The Effect of UHT and VAT Thermal Processing Systems on Whey Protein Denaturation and Gel Strength of yoghurt

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ABSTRACT

Milk was thermally processed by Ultra-High-Temperature (UHT, 132 to 154 ^{0}C for 0 to 12 sec) and Vat (VAT, 66 ^{0}C to 88 ^{0}C for 0 to 30 min) systems. Representative milks were fermented into gels by a traditional yoghurt fermentation procedure. Selected functional properties, i.e. whey protein denaturation, gel strength, and their interrelationship were studied. Denaturation behavior of the five whey proteins indicates different heat effects produced by the UHT and VAT heat treatments. The degree of whey protein denaturation increased at a faster rate in the UHT than in the VAT heat treatments. The UHT heat treatment resulted in yoghurt gels of lower strength than those of VAT heat treatments. A critical point was found in the optimal gel strength in the UHT (149 ^{0}C, 3.3 sec) heat treatment. An approximate correlation between the degree of whey protein denaturation and the gel strength was observed in the VAT heat treatment, whereas no such correlation was found in the UHT heat treatment. It appeared, therefore, that whey protein denaturation in the UHT and VAT heat treatments plays a markedly different role in the mechanism of yoghurt gelation. Moreover, the VAT but not the UHT heat treatment may provide an index of the gel strength.

Keywords: Whey proteins, gel strength, denaturation, UHT process, VAT, yogurt

INTRODUCTION

It is well known that different process variables can induce changes in the physical properties of milk proteins. Such variables affecting physical properties of proteins include heat treatment, protein content, acidity, etc. There is considerable controversy, however, regarding temperature-time relationships necessary to change the physical property of a product, e.g. stability [1].

Moreover, heating of milk proteins to temperatures exceeding normal pasteurization levels by UHT and VAT processing systems Influences the physical properties of yoghurt consistency [2]. Parnell-Clunies et al. [1] found the highest viscosity in VAT heat treatments (85 ^{0}C, 10-40 min), while UHT heat treatments (140 ^{0}C, 2-8 sec) gave remarkably lower viscosity.

There are many studies concerning the heat-denaturation of milk proteins but very few of them describe the relation of denaturation to other functional properties [3]. Elsewhere, it has been indicated that heat treatments of 78 ^{0}C for 1 hr or 80 ^{0}C for 30 min or 90 ^{0}C for 5 min is required for complete denaturation of milk proteins [4]. McKenzie [5] claimed that prolonged exposure to denaturation will cause extensive unfolding of proteins leading eventually to cleavage of disulfide groups and other complicated reactions which could affect the functional properties of the products. For example, excessive UHT heat treatment of milk resulted in co-precipitation of the denatured whey proteins with the caseins and loss of the desirable functional property, i.e. curd strength, of cottage cheese [6]. Also, it was claimed by these authors that the interaction of beta-lactoglobulin with caseins after a heat treatment improves the consistency of buttermilk gel. A statistical model was used to study the maximum range of factors and their effect on fouling using the minimum number of experiments. These factors included the amount and rate of the denaturation of b – lactoglobulin comparing the amount of b -lactoglobulin reaction with the fouling of a multichannel Plate heat exchanger. [7].

A number of factors such as milk quality, composition/ additives, and the processing conditions have been shown to influence the gelation of UHT treated milk [8]. Similar studies have shown that a gelation effect can be caused by severe distortion of casein micelles and their formation of a three-dimensional network or by the precipitation of casein micelles to form sediment [9]. Others found that there was no correlation between

gelation and protein breakdown in UHT processed milks [10]. But the exact mechanism of gelation in UHT milk is not clearly understood [11].

Sedlmeyer and Kulozik [12] investigated the effect of the heating temperature in the range of 120–139°C and the filling temperature after cooling down to 4–14°C prior to storage for 24 and 96 h at 4 °C on a system comprising milk and 400 ppm kappa-2 type of carrageenan. The textural properties and the stability of such systems mainly depend on the interaction of the carrageenan and the casein micelle surface which was assessed by means of the hysteresis loop area between the upward and downward flow curves upon variation of the shear stress. During storage, a further influence of the process parameters on structural development between casein and carrageenan was observed. Structure point analysis, small angle oscillatory rheology and particle size measurements were used for further explanation of the results of the hysteresis loop area.

