Introduction

The heat treatment of milk is an essential step of industrial dairy food processes, and can result in partial denaturation of whey proteins. As a result, free sulfide groups become available and can form an association on the surface of the casein micelle with κ-casein (κ-CN). This association can affect the functional properties of milk in downstream applications such as cheese, where it could negatively affect moisture content and texture, and in yogurt where it may improve textural properties and reduce wheying-off.

Early prediction of heat induced whey protein denaturation will allow milk batches to be used for their most suitable purposes. The economical impact this would have on the dairy industry, in addition to the non-existence of a simple, inline method of protein denaturation measurement, motivates this research. The goal of this project is the development of an optical sensor technology for inline determination of whey protein denaturation and subsequent association with κ-CN during milk heat treatment. Given the scattering properties of the micelles, light backscatter was chosen for optical measurement.

Preliminary experiments indicate that light backscatter response is proportional to heat treatment intensity, which suggests that light scatter could be used to determine the whey protein denaturation degree. The development of a successful method of whey protein denaturation determination will require: a) finding the adequate wavelength/s, b) developing a robust prediction algorithm, c) obtaining chemical data supporting the observed optical responses, and d) improving the current optical sensor configuration to better suit this methodology. Successful development of this optic sensor technology will aid in the decision-making process of dairy plants for the efficient use of raw milk and the assurance of a high quality product.

Objectives

The main goal of this research is to develop an inline light backscatter sensor technology to measure thermal denaturation of whey proteins during milk processing. The proposed measuring technique is being developed based on the existing correlation between the light backscatter ratio response in heat-treated milk and the degree of denaturation of its whey proteins. This correlation allows using a light backscatter ratio as an indicator of the interaction taking place on the surface of the casein micelle between thermally denatured whey proteins and κ-CN during heat exposure.

This Emerging Technology Proposal has the following specific objectives:

1. Set up a laboratory system for optical measurement of thermally processed milk samples. This would involve the improvement of the current optical system to better suit this methodology.
2. Analyze light backscatter response over a broad range of wavelengths for varying degrees of heat-treated milk.
3. Determine optical parameters (e.g., wavelength ratios) which are the best predictors of whey protein denaturation.
4. Obtain physical and chemical data to validate that whey denaturation occurred when light backscatter increased.

In this project proposal, a non-invasive optical technology for determination of milk's protein quality for improved dairy processing is proposed. The ultimate outcome of this research is the development of an optical sensor technology that can aid in the decision-making process of dairy plants for the efficient use of raw milk and the assurance of high quality dairy products.

Work performed

The project has progressed satisfactory, according to the Gantt Chart presented in the proposal. During the project duration all the specific goals and deliverables that were foreseen have been achieved successfully (100%). The results derived from the research project have contributed to develop solid bases for building an inline sensor prototype for whey protein denaturation determination and for future refinement of this optical technology. According to the project Gantt Chart, 100% of Objectives 1, 2, 3 and 4, have been successfully completed during the project. All the data obtained had been evaluated, and processed. The result obtained have been partially published (two peer-reviewed papers published, a third one under evaluation, 9 abstracts/conference papers presented and one more under evaluation). A number of peer-review papers and conference abstracts are planned (several of them are already in progress).

Results

The changes induced by heat treatment in light backscatter, front-face fluorescence, casein micelle size and different whey protein fractions (native whey proteins, total denatured whey proteins, denatured whey proteins attached to casein micelles, and denatured whey proteins forming soluble aggregates) were evaluated at different heating temperatures, heat exposure times and milk pHs in skim milk, low fat milk and whole milk.

Consistent with the literature was the observed decrease in casein micelle particle size with increasing pH (Figure 1). Additionally this relationship was accentuated at higher temperatures, where a higher percentage of the whey proteins in milk are denaturated. Change in particle size appears to fit well to a polynomial curve, with high R^2 values of 0.87 and 0.99 at 80°C and 90°C, respectively. The complete curve of the light backscatter spectrum as a function of pH and temperature is shown in Figure 2. There is an obvious relationship of peak intensity with respect to pH, as well as relationship with respect to temperature of heat treatment at each various pH values. With respect to light backscatter, it was also observed a marked decrease in maximum intensity with respect to an increase in pH (Figure 3) at both 80°C and 90°C. Additionally, there was a correlation between light backscatter and particle size, which was only significant at pH 6.3 (r = 0.87), while pH 6.7 and 7.1 proved poor correlation (r = 0.15 and 0.45, respectively, P > 0.05), which indicated attachment to casein micelle at pH 6.3, and a lack of attachment at lower pH values (Table 1).

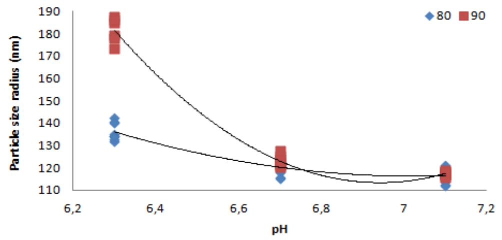

Figure 1. Particle size as a function of pH at temperatures 80°C and 90°C.

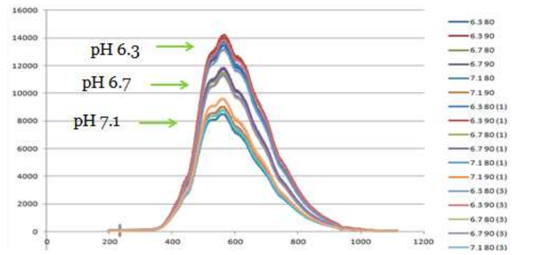

Figure 2. Light backscatter intensity (200-1100nm) as a function of milk pH at temperatures 80°C and 90°C.

