



Article

Performance Evaluation of Fiber Near-Infrared (NIR) Optic Probes for Quality Control of Curd Hardness in Cheese Produced by Spray-Dried Milk

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Abstract: This study aims to provide the dairy industry with a direct control model focused on milk coagulation by using multifiber probes to determine parameters in the curding process, such as cutting time, at a lower cost. The main objective of the research is to confirm that a multifiber NIR light scattering probe can be used to predict the elastic modulus of curd during milk coagulation in cheese production. Two randomized complete block designs were used with a 3×3 factorial arrangement of three protein levels (3%, 3.5% and 4%) and three wavelengths (870 nm, 880 nm and 890 nm). Using a multifiber probe at a wavelength of 880 nm allowed obtaining a better optical response of the sensor during enzymatic milk coagulation than the 870 nm. It showed greater sensitivity to variations in the protein content of the milk and lower variation in the response. The multifiber probe at a wavelength of 880 nm generated a NIR light backscatter profile like those obtained with other systems. The results showed that the prediction model parameters had a variation as a function of the protein content, which opens the possibility of improving the prediction model's performance substantially. Furthermore, the initial voltage obtained with the probe responded linearly to the different protein levels in milk. This fact would make it possible, at least theoretically, to estimate protein concentration with the same inline probe for G' determination, facilitating the incorporation of a corrective protein factor in the prediction models using a single instrument.

Keywords: multifiber probe; coagulation; prediction; spray-dried milk



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1. Introduction

Cheese production was approximately 21.86 million metric tons worldwide, while the USA manufactured over 6 million tons, in 2021 [1]. Cheese is a dairy product that has played an important role in human nutrition for centuries. Since then, the main objective has been and is today to transform a highly perishable product, such as milk, into another that has a long life and preserves its nutrients. In essence, cheese is made by removing water or whey, allowing the solids or curds to be controlled. Cheese is a healthy and diverse dairy product regularly consumed as part of a dish, as a snack, or as a premade meal. Cheese-making is a process in which the fat and casein of milk are concentrated in five steps: acidification, coagulation, whey separation, molding, and salting [2,3]. Several parameters impact the transformation of milk into cheese, and the knowledge of such factors could represent a competitive advantage for the cheesemaker. The chemical composition of the milk plays a crucial role in cheese manufacture due to the complex biochemistry and microbiology involved [4]. Cheesemaking technology permits the production of various

flavors, textures, and consistencies [5], which involves the meticulous monitorization of the curd cutting time.

Cheesemaking can be considered a sensitive process. Once the gelation point is reached, the curd's hardness increases with time. Therefore, selecting the optimal hardness moment for the cut is necessary, which induces the drainage process [6]. The cut should be performed when the curd is sufficiently cohesive but has not reached excessive hardening [7]. The changes in the optical properties of milk during coagulation have allowed, primarily using optical fibers, the development of a series of instruments based on determinations of reflection, absorbance, dispersion, and refraction of light [8]. These are continuous and non-destructive methods. Among the properties mentioned, light scattering is one of the properties that yields the best results for the study of gel formation. In a non-absorbing medium, it is possible to directly measure the portion of incident light that is dispersed at a certain angle or indirectly measure the portion of the light that has not interacted with the medium, that is, the transmitted light [9]. In milk, near-infrared light scattering is directly related to the speed of micellar aggregation and gel hardening, so this optical sensor technology has been proven as a valuable tool to control milk coagulation [10]. In particular, the fiber optic sensor near-infrared (NIR) scattering, CoAguLite, is an inline sensor that has been well-documented for monitoring milk coagulation and predicting gelation and cutting times [11] and is currently commercially available for inline control in cheesemaking, mainly in the United States. Several previous studies have shown that such a sensor is a promising instrument for inline monitoring of optical properties during milk coagulation, providing indirect information on relevant technological parameters in cheese production. Arango et al. (2018b) [12] obtained and validated curd cutting time prediction models using optical time parameters determined by calculating derivatives from the dispersion ratio profile as a function of time. Similarly, Salvador et al. (2019) [13] found that the optical light scattering sensor predicts well the time the gel reaches values of $G' = 30$ Pa.

