International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 2 (3): 196-206, 2007
ISSN 1811-9743

Rheological and Functional Properties of Whey Protein Concentrate and β-Lactoglobulin and α-Lactalbumin Rich Fractions

S. El-Shibiny, A.F. Farrag, G. El-Garawany and F.M. Assem
Department of Dairy, National Research Center, Dokki, Cairo, Egypt

Abstract: Whey from Edam cheese manufacture was concentrated by ultrafiltration (Concentration factor 20) and Whey Protein Concentrate (WPC) was fractionated into β-lactoglobulin (β-Lg) and α-lactalbumin (αLa) rich fractions by two different methods. The composition, emulsifying capacity and stability and foam capacity and stability of WPC and its fractions were determined. Also, the viscosity of WPC and β-Lg and α-La rich fractions were followed as affected by the addition of different concentrations of NaCl and $CaCl_2$. The results indicated that the yield and composition of the prepared fractions by the two methods were not identical. However, β-Lg fraction showed higher Emulsifying Capacity (EC) followed by (α-La) rich fraction compared with lowest EC for the original WPC solution. On the other hand, emulsion stability of WPC and its fractions was nearly the same. The foaming capacity of WPC, β-Lg and α-La increased slightly with increasing whipping time up to 15 min. α-La rich fraction showed the lowest foam capacity, as compared to β-Lg and WPC. Addition of sodium chloride decreased the viscosity of solutions of WPC and its fractions solutions with a linear relation between the NaCl concentration and the decrease in the viscosity. Addition of NaCl up to 50 mg/100 mL increased slightly the viscosity of β-Lg solution but further increase of NaCl decreased its viscosity. Addition of $CaCl_2$ was found to increase the viscosity of WPC solution reaching a maximum when 60 mg $CaCl_2$/100 mL and then decreased on further increase in the added $CaCl_2$. The viscosity of α-La solutions with different NaCl contents decreased with the increase in temperature. Also, addition of $CaCl_2$ up to 60 mg/100 mL to α-La solution increased its viscosity.

Key words: WPC, β-lactoglobulin, α-lactalbumin, emulsification, foaming, viscosity

INTRODUCTION

The last two decades have witnessed a growing interest in the use of whey proteins as functional ingredients in a wide range of food products due to their excellent nutritional and health attributes (McIntosch *et al*., 1998) and their versatile functional properties (Harper, 1991; Korhonen *et al*., 1998). Recently undenatured whey proteins were reported to enhance of immune statues through intercellular glutathione synthesis (Middleton *et al*., 2003).

Out of the different whey protein products, Whey Protein Concentrates (WPC) is the most widely utilized product nowadays. Ji and Haque (2003) found that the emulsion stability of WPC solutions was significantly different based on the source of whey. Also, Onwulata *et al*. (2004) reported that variations in the functionality of WPC from different manufacturers can be minimized by reducing their particle size distribution within a narrow range. The variability in the functional properties of the commercially available WPC is a limiting factor in their potential applications. Emulsifying properties of whey protein concentrate was greatly affected by the changes in pH and heat treatment before ultrafiltration (Fachin and Viotto, 2005). Recently, Dickinson and Parkinson (2004) and Parkinson and Dickinson (2004) showed that very small amounts of casein were able to

Corresponding Author: S. El-Shibiny, Department of Dairy, National Research Center, Dokki, Cairo, Egypt

exert a protective stabilizing effect on whey protein-stabilized emulsions when subjected to thermal treatment (e.g., at 90°C for 3 min). Effect of purified β-lactoglobulin and purified α-lactalbumin or mixture of purified β-Lg and α-La on the rheological properties of acid skim milk gels has been done (Bikker and Anema, 2003). The synergistic effect by which a very small amount of casein can confer stability to a whey protein-stabilized emulsion heated to 90°C has been studied by Parkinson and Dickinson (2007).

Viscosity data are often derived from single-point measurements. Morison and Mackay (2001) suggested that Einstein's equation can be used for whey protein concentrates up to 15% and proposed on empirical relationship for high concentrations. Remeuf *et al*. (2003) fortified heated yoghurt milk with WPC. After heating viscosity and graininess of yoghurt prepared from WPC-enriched milk increased. Solutions of Whey Protein Isolate (WPI) behaved as Newtonian fluids at concentration less than 10% WPI, while higher concentrations demonstrated a non-Newtonian behaviour (Patocka *et al*., 2006).

One of the proposals to solve this problem is to fractionate WPC into its component proteins or at least into more simple mixtures of whey proteins. This would develop products of variable and more consistent functional properties suitable for different uses.

