POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

http://journal.pan.olsztyn.pl
e-mail: pjfns@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2010, Vol. 60, No. 4, pp. 353-362

FUNCTIONAL AND THERMAL CHARACTERISTICS OF BUFFALO'S MILK PROTEIN PRODUCTS

Zakaria M.R. Hassan, Yehia A. Heikal

Food Science Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khaima, Cairo, Egypt

Key words: buffalo's milk protein products, functional properties, differential scanning calorimetry

Buffalo's milk protein products, total milk proteinate (TMP), rennet casein, and lactic acid casein were studied. The chemical composition was determined and some properties (water and oil absorption capacity, emulsion activity, foam expansion, and buffer capacity) of these products were also determined. The results indicated some differences in the chemical composition and electrophoresis bands of protein between total milk proteinate, rennet casein and lactic acid casein. The TMP was characterised by the lowest ash and moisture contents with highest protein contents compared to the other protein products. There is no remarkable effect of drying methods on the chemical composition within each type of protein products. Total milk proteinate contains some whey proteins which are high in alanine, cystine when compared with rennet casein. Lactic acid casein had high contents of amino acid proline. On the other hand, the rennet casein had a low content of sulfur containing amino acids cysteine and methionine. Differences between total essential amino acid (TEAA) of total milk proteinate and lactic acid casein were significantly ($p<0.05$) higher than those of rennet casein. Freeze dried total milk proteinate exhibited excellent foaming and emulsifying potential when compared with oven-dried caseinate. For all protein types, the maximum WAC can be seen for freeze-dried milk proteinates, whereas the minimum value was for oven dried rennet casein. The minimum value of emulsion capacity and surface tension was obtained at pH 4.5 and 2.5 in all types of proteinates, whereas the maximum values were found at pH 10.5. Relative viscosity of TMP solutions was higher than those of rennet and lactic acid casein. However, relative viscosity values tended to decrease with lowering or increasing the pH values of the solutions from the neutral pH value. Calorimetric analysis showed two major enthalpy changes in the tested caseinate samples. The first change occurred at peak temperature range of 92.2°C to 100.8°C for the moisture removal, while the second change occurred between 273.9°C and 314.6°C for protein degradation. The enthalpy values ranged between 218.3 to 268.4 J/g for moisture removal. Total milk proteinate showed two major peaks for protein degradation indicating the presence of whey proteins and milk caseins.

INTRODUCTION

The utilization of buffalo's milk protein in food system is limited principally because of their variable functional properties. Most of protein properties are affected by altering the structure, configuration and molecular weight of proteins. Information on protein functional and thermal properties are very important for food processing strategies and heat processing design. Functional properties of protein are those physicochemical properties, which affect protein behaviour in food systems during preparation, processing, storage and consumption. Also, they contribute to the quality and organoleptic attributes of food systems [Singh & Ye, 2009]. Functionality of food proteins has been evaluated by means of test procedures for solubility, water and oil binding, emulsifying, foaming and buffering capacity. Viscosity is one of the most important functional properties of food proteins. It is important for providing physical stability to emulsions [Jayasena *et al.,* 2010]. Proteins maintain their native structure by chemical forces such as hydrophobic, ionic, hydrogen, and disulfide bonds. The chemical bonds are highly dependent upon the environment. As environmental conditions change, some of the original bonds may be altered, and new bonds may form. The proteins then assume new conformations. During this process, rupture of hydrogen bonds may lead to endothermic reactions, and disruption of hydrophobic bonds may lead to exothermic reactions. Such enthalpy changes can be detected by differential scanning calorimetry (DSC) [Raikos, 2010]. DSC is used to investigate heat-induced conformational or structural changes of a broad range of food ingredients (biopolymers, proteins, fats, sugars, emulsifiers) in various physico-chemical conditions, and at various weight fractions of water [Dean *et al.,* 2001]. The surface tension of fluid dairy products is another fundamental physical property relating to the stability of foams, emulsions, and films as well as affecting industrial processes such as fractionation and concentration. Milk contains several surface-active components (*e.g.*, proteins, free fatty acids, and derivatives of the milk fat globule membrane) that affect both the surface properties (*e.g.,* surface tension) and bulk properties (*e.g.*, micelle and globule formation) [Kristensen *et al.,* 1997].

The present work was designed to shed some lights on the possibility of industrial application of buffalo's milk proteinates through study the effect of preparation and drying methods on some functional properties (emulsion and foaming properties, water and oil binding capacity, buffer capacity, relative viscosity and surface tension) of milk casein proteinates of buffalo's milk. Also, the thermal behavior of these proteinates was studied to establish a non-destructive method for the differentiation between the prepared protein samples.

Author's address for correspondence: Dr. Zakaria M.R. Hassan, Food Science Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khaima, Cairo, Egypt; e-mail: zakaria_rezkh@yahoo.com

MATERIALS AND METHODS

Materials

Buffalo's milk was obtained from the herd of Faculty of Agriculture, Ain Shams University and used for preparing the protein products. The chemical composition of the used buffalo's milk was as fallows: total solids – 15.68%, total protein – 3.84%, ash – 0.8%, fat – 6.14%, and lactose – 4.86. Total milk proteinate, rennet casein, and lactic acid casein were isolated from buffalo skim milk following the method of Morr [1985]. The prepared protein samples were divided each in two portions. One portion was dried in a laboratory hot air oven (Heraeus, Hanau, RAT360, Germany) at 60°C for 6 h, while the second portion was lyophilized at –40°C by freeze drying (LYPH-LOCK-4.5, Zirbus Technology, Germany, equipped with vial stoppering system and magnetic valve for vacuum regulation). Both drying methods are different in modes of heat transfer to the wet materials and in the physical structure of the dried products.

Chemical analysis

Moisture and ash contents were determined according to AOAC [2007]. Total nitrogen content was determined by Kjeldahl method as described by AOAC [2007]. The amino acids content was determined according to Cohen *et al*. [1989]. Protein efficiency ratio was calculated according to Alsmeyer *et al*. [1974].

Electrophoresis

The method is based on the separation of principle polypeptides according to their molecular size by applying the sodium dodecyl sulphate-polyacrylamide gel electrophoreses (SDS-PAGE). The own charges of the polypeptides will be affectively surcharged through the anionic SDS, so that anion with constant charge per a constant of mass arises. Through electrophoreses in a polyacrylamide gel, which works as a molecular sieve, the relative mobility of these SDS-protein complexes will be proportional to the logarithm of their molecular size. A standard curve of parallel separated standard proteins is applied to calculate the molecular size of each fraction. The preparation of solutions, cathode and anode buffers as well as pouring the collection gels and conducting the electrophoreses were carried out according to Rawel *et al.* [2003]. The used protein standards were obtained from PHARMCIA (Germany) covering molecular masses between 14,400 to 94,000 D. The electrophoresis equipment consists of an electrophoresis network and cells (Bio Rad Laboratories, Munich, Germany). The applied software was a Bio-Rad Scanner system, Version 1.1 (Bio Rad, Hercules; California, USA).

