Effect of Heat Treatment on Whey Proteins Denaturation in the Presence and Absence of Lactose

Jasim M.S. Al-Saadi*

* Food Science Department, Technical College of Agriculture, Halabja, Sulimmania, Iraq, e-mail: jasim_salih@yahoo.com

Abstract:

The effect of lactose on denaturation of whey proteins was investigated in cow milk, with and without lactose, heated at different temperatures ranged of (65-95)°C for 30 min. Compared with the milk without, the milk containing lactose showed a smaller increase in pH values and a higher concentration of whey proteins. Whey proteins percentage in milk samples with and without lactose heated at (65,70,75 ,80,85,90 and 95) °C for 30 min were(90.38,88,86.16 , 67.9,44,23,23.5 and 23.2%) and (82.85,75, 71.14 , 44.18,25.92,20.52 and 20%) respectively. It was concluded that heating cause formation new band located below β-Lg band in whey samples of both milk ,with and without lactose , and the intensity of this band increased with increment of heating temperatures and heating time.

Keywords: milk proteins, heat treatment, whey proteins , denaturation

I. Introduction:

Milk is heat treated in order to destroy pathogenic microorganisms, inactivate enzymes and extend its shelf life. It is also heat treated during the manufacture of dairy products such as yogurt to not only destroy microorganisms but also to alter the physical properties of the product, e.g., to increase viscosity. The most common types of heat treatment are pasteurization (63°C for 30 min or 72 °C for 15 sec), ultra high temperature (UHT) treatment (e.g., 135°C for 20 sec or 140°C for 5 sec.) and in-container sterilization (110 - 120°C for ~20 min). The structure of milk proteins undergoes changes during heat treatment and the extent of changes depends on the type of heat treatment employed [1]. Cow milk contains two major groups of proteins, casein and whey proteins. Caseins are phosphoproteins precipitated from raw milk at pH 4.6 at 20°C. They comprise approximately 80% of the total protein content in milk. The principal proteins of this group are classified into αs1-, αs2-, β- and κ-caseins [2]. Whey is a complex mixture of different proteins. In general, the main components include β-Lactoglobulin (55%),α-LA (24%), serum albumin (5%) and immunoglobulins (15%) [3].

When milk proteins are subjected to thermal processing, depending on the

heating conditions, No noticeable effects are observed on the casein micelle fraction due to heat treatment in the temperature range 70-100 °C [4].

The caseins have a mainly random coil structure and are therefore not susceptible to denaturation processes. But whey proteins may undergo a structural change, commonly known as denaturation, which is accompanied by protein unfolding and an exposure of hydrophobic groups.

During heat treatment, small aggregates of β-lactoglobulin are formed which, at increasing temperature or heating time enlarge, and larger denatured β-lactoglobulin aggregates are formed [5]. When the heating temperature and/or time is further increased, denaturation of α-lactalbumin begins, which forms complexes with large denatured β-lactoglobulin aggregates, and both proteins bind to the surface of casein micelles [6].

Thus, following the denaturation of whey proteins, there is a reaction between the two groups of protein also occur. Through thiol group-disulfide bond exchange reactions the whey proteins can interact with k-casein present at the exterior of the casein micelle. The initial step of this process is believed to be physical in nature, but the final interaction is often covalent, e.g. a disulfide linkage [5 , 7].

The kinetics of protein denaturation and aggregation is controlled by the heating conditions and the chemical environment . Heating temperature and pH being probably the most important factors in determining the rate and extent of protein denaturation and the degree of the subsequent interaction of whey proteins with casein micelles [8 , 9].

The aim of this study was to study the effect of lactose on denaturation of whey proteins during heat treatments of milk at different temperatures.

II. Materials and Methods:

A. Preparation of samples

Fresh bulk cow was provided by local dairy farm, Penang, Malaysia. Milk was skimmed by centrifugation (2500 g for 30 min at 5°C). The skim milk was mixed with TCA (24%) 1:1 for 30 min. The milk protein precipitate was collected using centrifugation and the liquid supernatant layer was discarded. The milk protein precipitate was washed twice with 12% TCA to remove traces of lactose, dissolved in distilled water with adding NaOH (2M) to pH 7, dialysed against tap water for 48 h and freeze dried [10].