Several methods have been developed for industrial fractionation of WPC. These methods produce mainly two fractions each rich in one of the major whey proteins i.e., β-lactoglobulin and α-lactalbumin (Pearce, 1983; Maubois *et al*., 1987; Mate and Krochta, 1994; Maubois and Olvier, 1997). However, little has been cited on comparing the composition and properties of fractions obtained by these methods.

Although several studies have been carried out on the functional properties of Whey Protein Concentrates (WPC), little has been done on the individual whey proteins fractions particularly β-Lg and α-La rich fractions. Therefore, the present research describe the fractionation of whey protein concentrates following the methods of Mate and Krochta (1994) and Maubois and Olivier (1997), respectively and comparing the composition and functional and flow properties of the obtained fractions.

MATERIALS AND METHODS

Sweet whey (pH 6.2) was obtained from the manufacture of Edam cheese at the Arab Dairy Co., Kaha. Whey was separated using a cream separator to remove residual fats and cheese fines. The separated whey was ultrafiltered using Carbosep S151 UF-pilot plant equipped with 6.3 m^2 zirconium oxide tubular membrane (Mol. Cut off 50,000 Da). Ultrafiltration was carried out at 30°C and 6 and 4 bar inlet and outlet pressure to a concentration factor of 20 (Total solids of whey protein concentrate 20%). Fractionation of Whey Protein Concentrate (WPC) was carried out using 100 mL of the WPC (Laboratory scale) and using 3 L of WPC (large scale) by the following methods:

Method I

The pH of the WPC was adjusted to 4.5 using 1 N HCl, heated at 55°C for 20 min with continuous stirring. The formed precipitate was separated by centrifuging at 10,000 g (Joan cooling centrifuge, France) for 20 min. The precipitate was considered as the α-lactalbumin (α-La) rich fraction while the supernatant was considered as the β-lactogloulin (β-Lg) rich fraction (Maubois and Olivier, 1997).

Method II

The WPC was adjusted to pH 2 with 1 N HCl and NaCl was added in it to give 7% (w/w) in solution. The salted acidified WPC was left to stand for 20 min at room temperature. The formed

precipitate was separated by centrifuging at 10,000 g for 20 min. The precipitate was considered as the α-La rich fraction and the supernatant as the β-Lg rich fraction. The separated fractions were dialyzed to remove the NaCl (Mate and Krochta, 1994).

Methods of Chemical Analysis and pH

The total solids content and ash content were determined (AOAC, 1975), total nitrogen (IDF, 1993), fat content by Gerber method (Ling, 1963) and lactose content (Barrnett and Tawab, 1957) and pH using a pH meter with combined electrode. The proteins in WPC and separated fractions were separated by SDS-polyacrylamide electrophoresis (Lamilli, 1970) and the relative content of β-Lg and α-La was assessed visually from the relative intensity of their separated zones..

Determination of Emulsion Capacity and Stability

The method of Pearce and Kinsella (1978) was adopted. Ten milliliters of corn oil were emulsified with 30 mL of an aqueous solution (0.5% protein) of WPC or its fractions for 3 min using a blender (Hamilton Model 600 Al, USA). One milliliter of the emulsion was diluted in a 1000 mL volumetric flask and made up to volume with 0.1% sodium dodecyl sulphate. The absorbance of the solution was measured at 500 nm using a spectrophotometer (Shimadzu UV-240, Japan). The Emulsifying Capacity (EC) was determined as emulsifying activity index by the following equation:

$$\text{EAI}(\text{m}^2\ \text{g}^{-1}) = \frac{2 \times 2.303 \times \text{absorbance at } 500\,\text{nm}}{25 \times \text{path length of cuvette (in meter)} \times \text{pretein concentration } (\text{g m}^{-3})}$$

To determine the Emulsion Stability (ES), the emulsion was held at room temperature and its EC was measured after 1, 2 and 3 days as described above. The changes in EAI indicates the emulsion stability.

Foam Capacity

The method of Patel *et al*. (1988) was adopted. Solutions of WPC, α-La and β-Lg (0.5%) were whipped separately for 5, 10 and 15 min using a blender (Hamilton AL, USA) at whipping speed. The foam capacities of these solutions were calculated from the equation:

$$\text{Foam capacity (\%)} = \frac{\text{Volume of whipped sample (including liquid)} - \text{Sample volume}}{\text{Sample volume}} \times 100$$

Viscosity Measurement

A coaxial cylinder viscometer (Buhlin V88, Sweden) equipped with C30 system which permits a gap of 1.5 mm between the two cylinders and attached to a workstation loaded with V88 viscometry programme was used. The measuring system was filled with about 40 mL 1% protein solution of WPC, β-Lg or α-La preparations, placed in a controlled temperature water bath, heated to the desired temperature before viscosity measurements. Measurement of viscosity was carried out at a shear rate of 265 1/s, at 2 min intervals.

