

RHEOMETRY AND SCANNING ELECTRON MICROSCOPY STUDY OF CASEIN CURDS ADDED WITH MESQUITE SEED GUM AND SOY PROTEINS

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Abstract — The rheological and micro-structural characteristics of curds added with mesquite seed gum (MESG) and soy protein (SP) were studied. Two types of curds were prepared either with 100% raw commercial milk or with a 50% low-fat milk and the effect of the addition of 0.15% (w/w) and 0.45% (w/w) of MESG and/or 0.3% (w/w) and 0.6% (w/w) soy protein content was analyzed. The addition of MESG and SP results in moisture of up to 64.0% compared to 43.2% in the control sample. The rheometric functions G' (storage modulus) and G'' (loss modulus), measured in the frequency sweep from 6.28 to 62.8 rad/s tend to decrease for curds prepared with 100% raw milk and the addition of MESG and/or SP. In the case of low-fat milk curds the opposite behavior was observed. The micro-morphology of the samples added with MESG-SP present a cavernous appearance that was not present in the control curd.

Keywords — Rheometry, microscopy, casein curds, mesquite seed gum, soy protein.

I. INTRODUCTION

The addition of carbohydrates and proteins to cheeses has been amply studied (Anonymous, 1989; Brummel and Lee, 1990; Messina and Messina, 1992; Kucukoner and Haque, 1995) regarding their rheology, textural and microstructural behavior, in order to improve the characteristics of low-fat cheeses. However, not enough information about fat replacer ingredients is available, and our understanding of the behavior of this material needs to be expanded (Bagley and Christianson, 1987). Fat replacers are additive compounds used to fully or partially replace fat in reduced foods (Ma *et al.*, 1997). Some carbohydrates have been developed for low-fat cheeses, such as Novagel (cellulose microcrystalline-guar), Stellar (starch), Salatrim, Dairy Lo and Simplese (Kucukoner and Haque, 1995; Kosmark, 1996). Also, natural proteins with

excellent nutritional and physical properties such as soy protein (SP) have been tested, but are used less frequently (Messina and Messina, 1992) although soy protein isolates could be incorporated into dairy products (Hokes, 1992).

Some natural hydrocolloids could be considered as fat replacers, such as mesquite seed gum (MESG) from *Prosopis sp.* Mesquite is a leguminous plant that grows naturally along the Mexican territory (Rzedowski, 1988); MESG has been shown to possess great potential applications in a variety of food products (Meyer *et al.*, 1986; Vázquez *et al.*, 1988; Romeo *et al.*, 1989; Figuereido, 1990; Bravo *et al.*, 1994; Hernández-Tinoco, 1998).

The sensory attributes of foods are closely related to their chemical composition, rheological behavior and microstructure (Tunick, 1989; Kaláb, 1993), and all affect customer appearance appreciation of the product. The combination of MESG and SP is thought to confer good physical properties to casein curd. The rheological behavior and microstructure of these added products provide information about the possible changes induced by the addition of these materials to the physical properties of the curd. Many materials in food industry are viscoelastic (Bagley and Christianson, 1987). These materials can be described by their rheological behavior in terms of a general constitutive equation which relates the stress state to the strain history it has experienced, rather than relying on simple relationships which are material-specific and applicable only over a limited range of conditions (Ferry, 1980). The aim of this work was study the effects of the addition of MESG and SP at different concentrations to casein curds to determine its rheological and microscopic characteristics.

II. MATERIALS AND METHODS

A. Experimental details

Two series of curds were prepared, one series (A) was prepared using pasteurized homogenized raw milk and another series (B) using 50% low-fat milk prepared with raw milk and powdered low-fat milk. The rennet was

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single-strength liquid from CUAMEX, S. A. The lyophilized starter-culture was from TEXEL, brand series MM-BT, MM100 supplied by CUAMEX. Both series included, besides the control, additions of 0.15% (w/w) and 0.45% (w/w) of MESG and 0.3% (w/w) and 0.6% (w/w) of SP as indicated in Tables 1 and 2. The mesquite seed gum was lyophilized and milled after being extracted in the laboratory as described by Ramos and Salazar (1995). The MESG was added by stirring it into the milk, and was then heated at 35 °C. Soy protein from Protein Technologies International (FXP 920, USA) was hydrated and mixed directly into the milk. The curds were prepared as follows: the starter culture was added to the milk and held for one hour until acidity decreased. A CaCl₂ solution and the rennet was incorporated into the milk and maintained for about one hour until coagulation. The curd was cut in cubes of 1 x 1 cm, held for another 10 min and heated to 35 °C for 10 min with stirring. Whey was drained for 4 hours in a manual press, the curd was packaged in polyethylene bags and stored at 4 °C.

