ACTION OF SOME BIOPOLYMERIC MIXTURES ON THE STRUCTURE FORMATION AND CONSISTENCY IN DAIRY ACID PRODUCTS

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Abstract: The structure formation by two industrial biopolymeric mixtures in yogurt was investigated. The stabiliser mixtures were: stabiliser Bekaplus Y3 containing modified starch, gelatin and pectin and stabiliser Bekaplus Y2 with whey protein and gelatin. For each stabiliser, three concentrations in their working domain (0.5 to 2 mg/l for Y3 and 0.3 to 0.7 mg/l for Y2) were tested. The microstructure formation during gel formation was analysed using optical microscopy and the structural analyses were correlated with rheological properties (consistency) and acidity.

The results indicate that the stabiliser type and quantity influence strongly the structure and the functional properties. In the case of using stabiliser Y3, a compact and resistant final coagulum can be obtained at the addition of 2 mg/l, whereas smaller quantities of stabiliser (0.5 to 1 mg/l) give weaker curdles with structures near the natural yogurt. The use of small quantities of stabiliser Y2 (0.3 mg/l) allows the formation of very small clusters giving a uniform structure. The increase of quantity of Y2 in the initial milk determines non-significant changes of the gel consistency, but high differences at the structural level, where aggregates looking as self-assembly structures are formed.

Keywords: yogurt, stabiliser, structure, consistency, acidity

INTRODUCTION

Proper structuring of multiphase foods may provide increased stability or protection as well (Aguillera, 2005). In such systems is very important to understand the relation between structure and functional properties (Cesaro et al., 1992) (Pelletier et al., 2001), the kinetic of structure formation and destruction and how they are influenced by the working conditions and the system composition (Aguilera and Stanley, 1999).

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Food biopolymers are used as functional ingredients in the food emulsions or gels in order to create microstructures which provide products with desirable textural and rheological properties (Nunes et al., 2006). In the case of yogurt, a dairy acid product, proteins and/or polysaccharides are added for the improvement of flow properties, texture or water retention (Sodini et al., 2004).

Additives as polysaccharides or gelatine have been used for the increase of stability or to provide a desirable texture in yogurt or to modify the rheology (Sodini et al., 2004). For example, the addition of a mixture of modified starch and pectin (0.6%) produce a firmer spoonable and more viscous yogurt; the use of 1% modified starch and gelatine gives a very firm custard-style product, with increased apparent viscosity (Hess et al., 1997).

At the obtaining of yogurt, gelling is the major process responsible for microstructure modification and is due to the physical structuration of casein micelles, with the contribution of fat globules and whey proteins (Lucey et. al., 1998). The addition of other biopolymers, like pectin, gelatine or modified starch, favours repulsive casein-polysaccharide interactions, which promotes a demixing phenomenon; the balance between demixing, aggregation/gelation of casein and casein/polysaccharide systems determines the microstructure of gels (Sodini et al., 2004). Pectin (0.3%) or gelatin (1.5%) form a secondary network, whereas starch (2%), a non-gelling polysaccharide, doesn't contributes to the formation of a secondary network (Sodini et al., 2004).

Process conditions influences also very strongly the characteristics of the final product. For example, whey proteins become an important cross-linking agent in milk heated above 70°C, (Lucey et al., 1997) the complexation of β-lactoglobulin with κ-casein giving the casein micelles a hairy or spiky appearance; during gelation, the altered casein micelles form branched chains rather clusters (Sodini et al., 2004). The rheological properties are also improved by a preliminary heating of milk or by fermentation at 43-45°C (Lucey et al., 1997).

This research aims to analyse the influence of two biopolymeric mixtures used industrially as stabilisers on the microstructural properties and consistency of cow milk during the process of obtaining set yogurt. One stabiliser contains gelatin, modified starch and pectin and the other whey protein and gelatin. For each stabiliser, three concentrations are used.

