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Original article

Simultaneous effects of gelatin and espina corona gum on rheological, physical and sensory properties of cholesterol-reduced probiotic yoghurts

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Summary The effects of gelatin (G) and espina corona gum (ECG) on rheological, physical and sensory properties of cholesterol-reduced probiotic yoghurts were studied. The results showed that it was possible to efficiently remove the cholesterol (> 85%) and the probiotic microorganism counts were > 7 $\log_{10}$ CFU.g^{-1}. The addition of G decreased flow behaviour index (n), while consistency index (K) increased with the addition of both thickeners. Thixotropy, initial shear stress of the clot to be deformed by mechanical action (A) and destruction rate of the structure (B) were enhanced by increasing G. ECG imparted greater creaminess, less grittiness and less astringency, while G gave more consistency. Both hydrocolloids helped to reduce acid taste and increased water retention index (> 95%). The optimum formulations were: 0.49% G – 0.41% ECG to obtain set yoghurts and 0.01% G – 0.43% ECG for stirred yoghurts, with desirable sensory, rheological and stability characteristics.

Keywords Hydrocolloids, rheology, sensory, thixotropy, water retention.

Introduction

Today, there is growing demand for low or nonfat dairy products in the market. This is related to the fact that milk fat has saturated fatty acids (SFAs) and cholesterol that have been associated with coronary heart disease (CHD) (Joint FAO/WHO Expert Consultation, 2003). While not all SFAs possess identical hypercholesterolaemic potency (Parodi, 2009), the cholesterol can generate, by exposure to heat and radiation, dozens of oxidised compounds (COPs) that are atherogenic and carcinogenic (Sieber, 2005).

The partial or total removal of fat from dairy products decreases the overall quality perceived by the consumer. Thus, it is desirable to extract only cholesterol without milk fat removal. Beta-cyclodextrin (β-CD) is proposed as an alternative to extract cholesterol with a yield around 90% (Lee *et al.*, 1999). The radius of β-CD's cavity accommodates highly specifically the cholesterol molecule and the lipid composition of milk was not affected with β-CD treatment (Alonso *et al.*, 2009).

Among commercial hydrocolloids used, gelatin (G) has long been used as gelling agent and its importance lies in the fact that it forms a gel meltable in the mouth (Koksoy & Kilic, 2004). Gums and stabilisers, like guar gum (GG), are used as stabilising and thickening agents (Major *et al.*, 2011). There could be a substantial market for new gums to substitute several of these hydrocolloids for a better price and suitable functionality or to complement them and generate synergies that improve the rheological and textural properties of the products. Specifically, espina corona gum (ECG) is a neutral galactomannan extracted from the seeds of *Gleditsia amorphoides*, a leguminous native tree that grows in Latin America. Because ECG has similar mannose/galactose ratio than GG and has the same physical, chemical, mechanical and technological characteristics of locust bean gum (LBG), some authors considered that ECG could replace them (Maiochi, 2006). ECG has approximately 70% carbohydrates in its composition and forms highly viscous dispersions in water. It was approved in Argentina to be used like thickener/stabiliser agent (Spotti *et al.*, 2012).

Knowing the instrumental parameters and sensory textural attributes provides important information for having a better understanding of the underlying processes during consumer's perception (Krzeminski *et al.*, 2013).

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Moreover, the presence of probiotic microorganisms has been traditionally associated with certain health benefits like hypocholesterolaemic potential (Ejtahed *et al.*, 2011), reduction in lactose intolerance (Ibarra *et al.*, 2012), among others. However, several factors have been reported to affect the viability of probiotic cultures in yoghurts during cold storage (Cruz *et al.*, 2012), and it is desirable to maintain viable probiotic cells above the minimum therapeutic level.

The aim of this work was to study the effects of the addition of gelatin and espina corona gum on rheological, physical and sensory properties of cholesterol-reduced probiotic yoghurts during storage. A multiple response optimisation was studied too.

