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DETERMINATION OF LACTULOSE AND FUROSINE CONTENTS DURING HEAT TREATMENT OF CAMEL MILK: AS A NEW METHOD FOR DISCRIMINATING THE DEGREE OF HEAT TREATMENT GIVEN TO CAMEL MILK

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ABSTRACT

The present study aimed to evaluate the thermal indicator markers (furosine and lactulose) as new suitable indicator markers in heat-treated camel milk under different thermal and storage conditions. The concentrations of lactulose and furosine were determined in raw camel milk and after pasteurized Low Temperature Long Time (LTLT), High Temperature Short Time (HTST) and boiling at various holding times and storage at 4 ºC or 30 ºC for 1, 7 and 14 days. The results showed that, lactulose content was not detected in raw camel milk. In all samples, lactulose and furosine contents were gradually increased with rising heat temperature. Lactulose content in camel milk, treated with LTLT, HTST and boiling ranged from 2.93 to 3.40, 3.7 to 4.8 and 12.1 to 14.75 mg/100 ml milk, respectively. Further, the average content of furosine in treated camel milk with LTLT, HTST and boiling ranged from 7.55 to 8.95, 11.23 to 13.85 and 28.85 to 34.90 mg/100 g protein, respectively. During storage, the lactulose and furosine contents of camel milk samples gradually increased up to 14 days of storage. Furthermore, the results showed that, there were significant differences ($P<0.05$) in lactulose and furosine content between boiling of camel milk samples and both of milk samples treated with LTLT and HTST. Also, there were significant differences ($P<0.05$) between heat process temperature and holding time within the same heat treatment in all camel milk samples. Lactulose and furosine concentrations of LTLT, HTST and boiling milk samples were noticeably increased during storage at 30ºC, compared to the storage at lower temperature (4ºC). Furosine formation was more much higher than lactulose content in all camel milk samples. The present study confirmed that, lactulose and furosine contents could be successfully used as suitable indicators to assess the heat load of camel milk.

Key words: Camel milk, heat indicators, lactulose, furosine.

INTRODUCTION

The camel (*Camelus dromedarius*) is of significant socio-economic importance in many arid and semi-arid parts of the world. Camel milk has an important role in human nutrition and in treatment of many serious diseases at different parts of the world, because it is rich in numerous bioactive substances (El-Agamy *et al.*, 2009). This milk has a very high concentration of mono-and polyunsaturated fatty acids , serum albumin , lactoferrin , immunoglobulin, vitamins (C and E), lysozyme, manganese and iron, as well as the hormone insulin. Therefore, camel milk can be used to treat diabetes, food allergies, Autism, psoriasis, gastrointestinal disorders, high cholesterol in the blood, strengthen the immune system and tuberculosis (El-Hatmi *et al.*, 2007; Al Haj and Al Kanhal, 2010; Kaskous, 2016). Recent studies showed that camel milk had anti-hepatic B and C, and anti-cancer properties (El-Fakharany *et al.*, 2012; Habib *et al.*, 2013).

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Camels were kept by nomadic pastoralists under traditional management. In most pastoral areas, camel milk is produced in the traditional way by hand milking, handled and transported for long distance where it is distributed to retailers under low hygienic conditions. Besides, the dust and flies at the milking site, especially if milk containers were left open and the uses of unclean water for milking process are responsible for contamination of milk, which caus food-borne diseases (Almaw and Molla 2000; Kenyanjui *et al.*, 2003; Younan, 2004; El-Ziney-Al-Turki, 2007).

Moreover, the natural antimicrobial factors in camel milk can only provide a limited protection against specific pathogens and for a short period. Such risk is higher when the milk is consumed in its raw state as is commonly practiced by the local producers (Benkerroum *et al.,* 2003; Karimuribo *et al.,* 2005; El-Zubeir and Nour, 2006).

The main reason for heat treatment of milk is to improve the keeping quality and safety of milk and products. Efficiency and effects of heat treatments are related to the temperature-time combinations, heating method utilized and milk pre-treatment conditions (Sakkas *et al.,* 2014). The heating techniques used would result in different degrees of damage nutritional properties. Heating process of milk causes significant differences in the physico-chemical state of its components, leading primarily to denaturation of certain protein fractions and the formation of Maillard reaction (Pizzoferrato *et al.*, 1998; Aragon *et al.*, 2002).

