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A further insight into the practical applications of exopolysaccharides from *Sclerotium rolfsii*

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Abstract

The ability of exopolysaccharides EPS I (after 48 h-cultivation) and EPS II (after 72 h-cultivation), produced by the fungus *Sclerotium rolfsii* ATCC 201126, to minimize the liquid separation (syneresis) experienced by cooked starch pastes during refrigeration was investigated. After comparing different techniques, the extent of syneresis was finally estimated by daily measurement of the liquid phase length (Δh) separated above the sedimented phase throughout the storage at 5 °C. The degree of syneresis was represented by $\Delta h/h_0$, where h_0 stands for the initial height of the sample dispersion. Proportions varying between 9.90/0.10 and 9.00/1.00 (w/w) for 2% (w/v) corn starch/EPS aqueous blends were evaluated against 2% (w/v) corn starch (CS) as control. Up to 20 days of refrigeration and for the highest tested proportion (9.00/1.00), syneresis could be completely inhibited or 91% reduced by EPS II and EPS I, respectively. EPS II was thereby selected as the optimal syneresis preventive and subsequent analysis of its rheological behaviour in distilled water, skimmed and whole milk confirmed the ability to increase viscosity with a non-Newtonian, pseudoplastic behaviour. Rheology of CS/EPS II blends, when compared to the separated CS and EPS II, also evidenced a desirable synergistic effect in the aforementioned solvents, as witnessed by the increase in viscosity, higher consistency coefficients and lower flow behaviour indexes. Additionally, EPS II was able to prevent syneresis without affecting pH, gelling properties, hardness or colour. These results revealed that scleroglucan might become a food-approvable hydrocolloid with prospective use as food stabilizer and water loss preventive.

Keywords: Scleroglucan; *Sclerotium rolfsii*; Syneresis; Starch; Rheology; Milk

1. Introduction

The worldwide food industry uses 70,000 tonnes of polysaccharides per year as thickening agents, stabilizers and texturisers. As the emerging food products become more complex and diverse, the requirement for new and versatile additives is stronger. Nowadays, different polysaccharides are used to modify food viscosity and texture. Additionally, polysaccharide gums constitute non-fat alternatives that may serve as a source of soluble dietary fibre with health beneficial effects at quite low levels. They are currently obtained from plants (starch, cellulose, pectin, guar gum), seaweed and crustaceans (alginate, carrageenans, chitosan) or microbial sources (xanthan gum) but the exploration for novel candidates still continues (Gimeno, Moraru, & Kokini, 2004; Sadar, 2004).

To this end, vast research has been carried out aimed at improving different aspects related to polysaccharide production. However, as the knowledge on how the structure falls into the physical properties for a given polysaccharide remains insufficient, its use becomes limited. Therefore, advances in the science of polysaccharides may be expected to render future developments in the field of applications.

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Starch is one of the most abundant and widely distributed components in foodstuffs. Its gelatinization is commonly achieved by cooking in the presence of water. On cooling, starch granules recrystallize to form a solid gel, a process known as retrogradation. It proceeds into two stages: the first one occurs quickly and involves amylose gelation. Thereafter, further cristallinity develops slowly in the amylopectin region (Yoshimura, Takaya, & Nishinari, 1998). Temperature decrease determines a reduction on the kinetic energy that facilitates the amylose molecules to associate and form a three-dimensional network. As a consequence, water is squeezed out of the gel (syneresis), while intermolecular interaction between amylose molecules becomes stronger and gel shrinks.

Syneresis negatively affects the functional and sensory properties of foods (Sikora, Juszczak, Sady, & Krawontka, 2003; Zheng & Sosulski, 1998) and should be minimized without interfering with the native properties of food products. This may be achieved by means of the addition of hydrocolloids, such as polysaccharides. They can act not only at the syneresis level but also on viscosity, gelling properties, and other sensory properties. Nevertheless, as no rules allow predicting the applicability of a given polysaccharide, each case needs to be investigated in particular (García-Ribera, Monteoliva-Sánchez, & Ramos-Cormenzana, 2002).

Diverse microbial polysaccharides may be potentially used as thickeners and gelling agents when mixed with water, milk or other water-containing foodstuff (García-Ribera et al., 2002). In view of the already known virtues of scleroglucan to maintain its physical properties in the presence of salts, at high temperatures or extreme pH (Fariña, Siñeriz, Molina, & Perotti, 2001), this work explored its capacity to prevent starch paste syneresis. Additionally, the rheological properties in different solvents and mixtures were also evaluated as an indication of its potential application in the food industry.

Since at the time of evaluating and comparing starch syneresis, highly fluctuating data were encountered in the literature, the reliability of already known techniques was questioned. On the other hand, cited starch concentrations were quite variable and no agreement on how they affected the degree of syneresis was found (Zheng & Sosulski, 1998). On this context, the development and optimization of a reliable syneresis quantification technique, sensitive enough and highly reproducible, was aimed. In addition, starch concentrations were selected so that our results could be compared within the range reported in the literature.

2. Materials and methods

2.1. Starch and polysaccharides

Normal corn starch (MAIZENA® Duryea, 99.4% purity), commercial grade and locally purchased from Refinerías de Maíz SAICF (Buenos Aires, Argentina) was used. Commercial grade cassava starch (ALdeMA, 98% purity) was purchased from Cooperativa Agrícola e Industrial San Alberto Ltda (Misiones, Argentina). Pullulan and carboxymethyl cellulose were obtained from Sigma Chemical Co. (St Louis, MO, USA).

2.2. Scleroglucan production

The polysaccharides EPS I and EPS II used in these studies were produced by the fungus *Sclerotium rolfsii*, isolated from rotten red pepper and currently catalogued as ATCC 201126, which was preserved as sclerotia in distilled water following the protocol previously described (Fariña, Siñeriz, Molina, & Perotti, 1996). Mycelium was obtained by germination of water-preserved sclerotia on malt Czapek agar plates incubated at 30 °C (Fariña et al., 1996). Seed cultures were prepared in 100 mL of PM_{20} liquid medium (Fariña, Siñeriz, Molina, & Perotti, 1998) placed in a 500-mL Erlenmeyer flask and inoculated with 10 mycelium-covered agar discs (~5-mm diameter) removed from a 2-day-old, malt Czapek-grown culture. Suspensions were aseptically homogenized (CB-6 Waring blender, minimum output, 10 s) and incubated at 30 °C on an orbital shaker at 220 rpm for 48 h. Resulting pre-inocula were first 5-fold diluted with fresh PM_{20} and further incubated for 48 h under the same conditions as above and then, used for inoculation (10%, v/v) of a 10-L stirred-tank reactor fitted with baffles and six-flat bladed Rushton turbine impellers (MicroFerm, New Brunswick Scientific Co.) with a working volume of 8 L.

