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Development of rheological and sensory properties of combinations of milk proteins and gelling polysaccharides as potential gelatin replacements in the manufacture of stirred acid milk gels and yogurt

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Abstract

Combinations of gelling polysaccharides (xanthan/locust bean gum [X/L], carrageenan and starch) and milk proteins (whey protein isolate [WPI], sodium caseinate and skim milk powder) were evaluated as potential gelatin replacers in acid milk gels. Gels with added X/L alone showed rheological (gelling and melting) and microstructural (typical casein network with thin strand-like structures) properties similar to those of gels with gelatin. Similar to the effect of adding gelatin, milk protein fortification enhanced water holding capacity (WHC) of the gels, with WPI being the most effective. Gels with combinations of polysaccharides (except carrageenan) and WPI were stronger and had higher WHC than gels with no stabilizer. In yogurt, the combination of WPI and X/L (WPI-X/L) produced similar effects on consistency, pseudoplasticity and apparent viscosity as gelatin and higher sensory scores for thickness and stickiness than gelatin; a lower score for smoothness was observed with WPI-X/L than with gelatin.

Key words: gelling polysaccharides, whey protein isolate, gelatin replacement, yogurt, rheology, sensory

1. Introduction

Stabilizers are used in the manufacture of yogurt, especially stirred yogurt, to help maintain its desirable textural properties and prevent syneresis. Among the stabilizers used, gelatin is preferred due to its unique properties (Kumar & Mishra, 2004). As explored in our previous study, gelatin showed gelling and melting properties below body temperature in acid milk gels and it increased the water holding capacity (WHC) of the gels (Pang et al., 2015b). However, finding alternatives to gelatin has gained considerable attention in recent years due to religious beliefs and vegetarian lifestyle choices (Karim & Bhat, 2008).

One of the most common approaches to replacing gelatin in yogurt manufacture is using alternative hydrocolloids. Considering the gelling function of gelatin, which provides yogurt both decent physical and sensory properties, polysaccharides that can form thermo-reversible gels could behave similarly to gelatin. Xanthan forms transparent thermo-reversible elastic gels when mixed with LBG, due to intermolecular binding between the galactomannan backbone of locust bean gum (LBG) and xanthan chains (Agoub et al., 2007; Zhan et al., 1993). Both ι- and κ-carrageenan undergo a coil-to-helix transition during temperature decrease, resulting in thermo-reversible gelation (Tye, 1988). This process also needs cations such as K^+ and Ca^{2+} that are present in milk (Drohan et al., 1997). Starch is a gelling agent that is used commercially in yogurt (Kalab et al., 1975). Therefore, these polysaccharides were investigated in this study.

Fortification with milk solids improves the properties of yogurt, including syneresis and texture (Modler et al., 1983). Skim milk powder (SMP) fortification is standard practice in yogurt manufacture (Karam et al., 2013). Yogurt fortified with whey protein concentrate (WPC) or sodium caseinate (NaCn) was reported to exhibit improved rheological and sensory properties (Damin et al., 2009; Marafon et al, 2011). Also, in our previous study (Pang et al., 2015b), pure whey protein gels showed extremely high WHC. Therefore, milk fortification with three milk protein ingredients (SMP, whey protein isolate [WPI] and NaCn) was studied. Combining certain types of milk proteins and polysaccharides rather than using them alone has been found to be more effective for replacing fat in yogurt (Teles & Flores, 2007); this approach may also apply to gelatin replacements. Hence, combinations of polysaccharides and milk proteins as gelatin replacements were also investigated.

The aim of this study were to 1). evaluate the physical properties of milk gels containing gelling polysaccharides, milk proteins and their combinations; and 2) assess further the potential gelatin replacers in the manufacture of stirred yogurt, based on their ability to form thermo-reversible gels and improve the water holding capacity of milk gels.

2. Materials and methods

2.1. Materials

The milk protein ingredients, whey protein isolate (WPI, protein 93.9%, moisture 4.7%, fat 0.3%, lactose 0.4% and ash 1.5%), sodium caseinate (NaCn, protein 88%, moisture 6%, fat 1.5%, lactose 1% and ash 3-6%) and low-heat skim milk powder (SMP, protein 33%, moisture 3.6%, fat 0.9%, lactose 54.7% and ash 7.8%) were obtained from Murray Goulburn Co-Operative Ltd (Melbourne, Australia). Xanthan (GRINDSTED 80 ANZ), and LBG (GRINDTED 246) were donated by Danisco, France. Hydroxypropyl distarch phosphate modified tapioca starch (NATIONAL FRIGEX) was provided by National Starch, Singapore. Carrageenan (GENULACTA type LRA-50, comprised of κ- and ι-carrageenan in ratio of 1:1) was kindly provided by CP Kelco ApS, Denmark. The acidulant, glucono-delta-lactone (GDL), was purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Methods