Fat content in the curds was measured following the procedure described by Gerber (NOM F-387, 1984) and protein content was determined using the micro-Kjeldahl-Cunning method described in NOM F-98 (1976). Moisture was established by weight difference after drying the samples at 100 °C during 24 hours in a stove (AOAC, 1997). Rennet-induced skimmed milk gels are

viscoelastic, and their small deformation rheological properties can be determined with the measurement of storage modulus (G') and loss modulus (G'') (Zoon *et al.*, 1988; Van Vliett *et al.*, 1989; Srinivasan and Lucey, 2002). The storage modulus (G') and loss modulus (G'') of the curds were determined with a stress-controlled rheometer by Paar Physica model LS100. The microstructure of the samples was observed with a Jeol 6300, scanning electron microscope (SEM). In order to preserve the structural characteristics of the samples for SEM observation, they were prepared following the Tunick and Shieh modified method (1995). The results shown up are the average of triplicate samples.

B. Rheometry

Dynamic measurements were carried out using a stress-controlled Rheometer model LS100 (Paar Physica, Germany), at 20 °C with a 20 mm plate-plate geometry and 2 mm gap. Storage modulus represent the elastic component and loss modulus the viscous component of a viscoelastic material. They measure the energy stored and recovered per cycle; or energy dissipated or lost as heat per cycle of sinusoidal deformation respectively, when different systems are compared at the same strain amplitude (Ferry, 1980). The viscoelastic lineal zone must be established by an amplitude sweep to find G' and G'' independence from the shear stress. Then, the

Table 1 - Composition of series A curds.

Curd	Concentration % (w/w)		%		
	MESG	SP	moisture	fat	protein
control	0.0	0.0	43.2 ± 1.2	32.0 ± 1.1	24.8 ± 1.1
1	0.15	0.3	64.0 ± 2.1	11.5 ± 0.3	24.5 ± 1.1
2	0.45	0.6	57.5 ± 1.3	23.0 ± 1.2	19.2 ± 0.7
3	0.15	0.6	62.7 ± 1.4	11.5 ± 0.3	25.5 ± 1.0
4	0.45	0.3	58.5 ± 1.3	20.1 ± 1.0	21.0 ± 0.8

The results are average of triplicate.

Table 2 - Composition of series B curds.

Curd	Concentration % (w/w)		%		
	MESG	SP	moisture	fat	protein
Control	0.0	0.0	30.7 ± 1.1	15.9 ± 1.1	53.3 ± 1.6
1	0.15	0.3	59.4 ± 1.9	5.0 ± 0.2	34.6 ± 0.8
2	0.45	0.6	36.1 ± 1.3	8.0 ± 0.2	54.8 ± 1.4
3	0.15	0.6	60.0 ± 1.2	6.1 ± 0.1	33.4 ± 0.7
4	0.45	0.3	59.5 ± 1.1	7.0 ± 0.2	33.3 ± 0.6

The results are average of triplicate.

frequency sweep is made to obtain the rheogram to describe the material. Measurements were made in triplicate.

C. Microscopy

Samples for scanning electron microscopy (SEM) were prepared with the Tunick and Shieh modified method (1995) as follows: the sample were removed from the interior of the cheese curd, and diced into rectangular blocks of approximately 5 X 2 X 2mm. These were immersed in 1% glutaraldehyde in phosphate solution (pH 7.2) at room temperature for 1 hr. Samples were then washed in phosphates buffer solution, dehydrated in a graded series of ethanol solutions, extracted with three changes in chloroform, transferred into ethanol, freeze-fractured in liquid nitrogen and dried at the critical point in carbon dioxide in a drying equipment (model Samdri 780A, USA). The dried blocks were mounted on a special plate for microscopy, coated with a thin layer of gold in a cold sputter etch unit (Denton Vacuum Inc, model desk II, USA) and examined by electron imaging in a scanning electron microscope (Jeol JSM-6300, Japan).

