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FTIR Determination of Protein Aggregates in Enzymatic Coagulation of Cow Milk

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Abstract: The study of Thermally Induced Aggregates (TIA) has been explored by analytical techniques due to the influence of variables such as cutting time, curd firmness and performance, and functional and sensory characteristics of the finished product. Cow milk (3% fat) was subjected to heat treatment by kinetic pasteurization. Heat treatments were applied at 72 ° and 78 °C with scan times from 10 to 200 s. It was observed that for the different coagulation times, the gel generated with raw milk had the highest force value (kg), followed by generated with unpasteurized milk to 72 ° C and finally over-pasteurization treatment of 78 ° C 15 s. The behavior of the absorbance spectra in the kinetics of pasteurization established that formation magnitudes βLG-κCN complex and βLG- βLG polymer depend on the intensity of the heat treatment. Formation of complex BLG-κCN is one of the most important factors causing an increase in rennet coagulation time because their presence decreases the rate of hydrolysis of κCN by steric hindrance at the specific site of action of the enzyme The measurement technique of the coagulation process by FTIR represents a first promising approach for the development of an alternative optical technique to track the changes associated with the enzymatic coagulation of milk and to determine the ideal cutoff of curd.

Keywords: Protein aggregates, infrared spectroscopy, pasteurization, enzymatic coagulation.

INTRODUCTION

Pasteurization of cow milk affect the enzymatic coagulation process because the denatured whey proteins β-lactoglobulin (βLG) interact among themselves and with the casein micelles; in the last case the specific reaction of κ-casein (κCN) and denatured βLG with the consequent formation of the complex βLG-κCN affects the rate of enzymatic hydrolysis[1]. The study of this Thermally Induced Aggregates (TIA) has been explored by analytical techniques due to the influence of variables such as cutting time, curd firmness and performance, and functional and sensory characteristics of the finished product. One of the most versatile analytical techniques currently employed is spectroscopy Fourier Transform Infrared (FTIR) which can be used to evaluate structural changes in the milk proteins as a result of external factors[2].

The aim of this work was to develop a methodology based on FTIR to analyze possible changes in the properties of enzymatic coagulation of bovine raw milk as a consequence to different heat treatments, comparing the results with a conventional technique (instrumental firmness).

METHODS

Spectroscopic analyses were obtained on a FTIR (Vertex 70 Bruker Optics) in the mid-infrared region MIR (400-4000 cm^{-1}) in ATR mode (Attenuated Total Reflectance).

Milk proteins standards: α, β, κ-caseins and β-lactoglobulin lyophilized (Sigma Aldrich) were dissolved in deionized water in the ratio normally found in milk with the purpose of determining the characteristic IR peaks associated with these standards.

Cow milk (3% fat) was subjected to heat treatment by kinetic pasteurization with the implementation of a heating system (Miracle PIKE Technologies, Madison, WI). Heat treatments were applied at 72 ° and 78 °C with scan times from 10 to 200 s. Subsequently, coagulation kinetics was developed from samples thermally treated at 72 °C and 78 °C for 15 s. To contain the sample an eppendorf tube was implemented. To perform the coagulation process, a sample was taken with 10 ml of milk pH of 6.2, which was subjected to a process of thermization at 32 °C for 20 min, subsequently was added 0.005 g of calcium chloride and 1 mL of microbial rennet (Cuamix, Chr Hansen, strength 1: 10000); 1 mL was introduced to the eppendorf tube to start the analysis by FTIR.

Results of FTIR were compared with instrumental texture tests, in which the textural properties of gel generated clotting times of 30, 35, 40 and 45 min samples heat treated at 72 ° C and 78 were evaluated ˚ C for 15 s through an analysis of texture with a texturometer TA.XT Plus, which was calibrated for measuring the degree of firmness with a level of penetration force (kg) over a period of time (s) (depth of 1.58 mm at a speed of 0.05 mm / s).

RESULTS

The results obtained from the spectral analysis of the protein standards (α, β, κ-casein and β-lactoglobulin) were focused on the amide I band (1600-1700 cm^{-1}) and the band 1700- 1750 cm^{-1}, (see Figure 1). According to these results was established that new peaks appeared after each thermal process and are associated with the generation of βLG-βLG polymer (1633-1636 cm^{-1} and 1643-1646 cm^{-1}) and to the complex κCN-βLG (1651-1652 cm^{-1} and 1710-1716 cm^{-1}).