The optimized culture medium (MOPT) for polysaccharide production contained (in g/L): sucrose, 150; $NaNO_3$, 2.25; $K_2HPO_4 \cdot 3H_2O$, 2; yeast extract, 1; monohydrate citric acid, 0.7; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.05 (initial pH adjusted to 4.5). The following operative conditions were maintained throughout the experiment: air flow rate, 0.5 vvm; stirrer speed, 400 rpm; temperature, 30 °C; pH uncontrolled. To study the functional properties of the exopolysaccharide (EPS) obtained at different cultivation times, two fermentations were carried out under the above conditions, one during 48 h (EPS I) and the other one, for 72 h (EPS II). At the end of fermentation, the EPSs were purified for their subsequent use.

2.3. Scleroglucan recovery and purification

Culture broth was homogenized in a CB-6 Waring blender, neutralized, and 3-fold diluted with distilled water. After heating at 80 °C for 30 min, it was centrifuged in a 6-L capacity continuous centrifuge (Typ 61-763 Lahr/Schwarzwald) at 17,000 rpm. The EPS from clear supernatant was cooled at 5 °C and precipitated by adding an equal volume

of 96% ethanol. This mixture was allowed to stand at 5 °C during 8 h to complete EPS precipitation.

The precipitate was recovered with a fine sieve (Macotest A.S.T.M. N° 60) and then, redissolved in distilled water. This crude EPS was further purified by 96%-ethanol reprecipitation (twice). Finally, the precipitated polymer was freeze-dried and milled to a whitish glucan powder, giving a purified preparation of the polysaccharide. Glucan powder was analyzed for protein content by the Folin–Lowry method using bovine serum albumin as standard. Reducing sugars were measured according to the Somogyi–Nelson method (Hodge & Hofreiter, 1962) with glucose as standard. Total carbohydrates were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with dextran as standard. For both EPSs the presence of reducing sugars was not detected, whilst the total sugars content was about 98% (w/w), indicating a high grade of purity as compared to other commercial scleroglucans (Wang & McNeil, 1996). The Folin–Lowry determination revealed 1.9 and 1.6% (w/w) protein contents for EPS I and II, respectively.

2.4. Preparation of starch pastes, polysaccharides and mixtures

Moisture contents of polysaccharides were: corn starch (CS), 11.46%; cassava starch (CaS), 12.61%; pullulan, 8.88%; carboxymethyl cellulose (CMC), 6.49%; EPS I, 12.56% and EPS II, 13.54%. Control samples of starches (CS or CaS) and samples containing additional polysaccharides, were obtained by separate preparation of starch and polysaccharide solutions, and when necessary, by subsequent mixing. The appropriate amount of starch powder to get the desired final concentration was first dispersed in cold distilled water and the resultant starch slurry was then cooked in a boiling water bath (10–15 min) with gentle manual stirring until the paste was ready. Blends with other polysaccharides (pullulan, CMC, EPS I and II) were prepared at different mixing ratios (see Section 3). To do this, the suitable amount of polysaccharide was first dispersed in distilled water and then stirred continuously (in a magnetic stirrer) until complete dissolution. In the case of EPS I and II, dissolution started with overnight hydration under magnetic stirring (~200 rpm) and continued at 400 rpm with heating (60 °C) for about 48 h (until constant viscosity). Starch and polysaccharide solutions were then mixed to obtain the proportion under study. If necessary, volume was corrected by means of addition of distilled water, and blend was left aside to reach room temperature (~25 °C).

The behaviour of EPS, CS and different blends of CS plus EPS was comparatively studied in three different solvents: distilled water (DW), skimmed milk (SM) and whole milk (WM). For each of these solvents, three kinds of solutes or mixtures were tested: (a) 2% (w/v) CS (as it was the concentration selected for syneresis tests), (b) 2% (w/v) CS + 2 g/L EPS II (corresponding to a 9.00/1.00 (w/w) proportion, and (c) 2 g/L EPS II. Milks (La Serenísima, skimmed and whole milks) were obtained from MASTELLONE HNOS. S.A. (Buenos Aires, Argentina). Reconstituted milks to be used as solvents were prepared from dry powders by addition of distilled water according to the proportions suggested by the manufacturer.

The obtained solutions or mixtures were subjected to both pH and rheological measurements. Sensory properties (colour, gelling ability, and transparency) were qualitatively evaluated by visual inspection by five different panelists selected from the staff and student population at PROIMI (three female and two male individuals, 33 mean age). Gelling ability was rated between − and +++, corresponding to nil and maximum degree of gelling, respectively. Transparency was 'all or none' qualified and colour was defined after consensus of the panelists. Samples were randomly presented in transparent plastic tubes with undisclosed codes and under white fluorescent lights. Samples were also aliquoted for syneresis observation and results rated between − (no syneresis) and ++ (high degree of syneresis).

After preparation, starch pastes or mixtures were always allowed to reach room temperature before being aliquoted. Microbial activity during refrigeration was prevented by adding sodium benzoate (Merck) to a final concentration of 1 g/L. All starch and polysaccharide concentrations referred in the present work are dry-powder based.

2.5. Observation of syneresis

Different techniques were compared and the standardization involved the use of two different starches: CS and CaS, and variable concentrations (2–4%, w/v).

2.5.1. Technique 1a

The starch samples or mixtures (starch + polysaccharide) were poured into screw-cap plastic tubes (16 mm diameter × 120 mm height) to reach a final volume of 15 mL. Tubes were held vertically at 5 ± 1 °C for 20 days, unless otherwise stated. The extent of syneresis was estimated by measuring the liquid phase length, Δh, separated above the sedimented phase. The degree of syneresis was represented by $\Delta h/h_0$, where h_0 stands for the initial height of the sample dispersion at time $t = 0$ days. Measurements were daily performed throughout storage at 5 °C. Results are averages of at least triplicate determinations.

The starch/polysaccharide (w/w) proportions used were:

$A = 9.90/0.10$, $B = 9.75/0.25$,

$C = 9.50/0.50$, $D = 9.25/0.75$, $E = 9.00/1.00$

In the case of pullulan and CMC, only proportions A, B and E were tested. For the EPSs, proportions C and D were

included in order to determine the minimal concentration able to retard or inhibit syneresis.

2.5.2. Technique 1b

Procedure was similar to Technique 1a, but storage of starch pastes was performed in different tubes, according to a protocol previously reported (Yoshimura, Takaya & Nishinari, 1998). Glass tubes with different dimensions (27 mm diameter × 200 mm height) were used and the rest of the protocol was as described for Technique 1a.

2.5.3. Technique 2

A conventional method of centrifugation was also evaluated (Zheng & Sosulski, 1998). Starch samples were poured into plastic tubes to reach a final volume of 15 mL. Tubes were refrigerated as in Technique 1a but, after 20 days, half of them were analysed according to Technique 1a and the other half were centrifuged at $4000 \times g$ for 10 min. Accordingly, the value of Δh was registered either without centrifugation or with centrifugation, $\Delta h'$. Also, the extent of syneresis was estimated by weighing the water released from the separated phase before applying the centrifugal force and afterwards. The amount of water released from the paste was pipetted out, weighed and expressed as percent of the original gel weight (Zheng & Sosulski, 1998).