The goal of this work is to establish the adequate type of stabiliser and the concentration for the obtaining of yogurts with specific microstructural characteristics and consistency.

MATERIALS AND METHODS

Materials

For the obtaining of yogurt, cow milk was used. The characteristics of the raw material are presented in Table 1. The stabilisers Bekaplus Y3 and Y2 were gently provided by the firma Liliput. Bekaplus Y3 contains gelatine, modified starch (E 1422) and pectin (E 440) (the ratio of components is not given) with 92% dry substance and pH 6.5. Bekaplus Y2 contains gelatine and milk protein (the ratio of components is, also, not given) with 90% dry substance and pH 7.1.

Table 1. The characteristics of cow milk used at the obtaining of yogurt

Characteristics	**Value**
Acidity, ºT	17
pH	6.57
Total nitrogen, %	0.519
Total protein, %	3.31
Casein, g/100 ml milk	2.61
Free aminoacids, %	0.95
Fat, %	3.72
Fat acidity, mg KOH/g	2.56
Density, g/cm^3	1.028

Methods

For the yogurt obtaining, cow milk was firstly standardised at 2.5% fat content and then pasteurised at 85°C for 30 min. At the beginning of pasteurisation, stabiliser was added under continous mixing, with prevention of aggregates formation. Milk was cooled at 45°C and starter culture Jointec E (2 unit/ 100 ml milk) was added. The samples were distributed in plastic cups (200 ml) and maintained for 3-4 h under quiescent (no agitation) conditions. The coagulated product was precooled to 20°C and afterwards cooled at refrigeration temperatures (5°C) to slow down the physical, chemical and microbiological degradation (Costin, 2005). A blind sample was also prepared, in the same way but without addition of stabiliser.
In all samples, the process was stopped when acidity reached the values 64-65°T. At this acidty, some typical characteristics in yogurt were observed:

syneresis at the surface, specific aroma and taste, formation of a compact gel structure.
The changes during curdling were analysed. Determinations at three levels were made:

- Physical- chemical level: acidity was measured by titration with NaOH 1N (A.O.A.C. method STAS 6353-85). Samples were analysed every 30 minutes.
- Functional level: gel consistency was determined using a consistometer build in the lab. The apparatus has a tronconical form (base diameter of 5 mm, overside diameter 100 mm, height 100 mm). Consistency was measured as the time needed to the fluid to flow through this apparatus. Because this measurement is destroying the gel, parallel sample were prepared and then one sample was analysed every 30 minutes.
- Structural level: optical microscopy, with a Kruss Optronic microscop with image capturing system and magnification power 40x10. These measurements were made for the initial samples (moment 0), for the samples after 210 minutes of lactic fermentation, when significant changes of consistency were obtained and in the final product.

The experimental design is presented in Table 2.

Table 2. Experimental design

Description			**Dosage, %**		
Input parameters	variable	Stabiliser Y3, mg/l	0.5	1	2
		Stabiliser Y3, mg/l	0.3	0.5	0.7
	constant	Fat content, %	2.5		
		Temperature, °C	45		
		Starter, unit/100 ml milk	2		
Output variables		Consistency, s			
		Acidity, °T			
		Structure, optical			

RESULTS AND DISCUSSIONS

The most important process taking place at the obtaining of yogurt is the lactic fermentation, when lactic acid acid is produced. This compound is responsible for the sour taste, specific for yogurt, and determines the decrease of pH and increase of acidity, with modifications at the microstructural level. The variation of acidity during curdling in samples with different concentrations of stabiliser is presented in Figure 1.

For all samples, acidity increases during fermentation, as result of lactic acid accumulation. This increase is depending on time and type of stabiliser added. So, acidity modifies very slowly in the firs 150 minutes in the blind sample and the samples treated with stabiliser Y2, the changes being more evident after 210 minutes of tempering at 45°C. The acidity increase is higher in the milk with Y3; after 210 minutes, the quantity of acid is very high in these samples.

