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Addition of gelatin enhanced gelation of corn–milk yogurt

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Abstract

Corn–milk yogurt set by a combination of sodium caseinate plus gelatin at concentrations of 0, 0.2, 0.4 and 0.6% (w/v) were studied. The quality of the gels was determined by measurement of acidity, syneresis, texture profile analysis, viscoelasticity, structure scanning electron microscope and microbiology. Texture profile analysis (TPA) showed that increasing levels of gelatin increased hardness, adhesiveness and springiness as well as the acidity of the products. Viscoelastic behaviour displayed similar trends to the TPA characteristics, the storage modulus was less frequency dependent than the loss modulus giving a loss tangent of 0.2 in the high gelatin systems, which might indicate a true gel system. The microstructure was dense and spongy-like with small air cells, in particular, those having a high concentration of gelatin (0.6%, w/v) gave a very firm structure which might impair palatability. The addition of a commercial gelatin at 0.4% (w/v) gave good acceptability for the product (little syneresis of the gels produced). While the gelatin used for this study had a bloom value of 246 g the authors acknowledge that a different commercial gelatin may well result in a different concentration being required.

Keywords: Corn–milk yogurt; Gelatin; Texture profile analysis; Viscoelastic property; Microstructure

1. Introduction

Yogurt is principally made from cow's milk by the protocooperative action of two homofermentative bacteria *Lactobacillus delbrüeckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (De Brabandere & De Baerdemaeker, 1999; Lourens-Hattingh & Viljoen, 2001; Lucey, 2002; Tamime & Robinson, 1999). However, there have been attempts to make this type of product from a variety of food resources, including soy milk, grape juice, a combination of mango pulp–soy milk and buffalo milk (Granata & Morr, 1996; Kumar & Mishra, 2004; Öztürk & Öner, 1999). In this study, corn milk was used as the raw material for yogurt making, which could lay a claim to health benefits and could exploit the potential market for corn milk.

Sweet corn (*Zea mays Saccharata*) and corn milk which is noted for its aroma and sweet taste, is classified in the Gramineae family (Pulham, 1997). Extracted corn milk is extensively manufactured in Thailand and processed using such techniques as pasteurization and sterilization. This type of drink is very popular, especially among health conscious consumers, since it has nutritional benefits over other types of vegetable drink, and is high in vitamin content, including 24 IU of vitamin A, 0.020 mg of vitamin B_1, 0.030 mg of vitamin B_2, 0.020 mg of vitamin B_6, 3.7 mg of vitamin C and 0.520 mg of niacin in every 100 g of corn milk, it is also low in saturated fat and cholesterol (USDA, 2004).

Texture is a prime characteristic of yogurt quality and the addition of a stabilizer, functioning as a gelling agent or thickener, such as gelatin or other hydrocolloids has been shown to provide good stability and desirable texture (Duboc & Mollet, 2001; Kumar & Mishra, 2004; Sodini, Remeuf, Haddad, & Corrieu, 2004), since they impart good resistance to syneresis and a smooth sensation in the mouth

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(Amatayakul, Halmos, Sherkat, & Shah, 2006; El-Sayed, El-Gawad, Murad, & Salah, 2002; Fiszman, Lluch, & Salvador, 1999; Lal, O'Connor, & Eyres, 2006), by binding with water to reduce water flow in the matrix space. Some may interact with protein in the food matrix and as a result, further increase hydration behaviour (Duboc & Mollet, 2001; Tamime & Robinson, 1999). Several workers have investigated animal-milk or soy-milk yogurts, but little work has been done on corn–milk yogurt. Therefore, this study was aimed to develop a corn–milk yogurt, set by a combination of gelatin and sodium caseinate, as a value added corn–milk product.

2. Materials and methods

2.1. Preparation of sweet-corn milk

The variety of sweet corn (ATS-5), harvested on the 23rd day after silking of the corn plant was purchased from Thaweesak Sweet Corn Group, Chiang Mai province, Thailand. To prepare corn milk, the sweet corn was husked, taken off the silk and cleaned. Seeds of the cleaned corn were shaved off from its cob. Milk from the separated seeds was extracted using a fruit extractor (Moulinex, Spain), and filtered through a clean muslin cloth to remove residual hull particles.