Studies clearly show that high protein concentration can greatly influence the formation mechanisms of whey protein gels. Fickak et al. [13] used a laboratory produced heat induced whey protein gels (HIWPG) and a pilot plant heat exchanger fouling/cleaning to investigate the effect of protein concentration on formation and cleaning of dairy fouling.

The Viscolab (Tetra Pak), an indirect ultra-high treatment (UHT) pilot plant, was used by Depypere et al. [14] to prepare dairy desserts containing kappa-carrageenan, skimmed milk powder (SMP), adipate cross-linked acetyl-substituted waxy maize starch, sucrose and water. Effects of varying concentrations of carrageenan, milk powder and starch on the dessert rheology were studied. They found that the dessert preparation in an UHT pilot plant involved a more intense heat treatment. As a result, a more extensive whey protein denaturation and subsequent complexation with casein micelles is believed to contribute to the rheological properties of the UHT desserts.

Therefore, it is of interest to determine the degree of whey protein denaturation induced by different processing systems and the correlation of denaturation to the gel strength. The current study examines the effect of two process systems i.e. continuous UHT and batch VAT heat treatments, on the denaturation of whey proteins with a view to further correlation with the mechanism of gel strength in yoghurt.

MATERIALS & METHODS

Thermal Processing

Raw whole milk was heat treated by two processing systems, i.e. a continuous UHT and a batch VAT, represented by the following temperature-process holding time combinations: UHT processing at temperatures 132 ^{0}C to 154 ^{0}C with an app. 6 ^{0}C increment for 0.0, 1.2, 3.3, 5.2, 9.0, and 12.0 sec and VAT processing at temperatures 66 ^{0}C to 88 ^{0}C with an app. 6 ^{0}C increment for 0.0, 5.0, 10.o, 20.0, and 30 min. Unheated milk was utilized as a control.

UHT and VAT heat treatments were carried out in a helically coiled tube, with an indirect heating system, automatic temperature control and in a steam jacketed batch type pasteurizer respectively. Different residence times were obtained by using holding tubes of varying length size and changing pump speeds in the UHT process system. All heat treatments were homogenized at 60 ^{0}C preheating temperature and an approximate 105 Kg/cm^2 operating pressure.

Preparation of Gel

After homogenization and cooling to approx. 43°C, milk was inoculated with 3% mixed yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus* at a 1:1 ratio) and incubated at 43 ^{0}C until a pH of 4.5 was obtained (Fig. 1). Then, it was stored at refrigeration temperature for further analysis.

Gel Strength Measurements

For relative comparisons between heat treatments of the two processing systems, yoghurt gels were subjected to a standard procedure (one day-old, unstirred 4 ^{0}C yoghurt gel strength measurement). A Cherry-Burrel curd tension meter was used to measure the strength of yoghurt gels. Total breaking energy (gel strength or curd tension values) was expressed in grams required to penetrate approx. 6 cm deep into the body of the yoghurt gel with a round plunger.

Whey Protein Separation

Diverse heat treated milks and the control (unheated) were acidified to pH 4.6 at 35 ^{0}C, centrifuged (1000 x g 15 min, 4 ^{0}C), and filtered (Whatman no. 1 paper) to an acid whey solution which was ten-fold concentrated by treating 10 ml with l.8 g lyphogen medium for 4 hrs prior to electrophoresis. The whey proteins were separated on cellulose acetate strips using the electrophoresis procedure of Morr [15] as modified by Bell and

Stone [16] for separating blood serum proteins. The electrophoretic strips were scanned at 525 nm with a DCD-16 densitometer (Gelman Instrument Co., Ann Arbor, Mi). The instrument was set to yield nearly full scale peaks for the beta-lactoglobulin. A peak of the control sample (raw, unheated whey proteins) was employed in each experimental set.