On the other hand, Arango and Castillo (2018a) [10] developed and patented a method for real-time monitoring of gel firmness, which consists of a mathematical model that relates the NIR scattering signal obtained through the sensor optical fiber with the elastic modulus of the gel. This method does not involve time parameters and, therefore, allows the collected light scattering ratio data to be introduced directly into the model to calculate the value of G' in real time [14].

The curd cutting time is a critical aspect that impacts the quality characteristics of cheese [15]. Cutting time can significantly affect cheese yield, and the curd can change fat, whey, and moisture contents. A high-moisture curd results from cutting the curd too late, and a poor yield could be caused by cutting the curd too early. The curd cutting time is typically monitored by instruments based on mechanical approaches, vibrational techniques, ultrasonic methods, electrical conductivity, hot wiring, and optical systems [16]. These apparatuses are used to examine the rheological characteristics of the curd. The curd cutting time could demand a more effective, inline, and non-destructive method.

The light backscatter sensor could be a possible option, and these optical systems have been suggested by various studies [17] since these systems are non-destructive, rapid, and effective. Optical technology developed by Payne et al. (1999) [18] was designed for cow milk cheese production, and Castillo (2000) [2] and Nicolau et al. (2015) [19] evaluated and adapted NIR (near-infrared) light backscatter technology, respectively, for goat and sheep milk cheese manufacture. Thus, developing an optical sensor is required in spray-dried milk cheese. When predicting the cutting time, the NIR practicality of cow's, goat's or sheep's milk cheese could vary from spray-dried milk cheese. Therefore, the objective of this study was to consider if an inline fiber optic sensor with NIR could be applied to monitor the cutting time of curd hardness milk cheese produced by spray-dried milk. The study represents an innovative approach for the dairy industry to monitor milk coagulation in cheese production, as it uses multifiber probes and specific wavelengths to predict curd elasticity during milk coagulation, optimizing the process economically. The study

employed randomized block designs and varying protein levels and wavelengths, with 880 nm showing superior sensitivity and stability in response.

2. Materials and Methods

2.1. Milk Preparation and Coagulation

The milk coagulation was prepared and performed according to Nicolau et al. (2015) [19], with slight modifications. Liquid milk was produced by mixing spray-dried, skim milk powder (Arla Foods company, Madrid, Spain) with distilled water at ~1:9 ratio, with small variation according to mass balance in order to achieve the target protein concentrations described in Section 2.2. The obtained milk was then vat-pasteurized at 65 °C for 30 min, and, after pasteurization, refrigerated at 4 °C until further use. For each sample, 500 mL of milk was collected and heated to 35 °C, and a calcium chloride solution (100 $\mu\text{L kg}^{-1}$) (35%, Laboratorios Arroyo, S.A. 39011, Santander, Spain) was incorporated. The rennet solution (300 $\mu\text{L kg}^{-1}$) (Chr. Hansen, Barcelona, Spain) (100% chymosin; CHY-MAXs EXTRA, 600 IMCU mL^{-1}) was then incorporated and blended vigorously into the milk solution at the indicated amount after thermal equilibrium was reached. Later, the milk samples were divided into two aliquots for the optical (80 mL) and visual cutting time (remaining milk) analyses. Chemical composition (protein, moisture, ash, fat, and lactose content) of the skimmed milk powder was determined and examined according to AOAC International (2005) [20] (Table 1).

Table 1. Chemical composition of Arla skimmed milk powder (g per 100 g).

Chemical Composition	g/100 g
Fat	1.25 \pm 0.13
Protein	32.50 \pm 1.55
Lactose	52.91 \pm 2.72
Minerals	7.75 \pm 0.83
Moisture	5.58 \pm 0.77

The methods used for milk analysis are the Kjeldahl analysis method (% protein), the Gerber analysis method (% fat), the oven method (% moisture), the Muffle Furnace method (% ash), and the method by difference at 100 (% lactose).