The degree of syneresis was then expressed by $\Delta h/h_0$, $\Delta h'/h_0$ (without and with centrifugation, respectively) where h_0 stands for the initial height; and water separation as the percentage gel weight (before and after centrifugation). All determinations were carried out at least in triplicate.

2.5.4. Technique 3

A modification of the protocol previously proposed by Zheng and Sosulski (1998) was followed. Net syneresis was determined in a three-stage mode by quantitating: *a*: 'free water-FW', which represents the water separated by centrifugation from freshly-cooked pastes, *b*: 'expelled water-EW', as the water spontaneously expelled from the gel during cold storage, and *c*: 'absorbed water-AW', corresponding to the water removed by centrifugation after elimination of expelled water in the contracted gel.

2.5.4.1. 3a: Determination of FW. Fresh starch pastes were prepared as described above and the FW content was determined in 5-cc B–D Plastipak Syringes (Becton Dickinson and Co., Rutherford, NJ, USA) following the design previously described (Zheng & Sosulski, 1998). Syringes Luer-Lok tips and plungers were removed prior to use. A single piece of Millipore AP 2004200 filter paper (Millipore Corp., Bedford, MA, USA) was placed on the bottom and 0.15–0.20 g Celite (diatomaceous earth, min. 95% as SiO_2, Sigma Chemical Co., St Louis, MO, USA) was then added on the top of the filter paper. The Celite layer was moistened with deionised water and further compacted with a glass rod to ensure the complete sealing at the bottom. Syringes were then placed in 15-mL COREX® glass centrifuge tubes (No. 8441-15, USA) and centrifuged at $4000 \times g$ for 10 min to remove the surplus of water. After the syringes were weighed (wt_0), 2.5 mL of starch sample were added to each and the syringe was weighed again (wt_1). The syringe was then centrifuged at $4000 \times g$ for 10 min before final weighing (wt_2). Free water was calculated as:

$$FW = [(wt_1 - wt_2)/(wt_1 - wt_0)] \times 100$$

2.5.4.2. 3b: Determination of EW. It consisted on weighing the water spontaneously expelled from the gels after a 20-day refrigeration cycle at 5 ± 1 °C. The value was expressed as percent of paste weight before determination of absorbed water by centrifugation.

2.5.4.3. 3c: Determination of AW. After refrigeration as above described (3b) and once expelled water was removed, the syringe plus sample was weighed (wt_1), centrifuged at $4000 \times g$ for 10 min, and reweighed (wt_2). Absorbed water in the gels was calculated by the same formula as for the free water.

Finally, net syneresis was calculated from the formula:

$$\text{Net syneresis} = (EW + AW) - FW$$

Whenever a centrifugation step was included, and according to previous optimization reports (Zheng & Sosulski, 1998), $4000 \times g$ for 10 min were the conditions adopted.

2.6. Rheology measurements

Rheological properties were assessed with a rotational viscometer with narrow gap concentric cylinders or spindles (Cannon LV 2000) equipped with a Temperature Controlled Unit TCU 1000 and a Small Sample Adapter. Measurements (for 8-mL samples) were carried out with a TL-5 spindle, at 25 °C and at shear rates between 0.396 and 79.2 1/s. Readings were taken after 2-min rotation and data herein presented are average of at least three measurements.

3. Results and discussion

3.1. Syneresis evaluation

Methods based on conventional centrifugation, determination of net syneresis (Zheng & Sosulski, 1998) and the periodic liquid phase quantification after gel contraction (Yoshimura et al., 1998) were the different techniques compared for syneresis estimation. The selected procedure was further optimized and standardized for the case under study, as described below.

A first observation indicated a significant influence of the mode of starch paste preparation on the subsequent behaviour along refrigeration. The use of mixing blades or motorized stirrers as previously described (Yoshimura et al., 1998; Zheng & Sosulski, 1998), either during starch paste cooking or mixture preparation, led to distorted and irreproducible results of syneresis.

Conversely, highly reproducible data could be obtained by means of cooking the starch paste with glass-rod manual agitation until thickening and, when appropriate, mixing it with the polysaccharide dispersion separately prepared. This finding was not unexpected since, as previously emphasized for wheat starch and xanthan mixtures, the preparation technique exerted a notable influence on the subsequent rheological behaviour. In that case, it was recommended the separate preparation of starch and polysaccharide dispersions followed by their mixing instead of the blend of powders prior to water addition (Mandala & Bayas, 2004).

The observation of syneresis according to Technique 1a, in 15-mL tubes, and by using 2% (w/v) CS (Fig. 1) showed the highest sensitivity; syneresis could be detected as early as at 2 days of refrigeration. With regard to the optimal starch system, when 2% (w/v) CS was replaced by either 3% (w/v) CS or the same concentrations of CaS, sensitivity was considerably reduced (appearance of Δh was retarded or inhibited). In addition, high $\Delta h/h_0$ values as those achieved according to Technique 1a with 2% (w/v) CS, also provided a wider margin for detecting polysaccharide effects on syneresis.

Moreover, the convenience of applying Technique 1a was related to the use of small sample volumes which did not require to be sacrificed for every measurement. Likewise, quantification of the liquid phase did not involve sophisticated materials or equipment, and results could be readily obtained. In conclusion, this methodology showed to be a reliable, highly accessible, inexpensive and labour-saving proposal.

When Technique 1 was performed with larger glass tubes (Technique 1b), as proposed in Yoshimura et al. (1998), a decrease on sensitivity was noted. Appearance of syneresis was detected just at day 5 for 2% (w/v) CS, and at day 7 for 3% (w/v) CS (Fig. 1). Consequently, and as the use of higher volumes of sample may restrict its use to the availability of large amounts of starch and polysaccharides, the convenience of using the 15-mL tube alternative was sustained.

The reduced or nil sensitivity when CaS pastes were used (Fig. 1) was not completely surprising. Existing reports have previously indicated that root and tuber starches can exhibit high stability to cold storage (Zheng & Sosulski, 1998) and, for the purposes of this work, the use of CaS pastes was ruled out.

It is important to highlight that the period of refrigeration (20 days) was selected so that our results could be compared to those reported in the literature (Yoshimura et al., 1998). In accordance, previous researchers already recommended the use of two weekly cycles of refrigeration in order to obtain reliable repetitive determinations on the stability of normal starches (Zheng & Sosulski, 1998).

Results applying conventional centrifugation after refrigeration (Technique 2) were confronted to those emerging from Technique 1a (Fig. 2). The first observation was that, by incorporating a centrifugation step, not only the water spontaneously expelled after gel contraction was quantified but also the water absorbed in the gel. This last one would be compelled to release by the application of a centrifugal force.