Qualitative analysis of the whey protein fractions was made according to the procedure of Puyol et al. [17] in which the whey protein fractions of the milk are identified by the rate of travel in the electric field relative to standard proteins. Purified samples of bovine albumin and bovine globulins were obtained from Calbiochem, Lajolla, CA, whereas alpha-lactalbumin, beta-lactoglobulln A and beta-lactoglobulin B were obtained from Sigma Chemical Co., St. Louis, MO.

Protein Denaturation Measurements

The whey protein denaturation was expressed as a percentage resulting from the relation of the curve area of each heat treated sample to the corresponding area of the unheated sample.

Quantitative analysis of protein denaturation was made by measuring the total area under the densitometer tracing curves using a 620005 compensating planimeter (Keuffer and Esser Co., NY).

RESULTS & DISCUSSION

Effect of heat treatment on whey protein electrophoresis

A typical densitometer tracing of separation of whey proteins of raw milk is shown in Fig. 2. There were 5 distinct electrophoretic peaks, corresponding to immunoglobulins (Igs), alpha-lactalbum in A (alpha-La), beta-lactoglobulin B (beta-Lg B), beta-lactoglobulin A (beta-Lg A), and bovine serum albumin (BSA).

Progressive decreases in the area of electrophoretic peaks occurred with an increase in process holding time, for example, at the UHT system (149 ^{0}C) and at the VAT system (82 ^{0}C) as you can see in Figures 3 & 4, respectively. Qualitative analysis of the areas under the electrophoretic peaks for the UHT treatments at 132 ^{0}C through 154 ^{0}C showed that these treatments with no process holding times did not severely denature the whey proteins (Fig. 5). A significant denaturation was observed at l49 ^{0}C and above as is evident in Figure 5. However, the same UHT treatments but with 12 sec process holding times yielded a complete denaturation for all the temperatures applied to whey proteins (Fig. 6).

On the other hand, VAT treatments at 66 ^{0}C for 0 through 30 min process holding times indicated only a small effect on whey protein denaturation. Figure 7 shows that at VAT heat treatments 66 ^{0}C through 88 ^{0}C with 0 sec process holding time. There was a severe denaturation effect somewhere between 82 ^{0}C and 88 ^{0}C temperatures (Fig. 7). When VAT heat treatments were applied at various temperatures with a constant 30 min process holding time, a substantial denaturation effect was obtained after 71 ^{0}C temperature (Fig. 8).

However, it is obvious from the results that the cumulative effect of temperature and process holding time on the area under the electrophoretic tracing curves was substantially different between the UHT and VAT heat treatments. This was found to be particularly apparent at the higher temperatures of both processing systems where the areas under the electrophoretic tracing curves were markedly affected by process holding times.

Effect of heat treatments on whey protein denaturation

Among whey protein properties, denaturation Induced by thermal processes is of considerable importance. A differential sensitivity of whey protein denaturation to UHT and VAT processes was observed. For example, a UHT heat treatment at 149 ^{0}C for 3.3 sec caused a 70% denaturation of the total whey proteins. Analytically, the result above was due to the denaturation of 80% Igs, 45% alpha-La, 70% beta-Lg B, 75% beta-Lg A, and 60% BSA. However, an amount equal to 95% denaturation of whey proteins was caused either by the UHT thermal processing (l49 ^{0}C, 6 sec) or by the VAT thermal processing (82 ^{0}C, 5 min).

Overall, the heat effect on whey protein denaturation indicates that alpha-La is the most resistant whey protein followed by the Igs, beta-Lg A and beta-Lg B. The results above are similar to other literature observations [18]. From the results in this study, it is obvious that the degree of total whey protein denaturation or the denaturation of the predominant beta-Lactoglobulins could provide an index of the UHT and/or VAT heat treatments applied to various processed milks and to other similar products. Table 1 shows the progressive denaturation (decrease in the peak area) of the whey protein, i.e. beta-lactoglobulins, as a function of UHT process holding time at 138 and 149 ^{0}C temperatures. However, two regions near 70 ^{0}C and 130 ^{0}C have been attributed to the denaturation and/or unfolding of residual protein structure of beta-lactoglobulins according to Kirn et al. [19].