The milk protein preparation was dissolved in simulated milk ultrafiltrate (SMUF) up to 3.5% (W/V) according to[11].and the solution was divided into two parts; 5% lactose was added to one part(which represent lactose concentration in cow's milk) and no addition was made to the other part.

B. Heat treatment trials

The reconstituted skim milk samples were divided in 100 ml portions and heated in water bath at 65, 70 ,75,80 ,85, 90 and 95 °C for 30 min. All experiments except the SDS- electrophoresis were carried out in triplicate.

C. Soluble nitrogen determination

The pH 4.6-soluble nitrogen (pH 4.6-SN) fraction was prepared according to [12]. The pH of milk was reduced to 4.6 using 1M HCl and sample allowed to stand

for 30 min .Sample were filtered through Whatman No. 1 filter paper and nitrogen contents were determined in aliquots of the by adding 10 mL of 24% (w/v) TCA to 10 mL of milk. The acidified samples were allowed to stand for 30 min at room temperature. They were centrifuged at 10,000g for 15 min and the supernatants filtrates by the micro-Kjeldahl method [13]. The 12% trichloroacetic acid–soluble nitrogen (TCA–SN) fraction was prepared filtered through Whatman No. 42 filter paper. The nitrogen contents were determined in aliquots of the filtrates by the micro-Kjeldahl method [13].

D. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The SDS-PAGE of heat treated milk samples was performed in the presence of 2-mercaptoethanol using a Bio-Rad System following the method described by [14]. Milk samples were mixed (1:2 v/v) with sample preparation buffer (1% SDS, 0.01% bromophenol blue and 30% glycerin in 0.05 M Tris · HCl buffer pH 6.8) and, after addition of 5% 2-mercaptoethanol, were heated at 95°C for 5 min. The samples were cooled and loaded onto an SDS gel made up of stacking and separating gels containing 5% and 15% acrylamide, respectively. The gel was run in a Mini-Protean system (Bio-Rad, Richmond, CA, US) at 200 V using a Bio-Rad power supply unit (Power Pac 3000, Bio-Rad, Richmond, CA, US). The protein bands separated on the gel were stained with a solution containing 0.l% Coomassie Brilliant Blue G-250, 50% methanol and 10% acetic acid, and destained with 1% acetic acid.

The destained gel was scanned using a densitometer (Bio-Rad GS800). Semi-quantitative protein estimation of the protein bands on the scanned image was performed using Quantity One software (Bio-Rad Laboratories). The relative quantities of proteins were estimated by measuring the intensity of their bands as a percentage of the total bands measured.

III. Results and discussion:

A. Changes in pH values During heat treatments

The pH value of the milk samples was 6.8 directly before heat treatment. The pH, it is value of the sample containing lactose decreased gradually until reached 6.68 after 30 min. of heating at 75°C, then the pH increased gradually to 7.6 after 30 min. of heating at 95 °C (Figure 1) .The decrease in pH values after heating at 65,70 and 75 °C is caused by acids formed in the Maillard reactions[15], or protein-protein reactions that result in the release of protons [16].

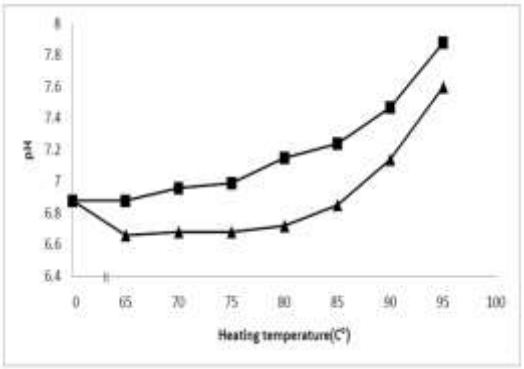

Fig.1:Changes in pH of milk samples after heating at different temperatures for 30 min. in the presence of lactose (▲) and in the absence of lactose (■).

The pH values of milk samples with and without lactose increased gradually with increasing heating temperature and reached to 7.6 and 7.88 respectively after 30 min of heating at 95 °C, this increase is attributable to deamidation of milk proteins [17]. The higher increase in pH values of milk samples without lactose is attributed to acids formed in the Maillard reactions in milks samples with lactose [15].

B. Change in whey proteins during heat treatments

Changes in whey proteins concentration in milk samples after heating at different temperatures for 30 min. with and without lactose are given in Figure 2.