2.2. Experimental Design

It is currently possible to monitor milk's enzymatic coagulation using NIR light scattering optical fibers. However, their configuration is relatively expensive. Therefore, the objective of this work was to evaluate a low-cost multifiber probe for the optic determination of the elastic modulus of the gel (G') during cheesemaking, using the prediction model proposed by Arango and Castillo (2018a) [10] on spray-dried milk. The study employed randomized block designs, and varying protein levels and wavelengths, with 880 nm showing superior sensitivity and stability in response. Before proceeding to the light scattering determinations, an experimental design in light transmission configuration was used and established to determine the impact of the cuvette wall NIR reflectance on the light backscatter measurements. Firstly, a factorial experiment ($2 \times 3 \times 3$) was selected as a first approach to examine the optical response in milk (3.5% protein using three different wavelengths of 780 nm, 880 nm, and 980 nm at IT of 100, 500, and 1000 ms under the two light conditions (presence and absence of halogen light). Any increase in the detected transmitted light, would suggest that light from the halogen source was able to reach the wall of the cell, increasing potential reflection interference when measuring light scatter. Secondly, another factorial experiment ($2 \times 2 \times 3$) was fixed and used to study the optical parameters in water and air using three different wavelengths of 780, 880, and 980 nm at 500 ms under two light conditions (presence and absence of halogen light). Here, part of the light travelling through the media (water or air) was expected to reach the detector. This ensures that absence of light detection when milk (3.5% protein) is used, rather than water or air, is due to absorption or scatter of light by the media, that prevent emitted light from reaching the cell wall in front of the light source. Once the cuvette wall reflectance

was excluded, five integration times (IT) (100 ms, 150 ms, 200 ms, 500 ms, and 1000 ms) were tested and examined at 880 nm to select an adequate IT for the multifiber probe. The first evaluation of the multifiber probe for monitoring milk coagulation (3.5% protein) was performed and studied using three different wavelengths of 870, 880, and 890 nm at an IT of 150 ms. The preliminary evaluation of the multifiber probe performance for monitoring milk coagulation was accomplished using three concentrations of milk protein (3%, 3.5%, and 4%) at 880 nm and 150 ms to confirm that light backscatter profile during coagulation was as expected. Thus, two randomized complete block designs were used with a 3×3 factorial arrangement of three protein levels (3%, 3.5%, and 4%) and three wavelengths (870 nm, 880 nm, and 890 nm).

2.3. Temperature Control

A NIR light backscatter measurement sample cell (Figure 1) consisting of a 20-mL space with a 28.96 mm diameter, equipped with a double jacketed for water circulation (lateral walls and bottom) to control temperature was used in this experiment. The sample cell was designed and built at Autonomous University of Barcelona (Barcelona, Spain). The materials used for the manufacturing of the cell were AISI 316L stainless steel (sample cell space), suitable for use in contact with food (milk in this case) and delrin high density polymer (structural base of the stainless-steel measuring cell). The design allowed a large area of contact with the heated water; and the materials were selected to achieve high heat transmission efficiency using one water inlet and three outlets. A Nerinox oxidation treatment was used inside of the sample cell in order to obtain an intense matte black tone which would minimize any measurement error as a result of light reflection. To fit the pieces of the sampling cell together, stainless steel screws and viton seals were used.