III. RESULTS AND DISCUSSION

A. Rheometry

The zone of linear viscoelastic behavior of the casein curds was in the interval between 255 Pa and 449 Pa of oscillatory shear stresses, where G' and G'' are independent of the shear. The effect of the dynamic rheology for series A and B curds was measured by monitoring changes in storage modulus (G') and loss modulus (G'') (Figure 1 and 2). In the four different formulations (curd 1,2,3 and 4) the behavior was similar; all were below the control, about 50% less at the lowest frequency (Figure 1). The G' values predominated over G'' in all of the measured frequency range, the plot showed frequency dependence of G' and G'' . The increase in G' is probably due to ongoing fusion of casein particles, due to rearrangement of both inter- and intramolecular forces (Roefs *et al.*, 1990; Van Vliet, 2000). Another author (Lucey, 2002) concludes that the casein particles also undergo rearrangement, fusion, and syneresis in the process of forming cheese curd; casein-based gels are inherently dynamic in nature, and the rearrangement process is involved in the syneresis of rennet-induced gels. As the proportion of SP (0.6% w/w to 0.3% w/w) diminishes with respect to the same concentration of MESG (0.15% w/w or 0.45% w/w) there is a trend of G' and G'' to decrease when the MESG increases, mainly at 0.45% w/w of MESG (Figure 1). The four curds have greater moisture content and smoother appearance than the control. This is a reason why G' and G'' moduli are below the control. Moisture plays an important role for viscoelasticity (Olson and Johnson,

1990; Messens *et al.*, 2000); and salt content is also important (Abdel-Hamid *et al.*, 2000). Research by Zhou and Mulvaney (1998) on a model system of water, casein, and milk fat showed that G' and G'' are primarily affected by milk fat content; in this study differences of G' and G'' were also present, where G' was greater than G'' , both moduli increased with ω .

In this study series B contained 50% less fat than series A (Table 1 and 2) and the storage and loss moduli are higher in series A than series B (Figures 1 and 2). Figure 2 shows the effect of MESG and SP on the dynamic moduli of series B casein curds, in this experimental condition an opposite effect is observed, G' and G'' values are larger than the control. The G' values predominated over G'' values in all the measured frequency range. The dependence of frequency produced an increase of viscoelasticity, as shown by the G' and G'' increase, as in the cheddar and mozzarella cheeses (Ustunol, 1995; Rowney *et al.*, 1999; Fenelon *et al.*, 2000). The curds of series B, looked more hard and dry and were different from the control. Table 2 also shows the results for moisture and fat of series B, the series A curds were highest in moisture and fat, and looked like the control curd. The viscoelastic behavior is similar in both series; however the fat content in the series B is smaller, approximately 50%. The magnitude of the moduli is bigger in the series A, a difference of a logarithmic cycle. Another study with Gouda cheese (Messens *et al.*, 2000) indicates that rheological properties returned to those of control samples; such changes were considered likely to be due to weakening of hydrophobic interactions, which are restored during ripening.

B. Microscopy

Scanning electron microscopy was used to study series A and B. Figure 3 shows the controls curds: (a) without MESG or SP, (b) added with 0.15% (w/w) MESG and (c) added with 0.6% (w/w) SP. Both curds, Figures 3b and 3c, show a mostly open structure and had a smoother continuous zone than controls. When mesquite seed gum, soy protein and casein micelles are mixed, the effect of addition becomes quite important, as shown in studies of gelation of casein-whey mixtures (Schorsch *et al.*, 2001). The continuous zones are a combination of casein with MESG or SP; moreover, the water occluded also contributes to the structure. The curds with SP are firm, porous like the control but with larger caverns, free spaces between flocculi, and a more open structure, that makes the sample fragile to mechanical handling.

