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ASSESSMENT OF CASEIN CONTENT IN MODEL SYSTEMS DURING HEAT TREATMENT

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Heat treatment affects the sensory, biophysical and nutritional properties of milk. To maintain and improve milk quality, an optimization of the heat treatment must be reached to ensure the microbiological safety of the product, while altering as little as possible its sensory and nutritional value. One of the major events occurring upon heating is protein denaturation. The rate of protein denaturation is governed by the immediate chemical environment of the reactants defined by the chemical composition of the system. The aim of the present work was to compare the kinetics of casein denaturation under different conditions, in order to define suitable markers to sensitively assess the degree of reaction in early stages accounting for the time/temperature effects. The results showed a clear dependence of protein denaturation on pH and calcium content, but temperature-time combination did not have a major impact on the extent of protein heat-induced changes.

Keywords: casein, denaturation, thermal indicators, kinetic

1. Introduction

Heat treatments of milk are widely used to modify the processing characteristic of milk in dairy products manufacturing for a variety of applications (Yüksel and Erdem, 2005).

To overcome the product processing drawbacks, as well as to keep safe heat treatment under control, certain criteria need to be defined. Such criteria not only must guarantee the correctness of heat treatment, but can also be applied for quality management in all stages of the production process (e.g. authenticity, HACCP, traceability) (Claeys, 2003). Moreover, these criteria must result in products on the market, that comply with their labeling in terms of processing.

Changes in milk composition influenced heat-induced interactions of proteins and thermal stability of milk. Many of these modifications cause changes in the solubility properties of milk proteins.

The accurate determination of the thermo-physical properties in milk is very important for the design, simulation, optimization, and control of food processing methods such as heat-treatment.

Caseins in milk of the genus *Bos* were defined as those phosphoproteins that precipitate from raw skim milk by acidification to pH 4.6 at 20°C. The caseins have biological function, including efficiently transporting inorganic calcium and phosphate to the neonate (Farrell, 1999). Inherent in this process are protein–protein interactions, protein–protein self-association and protein–salt interactions (Qi *et al*., 2004). The collective result of these interactions leads to the formation of stable colloidal complexes termed casein micelles (Swaisgood, 1992).

Protein concentration, heating temperature, and the type and concentration of salts present in milk protein systems are also important parameters in protein denaturation and aggregation (Parris *et al*., 1997).

The objective of this study was to follow the heat induced changes in milk model systems measured by changes in casein concentration for different pH and calcium concentrations.

2. Materials and methods

2.1. Preparation of reaction mixtures

Casein solutions (3.0 mg/ml) were prepared in 100 mL 0.07 M phosphate buffer at pH 7.5 and 6.6, with (0.2 M and respectively 0.02 M calcium chloride) or without Ca^{2+}. All solvents and chemical reagents were of analytical grade.

2.2. Heat treatments

Plastic tubes were filled with 1.5 mL of proteins solution. The thermal treatment experiments were performed in a thermostatic water bath kept to various constant temperatures (60-90°C) for preset time intervals (0 - 40 minutes). After the thermal treatment, the tubes were immediately cooled in ice water to prevent further reactions. The changes in absorbance were measured exactly in 2 min after the thermal treatment.

2.3. Protein content measurement

Measurement of absorbance at 280 nm where proteins show maximum absorption has been used to determine casein concentration. The procedure was described by Nakai *et al.*, (1964) as follow: for 0.5 mL (un)heated protein solutions, 0.5 ml butylamine was added. The solutions were held in a water bath at 65^0C for 5 minutes. Over that time period, 25 mL of diluting agent (3 g of ethylenediaminetetraacetic acid – EDTA and 3 g of Sodium lauryl sulfate - SLS in 500 ml water) was added. Five drops of HCl was used to neutralize the solution that was afterwards filtered through No. 42 Whatman paper. The optical density was read at 280 nm after 15 minutes using water as blank, after adding one drop 30% NaOH. Absorbance was measured in a UV–VIS GBC Cintra 202 spectrophotometer.

