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Effect of Thermal Treatments on the Fatty Acids Composition, Antioxidant and Anti-inflammatory Properties of Camel Milk

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Abstract: The present research was conducted to study heat treatment's effect on camel milk's physicochemical and biological activities. Milk samples were heated at 63°C for 30 minutes, 90°C for 3 minutes, and 100°C for 3 minutes. After heating, the physicochemical: pH, acidity, Fat, Dry matter, proteins, and fatty acids composition as well as the antioxidant and anti-inflammatory activities of all samples were determined. The antioxidant activity of camel milk was evaluated by different assays, including free radical-scavenging activity (DPPH and ABTS) and ferric-reducing power assay (FRAP). Results showed that the heat treatment process increased significantly ($p<0.05$) the viscosity, total solids, ash, and lactose content. Although, the monounsaturated fatty acids and particularly the oleic acid were significantly decreased after heat treatment. Heat treatment didn't show any significant effect on antioxidant activities. While a significant increase ($p<0.05$) was shown after boiling the milk in the anti-inflammatory activity. Therefore, pasteurization could be the greatest heating process to ensure the microbiological safety and the stability of the micronutrients in milk.
Keywords: Camel milk; Heat treatment; Fatty acids; Biological activities.

1. Introduction

Camel milk is considered an important source of nutrition in different arid rural communities of Africa and Asia. It contains all essential biomaterials such as; protein, fat, lactose, minerals, and vitamins. Therefore, in comparison with bovine milk, camel milk is known for its high concentration of polyunsaturated fatty acids and a great number of minerals and vitamins especially vitamin C; three to five times higher than bovine milk which is very essential in the Saharan region where the fruits and vegetables are scarce [1]. Hence, it is known for its higher digestibility which is may due to its smallest fat globules in comparison with cow, buffalo, and goat milk [2]. Moreover, it has greater antiviral and antibacterial properties than cow milk which may be due to its higher concentration of lactoferrin, lysozyme, and immunoglobulin [2, 3]; Camel milk was also reported other nutritional and therapeutic properties such as hypoallergic [3], anti-carcinogenic [4], antidiabetic properties [5,3] and has been recommended for children who are allergic from bovine milk due to its lack on β-lactoglobulin; the protein responsible for allergies in cow milk [6].

Heat treatment is included in most dairy industries to improve the bacteriological quality of dairy products to render them safe to drink and prolong their shelf life [7].

Most camel milk is consumed freshly. Therefore, to improve the microbiological quality and to extend the shelf life of milk, different heat treatments such as pasteurization and boiling for home use may be applied. On the other hand, in some regions such as central Asia and Gulf countries, heat treatment is used as a means to preserve camel milk and until now only a few studies have investigated the effect of heat processing on camel milk properties [8, 9].

For that reason, it is important to understand the modifications that happened in the composition, biological and functional properties of milk during the applied thermal treatments.

The purpose of this research was to study the effect of heat treatments at different temperatures on the composition of camel's milk. Antioxidant and anti-inflammatory activities of raw and thermal-treated milk were also taken into consideration.

2. Materials and methods

2.1 Milk samples

Fresh camel milk was obtained from a camel herd (Camelus dromedarius) belonging to the Livestock and Wildlife Laboratory, Arid Lands Institute (Medenine, Tunisia). Milk samples were subjected to three thermal treatments: Pasteurized (63°C for 30min), treated at 90°C for 3 min, and boiling at 100°C for 3 min. All samples were stored at -20°C until further analysis.

2.2 Physico-Chemical analysis of milk samples

The pH and viscosity values of samples were determined with the assistance of a pH-meter (Jenway, Staffordshire, United Kingdom) and a viscometer (Brookfield, model DV-E, MA, USA) respectively. The titrable acidity was analyzed by the method of [10]. The total solids in milk samples were determined by the standard oven drying method [11]. The ash content was measured by incineration in a muffle furnace at 560°C for 6h [11]. Mineral content was identified by atomic absorption spectrophotometry (Shimadzu AA-6800, Shimadzu, Germany). The protein content of milk samples was determined by the Kjeldahl method as total nitrogen content and converted to protein content by multiplying by a factor of 6.38 [11]. The Fat content of samples was measured using the Gerber method [11]. Lactose content was determined according to [12].

