TEXTURE ANALYSIS OF MILK PROTEIN GELS USING DIGITAL IMAGE ANALYSIS

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Keywords: Milk protein, Acid gel, Gel structure, Digital images, Glucono-delta-lactone, Bovine caseins.

Abstract: Sodium caseinate (NaCAS) is a very useful ingredient in food industry because of its nutritional and functional properties. Acidification produces a gel structure as a result of the dissociation and aggregation of caseinic fractions. Formation of these protein gels can be made by the slow reduction of pH through the addition of glucono-delta-lactone (GDL). Depending on its concentration and temperature, hydrolysis speed of GDL can affect the grade of hardness and elasticity of the formed gel. This study evaluated the effect on the formation and structure of protein gels induced by different relations of GDL through analysis of digital images obtained in an inverted conventional microscope and a confocal microscope. The entropy, smoothness and variance decrease with the added GDL quantity, but the uniformity increases. Results confirm that the texture depends on gelification speed, which is directly related to the amount of added GDL. This digital image analysis technique using conventional or confocal microscopy is, therefore, suitable and very useful for the texture analysis of acid gels formed by different GDL/NaCAS rates.

1 INTRODUCTION

The texture is a very important characteristic and its analysis is a very useful tool to quantify and classify objects or interest region in an image. Image texture is a quantification of the space variation of intensities that is impossible to define by its sensorial character. There are several textural parameters and algorithms proposed for the quantification of an image texture such as the co-occurrence matrix, statistical studies, the wavelet, etc. (Jensen, 1996) All these techniques can be useful to characterize a great variety of textures, but they can be unsuccessful when the textures do not show a periodic structure. A general assumption is that the relevant information is in the space relation inside the grayscale images.

Caseins (CN) represent the major protein component of bovine milk. The CN precipitate at pH 4.6 and may be resolubilized by increasing the pH. If the increase in the pH is carried out by the addition of NaOH it is possible to end up obtaining sodium caseinate (NaCAS). CN and NaCAS are extensively used in food industry because of their physicochemical, nutritional and functional

properties that make them valuable ingredients in complex food preparations. Casein gels are responsible for most of the rheological/textural properties (i.e. stretch, fracture) of cheese and other dairy products (Walstra, 1984; Mulvihill and Fox, 1989).

Dissociation and a further aggregation step of CN fractions due to NaCAS acidification results in the formation of a gel structure. A possible explanation to this observation is that as pH is adjusted towards the isoelectric point it causes a decrease of the repulsive interactions, resulting in a destabilization of the colloidal aggregates as pH drops slightly below 5 at a given temperature (Braga et al., 2006; Ruis et al., 2007). Nowadays, a process that has gained the attention of food industry is direct acidification by the addition of a lactone, such as glucono-δ-lactone (GDL) which allows to overcome some of the difficulties associated with the traditional process of using bacteria. In fact, the final pH of the system is a function of the amount of GDL added whereas starter bacteria produce acid until they inhibit their own growth as pH becomes lower (Ruis et al., 2007; de Kruif, 1997).

Depending on GDL concentration and temperature, hydrolysis speed of GDL can affect the grade of hardness and elasticity of the formed gel. The compactness and elasticity of gels formed at the end of the acidification process of NaCAS depend on the kinetic of the aggregation phenomena. As the aggregation process becomes slower the more easily a polypeptide chain could acquire different orientations leading to the formation of more compact gels with more elasticity and hardness (Nespolo et al., 2010).

The interaction mechanisms and rheological properties of obtained gels can be analyzed and characterized by optical techniques (Yan et al., 2008; Lucey, 2002).

In the present work, the gel structure formation kinetics and different compaction degree were evaluated by means of textural parameters from digital image analysis. In order to do this, images of bottom gel surface were obtained by conventional inverted microscopy and images of gel internal structure were obtained by confocal microscopy.

2 MATERIALS AND METHODS

2.1 Sample Preparation

In this work, the formation and structure of NaCAS gels (3%) induced by different GDL/NaCAS ratio (0.35; 0.5; 0.7 y 1.0) was evaluated by means of analysis of digital images obtained in an inverted optical and a confocal microscope.

