Pak. J. Biotechnol. Vol 15 (2) 577-582 (2018) *ISSN Print: 1812-1837*
www.pjbt.org *ISSN Online: 2312-7791*

PROCESSES OF STERILIZATION AND ULTRA-HIGH TEMPERATURE EFFECTS ON CHEMICAL COMPOSITION AND CARBOXYLIC ACID PROFILE IN BOVINE MILK

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Article received 9.12.2017, Revised 22.6.2018, Accepted 26.6.2018

ABSTRACT

The purpose of this study was to investigate the certain of pasteurization and extreme High-temperature procedures on adjacent and physicochemical aggregation, microbiological factors and fatty acid side view in bovine milk. Pastuerized, sterilized and fresh milks were submitted at a factory works nearby from the university. Specimens were collected toward resolution, protein, moisture, lactose, total fat, total solids, free-fat, dry extract, urea, calcium, phosphorus, pH, acidity, density, fatty acid profile, total bacterial calculation besides somatic cell calculation. Sterile as well extreme-high temperature milks reserved lactose also protein gratified alike fresh milk. Sterilization besides pasteurization changed milk combination in some measure, terminate whole fat besides whole solids also cumulative urea. The procedures redesigned heart short-chain fatty acids (6:0, 8:0 in addition to 10:0). Great dimensions in stearic acid (18:0), palmitic acid (16:1) and meristic acid (14:0) were set up in wholly analyzed milks. Lack in extended outstandingly milk adaptations mixture also fatty acid summary show meander complete the procedures devoid of changing the milk dietary rate.

Key words; Sterilization, UHT, Milk composition

INTRODUCTION

Several debates arise about the ingesting of milk and factory produces throughout maturity. Though, ingesting of milk offers great dietary rate, inclosing lactose, casein, vital fatty acids, vitamins in addition to minerals (Claeys, Verraes et al. 2014). Approximately Saturated Fatty Acids (SFA) labeled milk to have optimistic properties on healthiness; butyric acid (4:0) is a recognized modulating role of gene and can as well show a part in cancer inhibition, 8:0 and 10:0 might have a part in antiviral actions then 8:0 has been stated to interruption swelling development (Claeys, Verraes et al. 2014). Originated on an evaluation of epidemiological analyses, here appears to be unreliable relative among a great consumption of factory yields besides Cardiovascular disease (CVD) (Chaput, Klingenberg et al. 2011). Furthermore, Despite arguments, epidemiologic research approve the dietary significance of milk in the human nutrition and strengthen the probable character of its ingesting in avoiding numerous lingering circumstances like cardiovascular disease (CVD), approximately systems of cancer, fatness and diabetes(King, Bradford et al., 2014).

The impartial of this study was to control the belongings of extreme-high temperature sterilization and procedures on nearby and physicochemical arrangement, fatty acid profile and microbiological parameters milk in bovine. The principal purposes of heat action are to decrease the microbial residents, mutually spoilage and pathogenic, to deactivate enzymes and to reduce chemical responses and physical variations (Tamime 2009). It is fundamental to preserve milk adjusted at low temperatures; to keep this great dietary significance and give way it to heat actions for instance pasteurization besides Ultra-High Temperature (UHT) procedure (Anema 2014). proposed that this collection belongings comprises deprivation of lactose to organic acids and establishment of lactulose, denaturetion the whey of proteins, damage of enzymes and vitamins, hydrolysis of lipids and proteins and disruption of calcium/phosphorus balance. Some possessions of heating distress value besides technical possessions of milk. Additional things comprise heated aroma and dietary significance damage because of novel materials molded through Maillard reaction, which lasts throughout storing of heated milks (Sakkas, Moutafi et al. 2014). Human consumption covered by the production of milk heat treatment for the scale starting pasteurization to in-basin sterilization, with admiration to heat-encouraged and shelf life alterations in milk (Lorenzen, Clawin-RÄDecker et al. 2011). Though, Little investigation occurs with usage of pasteurization and sterilization procedures, specifically with affections to fatty acid outline besides milk chemical and physical possessions. The milk constituents have been described the effect of heat treatments. Though, the influence of treating and storing circumstances on the lipid outline of milk is not totally agreed besides is focus of argument, principally for the situation of fatty acids (Rodríguez-Alcalá, Harte et al. 2009).