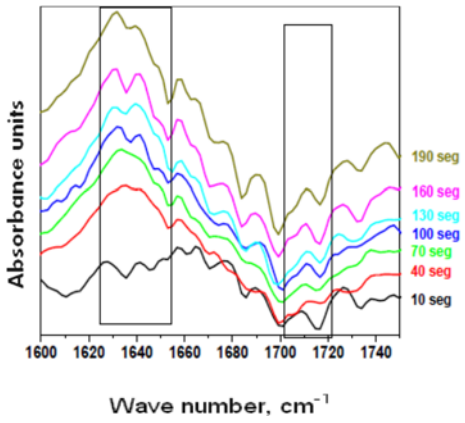

Fig.1: Spectrum BLG-KCN in water at 72 ° C.

FTIR spectral results of the kinetic standardized milk pasteurization (Figures 2 and 3) show that the raw milk (black line) presents a broad spectrum centered and well-defined wave number at 1645 cm^{-1}; meanwhile, the spectra corresponding to milk heat-treated at different temperatures (72 and 78 ° C) show formation of peaks at 1652 and 1710 cm^{-1} (higher wave numbers corresponding to βLG-κCN) and 1632 and 1643 cm^{-1} (lower wave numbers associated with βLG-βLG).

The results of instrumental firmness of gels at different times (30, 35, 40, 45 min and 50 min) corresponding to a typical profile curve gel firmness. It was observed that for the four different coagulation times, the gel generated with raw milk had the highest force value (kg), followed by generated with unpasteurized milk to 72 ° C and finally over-pasteurization treatment of 78 ° C 15 s.

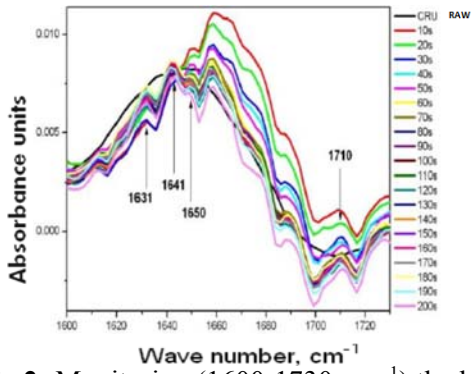

Fig.2: Monitoring (1600-1730 cm^{-1}) the heat treatment of milk (72 ° C 200s) with peltier system.

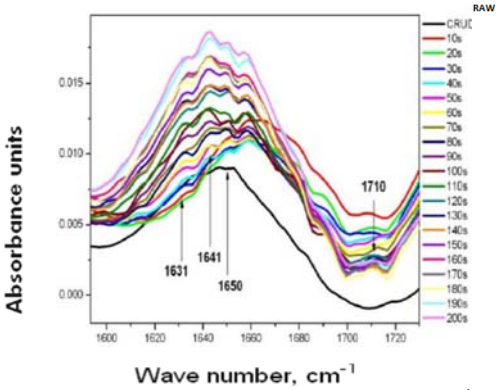

Fig.3: Monitoring (1600-1730 cm^{-1}) the heat treatment of milk (78 ° C 200s) with peltier system.

Texture analysis of corresponding values of firmness (maximum shear force in Kg) and the rate of cutting work, RCW (area under the curve in kg * s,); these results are shown in Table 1. This can be confirmed that the level of firmness (penetration force) decreases proportionally as the heat treatment of milk is intensified. According to the RCW value the optimum strength of raw milk is in a clotting time interval 30-35 minutes; meanwhile, the clotting time for the treatments of 72 °C 15 s and 78 °C 15 s started time ranges 42.09-50.5 min and 43.9-52.5 min respectively.

Table 1: Evolution of strength and work rate cut gels made from milk treated at 72 and 78 °C 15 s.

TIME (min)	RAW		72 °C, 15 s		78 °C, 15 s	
	Hardness (Kg)	RCW (Kg* s)	Hardness (Kg)	RCW (Kg* s)	Hardness (Kg)	RCW (Kg* s)
30	0.0268	0.21492	0.0258	0.19364	0.0252	0.18098
35	0.0315	0.24177	0.0288	0.20661	0.0269	0.19544
40	0.0342	0.26037	0.0298	0.20825	0.0291	0.20629
45	0.0385	0.27438	0.0318	0.22421	0.0308	0.21726
50	-	-	-	0.24017*	0.0329	0.23378
55	-	-	-	0.25613*	-	0.2503*

The results of kinetic clotting which was monitored by FTIR formation caseinomacropeptide (CMP) in the region of 1375-1410 cm^{-1} for raw milk and pasteurization treatment of 72 and 78 ° C 15 s, where is possible observed spectral changes associated with an evolution rate of enzymatic coagulation process.

In Figures 4, 5, 6 can observe different spectral behavior changes during the coagulation process. Featured in the case of raw milk (Figure 4) major changes in the region of 1390-1410 cm^{-1}, where the dotted lines tend to have two peaks at 1396 cm^{-1} and 1404 cm^{-1}, for the first time clotting kinetics which gradually merge into a single peak centered at 1400 cm^{-1}, which occurs at 32 min (solid cyan). This time reached coincides with the value at best firmly conditions shown in Table 1.