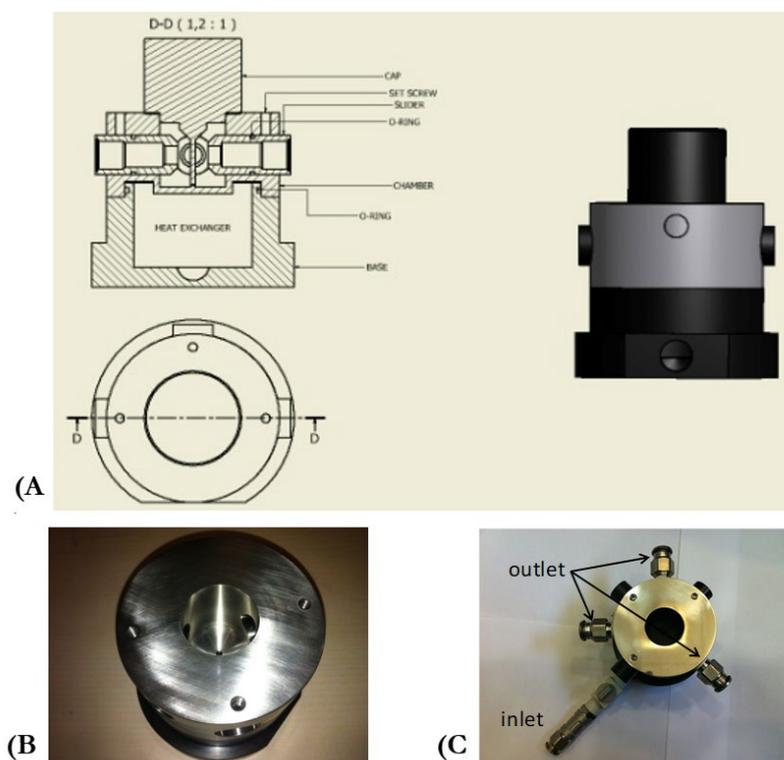


Figure 1. (A) Sampling cell with increased heat transfer with water circulation around the sample cell walls as well as the bottom. (B) Stainless steel sample cell before treatment. (C) Sample cell after Nerinox oxidation treatment for decreased light wall reflection.

The sampling cell was assembled with optical components, as described in Section 2.4, and connected to a water bath (Cubeta Inox 27L, OVAN, Suministros Grupo Esper, S.L, USA

2011) having a temperature management accurateness of ± 0.01 °C. The water bath was connected to the sample cell in order for water to flow from the bottom of the stainless steel part to the top, coming in contact with not only the bottom, but the sides of the sample cell. The temperature was verified by using a portable thermometer (RTD THERMO METER, HD 2127.2, Delta OHM, S.R.L, Italy). The water bath was furthermore utilized to hold the temperature of the milk for visual measurements of the cutting time.

2.4. Monitoring Light Backscatter Profiles

Milk coagulation was monitored coupling the temperature-controlled sample cell described above to a multifiber probe ($\frac{1}{4}$ diameter QR400-7-Vis-BX, Ocean Optics, Inc., Dunedin, FL, USA) (Figure 2), a fiber optic miniature spectrometer (Model HR4000; Ocean Optics, Inc.), and an halogen light source (LS-1; Ocean Optics, Inc.).



Figure 2. Multichannel fiber optic with six illumination fiber optics and one detection optic fiber (Photo obtained from Ocean Insight: <https://www.oceaninsight.com/products/fibers-and-probes/probes/reflectionbackscatter-probes/qr400-7-vis-bx/?qty=1>, accessed on 24 September 2023).

The optical parameters were determined, from the light intensity signal (bits) obtained using the spectrometer and examined similarly to Arango et al. (2018b) [12] (Figure 3) using Excel: the time passed from enzyme addition to the first maximum of the first derivative (min) (t_{max}), time from the enzyme addition to the first minimum of the second derivative (min) (t_{2min}), the value of the light scattering ratio (R) at time t_{max} (R_{max}), the value of the first derivative of R at time t_{max} (R_{1max}), the value of the second derivative of R at time t_{2min} (R_{2min}), and the spectral data was collected using OceanView 1.65 software (Ocean Optics, Inc., Dunedin, FL, USA).