2.2.1. Evaluation of stirred acid milk gels

i. Preparation of stirred acid milk gels

The acid milk gels were prepared as described previously (Pang et al., 2015b). The milk base dispersions for the control milk gel was prepared by reconstituting SMP (13.5% total solids [w/w]) in distilled water under continuous stirring for 30 min, to obtain a milk protein concentration of 4.5% (w/w). To prepare the fortified milk gels, the milk base dispersion was supplemented with WPI, NaCn or SMP at two levels of total solids (0.5 and 1%) (w/w). All dispersions were stored at 4 ˚C overnight before use and, where applicable, the polysaccharides were added the next day from stock solutions at various concentrations. The resulting treatments for stirred acid milk gels with milk protein fortifiers and polysaccharides are shown in Table 1. Note that all gels are denoted by the suffix “G”, for example, X/L-G refers to the X/L-containing milk gel.

Heat treatment at 95 ˚C for 10 min was applied to the dispersions in a water bath, with continuous stirring at 300 rpm. Water lost by evaporation was replaced with distilled water at the end of the heat treatment. The samples were cooled to 45 ˚C immediately using cold water, and 1.5% (w/w) glucono-delta-lactone (GDL) was added. The samples with GDL were immediately loaded onto a rheometer for rheological analysis. For other analyses, the samples were kept at 45 ˚C for 4 h, by which time the pH of the samples was ~ 4.6. The gels were then stirred using an overhead stirrer at 1200 rpm for 2 min and placed in cylindrical containers of diameter 11 cm and height 5 cm for texture analysis, and in 15 ml centrifuge tubes for measurement of water holding capacity. Gels were stored at 10 ˚C for 48 h before testing. For each treatment, two independent replicates were prepared for all analyses.

ii. Dynamic oscillatory rheology

The dynamic oscillatory rheology was carried out on the samples according to the method reported previously (Pang et al., 2015b). Aliquots of mixture solutions were poured onto the bottom plate of the rheometer equipped with a 4 cm, 2˚ cone-plate measuring system immediately after GDL was added. The measurements were performed in a four-stage process:

Acidification stage: measurement commenced at 45 ˚C and this temperature was maintained for 4 h, promoting formation of the milk protein gel;

Cooling stage: the temperature was lowered from 45 to 10 ˚C at a constant rate of 1 ˚C/min, to observe the gelling property of the hydrocolloids;

Annealing stage: the oscillatory tests were performed at 10 ˚C for 2.5 h to observe the maturation of the gelling samples;

Heating stage: the temperature was increased from 10 to 45 ˚C at 1 ˚C/min, to observe melting property of the hydrocolloids.

iii. Microstructure

The microstructure of the samples was observed using scanning electron microscopy according to the method described previously (Pang et al., 2014). Gels after 48 h storage at 10 ˚C were fixed with glutaraldehyde at room temperature, dehydrated with ethanol at room temperature and then dried with a CO_2 critical point dryer (Tousimis Automatic). Dried samples were platinum-coated and observed with a scanning electron microscope (JEOL 6610) at an acceleration voltage of 10 kV.

iv. Water holding capacity (WHC)

WHC was determined by the method reported previously with a modified centrifuge speed (Pang et al., 2015b). Samples were centrifuged at 200 *g* for 10 min at 10 ˚C and the WHC was defined as follows:

WHC (%) = 100 (MG weight – SE weight) / MG weight. Where MG = milk gel and SE= serum expelled.

2.2.2. Evaluation of yogurt products

i. Yogurt manufacture

Three yogurt samples (yogurt with no stabilizer (NY), yogurts containing 0.4% gelatin (GY), or WPI-xanthan-LBG [WPI-X/L-Y]) were prepared. Note that all yogurts are denoted by the suffix "Y", for example, GY refers to yogurt containing gelatin. The mixtures of SMP and stabilizers were prepared in the same way as in 2.2.1. The mixtures were heated to 95 °C for 10 min in covered steel containers and cooled to ~ 42 °C immediately. At this point, the mixtures were inoculated with 0.2 U/kg culture (YC-380; *Streptococcus thermophilus* and *Lactobacillus delbreuckii* ssp. *bulgaricus*, Chr. Hansen, Melbourne, Australia) and incubated at 42 °C until pH 4.6 was reached. Yogurts were then stirred at 1200 rpm for 2 min and cooled immediately using iced water. Yogurt samples were evaluated after 48 h storage at 4 °C. Yogurt production was performed in two independent replicates for all analyses.

ii. Rheology

Rheological properties of yogurt samples were determined using the same rheometer and geometry as in 2.2.1. Yogurts were stirred gently 10 times with a spoon and a small amount of sample was placed onto the bottom plate of the rheometer. Excess sample at the edge of the geometry was carefully wiped away without excessively disturbing the sample. The measurement temperature was 4 °C. For each treatment, triplicate measurements were taken and data processing was performed using the Rheology Advantage Data Analysis software package (Version 5.7.0, TA Instruments Ltd).