Functional properties

Emulsifying activity index (EAI) was determined by the turbidimetric method of Pearce & Kinsella [1978]. Foam expansion and foam stability was estimated by the procedure of Patel *et al*. [1988]. Buffer capacity was determined by the method of Salaün *et al.* [2007]. Buffering indices (dB/dpH) were calculated for each addition of acid and buffering curves were prepared by plotting these indices as a function of pH. Areas under buffering curves were integrated to estimate the intensity of buffering capacity.

Water and oil absorption capacities were estimated according to the method of Dekanterewicz *et al*. [1987]. Surface tension of the proteins was recorded using a tensiometer (DuNouy ring Tensiometer, Krüss-Instrument, No. 8158, Germany). The DuNouy ring used for determination was cleaned prior to each measurement by dipping the ring in diluted nitric acid then flaming until the ring was „red" hot in the oxidizing portion of Bunsen burner flame to remove organic materials. After cooling, the ring was then hung from the load cell and lowered to the base of sample container. The ring was pulled from the surface of sample and the force required to do so was recorded as surface tension values which were read directly from the instrument scale as dyne/m. Triplicate samples were prepared for measurements of each treatment. Viscosity of 1% casein solutions was measured using an Ubelhode Capillary glass viscometer according to Rao [1999]. Viscosity values were expressed as relative viscosity as follows:

η_{rel} = Flow time of casein solution/ Flow time of dist.water.

Differential Scanning Calorimetry (DSC) measurements

DSC measurements were performed on a NETZSCH STA 409 C/ CD (Netzsch, Selb, Germany) according to Czuchajowska & Pomeranz [1989] with a heating rate of 5°C/min in a temperature range from 30 to 450°C in a platinum pan under aerobic oxygen atmosphere.

Statistical analysis

All experiments have been carried out in triplicate. Means and standard deviation were calculated. To determine statistically significant differences between samples ($p<0.05$), the data were subjected to analysis of variance and appropriate means separation was conducted using Duncan's multiple range test using a statistical software program (SPSS for Windows Version 7.0).

RESULTS AND DISCUSSION

Major composition

The chemical composition of total milk proteinate, rennet casein and lactic acid casein from buffalo milk is shown in Table 1. Among the examined casein samples, total milk proteinate exhibited the lowest ash, moisture, sodium and phosphorus with the highest protein contents, compared with those of other casein samples. On the other hand, rennet casein possessed the lowest value of protein with highest ash, calcium, sodium and phosphorus contents. Lactic acid casein had high ash content compared with the total milk proteinate and high protein compared with rennet casein. There was no remarkable effect of the drying methods on the chemical composition within each type of protein products. The results are in agreement with those reported by Metwally & Smith [2001].

Amino acids

Amino acid composition of the milk protein products were compared as well (Table 2). The total milk proteinate had

TABLE 1. Chemical composition of buffalo milk casein samples.

Protein product	Moisture content (%)	Protein (%)	Ash (%)	Calcium	Sodium	Phosphorus
				(g /100g)		
Total milk proteinate						
Oven dried	4.47±0.26	88.62±1.08	3.23±0.16	1.06±0.08	0.01±0.002	0.68±0.057
Freeze dried	5.37±0.12	87.86±1.06	2.98±0.20	1.08±0.09	0.02±0.025	0.72±0.068
Rennet casein						
Oven dried	6.95±0.16	80.72±0.96	10.52±0.59	1.18±0.10	0.06±0.066	1.37±0.059
Freeze dried	5.31±0.11	81.79±1.04	10.64±0.61	1.01±0.08	0.07±0.008	1.46±0.057
Lactic acid casein						
Oven dried	5.81±0.15	83.50±1.52	3.65±0.25	1.00±0.07	0.02±0.037	1.09±0.06
Freeze dried	4.92±0.12	85.94±1.24	5.18±0.51	1.06±0.10	0.03±0.046	1.11±0.08

TABLE 2. Amino acid content (g/100 g) of freeze dried buffalo milk casein samples.

Amino acids	Total milk proteinate	Rennet casein	Lactic acid casein
Essential amino acids			
Phenylalanine	4.13±0.08	4.40±0.21	4.53±0.25
Valine	6.20±0.13	6.11±0.42	6.50±0.25
Leucine	8.60±0.15	8.23±0.29	8.56±0.33
Isoleucine	6.50±0.16	5.97±0.25	6.16±0.31
Methionine	2.97±0.09	2.32±0.18	2.43±0.21
Histidine	2.71±0.09	2.80±0.13	2.83±0.18
Threonine	4.04±0.08	3.25±0.20	3.91±0.17
Lysine	8.89±0.17	8.16±0.31	8.63±0.31
Total EAA	44.04±1.22[a]	41.24±1.04[b]	43.55±1.20[a]
Non-essential amino acids			
Arginine	2.75±0.14	3.51±0.30	3.09±0.20
Aspartic acid	6.86±0.18	5.81±0.21	6.74±0.39
Cystine	0.39±0.03	0.21±0.014	0.23±0.017
Alanin	3.19±0.13	2.93±0.18	3.02±0.13
Glutamic acid	22.73±1.02	21.91±0.82	22.11±0.86
Glycin	2.05±0.09	2.00±0.16	1.88±0.17
Proline	9.52±0.41	9.43±0.64	10.30±0.81
Serine	3.67±0.32	3.39±0.23	3.69±0.24
Tyrosine	4.02±0.17	3.94±0.19	4.00±0.21
Total NEAA	55.18±1.14[a]	53.13± 0.96[b]	55.06± 0.88[a]
Limiting score	99.22±2.04	94.37±1.54	98.61±1.23
PER	2.96±0.17	2.48±0.12	3.06±0.25

PER, Protein efficiency ratio, EAA, Essential amino acids, NEAA Non-Essential amino acids. Means in row with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

the highest concentration of alanine, cystine and methionine as compared with other products. The total milk proteinate contains some whey protein, which are high in alanine, cystine when compared to casein [Lampert, 1970]. Lactic acid casein had high contents of amino acid proline. On the other hand, the rennet casein had a low content of sulfur-containing amino acids: cysteine and methionine. The lower content of amino acid of rennet casein compared to the other casein products could be referred the to release of glucomacropeptide (fragment, 106-169) into the aqueous phase during manufacture of rennet casein [Fox, 1989]. The total essential amino acid (TEAA) of total milk proteinate and lactic acid casein were significantly ($p<0.05$) higher than those of rennet casein. Consequently, protein efficiency ratio (PER) of total milk proteinate and lactic acid casein were higher than those of rennet casein.