RESULTS AND DISCUSSION

Chemical Composition and pH

Table 1 shows the chemical composition of WPC and α-La and β-Lg rich fractions obtained from laboratory (100 mL) and large scale (3 L) experiments using the two methods of fractionation. The β-Lg rich fraction was found to contain higher protein, ash and lactose contents than that the α-La rich

Table 1: Composition of whey protein concentrate, α-lactalbumin and β-lactoglobulin prepared at pH 4.6*and 2.0**

	Large scale						Lab. scale					
	pH 4.6*			pH 2.0**			pH 4.6*			pH 2.0**		
Test %	WPC	α-La	β-Lg	WPC	α-La	β-Lg	WPC	α-La	β-Lg	WPC	α-La	β-Lg
TS	20.20	20.90	25.80	20.02	22.63	31.30	21.26	25.77	18.77	21.28	29.24	22.41
pH	6.48	4.60	4.60	6.48	2.00	2.40	6.21	4.50	4.40	6.20	1.89	2.09
Acidity	0.18	0.80	0.80	0.18	1.60	1.43	0.48	1.43	0.87	0.48	1.79	1.94
Fat	0.08	0.00	0.00	0.08	0.00	0.00	0.10	0.00	0.00	0.10	0.00	0.00
TP	15.20	16.00	19.30	15.30	17.90	23.40	11.48	11.50	12.30	11.48	12.00	13.20
TP/DM	76.20	76.50	74.70	76.40	78.90	74.70	54.00	44.63	65.18	53.95	40.87	58.90
Lactose	4.10	3.60	4.77	4.10	3.80	4.80	3.55	3.57	3.94	3.55	3.68	4.01
Lactose/DM	20.50	17.27	18.50	20.50	16.77	15.67	16.70	13.85	20.99	16.68	12.53	17.89
Ash	0.50	0.50	1.37	0.50	0.66	1.38	2.53	2.35	2.88	2.30	2.45	2.99
Ash/DM	2.48	2.40	5.30	2.48	2.90	4.53	11.92	9.12	15.34	10.61	8.35	13.34

*: At pH 4.6 Method of Maubois and Oliver (1997); **: At pH 2.0 method of Mate and Krochta (1994)

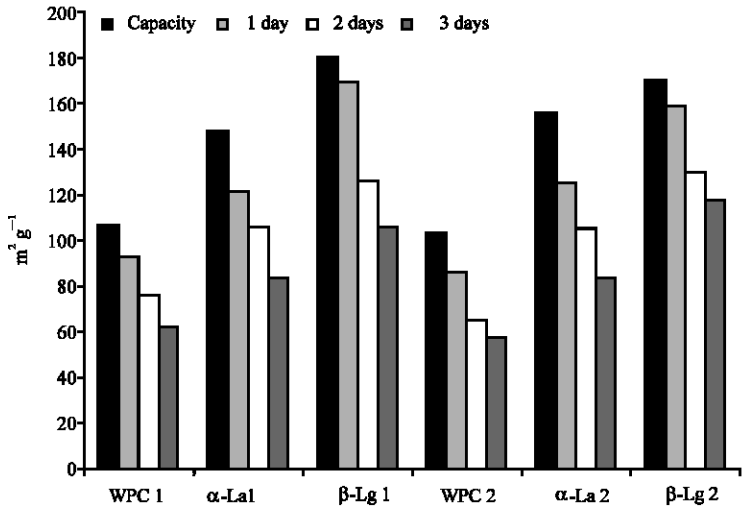

Fig. 1: Emulsifying capacity (m^2 g^{-1}) and stability for 3 days of WPC, α-lactalbumin and β-lactoglobulin

fraction obtained from the two methods of fractionation. The WPC and its fractions contained very low fat content and even not detected in the prepared fraction. Electrophoretic separation revealed that WPC, α-La and β-Lg rich fractions had almost the same protein pattern. However, the relative contents of the protein fractions were not the same in the α-La and β-Lg rich fractions. The former was characterized by high intensity of α-La zone while β-Lg rich fraction was characterized by high intensity of β-Lg zone. Both fractionation methods gave nearly the same results.

Emulsifying Capacity and Stability

It is obvious from the Fig. 1 results that the WPC fractions prepared by the two methods behaved similarly. Thus, β-Lg rich fraction had higher Emulsifying Capacity (EC) than the α-La fraction. β-Lg was reported to be more hydrophobic than α-La (Brown, 1984). β-Lg was found almost as surface active as β-casein in terms of the final lowering of the interfacial tension (Dickinson, 1997). Also, Yamauchi *et al*. (1980) found that β-Lg, immunoglobulin and lactoferrin selectively adsorbed on the surface of fat globules at pH 7, while the adsorption of α-La increased markedly at pH 5 or 3. This can explain the present finding as the emulsifying capacity was carried out at pH 7.