The flow behavior of the yogurt samples was characterized. The shear rate was varied from 0 to 100 s^{-1} and the shear stress was recorded at increasing shear rates (upward flow curve) followed by decreasing shear rates (downward flow curve). The resulting upward flow curve was fitted to the

Hershel-Bulkley model ($\sigma = \sigma_0 + K\gamma^n$), where σ = shear stress, σ_0 = yield stress, K = consistency index, γ = shear rate, and n = flow behavior index (Hassan et al., 1995; Paseephol et al., 2008). In addition, other parameters such as the area under the upward flow curve (A_{up}) and the difference in area under the upward flow curve and the downward flow curve (ΔA), as well as apparent viscosity (η_{app}) at $\gamma = 50\ s^{-1}$ were obtained.

Frequency sweep was also carried out to assess the viscoelastic properties of the yogurt samples, by increasing frequency from 0.01 to 10 Hz. The applied strain was 0.5% (within the linear viscoelastic range). The storage modulus (G') and loss modulus (G'') were recorded as a function of frequency, and loss tangent was calculated. The slopes of the resulting log–log plots for both G' and G'' were obtained for all yogurt samples.

iii. Sensory evaluation

Triangle test

Preliminary triangle tests as described in ISO 41:2004 (BS EN ISO 4120: 2004) were performed with the samples: (a) GY vs. NY, (b) GY vs. WPI-X/L-Y. Ten panelists were recruited for the analysis. To increase their discriminative ability, six one-hour training sessions were performed before the triangle tests to help them become familiar with the products and the mechanics of the triangle test, and to develop the vocabulary for yogurt description. Analyses were conducted in individual tasting booths under red lights. For each comparison, three randomly coded samples consisting of two of the same and one different sample were presented to the panelists at 10 °C in a randomized balanced order. Panelists were asked to taste the samples in the order given, identify the odd sample, provide written comments on the odd samples, and to note if they were guessing. A 5 min break was taken between the sets of comparisons. Panelists were provided with spring water for palate cleansing between samples. In the cases where the odd sample could not be identified, the panelists were forced to make a choice. For each test, replicates were conducted to improve the power of analysis and to be able to detect true discriminators.

Ranking test

Ranking tests were conducted on the yogurt samples using method as described in ISO 8587: 2006 (E) (BS ISO 8587:2006). The ranking test panel consisted of 38 untrained volunteers. They ranked the samples according to the attributes from highest intensity to lowest. The samples were provided in small cups randomly coded with three-digit numbers and presented simultaneously at 10 °C. Spring water was served to cleanse the palate between samples.

2.3. Statistical analysis

Minitab ver. 16 software (Minitab Inc., USA) was used for the statistical analysis. ANOVA was performed on the instrumental data, using $p < 0.05$ as the test of significance. The results of the triangle test were analysed using Chi-square distribution. Friedman analysis of variance was applied to the ranking data set and the significance of differences between samples was determined by the Fisher test (α=0.05).

3. Results and discussion

3.1. Evaluation of xanthan/LBG as a gelatin replacer in stirred acid milk gels

3.1.1. Rheology

The concentration of xanthan gum used in this study was based on the results obtained in preliminary trials and on the reported synergistic effect between xanthan and LBG being maximal at a ratio of 1:1 (Copetti et al., 1997); therefore the concentration and composition of the xanthan: LBG (X/L) mixture were chosen to be 0.01% [w/w] and 1:1, respectively. The effects of addition of X/L on the rheological properties of the milk gels during different stages are shown in Fig. 1. Fig. 1A shows the results of milk gels with or without X/L during the acidification stage; X/L had little effect on the G′ during this stage, that is, the curve for X/L-G was very similar to that of NG, the gel without added polysaccharides. During the cooling stage (Fig. 1B), the G′ showed an increase due to the reinforcement of the milk protein gels (Pang et al., 2015b) and possibly structural changes in the polysaccharides; X/L-G showed an inflection between 24 and 21 °C, which indicated structural change at these temperatures. The “coil-to-helix” transition of xanthan molecules during cooling has been reported previously and the non-specific interactions between the galactomannan backbone of LBG and xanthan chains can promote gel formation (Agoub et al., 2007; Zhan et al., 1993). The G′ values of all samples were quite stable during the annealing stage (data not shown). During the heating stage (Fig. 1C), the G′ of all samples showed a decrease with increasing temperature, possibly due to shrinkage of the milk protein gel particles as indicated by the decrease shown by NG (Pang et al., 2015b). Similar to gelatin containing gels (Pang et al., 2015b), X/L-G showed inflection during the heating stage, at ~19 to 26 °C. It is worth mentioning that these temperatures were below human body temperature, which could result in “melt-in-the-mouth” phenomenon, as shown by gelatin. Xanthan has been previously reported to form “melt-in-the-mouth” gels with konjac, another galactomannan (Agoub et al., 2007).