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade and obtained from Sigma-Aldrich Co. Ltd. (MO, USA) and Merck (Darmstadt, Germany).

Milk samples: The treating of milk in different phase (sterilized, raw and pasteurized) twelve lots of bovine milk were composed at a dairy factory in the area close to the university. In tetra pack apparatus the pasteurized milk was gathered by 30,000 L hG1 then in this procedure the milk was kept at 4°C.The similar portion of fresh milk was acquiesced to sterilization (130°C for 3 sec) and formerly too extreme-high temperature method (145°C for 2 sec). Each portion was tested three periods so as to realize examination in triplicates. Fresh, sterilized and UHT milk tasters were moved to sanitized tubes. After sampling and conveyed in thermal containers the tubes were directly cooled at 4°C.

Determination proximate and physicochemical composition and microbiological parameters: Protein, lactose, moisture, total fat, free-fat dry extract, total solids, phosphorus, calcium and urea were resolute by a Milko Scan (Milkoscan FT+, FOSS, Denmark) by Fourier transform infrared spectroscopy (FTIR). The Bacto Scan was worked according to the techniques suggested by the manufacturer (Pulinas, Spanu et al. 2017). Evaluates were done along with the processes mentioned by the industrialist (Foss and Analytical, 2008). Cytometric flow using for total bacterial count and somatic cell total were examined by Bacto Scan (Bacto scan H, FOSS, Denmark). The taster pH was evaluated via a Mettler-Toledo pH meter (Mettler-Toledo International Inc., Greifensee, Switzerland). Acidity and density were resolute according to (Hogsden and Harding 2012). All analyzes were recognized in triplicate.

Fat extraction and fatty acid profile: Lipid extraction followed the methodology $_{\text{suggested}}$ by (Park and Li 2012). About 5 ml of milk was disso-lved in 2 mL of ethanol, 4 mL of ultrapure water and 2 mL of NH4OH. This mixture went through shaking water bath at 70°C for 10 min. The extrac-ted fat residue was dissolved in 50 mL diethyl ether: petroleum ether (1:1, v/v). Then, the solution was evaporated to dryness in a 35°C water bath under nitrogen stream. The fat residue was melted in 5 mL chloroform:diethyl ether (1:1, v/v) and the solvent was evaporated under nitrogen stream. The residue was added 2 mL 6% BF3 and 1 mL toluene and heated in a 95°C oven for 50 min. After added 5.0mL ultrapure water, 1.0mL hexane and 0.2g Na_2SO_4, the solution was vortex-stirred for 1 min, followed by centrifugation at 4000 rpm for 2 min. The organic upper phase was improved and analyzed by Gas Chromatography (GC). Fatty Acid Methyl Esters (FAME) were examined on an Agilent GC unit (model G890N, Palo Alto, CA, USA) equipped with a flame ionization indicator. Fatty acids were separated using a DB-23 fused-silica capillary column (30×0.32 mm ID×0.25 μm film thickness, Agilent, USA). Supelco Inc. (Bellefonte, PA, USA) was developed documentation of heights achieved by contrast the taster of highest holding times thru those of FAME usual combination. Quantification of the FAME in milk lipids was completed by means of undecanoic acid (11:0) as an interior normal. Helium was utilized as the transporter gas and the injector divided ratio was 1:50. Afterward inoculation (1 μL), the primary column temperature of 70°C was detained for 0.5 min, amplified to 170°C (9°C minG1). At that point, it was amplified to 200°C (1°C min G1) and lastly augmented to 230°C (10°C min G1) and continued for 2 min. The FAME was stated as g/100 g of whole fatty acid gratified, presumptuous a straight association among highest area besides FAME weight. The examination was recognized in triplicate.

Statistical analysis: The model of statistical measured the usage type (UHT, sterilized and raw) a recurrent degree contained by the milk. The data were evaluated by means of the PROC Mixed Procedure of the Statistical Analysis Systems (SAS, 2004). Differences were measured significant at a p value below 0.05. Least squares mean (LS means), by means of the choice PDIFF (probability difference procedure), were strongminded in order to liken sets.