Materials and methods

Experimental design

The experiments were based on a three-level, two-factor factorial design. The independent variables were concentration of gelatin (X_1 =% G = 0–0.6% w/w) and espina corona gum (X_2 =% ECG = 0–0.5% w/w). These values were selected based on industry data and preliminary tests. The complete design, along with coded and uncoded variables, is given in Table S1.

Cholesterol-reduced probiotic yoghurt manufacture

Whole milk was prepared by dissolving 13% (w/v) of milk powder (Milkaut, Santa Fe, Argentina) in distilled water, then pasteurised (75 °C – 5 min) and homogenised at 150 bar in a two-stage homogeniser ST2 (120 bar first stage, 30 bar second stage) (SIMES S.A., Argentina). Milk was cooled at 10 °C and commercial β-CD (1% w/w) (Kleptose®, Roquette, France) was added and mixed for 20 min at 100 rpm. The β-CD–cholesterol complex was removed by centrifugation (Mistral 4 L, MSE, Crawley, UK) (Lee *et al.*, 1999). 10% w/w of sucrose (Ledesma SAAI, Jujuy, Argentina), 3% w/w of WPC35 (Milkaut S.A., Argentina) and 0.5% w/w of cassava-modified starch (Glutal S.A., Argentina) were added at 50 °C into supernatant fraction containing cholesterol-reduced milk. Also, espina corona gum (Nature Gum, Buenos Aires, Argentina) and gelatin (Bloom 223 g, PB Leiner, Santo Tomé, Argentina), in amounts depending on the factorial design, were incorporated (Table S1). The mixture was heated at 90 °C for 5 min. After cooling until fermentation temperature (40 ± 1 °C), calcium chloride and potassium sorbate (Ciccarelli, San Lorenzo, Argentina) were added. Additionally, $2.5.10^{-3\%}$ w/v of lyophilised yoghurt starter (YC-180 Chr. Hansen, Denmark) and 0.015% w/v probiotic culture (*Lactobacillus rhamnosus* SP1, Sacco S.R.L, Italy) were inoculated. At pH = 4.8, the fermentation was stopped. The final product was homogenised (100 bar), fast cooled and flavoured with vanilla (Givaudan, Buenos Aires, Argentina). The probiotic yoghurt samples were stored at 4 °C for 25 days.

Extraction and determination of cholesterol

For the extraction of cholesterol, four samples of milk (before and after the treatment with β-CD) were saponified. After that, cholesterol was extracted as described by Lee *et al.*, 1999. Cholesterol quantification was made by an enzymatic method using a commercial test kit (Colesterol Enzimático, Soc. de Bioq. de Santa. Fe, Argentina). The absorbance was measured at 510 nm with an UV-VIS spectrophotometer (Milton Roy, Ivyland, PA, USA), against the reagent blank and with an internal standard.

Rheological measurements

The rheological properties of the probiotic yoghurts were measured using a concentric cylinder rheometer Haake RV2, with MVII-sensor system and cells of 50 and 500 N.cm (Haake Mess-Tecknik, Vreden, Germany). All assays were performed in duplicate under controlled temperature (10 ± 0.5 °C) at 3 and 25 days after fermentation.

Shear stress vs. shear rate

Shear stress values were recorded with increasing and decreasing of the shear rate from 0–200 s^{-1} and 200–0 s^{-1}. Data from the upper and lower curves of the shear cycle were fitted to modified Casson's (eqn 1) and Herschel–Bulkley's (eqn 2) models to provide values of consistency index (K) and flow behaviour index (n),

$$\sigma^{0.5} = \sigma_o^{0.5} + K_c.\dot{\gamma}^{0.5} \quad (1)$$

$$\sigma = \sigma_o + K.\dot{\gamma}^n \quad (2)$$

where K_c is the consistency index (Pa.s) for modified Casson model, σ_o is the yield stress (Pa), σ is the shear stress (Pa), and $\dot{\gamma}$ is the shear rate (s^{-1}).