The concentration of whey proteins in unheated milk was 100% and this concentration decreased gradually with the increment of heating temperature in both milk samples with and without lactose. The rate and extent of the decrease were higher in milk samples without lactose than in the samples with lactose. Whey proteins percentage in milk samples with and without lactose heated at (65,70,75 ,80,85,90 and 95) °C for 30 min were(90.38,88,86.16 , 67.9,44,23,23.5and 23.2%) and (82.85,75, 71.14 , 44.18, 25.92 , 20.52 and 20%) respectively.

This decrease in whey proteins concentration in both samples is attributable to denaturation of whey proteins and their subsequent interactions with caseins, mainly via disulphide bridges [18], or formation of xenobiotic crosslinks arising from β-elimination and condensation reactions [19].

In the milk sample containing lactose, an additional type of whey protein–casein interaction occurs because of advanced Maillard reaction (MR) products [20].The higher Percentage of proteins in whey samples with lactose is attributed to the role of lactose in increasing milk viscosity which decrease heating effect on milk proteins [21].

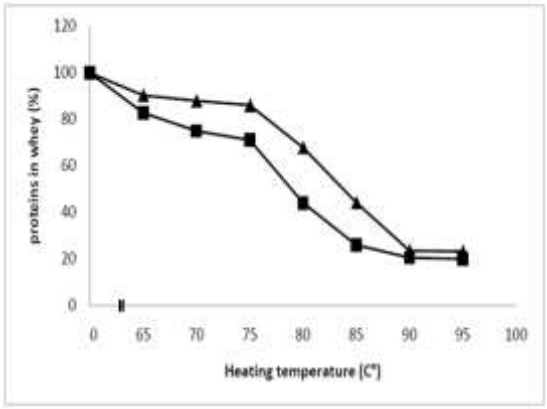

Fig. 2: Changes in whey proteins ratio after heating at different temperatures for 30 min. in the presence of lactose (▲) and in the absence of lactose (■).

C. Change in TCA-soluble nitrogen during heat treatments

Figure 3 shows that the TCA-SN increased in both milk samples during heating at different temperatures for 30 min. In unheated milk the TCA-SN values were 1% and this value increased with time of heating to 1.3% after 30 min. of heating at 95°C in both samples with and without lactose. The increase in TCA-SN during heating is largely attributable to the release of ammonia from milk proteins by deamidation [17].

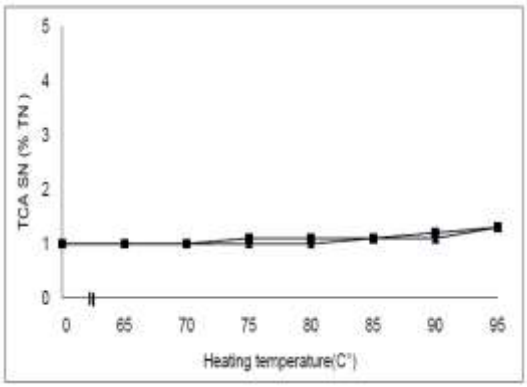

Fig.3: Changes in 12% trichloroacetic acid–soluble nitrogen (TCA–SN) in milk samples after heating at different temperatures for 30 min. in the presence of lactose (▲) and in the absence of lactose (■).

D. SDS-PAGE of heated milk samples

The SDS-PAGE electrophoretograms in presence of 2-mercaptoethanol (ME) of the milk samples after heating at different temperatures for 30 min. are shown in Figure 4. The electrophoretic patterns indicate that heat temperatures and presence of lactose had no effect on milk protein bands . While in Figure 5 which present SDS-PAGE electrophoretograms in absence of ME of the milk samples after heating at different temperatures for 30 min., a high molecular weight proteins bands were appeared .

These results indicate that disulphide bonds were responsible for the covalent bonds leading for formation of the polymeric milk proteins visible in Figure 5. The changes in electrophoretic patterns of the milk samples increased with heating temperature. The high-molecular-weight bands appeared in the samples with and without lactose.

