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Influence of Heat Treatments on Nitrogen Distribution in cow and Buffalo's Skim Milk

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Abstract

Cow and buffalo's skim milk were heated at 70-90°C instantly in order to study its effect on nitrogen distribution and contrary on whey protein denaturation. The obtained results showed that the cow skim milk had higher values of WPN, WN/TN & WPN/TN and lower values of TN, CN, NPN, C No., NPN/TN & WP denaturation than that of buffalo's skim milk in all treatments, respectively. The raw skim milk had higher values of WN, WPN, WN/TN & WPN/TN and lower values of TN, CN, NPN& C No. than that of heated skim milk in all treatments, respectively. In addition, the clotted cow milk had higher values of RCT and SY and lower value of CF than that of clotted buffalo's skim milk in all treatments. The increase of heat treatment of milk cause an increase of RCT and decrease of CF and SY in all treatments. Moreover, the control samples had higher and lower values of RCT and higher CF and SY than that of heated milk in all treatments. The decreases of pH values from 6.4 to 6.0 cause an increase of CF and then decrease with decreasing the pH up to 5.6 in clotted cow skim milk, while there were decreases of CF in clotted buffalo's skim milk up to pH 5.6 in all treatments.

Keywords: Heat treatments, Nitrogen distribution, cow and buffalo's Skim milk.

Introduction

Protein structure is profoundly affected by environmental factors such as pH, heat treatment, protein concentration, *etc*. The protein conformation is influenced by several processes, such as rupture of non-covalent and covalent interactions that may be occurring simultaneously as the solution is heated and the pH is adjusted (Phillips *et al*., 1994). The buffalo milk was also lower in β-lactoglobulin content and had the largest casein micellar size and fat globule size. Proportion of casein (CN) to whey protein was lower in cow milk and this milk was found higher in β-lactoglobulin and naturally occurring peptides (Islam *et al.,* 2014). The proteins contained in buffalo milk are more resistant to heat denaturation than those of cows' milk. Thus, buffalo's milk has a greater buffering capacity than cows' milk (Ahmad *et al*., 2008). The extensive research has been dedicated to the understanding of the heat induced aggregation of denatured whey proteins in milk or in its fractions as well as in model systems of individual proteins, especially β-lactoglobulin(Euston *et al*., 2007).

The rheological properties of milk gels, both chymosin and acid induced, are affected by the heat treatment applied to milk, and can result in irreversible changes in milk protein structure. Some of the changes involved are whey protein denaturation and aggregation, interactions of whey proteins with casein micelles, reactions between lactose and proteins, changes in casein micelle structure, transfer of soluble calcium and phosphate to colloidal phase, changes in fat globule membranes, and decrease in pH (Parnell-Clunies *et al*., 1988).

High heat treatment and heating time gives most significant changes. It causes whey protein denaturation which is an irreversible process. The mineral balance also changes during heat treatment. Calcium and phosphate becomes more insoluble and binds to the casein micellar structure. This is reversible for temperatures below 100°C, while severe heating can cause hydrolysis of phosphorserine of caseins and calcium phosphate can precipitate out of solution which is irreversible processes (Gaucheron, 2011). The reversible casein/whey protein complex formation in ultra-high temperature (UHT) milk was described by equilibrium between two main conformations of the protein, which is followed by the change of the proton-binding properties as a function of pH. The kinetic measurements supported the intermolecular and cooperative "all-or-none" change of casein micelles caused by the action of protons. The metastability of conformation is predominantly due to the energy barriers, preventing the equilibrium transition of the protonated milk protein system above a critical pH value. The overall equilibrium constant of the process depended on the heating strength and the pH value of milk (Mansour, 2013). The use of thermally processed milk in the production of rennet coagulated cheeses and also some of the potential alternatives available for in collusion of whey proteins in cheese, such as the addition of microparticulated whey proteins to cheese milk was studied (Prashanti and Hill, 2015).

Hence the present study was under taken to determine the influence of heat treatments on nitrogen distribution in cow and buffalo's milk andstudied effect of heat treatments on some rheological properties of coagulated cow and buffalo's skim milk.

Materials and Methods

Materials:

Whole fresh cow and buffalo'smilk: It was obtained from the herd of the Animal Production Department, Faculty of Agriculture, Al-

Azhar University (Branch of Assiut). Milk samples were defatted by centrifugation at 3000 xg for 20 min at 40°C, using Janetzki. T23 centrifuge.

Microbial Rennet: It was used as a powder obtained from DSM (France) with a commercial name (Fromase R 2200). Microbial rennet solution (1N) was prepared by dissolving 3 gm of powder rennet in 100 ml distilled water; it was used at a rate of 1.0 ml/L. milk (0.03 gm/L. of milk).