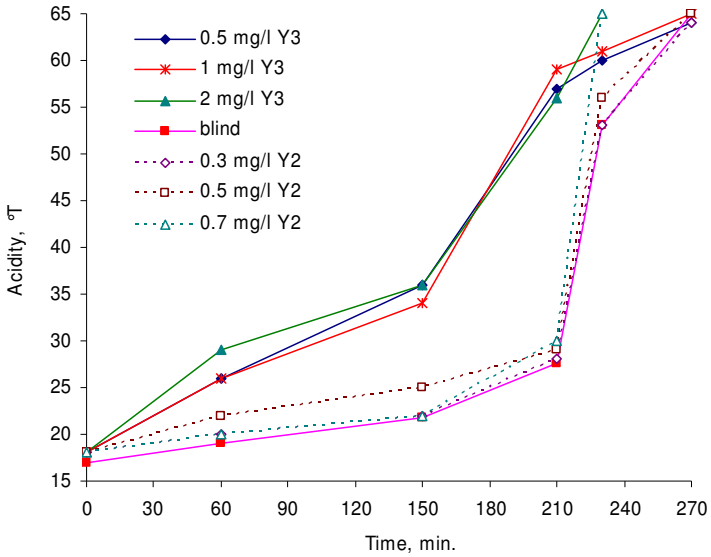


Figure 1. Evolution of acidity at the obtaining of yogurt with differerent composition of stabilisers Y3 and Y2, compared with the blind sample

The results obtained at the analysis of consistency during fermentation are presented in Figure 2. For a clearly differentiation from the blind sample, the consistency of samples with stabilisers added is presented as reported to blind. Compared with the acidity, the gel resistance doesn't increases in the first 150 minutes of maintaining at 45°C; no gel is formed. With the accumulation of lactic acid, the proteins restructuration occurs and the gel formation process is initiated; materials which are flow resistant are formed. Only after 150 minutes of tempering, the flow resistance of the product begins to increase. After 210 minutes, the consistency changes are evident in all the samples, including the blind.

For all samples, consistency increases, compared with the blind sample. The increase varies in time, depending on the type and quantity of stabiliser used. At the addition of stabiliser Y3, the increase of gel resistance is evident only

at concentrations higher as 1 mg/l; as figure 2a shows, the use of 2 mg/l Y3 determines the formation of final gels much more consistent as the blind sample.

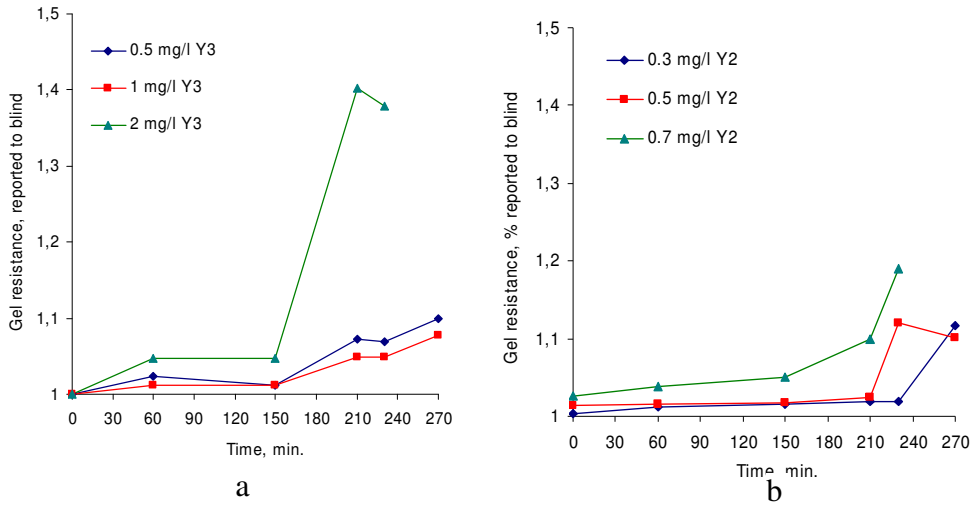
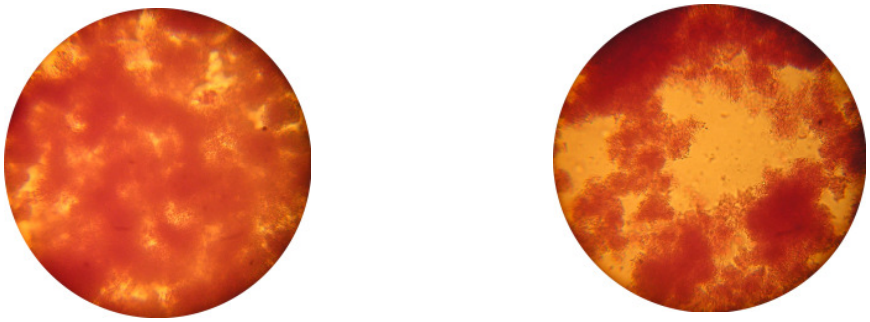