Effect of heat treatment on gel strength

Whey proteins have been demonstrated to participate in gel formation, while other studies have shown that heat treatment (conventional or UHT) of milk affects the rheological properties of yoghurt gel prepared from this milk [2]. However, a differential effect of VAT and UHT heat treatments on the gel strength of yoghurt made by the above processing systems was observed in this study. Gel strength measured by curd tension values ranged between 15 and 34.5 g for the UHT heat treatments and reached up to 85 g for the VAT heat treatments (Table 2). The strength of yoghurt gels prepared from UHT heat treatments was, thus, always lower than those prepared from VAT heat treatments. It is obvious, therefore, the following explanation: The higher holding times applied in the UHT heat treatments seem to have a specific effect on some kind of casein-whey protein-mineral interactions which in turn^ reduce the capacity of the network system to form high strength gels.

Since beta-lactoglobulins compose 40-60% of the normal whey proteins, they should have a major contribution to gel strength. Investigators have noted that beta-lactoglobulin is the most important predictor of gel strength at pH 8.0 but not at pH 4.6 – 6.5. It is, also, well known that beta-lactoglobulins are the major source of sulfhydryl groups in whey proteins, so it might be possible that the sulfhydryl-disulfide interchange can make an important contribution to the formation of a gel matrix.

Effect of heat denaturation on gelation

A rough correlation between the extent of whey protein denaturation and gel strength was found for the VAT heat treatments. For example, a heat treatment for 30 minutes at 66 ^{0}C yielded only moderate denaturation and a considerably higher degree of the gel strength. No such correlation was observed for UHT heat treatments in relation to the gel strength. Although a progressive increase in protein denaturation with increasing process holding time at 149 ^{0}C was found (Fig. 3), the strength of the yoghurt gel reached its maximum at 3.3 sec process holding time (Table 2). However, the degree of gel strength was found lower than the above at shorter or longer process holding times. It was, also, observed that the strength of yoghurt gels prepared from UHT heat treatments was lower than those prepared from VAT heat treatments regardless of the degree of denaturation.

The lack of correlation between whey protein denaturation and degree of gel strength of yoghurts may reflect the lesser contribution of the UHT heat treatments to the matrix formation of the gel.

The gelation of yoghurt is presumably a process which takes place in two stages. One corresponds to a slow unfolding (denaturation) of the proteins and the other to an aggregation (intermolecular disulfide) hydrophobia, hydrogen, and ionic bond reactions). During the second stage if it is sufficiently slow, a well ordered network may be formed due to intermolecular bind formation. If denaturation is too rapid and/or too extensive as with UHT heat treatments, there may be excessive local coagulation of protein and thus a weaker strength of gel results. The extent of denaturation of the whey proteins With progressive UHT process holding time resulted in an increased strength of gels to a maximum point (149 ^{0}C, 3.3 sec.) after which a decreased strength of gels started to occur. This could be a result of the sediment effect which is observed at the higher UHT heat treatments, which in turn could be the main factor for the reduction of gel strength in yoghurt.

The respective degree lactogloglobulins at two temperatures and progressively longer process holding times as shown in Table 1 was ranged between 56 and 96%. The degree of gel strength for the VAT heat treatments increased progressively during the increased holding process times, while for the UHT heat treatments subsequently declined after a certain point, i.e. 149 ^{0}C at 3.3 sec. According to de Witt and Swinkels (1980), the heat denaturation of a protein reflects the stability of the native structure. The increase in denaturation is correlated with an increase in yoghurt gel hardness for the VAT heat treatments, while denaturation of whey proteins was not associated with the degree of gel strength of yoghurt when UHT heat treatments were applied. These indicate that the high temperatures of the UHT could possibly have some specific effects on casein-whey protein or whey protein-mineral interactions which in turn may reduce the capacity o£ the casein-whey protein-mineral system to forma high strength gel. These results could, also, be due to factors such as the sedimentation, an effect which can be due to interactions and co-precipitation of whey proteins, caseins and minerals, especially at the higher UHT heat treatments where it was observed to be increased.