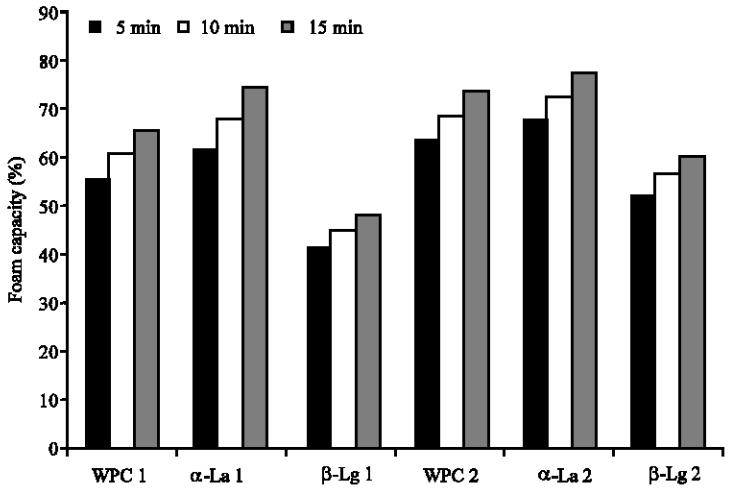

Fig. 2: Foam capacity (%) at different whipping time of WPC, α-Lactalbumin and β-Lactoglobulin

However, both α-La and β-Lg fractions showed higher EC than the original WPC solution which can be attributed to the removal of constituents impairing EC during the fractionation process. The EC of WPC and its fractions decreased during storage for 3 days. However, the rate of decrease of EC in WPC and its fractions during storage was nearly the same, which suggest almost similar stability of emulsions prepared the WPC and its fraction prepared by the two methods. This can be understood from the fact that the average size of fat globule is a major factor in the stability of the formed emulsion (Harper, 1991) and that the method used for the preparation of emulsion would yield fat globules of similar sizes.

Foam Capacity

From Fig. 2 it was observed that foam capacity was found to increase slightly by increasing the whipping time from 5 to 15 min. Comparing the foam capacity of WPC and its fractions α-La rich fraction showed the highest and β-Lg rich fraction the lowest foam capacity. These results were not in agreement with previous reports. Kim *et al.* (1987) found that foam overrun and gel strength were significantly correlated with β-Lg content. Also, Casper *et al.* (1999) reported that ovine whey protein contained higher β-Lg content and foam capacity than bovine and caprine WPC. Variations in the quantities of lipid, ash or proteose peptone and the degree of denaturation affect the foam capacity of WPC (Harper, 1991) which may explain the present results.

Changes in the Viscosity of Heated WPC Solution

Effect of NaCl

The viscosity of WPC solution was affected markedly by the percentage of the NaCl added (Fig. 3). Thus WPC solution without added NaCl showed the lowest viscosity compared to WPC solutions with added NaCl at the different temperatures.

Increasing the temperature of measurement from 50 up 70°C decreased the viscosity of WPC without added NaCl but further increase in the temperature to 80°C increased its viscosity. This can be attributed to heat denaturation and aggregation of β-Lg which show a maximum at 78°C (Foegeding *et al*., 2002). Similar results have been reported by Abd El-Salam *et al.* (1993).

Addition of 50 mg/100 mL of NaCl to WPC solution increased its viscosity from 5.46, 5.36, 4.96 and 6.56 mPas to 6.07, 7.01, 7.04 and 7.79 mPas at 50, 60, 70 and 80°C, respectively. Further increase

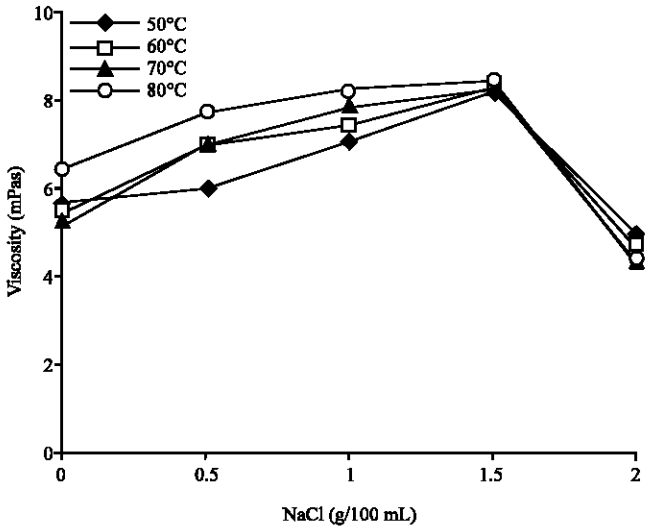

Fig. 3: Viscosity of WPC solution as affected by temperature and NaCl

of added NaCl to 100 and 150 mg/100 mL increased the viscosity of the WPC solution to 7.06, 7.51, 8.11 and 8.45 and 8.19, 8.31, 8.28 and 8.41 mPas at 50, 60, 70 and 80°C, respectively. However, addition of 200 mg/100 mL of NaCl to the WPC solution decreased its viscosity to 5.14, 4.82, 4.37 and 4.32 mPas, respectively.