The thixotropic behaviour was evaluated by calculating the hysteresis loop area between the upward and downward flow curves (TI = thixotropy index, Pa.s^{-1}).

Shear stress vs. shear time

Time-dependent rheological properties were investigated by shearing the sample at a constant shear rate ($\dot{\gamma}$ = 50 s^{-1}, used as shear rate that reflects the best correlation to describe sensorial perception) and shear stress (σ) was measured as a function of shear time (t), until an equilibrium state was reached (about 12 min). The experimental data were evaluated by fitting it with Weltman's model (eqn 3), where A (Pa) is the initial shear stress and B (Pa) the coefficient of thixotropic breakdown.

$$\sigma = A - B.\ ln\ t \quad (3)$$

Sensory evaluation

The samples were evaluated by a trained sensory panel of ten assessors (eight women and two men from 25 to 45 years old), all of who had used quantitative descriptive analysis (ISO, 1993) on regular basis over the past 2 years. The panel was calibrated in the use of the chosen attributes during six training sessions. During these sessions, panellists discussed and agreed upon the definitions (Table S2) and how to qualify the attributes on the scale using commercial yoghurts and following the recommendations of the International Dairy Federation (IDF Standard, 1997). Texture, taste and flavour descriptors were evaluated on a 10-cm unstructured line scale anchored with appropriate terms at the left and right ends. For texture attributes, the anchor points were: 1 = 'almost nothing', 9 = 'a lot' and for taste and flavour properties: 1 = 'barely', 9 = 'extremely' perceptible. Test samples, identified by a three-digit code, were presented to the panellists in a randomised order and at 10 °C after 3 and 25 days of storage. During tasting, the panel scored, in each unstructured scale, the perceived intensity of the descriptors. Then, the intensities of each attribute were measured in each scale, in order to assign a value for statistical analysis. The mean value of the scores between the panellists of each attribute was used for multiple regression analysis. All tests were conducted in duplicate and in a standardised room (ISO-8589, 1988).

Water retention index by centrifugation and siphon methods

A cup of fifty grams of each sample was centrifuged (Mistral 4L, MSE, Crawly, UK) at 500 g for 20 min at 4 °C, and the released whey was removed and the sample was reweighed. WRI_c was expressed as indicated in eqn 4.

$$WRI = 100 - [(w1 - w2)/w1].100, \quad (4)$$

where $w1$ (g) and $w2$ (g) are the weights before and after centrifugation process.

On the other hand, a cup of 50 g of each sample was taken from the cool room (4 °C), weighed and kept at an angle of approximately 45 °C to allow whey collection at the side of the cup. A needle connected to a syringe was used to siphon the whey from surface of the sample, and the cup of yoghurt was weighed again (Amatayakul *et al*., 2006). WRI_s was expressed as indicated in eqn 4.

Microbiological analysis

Enumeration of *L. rhamnosus* SP1 was made in triplicate after 3 and 25 days of storage at 5 °C. Colony counts were carried out using pour plate technique on MRS agar (Biokar Diagnostics, Beauvais, France) added with 0.15% w/v bile salts (Sigma-Aldrich, St. Louis, MO, USA) as described previously by Vinderola & Reinheimer (2000). Decimal dilutions of samples were transferred to Petri plates with already solidified media and incubated at 37 °C for 72 h in aerobic conditions.

Statistical analysis

The response surface methodology (RSM) was applied to the experimental data using Design expert 7.0 (Statease Inc., Minneapolis, MN, USA) for statistical analysis. The adequacy of the models was checked accounting for R^2, adjusted-R^2, F-value and the lack of fit (LOF). Numerical optimisation technique was used for simultaneous optimisation of the multiple responses.

Results and discussion

Cholesterol quantification

The cholesterol content before and after β-CD treatment was 14.391 ± 1.063 mg% and 1.971 ± 0.539 mg %, respectively. The average percentage of cholesterol extraction was 86.405 ± 2.736%.

