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Whey Protein Concentrates Added in a Beverage and Their Thermal Denaturation Studied by Liquid Phase Chromatography

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Abstract: Whey protein concentrates added in a beverage was subjected to various time-temperature treatments and the rates of denaturation of α-lactalbumin (α-LA) and α-lactoglobulin A and B (β-LG-A and β-LG-B) were monitored by Liquid Phase Chromatography. The denaturation reaction appeared to follow first order kinetics for α-LA while both β-LG-A and β-LG-B followed second order kinetics. All three denaturation reaction showed a break in the Arrhenius plot at approximately 90°C. Equations were derived relating temperature ($°K^{-1}$) to first order rate constants $k_1(s^{-1})$ for α-lactalbumin or kinetic constants $k_2(M^{-1}s^{-1})$ for both β-LG-A and β-LG-B. Denaturation of β-LG-B was slightly more rapid than β-LG-A at temperatures greater than 90°C.

Key words: Whey protein concentrates, thermal denaturation, liquid phase chromatography

INTRODUCTION

The heat treatment of milk by commercial processing procedures result in physico-chemical modifications of milk components. Whey protein concentrates denaturation is extensively studied and it is a food important modification. Cooked in the beverage, whey protein concentrates develop the mixture flavour (Koster, 1989) and texture (Dumay, 1988), increase heat stability flowing concentration, reduced colloidal stability upon freezing and coagulation of beverage by acid or enzymes.

Several techniques are used to study the kinetics of heat denaturation of whey proteins: Reaction kinetics of the denaturation of whey proteins (Dannenberg and Kessler, 1986), study in relation to gelation kinetics of heat- induced quantification casein-whey interactions in milk (Vasbinder-AJ *et al*., 2003), differential scanning calorimetric was used to study the thermal denaturation of β-lactoglobulin (Reklin and Launay, 1990), polyacrylamide gel electrophoresis was used to study irreversible thermal denaturation of α-lactalbumin (Chaplin and Lyster, 1986), using hydrophobic-interaction chromatography method to study thermal denaturation of whey proteins and its importance in technology (Benedek, 1989), using electrophoresis temperature between 70-150°C and time between 2-5400 s to determine yogourt texture (Dannenberg, F and Nestec (1990), using differential scanning calorimetry to study the state of water in protein-water systems (Lambelet *et al*., 1989). Hillier and Lyster (1979) studied whey protein denaturation in heated milk and cheese whey.

The kinetic data obtained by these different techniques are not totally in agreement. Mangi and Kakuda (1986) used Fast chromatography liquid to study whey protein denaturation. They have measured the rates of denaturation of α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) in heated skim milk and found (α-LA) to follow first order kinetics, while both variants of β-LG (A and B) followed second order kinetics, whiles both variants of β-LG (A and B) followed second order kinetics. A similar second order reactions for β-LG denaturation were reported by Mc Sweeney *et al*. (2004), Dumay and Cheftel (1989).

The results obtained by the first works on the kinetics of whey protein denaturation were dependent to the large degree on the method using heat denaturation and on the method to essay. Changes in the chemical, optical, electrophoretic or thermodynamic properties have been utilized to monitor protein denaturation. It is reasonable to assume that the results may be influenced

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by the essay procedure and also by the ability of some denatured proteins under certain conditions to renature (Matsudomi, 2003).

The availability of chromatographic procedure for whey protein analysis used by Mangi *et al.* (1986), offered another possibility for investigate of the kinetics of whey protein denaturation in the beverage and comparison of its effectiveness with other available technic.

MATERIALS AND METHODS

This study was conducted at the laboratory of Biophysico-chemical and Technology Alimentary of Compiegne University of Technology (France) in 2003. Whey protein concentrates was prepared at French EURIAL Company. It was added with a beverage and this beverage came from Gamboma in Congo Brazzaville. The beverage added with whey protein concentrates was prepared by centrifugation in the laboratory of Biophysico-chemical and Technology Alimentary of Compiegne University of Technology-France. The beverage was heat treated in sealed capillary tubes (90×1.6 mm). The tubes were filled by inverting them into a beaker of beverage placed inside a vacuum desiccator. The vacuum served to degas the beverage to prevent surface denaturation and to allow rapid filling of the tubes when the vacuum was realized. The open ends were sealed with a flame and sealed tubes were incubated in thermostatically controlled water bath or oil bath. The water bath was used for temperatures of 70 and 80°C while the oil bath covered the temperature range of 90 to 140°C. The temperature of the baths were monitored with a model Bat-12 thermocouple.