Electrophoresis characterization

Patterns of electrophoresis were performed to monitor the band of total milk proteinate, rennet casein and lactic acid casein (Figure 1 and Table 3). Significant differences ($p<0.05$) were found only in the peak intensity between oven-dried and freeze-dried protein samples. The electrophoresis patterns for the total milk proteinate (lane 1) seemed to be divided into five major regions including k-casein, β-casein, α_s-casein, α-lactalbumin and β-lactoglobulin. This appeared in molecular weight and peak intensity (Table 3). The electrophoresis patterns of lanes 2, 3, 4, 5 and 6 were characterised by disappearance of some bands in whey proteins region. It could be observed that the intensity of whey protein bands decreased compared to that of total milk proteinate pattern (lane 1). All of β-lactoglobulin fractions disappeared in fractionations pattern of TMP (lane 6). This may be due to the complex formation between whey protein and casein during preparation of TMP [Morr, 1985]. Although the intensity of whey protein bands decreased, no bands could be detected in the whey protein region (lanes 2, 3, 4, 5, 6). The results are consistent with those of Metwally [1997].

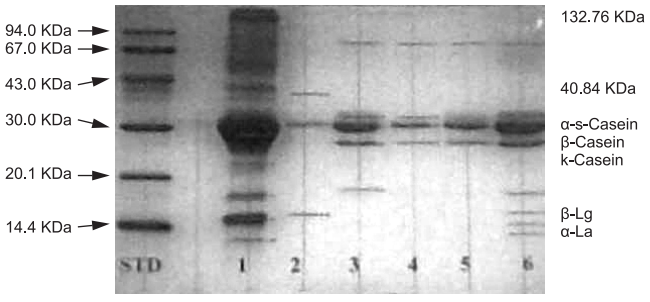

FIGURE 1. SDS Polyacrylamide gel electrophoresis patterns for total milk proteinate, rennet casein and lactic acid casein.
Lane (1) freeze dried and lane (6) Oven total milk proteinate.
Lane (2) freeze dried and lane (4) Oven rennet casein.
Lane (3) freeze dried and lane (5) Oven lactic acid casein.

TABLE 3. Molecular characteristics of casein samples

Treatment	Molecular weight (KDa)		Peak intensity		Relative Qty	
	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried
Total milk proteinate	75.9±1.1 32.9±0.77 29.8±0.73 26.3±0.61 17.9 ±0.53 15.7±0.49 14.4±0.37 13.5±0.37	132.8±1.23 40.8±0.74 28.4±0.65 22.4±0.53 17.7±0.57 15.0±0.50 13.0±0.49	1614.4±56.6[a] 2178.5±49.8[a] 3429.3±82.8[a] 3667.8±83.1[a] 828.1±44.9[a] 500.8±30.4[a] 478.8±24.5[a] 538.4±36.5[a]	3861.9±73.7[b] 2464.1±45.1[b] 3667.8±79.1[b] 1944.7±59.4[b] 2243.6±66.9[b] 3259.8±79.3[b] 1242.8±40.9[b]	6.20±0.39 17.6 ±0.73 47.5± 0.92 19.6 ±0.62 3.1 ±0.19 2.0±0.08 1.9±0.08 2.2±0.098	16.4±0.53 9.8± 0.37 46.1 ±0.85 6.2 ±0.41 6.5 ±0.46 13.4 ±0.69 1.6± 0.09
Rennet casein	76.1±0.97 32.2 ±0.78 30.4±0.79 26.6 ±0.71	77.5±1.1 32.5±0.81 30.0±0.73 26.4±0.65 18.4±0.48	1027.2±40.8[a] 1492.6±45.7[a] 2870.8±113.3[a] 1621.0±46.3[a]	1222.4±44.8[b] 1563.3±60.7[b] 3222.9±87.4[b] 2246.9±48.8[b] 566.6±27.1[b]	8.4±0.21 21.6±0.86 50.7±1.1 19.2±0.60	6.8±0.55 17.4 ±0.74 53.2±0.86 19.3 ±0.72 3.4±0.21
Lactic acid casein	38.9±0.85 30.9±0.57 15.5±0.47	76.4±0.84 32.5±0.73 30.6±0.61 26.6±0.49	1671.7±57.8[a] 597.9±24.8[a] 688.8±37.5[a]	892.9±32.8[b] 786.8±36.7[b] 2273.7±61.5[b] 976.5±39.0[b]	15.8±0.74 69.8±1.1 14.3±0.53	12.7±0.67 10.6±0.45 61.1±1.02 15.7 ±0.53

Means in column with different letters are significantly different ($p<0.05$) according to Duncan's multiple range test.

Emulsifying activity, surface tension and relative viscosity of casein solutions

The emulsion activity index (EAI) is an important factor for using protein preparations in manufacturing food emulsion. The EAI of emulsions prepared by homogenizing a solution of protein products at different rates with corn oil is shown in Table 4. Significant differences ($p<0.05$) were found only in EAI values between oven-dried and freeze-dried samples. The results clearly demonstrated that the emulsion activity and surface tension of casein samples progressively increased with increasing the pH values. The lowest surface tension values of total milk protein compared with those of the other casein samples may be attributed to the complex formed between casein and undenaturated whey protein. Rennet casein had lower surface tension than did lactic acid casein, which may be due to the release of a glucomacropeptide from k-casein which increased the hydrophobicties of rennet casein [Raikos, 2010]. It can be observed from the results that the emulsion capacity and surface tension were pH dependent. The minimum value of emulsion capacity and surface tension was obtained at pH 4.5 and 2.5 in all types of casein samples due to precipitation of protein, whereas the maximum values were

TABLE 4. Effect of drying method and environment pH on emulsion activity (EAI), surface tension (N/m) and relative viscosity of buffalo total milk proteinate, rennet casein and lactic acid casein.