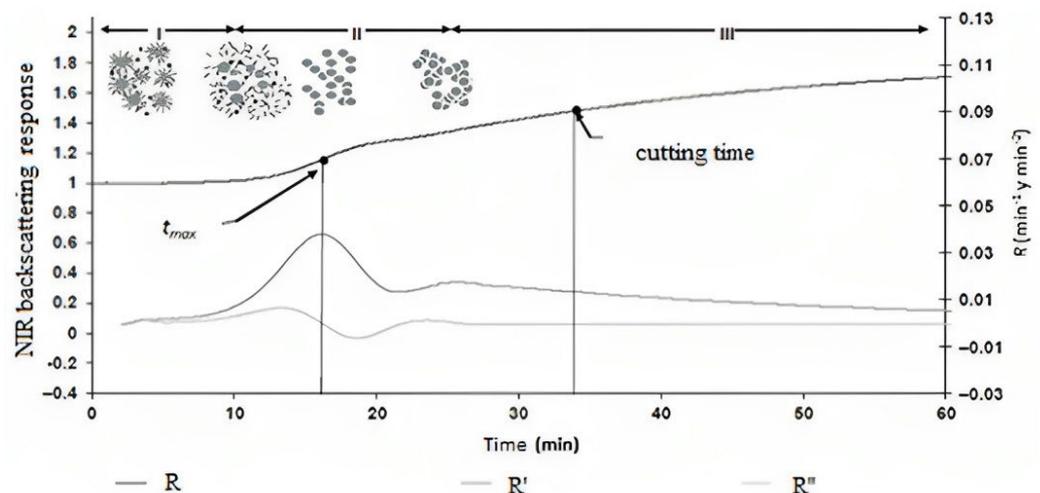


Figure 3. Profile of the light scattering ratio with its first and second derivatives as a function of time. (I) latency period, (II) sigmoidal period, (III) asymptotic period. The light scattering ratio = R , the first derivative of $R = R'$, the second derivative of $R = R''$, and the time elapsed from enzyme addition to the first maximum of the first derivative = t_{max} .

2.5. Statistical Analysis

The statistical analysis was processed and analyzed using the Statistical Analysis System, SAS 9.2 (Statistical Analysis System Institute, Inc., Cary, NC, USA 27513, 2003-2008). An analysis of variance (ANOVA) was performed using the general linear model (GLM). Least square means (LSM) were statistically different when $\rho < 0.05$.

3. Results and Discussion

3.1. NIR Light Scattering Ratio through the Cell

Tungsten halogen light is commonly used for monitoring milk curd formation [21] because it plays a critical role in achieving a good spectrum quality [22]. According to the results, the absence and presence of tungsten halogen light did not significantly ($\rho \geq 0.05$) affect the NIR light transmission (intensity; bits) in milk with 3.5% (Table 2), which shows that milk path length absorbs and scatters all the light arriving at the sample from the illuminating fiber optic and, subsequently, no light is travelling through the sample to reach the detector. On the other hand, as expected, it was observed that as the IT increases, the intensity detected by spectrometer increases. The IT of 100 ms was significantly ($\rho < 0.05$) lower than those of 500 ms and 1000 ms. A longer IT can lead to more electrons induced by the dark current (i.e., noise) since this current generates electron holes [23]. Additionally, longer IT also yield larger intensity response as the time during which a detector accumulates signals before they are read out is larger. Consequently, the integration of 1000 ms reported the highest intensity, and the 100 ms noted the lowest value. Although the relation between the IT and the intensity of response is, in many cases, linear, those two parameters can have a nonlinear relationship [24]. In milk, the intensity detected could be also affected by the fat/protein proportion, the fat globule size, and the milk variety [25]. The NIR transmission intensity on water and air at 780 nm, 880 nm, and 980 nm with an IT of 500 ms under two light conditions (absence and presence of halogen light) is presented in Table 3. As expected, the tungsten halogen illuminated samples had a significantly ($\rho < 0.05$) higher NIR light transmission intensity than the non-illuminated samples. The water samples did not significantly ($\rho \geq 0.05$) differ from air samples when the tungsten halogen light was used and applied. Results were as expected since most of the incident light was transmitted through the path length of air or water. Neither of these media can absorb or scatter significant light as it travels through the short pathlength (28.96 mm), and subsequently, a significant amount of light passes through either water or air to reach the detector. The NIR transmission intensity detected at 880 nm was significantly ($\rho < 0.05$) higher than at 780 nm and 980 nm in milk. The use of 880 nm in milk it is recommendable when the samples do not contain considerable amounts of fat (<3%), which could generate a >90% NIR light extinction. The milk used in this study had <0.15% fat, meaning that light scatter by fat particles was at acceptable levels.

