

Quaternion-Type Representation for Measuring the Rennet Coagulation Time of Milk by Color Image Sequences Processing

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ABSTRACT: The determination of the rennet coagulation time of milk by image sequences processing was performed using a computer vision system (CVS), consisting of a computer coupled with a transmitted light microscope equipped with a digital camera. Algorithms implemented with Matlab 2014 encoded the color image as a quaternion, computed and analyzed the histogram peak. The evolution of this last parameter was monitored as a function of the milk coagulation time, for different concentrations of calcium chloride (0.01–0.03 M) and for coagulation temperatures varying between 30 and 36°C. No statistically significant difference was observed in the measurements of the rennet coagulation time, neither by the Berridge method nor by the analysis of the image sequences, except for those at 36°C. The association of the optical microscopic method and the analysis of image sequences by a quaternion-type representation, made it possible to identify optical changes during gel formation and to accurately determine the rennet coagulation time of milk.

KEYWORDS: Image Sequences Analysis, Quaternions, Color Histogram, Histogram Peak, Optical Sensor, Rennet Milk Coagulation Time.

1. INTRODUCTION

The coagulation of milk is one of the most critical steps in cheese making, as it considerably influences the cheese yield as well as the quality of the product obtained [1, 2]. Monitoring the whole process of milk curd formation is a constant preoccupation for dairy researchers and cheese companies. Several methods have been proposed to control rennet coagulation of milk such as ultrasonic sensing methods which gained a real potential to control rennet coagulation of milk. It consists of propagating, in a quantity of milk, two types of sound waves with a certain frequency: shear and longitudinal waves [3]. In an aqueous solution, shear wave is damped, so only longitudinal wave can be used. Gunasekaran and Ay [4] dealt with ultrasounds to determine coagulum cutting time. Nassar *et al.* [5] used low-frequency ultrasonic technique to monitor milk coagulation time. Izbaim *et al.* [6] also, used ultrasounds to control the fermentation process of milk during yoghurt processing. Castillo Manuel [7] proposed a classification of some techniques used to monitor milk coagulation and/or the curd firmness. Klandar *et al.* [8] compared five methods developed, such as Berridge test, dynamic small amplitude oscillatory rheometer (DSAOR), near-infrared (NIR), piezoelectric rheometer and NIR spectroscopy, to control milk coagulation by following the rennet coagulation of two types of reconstituted skim milk. Conventionally, micelle aggregation was visualized by

macroscopic changes and measured using the method of Berridge [9]. Kübarsepp *et al.* [1] also controlled milk coagulation using Formagraph. The Formagraph, based on the drag force technique, was the first mechanical off-line instrument widely used. It is formed by a pendulum plunged into a bowl containing renneted milk as described by McMahan and Brown [10]. The clotting of milk is plotted on a photo sensible paper moving at the rate of 2 mm per min. It was capable to monitor rheological changes during milk coagulation, but its intrusive behavior was a drawback. Karacheviev *et al.* [11] evaluated the possibility for modelling milk coagulation kinetics using enzymes with different concentrations. Rheological devices such as the low-amplitude dynamic rheometer oscillatory provide very useful information on the gel formation process [12]. This indirect method, considered as a reference one, is often used to control rennet coagulation of milk. O'Callaghan *et al.* [13] successfully assessed rennet-induced coagulation of milk using a tuning-fork and torsional probes. Nevertheless, both devices were associated with high-frequency motion (from 10 to 50 Hz, respectively), which could disturb the coagulation process [8]. Another mechanical instrument like the Foodtexture Puff Device was used by Bamelis and De Baerdemaeker [14] to determine texture in order to evaluate milk coagulation. The Foodtexture Puff Device measures the dynamic deformation of the surface of milk during

coagulation after applying an air puff directed on this surface. A thermal on-line method, the hot wire probe, was particularly efficient at predicting gel time [15–17]. It is based on the hot wire probe plunged in the renneted milk, but it turned out to be unsuitable for monitoring the evolution of firmness.

Changes of the optical properties of milk during coagulation have permitted to develop several devices, more particularly since optical fiber became widespread [15]. The Optigraph is a device invented by Ysebaert society to control milk coagulation and used by Kübarsepp *et al.* [1]. It measures optical infra-red signal attenuation through milk sample during coagulation and gives time of coagulation and the firmness of coagulum. Klandar *et al.* [8] have signaled that optical methods such as light absorbance, transmission and reflectance over visible and near-infrared wavelengths, were employed to assess milk coagulation. Fagan *et al.* [18] have developed a light backscatter sensor technology for on-line monitoring milk coagulation and whey separation. For the online monitoring of fresh goat cheese during coagulation and syneresis, Rovira *et al.* [19] used a light backscatter sensor with a large field of view (LFV). Pytel *et al.* [20] compared two different methods (the visual method and the nephelo-turbidimetry method) for determination of rennet coagulation time. Beux *et al.* [21] reported that the milk coagulation capacity can be measured by means of optical devices, such as Near-Infrared and Mid-Infrared Spectroscopy. A non-destructive optical microscopic method was proposed by Lagaude *et al.* [22], to determine visual aggregation and rheometric gel times and to observe simultaneously structural changes during rennet-induced milk coagulation by images processing. As casein micelles cannot be visualized with an optical microscope, the focusing was realized on a bubble of air presenting a diameter of 100 μm . The resolution of the optical microscopic observation was sufficient to provide significant information about the formation of the gel network. However, images used were binary images acquired with a rate of one image every two min, which could lead to unsuitable surveying dynamic phenomenon and loss of some adequate information.