RESULTS AND DISCUSSION

Proximate and microbiological parameters and physicochemical composition: Proximate and microbiological parameters and physicochemical composition affected on treatments of bovine milk as illustrate in Table 1. The wet matter basis is described on a proximate composition. The total contents of fat, moisture, urea and total solids significantly affected by the treatments ($p<0.05$). Centrifugation caused the change in total fat after sterilization and pasteurization caused from calibration. This is completed inspite of the eliminate fat cream, observing by lawmaking. The damage to the fat globule membrane caused by the thermal action and milk homogenization process (O'Sullivan, Lorenzen et al. 2001), producing more contact to materials existing on the surface membrane (Collins, McSweeney et al. 2003). This possibly caused in discharge of urea, permitting its quantification, which might clarify the rise in contents of urea originate in raw, pasteurized and UHT milks, correspondingly.

Sterilization and UHT processing were not influenced the main milk nutrients (protein and lactose). The phosphorus (0.09±0.02%) and calcium (0.09± 0.01%) samples concentrations did not demonstration significant differences among the diverse treatments.(Thomas, Waterston et al. 2015) was described these results with the concentration variety establish in bovine milks count.

Table 1a: Proximate and microbiological parameters and physicochemical composition in fresh, pasteurized and UHT bovine milk. Analysis of different treatments in raw, sterilized and UHT bovine milk

Analysis	Treatments		
	Raw	**UHT**	**Sterilized**
Protein(g/100g)	3.19±0.05	3.23±0.05	3.22±0.05
Moisture (g/100g)	87.18±0.27[a]	87.60±0.54[b]	87.71±0.50[b]
Lactose(g/100g)	4.26±0.08	4.25±0.09	4.19±0.05
Total Fat (g/100g)	3.10±0.27[a]	2.85±0.22[b]	2.80±0.22[b]
Total acid(g/100g)	11.90±0.20[a]	11.02±0.21[b]	11.01±0.20[b]
Free fat dry extract(g/100g)	7.47±0.08	7.41±0.09	7.47±0.07
Calcium(g/100g)	0.10±0.02	0.09±0.01	0.10±0.02
Phosphorus(g/100g)	0.08±0.02	0.09±0.02	0.09±0.01
Urea(g/100g)	16.56±4.19[a]	24.67±3.81[c]	20.66±3.88[b]
N	12	12	12

On each line, the values with different letters are significantly different ($p<0.05$). Values are mean value±SD, UHT: Ultra high temperature, n: No. of samples.

Table 1b: Proximate and physicochemical composition and microbiological parameters in raw, sterilized and UHT bovine milk.

Analysis	**Treatments**		
	Raw	**UHT**	**Sterilized**
Physiochemical composition			
Acidity (% Lactic Cid)	0.22±0.08[a]	0.15±0.01[b]	0.15±0.02[b]
Density(g/ml)	6.47±0.41[a]	6.77±0.10[b]	6.77±0.12[b]
PH	6.47±0.41[a]	6.77±0.10[b]	6.77±0.12[b]
Micrbiological Parameters			
Somatic cell count(1000/ml)	619.79±144.78[a]	59.08±21.80[c]	365.33±92.95[b]
Total bacterial count (1000 UFC/ml)	6470.21±1903.57[a]	1031.83±739.86[b]	1047.58±413.81[b]
N	12	12	12

The free-fat dry extract presented a drop between UHT raw and milks according to Table 1. The physicochemical characteristics of UHT milk analyzed by (Simone, Marcelo Pinto et al. 2016) throughout its observed and industrialization alike consequences in this effort.

The pH increased with resulting decrease in acidity of milk by marketable sterilization and then pasteurization. This high pH can be explained by lower whey protein associating with the micelles. At pH 6.7 only about 30% of the denatured whey proteins are related with the casein micelle surface described by (Anema and Li 2003).

The animal healthiness and the hygiene circumstances are strongly influenced by milking somatic cell and total bacterial counts. As expected, the number of pathogenic bacteria and resilient pathogenic microorganisms aimed the decrease shelf life by increased the heat treatment. In this research, the observed of somatic cell and total bacterial count decreased by the heat of milks treatment.

Fatty acid profile: The influence of the actions on the fatty acid outline of milk fat is existent in Table 2. Raw, UHT and sterilized milks had closely like fatty acid outlines. It is recommended the little affects fatty acid profile by actions throughout the commercial sterilization and pasteurization procedures in milk (Pearce et al. 2001) furthermore assessed the arrangement and outline of pasteurized and raw milk fatty acids. The effect of pasteurization in raw milk found in mini dairies there is no significant difference ($p>0.05$).