Several heat-induced changes related to these modifications have been developed in recent years to determine the quality of milk. These so-called heat-damage markers indicators can be used to control and check the heat treatments given to milk. However, two types of chemical reactions may be used to assess heat treatments: type I-indicators include denaturation, degradation and inactivation processes of heat labile components. These indicators include milk enzymes (*e.g.,* alkaline phosphatase or lactoperoxidase), water and fat soluble vitamins (*e.g.,* cobalamin, pyridoxine, ascorbic acid) as well as whey proteins (*e.g.,* α-lactalbumin and β-lactoglobulin) (Walstra *et al.,* 2006). In contrast, type II-indicators describe the formation of substances that are (almost) not present in milk. Typical indicators are Maillard reaction products, such as lactulose, 5-hydroxymethylfurfural and furosine (Aragon *et al.,* 2002; Elliott *et al.,* 2003; Mayer *et al.,* 2010; Sakkas *et al.*, 2014).

With increasing interest in camel milk and its exportation to the western countries, reliable tests are needed to evaluate whether heat treatments, especially pasteurization, are performed effectively or not. Studies were carried out on the indigenous enzyme activity type of bovine, sheep and goat milk (Lorenzen *et al.,* 2010), but camel milk is different and suitable markers for proper pasteurization are not necessarily the same as in other milk producing species (Merin *et al.*, 2005). Lorenzen *et al.* (2011) investigated the activity of six different indigenous enzymes of camel milk regarding their usefulness as heat treatment indicators. They observed that alkaline phosphatase (ALP), Gamma-glutamyltransferase (GGT), Leucine arylamidase (LAP) and lactoperoxidase (LPO) are not suitable as a marker because residual activities were detected in pasteurized milk.

Lactulose does not occur naturally in raw milk; however, during heat treatment of milk, the presence of carbohydrates and salts can affect the isomerization progress of lactose to lactulose. Therefore, it can be used as an indicator of the severity of heat treatment of milk and to distinguish among raw, pasteurized, ultra-heat treated (UHT) and sterilized milk Mayer *et al.* (1996).

Furosine (e-N-2-furoylmethyl-L-lysine), an amino acid formed during acid hydrolysis of the main stable Amadori compound (e-deoxy-fructosyl-lysine), can be used to measure the initial steps of the Maillard reaction (Ferrer *et al.,* 2000; Tokus *et al.,* 2006). It is considered the most specific and the earliest indicator of the Maillard reaction related to the type and intensity of the food-processing conditions, as well as the storage conditions. During heated milk storage, its concentration increases depending on storage temperature, due to the continuous formation of lactulose-lysine through Maillard reaction. Therefore, furosine is a

suitable indicator to the severity of heat treatment and storage conditions of dairy products (Hernández *et al.*, 2002; Elliott *et al.*, 2005; Feinberg *et al.*, 2006).

No data are present in the literature on the content of thermal indicators markers (lactulose and furosine) in camel milk. Therefore, this study was aimed to evaluate the lactulose and furosine as new suitable indicator markers in raw and heat-treated camel milk under different thermal and storage conditions.

MATERIALS AND METHODS

Milk Samples

Fresh whole raw milk from healthy camel was obtained from a local farm in Al-Nigella areas, Matrouh Governorate, North West Coast, Egypt. Samples were collected from twelve female one-humped camels (*Camelus dromedaries*) after 15 postpartum and immediately cooled and refrigerated at 4ºC for 12 hr., until processed in the laboratory.

Concentration values of protein, fat, lactose and total solids in the raw camel milk that determined using a Lactoscan milk analyzer, (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) calibrated for camel milk were 3.25%, 3.2%, 4.5% and 12.15%, respectively. All analytical determinations were carried out in triplicate.

Heat Treatments of Milk

Three batches of camel milk (16 liter/ batch) were used in this study. Each batch of camel milk was divided into four portions. The first portion was kept as a control (raw milk), the second one was placed in a thermostatically controlled water bath at 65ºC for 30, 45 and 60 min (Low Temperature Long Time, LTLT) while the third portion heated at 72ºC for 15 s, 2 and 10 min (High Temperature Short Time, HTST) and the last portion boiling in the oil bath for 2, 5 and 10 min. All heated treatments were cooled immediately in ice bath and then stored at 4ºC (optimal storage temperature) and at 30ºC (harsh storage condition) and analyzed after 1, 7 and 14 days. All measurements were performed in duplicate.

Determination of Lactulose

Lactulose concentrations were determined enzymatically by the method described by Amine *et al.* (2000) and the reference methods of the spectrophotometric enzymatic test kit for 100 determinations 'Lactulose in Milk', (Sigma-Aldrich Co. LLC, US Catalog Number MAK182).