Several authors mentioned that automatic tracking of changes in dynamic objects can be surveyed using image sequences [23–28]. For this reason and because color processing is needed everywhere for new technologies, Djaowé *et al.* [29] studied the kinetic of milk coagulation using color image sequences with a rate of 1 image/s, so as to take into account the color information. But the approach to deal with color images was to process each R, G, B channel of the color image separately. The output vector will rarely be one of the input vector, which yields false color appearance, as reported by Lambert and Chanussot [30], hence the need to look for other approaches to produce visually pleasing color images. A quite recent successful approach in color image processing consists to encode the three R, G, B channel

components on the three imaginary parts of a quaternion [31–33]. The main advantages of the use of quaternion-type representation is that: i) a color image can be treated as a vector field [34], ii) The holistic, compact processing of vector-valued signals that are a function of one or more independent parameters (e.g. time, location, physical quantities) [35]. Here, the dimension of the algebra must be chosen in compliance with the dimension of the signal vector. This means that each vector-sample is treated as a whole rather than treating its components separately. Classically, the reason for this is that the sample as a whole conveys information (direction in vector space) that is lost if the components of the sample are processed independently [35]. To our knowledge, the quaternionic representation of the parameters of color image sequences, to determine the coagulation time of milk, has not yet been studied. Taking into account the technological difficulties in determining the accurate coagulation time of renneted milk, the objective of the present work is therefore, to assess this parameter by analysis of image sequences using a quaternion-type color histogram representation.

The rest of this article is organized as follows. First, we discussed some techniques previously developed to control milk coagulation. Then, the quaternion algebra is presented, so as to understand the color representation based on the quaternionic model, followed by the method of images sequence analysis developed using color histogram feature. We then presented results and discussion related to rennet milk coagulation times obtained by image sequences analysis, compared with the reference method of Berridge, and finally the article is concluded.

2. MATERIALS AND METHODS

2.1. Milk preparation and renneting

2.1.1. Raw cow milk collection and physicochemical characterization

Our experimental raw cow milk used was freshly provided by a breeder, less than an hour after milking, from Ngaoundere (7°21'N and 13°33'E) in the Adamawa region of Cameroon. Elevation in Ngaoundere ranges from 900 to 1100 m above mean sea level. Once in the laboratory, the milk sample was left to stand for 2 h at 18°C before using [36]. Physico-chemical parameters (Proteins, fat content, dry matter, ash, pH and water content) of our raw cow milk were determined in 100 g of milk according to the experimental protocol described by Mahieddine *et al.* [37]. pH was 6.98 ± 0.08 , close to pH obtained by Edima *et al.* (2013) [38] when analysing milk quality from Adamawa region. Water content was 82.76 ± 0.08 %, not far away from values obtained by Adetunji and Babalobi [39]. Proteins, fat rate, ash and dry matter were 3.89 ± 0.21 , 3.25 ± 0.01 , 0.56 ± 0.07 and 17.23 ± 0.09 respectively.

2.1.2. Reconstitution of skimmed milk powder

A control milk sample as a commercial low-heat skimmed milk powder (Régilait, Saint-Martin—Belle-Roche, France) was

dissolved in distilled water (120 g L^{-1}) and supplemented with (0.01, 0.02 or 0.03) M ($\text{CaCl}_2, 2\text{H}_2\text{O}$). The reconstituted skimmed milk was stirred for 30 min at 20°C and left to stand for 2 h. Before using, it was stored for 2 h at 20°C to restore mineral equilibrium and then for 30 min at the experimental temperatures ($30^\circ\text{C}, 33^\circ\text{C}, 36^\circ\text{C}$) [22].

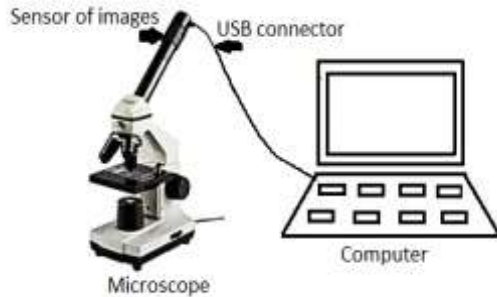


Figure 1: Experimental setup for capturing microscopic image sequences.

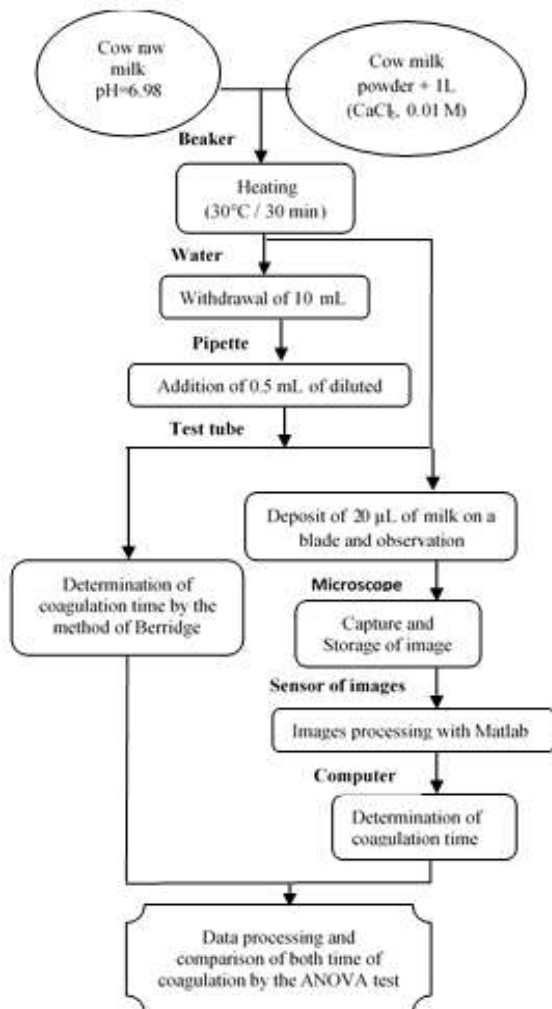


Figure 2: Flow Chart for experimental design of rennet-induced milk coagulation and image sequence processing.

