EFFECT OF BUFFALO AND COW SKIM MILK FLOURIDIZATION ON 1-SOME PHYSICO-CHEMICAL PROPERTIES

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ABSTRACT

The effect of added sodium fluoride (NaF, 5-20 ppm. F \circ) on some physico-chemical properties of buffalo's and cow's skim milk was investigated. Adding NaF (5-20 p.p.m. F \circ) had no significant effect (P \geq 0.05) on the pH. The level of soluble calcium and the electrical conductivity (EC) were significantly decreased (P \leq 0.05) by adding NaF to buffalo's skim milk, but apposite trend was observed with cow's skim milk. Added NaF increased the ionic fluoride, and this effect increased with increasing the concentration of NaF. The optical properties at 280 and 260 nm of both milks containing 15-20 p.p.m. F \circ) were higher than those of the control samples. Cow's skim milk containing 15-20 p.p.m. F \circ was higher ethanol stability at 85-90% than that of buffalo's samples. Fluoridated buffalo's skim milk (10-20 p.p.m. F \circ) was more stable towards 5 and 7.5 mmol/L Ca²⁺ compared with the control, but the corresponding cow's treatments were not influenced. Also, all fluoridated milk samples were heat stable. The rennet clotting time, firmness and synersis of buffalo's skim milk were higher significantly (P \leq 0.05) with adding 20 p.p.m. F \circ than those of cows.

Key words: buffalo's skim milk, electrical conductivity, firmness, Synersis heat stable, NaF, optical properties, Physico-chemical properties, Rennet clotting time.

1. INTRODUCTION

The only proved function of fluorine as a trace element in human nutrition is to improve the resistance of teeth to decay, apparently by improving the structure of teeth. Because of only important source of fluorine are tea and sea fish, unless the water contains 1 p.p.m. or more, if infants and young children often fail to obtain the benefits of fluorine when their teeth are being formed (Rugg-Gunn, 1993, Villa et al., 1998 and El-Gabry and Darwish, 2003). Therefore, the fluoridation of water in order to decrease incidence of dental caries (American Academy of Pediatrics, 1979, 1986. Al-Khateeb et al., 1991 and Levy et al., 1995).

Milk is the natural food for infants and children. The use of milk for fluoridation of children diet would ensure that infants and young children obtained the necessary amount of fluoride but also a useful supply of calcium, phosphorus, vitamin D, good quality protein and other important nutrients (Davis, 1975 a,b, Villa et al., 1989 and Bian et al., 1995). However, the fluoride content of milk may vary according to the fluoride content of animal feed, species of milk, drinking water, the surrounding environment, processing of milk and milk products and supplementation or fortification of milk with fluoride (Junkkarinen and Kreula, 1976, Shehata et al., 1985, Garrec and Plebin, 1986 and Wheeler et al., 1988 a,b). Flouride in milk exists in two chemical forms: as ionic or free fluoride and bound fluoride. Ionic fluoride refers to fluoride ion still in solution, while bound fluoride refers to the fluoride that has a complex with other elements or compounds (calcium or protein for example)

Fluoridation of milk is cheaper and easier than fluoridation of water and there is no evidence of any adverse effect on the processing quality of the milk. Also, milk fluoridation was effective in improving the dental health of children and that the protective effect was increasing by exposing children to fluoridated milk at an earlier age (Davis, 1979, Marino, 1995 and Banoczy, 1997, Bian *et al.*, 2004).

Sodium fluoride (NaF) is by far commonly used for large scale production of fluoridation milk. Fluoridated milk is produced with different concentration of fluoride but a typical value may be 5 ppm Fó (American Academy of Pediatrics, 1979 and 1986). Previous studies had investigated the supplemented milk with NaF and processing effect on the chemical nature, stability and milk protein interactions of fluoridated milk and milk products (Beddows, 1982, Beddows and Blake, 1974, 1982. Wheeler *et al.*, 1988a and Wieczorek et al., 1992). However, so far no work has been conducted on the physico-chemical properties of fluoridated buffalo's milk. Therefore, this study was planned to investigate the influence of added Fó as NaFó at different concentration (5-20 ppm.) on some physico-chemical properties of fluoridated buffalo's skim milk compared with those of cow's skim milk.