Reagents and Apparatus

The test combination contains the following:

a) Clarification Carrez I solution: 15 g of potassium hexacyanoferrate (II) trihydrate [$K_4Fe(CN)_6 . 3H_2O$] were dissolved in 100 ml of distilled water.

b) Clarification Carrez II solution: 30 g of zinc sulphate heptahydrate $ZnSO_4 . 7H_2O$ were dissolved in 100 ml of distilled water.

c) Buffer solution A: pH 7.5: 24 g of Na_2HPO_4, 4.3 g of $NaH_2PO_4.H_2O$ and 0.5 g of $MgSO_4 .7H_2O$ to 450 ml of distilled water. Adjusted to pH 7.6 with 1 M NaOH (40 g/l) and make the volume up to 500 ml with distilled water.

d) Buffer solution B: pH 7.6 : 14g of triethanolamine hydrochloride, $N(CH_2CH_2OH)_3$ HCl are mixed with 0.25 g of magnesium sulphate heptahydrate, ($MgSO_4.7H_2O$) in 100 ml of distilled water.

e) Buffer solution C: mix 40 ml of buffer solution B with 100 ml of distilled water.

Procedures

Sample preparation

Milk samples preparations were performed according to Pereda *et al.* (2009). For defatting, camel milk samples were centrifuged twice (1^{st}: 10 min, 5,000 g; 2^{nd} : 3 min, 4,000 g) and skimmed milk was kept frozen until further analysis. Ten milliliters of camel milk samples were pipetted into 50 ml conical flask and 3.5 ml of both clarification Carrez I and II solution were added; the resulting solution was stirred for 2–3 min, 13 ml buffer solution A, pH 7.5 were successively added. The solution was then well mixed for 2–3 min, left to rest for 30 min.,

filtered through a No. 1 Whatman paper (Whatman, Maidstone, UK).

Hydrolysis of camel milk lactulose

Five milliliters of the sample filtrate plus 50 ml of β-galactosidase (Sigma Aldrich, St Louis, USA) and 0.3 ml of citric/phosphate buffer were added in succession to each glass-stoppered test tube (15.0×1.0 cm). The test tubes were placed in a water bath at 40 ºC for 60 min then cooled in tap water. A blank was prepared for each camel milk sample, without adding the β-galactosidase.

Oxidation of glucose

The following step in the same volumetric flask 2 ml of buffer solution C, 0.1 ml of oxidation solution (20 mg glucose oxidase (Sigma) in 1 ml of water), 0.1 ml drop of Octanol, 5 ml of Na OH 0.33 M, 50 ml of H_2O_2 30% and 0.1 ml of catalase (Sigma) were successively added and then a second incubation at 40ºC for 15 min was necessary for further enzymatic reaction. Then after the volume was completed until 10 ml and was filtered. Subsequent reactions were accomplished in cuvettes, as described in the enzyme kit manual.

Quantification of lactulose

Lactulose was determined spectrophotometrically by using a scanning double-beam spectrophotometer Jenway 6850 spectrophotometer (Jenway Instruments, Beacon Road, Stone, Staffordshire, ST15 OSA, UK) at a wavelength of 340 nm. According to the Sigma-Aldrich lactulose manual. Lactulose assay was linear over the range of 4.8-480 mg l^{-1} in a milk sample. Analyses were done in duplicate for each camel milk sample and results were expressed as milligrams lactulose per 100 ml of milk.

Determination of Furosine

Furosine content was determined according to the high-performance liquid chromatography (HPLC) method described by Resmini *et al.* (1990), using an HPLC system (Dionex™ UltiMate™ 3000 RS systems –Thermo Scientific system).

Sample Hydrolysis

Two milliliters of camel milk sample were mixed with 6 ml of 10.6 M HCl and exposed to nitrogen for 2 min prior acid hydrolysis in sealed tubes for 23 hr., at 110ºC. After hydrolyzed, it cooled to room temperature, and was filtered through No. 42 Whatman paper (Whatman, Maidstone, UK). The hydrolyzed sample was evaporated and the dried sample was dissolved in 0.5 l water. This solution was passed through a pre-wetted Sep-pak C18 cartridge (Millipore) and washed with 5 ml water: acetonitrile: formic acid (95:5:0.2) before HPLC analysis.