Figure 2. Evolution of gel consistency, reported to the blind sample, during curdling of milk treated with stabilisers Y3 (a) and Y2 (b)

The addition of small quantities of additive Y2 (0.3 to 0.5 mg/l) gives gels having similar resistance with those obtained with Y3 in double amount (0.5 to 1 mg/l). The increase of Y2 concentration to 0.7 mg/l increases the gel consistency to 1.2 reported to the blind sample.

In figure 3 the gel structure formation in samples without additives is presented.



a b

Figure 3. Structure of the gel obtained without stabilisers.
a- after 210 minutes of fermentation; b- final gel (after 270 minutes of fermentation)

The presence of small proteins aggregates forming a dispersed network is observed after 210 minutes of fermentation (figure 3a). At the end of the process, aggregates associate and form a firm gel with voids (figure 3b).
The structure formation during fermentation with stabilisers is presented in figures 4 (for the samples added with Y3) and 5 (for the yogurt formed with Y2) in three important moments:

- I: the first set of images corresponds to the initial milk, when the gel structure doesn't exists (moment 0);
- II: the second set of images is aquired after 210 minutes of tempering, when enough lactic acid is accumulated and the gelling process was initiated;
- III: the third set of images corresponds to the final product, with the maximal acidity and consistency.

In the moment 0, the image analysis shows the fat globules and the insoluble stabilisers components, dispersed in the milk mass. It is the case of modified starch or gelatine, observed in the milk added with Y3 (figure 4a.I.); the starch granules are round or ovoidal, whereas the gelatine has irregular shape. With the increase of stabiliser concentration, the quantity of insoluble compounds increases (figures 4b.I and 4c.I). In the samples added with stabiliser Y2, no significant structural changes are observed (figures 5a.I 5b.I and 5c.I); the whey proteins are soluble in milk and, probably, the quantity of gelatine is not so high to be observed through optical microscopy.
After 210 minutes of lactic fermentation, the formation of casein micelles is observed (figures 4.II and 5.II). The micelles are dispersed in the milk mass.
The gel formed after 210 minutes of lactic fermentation at 45°C has different structures, depending on the type and concentration of stabiliser. The image analysis shows that:

- At the addition of a small quantity of Y3 (0.5 mg/l), a compact and quasi-uniform distributed structure is formed, with casein aggregates (figure 4a.II). A different result is obtained with smaller concentrations of Y2 (0.3 mg/l); as figure 5a.II shows, uniform structure is formed, with very small casein clusters.
- With the increase of Y3 quantity, other structures are obtained. In the case of addition of 1 or 2 mg/l Y3 (figures 4b.II and 4c.II), the micelles are small and remain dispersed; this behaviour can be attributed only to the components of stabiliser Y3, which promotes demixing (Sodini et al., 2004) or are enough big to block the aggregation and the formation of bigger casein micelles. In this way, new structures, different of the classical one (figure 3a), are obtained.