Table 2. NIR light transmission intensity (bits) through the cell using milk with 3.5% protein.

T.I. (ms)	Without Light	With Light	Wavelength (nm)
100	11.73 ± 1.68 ^{eA}	13.04 ± 2.76 ^{fA}	780
	15.40 ± 1.73 ^{dA}	16.22 ± 3.12 ^{eA}	880
	11.73 ± 1.70 ^{eA}	13.04 ± 2.78 ^{fA}	980
500	29.00 ± 3.01 ^{cA}	31.74 ± 3.19 ^{cA}	780
	33.01 ± 3.25 ^{bA}	34.53 ± 3.48 ^{bA}	880
	24.32 ± 3.21 ^{cA}	25.77 ± 2.97 ^{dA}	980
1000	28.03 ± 2.41 ^{cA}	33.28 ± 2.80 ^{cA}	780
	39.12 ± 2.18 ^{aA}	41.44 ± 2.95 ^{aA}	880
	27.44 ± 2.28 ^{cA}	29.03 ± 3.02 ^{cA}	980

^{a-f} Column means not containing a common letter are significantly ($\rho < 0.05$) different among treatments. ^A Row means not containing a common letter are significant ($\rho < 0.05$). T.I. = time of integration.

Table 3. NIR light transmission intensity (bits) through the cell using distilled water and air at 500 ms.

Medium	Without Light	With Light	Wavelength (nm)
Water	29.12 ± 1.68 ^{bA}	15,621.36 ± 2.76 ^{aB}	780
	32.86 ± 1.73 ^{aA}	15,625.67 ± 3.12 ^{aB}	880
	11.73 ± 1.70 ^{dA}	15,620.81 ± 2.78 ^{aB}	980
Air	29.71 ± 3.01 ^{bA}	15,633.97 ± 3.19 ^{aB}	780
	33.62 ± 3.25 ^{aA}	15,635.77 ± 3.48 ^{aB}	880
	25.00 ± 3.21 ^{cA}	15,630.55 ± 2.97 ^{aB}	980

^{a-c} Column means not containing a common letter are significantly ($\rho < 0.05$) different among treatments. ^{AB} Row means not containing a common letter are significant ($\rho < 0.05$).

3.2. Determination of Integration Time

At 880 nm with the tungsten halogen light, the effect of IT (100 ms, 150 ms, 200 ms, 500 ms, and 1000 ms) on the intensity signal is shown in Table 4; as expected, the greater intensity was at the longer times. An extended IT could induce electron holes [23]. IT above 200 ms exceeded the limit (~ counts) intensity and generated a saturation of the detector.

Table 4. Light backscatter intensity (bits) determinations under the presence of light at 880 nm using milk with 3.5% protein.

Time of Integration (ms)	Counts
100	3914 ± 10.34 ^d
150	5908 ± 13.45 ^c
200	7849 ± 15.74 ^b
500	16,023 ± 20.45 ^a

^{a-e} Column means not containing a common letter are significantly ($\rho < 0.05$) different among treatments.