Chromatographic Conditions

The analyses were performed in a C8 column (250×4.6 mm Alltech; Furosine-dedicated) with a linear binary gradient. The mobile phase was 5mM sodium heptane sulphonate with 20% acetonitrile and 0.2% formic acid at a flow rate of 1.2 ml/min. The UV detector was set at 280 nm. A calibration curve was obtained by plotting the peak areas versus the micrograms per milliliter of furosine dihydrochloride was supplied by Neosystem S.A. (Strasbourg, France) injected.

Statistical Analysis

Statistical analysis of experimental data was carried out using SPSS Statistics package (SPSS v.18, Chicago, II 60611, USA, 2012) for Windows. One-way analysis of variance (ANOVA) was applied to investigate differences between means of more than two groups. Differences were considered significant at ($P<0.05$).

RESULTS AND DISCUSSION

Table 1 shows the mean values and standard deviations (SD) of lactulose formation in the analyzed camel milk samples, classified according to heat treatment (pasteurized LTLT, HTST and boiling) and storage conditions at 4ºC or 30ºC for 1 , 7 or 14 days. The data obtained confirmed that, lactulose content was not detected in raw camel milk. There are several reports about the estimation of the lactulose content of raw milks. It was reported that, lactulose is formed in heated milks by degradation of lactose during isomerization reactions. The amount of lactulose in raw milk can be considered to be zero or absent, therefore

it can be considered a useful indicator to evaluate the thermal treatment applied to milk (Morales *et al.,* 2000; Adriana *et al.,* 2003; Claeys *et al.,* 2003).

Table 1. Changes of lactulose (mg/100 ml milk) content in camel milk samples under different combination of heat temperature and time during storage at 4ºC or 30ºC for 14 days

Heat treatment	Temperature/ time	Storage periods / storage temperature						Total main effects
		1 day		7 days		14 days		
		4 ºC	30 ºC	4 ºC	30 ºC	4 ºC	30 ºC	
Raw camel milk		**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	
Pasteurization LTLT	**65ºC/30 min**	2.93^{D}b±0.32	3.11^{E}b±0.13	3.20^{D}b±0.14	4.07^{C}a±0.07	3.30^{E}b±0.01	4.35^{E}a±0.07	3.49e±0.56
	65ºC/45 min	3.15^{D}b±0.35	3.30^{E}b±0.14	3.11^{D}b±0.13	4.35^{C}a±0.07	3.45^{E}b±0.07	4.68^{E}a0.04	3.67e±0.65
	65ºC/60 min	3.40^{D}c±0.14	3.40^{E}c±0.14	3.57^{D}c±0.06	4.45^{C}b±0.07	3.64^{E}c±0.03	4.75^{E}a±0.07	3.87e±0.56
Pasteurization HTST	**72ºC/15 s.**	3.70^{CD}b±0.28	3.70^{E}b±0.42	3.83^{CD}ab±0.02	4.05^{C}ab±0.21	3.89^{E}ab±0.01	4.40^{E}a±0.14	3.93e±0.31
	72ºC/2 min	3.80^{CD}c±0.14	3.90^{E}c±0.42	3.95^{CD}c±0.01	4.45^{C}ab±0.07	4.06^{E}bc±0.06	4.80^{E}a±0.14	4.16e±0.40
	72ºC/10 min	4.80^{C}c±0.42	4.85^{D}c±0.07	4.93^{C}bc±0.11	5.35^{C}ab±0.07	5.10^{D}bc±0.06	5.75^{D}a±0.07	5.13d±0.37
Boiling	**100ºC/2 min**	12.10^{B}b±0.71	12.80^{C}b±0.57	12.80^{B}b±0.99	15.20^{B}a±0.14	13.05^{C}b±0.49	16.35^{C}a±0.07	13.72c±1.65
	100ºC/5 min	13.80^{A}b±0.14	13.91^{B}b±0.15	13.90^{B}b±0.99	16.50^{A}a±1.27	14.30^{B}b±0.71	17.85^{B}a±0.78	15.04b±1.74
	100ºC/10 min	14.60^{A}b±0.99	14.75^{A}b±0.49	15.20^{A}b±0.57	17.15^{A}a±1.06	15.35^{A}b±0.64	18.70^{A}a±0.28	15.96a±1.64
		Total mean effects						
LTLT		3.16^{B}c±0.31	3.27^{C}cd±0.17	3.29^{B}cd±0.24	4.29^{B}b±0.18	3.46^{B}c±0.16	4.59^{B}a±0.20	
HTST		4.10^{B}b±0.59	4.15^{B}b±0.61	4.23^{B}ab±0.54	4.62^{B}ab±0.60	4.35^{B}ab±0.59	4.98^{B}a±0.63	
Boiling		13.50^{A}b±1.27	13.82^{A}b±0.94	13.97^{A}b±1.27	16.28^{A}a±1.16	14.23^{A}b±1.14	17.63^{A}a±1.13	

Mean (±SE). ^{a,b,c} Values in the same row having different superscripts differ significantly ($P < 0.05$).