For the different starch systems tested (CS and CaS, at 2 and 3% (w/v)), the correlation between values of $\Delta h/h_0$ vs. water separation as percentage of gel weight without centrifugation, and between values of $\Delta h'/h_0$ vs. water separation after centrifugation was good ($r=0.9985$ and 0.9981, respectively) (Fig. 2). However, when values coming from the spontaneous separation of water were confronted to those obtained after centrifugation (both for

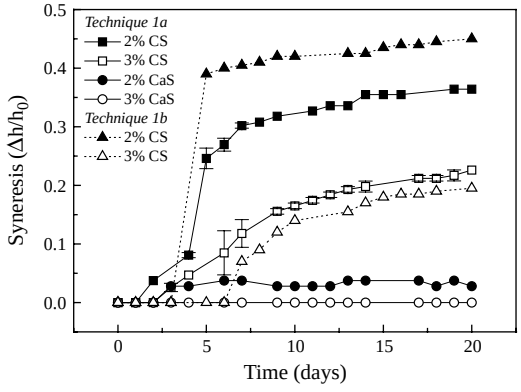

Fig. 1. Syneresis as $\Delta h/h_0$ vs. refrigeration time at 5 ± 1 °C for different starch systems according to Technique 1a (15-mL tubes) and 1b (20-cm tubes) (for details, see Section 2). Concentrations are expressed as percentage w/v. Results are the mean of at least triplicate determinations. CS: corn starch, CaS: cassava starch.

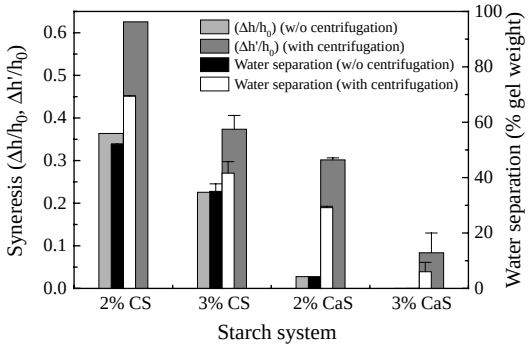

Fig. 2. Syneresis as $\Delta h/h_0$, $\Delta h'/h_0$ and water separation, after 20 days of refrigeration at 5 ± 1 °C for different starch systems according to Technique 1a (without centrifugation) and Technique 2 (by conventional centrifugation) (for details, see Section 2). Concentrations are expressed as percentage w/v. Results are the mean of at least triplicate determinations. CS: corn starch, CaS: cassava starch.

parameters $\Delta h/h_0$ vs. $\Delta h'/h_0$ and the water separation before vs. after centrifugation), no suitable correlation was found.

Based on the chemical definition of syneresis, that is "the spontaneous separation of liquid from a gel or colloidal suspension due to the contraction of the gel" (Lapedes, 1978), tests involving centrifugation have been previously criticized (Zheng & Sosulski, 1998). From the practical point of view, the use of a centrifugal force may be questioned in food systems because it would be compelling the release of water, as evidenced for 3% (w/v) CaS pastes (Fig. 2). In addition, expelled water (spontaneously released) may exhibit a different influence on the functionality and sensory properties of food from absorbed water. Consequently, Technique 1a would provide a more realistic assessment of the syneresis experienced by food products than Technique 2.

Results of net syneresis according to Technique 3 were also evaluated (Fig. 3). For the different CS concentrations tested: 2, 3 and 4% (w/v), values of FW, EW, AW and net syneresis decreased as the starch concentration increased. The slope of plotting water separation vs. starch concentration for net syneresis (slope = −8.06) was not as marked as those found for FW, EW and AW (slopes = −22.74, −17.13 and −14.61%, respectively) (Fig. 3). As a consequence, net syneresis revealed a lower sensitivity than the determination of the other estimated parameters.

From the practical point of view, it should be emphasized that when Technique 3 was applied, samples should be sacrificed for every measurement. Additionally, the three-stage determination of FW, EW and AW was really laborious and required much more material, equipment and preparation than the other tested techniques. Finally, efforts to shorten the period of refrigeration (to one weekly cycle) resulted in illogical values of net syneresis (data not shown).

Concluding, the overall evaluation of the above-described techniques indicated that quantification according to Technique 1a ($\Delta h/h_0$) was the most suitable for the assessment of polysaccharide effects on the syneresis experienced by CS pastes, as a model food system.

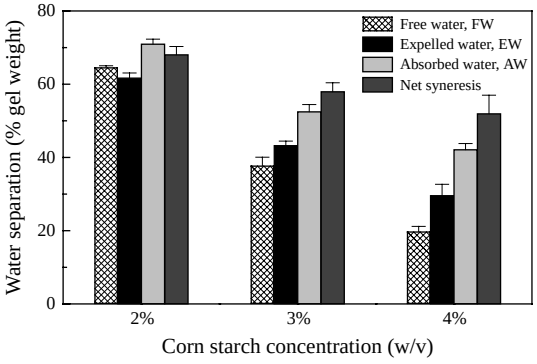

Fig. 3. Results of net syneresis according to Technique 3 for different corn starch concentrations after refrigeration at 5 ± 1 °C for 20 days (for details, see Section 2). Results are the mean of at least triplicate determinations.

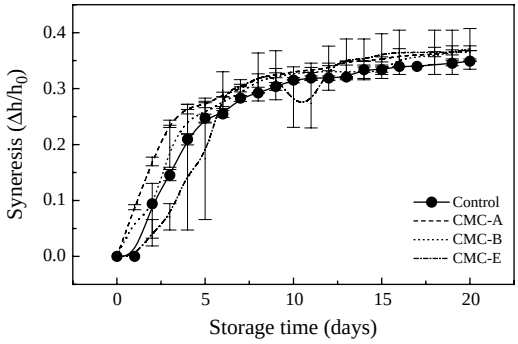

Fig. 4. Syneresis ($\Delta h/h_0$) vs. refrigeration time at 5 ± 1 °C for different blends of 2% (w/v) corn starch (CS) plus carboxymethyl cellulose (CMC) at different mixing ratios (CS/CMC, w/w): A = 9.90/0.10, B = 9.75/0.25, E = 9.00/1.00. Control: 2% (w/v) CS in water. Results are the mean of at least triplicate determinations.

3.2. Influence of polysaccharides on corn starch syneresis

It is already known that different polysaccharides may be used as food additives for either moisture retention, control of rheological properties, improving texture, limit or inhibit syneresis, slow down water diffusion or enhance other hydrocolloids performance. Positive effects may be attained at levels as low as 0.1–1% (Gimeno et al., 2004; Sadar, 2004; Sikora et al., 2003).

Results herein reported showed that CMC and pullulan were unable to retard or inhibit the extent of starch paste syneresis for the different concentrations tested (Figs. 4 and 5). All the starch/polysaccharide mixtures exhibited a similar behaviour against control starch (2% (w/v) CS).