Methods:

Heat Treated Milk Samples*:* Skim milk samples were divided into four portions for heating at 70, 80 and 90°C for the three heat treatments in the scale range of instantly and one portion non heated milk served as control.

Methods of Analysis*:*

- *pH measurement*: It was determined using a pH meter (model 68 ESD 19713), USA.

- The Total Nitrogen (TN), **Whey nitrogen (WN)**, and **Non protein nitrogen (NPN)** of the milk: were done by micro-Kjeldahl method according to (Devold*et al.* 2011).

- Whey Protein Nitrogen (WPN), **Caseino number (C No.)** and **Denaturation Percentages**: were estimated according to Manji and Kakuda (1987) as follow:

CN = TN - WN

WPN = WN - NPN

Caseino No. = [(TN - WN) / TN] × 100

Denaturation % = {WPN raw milk – WPN heated milk/WPN raw milk} × 100

- Rheological Properties of Curd:

Rennet Clotting Time (RCT): It was measured according to Berridge (1952).

Curd Firmness (CF): The penetration method described by Shalabi (1987).

Curd Syneresis (SY): The volume of whey expelled within 60 min from the curd of microbial rennet, was measured by the method described by Farooq and Haque (1992) as ml/250 ml milk.

The measurements of all previous tests were done in triplicate.

Results and Discussion

Datapresented in Table 1 illustrate the effect of heat treatments on the nitrogen distribution and whey protein denaturation in cow and buffalo's skim milk. The data revealed that the cow skim milk had higher values of WPN, WN/TN & WPN/TN and lower values of TN, CN, NPN, C No., NPN/TN & WP denaturation than that of buffalo's skim milk in all treatments, respectively. The low level of TN, CN & NPN in cow skim milk than that of buffalo's skim milk may be due to the high moisture content in cow skim milk (Harjinder*et al.,* 1996; Ahmad *et al.*, 2008; Ahmad *et al.*, 2013 and Islam *et al.*, 2014).

In addition, the raw skim milk had higher values of WN, WPN, WN/TN & WPN/TN and lower values of TN, CN, NPN& C No. than that of heat treated skim milk in all treatments, respectively owing to the denaturation effect of heat treatments. These results are similar to obtained by Hattem*et al.* (2011). Moreover, the data in the same Table revealedthose the TN, CN, NPN, C No., NPN/TN and WP denaturation were increased with the raising the heat treatment, while the WN, WPN, WN/TN & WPN/TN were reduced in the same treatments respectively. The same observation was reported by Parnell *et al.,*(1988) and Hattem*et al.*, (2011).

These data show thatthe clotted cow skim milk had higher values of RCT and SY than that of clotted buffalo's skim milk in all treatments, while the CF of buffalo's milk was higher than that of cow's one. In addition, the raising of heat treatment cause an increase in RCT in both of cow and buffalo milk, while the CF and SY were decreased in all treatments. Moreover, the control samples (unheated milk) characterized with the lower RCT values and higer CF and SY than that of heated milk in all treatments. These results are in agreement with those reported by Ismail *et al*. (2004). The increasing of RCT of heat treated cow and buffalo's milk may be due to the decrease in the soluble calcium ions due to its precipitation as tricalcium phosphate depending upon the temperature used (Amer*et al*., 1974 and Ismail *et al*., 2004).

Table 1. Effect of heat treatments on nitrogen distribution and whey protein denaturation in cow and buffalo's skim milk.

Properties %	**Raw milk**		**Heated milk**					
			70°C		**80°C**		**90°C**	
	Cow	**Buffalo**	**Cow**	**Buffalo**	**Cow**	**Buffalo**	**Cow**	**Buffalo**
TN	0.517	0.571	0.518	0.571	0.518	0.575	0.523	0.577
WN	**0.094**	**0.098**	**0.081**	**0.081**	**0.070**	**0.068**	**0.048**	**0.048**
CN	0.423	0.473	0.437	0.490	0.448	0.507	0.475	0.529
NPN	0.029	0.035	0.029	0.035	0.030	0.036	0.030	0.037
WPN	0.065	0.063	0.052	0.046	0.040	0.032	0.018	0.011
C No.	81.82	82.84	84.36	85.81	86.48	88.17	90.82	91.68
WN/TN	18.18	17.16	15.64	14.19	13.52	11.83	09.18	8.32
NPN/TN	5.61	6.13	5.59	6.13	5.79	6.26	5.74	6.41
WPN/TN	12.57	11.03	10.04	8.10	7.72	5.60	3.44	1.91
WP denaturation	00.00	00.00	20.00	26.99	38.46	49.21	72.30	82.54

Data presented in Table (2) illustrate the effect of heat treatments on rheological properties of clotted cow and buffalo's skim milk.