According to Table 2, oleic acid (18:1n-9), stearic acid (18:0), palmitic acid (16:0), and myristic acid (14:0) were predominant fatty acids.(Vieira, Cabral et al. 2017) described identical results were found in this dairy yields comprising whole milk. The essential monounsaturated fatty acid was Oleic acid in the tasters, causal to 25.4-26.2% of total fatty acids.

Short-chain fatty acids for instance caprylic acid (8:0) caproic acid (6:0) and butyric acid (4:0) were pretentious in UHT and pasteurized milks, nevertheless here were no changes among actions. These consequences could show in what way can reduction concentrations of butyric, caprylic and caproic acid by pasteurization and commercial sterilization processes. The effect of the low temperature past-

eurization procedure on the lipid outline of milk, did not discovery significant differences in SFA, as evaluated by (Melini, Melini et al. 2017). In this study, according to (Zivkovic, Wiest et al. 2009) the SFA (20:0, 8:0, 6:0 and 4:0) were influenced by treatments ($p<0.05$). stated there was no significant difference between sterilized and raw milk for all SFA evaluated.

Table 2: Fatty acid profiles of total lipids in raw, sterilized and UHT bovine mil*k*

Analysis	**Treatments**		
	Raw	**UHT**	**Sterilized**
4:0	2.93±0.95[a]	2.76±0.45[b]	2.77±0.45[b]
6:0	2.56±0.38[a]	2.27±0.25[b]	2.20±0.25[b]
8:0	1.56±0.16[a]	1.44±0.15[b]	1.43±0.10[b]
10:0	2.95±0.25	2.91±0.28	2.79±0.01
12:0	3.61±0.19	3.48±0.20	3.32±0.18
13:0	0.09±0.01	0.08±0.10	0.08±0.20
14:0	11.52±0.45	11.29±0.55	11.25±0.45
14:1 n-9	0.88±1.58	0.89±1.55	0.85±1.15
15:0	1.20±0.25	1.19±0.05	1.19±0.05
15:1	0.04±0.08	0.06±0.13	0.06±0.02
16:0	29.90±1.40	29.73±0.81	29.82±0.24
16:1 n-9	1.31±0.43a	1.44±0.08[b]	1.25±0.34[a]
17:0	0.42±0.25	0.46±0.23	0.59±0.07
17:1	0.22±0.17	0.19±0.01	0.19±0.01
18:0	12.54±0.85	12.98±0.75	12.95±0.10
18:1 n-9	25.41±1.35a	25.92±1.27[b]	26.20±1.19[ab]
18:2 n-6	1.81±0.25	1.69±0.25	1.72±0.51
18:3 n-3	0.56±0.02	0.55±0.25	0.58±0.10
18:3 n-6	0.06±0.06	0.05±0.01	0.06±0.03
20:0	0.14±0.01[a]	0.15±0.00[c]	0.16±0.00[b]
20:1	0.02±0.00	ND	ND
SFA	69.85±1.79[a]	67.92±1.66[b]	67.93±1.37[b]
MUFA	28.09±1.55[a]	28.095±1.45[b]	28.79±1.11[b]
PUFA	2.58±0.25	2.55±0.20	2.73±0.52
n-3	0.56±0.01	0.56±0.02	0.589±0.01
n-6	1.83±0.60	1.80±0.53	1.94±0.98
n-6/n-3	3.49±0.95	3.47±0.80	3.45±1.90
PUFA/SFA	0.05±0.02	0.05±0.01	0.05±0.02
n	12	12	12

The values on each line, with different letters are significantly different ($p<0.05$). Values are mean value±SD (% total fatty acids). MUFA: Monounsaturated fatty acid, SFA: Saturated fatty acid, PUFA: Polyunsaturated fatty acid. UHT: Ultra high temperature. ND: Not detected, n: No. of samples.

Main fatty acids in cereal grains then consequently transported into milk which reported by (Leiber, Kreuzer et al. 2005) was found higher levels in Oleic acid (18:1 n-9) afterward UHT action than in raw milk that nutrition founded on concentrates are predictable to rise the absorption of oleic acid and linoleic acid (18:2 n-6). On the opposing, it is well identified that a great ratio of polyunsaturated fatty acids (PUFA) found in fresh pasture with all principles present in milk in this research are near to those earlier initiate for milk founded on feeding of grass the cows (Capuano, van der Veer et al. 2014) , and α-linolenic acid being the main n-3 fatty acid in fresh grass (Elgersma, Tamminga et al. 2006).