- The increase of Y2 concentration in yogurt allows the possibility to form new structures. The micelles become bigger as with 0.3 mg/l, forming associations or aggregates. The aggregation is favorised by the increase of Y2 content (figures 5b.II and 5c.II).

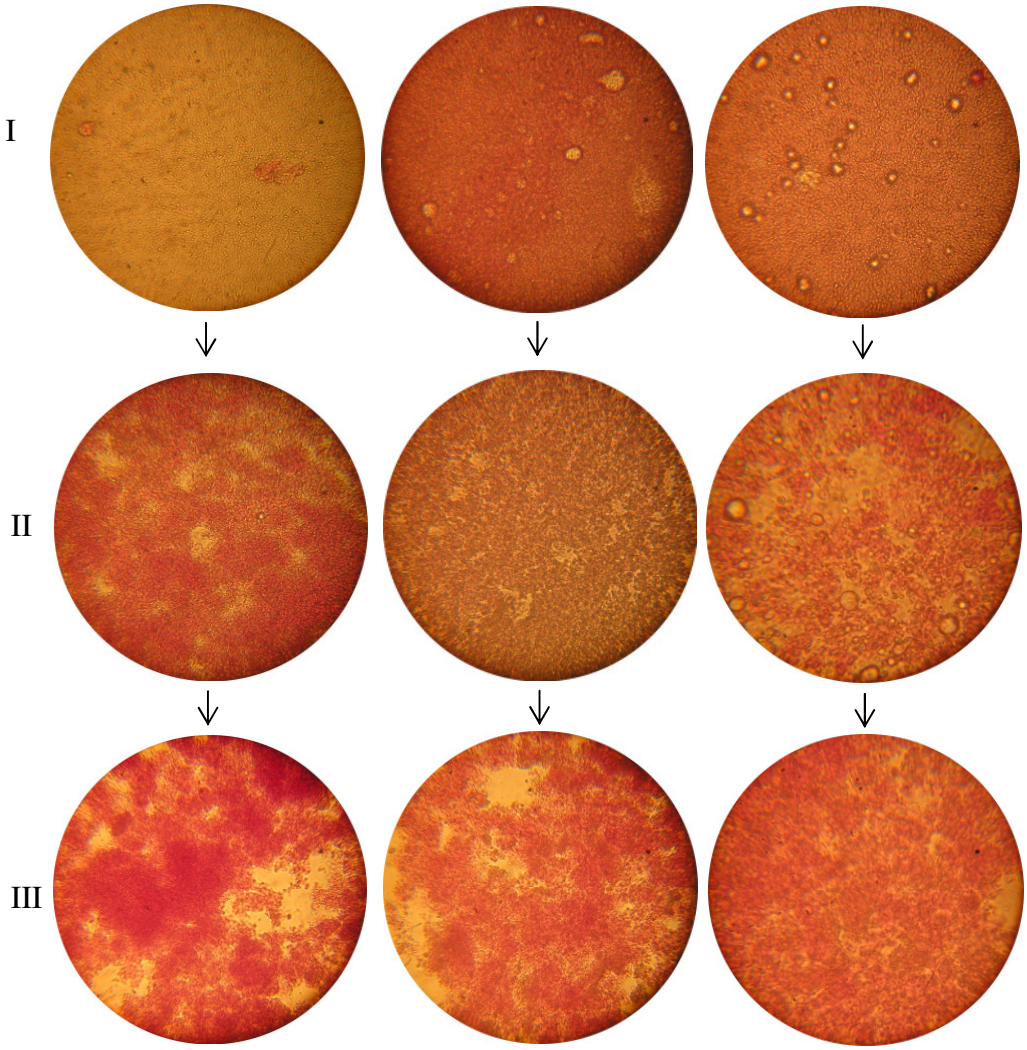


Figure 4. Structure evolution of gels obtained with addition of stabiliser Y3 during tempering at 45°C: a- 0.5 mg/l Y3; b- 1 mg/l Y3; c- 2 mg/l Y3.