Comparing changes in viscosity of WPC solutions as a function of temperature and NaCl up to 150 mg/100 mL suggest that NaCl enhanced the formation of larger size aggregates. This agreed with that reported by Ikeda (2003) who found that the size of primary aggregates and rate of aggregation of β-Lg increased with the increasing NaCl concentrations. The decrease in viscosity of WPC solution containing 200 mg/100 mL of NaCl may be explained on the basis that the size of the formed aggregates exceeded a maximum to form a precipitate at the low protein concentration of WPC studied.

Effect of $CaCl_2$

The addition of 20, 40 and 60 mg/100 mL of $CaCl_2$ to WPC solution (1%) increased its viscosity when heated to 50, 60, 70 and 80°C from 5.46, 5.36, 4.98 and 4.66 mPas for WPC without added $CaCl_2$ to 17.31, 17.95, 17.05 and 16.12 mPas when 60 mg/100 mL of $CaCl_2$ were added (Fig. 4).

The whey proteins seems to form aggregates on the addition of Ca which increases its viscosity. Hollar and Parris (1995) reported that at lower Ca concentration more soluble aggregates and less insoluble precipitate of whey proteins were formed when heated. As a role the formation of soluble aggregates in partially denatured whey proteins would stabilize the system and increase its viscosity.

Further increase in the added $CaCl_2$ to 80 and 100 mg/100 mL to WPC solution decreased its viscosity to 13.56, 13.90, 14.32 and 13.75 and 8.68, 7.28, 4.19 and 4.93 mPas at 50, 60, 70 and 80°C, respectively. This suggest the increase of the formation of the insoluble precipitate of denatured whey proteins at $CaCl_2 \geq 80$ mg/100 mL decrease the viscosity of WPC solution.

Changes in the Viscosity of Heated β-lactoglobulin Solutions

Effect of NaCl

The effect of added NaCl on the viscosity of heated β-Lg solution (Fig. 5) followed similar trend to its effect on heated WPC solution (Fig. 3). Thus the addition of 50 mg/100 of NaCl to β-Lg solution increased slightly its viscosity from 4.04, 4.03, 3.63 and 3.41 to 4.52, 4.12, 3.89 and

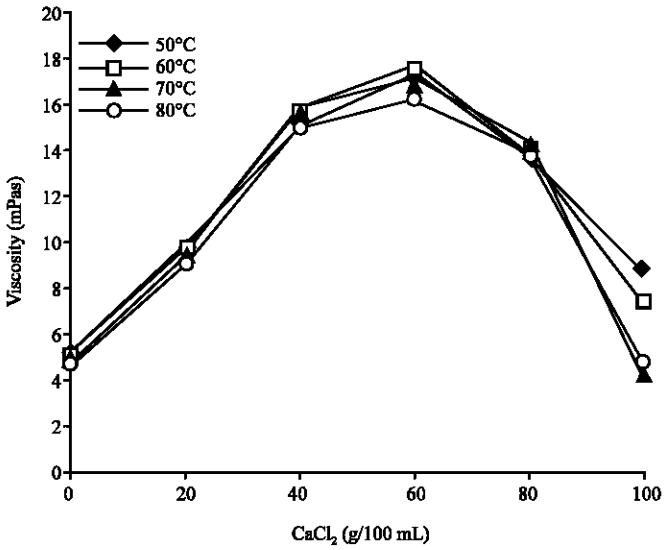

Fig. 4: Viscosity of WPC solution as affected by temperature and $CaCl_2$

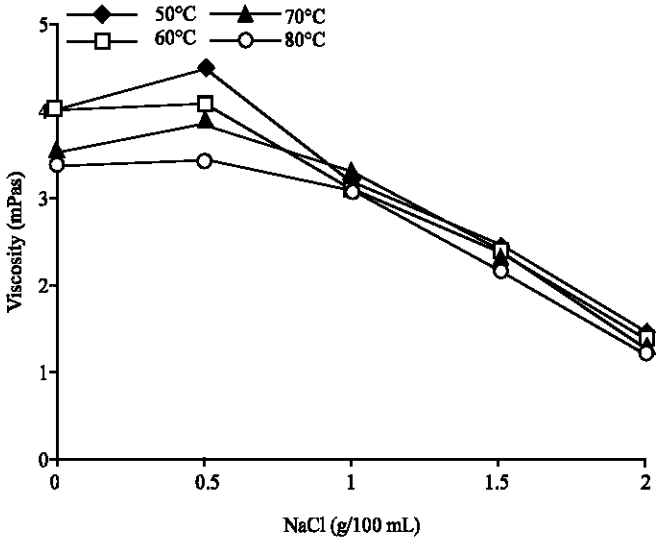

Fig. 5: Viscosity of β-lactoglobulin solution as affected by temperature and NaCl