2.1.3. Milk renneting

0.5 g of rennet (1/10,000 strength; 99.5% of activity due to the chymosin, the Etablissement Boll, Arpajon, France) was

diluted in 10 mL of distilled water before addition in milk. 0.5 mL of rennet was added in 10 mL of milk placed in a glass tube and vigorously mixed for 30 s. Three levels of coagulation temperature ($30^\circ\text{C}, 33^\circ\text{C}, 36^\circ\text{C}$), CaCl_2 content (0.01, 0.02, 0.03) M and rennet quantity (0.3, 0.5, 0.7) mL were combined, taking into account real operating ranges. All experiments were carried out in triplicate.

2.2. Milk clotting assessment methods description

2.2.1 Renneted milk microscopic image sequences acquisition

A transmitted light optical microscope (Biolux AL, $20\times - 1280\times$, 220 – 5.5 VS, 200 MA, Meade Instruments Europe GMBH & Co. KG, Rhede/Westf., Guetenbergstr.2, Germany) equipped with a digital photograph camera (resolution 640×480 pixels) connected to a HP computer type (2.20 GHz, 2.99 Gb RAM, 240 Gb DD, Hewlett–Packard Company) was used to observe milk aggregate during renneted milk coagulation (Fig. 1). Rennet-induced milk coagulation and images processing were carried out according to the chart flow in Fig. 2. A sample of rennet-induced milk ($20 \mu\text{L}$) was placed on a microscopic slide covered with a cover slide for examination. The image sequences were recorded for determined times at the same magnification ($40\times$) for each sample and were examined by image analysis to identify the histogram peak using Matlab software (2014). As casein micelles cannot be visualized with an optical microscope, the focusing was realized on light passing through milk and captured as images with a rate of 30 image/s. The acquisition process of milk image sequences was interrupted at determined time after the coagulation time was observed by the method of Berridge; depending on rennet level, added calcium and assay temperature.

2.2.1.1. Milk image sequences processing

2.2.1.2. Quaternion-type color image histogram representation

A quaternion $Q \in H$ (H refers to Hamilton who was first to discover this number) is a generalized complex number defined as:

$$Q = q_0 + q_1i + q_2j + q_3k \quad (1)$$

where q_0, q_1, q_2 and q_3 are real numbers; i, j and k are three imaginary units obeying following rules:

$$i^2 = j^2 = k^2 = ijk = -1 \quad (2)$$

$$ij = -ji = k; jk = -kj = i \text{ and } ki = -ik = j \quad (3)$$

The conjugate and modulus of a quaternion are respectively defined by:

$$Q^* = q_0 - q_1i - q_2j - q_3k \quad (4)$$

$$|Q| = (q_0^2 + q_1^2 + q_2^2 + q_3^2)^{1/2} \quad (5)$$

2.2.1.3. Milk image sequences analysis and color histogram determination

Images were analyzed following the block diagram shown in Figs. 3a, b. Rennet-induced milk coagulation images analysis in this paper was based on histogram images analysis. The procedure included two main parts: RGB histogram determination using quaternion algebra and color attributes definition and determination.

2.2.1.3.1. RGB histogram image determination

The histogram of each channel of a color image was calculated by the following relation:

$$h(m) = N_m \tag{6}$$

where N_m is the number of pixels corresponding to $m \in [0, 255]$, the gray level in an image.