Conversely to certain hydrocolloids which, because of their incompatibility with amylose, can adversely affect gel properties by promoting amylose gelation (Yoshimura et al., 1998), EPSs from *S. rolfsii* showed a marked ability to retard or inhibit starch retrogradation (Figs. 6 and 7). Up to 20 days of refrigeration, the highest proportion tested (E = 9.00/1.00 (w/w)) was able to inhibit syneresis completely in the case of EPS II, and significantly retard

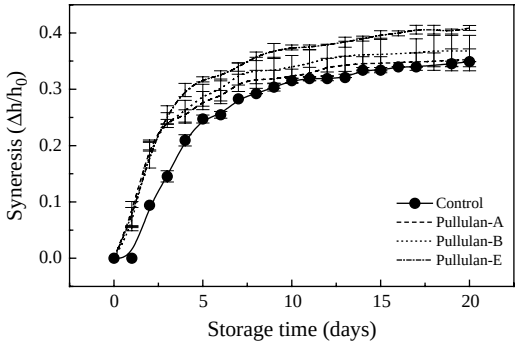

Fig. 5. Syneresis ($\Delta h/h_0$) vs. refrigeration time at 5 ± 1 °C for different blends of 2% (w/v) corn starch (CS) plus pullulan at different mixing ratios (CS/pullulan, w/w): A = 9.90/0.10, B = 9.75/0.25, E = 9.00/1.00. Control: 2% (w/v) CS in water. Results are the mean of at least triplicate determinations.

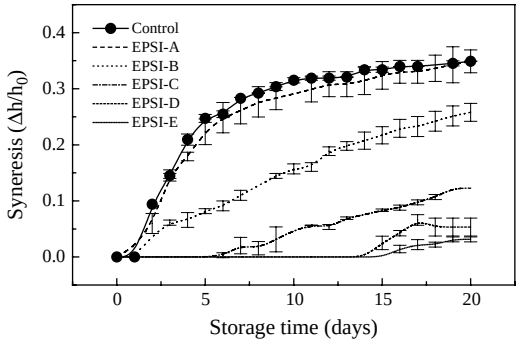

Fig. 6. Syneresis ($\Delta h/h_0$) vs. refrigeration time at 5±1 °C for different blends of 2% (w/v) corn starch (CS) plus EPS I at different mixing ratios (CS/EPS I, w/w): A=9.90/0.10, B=9.75/0.25, C=9.50/0.50, D=9.25/0.75, E=9.00/1.00. Control: 2% (w/v) CS in water. Results are the mean of at least triplicate determinations.

(for 16 days) and reduce it (91%), for EPS I. The other studied proportions showed lower capacity to retard or reduce syneresis, proportionally to the amount of EPS present in the mixtures (Figs. 6 and 7, Table 1).

Similar results were reported for konjac glucomannan which, even at small proportions, was capable to prevent CS syneresis during cold storage (Yoshimura et al., 1988). As suggested for konjac glucomannan, the ability of *S. rolfsii* EPSs may be linked to their high water-binding capacity. This property has been previously related to cell protection in low-moisture environments (Broadbent, McMahon, Welker, Oberg, & Moineau, 2003; Fariña et al., 2001). In addition, the steric hindrance caused by stiff, rod-like gum molecules may prevent the starch molecules from getting close to each other. Consequently, the more extended conformation would behave as a network with cavities penetrated by water molecules (Gimeno et al., 2004).

Finally, differences between EPS I and EPS II to prevent syneresis were not surprising as variations on their rheological behaviour were already reported. As previously suggested, conformational divergences may be implicated,

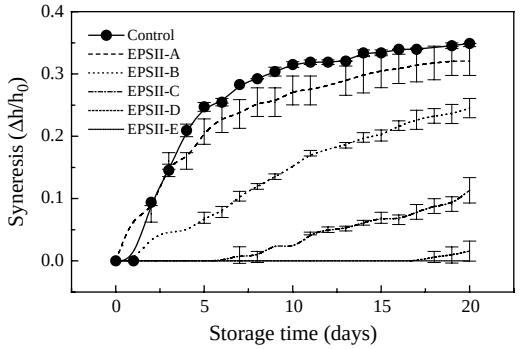

Fig. 7. Syneresis ($\Delta h/h_0$) vs. refrigeration time at 5±1 °C for different blends of 2% (w/v) corn starch (CS) plus EPS II at different mixing ratios (CS/EPS II, w/w): A=9.90/0.10, B=9.75/0.25, C=9.50/0.50, D=9.25/0.75, E=9.00/1.00. Control: 2% (w/v) CS in water. Results are the mean of at least triplicate determinations.

Table 1
Percentage of syneresis experienced by different blends of corn starch (CS) and *S. rolfsii* EPSs with respect to the syneresis of control CS in water (2%, w/v) after 20 days of refrigeration at 5±1 °C (referred as 100% syneresis)

EPS I		EPS II	
Mixture[a]	% Syneresis	Mixture[a]	% Syneresis
A=9.90/0.10	100.0 (ns)	A=9.90/0.10	91.9 (ns)
B=9.75/0.25	73.9***	B=9.75/0.25	70.3***
C=9.50/0.50	35.1***	C=9.50/0.50	32.4***
D=9.25/0.75	15.3***	D=9.25/0.75	5.0***
E=9.00/1.00	9.0***	E=9.00/1.00	0.0***

ns, value not significantly different from control ($P>0.05$). ***Value extremely different from control ($P<0.001$).
[a] Expressed as CS/EPS (w/w) proportion for a 2% (w/v) solution.

even when the primary and secondary structures were identical (Fariña et al., 2001).

3.3. Rheological behaviour of EPS, CS and CS/EPS blends in different solvents

Incorporation of hydrocolloids in dairy foods (e.g. ice cream, yogurt, sour cream) or beverages such as citrus-based drinks is a well-known strategy to provide viscosity, stability and water-holding capacity. As a consequence, mouth-feel, texture, visual and taste perception, storage stability, mechanical protection and prevention of syneresis may also become improved in the final products (Broadbent et al., 2003; Hassan, Ipsen, Janzen, & Qvist, 2003; Rankin & Brewer, 1998; Sikora et al., 2003). In addition, the high-temperature insensitivity of those added hydrocolloids may constitute an advantageous attribute at the time of heat processing or storage and distribution of foodstuffs to hot climates (Anderson, Daubert, & Farkas, 2002; Sadar, 2004).

Milk represents a complex rheological system where interaction of water, fat, proteins and lactose is facilitated. Among these interactions, for instance, the natural emulsification of fats when dispersed by a coating of casein is widely known. As already demonstrated, desirable effects can also be imparted to milk products by either the in situ production of EPS by lactic acid bacteria (Broadbent et al., 2003) or by adding commercial hydrocolloids, such as carrageenans in milk gels, flans, custards, whipped creams, etc. (Sadar, 2004).