3.46 mPas at 50, 60, 70 and 80°C, respectively. Further increase of added NaCl up to 200 mg/100 mL decreased the viscosity of β-Lg solution to 1.51, 1.43, 1.32 and 1.23 mPas at 50, 60, 70 and 80°C when 200 mg/100 mL of NaCl were added.

The NaCl seems to be able to modify the conformation of β-Lg and decreasing its thermal stability. Increasing added NaCl may mask some exposed ionic groups and alter the electric double layer to facilitate chain interaction between denatured β-Lg molecules (Youling *et al*., 1993).

Effect of $CaCl_2$

The addition of $CaCl_2$ to 1% β-Lg solution up to 60 mg/100 mL increased its viscosity (Fig. 6). Thus the viscosity increased from 4.04, 4.03, 3.63 and 3.41 mPas for WPC solution at 50, 60, 70 and

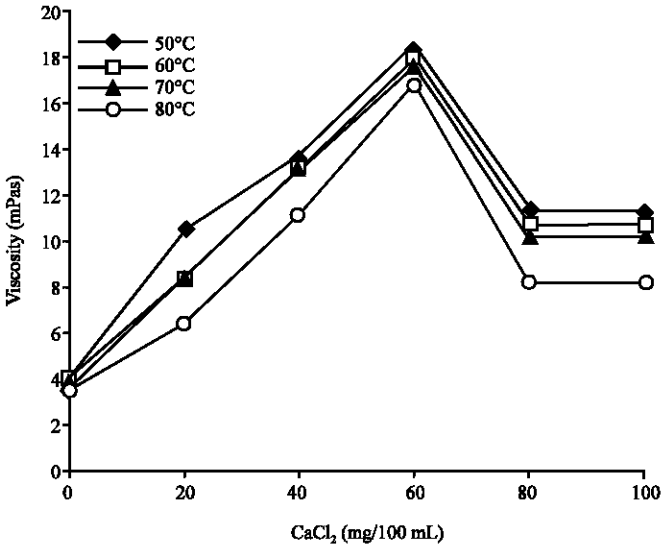

Fig. 6: Viscosity of β-lactoglobulin solution as affected by temperature and $CaCl_2$

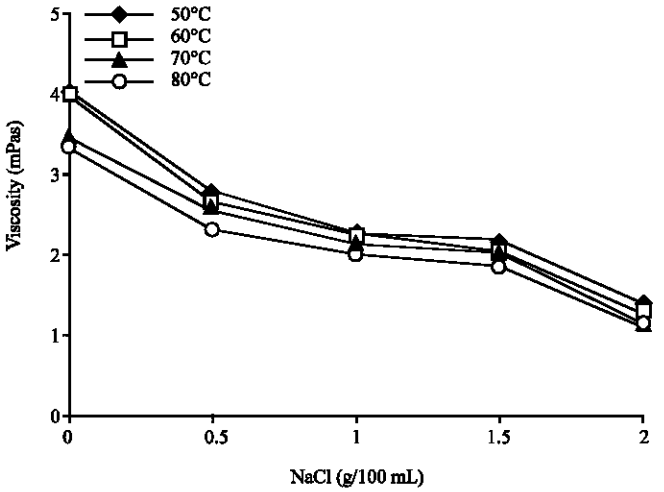

Fig. 7: Viscosity of α-lactoalbumin solution as affected by temperature and NaCl

80°C, respectively to 18.24, 17.82, 17.51 and 16.29 mPas for WPC solution with added 60 mg/100 mL of $CaCl_2$ in the same order. These results are online with that obtained for WPC solution. Addition of 80 and 100 mg/100 mL of $CaCl_2$ to β-Lg solution decreased its viscosity to 11.24, 10.46, 9.83 and 8.13 and 11.20, 10.41, 9.81 and 8.11 mPas at 50, 60, 70 and 80°C, respectively. Sherwin and Foegding (1997) reported that excess calcium inhibited in some way the aggregation of denatured whey proteins, which may explain the present results.