3. Results and discussions

3.1. Influence of pH

The effects of heat treatment on the components of milk are very important for the final product character, since they undergo modifications that affect sensory and nutritional quality of milk. The effects of the wide range of technological processes used in the dairy industry may be evaluated by determining several chemical compounds specifically related to such processes, either through degradation of original milk components or as result of reactions at high temperatures used.

There is a need to select and define potential thermal intrinsic indicators to evaluate the destruction of the target micro-organism associated with each heating process, taking into consideration the safety and quality aspects.

The European Regulation EC 2597/97 proposal suggests the following indicators to evaluate the heat-treatment of milk: denaturation, degradation and inactivation of heat labile components (whey proteins, enzymes and vitamins) and unspecific compounds formed during heat treatment (lactulose, hydroxymethylfurfural, furosine, etc.).

In the literature, there is a lack of data regarding the behavior of casein during heat-treatment of milk. A better understanding of the milk constituents behavior during heat-treatment is essential for the control and predicts functional and nutritional properties of the end-products and to optimize the thermal processing condition.

The mechanism and the profile of the reactions in milk during thermal treatment are very complicated. Therefore, in this study, we have been used a model systems to limit the scope.

Kinetics plots of the protein denaturation measured by residual concentration of casein heated in 0.07 M phosphate buffer, at pH 7.5 are given in Figure 1. The plots show that depending on the temperature-time

combination, the protein content gradually decreases until an equilibrium value was reached, especially within 75-90^0 C temperature range. At 65^{0}C and 70^{0}C, the kinetics of casein denaturation seems to follow a linear decrease (R = 94.5% and 96.8%, respectively).

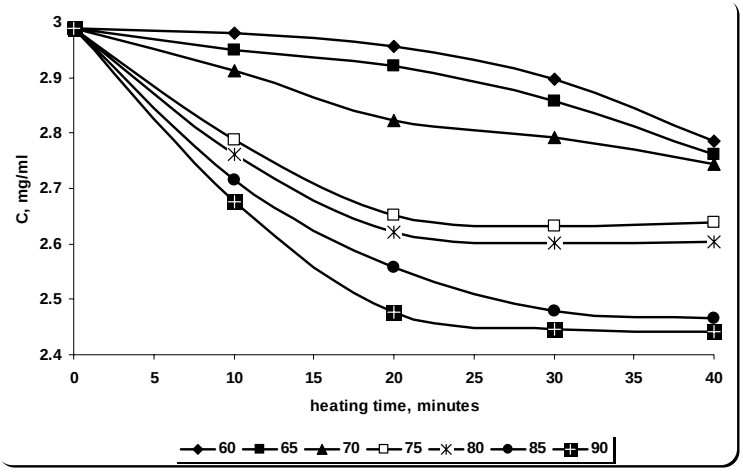

Figure 1. Time dependent changes in casein solutions heated in 0.07 M phosphate buffer, at pH 7.5 and different temperatures

As it can be seen, the plots show two temperature intervals: 60-70^{0}C with a minimal loss of casein (6.7-8%) and 75-90^{0}C with 11-18 % losses of protein content after 40 minutes of heating. That could be explained by the excellent heat resistance of casein in the absence of calcium and for a neutral pH.

Figure 2 shows the kinetics of heat induced changes in casein concentration in 0.07 M phosphate buffer for pH 6.5. For a physiological pH of milk and in the absence of Ca^{+2}, casein seems to be heat resistant in temperature ranges of 60-75^{0}C, with a minimal decrease in concentration (9 to 12%), even after 40 minutes of heating. More significantly heat-induced changes in protein concentration can be observed for a higher temperature range suitable to ultra high pasteurization (80-90^{0}C). Thus, after 40 minutes of heating at 90^{0}C, the concentration of protein was by ~20% lower than the initial value.