Table 1. Effect of UHT heat treatment at 138 ^{0}C and 149 ^{0}C on b-lactoglobulin.

Process Holding Time (sec)	Electrophoretic Area (sq. cm)		Denaturation (%)	
	138 ^{0}C	149 ^{0}C	138 ^{0}C	149 ^{0}C
0.0	5.7	4.2	40	56
1.2	3.6	2.2	62	77
3.3	1.9	1.7	80	82
5.2	1.0	1.2	90	92
9.0	0.9	0.4	91	96
12.0	0.7	--	93	--

Note: - Average values of triplicate measurements

- Area of raw (unprocessed) sample was shown 9.5 cm^2 value for the b-Lactoglobulin fraction.

Table 2. Effect of UHT and VAT heat treatments on gel strength of yoghurt.

HEAT TREATMENT	Process Holding Time (sec)	Process Temperature (^{0}C)	Gel Strength (g)
UHT	0.0	149	24
UHT	3.3	149	33
UHT	5.2	149	27
UHT	9.0	149	14
UHT	12.0	149	12
VAT	1800.0	82	86

Note: - Average curd tension values of at least triplicated measurements.

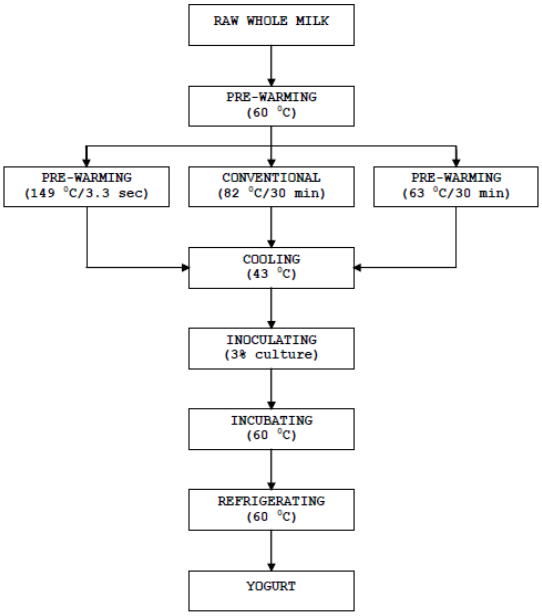

Figure 1: Flow diagram of traditional VAT and UHT yoghurt processing

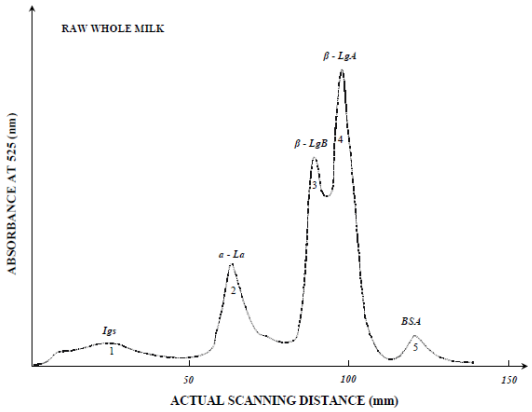

Figure 2: Electrophoretic pattern (densitometric tracing) of whey proteins in raw whole milk (unheated)

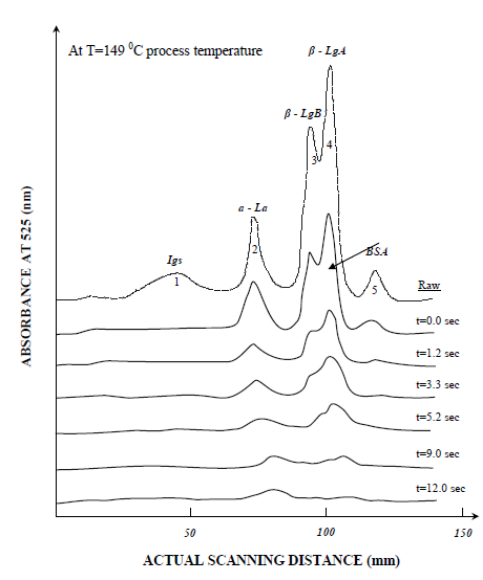

Figure 3: Electrophoretic patterns of whey proteins of whole milk thermally processed by the UHT system at 149°C temperature and 0 to 12 sec process holding times.