2.3 Fatty acids analysis

The milk fat was extracted by centrifugation of camel milk (3500×g, 20min, 4°C, centrifuge Sorvall Lynx 6000; Thermo Fisher Scientific, Waltham, MA, USA). Then the milk fat was subjected to methylation using a methanol KOH (2 N) solution. To extract the fatty acids methyl ester (FAME), the solution was mixed with hexane and the supernatant was analyzed using gas chromatography QP2010 Shimadzu (Tokyo, Japan) coupled to mass spectrometry. The Fatty acids were quantified and identified using FAME 37 internal standards [13].

2.4. Antioxidant activities

1) DPPH Radical Scavenging Activity

The determination of radical scavenging activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was determined according to [14] with minor modifications.

A volume of 500 µL of different samples was added to 500 µL of a methanol solution of DPPH (100 µM). The solution was then incubated in dark for 30min. Distilled water was used as a control. The absorbance was measured at 517 nm using an ultraviolet-Visible spectrophotometer.

The percentage of radical-scavenging activity was expressed as follows:

DPPH radical-scavenging activity (%) = (Ac – As/Ac) X 100

Ac: Absorbance of control and As: Absorbance of samples.

2) Determination of ABTS radical-scavenging activity

The ABTS radical scavenging activity of milk samples was determined according to the method of [**14**]. The ABTS radical caption was obtained by dissolving 7 mM of cation ABTS+ in 2.45 mM potassium persulfate. The mixture was incubated in the darkness for 16h at room temperature. Then it was diluted in 5mM sodium phosphate buffer, pH 7.4 to obtain an absorbance of 0.7 unit at 734 nm.

A volume of 100µL of each sample was added to 900 µL of the ABTS+• radical reagent, mixed, incubated for 10 min, and measured at 734nm using a UV-Visible spectrophotometer.

The percentage of ABTS radical-scavenging activity was calculated as follows:

ABTS radical-scavenging activity (%) = (Ac – As/Ac) X 100

Ac: Absorbance of control and As: Absorbance of samples.

3) Ferric reducing power assay

The ferric reducing iron was determined according to the method of [15] with minor modifications. Briefly, a volume of 1.25 mL of each milk sample was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1 % (w/v) potassium ferricyanide solution. A volume of 1.25 mL of 10 % of trichloroacetic acid was added after incubation of the mixture at 50°C for 20min. Then, the mixture was centrifuged at 3000 rpm for 10 min. Thereafter, a volume of 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1 % (w/v) ferric chloride. The absorbance was measured at 700 nm using a UV-Visible spectrophotometer.

2.5 Anti-inflammatory activity

The anti-inflammatory activity was determined against the enzyme 5-Lipo-oxygenase using the method of [16] with a minor modification. A volume of 80 µL of each samples was added to 600 µL of 100mM phosphate buffer (pH 7.4), 240 µL of linoleic acid (3.5 mmol/L), and 80 µL of 5-lipo-oxygenase. The mix was then incubated for 10 min at 25°C and the absorbance was measured at 234nm in UV-Visible spectrophotometer.

The percentage of inhibition of the 5-Lipo-oxygenase was defined as the anti-inflammatory activity.

2.6 Statistical analysis

Statistical analysis for the attained data was done using EXCEL STAT (Addinsoft 2014). Analysis of variance (ANOVA) followed by Tukey's test was used to classify the significant differences between the different samples. The GraphPad Prism software was used to prepare the graphs.

3. Results and discussion

3.1 Physico-chemical composition

As shown in table 1, a significant effect of heat treatment was shown on the viscosity of camel milk; the viscosity increased after boiling. This result may be due to the particular composition of the casein micelle of camel milk. Unheated camel milk illustrated the lowest amount of total solids (113.10±12.15 g/L) and ash content (10.16±0.95 g/L) which is in accord with [17] who found (99±1,189 g/L) and (6,8±0,96 g/L) for total solids and ash respectively for fresh camel milk. Therefore, heat treatment did not show any significant difference in fat and protein contents. The highest lactose content was attained in the treated milk at 100°C, which is in agreement with [18] who found that the lactose quantity was increased after heating camel milk.