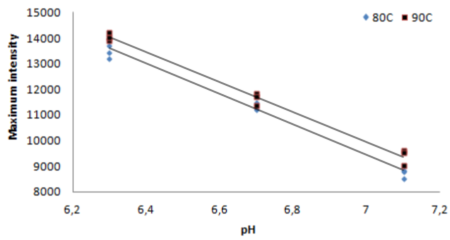

Figure 3. Maximum light backscatter intensity (approximately 570 nm) as a function of pH at temperatures 80°C and 90°C.

	Modelo			Fuente de variación		CORR
				Rep (GL=2)	T (GL=1)	
pH	Parámetros	R^2	F	F	F	r
6.3	PS	0.975722	26.79*	0.28	79.82*	0.87416*
	LB	0.989758	64.42*	24.91*	143.45*	
6.7	PS	0.588365	0.95	0.69	1.48	0.14976
	LB	0.854237	3.91	5.20	1.31	
7.1	PS	0.615256	1.07	1.45	0.30	0.45095
	LB	0.979229	31.43*	11.38	71.52*	

Table 1. Statistical analysis, *P<0,05; PS, particle size; LB, light backscatter.

Our results showed that changes in light backscatter signal are primarily based on the pH-dependent attachment of denatured whey proteins to the surface of the casein micelle. Thus the pH dependent formation of whey protein serum aggregates and casein/whey protein complexes was taken into consideration for the development of predictive models to determine whey protein denaturation during milk heat treatment.

Detailed statistical analysis of the experimental data allowed obtaining prediction models for casein micelle size and several different whey protein fractions in heat-treated milk, successfully, using just light backscatter predictors. Table 2 summarized the most relevant prediction models obtained. This information established the base for further development, design, and manufacturing of an inline optical sensor prototype for monitoring whey protein denaturation and casein particle size during heat treatment in skim milk and low fat milk, which will be novel and cheap. To this regard, we are preparing a patent disclosure application for evaluation by the UAB IP Office if a patent application procedure should be initiated to protect this interesting and novel finding. For this reason, no further details are presented about the developed models in the present report (wavelengths of each specific ratio), to avoid improper disclosure of potential IP rights generated in this research.

Table 2. Optical prediction of casein micelle size and several different whey protein fractions in heat-treated skim milk[a]

Predicted parameter	Light backscatter prediction model	Determination coefficient, R^2
Casein micelle diameter (CMD)	$CMD = \beta_0 + \beta_1 \cdot LBR_1 + \beta_2 \cdot LBR_1^2$	0.98
[Heated native whey protein] (HNWP)	$HNWP = \beta_0 + \beta_1 \cdot LBR_2 + \beta_2 \cdot LBR_2^2$	0.85
[Denatured whey protein] (DWP)	$DWP = \beta_0 + \beta_1 \cdot LBR_3 + \beta_2 \cdot LBR_3^2$	0.84
[Denatured whey protein soluble aggregates] (AWP)	$ADWP = \alpha_0 + e^{\beta_0 + \beta_1 \cdot LBR_4}$	0.86
[Denatured whey proteins bound to micelles] (BDWP)	$BDWP = \alpha_0 + e^{\beta_0 + \beta_1 \cdot LBR_5}$	0.97

[a] LBR_1 to LBR_5 correspond to five different optical ratios calculated by dividing the light backscatter intensities of two specific wavelengths between 200 and 1100 nm

Several interesting models based on front-face fluorescence have been also obtained, which represent complementary results that, in fact were not anticipated/foreseen in this project.

Conclusions

Results obtained in this project confirmed our initial hypothesis showing potential of light backscatter not only for real time quantification of denatured whey proteins but also for inline measurement of casein micelle size. Casein micelle size were found to be a function of the degree of attachment of denatured whey proteins to the surface of casein micelle, which was in turn a function of temperature (degree of denaturation) and pH (degree of denatured whey protein attachment to micelles). Due to the observed correlations, a number of predictive models using light backscatter ratios as predictors were obtained that allowed estimating casein micelle size and concentration of several different whey protein fractions in heat-treated milk.

Potential impact

It is evident that the concept for an inline optical sensor technology to indicate whey protein denaturation and casein micelle size in milk has potential as a technology for process control in gel formation of dairy products. The technology consists of a unique optical sensor, which will evaluate the light backscatter from a ratio of two wavebands. According to the results, the light scatter increase is proportional to thermal severity, as expected. Thus, the results obtained are extremely encouraging, showing that the proposed technology is feasible. Whey denaturation and aggregation can create gelation problems in fresh and aged cheese varieties. From a market standpoint, the classification of milk based in thermal damage could allow for more consistent high quality dairy products. The technology will allow for the ideal thermal exposure level dependent on milk's purpose.

A whey denaturation inline sensor technology is currently unavailable. The proposed sensor technology being developed will be highly significant for the dairy industry and will greatly advance the way industries determine and monitor thermal protein denaturation in milk and dairy products. This sensor technology will provide the dairy industry with a powerful tool to increase heat treatment control, which will have an impact in milk and dairy foods product consistency and quality. This new method, if further developed, validated and transfered will aid in the decision-making process of dairy plants to assist them to efficiently select the use of the milk in agreement with the real functionality of its whey proteins after heat treatment.