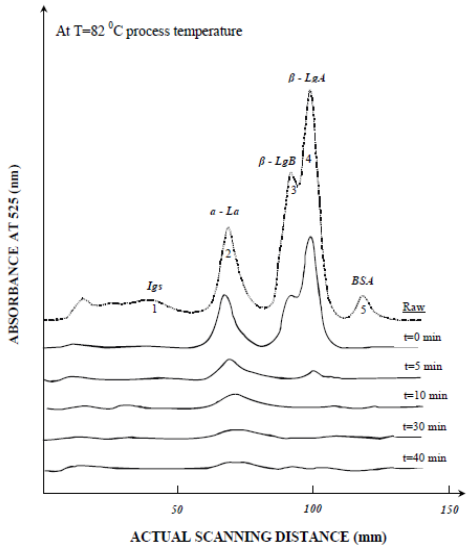

Figure 4: Electrophoretic patterns of whey proteins of whole milk thermally processed by the VAT system at 82°C temperature and 0 to 40 min process holding times.

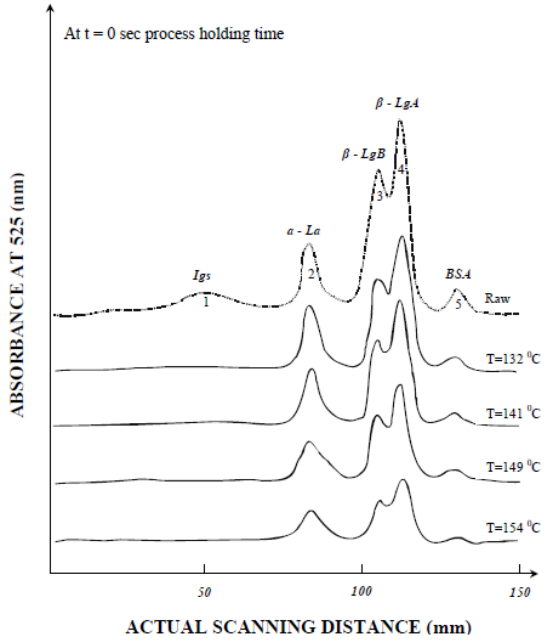

Figure 5: Electrophoretic patterns of whey proteins of whole milk thermally processed by the UHT system at 132°C to 154°C temperatures and a constant 0 sec process holding time

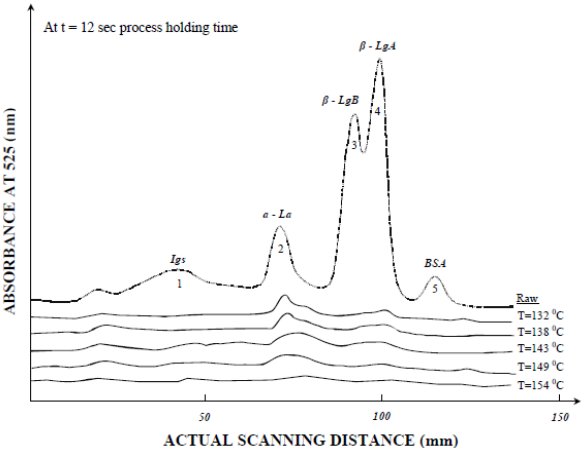

Figure 6: Electrophoretic patterns of whey proteins of whole milk thermally processed by the UHT system at 132°C to 154°C temperatures and a constant 12 sec process holding time.