3.1.2. Microstructure and WHC

Fig. 2 shows the microstructure of stirred acid milk gels with X/L after 48 h storage at 10 °C and a micrograph of NG included as a control. The typical casein network was maintained in both samples. These casein structures were consistent with those reported previously (Fiszman et al., 1999; Kalab et al., 1975).

In X/L-G, very thin strands connecting the casein micelle particles were observed (Fig. 2B). The organization of the network of chains and clusters of casein particles did not change. At a comparable X/L ratio (9:11), similar results were reported in acid milk gels by (Sanchez et al., 2000). The added polymers appeared as filamentous structures distributed on the surface of casein particles (Sanchez et al., 2000). It has also been reported that, like gelatin, X/L increases the consistency of yogurt (Keogh & O'kennedy, 1998), which might be related to the network it forms in the yogurt microstructure. The significant similarities of the strand-like structures between gels containing gelatin (Pang et al., 2015b) and X/L-G suggest that an X/L combination at an appropriate concentration is a potential replacement for gelatin in acid milk protein gels such as yogurt.

However, there was no significant increase in WHC ($p > 0.05$) over that of the control gels (NG) by addition of X/L (Table 2).

3.2. Evaluation of milk protein fortification as gelatin replacement in stirred acid milk gels

3.2.1. Rheology

The G′ of all gels fortified with milk proteins displayed trends similar to that of NG during the acidification, cooling and heating stages (Fig. 3). Fortification with WPI dramatically increased the G′ of milk gels compared to NG, even at 0.5% addition level. The G′ of WPI-G-1 was three times higher than that of NG. This is attributable to the interaction between κ-casein and denatured β-lactoglobulin, which greatly increases the density of gel-forming proteins in the gel matrix (Keogh & O'kennedy, 1998). A similar effect of whey protein fortification on acid milk gels has been previously reported (Lucey & Singh, 1997; Marafon et al., 2011). Addition of NaCn slightly increased the G′ of the milk gels compared to NG but the increase was independent of the concentration of NaCn added; addition of SMP had a negligible effect on the G′ of the acid gel at both concentrations used. It has been reported that NaCn increased the G′ of the final yogurt product, while no increase occurred with SMP supplementation (Damin et al., 2009). The effect of added proteins was different from that of addition of gelatin which interfered with milk gelation during the acidification stage (Pang et al., 2015b). However, this result was expected as the added milk ingredients are compatible with the SMP base in NG and do not cause depletion flocculation or phase separation like gelatin.

3.2.2. Microstructure and WHC

The micrographs of milk gels fortified with three types of milk proteins at two concentration levels are shown in Fig. 4. It can be seen that with fortification of WPI, the gels became more filamentous, especially at 1% concentration, and the casein particle clusters were less well-defined, compared with NG, which showed large and round clusters. The casein aggregates were linked by long filamentous chains instead of being fused into large aggregates in WPI fortified gels. Similar results were reported by Saint-Eve et al. (2006) and Akalin et al. (2012) who observed arrangements of casein micelles in long chains rather than fused aggregates in WPC-fortified yogurts. These chain-like structures are probably induced by the interaction between β-lactoglobulin and κ-casein during heating (Modler & Kalab, 1983; Sandoval-Castilla et al., 2004). At higher concentration of WPI, whey protein aggregates formed on the surface of casein micelles could affect the openness of the gel microstructure (Aziznia et al., 2008). NaCn-G (0.5 or 1) showed little difference in microstructure from NG (Fig. 4B, E). SMP-G (Fig. 4C, F) appeared to have a more compact casein particle network than NG, especially at 1% concentration.

Results of WHC of stirred milk gels with milk protein fortification are shown in Table 2. All three milk protein fortifiers increased the WHC of acid milk gels at the two concentrations studied with WPI being most effective. A high concentration of milk solids has been reported to prevent syneresis (Lazaridou et al., 2008). WPI greatly increased the WHC of milk gels, which is a major function of gelatin in milk gels (Pang et al., 2015b). It has been stated that attachment of whey protein molecules to the surface of the casein micelles can increase the entrapment of serum in gels (Keogh & O'kennedy, 1998). Similar results have been reported by Isleten and Karagul-Yuceer (2006) and Akalin et al. (2012), who observed the lowest syneresis in yogurt fortified with WPI among all milk ingredient fortifiers.

Thus, rheology and SEM analyses provided an assessment of the gelation properties and the structure of milk gels fortified with proteins. Fortification with WPI or NaCn increased the G′ of the gels to varying degrees while addition of SMP had negligible effect; micrographs showed no dramatic change from NG, except with a high concentration of WPI. Milk protein fortification also significantly increased WHC of the final gel, especially with WPI fortification. Hence, milk protein fortification alone had a similar effect on WHC of the gels as gelatin, but different effects on rheology and microstructure.