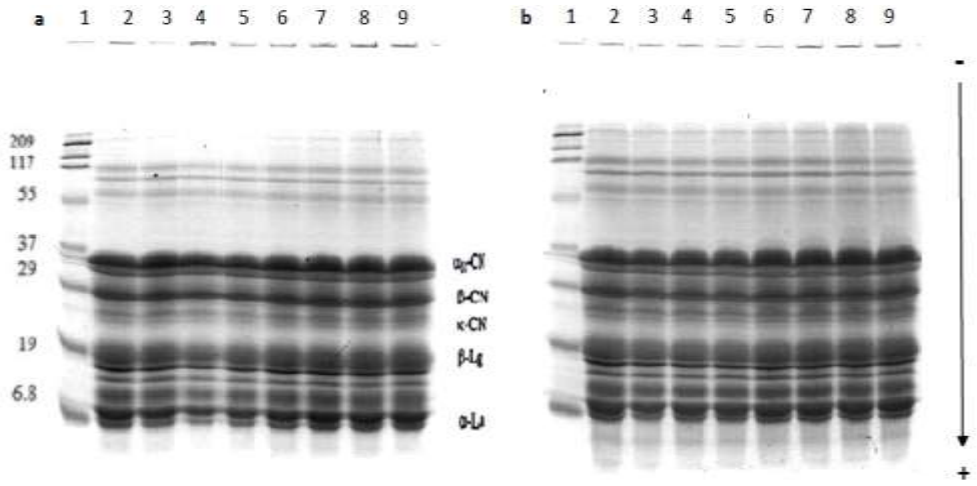

Fig. 4: SDS-PAGE of milk samples in presence of ME after heating at different temperatures for 30 min. in the presence of lactose (a) and in the absence of lactose (b) . 1, MW standards(KD), 2, unheated milk, 3, milk heated at 65 °C, 4, milk heated at 70 °C, 5, milk heated at 75 °C, 6, milk heated at 80 °C, 7, milk heated at 85 ° C, 8, milk heated at 90 °C and 9, milk heated at 95 °C.

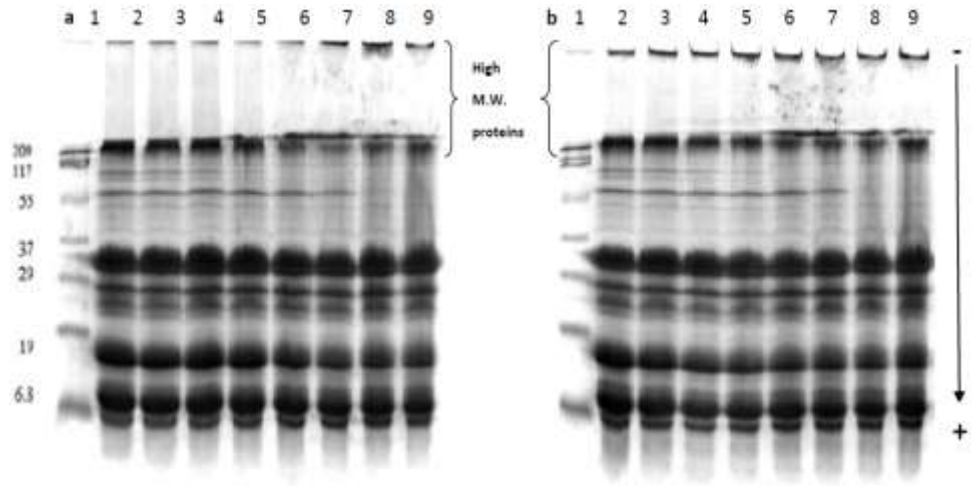

Fig. 5: SDS-PAGE of milk samples in absence of ME after heating at different temperatures for 30 min. in the presence of lactose (a) and in the absence of lactose (b) . 1, MW standards(KD), 2, unheated milk, 3, milk heated at 65 °C, 4, milk heated at 70 °C, 5, milk heated at 75 °C, 6, milk heated at 80 °C, 7, milk heated at 85 ° C, 8, milk heated at 90 °C and 9, milk heated at 95 °C.

Figure 6 showing the change in SDS–PAGE patterns of the pH 4.6 whey samples during heating at different temperatures for 30 min. A decrease in BSA ,β-Lg and α-La bands intensity was appear with the increment of heating temperatures of milk in both samples with and without lactose which is attributable to the denaturation of whey proteins and their subsequent interactions with caseins, mainly via disulphide bridges [18].