2.2. Stock culture

Freeze-dried 894 ATCC 19258 *S. thermophilus* and 892 ATCC 11842 *L. bulgaricus* (Thailand Institute of Scientific and Technological Research, Thailand) were grown in M17 broth (Merck, Germany) and MRS medium (Merck, Germany), respectively, at 37 °C for 18 h. A loop of each microorganism grown was transferred into 10 ml of litmus milk, a mixture of 16% (w/v) skim milk powder (Mission, Thailand), 2 ml of 1% (w/v) litmus concentration (BDH, England), 0.3% (w/v) yeast extract (Difco, USA) dissolved in distilled water. Sufficient calcium carbonate (Merck, Germany) was added to cover the bottom of the test tube which was then sterilized at 121 °C for 15 min and subsequently cooled to 37 °C. The inoculum was incubated at 37 °C for 18 h and stored at 5 °C (Sankhavadhana, 2001).

2.3. Mother culture

Mother culture was freshly prepared before the experiment, by inoculating a loop of stock culture in 100 ml of sterilized milk medium, which contained 16% (w/v) skim milk powder, and 0.1% (w/v) yeast extract subsequently sterilized at 121 °C. The inoculated culture was incubated at 37 °C for 18 h and kept at 5 °C until use (Sankhavadhana, 2001).

2.4. Corn–milk yogurt

Distilled water was added to the extracted corn milk in a ratio of 1:2, corn milk to distilled water, then mixed with 2% (w/v) lactose (Fonterra, New Zealand) and 4% (w/v) sodium caseinate (BBA, France) which contained 95.14 ± 0.28 mg calcium per 100 g. Subsequently, gelatin (Bloom 246 g, Gelita, New Zealand) was added to the mixture, at concentrations of 0, 0.2, 0.4 and 0.6% (w/v). The mixture was heated at 90 °C for 5 min, following by heating at 95 °C for 5 min (Raphaelides & Gioldasi, 2005) then cooled to 40 °C. Consequently 2% (v/v) of yogurt starter culture composed of *S. thermophilus* and *L. bulgaricus* at a ratio of 1:1 was inoculated. The inoculum was poured into 100 ml sterilized plastic cups and incubated at 40 °C until pH 4.4–4.6 was reached.

2.5. Chemical analysis

Chemical analysis for corn milk was performed following AOAC (2000) included No. 991.20 for protein content, No. 905.02 for fat content, No. 945.46 for ash measurement, No. 990.20 for moisture content. The Lane and Eynon method was used to determine the quantity of reducing sugar, invert sugar and sucrose (James, 1995).

2.6. Microbiological determination

For viable numbers of starter culture in the products, *S. thermophilus* was enumerated using M17 agar acidified to pH 6.8 by 1 M HCl (IDF, 1997) and subsequently incubated at 37 ± 1 °C for 48 h under aerobic condition. The number of *L. bulgaricus* was determined using MRS agar acidified to pH 5.4 with 100% glacial acetic acid then incubated at 37 ± 1 °C for 72 h under anaerobic condition (IDF, 1997).

2.7. Analysis of acidity and syneresis

Titratable acidity of the corn–milk yogurt was measured according to AOAC methods No. 947.05 (AOAC, 2000). The determination of syneresis was carried out 24 h after the completed fermentation (Wu, Hulbert, & Mount, 2001). The analysis was achieved using Whatman filter paper (number 1) on a Buchner funnel, on which was spread 20 g of yogurt as thin layer to cover the surface of the filter paper. The funnel was inserted into an Erlenmeyer flask connected to a vacuum pump and the yogurt was filtered under vacuum for 10 min. The weight of filtrate divided by the initial weight × 100 gave the percentage syneresis.

2.8. Texture profile analysis

Texture profile analysis (TPA) was carried out using a TA-XT Plus (Stable Micro Systems, UK) with a 5 kg load cell. Compression measurements were carried out using a 35 mm diameter cylinder probe to apply a 30% constant strain through a 100 ml cup-set yogurt. A speed of 0.5 mm s^{-1} was used during the pre-test, compression and relaxation of the specimens (Kumar & Mishra, 2004). The measurements were carried out at 10 ± 0.5 °C on three replicates.