The usefulness of polysaccharide ingredients in food products has not only been related to texture or viscosity improvement, but also to the reduction of syneresis and the achievement of suspending and stabilizing properties (Sadar, 2004; Sutherland, 2002). In the particular case of milk products, benefits would emerge not only from the inherent properties of the EPS but also, from its interaction with milk proteins that gives rise to network formation (Broadbent et al., 2003).

Whenever combinations of hydrocolloids are applied, synergies can be sought to gain additional advantages (Sadar, 2004; Sikora et al., 2003; Sutherland, 2002).

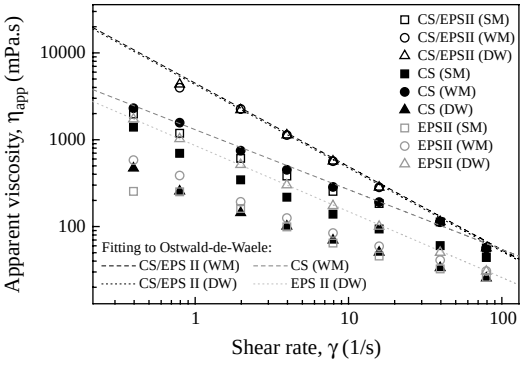

Fig. 8. Rheological behaviour of corn starch (CS, 2% w/v), EPS II (2 g/L) and CS/EPS II blends (2% w/v, 9.00/1.00 w/w ratio) in skimmed milk (SM), whole milk (WM) or distilled water (DW). Rheological characteristics of the solvents were: SM, $\eta_{app}=2.5$ mPa s, at 25 °C and 39.6 1/s; WM, $\eta_{app}=7.5$ mPa s, at 25 °C and 39.6 1/s and DW, $\eta_{app}=0.8904$ mPa s, at 25 °C. Measurements were carried out at $T=25$ °C, with a TL-5 spindle and for shear rates between 0.396 and 79.2 1/s. For the sake of clarity, only critical fittings were displayed and standard errors were not plotted.

On this basis, the occurrence of synergism between scleroglucan and CS was evaluated in the presence of different solvents of interest: skimmed milk (SM), whole milk (WM) and distilled water (DW). The rheological behaviour of these mixtures, as well as gelling, sensory properties and syneresis were assessed.

In accordance with the above results of syneresis (Fig. 7), EPS II was selected for further experiments. Considering the original rheology of the solvents, i.e. SM (apparent viscosity, $\eta_{app}=2.5$ mPa s, at 25 °C and 39.6 1/s shear rate), WM ($\eta_{app}=7.5$ mPa s, at 25 °C and 39.6 1/s shear rate) and DW ($\eta_{app}=0.8904$ mPa s, at 25 °C) (Weast & Astle, 1981), EPS II was able to increase viscosity and induce a non-Newtonian pseudoplastic behaviour in all cases (Figs. 8 and 9).

Nevertheless, the proportion of this increment was significantly different according to the solvent used. For EPS II alone, the decreasing order of viscosities was

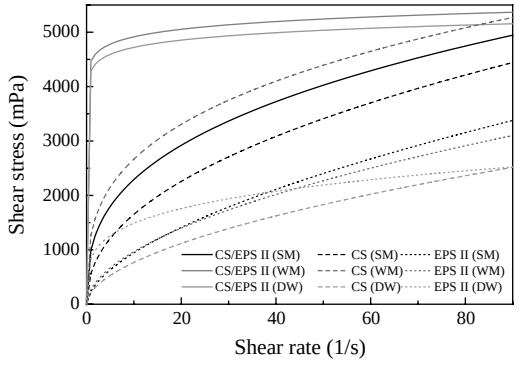

Fig. 9. Flow curves of corn starch (CS, 2% w/v), EPS II (2 g/L) and CS/EPS II blends (2% (w/v), 9.00/1.00 (w/w) ratio) in skimmed milk (SM), whole milk (WM) or distilled water (DW). Measurements were performed at $T=25$ °C, with a TL-5 spindle and for shear rates between 0.396 and 79.2 1/s.

Table 2
Rheological parameters of corn starch (CS, 2%, w/v), EPS II (2 g/L) and CS/EPS II blends (2%, w/v, 9.00/1.00 (w/w) ratio) dissolved in skimmed milk (SM), whole milk (WM) or distilled water (DW)

Composition	Ostwald-de-Waele[a]		Casson[a]	
	Consistency coefficient, K (mPa s^n)	Flow behaviour index, n (–)	Yield stress, τ_0 (mPa)	Casson's viscosity, K_c (mPa s)$^{1/2}$
CS/EPS II (SM)	1023.6±14.2	0.35±0.04	975.7±235.8	4.53±0.66
CS/EPS II (WM)	4482.4±0.8	0.04±0.02	4020.5±315.2	0.81±0.76
CS/EPS II (DW)	4305.7±26.3	0.04±0.02	4086.4±234.3	0.70±0.56
CS (SM)	586.4±8.6	0.45±0.03	430.4±18.6	4.35±0.07
CS (WM)	1306.2±19.8	0.31±0.03	1197.1±223.9	4.11±0.58
CS (DW)	221.5±3.4	0.54±0.02	159.2±16.0	3.68±0.10
EPS II (SM)	248.4±9.8	0.58±0.03	132.0±15.2	3.83±0.10
EPS II (WM)	285.5±6.3	0.53±0.02	207.2±15.5	3.98±0.08
EPS II (DW)	856.7±2.2	0.24±0.01	803.7±66.5	2.46±0.22

[a] Viscosity measurements were carried out at $T=25$ °C, with a TL-5 spindle and for shear rates between 0.396 and 79.2 1/s. Data were fitted to the rheological models of Ostwald-de-Waele and Casson.

DW > WM > SM, whilst in the case of CS was WM > SM > DW, and WM = DW > SM for CS/EPS II blends (Fig. 8). It should be noted that even the least pronounced pseudoplastic behaviour of CS/EPS II blends (i.e. in SM) led to viscosities (Fig. 8) and rheological parameters (Table 2) comparable to those characterizing CS in WM. This experiment also demonstrated the existing synergism between CS and EPS II, as evidenced by the highest viscosities attained when these two polysaccharides were mixed (Fig. 8).

The outstanding performance of EPS II was confirmed when the above results were confronted to those reported for jamilano, the EPS from *Paenibacillus jamilae* (García-Ribera et al., 2002). Viscosities of jamilano at 10 g/L were 14 mPa s in DW, 15.5 mPa s in SM and 80 mPa s in WM (values at 25 °C and 39.6 1/s shear rate) (García-Ribera et al., 2002). In the present work, a 5-fold lower EPS II concentration (2 g/L) led to viscosities of 50.3 mPa s in DW, 32.5 mPa s in SM and 41.0 mPa s in WM (at 25 °C and 39.6 1/s shear rate).