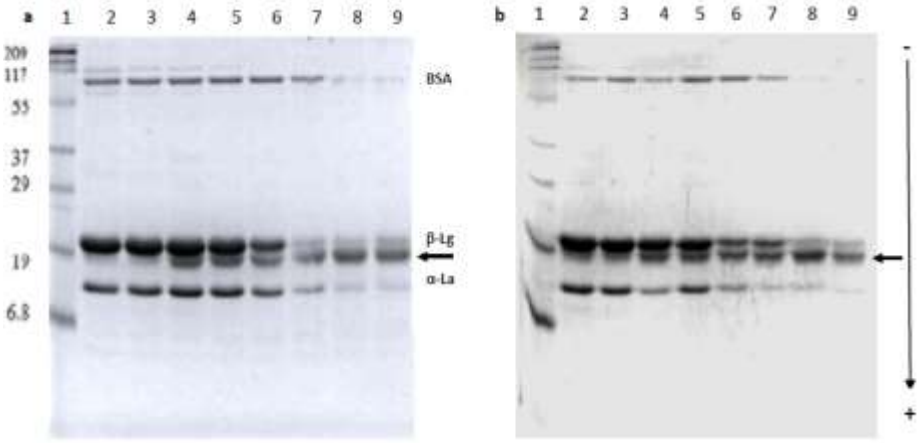

Fig. 6: SDS-PAGE of pH 4.6 whey samples after heating milk at heating at different temperatures for 30 min. in the presence of lactose (a) and in the absence of lactose (b) . 1, MW standards(KD), 2, unheated milk, 3, milk heated at 65 °C, 4, milk heated at 70 °C, 5, milk heated at 75 °C, 6, milk heated at 80 °C, 7, milk heated at 85 ° C, 8, milk heated at 90 °C and 9, milk heated at 95 °C.

Beside that we notice the appear of new band located below β-Lg band in whey samples of both milk ,with and without lactose , and the intensity of this band increased with increment of heating temperatures (Figure7) in the same time a decrease in β-Lg band intensity.

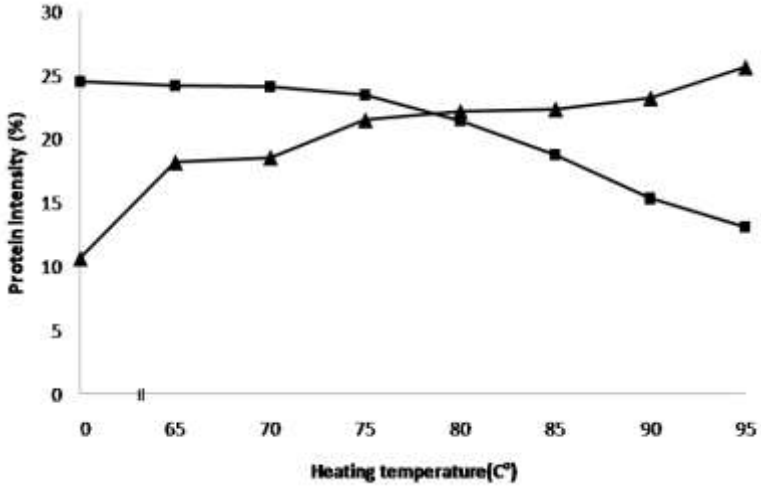

Fig. 7: Changes in β-Lg concentration (■) and new bad concentration (▲) after heating at different temperatures for 30 min.

The effect of heating time on 90 °C on formation of the new protein band is shown in Figure 8.The intensity of this protein band increased with increment of heating time . The formation of this band was not recorded in any research before, and the reason behind it formation is not clear.

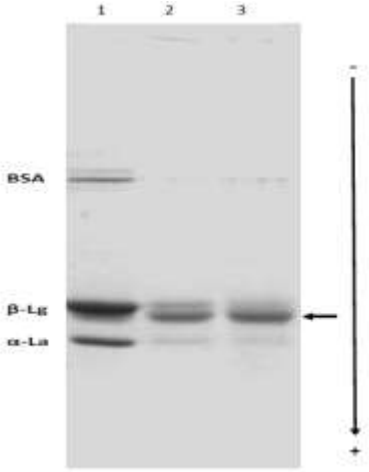

Fig.8: SDS-PAGE of pH 4.6 whey samples after heating milk at 90 °. 1,unheated milk, 2, milk heated at 90 °C for 30 min., 3, milk heated at 90 °C for 60 min.

IV. Conclusions:

During heat treatment of milk, In the presence and absence of lactose major changes occurred .Milk pH increased and the higher increase in pH values was in milk samples without lactose .Major changes in the nitrogen distribution occur.