2.9. Microstructure

Samples of corn–milk yogurt (0.3 g) were cut approximately 1 cm below the surface and mixed with 0.3 g of 3% aqueous agar solution at 45 °C (Oxoid cod L13). The mixtures were solidified by cooling at 20 °C, subsequently cut into 1 mm cubes and fixed with 2.5% glutaraldehyde solution in phosphate buffer (0.1 M, pH 7.3). The fixed samples were washed with phosphate buffer, and postfixed with 1% osmium tetraoxide solution in phosphate buffer. The postfixed samples were washed with phosphate buffer and dehydrated in a graded ethanol series (15%, 30%, 50%, 70%, 80%, 95% and 100%). Dehydrated samples were dried with a Pelco CPD-2-critical point drier (Ted Palla Co., Redding, CA, USA). Dried sections were sliced and mounted on aluminum SEM stubs for gold coating with a Fine Coat Jeol-JFC-1100 (Jeol Ltd., Akishima, Japan). The microstructure of the samples was examined by a scanning electron microscope Jeol JSM-5910LV (Jeol Ltd., Tokyo, Japan), at a magnification of 5500× (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004).

2.10. Rheological measurement

Oscillatory testing were performed using a control stress Rheometer AR 2000 (TA Instruments-Waters, Inc., New Castle, DE USA), using a 60 mm 2° steel cone and plate system (truncation 54 μm, TA Instruments Ltd.) with a sample gap of 900–1000 μm. (Afonso & Maia, 1999). Excess sample was trimmed off prior to the analysis. Initially, a strain sweep, at a constant frequency of 1 Hz, with a displacement ranging from 1×10^{-4} to 0.1 rad, was carried out, so that the linear viscoelastic region of the samples could be determined. A constant displacement of 0.002 rad was selected (Fig. 1) for the frequency sweeps which were performed at 0.01–1 Hz. The measuring system was thermostatted at 10 °C, all experiments were carried out in triplicate.

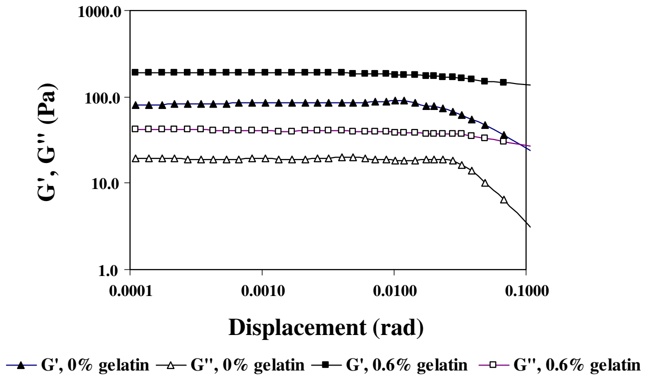

Fig. 1. Strain sweep at constant frequency of 1 Hz of corn–milk yogurt with 0.6% (w/v) added gelatin compared with the control (no added gelatin).

2.11. Statistical analyses

Statistical analysis was performed using a SPSS program version 10.0.1. All data were determined for an analysis of variance using a completely randomized design. If the F value from the analysis of variance was significant, a least significant difference (LSD) was then used to determine differences among treatment means.

3. Results and discussion

3.1. Chemical composition of corn milk

The chemical compositions of corn milk are as follows, $2.56 \pm 0.25\%$ (w/w) protein, $1.05 \pm 0.12\%$ (w/w) fat, $0.63 \pm 0.06\%$ (w/w) ash, $82.06 \pm 0.03\%$ (w/w) moisture, $3.78 \pm 0.10\%$ (w/w) reducing sugar, $4.19 \pm 1.11\%$ (w/w) sucrose and $8.18 \pm 1.07\%$ (w/w) invert sugar.

3.2. Starter culture counts and total acidity

The amounts of starter culture grown in the corn–milk yogurt were in the range 11.70–11.88 log cfu/ml and 9.13–9.50 log cfu/ml for *S. thermophilus* and *L. bulgaricus*, respectively, for the various levels of added gelatin. In Fig. 2, it can be seen that an increase in the concentration of gelatin, significantly increased product acidity ($P < 0.05$) although at gelatin concentrations of 0.4% and 0.6% (w/v), the acidities were similar. These results agree with those of Kumar and Mishra (2004) who found that the acidity of a mango soymilk set yogurt increased with increasing levels of added gelatin.