Despite the amount of polysaccharide can certainly affect milk viscosity, the functional impact of a given polysaccharide on milk products is largely determined by its molar mass, monosaccharide composition and linkage type. Further effects may arise from the interaction of EPSs with milk proteins; this will determine the viscosity of the final product (Broadbent et al., 2003). It is worth to mention that polysaccharide shape also plays an important role. Particular conformations adopted by individual polysaccharide chains make possible the association with water,

with themselves or other polymers, and this fact can affect network development and rheological behaviour (Morris, 1986).

Within a polymer network, domains with a particular folding exhibit a particular shape and this entails a particular property. That folding will be coordinated by the nature and linkage type of neighbouring sugar moieties (Kath, Lange, & Kulicke, 1999). Regular shapes, such as helical or ribbon-like, can in many circumstances line up with one another and form interchain physical bonds giving rise to network cavities where water molecules are trapped. Conversely, areas of irregular conformation cannot be associated. Depending on the nature of the junction zones, they will be more or less easily disrupted by shear forces. Destruction of that network will therefore lead to the release of water and a viscosity fall (Morris, 1986).

The stiffness of the scleroglucan linear structure resides in the self-association between individual chains to form a triple helix (Bluhm, Deslandes, Marchessault, Pérez, & Rinaudo, 1982). This event provides architectural stability to the polysaccharide and confers exceptional viscosity to its aqueous solutions (Pérez & Vergelati, 1985). Additionally, the protruding β-1,6-linked residues prevent intermolecular association beyond the triple helical stage and consequently, avoids precipitation (Bluhm et al., 1982).

At industrial level, the incorporation of additives in foodstuffs became a widespread practice to optimize flow behaviour. Apparent viscosity and the relationship between flow behaviour and shear rate are directly connected to the quality of the final food product. Principally for the food industry, selection of the best additive is essential. To this end, rheological parameters such as the consistency coefficient (K) and flow behaviour index (n) from the Power law model are particularly valuable (Gómez-Díaz & Navaza, 2002a, 2002b; Sikora et al., 2003).

For that reason, rheology of CS and EPS II was independently evaluated for the different solvents tested and then, the same procedure was applied to the polymer blends. Rheological data were presented in the form of flow curves (Fig. 9) and fitted to the Ostwald-de-Waele and Casson models. All EPS II, CS and CS/EPS II dispersions, showed different levels of non-Newtonian, pseudoplastic behaviour (Figs. 8 and 9, Table 2)

$$\text{Ostwald-de-Waele model}: \ \eta = K\gamma^{(n-1)}$$

where η represents the apparent viscosity, γ the shear rate, K the consistency coefficient and n the flow behaviour index

$$\text{Casson model}: \ \tau^{1/2} = \tau_0^{1/2} + K_c\gamma^{1/2}$$

where τ represents the shear stress, γ the shear rate, τ_0 the yield stress and K_c the Casson's viscosity.

The parameter values estimated by applying the above models confirmed the improvement on the rheological behaviour consequent on the synergism between CS and EPS II (Table 2). The most pronounced pseudoplastic behaviour was characterized by the highest values of K and τ_0, and the lowest values of n and K_c. Trends on these parameters with respect to the solvent used were similar to those observed for η_{app} measurements (see above).

Differences on the rheological behaviour as a function of the employed solvent were not easy to explain. Nevertheless, the best performance of EPS II when dissolved in water was not surprising as viscosity is seriously affected by scleroglucan solubility and this one is favoured in the presence of water (Fariña et al., 2001). On the other hand, and similarly to the behaviour previously reported for jamilano (García-Ribera et al., 2002), CS showed the highest viscosities when dissolved in whole milk. Reasonably, blends where both polysaccharides, EPS II and CS were present, behaved quite similarly in DW and WM (Figs. 8 and 9). In accordance, comparable values of η_{app}, K, n (Ostwald-de-Waele model), τ_0 and K_c (Casson model) were encountered both in DW and WM (Table 2).

The explanation for divergences on the preferred solvent may be related to the polymer nature and/or electrical charge. These features may influence not only the complex interactions between polysaccharide and the components of milk but also, the synergism with other present polymers. For instance, negatively charged gums (CMC, carrageenans, alginate, xanthan) may exhibit repelling forces against the phosphate groups on potato starch thereby causing a decrease on the pasting peak viscosity (Shi & BeMiller, 2001).

Likewise, the adverse influence of high sugar levels on polysaccharide behaviour has also been previously referred. This phenomenon may be a result of the competition for water by different sugars (mono-, di- and oligosaccharides) against hydrocolloids. High salt levels can also compete for water and may decrease viscosity if EPS hydration is compromised (Sikora et al., 2003).

In the present case, the main differences in the composition of milks related to a 7% lower content of lactose, a 30-fold higher content of fats in WM than in SM, and the presence of lecithin as emulsifier in WM. Minor discrepancies comprised calcium and protein contents. All sorts of solids contained in milks (e.g. proteins, fats, salts, sugars, etc.) may be expected to promote variations in viscosity and/or textural properties of hydrocolloid-containing systems when confronted to DW (Sikora et al., 2003).

3.4. Synergism and sensory properties of CS/EPS blends in different solvents

As above discussed, the combination of CS with EPS II not only enabled syneresis prevention but also improved the rheological properties of the mixture, in comparison to the polysaccharides alone (Figs. 8 and 9, Table 2). In addition, CS/EPS II blends exhibited gelling characteristics and adequate sensory properties (colour, aroma, texture). No significant variations were imparted to the pH of the mixture (Table 3). Similar behaviour was found in the three solvent

Table 3
Physicochemical and sensory properties of corn starch (CS, 2% (w/v)), EPS II (2 g/L) and CS/EPS II blends (2% (w/v), 9.00/1.00 (w/w) ratio) dissolved in skimmed milk (SM), whole milk (WM) or distilled water (DW)

Composition	pH	Gelling ability	Gel colour	Transparency	Syneresis $\Delta h/h_0$ (Technique 1a)
CS/EPS II (SM)	6.62	+++	White-cream	−	−
CS/EPS II (WM)	6.61	++	White-cream	−	−
CS/EPS II (DW)	5.38	+++	White	−	−
CS (SM)	6.65	−	Opaque white	−	++
CS (WM)	6.64	+	Opaque white	−	++
CS (DW)	5.07	++	White	−	+
EPS II (SM)	6.64	−	White	−	−
EPS II (WM)	6.56	−	White-cream	−	−
EPS II (DW)	6.13	−	Slightly whitish	+	−

systems tested which, in the case of milks, could be promising for the potential application in dairy products manufacture.

Comparing to the literature, 1% (w/v) jamilano/carrageenan mixtures (containing 5 g/L jamilano +4.5 g/L carrageenan), were similarly able to prevent syneresis (García-Ribera et al., 2002). However, gelling ability, viscosity, hardness and transparency of these blends were negatively affected. Apart from that, the required amount of jamilano was twice of that herein suggested for EPS II (2 g/L).