2. MATERIALS AND METHODS 2.1. Materials

2.1.1 Milk samples

Fresh raw bulk of both buffalo's and cow's milk obtained from the herds of the Fac. Agric., Cairo Univ. The milk samples were separated twice using a cream separator. The residual fat was removed by centrifugation at 3000 rpm for 15 min. at 5°C.

2.1.2 Sodium fluoride solution (NaF) Analytical grade sodium fluoride (Sigma Chemical Co.) was dried and 2.210 g weighed, dissolved in distilled-deionized water and made to 1L (1000 mg Fố /L).Fluoride standards: Appropriate dilutions of prepared NaF solution were used to give standard solutions in distilleddeionized water of 30 to 0.01 mg Fô/L.

2.1.3. Preparation of fluoridated milk

Buffalo's and cow's skim milk samples of known added fluoride concentrations (5, 10, 15 and 20 p.p.m F \circ) were prepared by the addition of appropriate volumes of aqueous sodium fluoride (1000 ppm F \circ) to known volumes of milk with stirring. Samples were left standing for one hour to equilibrate at room temperature 25°C. The control samples were prepared without adding NaF.

2.2. Methods of analysis

The fluoridated and non-fluoridated milk samples were analysed for pH and titratable acidity. Electrical conductivity (EC) was measured by digital conductivity meter (Henkel Canada Ltd), automatic correction of the conductivity measured to the reference temperature (25°C) and reported as mil/mhos/cm (µs/cm). Soluble calcium was determined by using the EDTA method as described by Natailianas and Whiteny (1964). For the determination of ionic fluoride, the samples after casein were adjusted to pH 5.5 precipitation (Wheeler et al., 1988b), Spadns method was used according to the standard method for the examination of water and waste water (1989).

2.2.1 Optical properties measurements

The method of Nakai and Le (1970) was followed. Five ml of 97% acetic acid were added to 0.05 ml of fluoridated milk samples in test tube with ground-glass stopper and shaked to ensure complete solubility. The absorbance of the diluted solutions was measured at 260 nm and 280 nm that indicate aromatic amino acids using a Unicam 8625 UV/VIS spectrophotometer (Ati Laicam, England)

2.2.2. Determination of milk stabilities 2.2.2.1. Ethanol stability

Equal volumes of milk samples and aqueous ethanol solutions were mixed in test tubes and examined for immediate precipitation. The minimum concentration (v/v %) of added ethanol solution which caused the immediate formation of visible particulate matter was defined as the ethanol stability (Horne and Parker, 1980).

2.2.2.2. Ca²⁺ stability

Equal volumes of milk samples and $CaCl_2$ solutions were mixed with mechanical stirring, to give a final Ca^{2+} concentration of 30 to 5 mM/L and examined for clots or fine particular material.

2.2.2.3. Heat stability

The heat stability of fluoridated milk (5 to 20 ppm F°) was determined at 125°C for 30 min. in thermostatically controlled oil bath for clots or fine particular material.

2.2.3 Determination of coagulation properties

2.2.3.1. Determination of rennet clotting time (RCT)

Twenty five ml of the fluoridated milk samples were coagulated at 35°C using Chymax (Pfizer Inc., Milwaukee, WI, U.S.A) at enzyme/substrate (E/S) of 0.008 and RCT were determined according to Berridge (1952).

2.2.3.2 Determination of curd firmness

The firmness of the rennet coagulum expressed in newtons (N) at 10 mm, was measured using Accu Forsc® II Ametek texture analyzer (Manfield&Green Division, U.S.A.) as described by Tamime *et al.*, (1989).

2.2.3.3. Determination of curd Synersis

The volume of whey expelled from the rennet curd in 60 min after cutting at 35°C was measured according to Marshall (1982). 2.2.4 Statistical analysis

The fluoridated and non-fluoridated milk samples were triplicated, and duplicate were performed on each replicate. Analysis of variance was performed by the SAS general liner methods (SAS, 2000) and differences were considered significant at P ≤ 0.05 .