According to the team's experience and the recommendation by the spectrometer manufacturer (Ocean Optics Inc.), the maximum intensity response is ~ counts, and it is recommended to have ~35% of the maximum possible signal during measurements. To sum up, the IT of 150 ms reported the best voltage value (5908 counts), and this IT was selected to perform the coagulation tests. The selected IT value left a considerable margin without saturating the detector since coagulation typically produces an increase in the voltage response of ~50% in skimmed cow's milk [23]. The advantages of fiber optic sensors solve many of the drawbacks traditional measurement devices present. In the case of voltage and current measurement, characteristics such as the dielectric character of the optical fiber and its immunity against electromagnetic interference are fascinating, since they eliminate the need to incorporate complex insulation systems, which often include dangerous substances or contaminants.

3.3. Visual Cutting Time

A significant and linear increase ($R^2 = 0.999$) in I_0 (intensity) was observed with increasing protein concentration (Table 5). In addition, the optical parameter, t_{2min} , decreased with increasing protein concentration, showing significant differences between 3%, 3.5%, and the same trend, but less marked, between 3.5% and 4%. At typical experimental conditions, when protein concentration is increasing at constant enzyme concentration, a decrease in the enzyme/casein ratio is expected, that causes enzyme saturation by the substrate. Thus, a longer time is required to generate sufficient hydrolysis of the κ -casein and induce micelle aggregation [26,27]. However, in this experiment, this did not appear to be the case as t_{max} was not significantly different as a function of protein but t_{2min} was, in fact, decreasing significantly with increasing protein concentration. The lack of increase in t_{max} with the rise in protein concentration was attributed to the relatively high amount of chymosin (300 $\mu\text{L kg}^{-1}$) that was used for the experiment (i.e., unlikely the enzyme was saturated by the substrate). On the other hand, an increasing concentration of protein

was likely inducing a faster rate on aggregation on destabilized casein micelles, which is confirmed by a shorter t_{2min} value.

Table 5. Influence of the protein content on the evaluated light backscatter coagulation parameters at 880 nm.

Optic Parameters	Protein Content (%)		
	3%	3.5%	4%
I_0 (bits)	434 ± 5.98 ^a	472 ± 7.96 ^b	506 ± 8.67 ^c
t_{max} (min)	4.81 ± 0.54 ^a	4.20 ± 0.32 ^a	4.47 ± 0.27 ^a
R_{max} (dimensionless)	1.04 ± 0.02 ^a	1.04 ± 0.01 ^a	1.06 ± 0.02 ^b
R_{1max} (dimensionless)	0.0278 ± 0.001 ^a	0.0331 ± 0.001 ^b	0.0391 ± 0.001 ^c
R_{2min} (dimensionless)	−0.0064 ± 0.001 ^a	−0.0105 ± 0.001 ^b	−0.0107 ± 0.001 ^b
t_{2min} (min)	13.83 ± 2.34 ^a	6.09 ± 2.07 ^b	5.65 ± 1.75 ^b

^{abc} Row means not containing a common letter are significantly ($p < 0.05$) different among treatments.

A significant and linear ($R^2 = 0.998$) decrease in I_0 was observed with increasing wavelength (Table 6). In addition, the optical parameter, t_{2min} , showed significant differences between 870 nm, 880 nm, and 890 nm. The data obtained using the selected multifiber probe to monitor milk coagulation showed that both the light scatter profiles and resulting derivatives were similar to those obtained with other devices in previous studies [28]. Therefore, it is possible to determine the optical parameters and analyze their behavior as has been done in previous works.

Table 6. Influence of the wavelength on the evaluated optical coagulation parameters using milk with 3.5% protein.

Optic Parameters	Wavelength (nm)		
	870 nm	880 nm	890 nm
I_0 (bits)	531 ± 5.87 ^a	468 ± 6.76 ^b	413 ± 4.77 ^c
t_{max} (min)	4.34 ± 0.39 ^a	4.68 ± 0.34 ^a	4.46 ± 0.21 ^a
R_{max} (dimensionless)	1.04 ± 0.04 ^a	1.05 ± 0.03 ^a	1.05 ± 0.02 ^a
R_{1max} (dimensionless)	0.034 ± 0.001 ^a	0.032 ± 0.001 ^a	0.033 ± 0.001 ^a
R_{2min} (dimensionless)	−0.00890 ± 0.001 ^a	−0.00906 ± 0.001 ^a	−0.00968 ± 0.001 ^a
t_{2min} (min)	6.09 ± 1.89 ^a	8.70 ± 2.61 ^{ab}	10.79 ± 3.05 ^b

^{abc} Row means not containing a common letter are significantly ($p < 0.05$) different among treatments.