Treatment	EAI (m^2g^{-1})		Surface tension (N/m)		Relative viscosity (sec) η_{rel}	
	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried
Total milk proteinate						
pH						
2.5	43.48±0.86[a]	66.33±1.11[b]	57.5±0.28	57.5±0.23	1.93±0.007	1.93±0.02
4.5	9.91±0.45[a]	11.05±0.81[b]	57.0±0.40	57.0±0.28	1.80±0.03	1.80±0.05
6.5	28.00±0.95[a]	46.80±0.98[b]	58.5 ±0.16	58.5±0.25	2.00±0.03	2.00±0.06
8.5	48.64±1.07[a]	51.22±0.99[b]	58.5 ±0.41	58.5±0.20	1.93±0.02	1.93±0.04
10.5	76.64±1.1[a]	90.12±1.53[b]	58.75±0.29	58.5±0.21	1.86±0.02	1.93±0.02
Rennet casein						
pH						
2.5	30.58±0.74[a]	45.96±0.98[b]	56.83±0.25	56.83±0.14	1.80±0.03	1.80±0.03
4.5	6.47±0.40[a]	8.58±0.84[b]	57.00±0.25	57.0±0.25	1.80±0.04	1.80± 0.03
6.5	10.26±0.79[a]	29.48±0.85[b]	59.25±0.16	59.25±0.16	1.86±0.03	1.86 ±0.02
8.5	23.58±0.84[a]	33.74±0.83[b]	59.75±0.29	59.75±0.20	1.80±0.02	1.80 ±0.02
10.5	42.06±1.14[a]	53.28±0.91[b]	60.25±0.26	60.00±0.41	1.80±0.035	1.80± 0.03
Lactic acid casein						
pH						
2.5	33.90±0.82[a]	39.11±0.87[b]	57.0±0.22	57.0±0.21	1.75±0.02	1.75± 0.025
4.5	8.95±0.68[a]	6.16±0.50[b]	58.5±0.36	58.5±0.25	1.88±0.02	1.88± 0.025
6.5	26.17±0.90[a]	45.48±0.75[b]	59.5± 0.36	59.5±0.34	1.93±0.02	1.93± 0.03
8.5	36.48±0.85[a]	53.80±0.82[b]	60.0±0.37	60.0±0.29	1.95±0.024	1.95± 0.025
10.5	59.59±1.06[a]	65.27±0.82[b]	61.0±0.37	60.25±0.12	1.97±0.014	1.95±0.03

Means in column with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

found at pH 10.5. Also, the surface tension of casein samples was higher at pH 10.5 than at pH 2.5. By shifting the pH away from the pI, the emulsion capacity was greatly enhanced in both acidic and alkaline sides. The results show that the total milk proteinate had the highest values of emulsion activity index, while rennet casein had the lowest values. This may be due to the undenatured whey protein included in TMP as reported by Morr [1982]. In addition, whey proteins enhance the emulsion activity index [Pearce & Kinsella, 1978]. On the other hand, it can be seen that the freeze-dried casein samples showed higher EAI when compared with the oven-dried casein samples. Relative viscosity of TMP solutions was higher than those of rennet and lactic acid casein. However, relative viscosity values tend to decrease with lowering or increasing the pH values of the solutions from the neutral pH values. This could be referred to the changes in casein solubility with changes in the pH value.

Water and oil absorption capacity

Results of water absorption capacity (WAC), oil absorption capacity (OAC) and water and oil absorption index (WOAI) are shown in Table 5. Significant differences ($p<0.05$) between oven dried and freeze dried samples were found in water absorption capacity (WAC), oil absorption capacity (OAC), while no significant effect of drying method was found for water and oil absorption index (WOAI). For all casein samples, the maximum WAC can be seen for freeze-dried milk proteinates, whereas the minimum value was for oven-dried rennet casein. Total milk proteinates showed a higher water absorption capacity than the rennet casein and lactic acid casein. This result may be due to the whey protein incorporated in the final products. Snoeren *et al.* [1982] reported that whey proteins denaturation serves to increase the water hydration from 0.32 to 2.30 g water/g protein. On the other hand, OAC showed the maximum values of 1.83 for freeze-dried milk rennet casein. The WOAI was between 1.10 to 2.07 for oven-dried and 1.20 to 2.00 for freeze-dried casein samples. The ratio between oil and water absorption index (WOAI) was calculated to find out if the index agreed with general conclusion reported by Dekanterewicz *et al*. [1987], who mentioned that the maximum emulsion capacity was achieved when the WOAI was nearly 2.0. Based on the WAC and AOC values given in Table 5, it could be concluded that the total milk proteinate was ranked as the best emulsifying product. The WOAI shifted towards the hydrophobic side, when it was less than 2.0 and the best example for that is rennet and lactic acid casein.

Foam expansion and stability

The effect of environmental pH, drying system and addition of carboxyl methyl cellulose (CMC) to total milk proteinates, rennet casein, and lactic acid casein on the foam expansion (FE) and foam volume stability (FVS), was presented in Table 6. Significant differences ($p<0.05$) in foam expansion and foam volume stability values were found between the oven-dried and freeze-dried samples, as well as to the addition of CMC. It could be seen that the minimum values of FE, obtained at pH 4.5, were 85% and 90% for oven-dried and freeze-dried total milk proteinate, respectively. Rennet casein had higher foam expansion (FE) values at pH 4.5 than did lactic acid casein and TMP, respectively. The values were increased to 270% and 400% at pH 8.5 in the same order. The foam expansion was higher at pH 2.5 for freeze-dried protein samples than for the oven-dried samples, which reached 450% and 200%, respectively. Rennet casein had higher foam expansion (FE) at pH 2.5 than lactic acid casein and TMP, respectively. These results indicated that FE was pH dependent. The foam expansion was greatly enhanced in both acidic and alkaline sides. The FE values of the total milk proteinates were decreased compared with other casein

TABLE 5. Water absorption capacity (WAC), oil absorption capacity (OAC) and water-oil absorption index (WOAI) of buffalo total milk proteinate, rennet casein and lactic acid casein.

Treatment	WAC (g water/g protein)		OAC (g oil/g protein)		WOAI (g water/g oil)	
	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried
Total milk proteinate						
Whole TMP	2.80±0.09 [a]	3.60±0.14[b]	1.35± 0.05[a]	1.80±0.12[b]	2.07±0.09 [a]	2.00±0.09[a]
Particle size 0.10	3.20±0.14	4.20±0.18	1.80±0.14	2.60±0.14	1.77 ±0.11	1.61±0.08
0.25	2.40±0.09	3.20±0.13	1.50±0.11	2.16±0.10	1.60± 0.11	1.48±0.08
0.50	2.20±0.13	2.80±0.14	1.40±0.12	1.90±0.11	1.57±0.07	1.47±0.09
Rennet casein						
Whole RC	2.20±0.13[a]	2.40±0.09[b]	1.52±0.11[a]	1.83±0.10[b]	1.44±0.09[a]	1.31±0.09[a]
Particle size 0.10	2.30± 0.07	2.60±0.15	1.82±0.12	1.90±0.09	1.26±0.09	1.36±0.07
0.25	1.90±0.11	2.10±0.12	1.55±0.11	1.69±0.10	1.22±0.10	1.24±0.06
0.50	1.60±0.10	1.80±0.13	1.45±0.10	1.50±0.10	1.10±0.09	1.20±0.07
Lactic acid casein						
Whole LAC	2.40±0.13[a]	2.80±0.15[b]	1.40±0.10[a]	1.70±0.08 [b]	1.70±0.11[a]	1.64± 0.09[a]
Particle size 0.10	2.80 ±0.14	3.00±0.14	1.70±0.07	1.80±0.13	1.64± 0.09	1.66±0.09
0.25	2.00±0.11	2.80±0.14	1.40±0.07	1.71±0.10	1.42± 0.07	1.63±0.09
0.50	1.80±0.14	2.10±0.13	1.35±0.08	1.40±0.10	1.33±0.08	1.50±0.09

Means in row with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

TABLE 6. Effect of drying system and environment pH on the foam expansion (FE%) and foam volume stability (FVS% after 15 min) of buffalo total milk proteinate, rennet casein and lactic acid casein without and with CMC at different pH.