Figure 4 shows the four different structures for the formulations of series A. Micrographs 4b and 4d are similar, they have a smooth and open structure, the size of flocculi is similar, and their moisture content is also similar, about 58%. Moreover, they have the same MESG content (0.45%). Micrographs 4a and 4c, are similar as

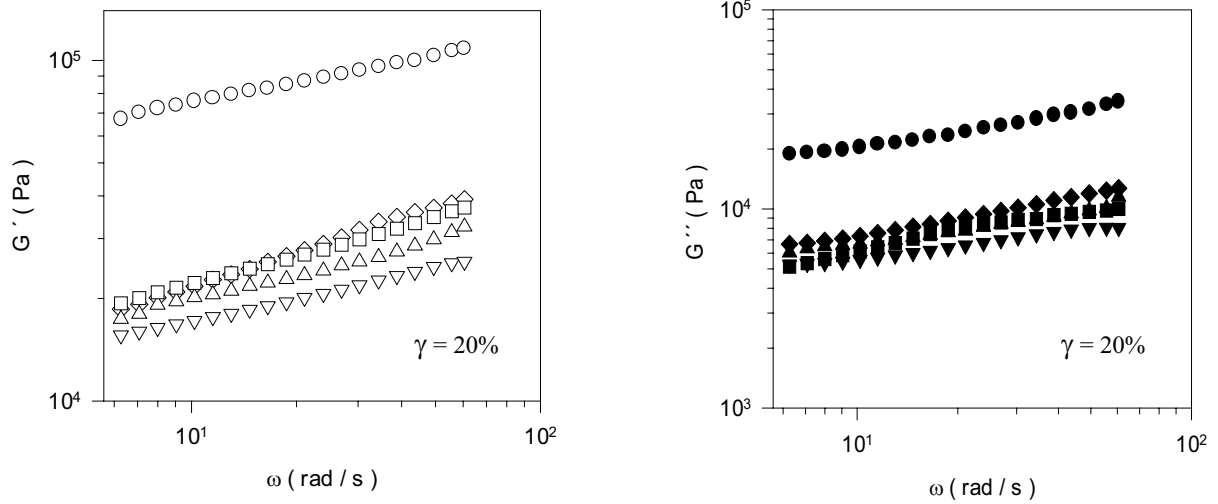


Fig. 1 - Effect of MESG and SP on dynamic moduli of series A casein curds. Control (\circ , \bullet), curd 1 [0.15% (w/w) MESG and 0.3% (w/w) SP] (\square , \blacksquare), curd 2 [0.45% (w/w) MESG and 0.6% (w/w) SP] (\triangle , \blacktriangle), curd 3 [0.15% (w/w) MESG and 0.6% (w/w) SP] (\diamond , \blacklozenge) and curd 4 [0.45% (w/w) MESG and 0.3% (w/w) SP] (∇ , \blacktriangledown). Torque amplitude = 0.3 mNm.