Changes in the Viscosity of α-lactalbumin Solution

Effect of NaCl

The viscosity of α-La solutions with different NaCl contents was found to decrease with the increase of temperature (Fig. 7). This can be explained on the basis the α-La resisted denaturation under

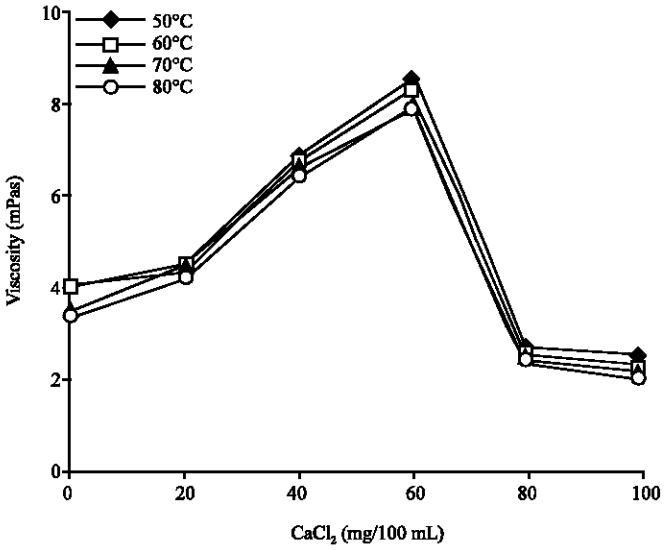

Fig. 8: Viscosity of α-lactoalbumin solution as affected by temperature and $CaCl_2$

the heating conditions used (Foegeding *et al*., 2002). The observed decrease in the viscosity can be attributed to the general effect of temperature on viscosity.

The addition of increasing NaCl concentrations to the α-La solution decreased its viscosity and the rate of decrease can be related to the percentage of NaCl added. Thus the viscosity of α-La solution was 3.99, 3.95, 3.46 and 3.28 mPas at 50, 60, 70 and 80°C, respectively that decreased to 2.78, 2.64, 2.56 and 2.25 and 1.38, 1.22, 1.07 and 1.05 mPas when 50 and 200 mg/100 mL of NaCl were added. The addition of NaCl would increase the repulsive forces and decreases the aggregation of α-La molecules and in turn decreases the viscosity of its solution.

Effect of $CaCl_2$

The addition of $CaCl_2$ up to 60 mg/100 mL of α-La solution increased its viscosity (Fig. 8). Thus the viscosity of α-La solution increased from 3.99, 3.95, 3.48 and 3.28 mPas at 50, 60, 70 and 80°C, respectively to 8.78, 8.47, 7.89 and 7.85 mPas in the same order when 60 mg/100 mL of $CaCl_2$ were added. However, further increase of added $CaCl_2$ to 80 and 100 mg/100 decreased the viscosity of α-La solution to 2.71, 2.49, 2.36 and 2.25 and 2.61, 2.22, 2.15 and 1.95 mPas in the same order. This can be attributed to decreased aggregation of α-La molecules (Sherwin and Foegeding, 1997).

REFERENCES

Abd El-Salam, M.H., S. El-Shibiny and H.M. El-Etriby, 1993. Rheological properties of whey protein concentrate as affected by heating conditions. IDF Special Issue No. 9303: 242-250.

AOAC, 1975. Official Methods of Analysis. 12th Edn., Association of Official Analytical Chemists, Wachington, DC.

Barrnett, A.J.G. and A. Tawab, 1957. A rapid method for the determination of lactose in milk and cheese. J. Sci., Food Agric., 8: 437-441.

Bikker, J.F. and S.G. Anema, 2003. Effect of individual whey proteins on the rheological properties of acid gels prepared from heated skim milk. Int. Dairy J., 13: 401-408.

Brown, E.M., 1984. Interactions of β-lcatoglobulin and α-lactalbumin with lipids: A review. J. Dairy Sci., 67: 713-722.

Casper, J.L., W.L. Wendorff and D.L. Thomas, 1999. Functional properties of whey protein concentrates from caprine and ovine specialty cheese whey. J. Dairy Sci., 82: 265-271.

Dickinson, E., 1997. Properties of emulsion stabilized with milk proteins: Overview of some recent developments. J. Dairy Sci., 80: 2607-2619.

Dickinson, E. and E.L. Parkinson, 2004. Heat-induced aggregation of milk protein stabilized emulsions. Sensitivity to processing and composition. Int. Dairy J., 14: 635-645.

Fachin, L. and H.W. Viotto, 2005. Effect of pH and heat treatment of cheese whey on solubility and emulsifying properties of whey protein concentrate produced by ultrafiltration. Int. Dairy J., 15: 325-332.

Foegeding, E.A., J.P. Davis, D. Doucet and M.K. McGuffey, 2002. Advances in modifying and understanding protein functionality. Trends Food Sci Technol., 13: 151.