Treatment	Foam expansion (FE%)						Foam volume stability (FVS%)					
	Without CMC		With 0.5% CMC		With 2.0% CMC		Without CMC		With 0.5% CMC		With 2.0% CMC	
	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried	Oven dried	Freeze dried
Total milk proteinate												
pH												
2.5	200±8.18^{A}	235±9.53^{B}	260±8.17^{B}	485±21.65^{a}	230±9.42^{b}	330±14.29$^{a\,b}$	71±5.72^{A}	33±3.68^{a}	105±9.81^{A}	25±3.68^{b}	74±4·92^{A}	55±4.50^{b}
4.5	85±4.08^{A}	90 ± 4.90^{B}	145±8.56^{B}	170±11.02^{a}	120±9.39^{b}	180±11.52$^{a\,b}$	0.0±0.0^{A}	0.0±0.0^{a}	23±3.26^{A}	37±5.13^{b}	116±9.03^{A}	46± 3.68^{b}
6.5	215±7.76^{A}	280±8.16^{B}	265±11.05^{B}	350±16.33^{a}	260±11.03^{b}	380±16.8$^{a\,b}$	90±7.36^{A}	80±6.59^{a}	76±6.94^{A}	27±4.08^{b}	66±6.54^{A}	32±3.14^{b}
8.5	270±10.2^{A}	400±14.73^{B}	275±9.27^{B}	495±22.48^{a}	240±13.07^{b}	340±13.95^{ab}	76±6.94^{A}	66±5.73^{a}	90±7.36^{A}	11±1.70^{b}	26±3.68^{A}	27±2.57^{b}
Rennet casein												
pH												
2.5	300±10.28^{A}	450±16.81^{B}	470±13.95^{B}	500± 26.77^{a}	490±15.12^{b}	285±11.74$^{a\,b}$	80±7.36^{A}	90±6.13^{a}	42±5.71^{A}	21±2.86^{b}	112±8.22^{A}	18±1.84^{b}
4.5	180± 5.74^{A}	185±7.36^{B}	29±2.86^{B}	240±13.14^{a}	360±15.06^{b}	360±16.33$^{a\,b}$	85±8.16^{A}	83±6.12^{a}	98±8.22^{A}	52±4.49^{b}	71±4.89^{A}	40±3.26^{b}
6.5	140±6.53^{A}	150±6.59^{B}	280±11.84^{B}	375±21.31^{a}	300±17.20^{b}	360±14.04$^{a\,b}$	69±7.34^{A}	30±4.08^{a}	55±6.59^{A}	27±3.69^{b}	94±7.34^{A}	29±2.79^{b}
8.5	245±8.16^{A}	350±12.25^{B}	325±14.72^{B}	450±17.19^{a}	550±24.94^{b}	330±10.21$^{a\,b}$	81±6.55^{A}	84±7.36^{a}	55±6.35^{A}	29±3.30^{b}	98±8.22^{A}	29±2.61^{b}
Lactic acid casein												
pH												
2.5	350±14.34^{A}	370±9.42^{B}	450±16.32^{B}	480±21.70^{a}	395±17.15^{b}	390±14.73$^{a\,b}$	81±6.13^{A}	70±4.50^{a}	20±2.87^{A}	22±2.45^{b}	29±3.68^{A}	26±2.66^{b}
4.5	150±6.12^{A}	150±7.36^{B}	280±11.86^{B}	325±11.84^{a}	285±15.57^{b}	340±15.94$^{a\,b}$	57±5.71^{A}	80±6.53^{a}	100±7.76^{A}	26±3.66^{b}	22±2.86^{A}	80±3.57^{b}
6.5	395±14.76^{A}	410±6.59^{B}	415±17.57^{B}	335±15.94^{a}	395±16.75^{b}	355±16.50$^{a\,b}$	60±5.31^{A}	90±7.12^{a}	50±6.12^{A}	31±3.27^{b}	21±2.54^{A}	66±3.47^{b}
8.5	325±9.81^{A}	320±12.25^{B}	460±19.00^{B}	420±18.41^{a}	340±14.34^{b}	435±17.15$^{a\,b}$	85±6.55^{A}	83±6.94^{a}	21±3.29^{A}	24±2.86^{b}	83±4.92^{A}	21±2.28^{b}

CMC= Carboxy methyl cellulose. Means in column with capital as well as with small letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

samples. On the other hand, the freeze-dried casein samples showed a high value of FE in comparison with the oven-dried samples. The freeze-dried casein samples showed poor foam stability than the oven dried samples. The added CMC improved the foam expansion of casein samples but no significant differences ($p<0.05$) were found between the addition of 0.5 or 2% CMC. The results are in agreements with findings reported by Metwally [1997].

Buffer capacity

Buffer index (BI) *versus* pH values of buffalo casein samples, total milk proteinate, rennet casein, and lactic acid casein, as titrated with 0.1N HCl from pH 10.0 to 3.0, are illustrated in Figure 2. Buffer intensity curves of all casein samples followed the same trend in the peak. From the data, it could be generally concluded that the buffer index of buffalo casein samples showed a broad BI peak at pH range from 3.8 to 6.0. At acidic side, the BI decreased progressively and reached a first minimum value at pH about 3.5. As pH was further lowered, an increase in BI was observed at pH 3.0. At alkaline side, the second minimum value of BI was observed at pH range from 7.0 to 8.0, and then an increase was noticed again at pH about 10.0. From the results presented, it could be observed that the buffer index values of lactic acid casein were slightly lower than that of TMP and rennet casein. Different behavior of casein samples in buffer index could be referred to the differences in the form and nature of protein fractions, previously discussed in the electrophoresis patterns of protein samples used in the present work and agree with the results obtained by Metwally & Awad [2001]. The buffer index at pH 3.0 of TMP was about 0.4, while it was about 0.7 for rennet casein or lactic acid casein. The buffering capacity at the maximum point was higher for the freeze-dried casein samples than for the oven-dried casein samples. The results are in the same trend given by Salaün *et al.* [2005], who reported that the maximum buffering capacity of acid casein was in the range of pH 5-6. These results also coincide with those of Morr *et al.* [1973], who reported that the buffer capacity was low at PI region and this may be due to the protein which was insoluble. This report may explain the reason of obtaining the minimum peak in the acidic side.