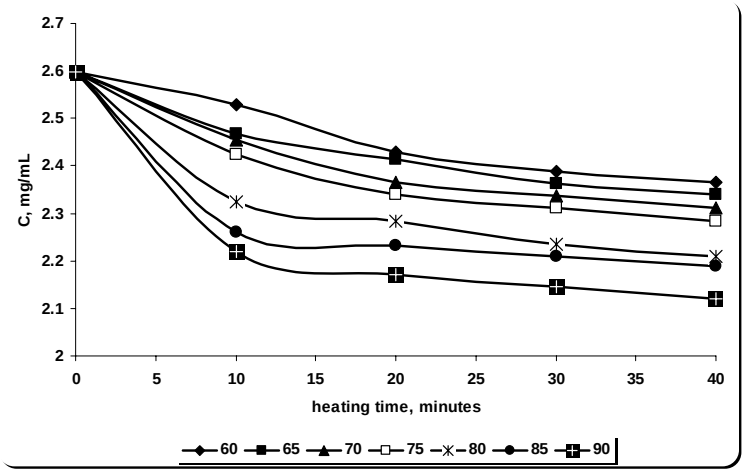

Figure 2. Time dependent changes in casein solutions heated in 0.07 M phosphate buffer, at pH 6.5 and different temperatures

It should be mentioned that, in the native state, the casein is rather insoluble in water. It displays a partial solubility at certain ionic strength and pH. That is why, in this study, the initial protein concentration is different, as it can be seen. Although, to compare the dynamics of casein denaturation, we have used the parameter C_0/C_f (initial concentration: final concentration at certain temperature –time combination) to quantitatively describe the heat-induced changes.

The decreasing tendency of the casein concentration during heat-treatment revealed little changes in the protein content, indicating that casein displays thermal resistance when the solutions are heated both at pH 6.5 and 7.5.

To compare the dynamics of casein content changes during thermal treatment, Figure 3 shows the C_f/C_0 rapport which describes the changes in solutions heated at pH 6.5 and 7.5, at different temperatures for 40 minutes.

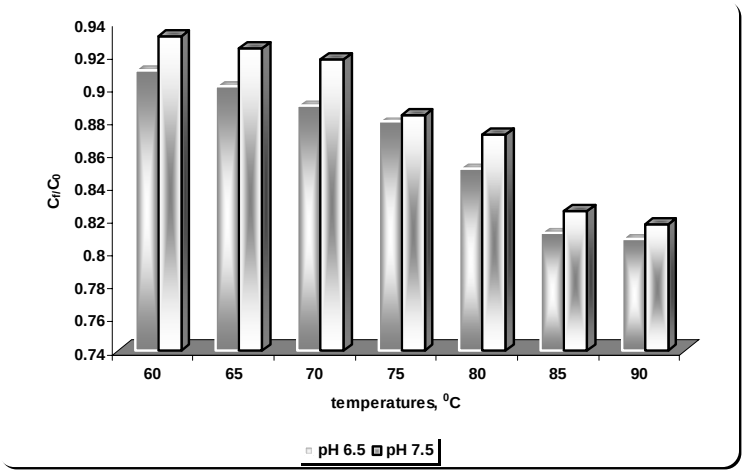

Figure 3. Changes in protein content after 40 minutes of heating as a function of temperature and pH

It can be seen, the heat-induced changes in casein concentration are more advanced for both pH values, especially for higher temperature range (80-90^{0}C), being by ~20% higher compared with the values calculated at time 0 (native state of protein).

3.2. Influence of Ca^{2+} concentrations

Although much has been reported on the role of Ca^{2+} in systems based on milk protein on the formation of gels, the effect of Ca^{2+} on the protein denaturation or formation of complexes is not fully understood.

The protein content in casein solutions heated at pH 7.5, in the presence of 0.02 M $CaCl_2$ at different temperatures, as a function of time, is given in Figure 4. Similar decrease tendencies were observed in these model systems.