Rheological properties

Table S1 summarises the average values for upper and lower flow curves (K_u, K_l, n_u, n_l), TI, A and B parameters for each experience, at 3 and 25 days of storage at 5 °C. The estimated regression coefficients are shown in Table S3. Suitable polynomial models were obtained (generally, R^2, adj-R^2 > 0.90) to predict the relationship of the parameters with coded variables and the lack of fit test was nonsignificant ($P > 0.05$) in all cases.

Flow curves and thixotropy

The flow curves of shear stress variation with shear rate showed that all yoghurt samples presented non-Newtonian shear thixotropic behaviour ($n < 1$), indicating the viscosity dependence on shear rate. As expected, K_u increases with increasing hydrocolloid dosage resulting in a firmer coagulum with more consistency and viscosity. Gelatin (G) was the main factor affecting K_u values, exerting a positive effect on this parameter, while, for n_u, this effect was negative, as revealed by the respective coefficients of the linear terms of the equations (Table S3). A positive effect was observed with ECG in both parameters. Generally, K_u values increased < 10% in most samples during storage, while n_u decreased. The same effect of G and ECG over K_u and n_u was observed at the final shelf life (Fig. S2a and d). According to Tamime and Robinson (2000), it is a typical physical change of yoghurt in refrigerated storage, improving the viscosity and consistency as a consequence of hydration of the macromolecules and added stabilisers. It was reported that the addition of G to the yoghurt forms flat sheets or surfaces which interacted with the casein matrix, enclosing granules of casein in several zones (Fiszman *et al.*, 1999).

Hysteresis area (*TI*) was larger for those samples manufactured with the addition of both thickeners (G mainly), increasing an average of 6 times the area of sample 5 (maximum levels of G and ECG) compared with the sample 2 (without G and ECG) at 3 days (Table S1). These differences between the areas were more noticeable at 25 days (Fig. S2c) probably due to noncovalent bonds formation that promotes thixotropy. The prediction models (Table S3) obtained for *TI* at 3 and 25 days supported this observation. Thus, yoghurt samples with a higher content of both hydrocolloids (e.g. sample 5) were more susceptible to structural breakdown due to the application of a shear stress (this should be taken into account during industrial processes).

Time-dependent flow behaviour

All samples showed thixotropic behaviour, because apparent viscosity decreased gradually when samples were subjected to a constant shear rate (50 s^{-1}), imitating as it would be structural degradation in mouth during yoghurt consumption. The samples with the highest G level (0.6%w/w) showed the highest *A* and *B* values and these values tended to increase during storage (Table S1). ECG influenced positively too but with less significance. Hence, those samples with more G had a stronger resistance to start up of shear (> *A*) and were more susceptible to structure loss during shearing (> *B*) (Table S3 and Fig. S2e and b, respectively). These results are in agreement with those reported by Basak & Ramaswamy (1994), Fiszman *et al.* (1999), among others. ECG and G are hydrophilic hydrocolloids, which increase significantly the system viscosity and improve texture. ECG probably acts as filler between milk proteins network, establishing hydrogen bonds, principally, because it is a neutral polysaccharide (Spotti *et al.*, 2012).

From an academic point of view, rheological tests provide useful information for understanding the application of appropriate models in this type of product and how affects hydrocolloids the rheological parameters studied. From an industrial point of view, yoghurt rheological characterisation is required for quality control, design and evaluation of processing equipment, unit operations and process parameters, elucidation of the relationship between structure and textural properties and ensures consumer acceptability.

Sensory analysis

The average data for all sensory descriptors are summarised in Table S1 and the regression coefficients of mathematical models in Table S3 for 3 and 25 days of storage. Nonmathematical models were found for *creamy* (*Cre*) and *sweet* (*Sw*) taste.