Table 2. Effect of heat treatments on rheological properties in clotted cow and buffalo's skim milk.

Properties	Unheated milk (Control)		Heated milk (°C)					
			70		80		90	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
RCT(min)	12	10	16	12	18	15	20	17
CF (gm)	22.3	26.2	20.0	23.1	17.5	20.1	15.1	16.9
SY (ml)	135	120	113	105	85	74	75	64

RCT: Rennet Clotting Time CF: Curd Firmness SY: Curd Syneresis

Data presented in Table (3) illustrate the effect of pH values on rheological properties of clotted cow and buffalo's skim milk heated at 90°Cinstantly. The data observed that the clotted cow milk had a higher values of RCT and SY, while was shows lower CF than that of clotted buffalo's skim milk in all treatments. In addition, it will be observed that the decreasing of the pH value led to lowering the RCT the control samples (unheated milk) had higher RCT value in both of cow's and buffalo's milk as compared with heated milk at low pH values. The CF and SY were also higher in unheated milk than that of heated milk in all treatments. These results are in harmony with those reported by (Najera *et al.*, 2003).

Table 3. Effect of pH changes on rheological properties in clotted cow and buffaloes skim milk at 90°C instantly.

Properties	Unheated milk (Control) (pH 6.8)		Heated milk pH values					
			6.4		6.0		5.6	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
RCT(min)	12.0	10.0	12.5	11.0	11.0	9.0	8.0	7.0
CF (gm)	22.3	26.2	16.2	24.5	20.5	22.1	15.5	15.8
SY (ml)	135	120	86	80	85	74	69	68

Moreover, the decreases of pH values from 6.4 to 6.0 cause an increase of CF and then decrease with decreasing of pH up to 5.6 in clotted cow skim milk, while there were decreases of CF in clotted buffalo's skim milk up to pH 5.6 in all treatments. This may be attributed to the difference of calcium content of cow and buffaloes skim milk.

Conclusion

Our results showed that heat treatment is higher than 65°C starting 70°C had an impact clearly on nitrogen content of milk proteins of cow and buffalo's and a temperature of 90°C resulted in denaturation rate for whey proteins for both of cow and buffalo's. The heat treatments of milk were to avoid the lost of whey proteins in whey and kept them in the curd. Lowering the pH value of heated milk resulted in high activity of enzymatic coagulation of milk and gives good rheological properties of the resultant curd.

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تأثير المعاملات الحرارية على توزيع النيتروجين في اللبن البقرى والجاموسي

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الملخص

تم معاملة اللبن الفرز البقري والجاموسي حراريا على درجات حـرارة ٧٠ ، ٨٠ ، ٩٠°م لحظيا لدراسة تأثير هذه المعاملات علي توزيع النتروجين والتغير في طبيعة بروتينات الشرش ، وقد أظهرت النتائج المتحصل عليها مايلى :–

١– زيادة قيم كلا من نيتروجين بروتينات الشرش ، نسبة نتروجين الـشرش/النتـروجين الكلي ، نتروجين بروتينات الشرش/النتروجين الكلي ونقص كلا من النتروجين الكلي ، نتروجين الكازين ، النتروجين الغير بروتيني ، رقم الكازين ، النتروجين الغير بروتيني/النتروجين الكلـي في عينات اللبن الفرز البقري عنها في عينات اللبن الفرز الجاموسي .

٢– زيادة قيم كلا من نتروجين الـشرش ، نتـروجين بروتينـات الـشرش ، نتـروجين الشرش/النتروجين الكلي ، نتروجين بروتينات الشرش/النتـروجين الكلـي ونقـص كـلا مـن النتروجين الكلي ، نتروجين الكازين ، النتروجين الغير بروتيني ، رقم الكازين في عينات اللـبن الخام عنها في اللبن المعامل حراريا .

٣– زيادة قيم كلا من زمن التجبن ، طرد الشرش ونقص قيمة صلابة الخثرة فـي خثـرة اللبن الفرز البقري عنها في خثرة اللبن الفرز الجاموسي.

٤– بزيادة مدة تسخين اللبن يحدث زيادة في زمن التجبن ونقص فـي كـلا مـن طـرد الشرش، صلابة الخثرة.

٥– زيادة قيم صلابة الخثرة ونقص قيم طرد الشرش في الخثرة الناتجة من لبن خام عنها في الخثرة الناتجة من لبن مسخن.

٦– نقص رقم الحموضة في الخثرة الناتجة من لبن فرز بقري من ٦,٤ إلى ٦ أدى إلـى زيادة قيم صلابة الخثرة ثم تتناقص القيم حتى رقم حموضة ٥,٦ ، بينما كان هناك نقص واضح في قيم صلابة الخثرة الناتجة من لبن فرز جاموسي حتى رقم حموضة ٥,٦ .