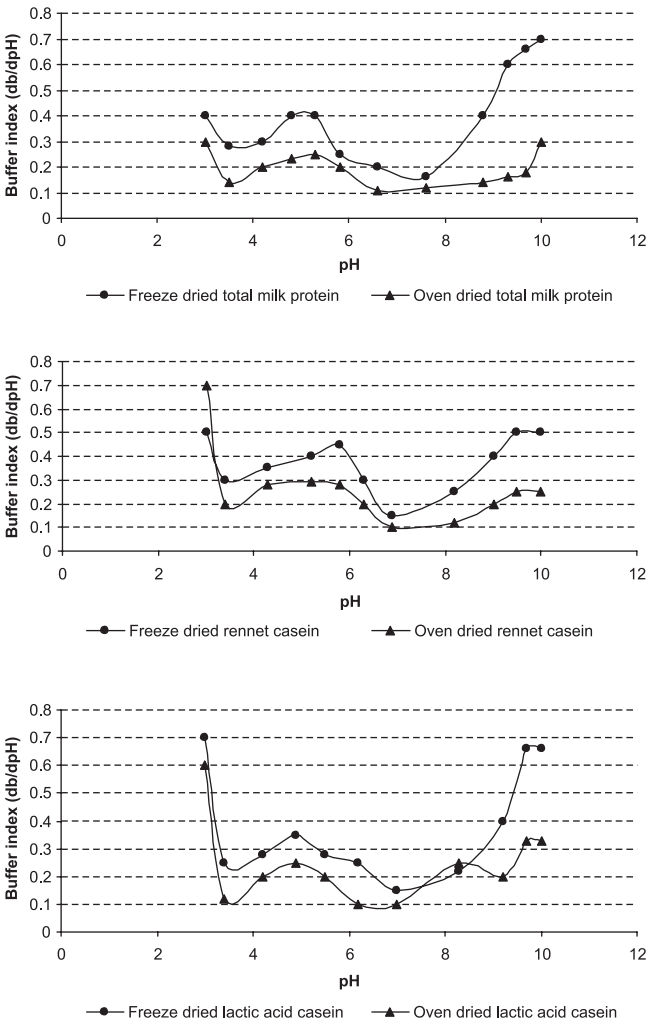

FIGURE 2. Buffer intensity curve for total milk proteinate, rennet casein and lactic acid casein.

Thermogravimetry of casein samples

Figure 3 shows results of the gravimetrical thermal analysis (TG) of the casein samples carried out in the range of 30 to 450°C under oxygen flow. As seen, all TG-curves showed two different reactions, of which the first one is characteristic for the moisture removal and lasted from the start of heating until removal of the moisture and flatten of the mass change curves. The second reaction is a negative sloping part indicating the loss of the organic matter in the casein samples. Under these conditions, the change in mass as well as the necessary endothermic or exothermic energies and the corresponding temperature peaks and ranges were registered and included.

Table 7 shows the data obtained from the thermograms of the tested casein samples. The results showed that the highest moisture level was that of oven-dried lactic acid casein (7.02%). Furthermore, it could be observed that the freeze-dried samples showed, in general, lower moisture content than the oven-dried ones. The energy required to remove moisture ranged from 261.5 to 362.6 J/g depending on the moisture content and the strength of the water binding in the casein samples. These energy values exceeds by 154% to 268.7% the energy needed to evaporate free water (2430 J/g H_2O), indicating that the moisture content of the casein samples is mainly in the form of bound and monolayer water rather than in the form of multilayer or condensed water. This phenomenon was more observed in the total milk proteinates samples than did in rennet or lactic acid casein samples. The peak temperatures of moisture removal were in the range of 62.1°C to 86.4°C, depending on the width of temperature (and time-) range of the moisture removal section of the TG curves. The second section of the TG-curve starts at the end of the moisture removal section, which is characteristic for the thermal degradation of the organic components in casein samples. As seen, all casein samples (except the oven-dried total milk proteinate) showed a single peak of degradation at a very narrow temperature range between 201.1°C and 215°C indicating the degradation of casein protein. However, the oven-dried total milk proteinate samples showed two