The odd-numbered fatty acids (17:0, 15:0 and 3:0) in this study found that, expressive 2% of total fatty acids did not existing changes after commercial sterilization and pasteurization processes. Propionyl-CoA as a precursor used in endogenous chain elongation in the mammary gland (Fievez et al. 2012) clarifies the incidence of C11:0, C9:0, C7:0, and C5:0 in milk and enhances to odd-chain fatty acids moved from the duodenum (C13:0, C15:0 and C17:0). Dairy of cows and Odd-fatty acids in milk might be reflecting rumen role (e.g., ruminal fermentation design, comprising intestinal movement of microbial protein, methane, and acidosis) (Fievez, Colman et al. 2012).

In this study, lauric acid (12:0) did not existing changes afterward sterilization and UHT handling according to Table 2. Lauric acid may have antibacterial and antiviral functions and might act as an anti caries and anti-plaque agent (Brumini, Criscione et al. 2016).

Regarding partial sums of fatty acids (Table 2), the UHT milk affected significantly the monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). Raw milk had advanced quantities of total fat then subsequently greater SFA levels. The Increments gratified of SFA were greater than those of PUFA as fatness increased (German and Dillard 2006). The fresh milk, related with the UHT, had higher relative proportions of MUFA ($p<0.05$), but lower percentages ($p<0.05$) of SFA. The n-6 PUFA, n-3 PUFA and PUFA did not present differences between treatments.

There was no discovered difference between treatments ($p>0.05$) for the PUFA/SFA and n-6/n-3 ratio. According to current nutritional approvals, for the PUFA, the n-6/n-3 proportion should not exceed 4.0 (Kalogeropoulos et al., 2010), and the PUFA/SFA relation in human nutrition should be over 0.45). This latest index has been the issue of some discussion. (Dawczynski, Schubert et al., 2007) have planned that it is extra significant to assess the entire quantities of nutritional PUFA than their particular proportion. Furthermore, (Mozaffarian and Wu 2011) projected that the position of n-3 PUFA could be enhanced by reducing the consumption of n-6 PUFA or through increasing dietary consumption of n-3 PUFA and that joining both approaches would be greatest active. In the current research, the n-6/n-3 and PUFA/SFA percentages were inside the suggested guiding principle on behalf of the human nutrition, in spite of the PUFA/SFA being much under 0.45. The PUFA/SFA reproduced the detail that PUFA

and SFA are further rich in the phospholipid and triacylglycerol portions (Scollan, Hocquette et al. 2006). (Paixão, Rodrigues et al., 2014) reported similarly reliable with the fat portion of milk proposals 98% triacylglycerol and about 1% phospholipids.

The results of this research come to an agreement with those issued by (Claeys, Verraes et al., 2014). The results show that UHT and sterilization treating does not considerably affect the fatty acid outline of milk. These authors suggested alike to what happened in this research when fresh milk was submitted to extreme-high temperature (140°C for 3 sec) that thermal degradation of milk lipids is commonly not experiential, for the reason that the temperature required for non-oxidative decomposition of fatty acids (>200°C) is well external the range in which milk yields are heated. Furthermore after heating alterations detected in the fatty acid content of milk seemed to be less applicable than the well-recognized report that ruminant milk lipid structure is influenced by environmental factors (e.g., diet and management) and genetic (e.g., breed), as projected by (Caroli, et al., 2009). It can therefore be supposed that heating has a slight influence on the dietary rate of milk fat, which clarifies the small differences originate in the fatty acid outlines of the milk examined.

CONCLUSION

Raw milk similar to sterilization and extreme-high temperature milks reserved protein and lactose gratified. The composition of the milk slightly altered with sterilization and ultra-high temperature processes, declining entire solids and total fat and increasing urea. These procedures altered important short-chain fatty acids (8:0, 6:0 and 4:0). All milks analyzed were found high sizes of myristic acid (14:0), oleic acid (18:1 n-9), stearic acid (18:0) and palmitic acid (18:0). The close composition and fatty acids outline in raw bovine milk does not significantly change with the ultra-high and sterilization temperature processes, concerning its possible nutritious possessions and subsequent welfares for health of human.

ACKNOWLEDGMENTS

The author acknowledged all those technical staff in the laboratory of university and all the workers in the Diary Factory.

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