The tubes were incubated at 8 different temperatures. The reaction was controlled by removing 10 tubes from the bath at 7 timed intervals and immersing them in ice water. Their contents were pooled and centrifuged in 8×49 mm ultra clear tubes at 100 g for 40 min. The clear supernatant was collected and held at 4°C until analyzed. All samples were analyzed within 48 h. The experiment was performed in triplicate on 3 different batches of mixture.

Quantitation of whey protein concentrates: Liquid phase chromatography on an anion exchange column was used for the quantitation of undenatured whey proteins (Mangi and Kakuda, 1986). Three injections of 100 μL each were used to essay each supernatant. The levels of α-LA and β-LG A and B remaining in solution, expressed as the percentage of undenatured protein, were calculated by dividing the area of each protein peak obtained from heated samples by the erea of the corresponding protein peak obtained from unheated beverage.

The data for percentage undenatured protein versus time data were fitted to either a first order equation (Log C/C_O = -kt/2.303) or a second order equation ($Co/C = 1+ CoK_2t$). The best fit was determined by linear regression using the least squares procedure.

RESULTS AND DISCUSSION

Kinetics constants: The rates of thermal denaturation of α-LA and β-LG-B in beverage were determined over a temperature range of 70-140°C. The data at each temperature were analyzed graphically using linear regression to obtain the best fit and to determine the order of reaction. Figure 1 shows typical progress curves of% residual (undenatured) protein versus time of α-LA, β-LG-A and β-LG-B at 80°C. Similar results were obtained at all other temperatures investigated. A linear relationship was obtained when the integrated form of the first order rate equation was used to plot the data of α-LG-A (Fig. 2) in contrast to the slight curvature seen when the data was plotted as a second order reaction. Using this procedure the denaturation of α-LG-A in the beverage at all temperatures studied was found to be first order.

First order plots of β-LG-A and were not linear, however, good linearly was obtained when the data for β-LG-A and B were plotted as a second order reaction (Fig. 3). Similar results were obtained at all other temperatures studied for both β-LG-A and B. The second order rate constants were calculated from the slopes of the linear regression lines and expressed as C_ok_2. Second

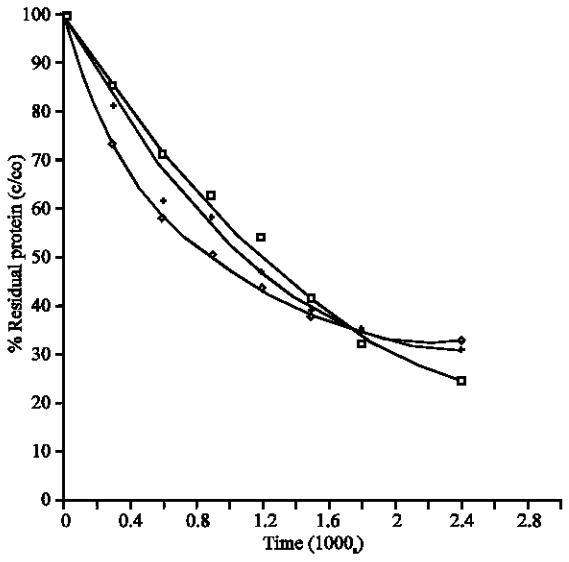

Fig. 1: Plot of % residual (undenatured) α-lactalbumin (□); β-lactoglobulin A (+) and β-lactoglobulin B (◇) versus time in skim milk heated at 80°C