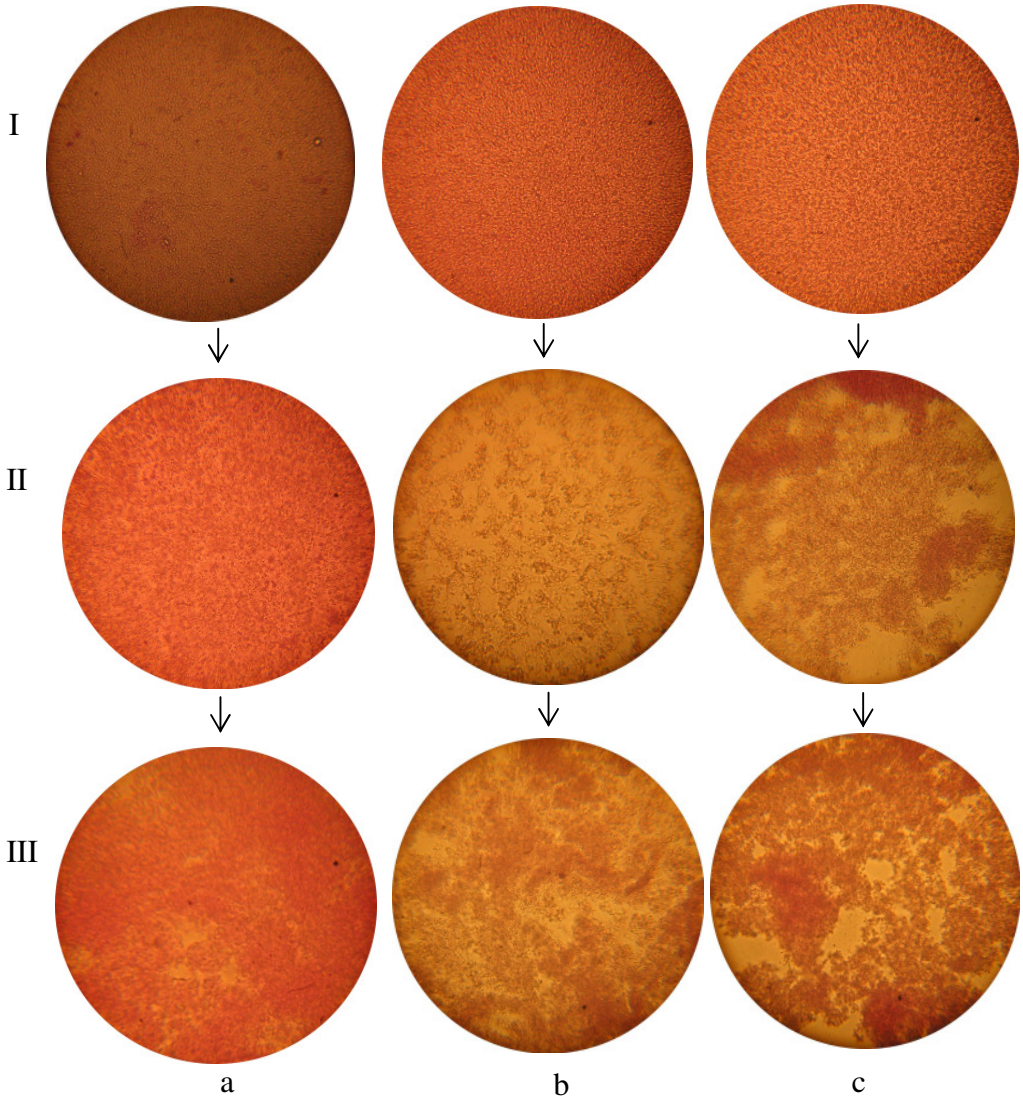


Figure 5. Structure evolution of gels obtained with addition of stabiliser Y2 during tempering at 45°C: a- 0.3 mg/l Y2; b- 0.5 mg/l Y2; c- 0.7 mg/l Y2.