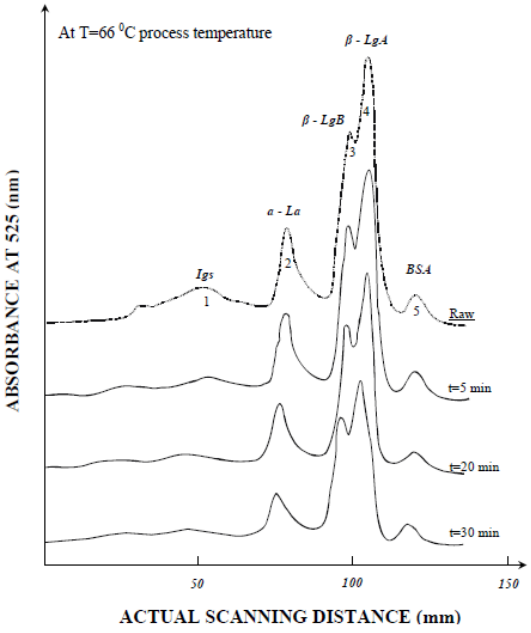

Figure 7: Electrophoretic patterns of whey proteins of whole milk thermally processed by the VAT system at different process holding times of 5, 20, 30 min and at constant process temperature of 66 °C.

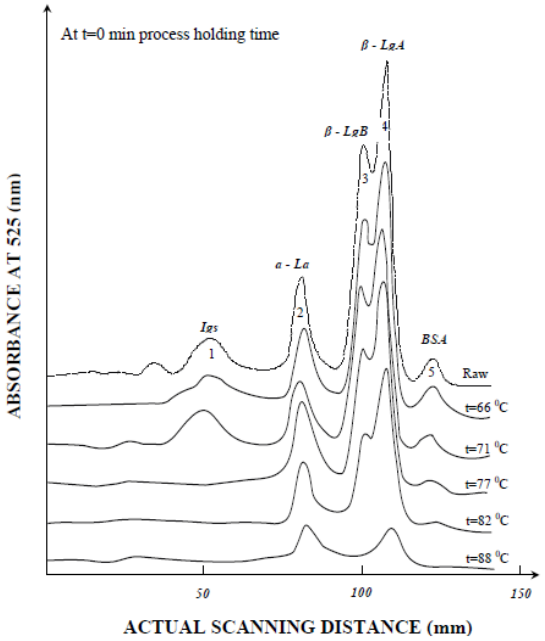

Figure 8: Electrophoretic patterns of whey proteins of whole milk thermally processed by the VAT system at 66°C to 88°C temperatures and a constant 0 min process holding time.

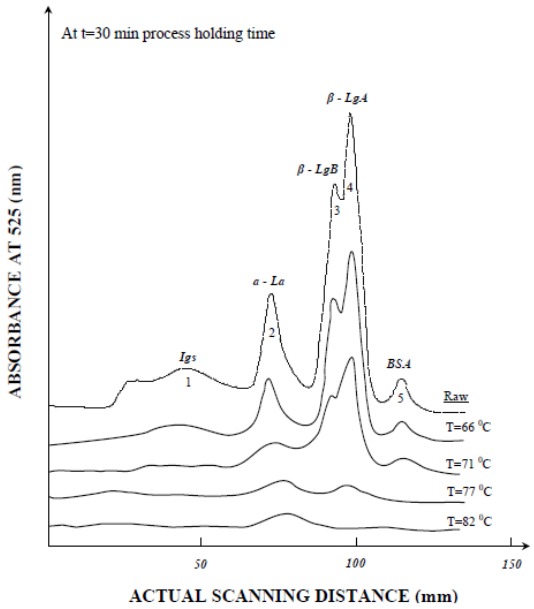

Figure 9. Electrophoretic patterns of whey proteins of whole milk thermally processed by the VAT system at 66°C to 82°C temperatures and a constant 30 min process holding time

CONCLUSION

The results of this study indicate that electrophoresis on cellulose acetate membranes provides a relatively simple and quick procedure for separating and measuring the relative quantities of protein denaturation induced by different thermal processing operations. Denaturation of the whey proteins induced by UHT and VAT processing systems was dependent on thermal processing systems and thermal processing parameters (process holding time and temperatures). A rough correlation between extent of whey protein denaturation and gel strength was found for the VAT heat treated whey proteins, while no such correlation was observed in the UHT heat treated whey proteins. On the other hand, the strength of gels prepared with the UHT process was always lower than those prepared with the VAT process. This could lead to production of light strength gels with an application to drinkable or semi-liquid products.

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