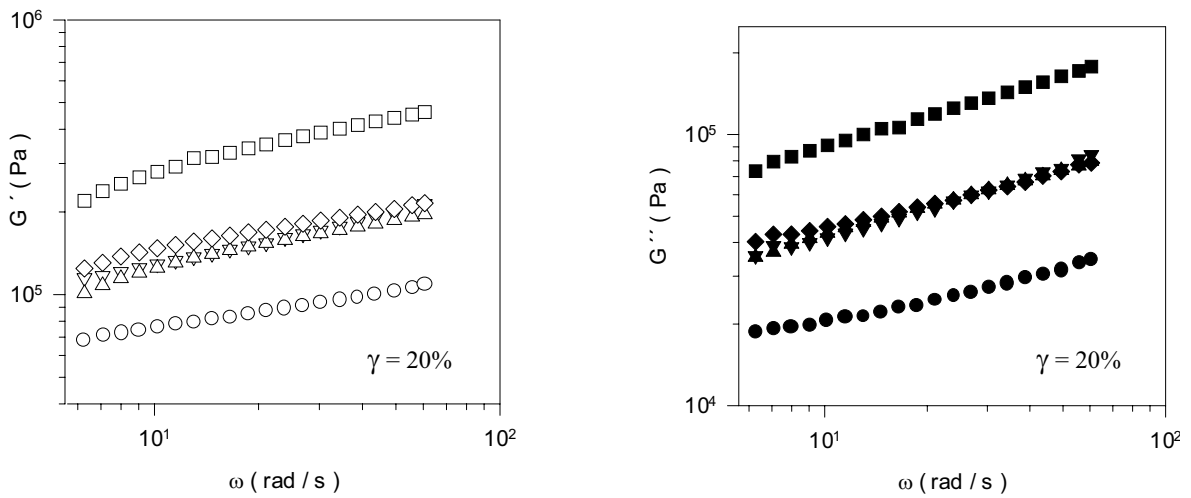


Fig. 2 - Effect of MESG and SP on dynamic moduli of series B casein curds, with standardized milk at 50%. Control (\circ , \bullet), curd 1 [0.15% (w/w) MSG and 0.3% (w/w) SP] (\square , \blacksquare), curd 2 [0.45% (w/w) MESG and 0.6% (w/w) SP] (\triangle , \blacktriangle), curd 3 [0.15% (w/w) MESG and 0.6% (w/w) SP] (\diamond , \blacklozenge) and curd 4 [0.45% (w/w) MESG and 0.3% (w/w) SP] (∇ , \blacktriangledown). Torque amplitude = 0.3 mNm.

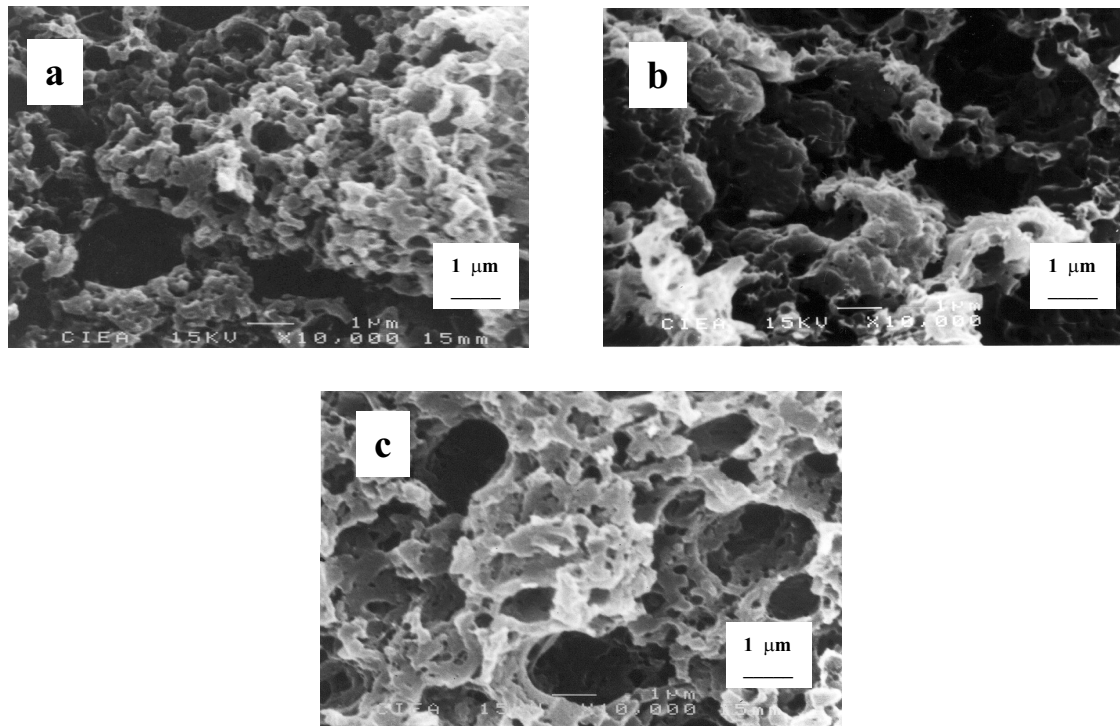


Fig. 3 - Micrographs of control curds. a) casein curd without MESG or SP. b) casein curd added with 0.15% (w/w) MESG. c) casein curd added with 0.6% (w/w) SP. Magnification 10 000 X.