Means (±SE). ^{A,B,C} in the same column with different superscripts letters are significantly differ ($P<0.05$).

ND = Not detected LTLT = Low Temperature Long Time HTST = High Temperature Short Time

On the other hand, after heat treatment, the lactulose content of treated camel milk increased gradually with rising heating temperature. From 65 to 72ºC, lactulose content increased slowly while increasing the temperature to boiling resulted in marked changes in lactulose of camel milk.

As the heat treatment changed, lactulose in camel milk heated at 65or 72ºC and boiling ranged from 2.93 to 3.40, from 3.7 to 4.8 and 12.1 to 14.75 mg/100 ml milk, respectively. These results were higher than those reported by De Rafael *et al.* (1996), Pellegrino *et al.* (1996) and Villamiel *et al.* (1999) who found that lactulose concentration ranged from 0 mg/l to 5.8 mg/l in commercial milks labeled as pasteurized whereas Feinberg *et al.* (2006) reported 1.5 ± 1.2 mg/100 ml lactulose for milk heated at 74 ºC for 30 s. Also, Olano *et al.* (1989) found levels of 0.52 mg/ 100 ml of lactulose in milk treated at 63ºC for 30 min. The variation in lactulose content of camel milk can be related to the low content of protein, especially casein and higher α-Lactalbumin (α-La) where the formation of lactulose is highly dependent on protein concentration.

The major whey soluble protein in camel milk mainly consisted of α-Lactalbumin (α-La) with an average concentration of ca. 7.2 g/l, which is five-fold higher than in cow's milk (Conti *et al.*, 1985; Beg *et al.*, 1986; Farah, 1986 and Kappeler *et al.*, 1998). Also, camel milk had a lower content of protein, especially casein (Ramet, 2001; Mehaia, 2006; El-Zubeir and Jabreel, 2008).

Typically, lactulose occurs in heated milk as free lactulose or as ε-N deoxylactulosyl-L-lysine covalently bound to milk protein. Martinez-Castro *et al.* (1986) and Andrews and Prasad (1987) proposed that the formation of lactulose in heated milk proceeds exclusively by the α-Lactalbumin transformation, catalyzed by the milk salt system.

Greig and Payne (1985) proposed that the decrease of lactulose formed with the increase of protein concentration are due to increased formation of lactosyl-amino compounds and concluded that casein, or a component of casein, inhibits the formation of lactulose. Similar results were found by Andrews and Prasad, (1987) who showed that an increasing amount of protein resulted in a decrease in lactulose concentration. From these results it can be concluded that, a small amount of protein in camel milk increased the lactulose level. The more intense the thermal processing of the camel milk (pasteurized LTLT < HTST < boiling), the higher lactulose concentrations.

Moreover, the values of lactulose content gradually increased during storage period in all tested samples. The maximum concentration of lactulose was found in LTLT, HTST and boiled milk at the end of 14 days of storage at 30 ºC it was 4.75, 5.75 and 18.7 mg/100 ml milk, respectively.

On the other hand, LTLT, HTST and boiled treated samples showed different attenuated during conditions storage temperatures (4 and 30ºC). At higher storage temperatures (30ºC) the lactulose concentrations in all camel milk samples steadily increased through storage periods, while at 4ºC remained constant. Average increases in concentrations of lactulose are highly dependent on the storage temperature that is the concentrations of lactulose in all samples noticeably increased only at 30ºC. Andrews (1989), Berg and van Boekel (1994) and Claeys *et al.* (2002) affirmed that lactose isomerization depends on the initial concentration of lactose and storage temperature.

Furthermore, the results also show that, the values of lactulose in boiled camel milk were significantly ($P<0.05$) higher than that LTLT and HTST camel milk treated samples.