3.3. Syneresis

Syneresis of the corn–milk yogurt was affected by the addition of gelatin as shown in Fig. 3. Increased levels of gelatin significantly reduced the extent of syneresis ($P < 0.05$). This might be due to effective immobilization of the aqueous phase by the gelatin in the yogurt network

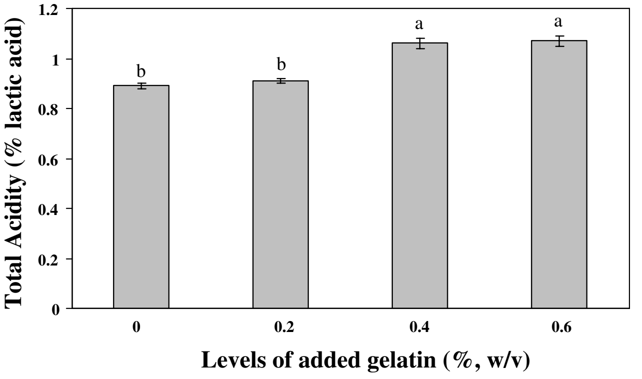

Fig. 2. Total acidity of corn–milk yogurt with different concentrations of gelatin addition.

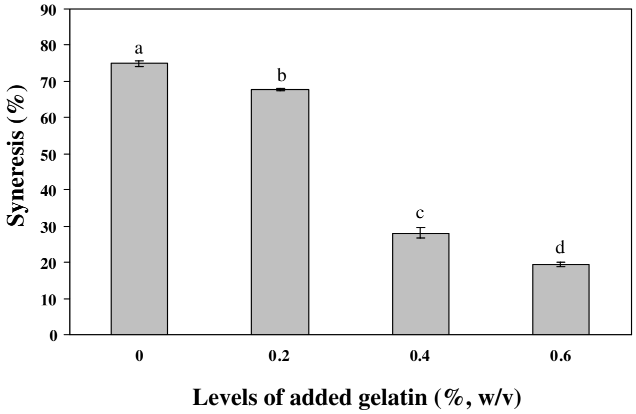

Fig. 3. Syneresis of corn–milk yogurt with different concentrations of gelatin addition.

which thus significantly reduced the susceptibility to syneresis (Fiszman et al., 1999; Keogh & O'Kennedy, 1998; Modler & Kalab, 1983). It is worth noting that large amounts of gelatin would induce the formation of a gel network with the sodium caseinate in the yogurt mixture (Lal et al., 2006).

3.4. Textural characteristics

Fig. 4 shows the effect of gelatin addition on hardness, adhesiveness and springiness, nearly all of these parameters