For texture attributes, *sensorial consistency* (*SCo*) was the attribute that presented most variation in scores between samples after 3 days of storage. The highest values were obtained for those samples that had greatest amount of G (samples 3, 5 and 9). The minimal value was to sample 2 (without G and ECG). In multiple regression equations (Table S3), it was observed the direct influence of both hydrocolloids, with G having the strongest effect in the response. *Creaminess* (*Cre*) scores also presented variation between samples and the same positive effect for both factors (Table S3). Quadratic term of G defined the curvature of the surface response, allowing better model performance. *Grittiness* (*Gri*) and *astringency* (*Ast*) scores were lower than 3, indicating that these attributes were not found in high intensity. Additionally, more ECG content decreased *Gri* and *Ast* (Table S3). After 25 days, most texture descriptors presented similar behaviour and the same dependence for variables than at 3 days (Fig. S3a, b, d and e). Generally, the perception was increased at the end of shelf life, which was reflected in higher average values. *Cre* is essential to many dairy products as it relates positively to product liking. Consumers seem to consider a ‘creamy’ product when it has a high fat content, dairy flavour and a viscous, slippery, greasy and mouth-coating texture (Johansen *et al.*, 2008). Yoghurt with high degree of *Gri* and *Ast* is not accepted by consumers; hence, it is important that the use of hydrocolloids results in desirable changes in terms of texture or mouthfeel (Gallardo-Escamilla *et al.*, 2007). In our work, the increase in scores of texture attributes during storage depends on the water-holding capacity of proteins added to probiotic yoghurts (like WPC and thickeners agents) as it was described by Akalin *et al.* (2012) and Marafon *et al.* (2011).

The *acid* taste (*Ac*) was less detected in those samples with more G and ECG levels at 3 and 25 days (Table S3 and Fig. S3c), possibly due to damping capacity of these additives. As expected, the perception of this attribute increased at the end of storage time, because of postacidification process.

Creamy taste (*Cr*) was significantly higher in samples with more ECG content. Galactomannans, like ECG, are used in low-fat formulations as they act as thickening agents for lubricity and flow control, which helps to give perceived fat-like properties (e.g. creamy mouthfeel). In case of gelatin, it traps fat globules and promotes *Cr* perception during consumption (Roller & Jones, 1996).

Sweet taste (*Sw*) was perceived at similar intensity ('slightly' and 'moderately' perceptible) in the different samples. The addition of sucrose and vanilla flavouring in adequate quantities helps to enhance sweet taste in the samples.

Generally, '*cooked milk*' (*CM*) and *rancid* (*Ra*) flavours were not detected, the exceptions were samples 8 and 6, respectively, but the intensity was 'little perceptible' in both cases (data not shown).

Water retention index (WRI)

Comparing both methods, spontaneous WRI (*WRIs*) represents real values for those products present at the market, while centrifugation forces (*WRIc*) affect the structure of the yoghurt, so represents the syneresis of broken curd. *WRIs* was 100% after 3 days of storage, but a slight decrease in this parameter was observed at the end of shelf life in many samples. However, expulsion of aqueous phase induced by centrifugation, generally, was more evident (< *WRIc*) in contrast to *WRIs* at the two periods of study (Table S1). Increased syneresis with storage time is usually associated with casein network rearrangements that promote whey expulsion (Amatayakul *et al.*, 2006). In both determinations, *WRI* decreased when the hydrocolloid concentration decreased too. At 25 days of storage, a mathematical model was found for *WRIc*, showing that a more content of ECG and G increases *WRIc* (Table S3). Hydrocolloids and whey proteins (like WPC35) have high water-binding capacity and may act synergistically in retaining water in the gel structure (Fiszman *et al.*, 1999). So, the addition of ECG and G achieved greater stability of the clot, which is a relevant quality parameter because whey separation is an undesirable defect in dairy products.