There were a significant differences ($P<0.05$) in lactulose content between boiled camel milk and both of milk treated with LTLT and HTST during storage. Also, there were a significant differences ($P< 0.05$) between heating temperatures and time within the same storage temperature. Several studies have also reported that the amount of lactulose can be related to the heating conditions mainly (time and temperature) and storage temperatures of the process and product moisture (Resmini and Pellegrino, 1994; Montilla and Olano, 1997 ; Kulmyrzaev and Dufour, 2002).

Furosine contents (expressed as mg/100 g protein) in raw and heat treated camel milk (pasteurized LTLT, HTST and boiled during storage at 4ºC or 30ºC up to14 days are reported in Table 2.

Table 2. Changes of furosine (mg/100 g protein) content in camel milk samples under different combination of heat temperature and time during storage at (4ºC or 30ºC) for 14 days

Heat treatment	Temperature/ time	Storage periods / storage temperature						Total main effects
		1 day		7 days		14 days		
		4 ºC	30 ºC	4 ºC	30 ºC	4 ºC	30 ºC	
Raw camel milk		3.70^{I}a±0.28	3.75^{H}a±0.07	3.75^{H}a±0.21	4.05^{F}a±0.21	3.75^{G}a±0.07	4.10^{H}a±0.01	3.85h±0.21
Pasteurization LTLT	**65ºC/30 min**	7.55^{H}c±0.21	7.75^{G}bc±0.21	7.90^{G}bc±0.01	8.05^{EF}ab±0.21	8.15^{F}ab±0.07	8.35^{G}a±0.07	7.96g±0.30
	65ºC/45 min	8.35^{FG}b±0.21	8.30^{G}b±0.14	8.45^{FG}b±0.21	8.65^{EF}ab±0.07	8.70^{F}ab±0.01	9.05^{FG}a±0.21	8.58fg±0.29
	65ºC/60 min	8.95^{F}b±0.64	9.15^{F}ab±0.35	9.10^{F}ab±0.28	9.40^{DE}ab±0.14	9.25^{F}ab±0.07	9.85^{E}a±0.07	9.28f±0.39
Pasteurization HTST	**72ºC/15 s.**	11.23^{E}b±0.25	11.35^{E}b±0.21	11.40^{E}b±0.28	12.2^{CD}a±6.62	11.65^{E}b±0.07	12.70^{E}a±0.14	11.76e±0.58
	72ºC/2 min	13.20^{D}b±0.99	13.30^{D}ab±0.14	13.55^{D}ab±0.07	13.85^{C}ab±0.49	13.80^{D}ab±0.01	14.50^{D}a±0.01	13.70d±0.56
	72ºC/10 min	13.85^{D}a±0.49	13.85^{D}a±0.49	14.05^{D}a±0.21	14.55^{C}a±0.07	14.15^{D}a±1.06	14.75^{D}a±0.21	14.20d±0.53
Boiling	**100ºC/2 min**	28.85^{C}d±0.35	28.95^{C}d±0.78	29.80^{C}cd±0.14	31.40^{B}b±0.28	30.85^{C}bc±0.92	33.20^{C}a±0.42	30.51c±1.64
	100ºC/5 min	32.60^{B}c±0.71	33.45^{B}bc±0.07	33.05^{B}bc±0.21	34.35^{AB}b±0.21	33.85^{B}bc±0.07	36.65^{B}a±1.20	33.99b±1.44
	100ºC/10 min	34.90^{A}c±0.42	35.15^{A}c±0.07	35.50^{A}c±0.99	37.96^{A}ab±0.08	36.40^{A}bc±1.41	39.40^{A}a±0.42	36.55a±1.79
		Total mean effects						
Raw camel milk		3.70^{D}a±0.28	3.75^{D}a±0.07	3.75^{D}a±0.21	4.05^{D}a±0.21	3.75^{D}a±0.07	4.10^{D}a±0.01	
LTLT		8.28^{C}a±0.70	8.40^{C}a±0.66	8.48^{C}a±0.56	8.70^{C}a±0.62	8.70^{C}a±0.49	9.08^{C}a±0.68	
HTST		12.76^{B}a±1.32	12.83^{B}a±1.20	13.00^{B}a±1.27	13.54^{B}a±3.26	13.20^{B}a±1.30	13.98^{B}a±1.01	
Boiling		32.12^{A}b±2.76	32.52^{A}b±2.89	32.78^{A}b±2.60	34.57^{A}ab±2.94	33.70^{A}ab±2.60	36.42^{A}a±2.84	

Mean (±SE). ^{a,b,c} Values in the same row having different superscripts differ significantly ($P < 0.05$).

Means (±SE). ^{A,B,C} in the same column with different superscript letters are significantly differ ($P<0.05$).