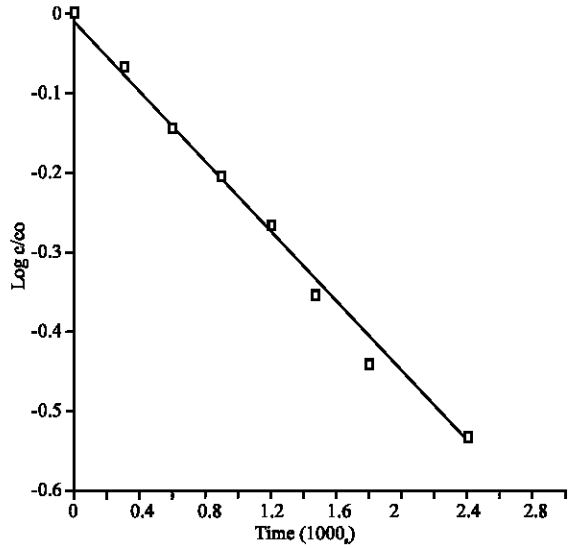

Fig. 2: First-order reaction plot for the denaturation of ∞-lactalbumin in skim milk heated at 80°C

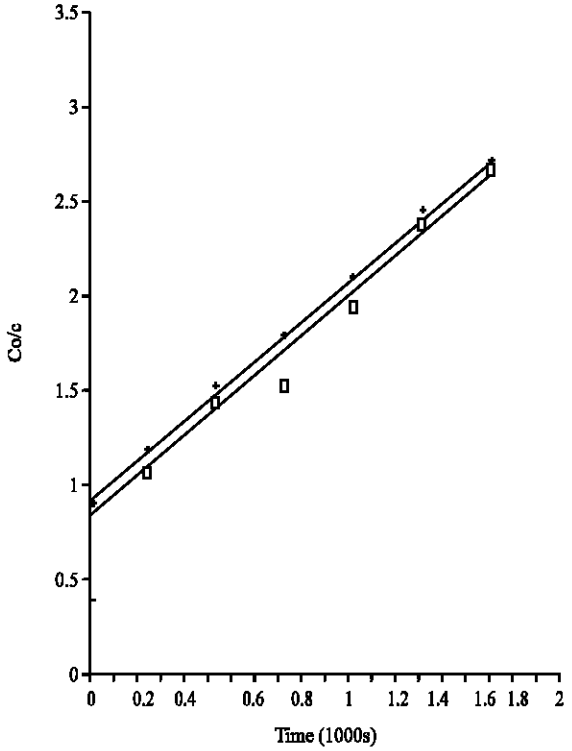

Fig. 3: Second-order reaction plot for the denaturation of β-lactoglobulin A(+) and β-lactoglobulin B (□) in skim milk heated at 80°C

order kinetics was also found by (Mangi *et al*., 1985) while (Gough and Jenness, 1962) reported the denaturation of β-LG to follow first order kinetics.

Temperature dependence: The temperature dependence of results for α-LG-A and β-LG-B denaturation in the

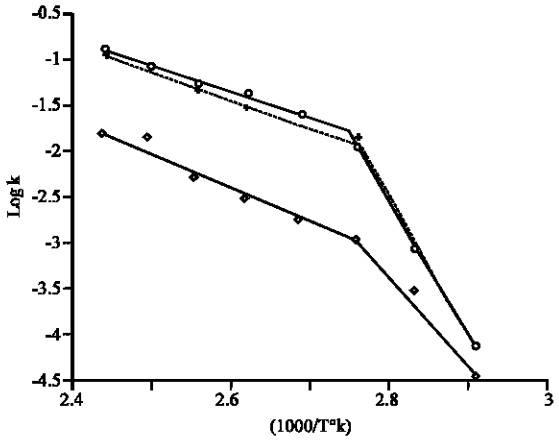

Fig. 4: Arrhenius plots of kinetic constants for α-lactalbumin (◇), β-lactoglobulin A(+) and β-lactoglobulin B (O), Each point represents an average of 3 determinations