Harper, W.J., 1991. Functional properties of whey protein concentrates and their relationship to ultrafiltration. IDF Special Issue No. 9201: 77-108.

Hollar, C.M. and N. Parris, 1995. Factors affecting the denaturation and aggregation of whey proteins in heated whey protein concentrate mixtures. J. Dairy Sci., 78: 260.

IDF, 1993. Determination of total nitrogen of milk. 20A, International Dairy Federation, Brussel, Belgium.

Ikeda, S., 2003. Heat-induced gelation of whey proteins observed by rheology, atomic force microscopy and raman scattering spectroscopy. Food Hydrocolloids, 17: 399.

Ji, T. and Z.U. Haque, 2003. Cheddar whey processing and source: 1- Effect of composition and functional properties of whey protein concentrates. Int. J. Food Sci. Technol., 38: 453-461.

Kim, Y.A., G.W. Chism and M.E. Mangino, 1987. Determination of the β-Lactaglobulin and α-lactalbumin and bovine serum albumin of whey protein concentrates and their relation to protein functionality. J. Food Sci., 52: 124-127.

Korhonen, H., A. Pihlanto-Leppala, P. Rantamaki and T. Tupasela, 1998. The functional and biological properties of whey proteins: Prospects for the development of functional foods. Agric. Food Sci., 7: 283-296.

Lamilli, M.K., 1970. Cleavage of structural proteins during the assembly the head of bacteriophage T4. Nature, 277: 680-685.

Ling, E.R., 1963. A Text Book of Dairy Chemistry. Vol. II Practical, Chapman and Hall, London, pp: 98.

Mate, J.I. and J.M. Krochta, 1994. β-lactoglobulin separation from whey protein isolates on a large scale. J. Food Sci., 59: 1111-1114.

Maubois, J.L., A. Pierre, J. Fauquant and M. Poit, 1987. Industrial fractionation of main whey proteins. IDF Bull. No. 212: 154-159.

Maubois, J.L. and G. Ollivier, 1997. Extraction of Milk Proteins. In: Food Proteins and Their Applications. Damodaran, E. and A. Paraf (Eds.), Marcel Dekker Inc., New York, pp: 579-595.

McIntosch, G.H., P.J. Royle, R.K. LeLeu, G.O. Regester, M.A. Johnson, R.L. Grinsted, R.S. Kenward and G.W. Smithers, 1998. Whey proteins as functional food ingredients? Int. Dairy J., 8: 425-434.

Middleton, N., J.R. Reid, T. Coolbear and P. Jelen, 2003. Proliferation and intracellular glutathione in Jurkat T cells with concentrated whey protein products. Int. Dairy J., 13: 565-573.

Morison, K.R. and E.M. Mackary, 2001. Viscosity of lactose and whey protein solutions. Int. J. Food Properties, 4: 441-454.

Onwulata, C.I., R.P. Konstance and P.M. Tomasula, 2004. Minimizing variations in functionality of whey protein concentrates from different sources. J. Dairy Sci., 87: 749-756.

Parkinson, E.L. and E. Dickinson, 2004. Inhibition of heated-induced aggregation of a β-lactoglobulin-stabilized emulsion by very small additions of casein. Colloids Interfaces B, 39: 23-30.

Parkinson, E.L. and E. Dickinson, 2007. Synergistic stabilization of heat-treated emulsions containing mixtures of milk proteins. Int. Dairy J., 17: 95-103.

Patel, P.D., M.A. Stripp and C.J. Fry, 1988. Whipping test for the determination of foaming capacity of proteins, a collaborative study. Int. J. Food Sci. Technol., 23: 57.

Patocka, G., R. Cervenkova, S. Narine and P. Jelen, 2006. Rheological behaviour of dairy products as affected by soluble whey protein isolate. Int. Dairy J., 16: 399-405.

Pearce, K.N. and J.E. Kinsella, 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J. Agric. Food Chem., 26: 716.

Pearce, R.J., 1983. Thermal separation of β-lactoglobulin and α-lactalbumin in bovine cheddar cheese whey. Aust. J. Dairy Technol., 38: 144-149.

Remeuf, F., S. Mohammed, I. Sodini and J.P. Tissier, 2003. Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yoghurt. Int. Dairy J., 13: 773-782.

Sherwin, C.P. and E.A. Foegeding, 1997. The effect of $CaCl_2$ on aggregation of whey proteins. Milchwissenschaft, 52: 93.

Yamauchi, K., M. Shimizu and T. Kaniya, 1980. Emulsifying properties of whey proteins. J. Food Sci., 45: 1237-1242.

Youling, L.X., A.D. Karl and W. Liping, 1993. Thermal aggregation of β-lactoglobulin; effect of pH, ionic environment and thiol reagent. J. Dairy Sci., 76: 70.