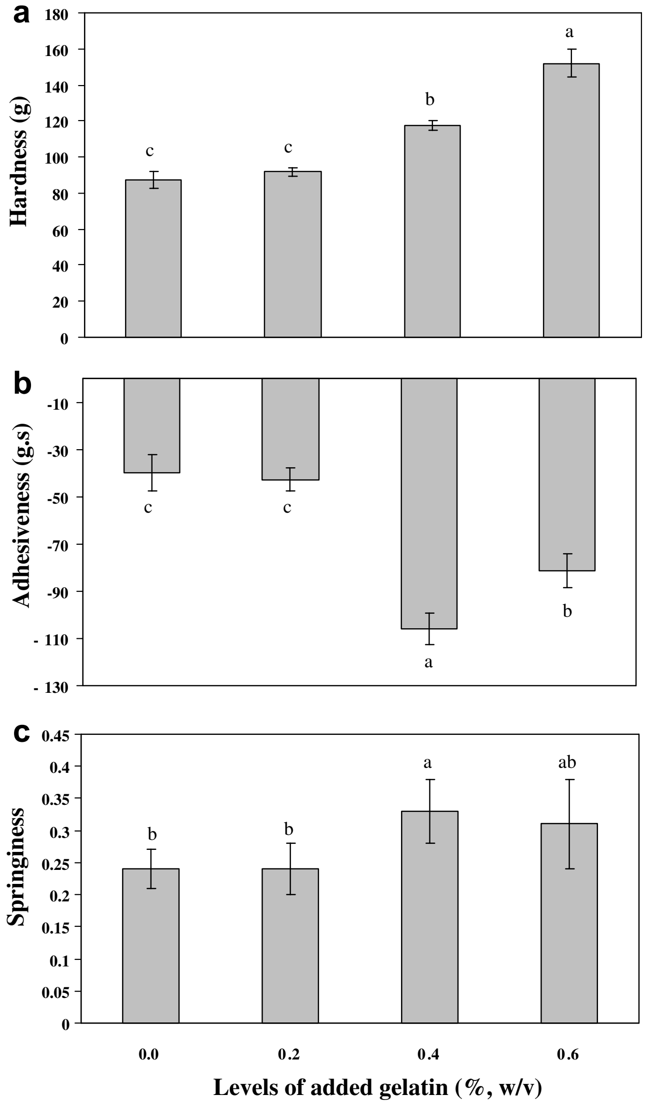

Fig. 4. Texture profile analysis of corn–milk yogurt with different concentrations of gelatin addition.

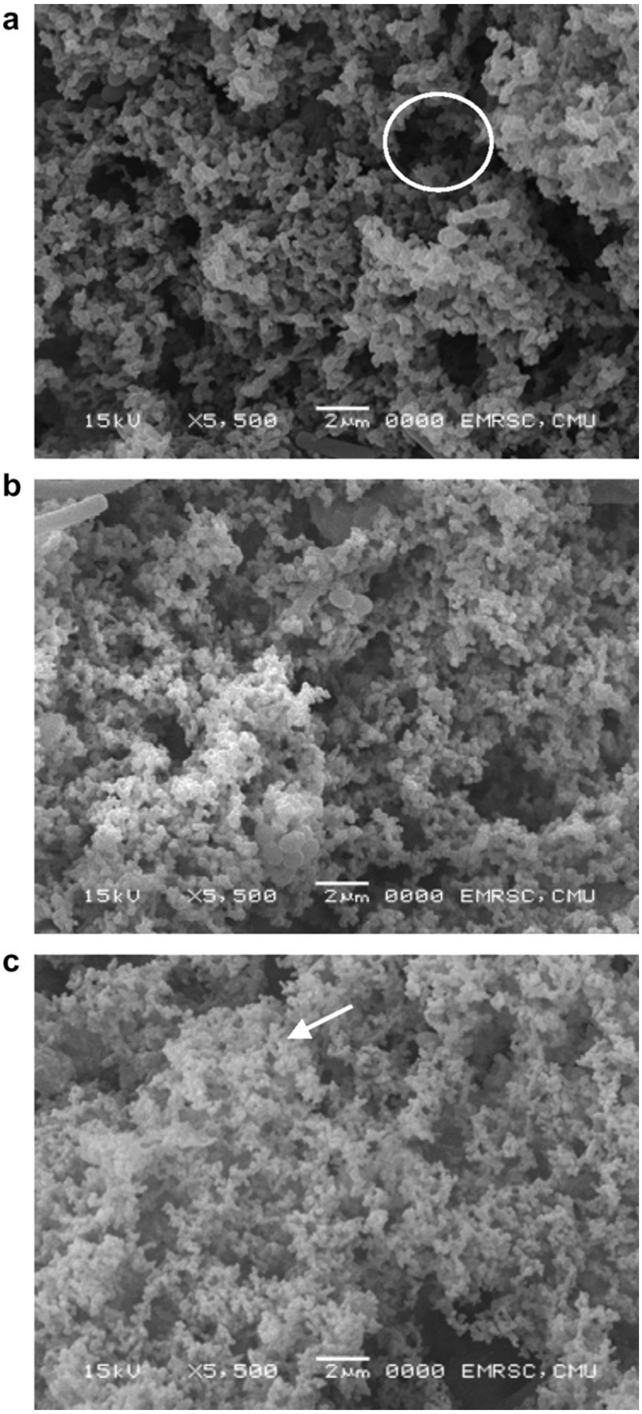

Fig. 5. Scanning electron micrographs of corn–milk yogurt with different levels of gelatin addition 0% (a), 0.4% (b), 0.6% (c) (light circle is air cells, and the light arrow indicates a dense and highly branched-structure).

significantly increased with increasing gelatin concentration ($P < 0.05$). However, too high a concentration of a stabilizer, such as gelatin (0.6%, w/v) can impair the palatability of a natural yogurt gel (Lucey, 2004). Therefore, a medium concentration 0.4% (w/v) of gelatin could be appropriate to ensure good textural quality. At this concentration the structure has a medium density of branched-structure as shown by the electron micrographs (Fig. 5).