3. RESULTS AND DISCUSSION 3.1. Some physico-chemical properties

The results obtained in Table (1) show that the addition of 5-20 ppm $F\circ$ (as NaF) had no significant effect on the pH of buffalo's and cow's skim milk samples. These results are in agreement with (Beddows, 1982) who reported that addition of up to 1000 ppm $F\circ$ (as NaF) to skim milk did not significantly affect pH and did not result in precipitation even after standing for 24 h at 20°C.

The concentration of soluble calcium in buffalo's skim milk was significantly decreased and the opposite trend was observed in cow's skim milk with adding 15-20 ppm F \circ (Table 1).The change in the level of soluble calcium by adding NaF may be attributed to dissociation of ionized NaF when dissolved in milk, thus NaF \leftrightarrow Na⁺ + F \circ and the Ca⁺⁺ would be found and precipitates as CaF₂ or added fluoride forms a reversible ionic complex with milk protein. However, the fact that an increase in ionic strength (*i.e.*, on adding NaF at higher concentration) leads to a reduction in the ion activity coefficient of calcium phosphate which causes an increase in solubility and dissociation of calcium phosphate or exchange with Ca⁺⁺ attached to micelles resulting an increase in soluble calcium (Davis, 1975a, Beddows and Blake 1982 and Wieczorek *et al.*, 1992).

The analysis of variance of soluble calcium proved that there were significant differences (P \leq 0.05) between the two species of milk.

Table (1) also, shows that adding up to 20 ppm F \circ (as NaF) progressively increased the ionic F \circ content from 1.95 and 1.85 to 11.95 and 9.95 ppm for buffalo's and cow's skim milk respectively. These obtained different results may be due to difference in ionic strength of NaF added and differences in the composition of casein micelles between buffalo's and cow's milk, In this respect previous published studies indicated about 85% of NaF added to skim milk remained in solution and release in ionic form after precipitation of the protein and value of ionic fluoride were 46-61% of the total fluoride (Wheeler *et al.*, 1988b).

As regards electrical conductivity (EC) of milk that is due mainly to its salt fraction and the protein contribution is of minor importance whereas fat and lactose can not conduct current. On the other hand, the distribution of salt fractions between soluble and colloidial phase has an important effect on the overall milk conductivity (Mabrook and Petty, 2003).

Data in Table (1) illustrate that the control sample of cow's skim milk had higher EC than that of buffalo's sample. These results are in good agreement with those reported earlier (El-Shibiny and Abd El-Salam, 1973, El-Shabrawy and Haggag, 1980, Therdthai and Zhou, 2001). Adding up to 20 ppm F⁶ progressively reduced and increased the EC of buffalo's and cow's samples. respectively. NaF induced reduction in the EC may be attributed to increase the ionic strength, interfered with mineral fractions of milk therefore decreased EC. However, the increase of EC through the addition of NaF may be aresult of increase concentration of monovalent cation or increase soluble calcium (Sharma and Sindhu, 2001). Statistical analysis (Table 1). revealed a significant effect I. H. I. Abd El-Ghany.....

F ं as		pН	·	Soluble calcium mg/100ml			Ionic F p.p.m.			EC (µs/cm)		
NaF (p.p.m.)	BSM	CSM	Mean	BSM	CSM	Mean	BSM	CSM	Mean	BSM	CSM	Mean
Control	6.60	6.700	6.680	65.235 ^A	46.240 ^D	55.738 °	1.950 ^F	1.850 ^F	1.900 ^f	4.815 ^D	5.335 ^C	5.075 °
5	6.665	6.740	6.703	66.235 ^A	45.880 ^D	55.558 °	4.000 ^E (80.00)	2.050 ^F (41.00)	3.025 ^d	4.745 ^E	5.615 ^B	5.180 ^a
10	6.675	6.735	6.705	66.315 ^A	45.880 ^D	56.089 ^{bc}	8.550 [°] (85.5)	7.250 ^D (7.25)	7.900 °	4.670 ^F	5.610 ^B	5.140 ^b
15	6.655	6.730	6.693	63.090 ^B	50.905 ^C	56.998 ^{ab}	8.550 [°] (57.00)	9.000 [°] (60.00)	8.775 ^b	4.665 ^E	5.640 ^B	5.153 ^b
20	6.675	6.740	6.707	63.096 ^B	51.260 [°]	57.175 ^a	11.950 ^A (60.00)	9.950 ^B (50.00)	10.950 ^a	4.335 ^G	5.680 ^A	5.008 ^d
Mean	6.666 ^b	6.729 ^a		64.590 ^a	48.030 ^b		7.00 ^a	6.020 ^b		4.646 ^b	5.576 ^a	
S.E	0.0040	0.0094			0.4585					0.0555	0.0410	