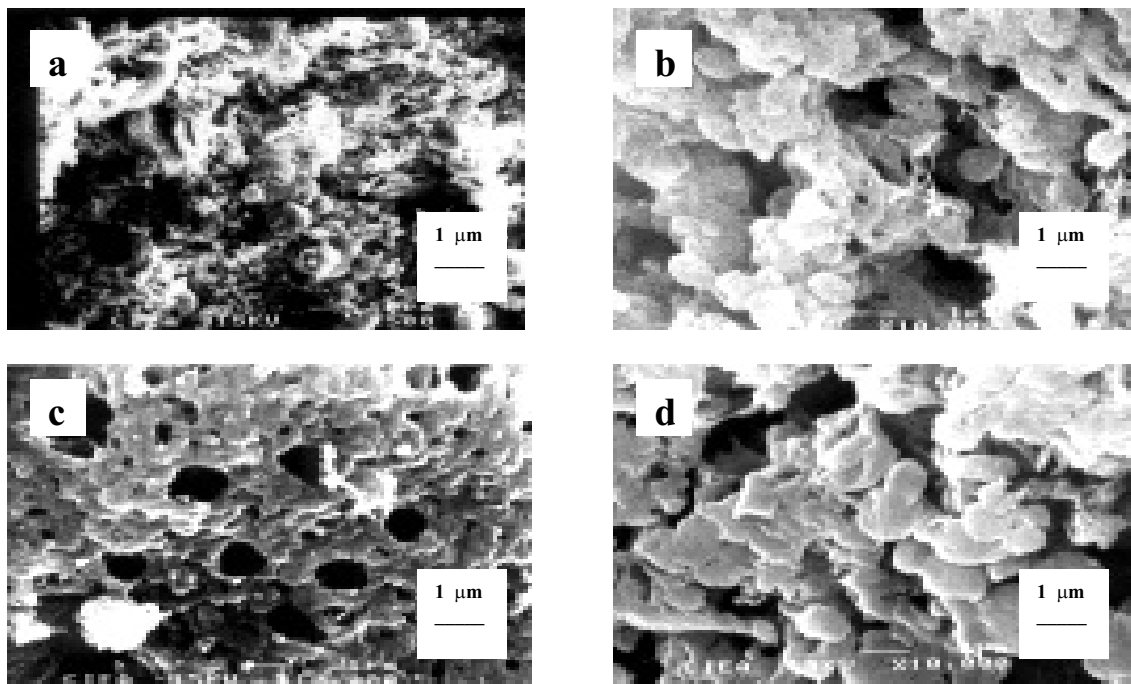


Fig 4 - Micrographs of series A curds added with MESG and SP. a) 0.15% (w/w) MESG and 0.3% (w/w) SP. b) 0.45% (w/w) MESG and 0.6% (w/w) SP. c) 0.15% (w/w) MESG and 0.6% (w/w) SP. d) 0.45% (w/w) MESG and 0.3% (w/w) SP. Magnification 10 000 X.