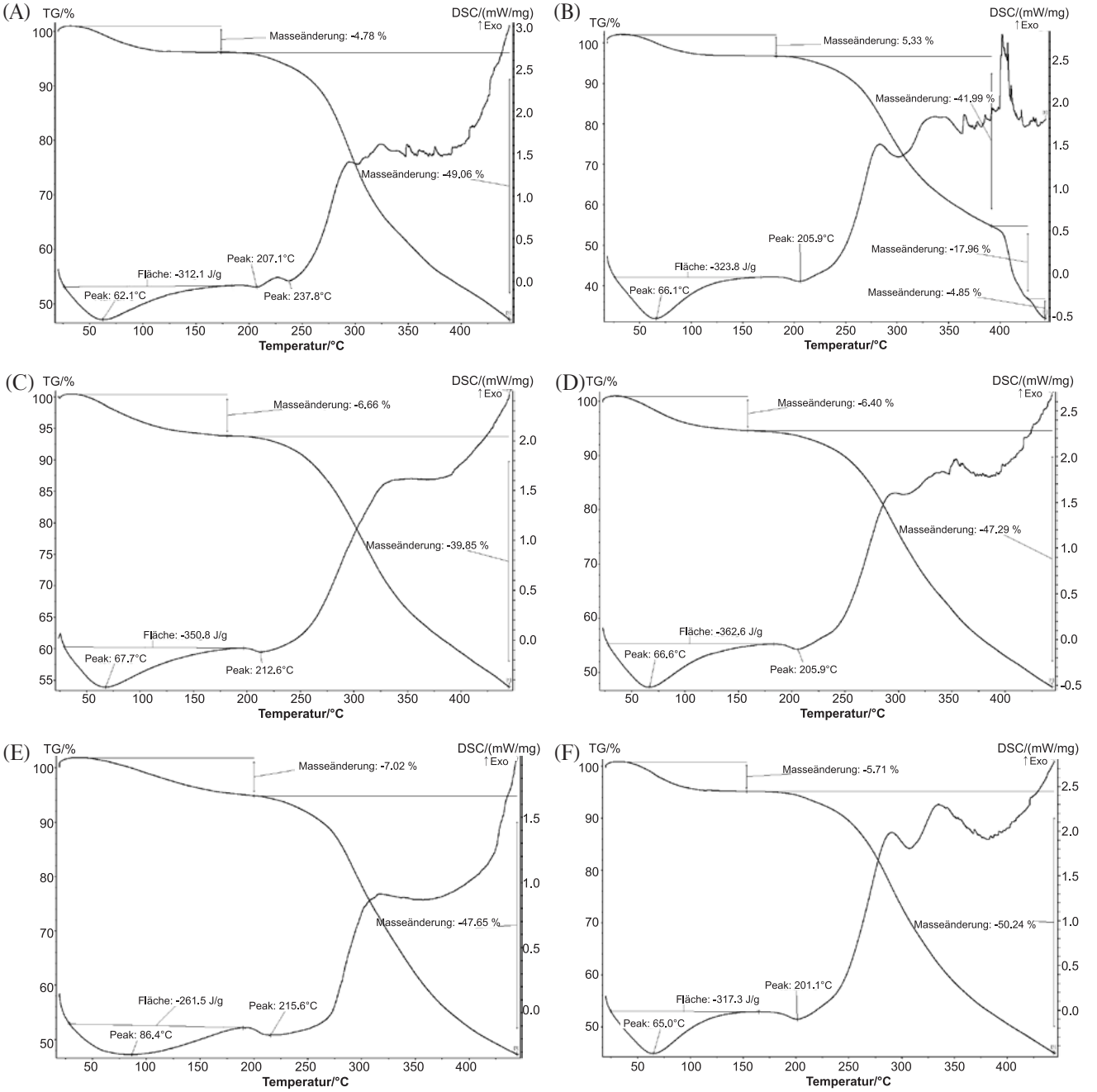

FIGURE 3. Thermo gravimetrical analysis curves of buffalo's milk caseinate powder.
Oven (A) and freeze dried (B) total milk proteinate. Oven (C) and freeze dried (D) rennet casein. Oven (E) and freeze dried (F) lactic acid casein.

TABLE 7. Thermogravemetric parameters of casein samples.

Protein product	Stage of moisture removal			Degradation stage of organic matter	
	Mass change (%)	Peak temperature (°C)	Exothermic energy (J/g)	Peak temperature (°C)	Mass change (%)
Total milk proteinate				207.1±2.03	
Oven dried	4.78±0.13	62.1±1.20	312.1±2.05	237.8±2.80	49.06±0.98[a]
Freeze dried	5.33±0.14	66.1±1.12	323.8±2.48	205.9±1.52	64.8±1.27[b]
Rennet casein					
Oven dried	6.66±0.15	67.7±1.02	350.8±2.80	212.6±2.04	39.85±1.23[a]
Freeze dried	6.40±0.12	66.6±1.14	362.6±2.46	205.0±2.05	47.29±1.00[b]
Lactic acid casein					
Oven dried	7.02±0.21	86.4±1.23	261.5±1.65	215±2.02	47.65±1.10[a]
Freeze dried	5.70±0.14	65.0±1.02	317.3±2.04	201±2.86	50.24±1.23[b]

Means in column of each group with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

distinguishing peaks at 207.1°C and 237.8°C, which may be due to the presence of casein protein and whey proteins. At the end of heating range (450°C), the total change in mass of protein samples was in the range of 47.29% to 50.24%, except for the oven-dried rennet casein (39.85%) and the freeze-dried total milk proteinate (64.8%), which corresponds with their protein content.

Differential Scanning Calorimetry (DSC) of casein samples

Figure 4 shows DSC diagrams of the tested casein samples. The thermograms are characterised by the presence of some thermal peaks. The first peak temperature was in the range of 92.2°C to 100.8°C and the energy value between 218.3 to 268.4 J/g. These peaks are characteristic for energy required to remove the moisture from the casein samples, with rennet casein and oven-dried acid casein samples showing the need for higher energy values, than did the freeze-dried acid casein or total milk proteinate. The total energy demand is well correlated with the moisture content of the casein samples given in Table 7. The second groups of peaks were in the range of 273.9°C to 314.6°C characterising the degradation of protein molecules present in the casein samples. Acid casein and rennet casein showed a big peak at 313.1°C, 313.6°C and 314.6°C and a very minor peak at 273.9°C and 282.1°C. On the other hand, the total milk proteinate showed two major peaks at 303.2°C and 286.7°C. This means that both acid and rennet casein consists principally from milk casein combined with traces of whey protein, while total milk proteinate contains a considerable amount of whey proteins. The DSC diagrams give a rapid test to differentiate between the different casein samples and their preparation methods with the help of the obtained peaks of the thermal degradation. These results agree with the observations of Gloyna *et al.* [1991]. They mentioned that amino acids and proteins did not show any considerable thermal degradation in the temperature range of 230 to 300°C and their thermal peaks appeared strongly at temperature exceeding 300°C. This method was also applied to differentiate between α and β-lactose in whey powder and their concentrations [Ross, 1978].

CONCLUSIONS

This work showed the influence of different buffalo's protein sample preparation and drying methods on some of their functional and thermal properties. There is no remarkable effect of drying methods on the chemical composition within each type of protein products. Total milk proteinate and lactic acid casein contained more essential amino acids than the rennet casein. Drying methods clearly affected the peak intensity of the molecular weight characteristics of buffalo's milk casein samples as well as emulsion activity index values. Also, the freeze-dried samples showed higher WAC and OAC values as well as thermal mass change compared with the oven-dried samples.