Table (1): Influence of added NaF (5-20 p.p.m. F ර) on some physico-chemical properties of buffalo's and cow's skim milk.

BSM= Buffalo's skim milk. EC=Electrical conductivity.

CSM= Cow's skim milk.

Different superscripts (A,B,C, a,b,c,...) at the same column are significantly different ($P \le 0.05$).

Figures in parenthesis indicate proportion % of added fluoride in ionic form.

S.E = Standard Error.

 $(P \le 0.05)$ of adding NaF on the EC of both milk samples.

3.2. Optical properties

Data in Table (2) show that the optical properties (optical density at 280 and 260 nm) of buffalo's and cow's skim milk containing 5-20 ppm F^{-} (as NF) were higher significantly than those of the control samples. The cow's samples had higher optical properties values than the corresponding values of buffalo's samples. The effect of NaF on optical properties changes may be attributed to interactions between NaF and tyrosine, histidine, phenylalaline and tryptophan residues.

Results in Table (3) indicate that cow's samples of skim milk containing 15-20 ppmF (as NaF) were stable towards ethanol at 85-90% compared with the control and buffalo's samples. This may be attributed to the higher effect of ionic and Na⁺ for cow's strength given by Fó milk than that of buffalo's milk. Chavez et al. (2004) observed that the relation between ionic strength and casein was more important to alcohol stability than to coagulation stability. Horne and Parker (1981) proposed a mechanism for ethanolinduced precipitation in which the dielectric strength of the micelles medium plays an important role. While, Mohammad (1989) concluded that the low ethanol stability of buffalo milk as compared with bovine milk, may be attributed to both the higher Ca⁺ concentration in buffalo milk and the different proportion of the individual caseins present in both types of milk.

The sensitivity of fluoridated milk samples to Ca⁺⁺ is shown in Table (4). Added NaF (5-20 ppm F \circ) had no effect toward sensitivity to Ca⁺⁺ for cow's milk. Buffalo's skim milk samples containing 10-20 ppm F \circ were more stable toward 5 and 7.5 mM/L Ca⁺⁺ compared with control samples. Changes in the physicochemical properties of casein micelles were deduced by comparing their zeta potential values in the absence and presence of cations and the net negative charge on casein micelles decreases with increasing calcium content (Philippe *et al.*, 2005).

It was found that all milk samples were heat stable at 125°C for 30 min irrespective of the type of milk and any concentration of F_{\circ} . No problems of heat stability would be involved in the use of fluoridized milk as mentioned by several workers (Davis, 1975a,b, Beddows and Blake, 1974, 1982 and Wheeler *et al.*, 1988a).

3.3. Rennet coagulation properties

Effect of NaF treatments on the rennet coagulation properties of milk samples is shown in (Table 5). The rennet clotting time (RCT) of the control buffalo's skim milk was shorter than that of cow's skim milk. This phenomenon probably is due to the higher calcium content, low level K-casein and larger size of casein micelles in the former than the latter (Ibrahim et al., 1973 and Amer et al., 1981). Adding (5-20 p.p.m. F as NaF) significantly (P≤0.05) increased the RCT of buffalo's and cow's skim milk compared to the control samples. At 20 ppm F changes rate of the RCT were 69.72 and 41.3% in the buffalo's and cow's milk samples respectively. The findings could be explained by association of NaF to form $NaF \leftrightarrow Na^+ + F \circ$ and Na^+ may cationic exchange taking place between Na⁺ and leading to form sodium calcium Ca⁺⁺ paracesinate which is more hydrophilic and requires longer time for clotting than calcium paracaseinate. Fílourine tightly ionic complex which bound reversible larger casein micelles in buffalo's milk than small casein micelles in cow's milk.