The analysis of mineral content presented in table 2 revealed a non-significant effect of heat treatment on mineral composition of milk. Therefore, calcium, K, and Na levels decreased at high temperatures, in this context, [**19**] found also that calcium decreased after heating 30 min at 80°C.

Table 1. Effect of different thermal treatment on physical-chemical composition of camel milk

	control	63°C	90°C	100°C
pH	6.47 ± 0.15	6.45 ± 0.12	6.41 ± 0.11	6.41 ± 0.13
Acidity (D°)	18.62 ± 0.69	18.60 ± 1.19	18.58 ± 0.96	18.53 ± 0.38
Viscosity	3.146 ± 0.65^{b}	3.50 ± 0.30^{ab}	4.30 ± 0.89^{ab}	15.58 ± 7.69^{a}
Total solids (g/L)	113.10 ± 12.15^{b}	117.78 ± 13.96^{ab}	124.26 ± 8.82^{ab}	128.52 ± 13.08^{a}
Ash (g/L)	8.39 ± 0.64^{b}	8.54 ± 0.73^{b}	8.74 ± 0.67^{b}	10.16 ± 0.95^{a}
Fat (g/L)	38.43 ± 4.98	37.48 ± 4.06	33.66 ± 4.41	34.61 ± 4.78
Proteins (g/L)	30.75 ± 2,97	33.00 ± 2,22	28,57 ± 3,18	29.22 ± 3.98
Lactose	35.19 ± 2.16^{ab}	33.72 ± 3.13^{b}	36.96 ± 6.10^{ab}	41.55 ± 4.01^{a}

Table2. Mineral composition (g/L) of camel milk subjected to different heat treatment

	Fresh milk	63°C	90°C	100°C
Ca	1.41 ± 0.02	1.33 ± 0.08	1.28 ± 0.24	1.27 ± 0.08
K	1.69 ± 0.02	1.33 ± 0.12	1.37 ± 0.21	1.32 ± 0.23
Mg	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.02
Na	0.59 ± 0.08	0.42 ± 0.03	0.45 ± 0.09	0.43 ± 0.07

3.2 Fatty acids composition

The average relative fatty acid compositions of control and heated camel milk are summarized in Table 3.

As illustrated in table 3, the content of short-chain fatty acids (SCFA) in this study was represented by caproic (C6:0) and caprylic (C8:0) acids. These results were partially in agreement with those reported by [20], who showed that camel milk is C4:0-C6:0 free. [21] has reported a smaller amount of these FAs compared with the bovine milk fat. The nature of camel feeding and the fast metabolizing of these FAs by camel tissues before being evacuated in the milk [20] could explain this lower concentration.

Fifty percent of the total fatty acid composition is presented by SFA. Heat treatment didn't show any significant effect on SFA amount while the monounsaturated fatty acids (MUFA) were significantly higher in the unheated (44.07 ± 3.65) milk compared to treated milk (40.46± 4.9 (63°C); 38.78 ± 2.71 (90°C) and 37.46 ± 2.15 (100°C)). In fact, monounsaturated fatty acids are well known for their oxidative stability and healthful properties as the aptitude of reducing level cholesterol [22]. Particularly the oleic acid (C18:1 cis9) as the most abundant FA was significantly affected by heat treatment while it has been reported that the oleic acid plays an important role in human health such as metabolic troubles, cardiovascular or autoimmune diseases, skin injury, and cancer disease [23, 24].

The PUFAs play a crucial role in the growth of the neonatal brain, as well as the retina and cognitive functions [25]. Among PUFAs, linoleic acid (LA) and alpha-linoleic acid (ALA) are respectively the major n-6 PUFA and n-3 PUFA in milk. In fact, the proportion of ALA was found to be higher in camel milk than in human and cow milk. It has been reported that ALA showed a protective effect against coronary heart disease and a positive effect on neurological activity [25].