Trials between carrageenan and another widespread stabilizer, xanthan, in a concentration similar to that of EPS II, allowed increasing viscosity with no loss of gelling ability, hardness and transparency (García-Ribera et al., 2002). Despite that, syneresis could not be prevented at all and gel colour was modified from grey to cream. Unfortunately in that investigation, the evaluation of the mentioned properties in skimmed or whole milk was not considered.

Concluding, there are no rules for predicting EPS behaviour. This is a multifactorial response where EPS concentration, macromolecular characteristics, structure, charge, and solvent composition all interlace mutually. Then, it seems relevant to emphasize that the real influence of a given polysaccharide on milk or other food products needs to be assessed for each case in particular. Research advances on the use of EPSs in the food industry will boost their widespread application and may offer added value and innovation to the foodstuff.

4. Conclusions

The synergistic behaviour observed when scleroglucan was combined to starch pastes and the ability to prevent syneresis whilst gelling properties, hardness and whiteness of these blends remained, extend the possibilities for practical applications. Considering the increase in viscosity when scleroglucan was added to solvents as water, whole or skimmed milk, and the pseudoplastic behaviour imparted to the respective solutions, this biopolymer may be taken into account for applications in dairy and other food products. Further studies on the synergism and compatibility of scleroglucan with other stabilizers should be therefore encouraged. It may thus be possible that, in the light of these findings, novel areas for the utilization of EPSs from *S. rolfsii* ATCC 201126 will be considered.

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References

Anderson, A. D., Daubert, C. R., & Farkas, B. E. (2002). Rheological characterization of skim milk stabilized with carrageenan at high temperatures. *Journal of Food Science*, *67*, 649–652.

Bluhm, T. L., Deslandes, Y., Marchessault, R. H., Pérez, S., & Rinaudo, M. (1982). Solid-state and solution conformation of scleroglucan. *Carbohydrate Research*, *100*, 117–130.

Broadbent, J. R., McMahon, D. J., Welker, D. L., Oberg, C. J., & Moineau, S. (2003). Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. *Journal of Dairy Science*, *86*, 407–423.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Fariña, J. I., Siñeriz, F., Molina, O. E., & Perotti, N. I. (1996). Low-cost method for the preservation of *Sclerotium rolfsii* PROIMI F-6656: Inoculum standardization and its use in scleroglucan production. *Biotechnology Techniques*, *10*, 705–708.

Fariña, J. I., Siñeriz, F., Molina, O. E., & Perotti, N. I. (1998). High scleroglucan production by *Sclerotium rolfsii*: influence of medium composition. *Biotechnology Letters*, *20*, 825–831.

Fariña, J. I., Siñeriz, F., Molina, O. E., & Perotti, N. I. (2001). Isolation and physicochemical characterization of soluble scleroglucan from *Sclerotium rolfsii*. Rheological properties, molecular weight and conformational characteristics. *Carbohydrate Polymers*, *44*, 41–50.

García-Ribera, R., Monteoliva-Sánchez, M., & Ramos-Cormenzana, A. (2002). Study of the use of a new exopolysaccharide obtained from *Paenibacillus jamilae* in food additives. *Ars Pharmaceutica*, *43*, 149–157.

Gimeno, E., Moraru, C. I., & Kokini, J. L. (2004). Effect of xanthan gum and CMC on the structure and texture of corn flour pellets expanded by microwave heating. *Cereal Chemistry*, *81*, 100–107.

Gómez-Díaz, D., & Navaza, J. M. (2002a). Rheological characterization of aqueous solutions of the food additive carboxymethyl cellulose. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *1*(1) [ISSN: 1579-4377].

Gómez-Díaz, D., & Navaza, J. M. (2002b). Effect of the food stabilizers blends composition upon the rheological behaviour. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *1*(3) [ISSN: 1579-4377].

Hassan, A. N., Ipsen, R., Janzen, T., & Qvist, K. B. (2003). Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *Journal of Dairy Science*, *86*, 1632–1638.

Hodge, J. E., & Hofreiter, B. T. (1962). Determination of reducing sugars and carbohydrates. In R. L. Whistler, M. L. Wolfrom, J. N. BeMiller, & F. Shafizadeh, *Methods in carbohydrate chemistry* (vol. I) (pp. 380–394). New York: Academic Press, 380–394.

Kath, F., Lange, S., & Kulicke, W.-M. (1999). Influence of the glycosidic linkage on the solution conformation of glucans. *Die Angewandte Makromolekulare Chemie*, *271*, 28–36.

Lapedes, D. N. (Ed.). (1978). *Dictionary of scientific and technical terms*2nd ed.. New York: McGraw-Hill.

Mandala, I. G., & Bayas, E. (2004). Xanthan effect on swelling, solubility and viscosity of wheat starch dispersions. *Food Hydrocolloids*, *18*, 191–201.

Morris, E. R. (1986). Molecular interactions in polysaccharide gelation. *British Polymer Journal*, *18*, 14–21.

Pérez, S., & Vergelati, C. (1985). Unified representation of helical parameters: Application to polysaccharides. *Biopolymers*, *24*, 1809–1822.

Rankin, S. A., & Brewer, J. L. (1998). Color of nonfat fluid milk as affected by fermentation. *Journal of Food Science*, *63*, 178–180.

Sadar, L. N. (2004). Rheological and textural characteristics of copolymerized hydrocolloidal solutions containing curdlan gum. Master of Science Thesis, Faculty of the Graduate School of the University of Maryland, College Park, MD 20742.

Shi, W., & BeMiller, J. N. (2001). Effects of food gums on viscosity of starch suspensions during their pasting. Proceedings of the 2001 AACC annual meeting, Charlotte, NC, USA.

Sikora, M., Juszczak, L., Sady, M., & Krawontka, J. (2003). Use of starch/xanthan gum combinations as thickeners of cocoa syrups. *Nahrung/Food*, *47*, 106–113.

Sutherland, I. (2002). A sticky business. Microbial polysaccharides: Current products and future trends. *Microbiology Today*, *29*, 70–71.

Wang, Y., & McNeil, B. (1996). Scleroglucan. *Critical Reviews in Biotechnology*, *16*, 185–215.

Weast, R. C., & Astle, M. J. (1981). *The viscosity of water 0 °C to 100 °C*. 62nd ed. *CRC handbook of chemistry and physics*. Boca Raton, FL: CRC Press Inc..

Yoshimura, M., Takaya, T., & Nishinari, K. (1998). Rheological studies on mixtures of corn starch and konjac-glucomannan. *Carbohydrate Polymers*, *35*, 71–79.

Zheng, G. H., & Sosulski, F. W. (1998). Determination of water separation from cooked starch and flour pastes after refrigeration and freeze-thaw. *Journal of Food Science*, *63*, 134–139.