3.3. Evaluation of combinations of WPI and polysaccharides in stirred acid milk gels

WPI can improve WHC effectively and X/L can induce gel microstructure and rheology characteristics similar to those produced by gelatin. A combination of WPI and X/L was therefore investigated to evaluate its potential as gelatin replacer in acid milk gels. In addition, starch and carrageenan, which showed gelling properties in our previous study (Pang et al., 2015a) and hence had potential as gelatin replacements, were also studied in combination with WPI at 0.2 and 0.05% concentrations, respectively. Considering that the G′ was increased dramatically by WPI addition, even at 0.5% fortification, in this study, part of the SMP in NG was replaced with 0.5% WPI (WPI-G (R)) while maintaining the same amount of total protein, instead of fortifying the milk gel with 0.5% WPI solids. The results of WPI-G (R) are also presented as a reference.

3.3.1. Rheology

The combination of polysaccharides and WPI, except that of carrageenan and WPI, resulted in higher G′ of the gels than that of NG (Fig. 5). Combining WPI and carrageenan had a negative effect on the milk gels, as G′ was lower than that of NG. Hence, the reinforcing effect of WPI fortification on the milk gel was lost when combined with carrageenan. The interaction between the highly sulphated carrageenan and milk proteins could have prevented the interaction between the caseins and whey proteins (Hemar et al., 2002). Interestingly, a similar effect was reported for a combination of NaCn or whey proteins and starch (Roberts et al., 2000; Sandoval-Castilla et al., 2004). This was attributed to the fact that the polysaccharides did not integrate into the protein network and inhibited the whey protein–casein interaction and casein aggregation. In these studies, the starch concentration was much higher than that used in our study and a different type of starch was used, which could explain why no such effect was observed in our study with WPI-S-G.

No obvious inflection was observed for any sample during the cooling stage. This is likely to be due to either the higher G′ value that masks any small changes caused by structural changes in polysaccharides or interactions between the polysaccharides and WPI. During the heating stage, a very small inflection was observed for both WPI-X/L-G and WPI-S-G at ~ 23 and 25 °C, respectively (Fig. 5D). Starch and WPI might have some synergistic effect, as neither component showed any inflection during heating when they were used alone at this concentration.[31] Further study needs to be done to elaborate these results.

3.3.2. Microstructure and WHC

Fig. 6 shows the micrographs of milk gels with combinations of WPI and polysaccharides. For WPI-S-G, it was difficult to distinguish the starch gel structure from the milk gel structure. It has been observed that addition of 1% modified tapioca starch resulted in a relatively open and loose structure in yogurt (Sandoval-Castilla et al., 2004). This may be related to the water and space competition between milk proteins and starch at such a high concentration of starch; this did not occur in our study at the level of starch used (0.2%). As expected, WPI-X/L-G showed thin strands connecting the casein aggregates throughout the entire structure, similar to that of gels containing gelatin as previously reported (Pang et al., 2015b). The micrograph of the WPI-C-G showed large aggregates distributed throughout the entire network, which could be due to the strong interaction between carrageenan and milk proteins (Hemar et al., 2002). In addition, the combinations of WPI and polysaccharides significantly increased the WHC of the milk gels.

Thus, the combination of WPI and polysaccharides could remedy the disadvantages of these components when used alone, and lead to products with characteristics closer to those of gels with added gelatin. However, the WPI-carrageenan combination did not yield promising results. More satisfactory results could be obtained by optimizing the protein: gum ratio to maximize the interaction between the hydrocolloids and proteins.

3.4. Evaluation of WPI-X/L in yogurt

From the results of the stirred milk gels, the combination WPI-X/L was most similar to gelatin. Therefore, it was further evaluated in cultured yogurt using both physical and sensory techniques.

The yogurt with WPI-X/L (WPI-X/L-Y) was compared with yogurt with no stabilizer (NY) and yogurt with 0.4% gelatin (GY).

3.4.1. Rheology

Flow behavior of yogurts

The upward flow curves were fitted to the Herschel-Bukley model and the resulting parameters are shown in Table 3. The model satisfactorily fitted the experimental data for all samples, showing R^2 values generally above 0.96 (data not shown). WPI-X/L-Y exhibited the highest yield stress, indicating the highest shear stress required to trigger the flow of yogurt. GY showed the lowest yield stress, even lower than NY, which accorded with the results reported previously (Pang et al., 2015b), which showed that gelatin tended to decrease the G′ of acid milk gels during gelation. The consistency coefficient (K) was highest for GY, followed by WPI-X/L-Y, with NY showing the lowest values. The results were generally in agreement with the previous research, showing that gelatin and X/L both increased K (Ares et al., 2007; Keogh & O'kennedy, 1998). As for the flow index (n), which is a measure of deviation of shear thinning fluids from Newtonian flow, NY showed a higher value (0.61) than WPI-X/L-Y and GY (0.46 and 0.32, respectively), indicating that addition of gelatin and WPI-X/L increased the pseudoplastic behavior of yogurt. Similar results were reported for gelatin and X/L in yogurt by Keogh and O'kennedy (1998) and Ares et al. (2007). They also concluded that greater shear thinning occurred with an increase of K.