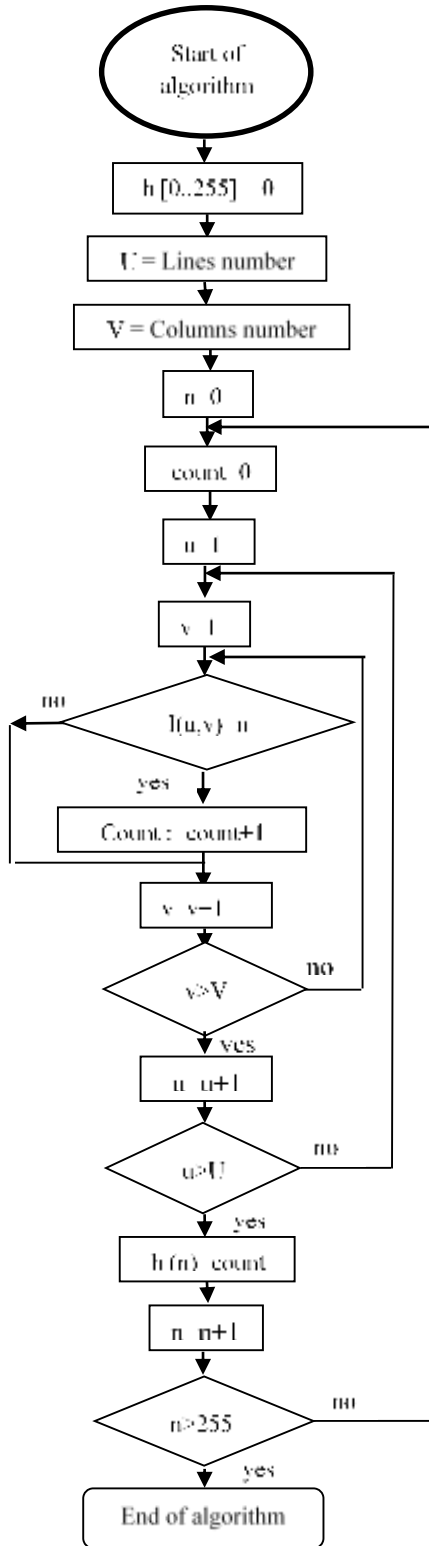


Figure 3a: Algorithm for computing the color channel histogram h of an image.

Let I^{RGB} be a color image with the spatial resolution of $N \times M$ pixels. In this way; it is represented by a set of $N \times M$ matrix related to each color channel:

$$I^{RGB}(x, y) = (I^R(x, y), I^G(x, y), I^B(x, y)) \tag{7}$$

where $x = 1, 2, \dots, N$ and $y = 1, 2, \dots, M$ are the spatial coordinates of the I^{RGB} image; I^R, I^G and I^B represent the red, green and blue channels of the color image, respectively.

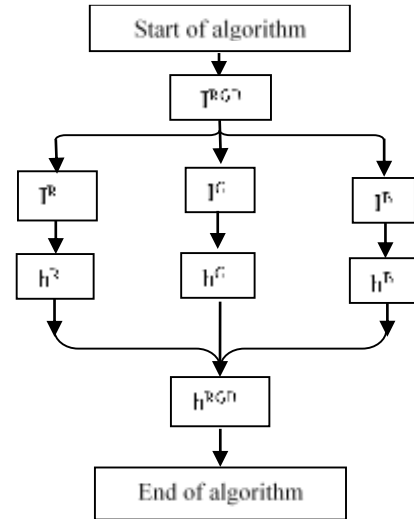


Figure 3b: Algorithm for computing the color image histogram encoded using quaternion

2.2.1.3.2. Quaternion-type RGB histogram image representation

Histogram of each color channel of an image was computed according to the algorithm described on the Fig.3a. RGB histogram (h^{RGB}) of the entire color image was encoded as a quaternion as shown in Fig.3b as follow:

$$h^{RGB}(m) = h^R(m)i + h^G(m)j + h^B(m)k \tag{8}$$

where h^R, h^G and h^B represent the red, green and blue histogram of each channel of the color image respectively, and i, j, k defined by relations (2) and (3).

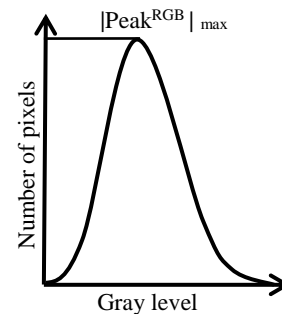


Figure 4a: Feature definition on histogram of an image

2.2.1.3.3. Quaternion-type RGB histogram image feature determination

One feature was defined and determined on the modulus of the quaternionic color histogram, computed according to relation (9):

$$|h^{RGB}| = ((h^R)^2 + (h^G)^2 + (h^B)^2)^{1/2} \tag{9}$$

This feature was the histogram peak denoted $Peak^{RGB}$ as shown in Fig. 4a and computed according to the flow chart algorithm described in Figs. 4b, c for image sequences.

$$Peak^{RGB} = |h^{RGB}|_{max} \quad (10)$$

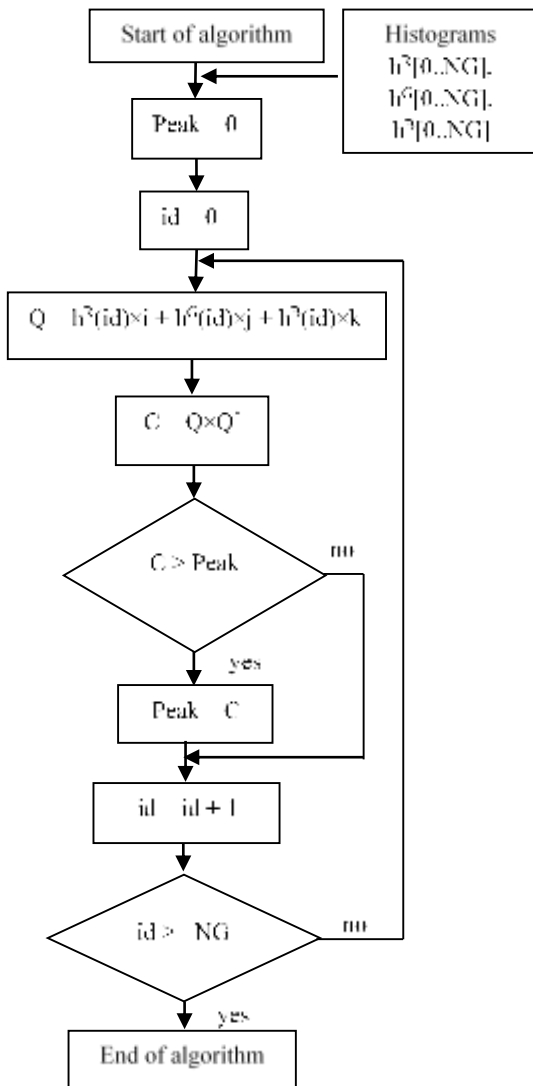


Figure 4b: Algorithm for computing the peak of histogram of an image with quaternion modeling of histogram's vector