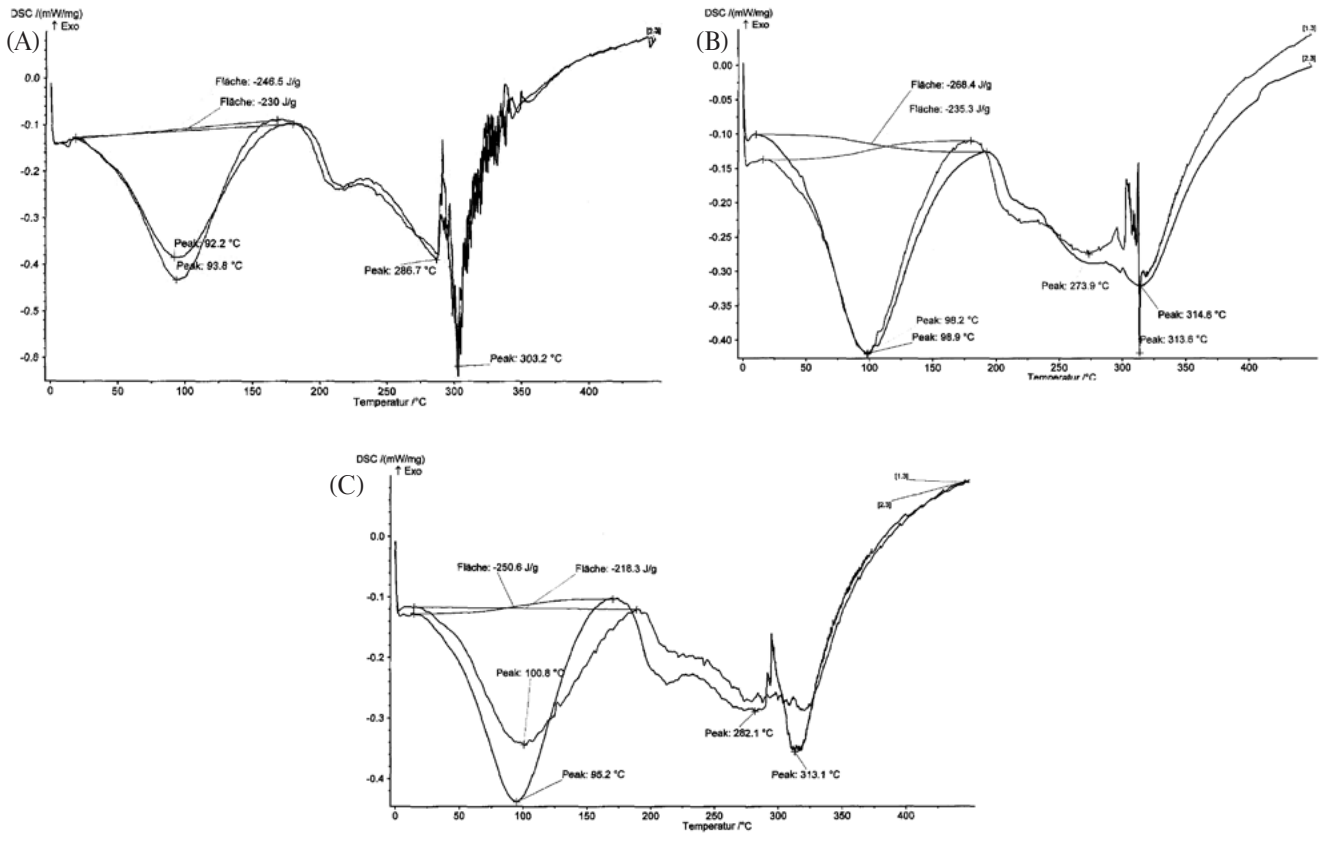

FIGURE 4. Differential scanning calorimetric (DSC) curves of buffalo's milk caseinate powder.
(A) Oven and freeze dried total milk proteinate. (B) Oven and freeze dried rennet casein. (C) Oven and freeze dried lactic acid casein.

REFERENCES

1. Alsmeyer H.R., Cuningham A.E., Happich M.L., Equation predict PER from amino acid analysis. Food Technol., 1974, 28, 34–40.
2. AOAC, Association of Official Analytical Chemists. Official Methods of Analysis. 2007, 18th Ed., Chapter 33, pp. 7, 10–14, Benjamin Franklin Station Washington, D.C., USA.
3. Cohen S.A., Meys M., Tarvin T.L., A Manual of Advanced Techniques for Amino Acid Analysis. 1989, Waters Company, USA.
4. Czuchajowska A., Pomeranz Y., Differential scanning calorimetric, water activity and moisture contents in crumb center and near-crust zones of bread during storage. Cereal Chem., 1989, 66, 305–309.
5. Dean A.L, Mariette F., Lucas T., Marin M., Assessment of the state of water in reconstituted milk protein dispersions by Nuclear Magnetic Resonance(NMR) and Differential Scanning Calorimetry (DSC). Lebens. Wiss. Technol., 2001, 34, 299–305.
6. Dekanterewicz R.J., Elizalde E.B., Pilosof R.M.A., Bartholomi B.G., Water-oil absorption index (WOAI): A simple method for predicting the emulsifying capacity of food proteins. J. Food Sci., 1987, 52, 1381.
7. Fox P.F., Development in Dairy Chemistry. Chapter 4. Functional Milk Proteins. 1989, Elsevier Appl. Sci., London and New York.
8. Gloyna D., Schreck S., Schierbaum F., Thermoanalytical studies on starch and starch containing systems. Starch/Staerke, 1991, 43, 431–437.
9. Jayasena V., Chih H.J., Nasar-Abbas S.M., Functional properties of sweet lupin protein isolated and tested at various pH levels. Res. J. Agric. Biol. Sci., 2010, 6, 130–137.
10. Kristrnsen D., Jensen P.Y., Madsen F., Birdi K.S., Rheology and surface tension of selected processed dairy fluids: Influence of temperature. J. Dairy Sci., 1997, 80, 2282–2290.
11. Lampert L.M., The milk proteins. 1970, *in*: Modern Dairy Products. Eurasia Publishing House (P) LTD., Ram Nagar, New, p. 37.
12. Metwally A.I., Comparative study between functional properties of spray dried buffalos ultrafiltrated skim milk retentate and total milk proteinate. Annals Agric. Sci., Ain Shams Uni. Cairo, 1997, 42, 313–320.
13. Metwally A.I., Awad R.A., Buffer intensity and function properties of low and high heat milk powders. Egyptian J. Dairy Sci., 2001, 29, 19–28.
14. Metwally A.I., Smith D.M., Surface activity and amino acid and mineral contents of buffalo milk protein products. Proc. 8th Egypt. Conf. Dairy Sci. & Techn., 2001, pp. 211–222.
15. Morr C.V., Functional of heated milk proteins in dairy and related foods. J. Dairy Sci., 1985, 68, 2773–2781.
16. Morr C.V., Functional of milk protein and their use as food ingredients.1982, *in*: Development in Dairy chemistry. 1- Protein (ed. P.F. Fox). Applied Science. London and New York, pp. 375–399.
17. Morr C.V., Swenson E.P., Richter L.R., Functional characteristics of whey protein concentrate. J. Food Sci., 1973, 38, 324–330.
18. Patel P.D., Stripp M.A., Fry C.J., Whipping test for the determination of foaming capacity of protein: A collaborative study. Int. J. Food Sci. Technol., 1988, 23, 57–63.
19. Pearce K.N., Kinsella J.E., Emulsifying properties of proteins: Evaluation of a turbid- metric technique. J. Agric. Food. Chem., 1978, 26, 716–723.
20. Raikos V., Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. Food Hydrocoll., 2010, 24, 259–265.
21. Rao M.A., Rheology of fluid and semisolid foods. 1999, Aspen Puplication, Maryland, USA, pp. 87–88.
22. Rawel H.M, Rohnes S., Krall J., Influence of a sugar moiety (rhamnosylglucoside) at 3-O position on the reactivity of quercetin with whey protein. Int. J. Biol. Macromol., 2003, 32, 109––120.
23. Ross K.D., Rapid determination of α- lactose in whey protein by differential scanning calorimetry. J. Dairy Sci., 1978, 61, 255––259.
24. Salaün F., Mietton B., Gaucheron F., Buffering capacity of dairy products. Int. Dairy J., 2005, 15, 95–109.
25. Salaün F., Mietton B., Gaucheron F., Influence of mineral environment on the buffering capacity of casein micelles. Milchwissenschaft, 2007, 62, 20–23.
26. Singh H., Ye A., Interactions and functionality of milk proteins in food emulsions. 2009, *in*: Milk Proteins: from Expression to Food (eds. A. Thompson, M. Boland, H. Singh). Academic Press, San Diego, CA,). pp. 321–345.
27. Snoeren T.H.M., Damman A.J., Klok H.J., The viscosity of skim milk concentrate. Neth. Milk Dairy J., 1982, 36, 305.

Received July 2009. Revision received March and accepted August 2010.