The effect of stabilizers on the apparent viscosity (η_{app}) at a shear rate of 50 s^{-1}, which was reported as an effective oral shear rate (Marcotte et al., 2001), and on the area of the hysteresis loop (ΔA), an indication of structural breakdown and rebuilding during shearing (thixotropy), is shown in Table 3. η_{app} was higher for WPI-X/L-Y and GY than for NY. Gelatin was reported to increase the apparent viscosity of yogurt due to the interaction between gelatin and milk proteins (Ares et al., 2007; Teles & Flores, 2007). Higher apparent viscosity of yogurt with whey protein fortification was observed previously (Isleten & Karagul-Yuceer, 2006). WPI-X/L-Y showed the highest ΔA and no significant difference was observed between the other two yogurts, indicating WPI-X/L-Y was more susceptible to structural breakdown by the application of shear stress and that restructuring of the protein aggregates into a coherent network structure after shearing was more difficult than in the other yogurts (Ares et al., 2007; Ramaswamy & Basak, 1992).

Viscoelastic properties of yogurts

The viscoelastic properties of the yogurts were also studied and the results at a frequency (ω) of 1 Hz are shown in Table 4. The WPI-X/L-Y showed the highest G′ and G″, while there was no significant difference between NY and GY, indicating firmer yogurt gels were formed with WPI-X/L. Whey protein fortification has also been reported to induce higher yield stress and higher G′ due to the increased protein–protein interaction caused by addition of WPI (Isleten & Karagul-Yuceer, 2006; Lee & Lucey, 2006; Marafon et al., 2011). Thus, the combination of WPI-X/L enhanced the properties of yogurt, in terms of consistency, pseudoplasticity and apparent viscosity, similar to 0.4% gelatin. Consistency is an important property for stirred yogurt, especially for flavored yogurt where the added flavor ingredients generally decrease the consistency, which is the reason why flavored yogurts generally contain stabilizers (Ramaswamy & Basak, 1992). It was reported that a higher consistency index and higher pseudoplasticity led to higher acceptability by sensory panelists for lactic beverages (Penna et al., 2001). On the other hand, different from gelatin, WPI-X/L-Y exhibited higher yield stress and G′ than NY, which are strongly related to gel strength. Yield stress was reported to correlate very well with the initial firmness of yogurt assessed by sensory evaluation (Harte et al., 2007). As discussed below, the gel strength could be adjusted by optimizing the ratio of milk proteins or using different types of whey protein ingredients as fortifiers.

3.4.2. Sensory

Triangle test

The primary triangle tests were conducted to test whether overall differences existed between the samples. Comments about the samples were required from the panelists for further investigation of samples that were perceived to be different. At least eight out of ten panelists correctly identified the odd sample for both comparisons between GY and NY, and nine out of ten between GY and WPI-X/L-Y. Chi-square distribution showed that GY was perceived to be significantly different from NY and WPI-X/L-Y ($p<0.001$) (data not shown). From the comments of the panelists, the differentiation was mainly based on thickness, smoothness and stickiness.

Ranking test

Since thickness, smoothness and stickiness were the most differentiated attributes according to the triangle tests, and they are important texture attributes of yogurt, they were further investigated using the ranking test. Friedman analysis of results from the ranking test showed significant differences ($\alpha=0.05$) in all these three attributes. Therefore, Fisher's test on the results of thickness, smoothness and stickiness was performed. The ranking sums of the samples are shown in Fig. 7. For thickness, WPI-X/L-Y showed the highest values, and significantly ($p < 0.05$) lower ranking was given to yogurt with gelatin (GY) and yogurt without any stabilizer (NY). Milk protein fortification has been reported to increase the oral viscosity of yogurt (Isleten & Karagul-Yuceer, 2006; Marafon et al., 2011; Penna et al., 1997). For smoothness, GY and NY showed significantly ($p < 0.05$) higher ranking than WPI-X/L-Y and no significant difference was perceived between GY and NY. For stickiness, the significantly ($p < 0.05$) highest ranking was for the WPI-X/L-Y; GY and NY showed no significant difference.

The ranking results for thickness are somewhat at odds with the comments of panelists from the triangle test where 9 out of 10 panelists commented that GY was thicker and smoother than NY (results not shown). This might indicate that the differences between GY and NY based on the three attributes, especially thickness and smoothness, could not be detected by panelists used for ranking test, but could be detected by panelists used for triangle test who were familiarized with the products. Familiarity with the product and its attributes seemed to play an important role in detecting small differences (Barcenas et al., 2004). Oral viscosity has been evaluated for yogurt with and without gelatin by Ares et al. (2007), using a trained panel. The results showed that higher sensory viscosity was obtained by addition of gelatin.