The differences between the structures formed in yogurt at the addition of stabilisers depend on the lactic acid formed, also. As figure 1 show, the presence of stabiliser Y3 allows the production of a higher quantity of lactic acid, which determines the casein precipitation and the aggregation.

The process is considered ended after 270 minutes of fermentation for the samples treated with 0.3 to 0.5 mg/l Y2 and with 0.5 to 1 mg/l Y3 and after 230 minutes for the milk added with 0.7 mg/l Y2 and 2 mg/l Y3. As figures 4.III and 5.III show, the micelles association is different from the blind sample, depending on the type and concentration of stabiliser:

- With the increase of quantity of stabiliser Y3 added in milk, the final gel has a more uniform structure, with small aggregates which become closer. This structure can be very well correlated with the consistency measurements (figure 2a); with the increase of stabiliser concentration the product become more compact and the consistency increases.
- The addition of small Y2 quantity (0.3 to 0.5) in yogurt gives a more compact final gel structure with very small voids or spaces filled with liquid (figures 5a.III and 5b.III). This behaviour could be attributed to two factors: technology of yogurt obtaining and whey proteins from Y2, which act as cross-linking agents with casein during the pasteurization at 85°C for 30 min (Lucey et al., 1997). The final clusters are maintained together by the bounds between casein and whey proteins. With the increase of Y2 concentration, this system is probably destabilised.

CONCLUSIONS

In this paper the structure formation by some industrial biopolymeric mixtures in yogurt is investigated. Two types of stabiliser mixtures are used: Y2 containing gelatine and whey protein and Y3 containing modified starch, gelatine and pectin, each stabiliser type with three concentrations in their working domain (0.3 to 0.7 mg/l for Y2 and 0.5 to 2 mg/l for Y3). The percentage of each compound of stabilisers Y2 and Y3 is not given.

The stabiliser type and concentration influence significantly the evolution in time of all the studied characteristics. Especially the structure and gel consistency are influenced, whereas acidity doesn't registers very significant changes, compared with the blind sample (without stabilisers).

In all cases, the modifications begin to occur after around three hours of fermentation at 45°C, when the lactic acid begins to be accumulated in larger amounts. When typical characteristics for yogurt are observed, the gelling process is finished.

The correlated analysis of the three characteristics studied (acidity, consistency and structure) shows that:

- For the samples treated with Y3, the addition of small quantities of stabiliser Y3 (0.5 mg/l) gives a gel with structure quite similar with the blind sample. Gel resistance and acidity increase are very low compared with the blind sample. In the milk added with 2 mg/l Y3, consistency increases after 150 minutes of fermentation, with a maximal value after 230 minutes, when the gel stabilises and the structure is uniformed, as the microscopical analysis shows. The stabiliser Y3 is not completely embedded in the casein micelles.
- In the case of samples added with Y2, acidity and consistency don't increase compared with the blind sample, excepting the samples with the highest stabiliser concentration (0.7 mg/l). The changes at the structural level are quite high for all the concentrations tested; small concentrations of Y2 (0.3 to 0.5 mg/l) allow the formation of small clusters which remain interconnected, whereas a higher concentration (0.7 mg/l) gives rather aggregates than clusters.

When a final gel with compact structure, viscous, consistent and without voids is desired, the use of high concentrations of Y3 (2 mg/l) is recommended; the addition of very small quantities of Y2 (0.3 to 0.5 mg/l) allows the obtaining of a gel with compact structure and without voids, but with low consistency.

For the obtaining of a gel with similar structure, but with higher consistency, the use of very small amounts of Y3 is recommended.

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