The pH 4.6 soluble nitrogen decreased with increment of heating temperature and this can be attributed to whey proteins cross-linking with casein via S-S bonds .. The changes were greater in the samples containing lactose than in those with no lactose. Heating cause formation of new band located below β Lg band in whey samples of both milk, with and without lactose, and the intensity of this band increased with increment of heating temperatures and heating time.

.

References

[1] H.S. Singh, "Heat-induced interactions of proteins in milk. Protein and fat globule modifications" IDF seminar, special issue 9303. 191, (1993).

[2] D. W. S. Wong, W. M. Camirand and A. E. Pavlath, "Structures and Functionalities of Milk Proteins" Critical Reviews in Food Science and Nutrition, 36 , pp.807-844, (1996).

[3] H.E. Swaisgood, " Review and update of casein chemistry" Journal of Dairy Science, 76, pp.3054-3061,(1993).

[4] A. J. R. Law, D. S. Horne, J. M. Banks and J. Leaver "Heat induced changes in the whey proteins and caseins" Milchwissenshaft, 49, pp.125-129, (1994).

[5] H. D. Jang and H. F. Swaisgood " Disuiphide bond formation between thermally denatured β-lactoglobulin and k-casein micelles" Journal of Dairy Science, 73, pp.900-904,(1990).

[6] P. F. Fox, " Advanced dairy chemistry. I. Proteins" Applied Science Publishers , London ,(1992).

[7] A. R. Hill, " The β-lactoglobulin-k-casein complex" Canadian Institute of Food Science and Technology Journal, 22, pp.120-123, (1989).

[8] M. Corredig and D. Dalgleish, " The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk" International Dairy Journal, 9, pp.233-236,(1999) .

[9] S. G. Anema and Y. Li, "Effect of pH on the association of denatured whey proteins with the casein micelles in heated reconstituted skim milk" Journal of Agricultural and Food Chemistry, 51, pp.1640−1646 ,(2003).

[10] J. M. S. Al-Saadi , A. M. Easa and H.C. Deeth "Effect of lactose on cross-linking of milk proteins during heat treatments" International Journal of Dairy Technology, 63 ,pp. 1-6, (2013).

[11] R. Jenness and J. Koops" Preparation and properties of a salt solution which simulates milk ultra-filtrate" The Netherlands Milk and Dairy Journal, 3,pp. 153–164,(1962).

[12] A. L, Kelly and J. Foley " Proteolysis and storage stability of UHT milk as influenced by milk plasmin activity, plasmin/β-lactoglobulin complexation, plasminogen activation and somatic cell count" International Dairy Journal ,7, pp.411–420,(1997).

[13] Association of Official Analytical Chemists "Official methods of analysis", 13th ed. pp 47021-47023,(1980).

[14] U. K. Laemmli "Cleavage of structural proteins during the assembly of the head of bacteriophage T4" Nature, 227 ,pp.680-685,(1970).

[15] A.T. Andrews "Properties of aseptically packed ultra-high-temperature milk. III. Formation of polymerized protein during storage at various temperatures" Journal of Dairy Research, 42,pp. 89-99,(1975).

[16] N. Venkatachalam, D. J. McMahon and P. A. Savello "Role of protein and lactose interactions in the age gelation of ultra-high-temperature processed concentrated skim milk" Journal of Dairy Science,76,pp.1882-1894,(1993).

[17] H.T. Wright" Nonenzymatic deamidation of asparaginyl and glutaminyl residues in proteins" Critical Reviews in Biochemistry and Molecular Biology, 26,pp. 1-52,(1991).

[18] D. G. Dalgleish and J. A. Hunt" Protein-protein interactions in food materials"In Ingredient Inter-actions. Effects on Food Quality (Ed. A.G. Gaonkar). Marcel Dekker, New York, pp. 199-233,(1995).

[19] M. van Boekel "Heat-induced deamidation, dephosphorylation and breakdown of caseinate " International Dairy Journal, 9,pp. 237-241,(1999).

[20] L. Pellegrino, M. van Boekel, H. Gruppen, P. Resmini and M. A. Pagani " Heat-induced aggregation and covalent linkages in β-casein model systems" International Dairy Journal, 9,pp. 255-260,(1999).

[21] J. M. S. Al-Saadi and C. H. Deeth "Cross-linking of proteins in UHT milk during storage at different temperatures" Australian Journal of Dairy Technology, 63,pp. 93-99,(2008).