2.2.1.3.4. Histogram peak in image sequences modelling

All Data (change in the histogram peak as a function of coagulation time) were means values of three replicate experiments produced to provide information on the kinetic parameters which characterize the kinetic model currently used to describe milk coagulation. Data were fitted with SigmaPlot 2012. The output of this analysis is expressed by a curve whose width decreases with the curd firmness. The model of type «four-parameter logistic equation» is a sigmoid ($R^2 > 0.9$):

$$Peak(t) = Peak_{min} + \frac{Peak_{max} - Peak_{min}}{1 + (\frac{t}{T_C})^\beta} \quad (11)$$

where t represents the time, T_C is the corresponding time to the apparition of first flocks in the test tube. $Peak_{max}$ and $Peak_{min}$

are the maximum and the minimum of the number of pixels respectively, and β describes the slope of the curve.

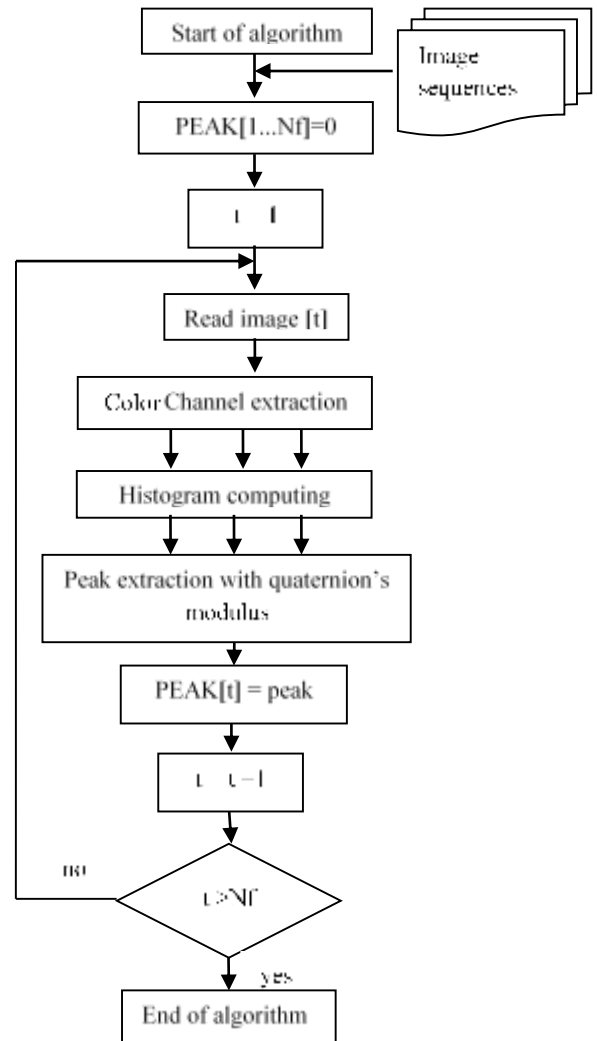


Figure 4c: Algorithm for computing the peak of histogram of milk's sequence of images.

2.2.1.3.5. Milk clotting time calculation according to image sequences analysis

As image sequences were acquired with a rate of 30 images/s, the time of coagulation depends on the number of images at the moment of coagulation:

$$T_C = \frac{N_C}{30} \quad (12)$$

where N_C is the corresponding number of images to the apparition of first flocks in the test tube.

2.2.2. Milk clotting time according to the method of Berridge

The reference method of Berridge consists of observing the coagulation of milk in a test tube. It was carried out by putting 10 mL of milk and 0.5 mL of coagulant solution in a test tube plunged in a water bath at 30°C [9]. The time of coagulation (T_C) is the period elapsed between the introduction of the rennet in the test tube and the moment where a thin film begins to form itself on the inner side of the tube.

2.3. Effects of rennet level, calcium level and assay temperature on rennet coagulability of milk

To test the effect of rennet level on milk coagulation, the 0.01 M calcium-supplemented our raw cow milk and the control skim milk powder were used and incubated for 1 h at 30°C. Before use, raw cow milk and skim milk substrates supplemented were treated with three different rennet quantities (0.3, 0.5 or 0.7) mL.

To test the effect of calcium level on rennet coagulation, raw cow milk and skim milk substrates supplemented with 0.01, 0.02 and 0.03 M calcium were prepared as described in Section 2.1.1. Before use, samples were incubated for 1 h at 30°C.

To test the effect of assay temperature on rennet coagulability, the 0.01 M calcium-supplemented raw cow milk and skim milk were used. Before use, samples were incubated for 1 h at the experimental temperatures, i.e. 30°C, 33°C, or 36°C, to restore the mineral equilibrium.

2.4. Statistical analysis

All experimental data were presented as the means of three independent experiments. Standard deviations were calculated using Excel software 2010 (Microsoft, Redmond, WA, USA) and ANOVA test was carried out using Statgraphics Centurion XVI (16.1.11). Curves fitting was performed using sigmaplot 2012.

3. RESULTS AND DISCUSSION

Standardization of the microscopic parameters including the lighting, the luminosity and the magnification was optimized to obtain good sensitivity and repeatability of the images. Before images acquisition, microscope and camera were left to stand on for 1 h to reach the thermal stability of the system and to avoid some variation while acquiring many images [40].