Casein did not show significant change in the temperature range of 60 to 80^{0}C. At 60 and 65^{0}C the decrease in protein content seems to have a linear tendency (R=98.7% and 96.6%, respectively). The maximum denaturation was observed at 85 and 90^{0}C, after 40 minutes of holding at constant temperature, with a maximum loss of casein of 19.2%.

Increasing the Ca^{2+} content (0.2 M calcium chloride) caused a decrease in casein concentration, especially for a higher temperature range. Protein showed almost the same behavior at 85 and 90^0C. The results are depicted in Figure 5.

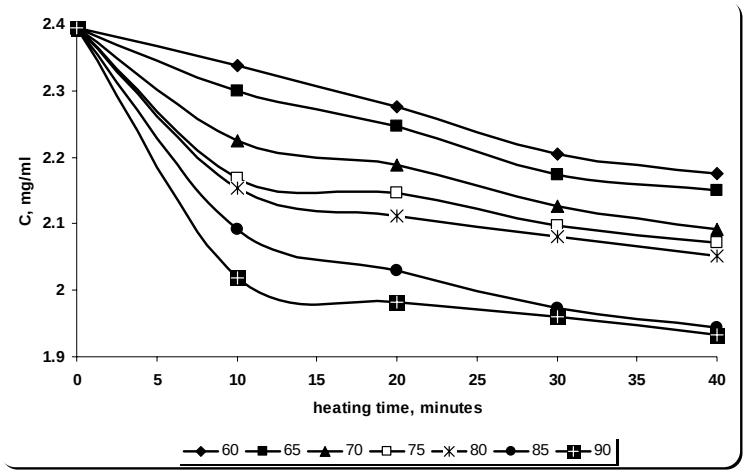

Figure 4. Time dependent changes in casein solutions heated in 0.07 M phosphate buffer, with 0.02 M $CaCl_2$, at pH 7.5 and different temperatures

For 60-75^0 temperature range, the residual casein was lower with 9.6-14% after 40 minutes of heating. At 80-90^0C, the denaturation was much more pronounced, the concentration of the protein in solutions after 40 minutes of holding was lower by 16-21.5% compared with the native state.

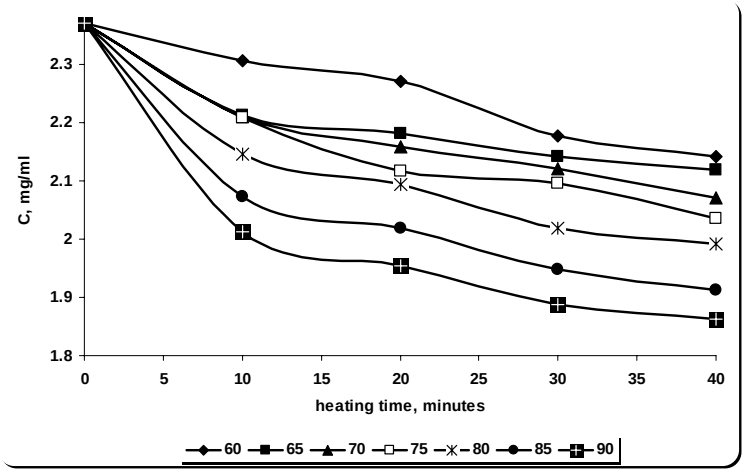

Figure 5. Time dependent changes in casein solutions heated in 0.07 M phosphate buffer with 0.2 M $CaCl_2$, at pH 7.5 and different temperatures

In order to evaluate the influence of calcium concentration, Figure 6 shows the C_f/C_0 values for the results obtained after maintaining the solutions for 40 minutes, at different constant temperatures.

It can be seen that the temperature did not have a major impact in protein heat-induced changes in whole range studied. The calcium content of samples had a significant effect on protein denaturation and aggregation, increasing calcium content leading to a higher loss of casein.