3.5. Microstructure

Fig. 5a–c show the microstructures of corn–milk yogurts as affected by gelatin concentration. At higher concentrations of gelatin (c) the micrographs show a dense, highly branched-structure and homogeneous spongy-like interior with few air cells. At the intermediate concentrations of gelatin (b) the structure is less dense and less branched and the spongy-like interior contains more air cells. The control sample (no added gelatin) shows the most open structure (Fig. 5a). These results agree with those of Fiszman et al. (1999) who investigated the effect of gelatin addition on the microstructure of acidic milk gels and yogurt. They found that the smooth bridge of gelatin with a double network structure seemed to be located inside the casein micelles, which could retain the aqueous phase more efficiently, thus reducing syneresis.

3.6. Rheological properties

Fig. 6 and Table 1 show the viscoelastic behaviour of the corn–milk yogurts, at the lowest concentration of added gelatin (0.2%, w/v) both the storage (G') and loss moduli (G'') exhibit a similar pattern to that of the untreated (no added gelatin) sample. Whereas in the samples having larger amounts of gelatin, both moduli increase significantly ($P < 0.05$). Probably due to a higher cross-link density induced by stronger gelation of the system (Apichartsrangkoon, 2002; Apichartsrangkoon, 2003; Apichartsrangkoon, Bell, Ledward, & Schofield, 1999; Apichartsrangkoon & Ledward, 2002). It also worth noting that the overall G' plots are less frequency dependent than the G'' plots and their loss tangent (ratio of G''/G') are as low as 0.2 which is an indication of true gel behaviour with solid-like structures (Ross-Murphy, 1984). These results are in accordance with the TPA (Fig. 4) and microstructure (Fig. 5) results.

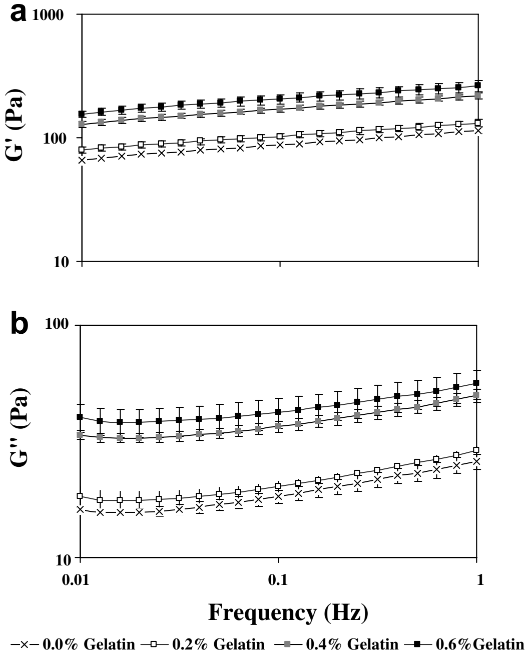

Fig. 6. Dynamic viscoelastic behaviour of corn–milk yogurt with different concentrations of gelatin addition.

Table 1
Viscoelastic behaviour of corn–milk yogurt with various amounts of added gelatin measured at a frequency of 0.1 Hz

Level (%)	G′ (Pa)	G″ (Pa)
0.0	87.56 ± 5.28^c	18.32 ± 1.22^c
0.2	102.82 ± 5.58^c	20.16 ± 1.06^c
0.4	170.33 ± 8.43^b	36.59 ± 2.05^b
0.6	206.40 ± 17.43^a	42.22 ± 6.09^a

Means in each column having the same superscripts are not significantly different $P < 0.05$.

4. Conclusion

Even though, the highest amount of additional gelatin (0.6%, w/v) gave the firmest structure, it led to a very dense and compact structure which could impair palatability. Thus the level of additional gelatin at 0.4% (w/v) was suggested for corn milk yogurt making. However, this optimum concentration will depend on the bloom specification of the gelatin used. Although sensory tests would be needed to confirm this, it is readily apparent that the addition of gelatin to the corn–milk mixture can yield yogurts with characteristics that are associated with good eating quality.

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