3.1. Histogram peak in milk image sequences without rennet

Fig. 5 shows a relatively constant variation of the histogram peak in milk image sequences. The fluctuations of the histogram peak around mean value with Standard Deviation of $20\ 042 \pm 1\ 767$ can be related to brownian agitation of the colloidal particles of milk. All particles in milk are subject to random motion caused by their thermal energy. Brownian motion is one of mechanical properties of colloid particles [41] that causes scattering light intensity by milk particles.

3.2. Histogram peak in rennet-induced milk image sequences

For rennet coagulation measured under the chosen microscopic conditions ($40 \times$ magnification), the changes in the histogram peak were followed as a function of the coagulation time of cow milk and skim milk powder. Figs. 6a, b show curves of variation of the RGB color histogram peak in images milk coagulation supplemented with 0.01 M calcium for three rates of the rennet (0.3 mL, 0.5 and 0.7 mL) at 30°C.

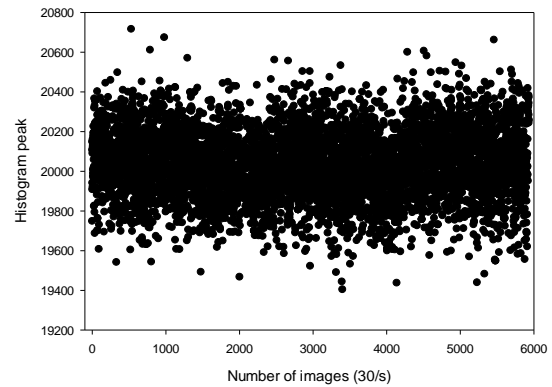


Figure 5: Evolution of the maximum number of pixels in not rennet-induced milk images

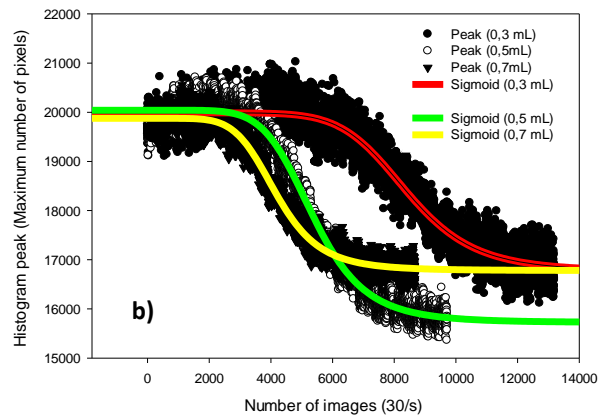
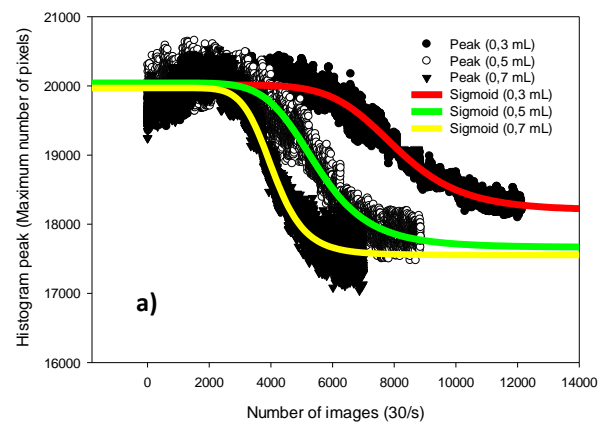


Figure 6: Effects of rennet level on milk coagulation:
a) Raw cow milk
b) Skim milk powder.

Figs. 7a, b show curves of evolution of the RGB color histogram peak in images milk coagulation supplemented with 0.01 M calcium for 0.3 mL of rennet at three experiment temperatures (30, 33 and 36)°C. While Figs. 8a, b show plots of variation of the RGB color histogram peak in images milk coagulation supplemented with CaCl_2 (0.01, 0.02 and 0.03) M for 0.3 mL of rennet at 30°C. The changes in these parameters as a function of coagulation time show that curves obtained presented three phenomena: (i) a relatively constancy in the histogram peak, (ii) a rapid decrease and (iii) a stabilization of

the parameters. The photometric coagulation measurement measures the relative transmission of light in the range of visible, which is passed through a coagulating milk sample. A camera detects the transmitted signal and the relative transmission is evaluated through image sequences analysis (histogram peak). The latter parameter decreases over the coagulation process due to increased absorption and scattering of the light as the milk structure changes [42]. For milk supplemented with 0.01 M at 30°C, the histogram peak began to deviate from the baseline after approximately 66.67 sec after the addition of rennet, decreased rapidly and then levelled off after approximately 300 sec. The midpoint of this transition occurs after approximately 200 sec after addition of 0.3 mL rennet, even in our raw cow milk and the control skim milk powder. At approximately 66.67 sec, the histogram peak curve shows a decrease. This could be attributed to the beginning of the formation of the network from gel precursors, where the aggregates of micelles form small patches/particles of gel. This leads to the observed increase of light scattered, as the size of the particles became comparable with the light wavelength (850 nm) [42]. According to Hardy and Fanni [43], generally, light diffusion caused by suspended particles in a liquid medium follows Rayleigh’s law for particles smaller than about 1/4 the wavelength of light. For these particles, diffusion is proportional to the cube of their diameter. As the particle diameter increases, diffusion decreases inversely with the square of the particle diameter. According to these laws, diffusion reaches a maximum level when particle size becomes about 1/4 the wavelength of light.