beverage are shown in Fig. 4. For all three whey protein (α-LG-A, β-LG-A and β-LG-B) a change in temperature dependence occurred at about 90°C. Mangi and Kakuda, (1986) observed a similar change in the Arrhenius plots for β-LG and (α-LG-A. Accordingly, the data were fitted to two linear equations using the least squares procedure for the temperature ranges of 70 to 90°C and 90 to 140°C (Table 1). Activation energies were calculated from the data of (Hillier and Lyster, 1979) as 14,8 kcal mol^{-1} for α-LA, 8.7 kcal mol^{-1} for β-LG-A and 7.6 kcal mol^{-1} for β-LG-B for temperatures ranging from 95 to 150°C. These estimations are in good agreement with our values of 17.2 kcal mol^{-1} for α-LA, 11.1 kcal mol^{-1} for β-LG-A and 11.1 kcal mol^{-1} for β-LG-B for temperature ranging from 90 to 140°C.

In the beverage investigated, β-LG-A appeared to be slightly more heat stable than β-LG-B above 90°C (Fig. 4). This is in constant to the findings of Dumay and Cheftel (1989) who observed greater stability with β-LG-B at temperatures above 95°C Below 0°C, variants show about the same heat stability with β-LG-B at temperatures above 95°C. Below 90°C, variants show about the same heat stability. The experimental technique of heating small volumes of mixture in thin capillary tubes was described by (Dumay and Cheftel, 1989). This technic provides a large surface area to volume ratio which minimizes both come-up and cool-down times. The collection of precise and accurate kinetic data is facilitated by this procedure. The utilization of the liquid phase Chromatography method has certain advantages over the previously used immunological techniques. This chromatographic technic allows for the fast and accurate quantitation of whey protein in the beverage with only an ultracentrifugation step prior to analysis. With this procedure, no pH adjustment to 4.6 was necessary. The Arrhenius plots (Fig. 4) shows the same trends observed by (Mangi *et al*.,

Table 1: Liner arrhenius equations and activation energise for thermal denaturation of α-lactalbumin and β-lactolobulin A and B

Protein	Temp. range (°C)	Linear equation	MSE	R^2	Ea (kcal mol^{-1})
α-LA	70-90	log k1 = 22.61 - 9.27(10^3/T)	±0.116	0.98	42
α-LA	90-140	log K1 = 7.27-3.76(10^3/T	±0.089	0.96	17
β-LG-A	70-90	log k2 = 37.92-14.45(10^3/T)	±0.053	0.99	66
β-LG-A	90-140	log k2 = 4.81-2.41(10^3/T)	±0.069	0.97	11
β-LG-B	70-90	log k2 = 35.22-13.51(10^3/T)	±0.059	0.99	62
β-LG-B	90-140	log k2 = 5.05-2.48(10^3/T)	±0.058	0.96	11

1985). The slopes of the log k vs 1/T plots change at temperature above 90°C. This change in slope is due to a drop in the rate protein denaturation which implies a more stable protein at temperature above 90°C. One possible explanation for this behaviour would require a temperature dependent change in mechanism.

If two consecutive reactions were involved in the denaturation process and if these two reactions have different activation energies, then the Arrhenius plots will concave downwards if the lower activation energy controls at the higher temperature (Mc Sweeney *et al*., 2004). Also, if the reactions were diffusion controlled, then the rate of diffusion may become limiting at higher temperatures. Since similar results were obtained for all three proteins, the phenomenon was not dependent on the type of protein. Another possible reason for the change in slope could be related to an increase in pressure. It has been shown the high pressures can stabilize proteins at high temperatures (Matsudomi, N. 2003; Vasbinder *et al*., 2003). These pressure effects, however, may not be significant at the pressure levels generated inside sealed capillary tubes.

CONCLUSIONS

One of the practical applications of the results obtained in this study could be to use the derived equations to predict the effects of heat treatment on α-LG-A, β-LG-A and β-LG-B. The amount of denaturation expected after processing milk through a system of known time-temperature profile. The general agreement of results from this study, with other published data, confirms that the convenient liquid phase chromatography method is sensitive accurate and suitable for assaying large numbers of samples.

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