An aqueous solution of bovine caseinate (NaCAS) 3% w/w was prepared from the commercial drug (Sigma Co.). The gelification process was started adding GDL on 5 g of NaCAS solution, at 35 ºC. The different amounts of GDL corresponded to different relations GDL/NaCAS (0.35, 0.5, 0.7 and 1) according to:

$$R = \frac{\% \; GDL}{\% \; CN} \qquad (1)$$

To obtain the microscopic images, 80 μL of each sample at different R were placed in compartments of the LAB-TEK II cells. The samples were made in duplicate maintaining the incubation temperature at 35ºC.

2.2 Stained Protocol for Confocal Microscopy

The gels were stained with Rhodamine B. In order to do, a dilution 1/50 v/v of 1 mL of Rhodamine B at 0.01% in 49 mL of bidistilled water was prepared. This medium was used to dissolve NaCAS to obtain a solution at 3%.

2.3 Image Acquisition and Analysis

Transmission images of gels were obtained using a conventional inverted microscopy (Union Optical) with an objective 100x and a digital camera (Canon PowershotA640) with a zoom 9.1x, for the different GDL/NaCAS ratios.

Images of internal structure of gels stained with Rhodamine B were obtained using a confocal microscope (Nikon EZ-C1.) at (14.25 ± 0.05) μm on the glass slide (inner the gel).

The effect on the formation and structure of protein gels induced by different R was assessed using analysis of microscopic digital images. Previous to the analysis, the images were transformed to numerical 8 bit formats RGB (Red, Green, Blue) where each of them corresponds to level color. All images were normalized to grayscale by following transformation:

$$Y = 0.299 \, R + 0.587 \, G + 0.114 \, B \qquad (2)$$

Then, the values were normalized by mean of the transformation:

$$N = (L-1)\frac{Y - Y_{min}}{Y_{max} - Y_{min}} \qquad (3)$$

where L is the maximum gray level. It can see in Figures 2 and 3.

In this numerical representation a simplest statistical approaches were used. From normalized gray level it obtained its histogram $p(N_i)$, where $i = 1 \dots L-1$ and L is the maximum gray level. As well as texture estimations of obtained images, the Shannon entropy S, smoothness R and uniformity U were studied and they were defined by the following equation (Gonzalez and Woods, 2002; Haralick, 1979):

$$S = -\sum_{i=0}^{L-1} p(N_i)\log_2(p(N_i)) \qquad (4)$$

$$R = 1 - \frac{1}{1 + \sigma^2(N)} \qquad (5)$$

$$U = \sum_{i=0}^{L-1} p^2(N_i) \qquad (6)$$

where $\sigma^2(N)$ is the variance. Because the $p(N_i)$ have values in the range form 0 to 1 and their sum is

equals 1, measure U is maximum for an image in which all gray level are equal. Instead entropy is a measure of variability and is 0 for a uniform image.

3 RESULTS AND DISCUSSIONS

Figure 1 shows the pictures registered at different times after GDL addition for the visualization of gelification kinetics.
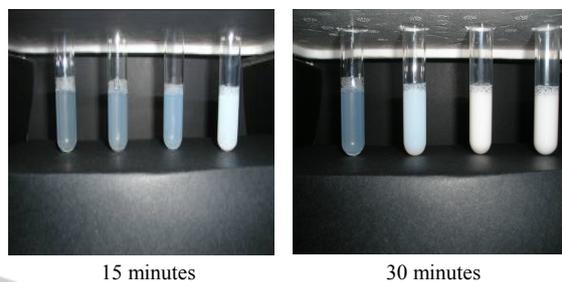
Figure 2 and 3 show respectively the transmission and confocal images of gels obtained for R = 0.35, 0.5, 0.7 and 1.0. From these images, it is possible to observe differences in the internal microstructure of gels. These observations can be quantified by the texture parameters as shown in tables 1 and 2.

15 minutes   30 minutes

Figure 1: Visualization of gelification kinetics as a function of GDL/NaCAS ratios (0.35, 0.5, 0.7 and 1.0).