The major difference between GY and WPI-X/L-Y was that GY was smoother. Several approaches can be taken to further improve smoothness of yogurts containing WPI-X/L. It was reported that fortifying yogurt with milk protein or severe heating tended to cause a granular texture and increase chalkiness (Isleten & Karagul-Yuceer, 2006; Sodini et al., 2004). However, another study showed that yogurts fortified with ion exchange-WPC and electrodialysis-WPC at 1% showed smoothness similar to gelatin-containing yogurt, while yogurt fortified with ultrafiltration-WPC was coarser than gelatin-containing yogurt (Kalab et al., 1975). It was also found that fortification with microparticulated whey protein resulted in a high creaminess score, comparable to high-fat yogurt (Janhoj et al., 2006). Therefore, better sensory properties could be achieved by using modified whey protein ingredients and optimizing the concentration. Also, greater thickness was perceived in WPI-X/L-Y than in GY. Sensory thickness was reported to increase with increasing levels of added milk protein fortifiers (Kalab et al., 1975). Further optimization of the applied concentration of WPI and the gums will be required to obtain the desired thickness.

4. Conclusions

The gelling polysaccharide mixture, X/L, introduced rheological and microstructural characteristics in acid milk gels similar to those produced by gelatin, and WPI showed a great ability to increase WHC. The combination of WPI and gelling polysaccharides (starch, carrageenan and X/L) induced stronger gels with higher WHC, except the combination with carrageenan, and WPI-X/L developed similar microstructure and showed similar inflection in the rheology curves during heating as gelatin. The combination of WPI-X/L showed promise as a replacer for gelatin in yogurt. However, the cultured yogurt containing WPI-X/L, WPI-X/L-Y, obtained the highest scores for sensory thickness and stickiness, which could also be related to its high gel strength induced by addition of WPI; WPI-X/L-Y achieved a lower smoothness score than GY. Further optimization of the concentrations of WPI and polysaccharides, and possibly the type of WPI in WPI-X/L, may bring it closer to gelatin in its effects on the properties of yogurt.

In this study, we show that the combination of techniques used, dynamic oscillatory rheology, scanning electron microscopy, texture profile analysis and WHC determination, was very useful for evaluating ingredients as gelatin replacers in yogurt.

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List of figure captions

Fig. 1. Changes in G′ of NG (—) and X/L-G (…). A, the acidification stage at 45˚C; B. the cooling stage from 45 to 10˚C; C. the heating stage from 10 to 45˚C. For definition of treatment codes see footnote to Table 1.

Fig. 2. SEM micrographs of NG (A) and X/L-G (B). Scale bars in the images are 1 µm. For definition of treatment codes see footnote to Table 1.

Fig. 3. Changes in G′ of NG (—), WPI-G-0.5 (— - - —), WPI-G-1 (…), NaCn-G-0.5(— —), NaCn-G-1 (－··－), SMP-G-0.5 (—·—), SMP-G-1(－－). A, the acidification stage at 45˚C; B. the cooling stage from 45 to 10˚C; C. the heating stage from 10 to 45˚C. For definition of treatment codes see footnote to Table 1.

Fig. 4. SEM micrographs of WPI-G-0.5 (A), NaCn-G-0.5 (B), SMP-G-0.5 (C), WPI –G-1 (D), NaCn-G-1 (E) and SMP-G-1 (F). Scale bars in the images are 1 µm. For definition of treatment codes see footnote to Table 1.

Fig. 5. Changes in G′ of NG (—), WPI-G (R) (— —), WPI-S-G (…), WPI-C-G (－··－) and WPI-X/L-G (－－). A, the acidification stage at 45˚C; B. the cooling stage from 45 to 10˚C; C. the heating stage from 10 to 45˚C; D: the inflection points for WPI-S-G and WPI-X/L-G during heating stage. For definition of treatment codes see footnote to Table 1.

Fig. 6. SEM micrographs of WPI-G (R) (A), WPI-S-G (B), WPI-X/L-G (C) and WPI-C-G (D). Scale bars in the images are 1 µm. For definition of treatment codes see footnote to Table 1.

Fig. 7. Results of ranking test of the yogurts according to attributes of sensory thickness, smoothness and stickiness. Different letters on top of bars mean significant difference within bar groups ($p < 0.05$). For definition of treatment codes see footnote to Table 3.