These presented results showed that milk optical parameters varied during rennet-induced coagulation. The survey of the coagulation of milk and the proteins of the soy from the color was already carried out by some researchers [15, 44]. The results obtained by Dybowska and Fujio [44], on the optical analysis of the coagulation of proteins of soy with the glucono- δ -lactone, permitted to understand that L^* , a^* and b^* values grow during the time and decrease according to the pH during the coagulation. The dimension of soy proteins aggregated increased with the increase of the intensity of light reflection. Research work performed by Lagaude *et al.* [22] on the optical method of characterization of rennet milk coagulation proved that the number and the average area of particles varied during rennet coagulation. They demonstrated that the number of particles decreased while the average area of particles increased during the same time. The increase of the intensity of light reflection, consequently reduces the light intensity transmitted through the renneted milk image sequences.

3.3. Coagulation time determination

3.3.1. Method of Berridge

All experiments were carried out with two different methods using raw cow milk and skim milk powder. The times of coagulation were determined for three different quantities of rennet and presented in Table 1. Table 2 presented values

obtained for different added calcium concentrations, while Table 3, contains values for three different assay temperatures. Times corresponding to the same quantity of rennet, the same added calcium and the same assay temperature present no statistical meaningful difference ($P < 0.05$).

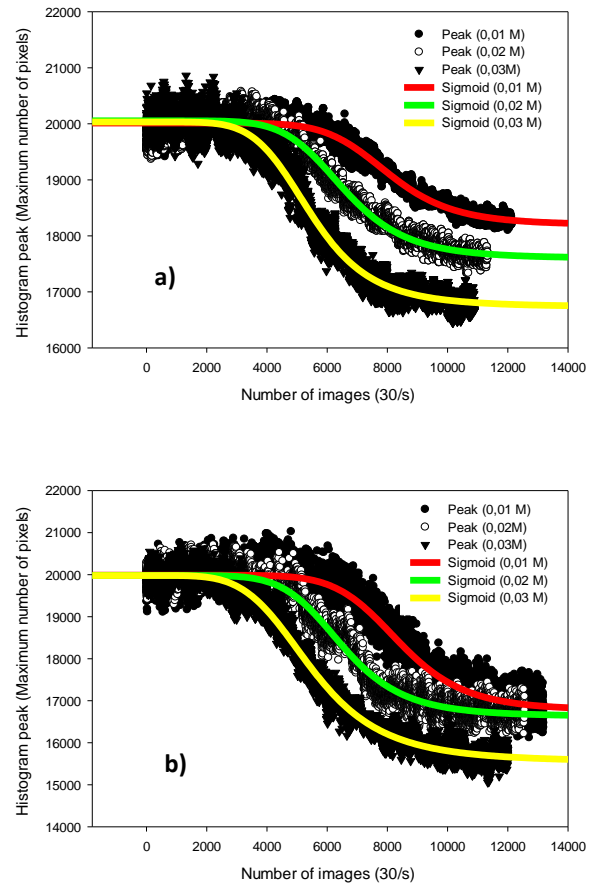


Figure 7: Effects of added calcium on the histogram peak, as measured by the image sequences histogram analysis method, as a function of time in rennet-treated milk added calcium levels were: 0.01 M, 0.02 M, 0.03 M:

- a) Raw cow milk
- b) Skim milk powder.

3.3.2. Method based on image sequences processing

T_F was determined as shown in Tables 1, 2 and 3 according to the approach previously described for three quantities of rennet, different added calcium concentrations and three different assay temperatures, respectively.

3.3.3. Rennet-induced milk coagulation times analysis

The histogram peak in image sequences enable the determination of the milk coagulation time (T_C) both, by the method of Berridge and by the approach described in this paper. It resulted from Tables 1, 2 and 3 that no statistically significant differences were found in rennet coagulation time measurements in either of the studied methods ($P > 0.05$), while at 36°C, there was a significant difference. There occurred statistically non-significant differences in most of the measurements, comparing both methods. The results show that

the proposed optical method based on measurement of changes in histogram peak is a potential objective method for T_C measurement.

Table 1: Effect of rennet level on T_C values as obtained for rennet-treated milk at 30°C with added Calcium of 0.01 M.

Milk coagulation times (s)				
Rennet quantity (mL)	Berridge method		Images sequences analysis	
	Raw cow milk	Skim milk powder	Raw cow milk	Skim milk powder
0.3	272.33±4.04 ^a	277.30±3.05 ^d	269.50±3.19 ^a	273.23±5.19 ^d
0.5	188.33±3.06 ^b	182.67±4.16 ^e	182.49±4.08 ^b	179.13±4.83 ^e
0.7	141.33±3.21 ^c	148.67±3.50 ^f	136.10±2.73 ^c	140.35±4.73 ^f

abcdef means with different superscripts within each row are different ($P < 0.05$).

Table 2: Effect of added calcium on T_C values as obtained for rennet-treated milk

Milk coagulation times (s)				
Calcium addition (M)	Berridge method		Images sequences analysis	
	Raw cow milk	Skim milk powder	Raw cow milk	Skim milk powder
0.01	272.33±4.04 ^a	277.30±3.05 ^d	269.50±3.19 ^a	273.23±5.19 ^d
0.02	222.33±3.06 ^b	223.00±5.29 ^e	221.66±2.78 ^b	217.57±4.30 ^e
0.03	187.67±2.52 ^c	184.33±4.04 ^f	182.76±5.67 ^c	181.20±3.17 ^f

abcdef means with different superscripts within each row are different ($P < 0.05$).