LTLT = Low temperature long time HTST = High temperature short time

As can be seen in Table 2, quantifiable amounts of furosine were detected in raw camel milk (3.70 mg/100 g protein). Similar results were reported by Van Renterghem and De Block (1996) and Resmini *et al.* (2003). This amount of furosine in raw milk probably due to sugar-protein interaction during milk synthesis (Resmini *et al.,* 1990; Pereda *et al.,* 2009). Furthermore, the low quantity of furosine in raw camel milk is related to the first stages of the Maillard reaction. It is the stable product produced by the acid hydrolysis of unstable lactulose-lysine, which is accumulated in heat-treated milk.

After heating the furosine content increased gradually with increasing the heat temperature. The average of furosine content in camel milk treated at 65, 72ºC and boiling, ranged between 7.55 to 8.95 mg/100 g protein, 11.23 to 13.85 mg/100 g protein and 28.85 to 34.90 mg/100 g protein, respectively. The obtained data of furosine contents were within the range values for pasteurized market milks reported by Birlouez-Aragon *et al.* (1998) who found furosine content of the category high-pasteurized market milks ranged from 17.0 to 52.9 mg/100 g protein while Villamiel *et al.* (1999) showed 10.1–31.4 mg furosine per 100 g protein. Moreover, Mayer *et al.* (2010), Lorenzen *et al.* (2011) and Sakkas *et al.* (2014), reported that in milk samples treated with high-pasteurization the furosine concentration was ranged between 3.3–68.8 mg/100 g protein and it was 7 times higher than that in mildly pasteurized milk.

In the present study, the furosine content in camel milk treated at heating temperature 65ºC to 75ºC increased slowly while from 75ºC to boiling increased steeply (Table 2). After boiling, the initial concentration of furosine was almost duplicated being dramatically increased thereafter.

The formation of furosine is highly dependent on protein concentration (positively correlated), and is therefore expressed as mg/100 g protein (Montilla and Olano, 1997; Rattray *et al.,* 1997).

Pellegrino (1994) observed higher furosine formation in skimmed than in whole cow's milk, which was explained by the difference in heat load between whole and skimmed milk. On the other hand, camel milk had a lower content of protein, especially casein (Ramet, 2001; Mehaia, 2006; El-Zubeir and Jabreel, 2008). From these results, it can be concluded that, a small amount of protein in camel milk increased the furosine level.

During storage, the furosine contents of camel milk samples gradually increased during 14 days of storage and thus, the values were also dependent on the storage period (Table 2). The maximum concentration of furosine was found in LTLT, HTST and boiled milk at the end of 14 days storage period at 30ºC being 9.85, 14.75 and 39.40 mg/100 g protein, respectively. Claeys *et al.* (2003), Elliott *et al.* (2005) and Feinberg *et al.* (2006) reported that, during heated milk storage, its concentration increases depending on storage temperature and period, due to the continuous formation of lactulose-lysine through Maillard reaction.

The results showed significant differences ($P<0.05$) between storage temperature and type of milk samples. At higher storage temperature (30ºC), the formation of furosine was faster than at lower temperatures (4ºC). At higher storage temperature (30ºC), the furosine concentrations in LTLT, HTST and boiled camel milk steadily increased through storage periods, while those stored at (4ºC) remained constant. The amount of furosine newly formed was, however independent of the furosine content immediately after heating and negligible at 4ºC (Nangpal and Reuter, 1990; Pellegrino *et al.*, 1995; Rattray *et al.*, 1997).

Comparing the concentration of lactulose and furosine formed during heat treatment, furosine formation was much higher than lactulose in all samples. Several researchers (Resmini and Pellegrino, 1994; Pellegrino *et al.,* 1995; Montilla and Olano, 1997) have also indicated that the heat-treated milks normally show lower values of lactulose than furosine.

Conclusion

The results showed that, in raw camel milk lactulose content was not detected; while quantifiable amounts of furosine (3.70 mg/100g protein) was detectable. After heat treatments, lactulose and furosine contents gradually increased with rising heating temperature. Lactulose and furosine concentration of LTLT,

HTST and boiled milk noticeably increased during storage at 30ºC, compared to lower storage temperature (4ºC). The provided data on pasteurized LTLT, HTST and boiled camel milk indicated a tremendous impact of different heat treatments on the heat load of camel milk. In particular, furosine and lactulose were found to be appropriate as heat load indicators of different processed milk. Moreover, these results may be of importance in the current context of lacking legislation regarding threshold levels for the heat load of camel milk. Proposed upper limits for maximum tolerable heat load of pasteurized LTLT, HTST and boiling camel milk could be used in the near future, to avoid unnecessary over-processing of these valuable dairy products (to meet the consumer demand for minimally processed foods). In this study it was successfully shown that the lactulose and furosine are most suitable new indicators for detection heat treatments given to camel milk. Further studies are needed on Maillard reaction kinetics in camel dairy products to understand its effects on physical and chemical properties and nutritional value of camel milk products.