(a) R = 0.35   (b) R = 0.5   (c) R = 0.7   (d) R = 1
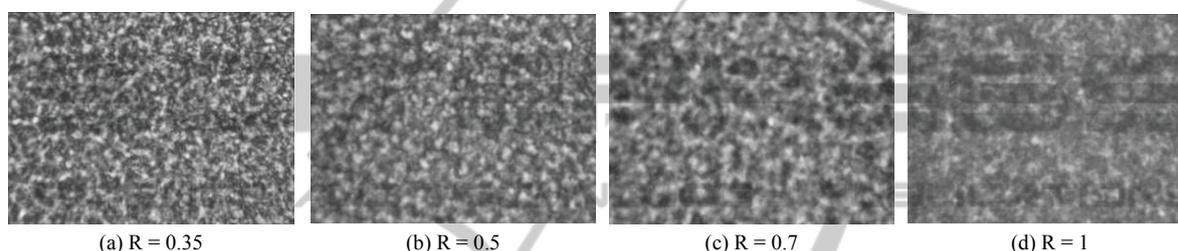
Figure 2: Images of gels obtained using a conventional inverted microscopy (Union Optical) with an objective 100x and a digital camera (Canon PowershotA640) with a zoom 9.1x, for the different GDL/NaCAS rates.
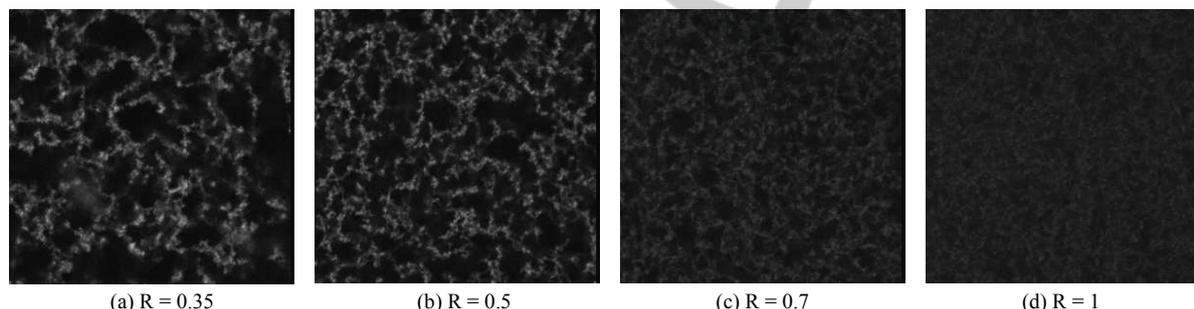

(a) R = 0.35   (b) R = 0.5   (c) R = 0.7   (d) R = 1

Figure 3: Images obtained by confocal microscopy inner the gels at (14.25 ± 0.05) µm on the slide.

Table 1: Texture parameters for the different GDL/NaCAS ratios. Digital images obtained by conventional inverted microscopy of acid gels on the slides.

R	Entropy	Smoothness	Variance	Uniformity
0.35	7,68 ± 0,01	0,046 ± 0.001	3100 ± 100	0,0050 ± 0,0001
0.5	7,58 ± 0,01	0,034 ± 0.001	2300 ± 100	0,0058 ± 0,0001
0.7	7,47 ± 0,09	0,029 ± 0,004	2000 ± 200	0,0065 ± 0,0006
1	7,39 ± 0,03	0,026 ± 0,001	1750 ± 50	0,0069 ± 0,0002

Table 2: Texture parameters for the different GDL/NaCAS ratios. Digital images obtained by confocal microscopy of inner structure of acid gel measured at 14 µm on glass slides.

R	Entropy	Smoothness	Variance	Uniformity
0.35	5.2 ± 0.1	0.012 ± 0.001	800 ± 90	0.038 ± 0.004
0.5	5.4 ± 0.2	0.014 ± 0.003	940 ± 100	0.030 ± 0.003
0.7	4.6 ± 0.1	0.014 ± 0.001	830 ± 100	0.051 ± 0.004
1	4.4 ± 0.1	0.015 ± 0.001	980 ± 90	0.056 ± 0.005

4 CONCLUSIONS

Results show that the structure compaction, texture and size of internal interstices depend on the gelification rate, which is related to the GDL added to the solution.

The entropy decrease and the uniformity increases with the added GDL in the images obtained using both microscopes, being the entropy the parameter that has more high precision. Results confirm that the texture depends on gelification speed, which is directly related to the amount of added GDL. Therefore, the present digital image analysis technique is suitable and very useful to characterize the texture of NaCAS acid gels formed by different GDL ratios.

The present analysis technique of digital images obtained by conventional and confocal microscopy is suitable for a structural study of acid protein gels and can be useful to evaluate the texture of dairy products.

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