess *et al.,* 2010). Moreover, the insufficient heat-treatment attributed to raw as well as the slow cooling and the poorly cleaned and disinfected installations promote the presence of thermophilic flora (Mourgues *et al.,* 1983). Furthermore, milking machines bring more heat resistant flora and coliforms than manual milking (Monsallier *et al.,* 2016). Otherwise, reconstituted milk samples show an acceptable mesophilic and thermophilic flora concentrations, which is found within the recommended standards (OJAR, 1998).

Effect of heat-treatment on milk sensory qualities

The samples acceptability was measured on a 9 categories hedonic scale before and after heat treatment (Table 3). Heat-treatment decreased the mean sensory color score for both milk types. This decrease was more significant for raw milk than reconstituted milk related to the increase in the number of samples with the “creamy white” color that appeared after heat treatment. Changes in milk color after heat treatment are mainly due to Maillard reactions where the assessment of the brown compounds indicates the severity of heat treatment (Pagliarini *et al*., 1990). However, good quality milk has a matte white color, which is largely due to the fat and carotene pigments (Fredot, 2012).

The viscosity decreased significantly for both milk types after heat-treatment ($p>0.05$). Indeed, the lowest mean scores were recorded in raw and reconstituted milk samples after pasteurization compared to their mean scores before heat-treatment, with averages of 5.81 and 5.13, respectively. Therefore, a significant number of panelists report that milk after pasteurization has become less viscous which can be linked to the wetting that has already been raised in the physicochemical part. Furthermore, the fat content has a large effect on milk viscosity which increased significantly with the increase of fat content (Li *et al*., 2018). Likewise, the flavor of raw milk was negatively affected by heat-treatment ($p>0.05$) with a cooked flavor felt by the panelists felt (Walstra *et al*., 2005). The heat-treatment increased the average scores for odor and taste, as opposed to color, viscosity and flavor. As a result, the highest scores were significantly recorded in raw and reconstituted milk after pasteurization. Generally, a large proportion of panelists perceived a lactic and animal odor before treatment for both raw and reconstituted milk. However, the unpleasant aroma and taste in pasteurized milk are generally the result of bacterial growth and characteristic of milk spoilage (Simon and Hansen, 2001). Consequently, the color, viscosity, odor and taste are important sensory attributes of milk that are

Table 2: Effect of pasteurization on some indicators of milk bacteriological qualities.

Milk type	WRM		PRSM	
Treatment	Before HT	After HT (85°C/30 s)	Before HT	After HT (72°C/15 s)
Flora (CFU ml^{-1})	TMAF	THF	TMAF	THF
n	32	32	32	32
MC (CFU ml^{-1})	9.38×10^5	5.62×10^4	2.14×10^4	2.02×10^4
(%S > LC)	90	45	0	0
Standard (CFU ml^{-1})	10^5	3×10^4	3×10^4	3×10^4
Reference	(OJAR, 1998)	(OJAR, 1998)	(OJAR, 1998)	(OJAR, 1998)

TMAF: Total Mesophilic Aerobic Flora; **THF:** Thermofilic Flora; **MC:** mean count; %S < **LC:** % of samples with a charge below the legal criterion, **n:** Number of samples; Standard: Threshold limit of acceptability beyond which the results of milk received at the factory are not considered satisfactory; **S:** second; **HT:** Heat Treatment.

Table 3: Effect of pasteurization on some attributes of milk sensory[1] qualities.

Attribute	WRM		PRSM	
	Before HT	After HT (85°C/30 s)	Before HT	After HT (72°C/15 s)
Color	7.93±0.89	7.71^b±1.05*	8.13±0.83	8.06^y±0.84#
Viscosity	6.95±1.06	5.81^b±1.01*	6.40±1.25	5.13^y±1.42#
Odor	4.20±1.05	5.28^b±0.61*	4.40±1.16	5.55^y±0.92#
Taste	4.86±0.96	5.64^b±0.54*	4.96±0.66	5.23^y±0.51#
Flavor	6.22±0.74	3.63^b±0.22*	5.96±0.36	5.44±0.63

[1]**Hedonic Scores: 9** = Like Extremely, **8** = Like Very Much; **7** = Like Moderately, **6** = Like Slightly, **5** = Neither Like Nor Dislike, **4** = Dislike Slightly, **3** = Dislike Moderately, **2** = Dislike Very Much, **1** = Dislike Extremely. * and # indicate values significantly different from within the same group before and after heat treatment (Student t-test, $p<0.05$).

influenced by several factors, such as milk composition, cow feed and metabolism, environmental factors and processing conditions.

CONCLUSION

This study highlighted the potential pasteurization effects on the physicochemical, bacteriological and sensory qualities of raw and reconstituted milk. Pasteurization ensures the hygienic safety of the milk, but can denature the primary organoleptic and/or nutritional characteristics of the milk before treatment. Good agricultural, hygienic and farming practices can preserve the quality of raw milk. Moreover, optimization of the pasteurization process can conserve milk nutritional and organoleptic qualities and decrease treatment costs. Alternative technologies such as non-thermal treatments must be adopted for a possible production of industrial milk that is safe and perceived as fresh.

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