Results in Table (5) show that adding NaF (10-20 p.p.m. F \circ) leads to significantly increase (P \leq 0.05) the firmness of buffalo's skim milk samples, but the firmness of cow's skim milk was not affected with adding NaF.

Data given in Table (5) show that the control cow's skim milk had higher synersis (whey volume expressed as a function of the incubation time of the cut curd) than that of buffalo's skim milk. This is attributed to the lower total solid of cow's milk than buffalo's milk and differences in structure composition of casein micelles in both milk (Abd El-Salam et al., 1978 and Hofi et al., 1978). Calvo and Balcones (2000) indicated that the differences in drainage rate amount curds made from bovine, ovine and caprine milk could have been due to a different formation of the reticulum of casein micelles of casein and the salt composition can influence the coagulum structure. While, Renault et al. (1997) found that

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Table (2): Influence of added NaF (5-20 ppm F \circ) on the optical properties of buffalo's and cow's skim milk .

F໌ as NaF (n.n.m.)	Optical o	lensity at 280	nm	Optical density at 260 nm				
(p - p)	BSM	CSM	Mean	BSM	CSM	Mean		
Control	0.405	0.460	0.433 ^d	0.344 ^F	0.381 ^C	0.361 °		
5	0.464	0.563	0.513 °	0.360 ^E	0.410 ^B	0.385 ^b		
10	0.480	0.575	0.527 ^b	0.368 ^D	0.430 ^A	0.399 ^a		
15	0.479	0.575	0.526 ^b	0.366 ^{DE}	0.434 ^A	0.399 ^a		
20	0.490	0.599	0.545 ^a	0.370 ^D	0.435 ^A	0.402 ^a		
Mean	0.464	0.554		0.361 ^b	0.418 ^a			
S.E	0.0103	0.0162		0.0035	0.0068			

BSM= Buffaloe's skim milk.

CSM= Cow's skim milk.

Different superscripts (A,B,C, a,b,c,...) at the same column are significantly different (P≤0.05).

S.E = Standard Error.

Table (3): Influence of added NaF (5-20 ppm F☉) on ethanol stability of buffalo's and cow's skim milk.

Eó as NaE		Buffaloe's skim	Cow's skim milk							
F ○ as NaF (p.p.m)	Alcohol concentration %									
	95	90	85	80	95	90	85	80		
Control	+	+	+	-	+	+	+	-		
5	+	+	+	-	+	+	+	-		
10	+	+	+	-	+	+	+	-		
15	+	+	+	-	+	+	+	-		
20										

Table (4): Influence of added NaF (5-20 ppm F⁶) on the sensitivity to Ca⁺⁺ of buffalo's and cow's skim milk.

F் as NaF (p.p.m.)	Species of skim	Ca ⁺⁺ concentration			
	milk	5 mM/L	7.5 mM/L	10mM/L	
Control	BSM	+	+++	+++	
Colluloi	CSM	-	-	-	
5	BSM	+	+++	+++	
5	CSM	-	-	-	
10	BSM	-	++	+++	
10	CSM	-	-	-	
15	BSM	-	+	+++	
15	CSM	-	-	-	
20	BSM	_	+	+++	
20	CSM	_	_	-	

BSM= Buffaloe's skim milk.

CSM= Cow's skim milk.

- = Non clotting + = Fair clotting

++ = Medium clotting. +++ = Strong clotting.

F´ as NaF (p.p.m)	Rennet clotting time (min.)			F	'irmness (N)		Volume of whey (ml) expressed from 100 ml of cut curd in 60 min.		
	BSM	CSM	Mean	BSM	CSM	Mean	BSM	CSM	Mean
Control	1.225 ^G	2.420 [°]	1.822 °	0.185 ^C	0.155 ^C	0.170 ^b	3.950 ^H	39.000 ^E	21.475 ^e
5	2.295 ^D	3.305 ^B	2.800 ^a	0.190 ^C	0.155 ^C	0.173 ^b	4.300 ^H	63.000 ^A	33.650 ^{cd}
10	2.150 ^{EF}	3.325 ^B	2.738 ^b	0.240 ^B	0.155 ^C	0.198 ^{ab}	4.300 ^H	59.000 ^B	31.650 ^d
15	2.195 ^E	3.375 ^B	2.785 ^{ab}	0.240 ^B	0.155 ^C	0.198 ^{ab}	35.000 ^{EF}	57.000 ^B	46.000 ^b
20	2.080 ^F	3.485 ^A	2.783 ^{ab}	0.310 ^A	0.155 ^C	0.233 ^a	49.000 [°]	55.000 ^B	52.000 ^a
Mean	1.989 ^b	3.182 ^a		0.234 ^a	0.155 ^b		19.310 [°]	54.60 ^a	
S.E	0.1297	0.1289		0.0171	0.0017		6.3530	2.7976	