Survival of probiotic microorganisms during storage

In our work, average counts of *L. rhamnosus* SP1 were 7.89 ± 0.19 $\log_{10}$ CFU.g^{-1} (at day 3) and 7.66 ± 0.12 $\log_{10}$ CFU.g^{-1} (at day 25), ensuring the minimum dose required in the product before consumption (Vinderola & Reinheimer, 2000). These results were comparable with other studies for yoghurts (Cruz *et al.*, 2012) and other dairy products like fresh cheeses (Gomes *et al.*, 2011) and dehydrated desserts (Trujillo-de Santiago *et al.*, 2012). In the last two products, the probiotic counts were significantly reduced by industrial processes such as whey drainage or drying processes, respectively, which increases the production costs. Additionally, the number of probiotic bacteria per portion is higher in the case of yoghurts and its consumption is more pleasant (Gomes *et al.*, 2011). This reinforces the fact that yoghurts remain the most popular vehicles for delivery of probiotics. Loss of viability of probiotic bacteria in yoghurts during storage was reported in many publications, due to the reduction in pH during postacidification (Shah, 2000).

Multiple responses optimisation

To obtain different formulation for set- and stirred-type yoghurts, the numerical optimisation test (through desirability function) was used. The desired goals for each factor and responses were chosen at 25 days (to guarantee the sensory and stability of the product at end of shelf life). In general, it was desirable to achieve adequate *Cre* (score more than 5), low *Gri* and *Ast* (scores less than 2), adequate *Ac* taste (score 3–5), higher *WRI* and the rheological parameters varying in accordance for the type of yoghurt. Some authors describe that too high concentration of thickeners can impair the palatability of a natural yoghurt gel (Supavititpatana *et al.*, 2008), and because of that, it was decided to minimise G and ECG concentration. Thus, the optimum formulation was 0.49% G and 0.41% ECG to obtain set-type yoghurt with desirable sensory and stability characteristics. On the other hand, the addition of 0.01% G and 0.43% ECG were the predicted values for stirred-type yoghurt (Table S4).

Conclusions

In the present study, it was achieved to develop a functional dairy product with adequate rheological, physical and sensory characteristics during shelf life. Good extraction levels of cholesterol were obtained and probiotic bacteria counts were maintained at recommended levels. The mathematical models obtained here helped to elucidate that G is the most influential variable on rheological parameters, while ECG on sensory descriptors, features that were preserved during storage, ensuring texture stability and sensory quality of the products. Susceptibility to syneresis is inversely related to the amount of stabilisers added. The desirability function method was adequate to find the optimum conditions to obtain stirred and set types of yoghurts. Our results are useful from an economical, textural and sensory point of view for dairy industry, because ECG has the potential of becoming an important commercial gum as a novel source of food hydrocolloid, with priced fairly minor. Also, consumer's acceptability studies would be necessary to complete the product development.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Variation (mean scores) of descriptors: (a) sensorial consistency, (b) creaminess, (c) grittiness, (d) astringency, (e) acid taste, (f) creamy taste and (g) sweet taste at 3 (grey) and 25 (black) days of storage for the experimental design experiences.

Figure S2. Response surface and contour plots for: (a) = consistency index for upper curve (K_u), (b) = coefficient of thixotropic breakdown (B), (c) = thixotropy index (TI), (d) = flow behaviour index for upper curve (n_u) and (e) = resistance to initial shear stress (A) as a function of gelatin (G) and espina corona gum (ECG) concentration at 25 days of cold storage.

Figure S3. Response surface and contour plots for: (a) = sensory consistency (SCo), (b) = astringency (Ast), (c) = acid taste (Ac), (d) = creaminess (Cre) and (e) = grittiness (Gri) as a function of gelatin (G) and espina corona gum (ECG) concentration at 25 days of cold storage.

Table S1. Experimental design in coded and uncoded variables and experimental data for all responses during shelf life.

Table S2. Sensory descriptors and definitions.

Table S3. Regression coefficients of the second-order polynomial model for the response variables (analysis has been performed using coded variables).

Table S4. Optimisation criteria for different factors and responses and solutions for optimum conditions to obtain stirred- and set-type yoghurts.