These findings signaled that the heat treatment could significantly modify the composition of some fatty acids of camel milk. [22] showed that the FA profile was affected by boiling when he studied the effect of heat treatment on cow and buffalo milk. While [23] reported that pasteurization didn't affect the fat content and the fatty acids composition of human milk.

Table 3. Fatty acid composition of fresh and heated camel milk. Data expressed as g/100 g of total FA.

	Fresh milk	Pasteurized milk at 63°C	Pasteurized milk at 90°C	Boiled milk at 100°C
C6:0	0.08 ± 0.04	0.07 ± 0.02	0.09 ± 0.05	0.09 ± 0.03
C8 :0	0.06 ± 0.03	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.01
C10 :0	0.06 ± 0.02	0.062 ± 0.02	0.05 ± 0.02	0.06 ± 0.01
C12 :0	0.55 ± 0.17	0.57 ± 0.19	0.54 ± 0.18	0.53 ± 0.17
C13	0.04 ± 0.002	0.05 ± 0.001	0.05 ± 0.009	0.04 ± 0.01
C14	8.46 ± 2.40	7.92 ± 1.66	8.17 ± 1.19	8.08 ± 1.47
Cis9 C14 :1	0.55 ± 0.36	0.38 ± 0.17	0.37 ± 0.09	0.36 ± 0.12
C15	1.13 ± 0.36	1.183 ± 0.41	1.01 ± 0.51	1.03 ± 0.32
Cis 10 C15 :1	0.34 ± 0.04	0.31 ± 0.17	0.40 ± 0.11	0.43 ± 0.004
C16	27.21 ± 5.03	26.02 ± 2.28	26.39 ± 2.33	27.01 ± 2.09
Cis9 C16 :1	7.06 ± 2.65	5.78 ± 1.48	5.52 ± 1.38	5.64 ± 1.29
C17	0.74 ± 0.15	0.75 ± 0.25	0.72 ± 0.28	0.71 ± 0.26
Cis 10 C17 :1	0.49 ± 0.18	0.44 ± 0.21	0.34 ± 0.27	0.38 ± 0.24
C18	18.59 ± 1.65	17.53 ± 2.99	17.64 ± 2.76	17.88 ± 2.07
trans9 C18 :1	5.23 ± 1.29	4.45 ± 1.24	4.54 ± 1.35	4.32 ± 1.69
Cis 9 C18 :1	**32.93 ± 2.93^{a}**	**31.89 ± 3.38^{ab}**	**28.62 ± 2.89^{ab}**	**27.55 ± 1.59^{b}**
9t 12t C18 :2	0.33 ± 0.17	0.31 ± 0.14	0.31 ± 0.14	0.19 ± 0.09
9c 12c C18 : 2	2.57 ± 0.74	2.65 ± 0.79	2.51 ± 0.59	2.52 ± 0.46
9c 12c 15c C18 : 3	1.09 ± 0.37	1.06 ± 0.41	1.05 ± 0.49	0.84 ± 0.49
C20 :0	0.44 ± 0.11	0.51 ± 0.13	0.478 ± 0.08	0.48 ± 0.09
11 cis C20 :1	0.18 ± 0.05	-	-	0.25 ± 0.07
11c 14c C20 :2	-	0.09 ± 0.02	-	-
8c 11c 14c C20 : 3	0.17 ± 0.001	0.07 ± 0.002	-	0.17 ± 0.002
5c 8c 11c14c C20 :4	0.29 ± 0.08	0.28 ± 0.13	0.24 ± 0.12	0.25 ± 0.09
5c. 8c. 11c. 14c. 17c C20 :5	0.21 ± 0.002	0.18 ± 0.001	-	-
C21 :0	0.09 ± 0.03	0.11 ± 0.05	0.08 ± 0.02	0.09 ± 0.03
C22 :0	-	0.1 ± 0.054	0.11 ± 0.06	0.10 ± 0.04
13 Cis C22 : 1	0.10 ± 0.001	0.23 ± 0.15	0.15 ± 0.001	0.09 ± 0.06
SFA	56,25 ± 5,43	54,69 ± 4,12	55,78 ± 4,87	55,25 ± 2,54
MUFA	**44.07 ± 3.65^{a}**	**40.46± 4.9^{ab}**	**38.78 ± 2.71^{ab}**	**37.46 ± 2.15^{b}**
PUFA	4.13 ± 1.35	4.34 ± 1.23	2.94 ± 1.03	3.72 ± 0.93
SCFA	0.10 ± 0.06	0.12 ± 0.04	0.10 ± 0.02	0.14 ± 0.04
MCFA	10.68 ± 3.15	10.23 ± 2.26	10.34 ± 1.82	9.89 ± 1.50
LCFA	85.19 ± 9.12	83.83 ± 9.98	83.29 ± 7.74	84.12 ± 7.52
Total n-6	3.05 ± 0.94	2.89 ± 0.66	3.01 ± 0.58	3.05 ± 0.38
Total n-3	1.33 ± 0.18	1.33 ± 0.16	1.19 ± 0.28	0.98 ± 0.48
n-6/n-3	2.20 ± 0.66	2.19 ± 0.87	2.59 ± 0.53	2.7 ± 0.092

* SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids, PUFA, poly-unsaturated fatty acids; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; n–6/n–3, omega6/omega3.

There is no significant difference between omega 3 and omega 6 and the n-6/ n-3 ratio between raw, pasteurized, and boiled milk. In this context, [24] reported that the pasteurization and Ultra High Temperature (UHT) didn't involve the n-6/ n-3 ratio of cow milk fatty acids. Although, the higher ratio of n-3 was in unheated and low pasteurized camel milk which is well known for greater health benefits [26]. This was confirmed by the n-6/ n-3 ratio which is the lowest compared to the two other heat treatments.

Despite the high levels of saturated fatty acids, camel milk is still a great source of essential fatty acids. In fact, the heat treatment didn't affect significantly the most analyzed fatty acids which are in accord with [24], however it must be careful with the processing since it was found a decrease in the essential fatty acids after the heat treatment.

3.3 Anti-oxidant activities

The DPPH, ABTS radical-scavenging activities and ferric reducing power of raw and heated camel milk are shown in Figure 1.

Figure 1 showed that camel milk can donate hydrogen or electrons able to transform the free radicals into more stable products. The heat treatment of milk didn't affect significantly the antioxidant activity of milk. However, the different assays (DPPH and ABTS) showed the decrease of the radical scavenging activities compared to fresh milk, which is in accord with [**27**] who found that the pasteurization at 63°C for 30min could affect significantly the DPPH and ABTS radical scavenging activities of camel milk.

The difference in the percentages of radical scavenging activities for ABTS and DPPH of raw milk (52.64% vs 14.74%) could be due to the solubility and diffusivity of radicals. DPPH is soluble only in alcoholic solution while ABTS is soluble in both aqueous solution and hydrophobic organic solution [28].

Figure 1c showed that the heat treatment didn't have a significant effect on the reducing power assay. However, after pasteurization and boiling, the ferric reducing power decrease in comparison with raw milk which is in agreement with [22] who reported that pasteurization and boiling decrease the reducing power of cow and buffalo milk.

The antioxidant activities slightly decreased after the heat process but no significant changes were observed. [29] have reported that only a severe heat treatment (more than 2 h at 90°C or 15 min at 120°C) could decrease the antioxidant properties of milk.

Indeed, the antioxidant activity of milk may be due to the contribution of its compounds, such as vitamin C, conjugated linoleic acid, casein, lysozyme, and lactoferrin [27, 29].

Moreover, a study of [30] reported that camel milk has shown heat treatment stability more than cow milk which is an advantage for the commercial production and processing of dairy products.