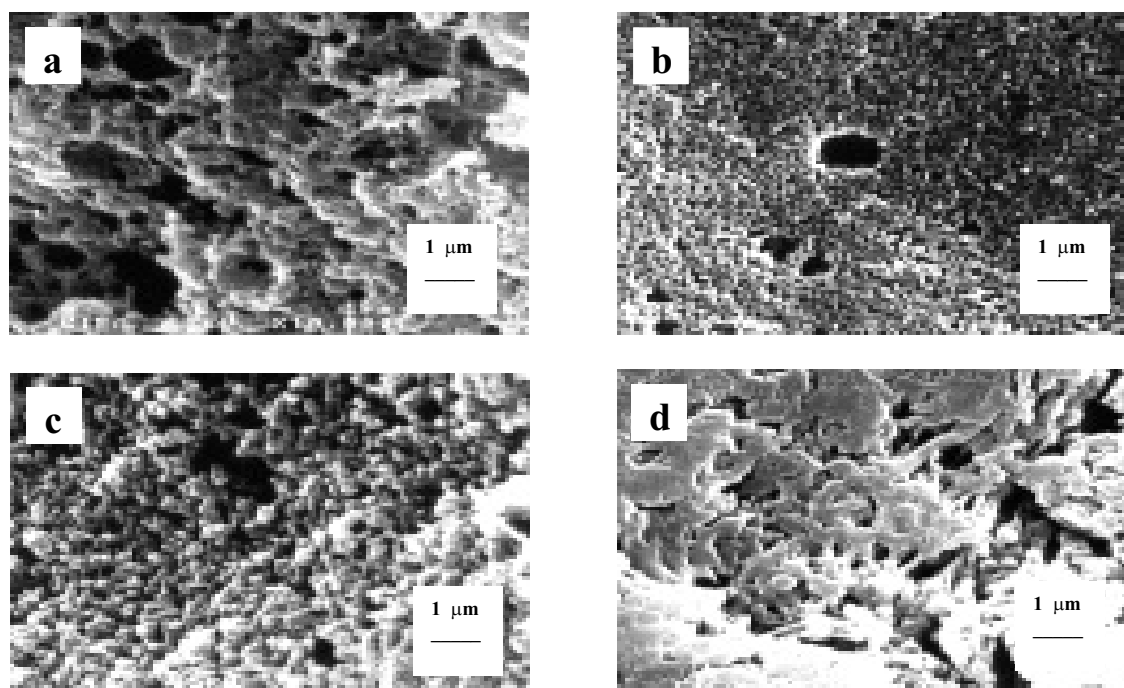


Fig. 5 - Micrographs of series B, with standardized milk at 50%, formulated curds added with MESG and SP. a) 0.15% (w/w) MESG and 0.3% (w/w) SP. b) 0.45% (w/w) MESG and 0.6% (w/w) SP. c) 0.15% (w/w) MESG and 0.6% (w/w) SP. d) 0.45% (w/w) MESG and 0.3% (w/w) SP. Magnification 10 000 X.

well, they have a compact and closer structure, the size of the pore is smaller than for 4b and 4d. As in the last case, they share the same MESG content (0.15%). The mentioned data show that with a smaller content of MESG, 0.15%, the structure is nearly closed and the size of the pores is smaller. The same as in other studies (Srinivasan and Lucey, 2002), clearly defined pores and a fractal structure are present.

Figure 5 shows the series B curds, the ones with standardized 50% low-fat milk. All micrographs show a closer and more compact network than series A curds; micrographs 5b and 5c have smaller pore size, than 5a and 5d, the curds had too much apparent interconnectivity and agreed with the rheological results which showed that these curds were strong, the values of G' and G'' were higher; also, the curds had the same SP content. As MESG concentration became higher the size of the flocculi became smaller. Therefore, the presence of MESG determines the final structure of the curd. Other studies indicated (Mellema *et al.*, 2000) that the topography of the casein curd is the result of the collision of fat globules with other molecules, as can be MESG or SP.

The lower values of G' and G'' correspond to smooth and open structures, and larger values of G' and G''

correspond to continuous and closed structures. Other studies (Zhou and Mulvaney, 1998; Wendin *et al.*, 2000) indicated that sensory properties and microstructure were affected by fat content; however, results demonstrate that it is possible to produce a lower fat product similar to commercial cheese.

In this study, G' and G'' were evaluated to investigate the influence on the physical properties and microstructure of acid-induced milk gel; scanning electron microscopy showed that, in the case of the control, the acid-induced gel consisted of a coarse three dimensional network of casein particles joined together in large clusters; whereas the other gels consisted of a finer network of smaller cluster of casein particles. The MESG seems to rule the final structure because of the way it combined with casein and occluded water in its structure. Niki *et al.*, (2001) and Osman *et al.*, (2001) indicate that the properties of particle-filled composites are generally determined by the component properties, composition, structure, particle-particle interaction and particle-matrix interaction. The extent to which particles agglomerate depends on the balance between the attractive and repulsive forces among the particles and between them and the matrix, as well as on the processing conditions.

IV. CONCLUSIONS

In this study it was possible to incorporate MESG and SP to casein curds. All the casein curds added with mesquite seed gum and soy proteins showed viscoelastic behavior. The one in which G' was higher than G'' showed a more solid-like gel character. The storage modulus (G') and the loss modulus (G'') were lower in reduced fat casein curd, series B. The examination of the gel structure by scanning electron microscopy showed that different types of structures are formed depending on the incorporation of MESG or SP. When the MESG level was high the microstructure of casein curds was altered, large changes in the microstructure of casein curds were only observed when the MESG or SP levels became high.

V. ACKNOWLEDGMENTS

The authors want thank to P. Méndez C., M. Márquez R., L. Rojas M. and B. Soto G., for technical assistance, from CINVESTAV-IPN, and CONACyT for financial support (Project FOSIVILLA 9606117).

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Received: March 2, 2002.

Accepted for publication: November 19, 2003.

Recommended by Subject Editor G. Meira.