The coagulation monitoring instruments with the most development and commercial use correspond to hot wire methods and those that use light in the near-infrared. With the help of the principle of the hot wire method, the Optiset was installed in more than 100 cheese vats in the early years of the 90s. It was noted then that the system improves quality and consistency. The other system, widely used commercially, directs infrared light at the milk through a fiber optic cable (fiber optic reflectance). The first systems using CoAgulite technology, were installed in 1993 by a company from Kentucky (USA). Fagan et al. (2008) [29] presented a “novel light backscatter” for continuous online monitoring of the coagulation and syneresis to improve the control of the moisture content of the curd. The technology proposal shows not only its usefulness to control clotting and syneresis, but also that it could be used to control the humidity of the curd, the fat losses, and curd yield, improving process control during cheesemaking [29]. Inline near-infrared sensors have been used to predict essential responses in syneresis, for example, the yield of whey, fat, and solids in whey and moisture content in curds [29]. Mateo et al. (2009) [30] reported validating a syneresis sensor in the composition range of milk and process parameters. Taifi et al. (2006) [31], using a non-intrusive ultrasonic method, studied the characterization of the syneresis and the firmness of a dairy gel. When measuring the electrical impedance, Li et al. (2010) [32] characterized the coagulation process of soy milk as having

unique importance in preparing tofu. Currently, a novel system based on reflection scans (1000–1850 nm) in the near-infrared (NIR) is proposed to determine automatic cutting time [33].

4. Conclusions

In cheese, choosing the appropriate moment for cutting the curd is important due to its effects on the product's yield, maturation, and useful shelf-life. The optical time parameters (t_{max}) and response parameters (R_{max} , R_{1min} and R_{2min}) generated from the light scatter curve (R), unlike the initial intensity (I_0), do not present significant statistical differences as a function of wavelength. This indicates that I_0 is inversely proportional to the wavelength. This was likely because any of the three selected wavelengths corresponded to the maximum light backscatter peak (i.e., the peak of maximum light scatter response was likely located at a wavelength slightly shorter than the wavelengths at which measurements were taken). However, this inversely proportional relationship did not translate clearly into a significant effect on the parameters of the coagulation process. This circumstance is preferable when designing a coagulation sensor, as the three wavelengths generate similar coagulation parameters but with a more intense response at a shorter wavelength. However, other parameters concerning the suitability of the signal should be considered in future studies to identify the wavelength that contains the highest level of information about coagulation and the highest degree of response.

In the coagulation monitoring process as a function of protein concentration, no significant differences are seen in t_{max} with this new probe, but they are seen in t_{2min} , which is close to the beginning of the hardening reaction. This indicates that hydrolysis and the beginning of aggregation did not change significantly with increasing protein concentration, likely due to the relatively high concentration of enzyme added, but the beginning of hardening did advance with increasing protein concentration, as a result of increasing rate of casein micelle aggregation with increasing protein concentration. The I_0 response, increases linearly with protein concentration. This could allow the G' estimation probe to be used to estimate the protein concentration in milk.

The multifiber probe evaluated with different protein levels represents an improvement in the inline monitoring process of milk coagulation, as it presents a lower cost and provides a coagulation profile like those of previously evaluated probes, which allows the generation of coagulation parameters. Those parameters respond as expected to a coagulation factor relevant to gel hardness as protein concentration. The light backscatter ratio obtained by the multifiber probe at the wavelengths used indicates that it can successfully adapt to the real-time prediction models of the elastic modulus of the gel, G' , previously established by other studies.

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