Table (5): Influence of added NaF (5-20 p.p.m. F ´) on the clotting rennet properties of buffalo's and cow's skim.

BSM= Buffalo's skim milk.

CSM= Cow's skim milk.

N= Newton.

Different superscripts (A,B,C, a,b,c,...) at the same column are significantly different ($P \le 0.05$).

S.E = Standard Error.

synersis could be influenced by changes in the protein conformation essentially due to hydrophobic bounding.

Buffalo's and cow's skim milk contained 20 and 5 ppm F⁶ (as NaF) respectively had the highest significantly increased synersis compared with other treatments. The pattern of synersis rate was significantly different (P≤0.05) among the buffaloe's and cow's milk. The alteration of synersis by adding NaF may be due to the different modification in the distribution of calcium between aqueous and colloidial phases and rearrangement of the protein network occurring after adding NaF. The intensity of these changes depends on the mature casein and concentration of NaF. Previous studies showed that addition of up to 300 mM/L NaCl did not affect the volume of whey expressed from the curd, but at 500 mM//L there was slight increase in the synersis (Grufferty and Fox, 1985).

It appears that the key-mechanism through which added NaF induces modification in the physico-chemical properties of milk.

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تأثير إضافة الفلوريد للبن الفرز الجاموسي والبقرى أولا: على بعض الصفات الطبيعية والكيميائية

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الملخص

أجرى هذا البحث بهدف دراسة تأثير إضافة فلوريد الصوديوم بتركيزات مختلفة (5-20 جزء في المليون فلورين) على الصفات الطبيعية والكيميائية للبن الفرز الجاموسي والبقري. وقد أظهرت الدراسة النتائج الأتبة

لم تؤد إضافة فلوريد الصوديوم (5-20 جزء في المليون فلورين) إلى حدوث تغير في ال pH مع حدوث انخفاض معنوي في الكالسيوم الذائب ودرجة التوصيل الكهربي للبن الجاموسي ولكن حدث العكس في اللبن البقري.

إزداد تركيز الفلورين الايونى في كل من اللبن الجاموسي والبقرى بزيادة تركيز فلوريد الصوديوم المضاف

تزداد قيم الكثافة الضوئية عند طول موجي 260 و 280 نانوميتر للبن الجاموسي والبقرى المضاف لـه فلوريد الصوديوم بتركيزات مختلفة (5-20 جزء في المليون فلورين) بالمقارنة بالكونترول.

كان اللبن الفرز البقرى المحتوى على 15-20 جزء في المليون فلورين أكثر ثباتا للتجبن بالكحول عند تركيز 85- 90 % بالمقارنة باللبن الفرز الجاموسي في حيَّن لم تتأثر درجة الحساسية لايونات الكالسيوم المضاف بعكس اللبن الجاموسي فقد كان أكثر ثباتا لأيونات الكالسيوم (5 – 7.5 مللي مول/لتر) بالمقارنة بالكونتر<u>ول.</u>

إزداد زمن التجبن والصلابة ومعدل التشريش للبن الفرز الجاموسي زيادة معنوية عند تركيز 20 جزء في المليون فلورين بالمقارنة بمثيلاتها للبن الفرز البقري.

وعلى ذلك يتضح من هذه الدراسة أن إضافة فلوريد الصوديوم للبن يؤدى إلى حدوث تغيرات طبيعية وكيميائية لها أهمية تكنولوجية. المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (59) العدد الثالث (يوليو 2008):187-196.