Table 3: Effect of coagulation temperature on T_C values as obtained for rennet-treated milk

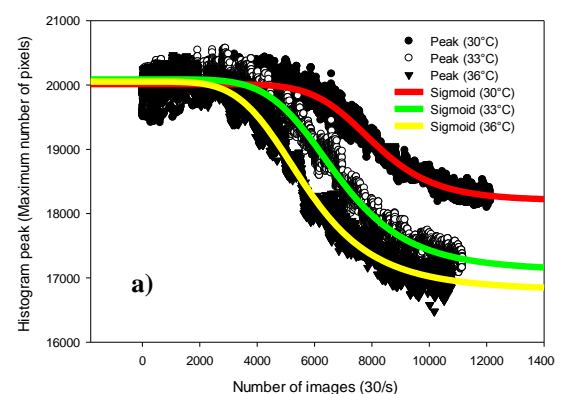
Milk coagulation times (s)				
Temperature (°C)	Berridge method		Images sequences analysis	
	Raw cow milk	Skim milk powder	Raw cow milk	Skim milk powder
30	272.33±4.04 ^a	277.30±3.05 ^d	269.50±3.19 ^a	273.23±5.19 ^d
33	231.67±4.51 ^b	228.50±4.04 ^e	226.60±5.60 ^b	224.07±2.12 ^e
36	196.67±3.06 ^c	196.33±3.51 ^f	186.37±4.33 ^c	187.83±3.06 ^f

abcdefgh means with different superscripts within each row are different ($P < 0.05$).

The proposed method seems very robust, objective and more accurate than the Berridge method which is subjective because it depends on an operator’s skill to consistently

identify milk flocculation. It should be noted that the times obtained by image sequences analysis were slightly lower than the times determined by the method of Berridge. It means that image sequences analysis is very sensitive to the phenomenon and quickly detects milk coagulation time before the method of Berridge.

To validate the approach dealt with image sequences analysis method using histogram quaternion-type representation, the effect of the rennet level, assay temperature and calcium concentration on the enzymatic coagulation of raw cow and skim milk powder was followed using both the image sequences analysis method and the Berridge method (Tables 1, 2, 3). These factors are known to affect the rennet coagulation: the addition of $CaCl_2$ to the milk in the concentration range of 0–10 mM increases the overall enzymatic coagulation rate [22, 45, 46] and increasing the temperature from 26°C to 40°C reduces the gelation time [12, 22, 46]. Indeed, it is well known that temperature affects both the primary (enzymatic hydrolysis) and secondary (aggregation reaction) phase of milk [47]. The results presented in Figs. 7a,b and Figs. 8a,b are in agreement with those reported in the literature cited above. Although method carried out by Djaowé *et al.* [29] was reliable to calculate milk coagulation time, however this marginal method is faced some kind of limitation: As stated by Alfsmann *et al.* [35], the sample as whole conveys information, that could be lost if the components of the sample are processed independently. In addition, according to Wang *et al.* [48], the quaternion-based models treat color image as a multivector in the quaternion form and preserve the relationships of the multiple channels to avoid the loss of structures information among different channels. According to Xu *et al.* [49] too, differing from traditional separated and concatenated monochrome models, which consider only a fraction of the subspaces that are needed for completely representing a vector-sensor array, the quaternion-based model can preserve the whole information of a 3D vector array. Furthermore, comparing to the tensor-based model, the quaternion-based model not only preserves the correlation among channels, but also the orthogonal property for the coefficients of different channels, which achieves a structured representation.



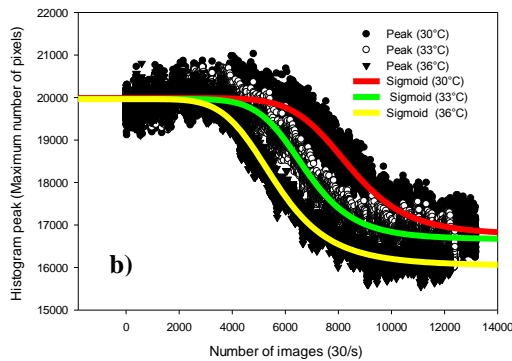


Figure 8: Effects of temperature on the histogram peak, as measured by the image sequences histogram analysis method.
 a) Raw cow milk
 b) Skim milk powder.

4. CONCLUSION

The rennet coagulation of milk was followed using optical microscopy coupled with image sequences analysis using quaternion type representation. The decrease in the histogram peak observed by this microscopic image sequences analysis method allowed the determination of one important information for controlling curd formation: the coagulation time (T_C). The determination of T_C measured using Berridge method and the image sequences analysis method described herein gave values not significantly different even if the milk samples were supplemented with calcium (0.01–0.03 M) or coagulated at different temperatures (30–33°C), except at 36°C where values of coagulation times are significantly different. It should be noted that the times obtained by image sequences analysis were slightly lower than the times determined by the method of Berridge. It means that image sequences analysis is very sensitive to the phenomenon and quickly detects milk coagulation time before the method of Berridge. The proposed method seems very robust, objective and more accurate than the Berridge method which is subjective because it depends on an operator’s skill to consistently identify milk flocculation. Due to the accuracy and the simplicity of the experimentation, this method could be easily automated and applied to characterize milk clotting enzymes and to study changes in the physical state of other polymers.

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