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تقديرمحتوى اللاكتيولوز والفيوروسين خلال المعاملة الحرارية للبن النوق كطريقة جديدة لتمييز درجة المعاملة الحرارية التي تعرض لها لبن النوق

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الهدف من هذه الدراسة تقدير تركيز كلا من (اللاكتيولوز والفيوروسين) وتقييمها كأدلة مناسبة جديدة لتمييز درجة المعاملة الحرارية التى تعرض لها لبن النوق، خلال الدراسة تم تقديرمحتوى اللاكتيولوز والفيوروسين في عينات لبن النوق الخام وبعد المعاملة الحرارية بثلاث طرق وهى البسترة بطريقة LTLT و HTST والمعاملة بالغليان تحت أوقات حجز مختلفة ثم تقسيم كل عينات المعاملات وتخزينها على درجة حرارة الثلاجة (٤مº) ودرجة حرارة (٣٠مº) وإجراء التقدير على فترات زمنية ١، ٧ و١٤ يوم، أظهرت النتائج أن لبن النوق الخام لا يحتوى على اللاكتيولوز في حين وجد انه يحتوى على كمية من الفيوروسين مقدارها ٣.٧٠ملليجرام /١٠٠جرام بروتين، وأشارت النتائج إلى حدوث ارتفاع تدريجى لمحتوى كل من اللاكتيولوز والفيوروسين في جميع العينات مع ارتفاع درجة حرارة المعاملة وزيادة وقت الحجز لنفس المعاملة، وتراوح متوسط محتوى لبن النوق من اللاكتيولوز والمعامل بالبسترة بطريقة LTLT ، HTST والمعاملة بالغليان ما بين ٢.٩٣-٣.٤٠ ملليجرام/١٠٠ مل لبن ، ٣.٧- ٤.٨ ملليجرام/ ١٠٠ مل لبن و١٢.١٠- ١٤.٧٥ ملليجرام/ ١٠٠ مل لبن على التوالي، من ناحية أخرى تراوح متوسط محتوى لبن النوق من الفيوروسين والمعامل بالبسترة بطريقة LTLT، HTST والمعاملة بالغليان ما بين ٧.٥٥- ٨.٩٥ ملليجرام/ ١٠٠ جرام بروتين ، ١١.٢٣- ١٣.٨٥ ملليجرام /١٠٠ جرام بروتين و ٢٨.٨٥-٣٤.٩٠ ملليجرام/١٠٠ جرام بروتين على التوالي، وأظهرت النتائج وجود فروق معنوية ($P<0.05$) في تركيز كل من اللاكتيولوز والفيوروسين في جميع العينات بين لبن النوق المعامل بالغليان وكلا من اللبن المعامل بطريقة LTLT وHTST خلال ١٤ يوما من التخزين، أيضا كانت هناك فروق معنوية ($P<0.05$) بين درجات المعاملة الحرارية ووقت الحجز داخل نفس درجة المعاملة الحرارية في جميع عينات لبن النوق، وأشارت النتائج إلى زيادة تركيز كل من اللاكتيولوز والفيوروسين لكل المعاملات الحرارية زيادة معنوية ($P<0.05$) بشكل ملحوظ في نهاية التخزين لمدة ١٤ يوم على درجة حرارة (٣٠مº) بينما كانت هناك زيادة محدودة أثناء التخزين على درجة حرارة الثلاجة (٤مº)، وأوضحت هذه الدراسة أنه من خلال تقدير تركيز كل من اللاكتيولوز والفيوروسين في لبن النوق يمكن التمييز بين اللبن المعامل وغير المعامل حراريا ويمكن التفرقة بين درجات المعاملة الحرارية التى تعرض لها لبن النوق كذلك تم التأكيد على أنه يمكن استخدام اللاكتيولوز والفيوروسين بنجاح كمؤشرات مناسبة لتقييم الحمل الحراري التى تعرض لها لبن النوق.

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