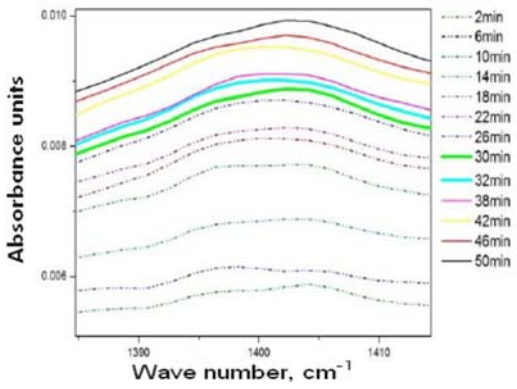

Fig.4: FTIR spectra of the kinetics of raw milk coagulation.

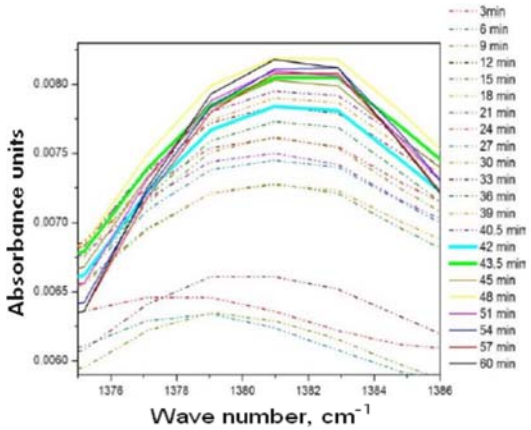

Fig.5: FTIR spectra of the kinetic clotting milk at 72 ° C 15 s.

For coagulation kinetics heat treatment at 72 ° C 15 s and 78 ° C 15 s, have a similar behavior in the spectral region of 1375-1385 cm^{-1}, connecting two peaks within this region. In the treatment of 72 ° C 15 s (Figure 5) peaks at 1380 cm^{-1} and 1382 cm^{-1} are shown, being the peak at 1380 cm^{-1} which has a higher intensity of absorbance during the first minutes of coagulation process (dotted lines) until gradually the peak of 1382 cm^{-1} is matched in intensity over time of between 42 and 43.5 min (solid line of cyan and green). Again this time coincides with firmness conditions shown in Table 1.

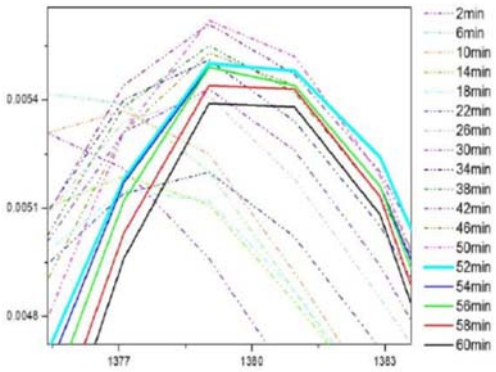

Fig.6: FTIR spectra of the kinetic clotting milk at 78 ° C 15 s.

In the treatment of 78 ° C 15 s (Figure 6) the same behavior in absorbance associated with the peaks at 1379 cm^{-1} and 1380 cm^{-1} is observed, which occurs 52 minutes to equate the intensity of absorbance (solid cyan). This time coincides with the conditions of firmness for cheeses made from milk treated at 78 °C 15 s shown in Table 1.

CONCLUSIONS

FTIR spectroscopy technique can differentiate formation βLG-βLG polymer and βLG-κCN complex by analyzing the peaks between 1631-1641 cm^{-1} and 1652-1720 cm^{-1} present in the band corresponding to amide I.

The behavior of the absorbance spectra in the kinetics of pasteurization established that formation magnitudes βLG-κCN complex and βLG- βLG polymer depend on the intensity of the heat treatment; at mild heat treatment predominates formation of βLG-κCN complex and increasing the intensity of heat treatment formation of polymer βLG-βLG is dominant; this is assumed to occur because there is increased availability of reactive sites (thiol group) in denatured βLG structure, which can interact with other βLG molecules denatured through an exchange reaction of disulfide bonds. The opposite happens in the protein interaction βLG-κCN where the thiol group of Cys160 residue of βLG is specific for reaction with the κCN by reactions of thiol disulfide exchange. Formation of complex BLG-κCN is one of the most important factors causing an increase in rennet coagulation time because their presence decreases the rate of hydrolysis of κCN by steric hindrance at the specific site of action of the enzyme.

Prediction of curd cutting time by FTIR might be related to the values of shear strength of gel experimentally determined by instrumental texture test.

The measurement technique of the coagulation process by FTIR represents a first promising approach for the development of an alternative optical technique to track the changes associated with the enzymatic coagulation of milk and to determine the ideal cutoff of curd.

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