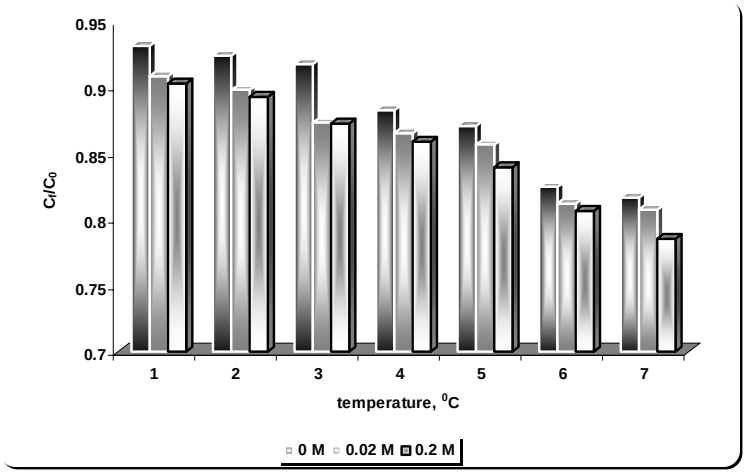

Figure 6. Changes in protein content after 40 minutes of heating as a function of temperature and calcium concentration

The caseins are stable during the principal processes to which milk is normally subjected. They are very stable for high temperature, coagulating only at 140^0C/15-20 minutes, at the physiological pH of milk. This phenomenon is not due to the protein denaturation, but to major changes which occur in the milk systems as a result of high temperature treatment (a decrease of pH mainly due to the formic acid, dephosphorylation of the casein, denaturation of whey proteins, etc). Also, caseins are stable for high calcium concentration, at least up to 200 mM at temperature up to 50^0C (Fox, 2003).

Bifactorial analysis of variation (ANOVA) was used to confirm the influence of pH and calcium concentration upon the variation of protein content in solutions after heat-treatment. The statistical analysis showed that both pH and calcium chloride concentration have a significant influence on the protein content after thermal treatment (data not shown).

Qi *et al*., (2004) suggested that β-casein has a rigid molecule and proposed the term of "native pre-molten globules" to describe the thermal and alkaline denaturation of the protein. Also, referring at the excellent stability of caseins, Uversky (2002) and Tompa (2002) suggested the terminology of "native unfolded" proteins.

4. Conclusions

To overcome the product over processing, as well as to control the heating system adequacy on the safety level, certain criteria need to be defined. Not only must such criteria guarantee the correctness of the heating treatment, but they can also be applied for quality management on the level of all the stages of the production process (e.g. authenticity, HACCP, traceability). Moreover, these criteria must result in products on the market that comply with their labeling in terms of processing.

The results showed in this study suggest that the pH has an important influence on casein behavior within the temperature range of $80\text{-}90^0$C. Also, the presence of calcium is very important for the aggregation of

protein, increasing the Ca^{2+} content for a higher time-temperature combination leading to an advanced denaturation rate of casein. When the calcium ion concentration is decreased the possibility for the formation of compact micelles also decreases. In the absence of calcium ions, the huge micelles break into the submicelles which prove to have a more open structure than whole micelles do.

It is very important to mention that for both sets of experiments, the time-temperature combination did not have a significant effect on casein, which leads us to conclude that this major milk protein displays an excellent thermal resistance. That is why we did not recommend using this protein as an intrinsic thermal indicator to differentiate various heat processes applied in milk and dairy industry. The development of universal and well adapted indicators for milk and dairy products by correlating biochemical changes and microbiological criteria should give the opportunity to evaluate quality and safety, as well as the authentication and traceability of the end-products.

Further studies are needed to provide insights into the mechanism of protein denaturation in milk. These specific and complex issues are some of the main objectives of our Research Project PN-II-ID-PCE-2008-2, Idea, ID 517 – *Research resulting in analytical systems for Romanian milk and dairy products traceability in order to comply with European quality and safety criteria*.

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