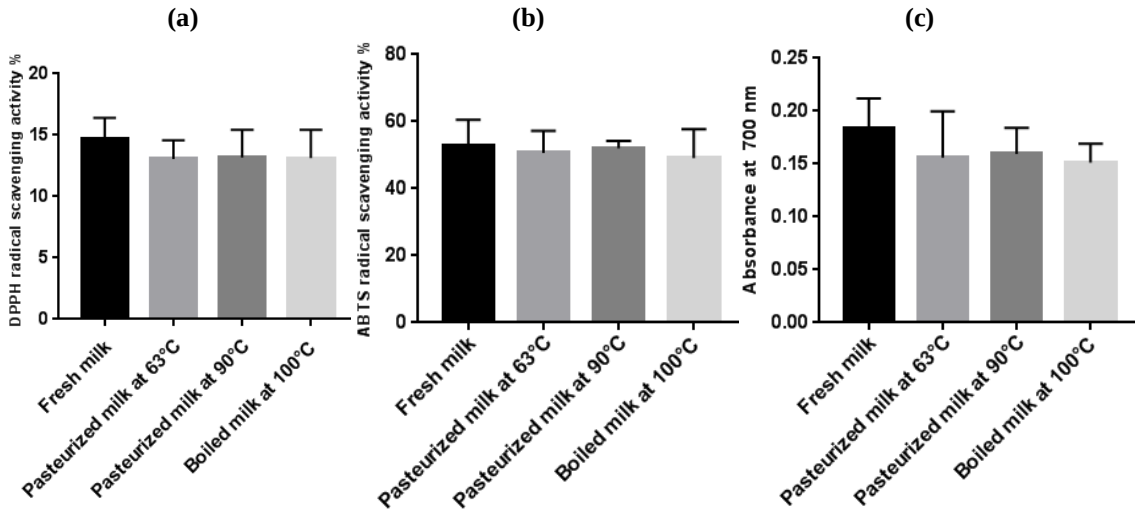

Figure 1. Antioxidant activity of raw and heat treated camel milk samples (a) DPPH radical-scavenging activity, (b) ABTS radical scavenging activity and (c) ferric reducing power assay

3.4 Anti-inflammatory activity

The anti-inflammatory activities of raw and heat-treated camel milk are presented in figure 2.

The result of the 5-lipoxygenase inhibition showed a significant effect of heat treatment on the anti-inflammatory activity.

In comparison to the control group, boiled camel milk showed a significant decrease ($p<0.05$) in the percentage of inhibition (30.69±2.04 vs 21.74±2.76). The significant decrease may possibly due to the degradation of bioactive micronutrients (fatty acids, peptides…) responsible for the anti-inflammatory activity. Therefore, an alternative treatments such as low-heat or non-thermal treatments could be considered to preserve the dietary and therapeutic values of camel milk.

In Vivo Study of [31] in rats confirmed the use of camel milk as an adjuvant cure for treating different chronic pain and inflammatory diseases.

Indeed, proteolytic digestion of beta-casein could be precursors of an important number of β-casomorphins defined as a group of peptides with opioid properties [32]. These opioid peptides have been evaluated in different animal and human models which proves an efficacy effect against pain [33].

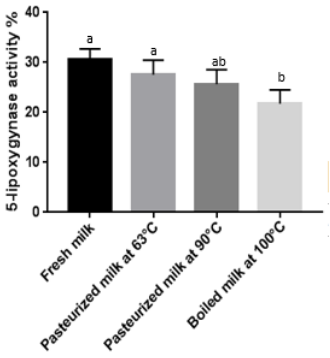

Figure 2. Anti-inflammatory activity of raw and heat-treated camel milk samples

4. Conclusion

Over the years, heat treatment has become an essential step in the dairy industry. The instability of camel components will have implications on the characteristics and nutritional value of dairy products. In fact, pasteurization can be used as a safe method for effective preservation of the quality of camel milk and extend its shelf life.

Therefore, these results are expected to be of interest not only for milk-product industrials processing but also for food formulation containing camel milk as an ingredient. More in vivo and in vitro studies are required to understand how heat process of milk affects their benefits for human health to enhance the protection and the usefulness of these components in dairy industries.

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