Table 1. Codes of the stirred acid milk gels with different treatments and levels of addition of ingredients

<table>
<tr><th rowspan="2">Treatments code</th><th>Starch</th><th>Carrageenan</th><th>Xanthan</th><th>LBG</th><th colspan="2">WPI</th><th colspan="2">NaCn</th><th colspan="2">SMP</th></tr>
<tr><th colspan="10">Concentration (%, w/w)</th></tr>
<tr><td>NG</td><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">13.5</td></tr>
<tr><td>X/L-G</td><td>-</td><td>-</td><td>0.005</td><td>0.005</td><td colspan="2">-</td><td colspan="2">-</td><td colspan="2">13.5</td></tr>
<tr><td>WPI-G- (0.5 / 1)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0.5</td><td>1</td><td colspan="2">-</td><td colspan="2">13.5</td></tr>
<tr><td>NaCn-G-(0.5 / 1)</td><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td><td>0.5</td><td>1</td><td colspan="2">13.5</td></tr>
<tr><td>SMP-G-(0.5 / 1)</td><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">-</td><td colspan="2">-</td><td>14</td><td>14.5</td></tr>
<tr><td>WPI-G (R)</td><td>-</td><td>-</td><td>-</td><td>-</td><td colspan="2">0.5</td><td colspan="2">-</td><td colspan="2">12.15</td></tr>
<tr><td>WPI-S-G</td><td>0.2</td><td>-</td><td>-</td><td>-</td><td colspan="2">0.5</td><td colspan="2">-</td><td colspan="2">12.15</td></tr>
<tr><td>WPI-C-G</td><td>-</td><td>0.05</td><td>-</td><td>-</td><td colspan="2">0.5</td><td colspan="2">-</td><td colspan="2">12.15</td></tr>
<tr><td>WPI-X/L-G</td><td>-</td><td>-</td><td>0.005</td><td>0.005</td><td colspan="2">0.5</td><td colspan="2">-</td><td colspan="2">12.15</td></tr>
</table>

NG: control milk gel with no addition of stabiliser or milk protein; X: xanthan; L: locust bean gum; X/L-G: X /L-containing milk gel; WPI: whey protein isolate; WPI-G-0.5/1: WPI-fortified milk gel at concentration 0.5 or 1% (w/w); NaCn: sodium caseinate; NaCn-G-0.5/1: NaCn-fortified milk gel at concentration 0.5 or 1% (w/w); SMP: skim milk powder; SMP-G-0.5/1: SMP-fortified milk gel at concentration 0.5 or 1% (w/w); WPI-G (R): milk gel with 0.5% WPI (w/w) replacing same amount of protein from SMP; WPI-S-G: milk gel with combination of WPI and starch; WPI-C-G: milk gel with combination of WPI and carrageenan; WPI-X/L-G: milk gel with combination of WPI and X/L.

Table 2. Water holding capacity (WHC) of acid milk gels with all treatments

Blocks	Treatment code	WHC (%)
Milk gel with no stabiliser or X/L	NG	87.6±3.9^{a}
	X/L-G	89.2±0.7^{a}
Milk gels with milk protein fortification at two concentrations	NG	87.6±3.9^{d}
	NaCn-G-0.5	96.3±0.5^{bc}
	NaCn-G-1	95.1±0.8^{c}
	WPI-G-0.5	98.4±0.4^{ab}
	WPI-G-1	99.5±0.3^{a}
	SMP-G-0.5	97.2±0.1^{bc}
	SMP-G-1	97.1±0.4^{bc}
Milk gels with combinations of WPI and polysaccharides	NG	87.6±3.9^{d}
	WPI-G (R)	97.7±0.3^{a}
	WPI-S-G	97.9±0.1^{a}
	WPI-C-G	91.5±0.7^{bc}
	WPI-X/L-G	95.3±0.6^{ab}

Means were compared within each block. Different letters mean a significant difference ($p < 0.05$). For definition of treatment codes see footnote to Table 1.

Table 3. Rheological parameters from flow curves of yogurts

Sample code	Yield stress σ_0 (Pa)	Consistency coefficient K (Pa.s)	Flow behavior index (n)	Area under up curve A_{up} (1/s.Pa)	Area difference ΔA	Apparent viscosity (50 s^{-1})
NY	10.03 ± 1.44^{b}	2.11 ± 0.97^{c}	0.61 ± 0.17^{a}	3192 ± 558^{c}	910 ± 228^{b}	0.66 ± 0.11^{b}
GY	6.34 ± 1.75^{c}	10.72 ± 2.54^{a}	0.32 ± 0.05^{c}	4214 ± 572^{ab}	1021 ± 148^{b}	0.84 ± 0.12^{a}
WPI-X/L-Y	19.28 ± 3.27^{a}	5.22 ± 2.79^{b}	0.46 ± 0.14^{b}	4757 ± 1031^{a}	1388 ± 326^{a}	0.94 ± 0.17^{a}

Means within a column with different letters are significantly different ($p < 0.05$). NY: control yogurt with no addition of stabiliser or milk protein; GY: Gelatin-containing yogurt; WPI-X/L-Y: yogurt with combination of WPI and X/L.

Table 4. Viscoelastic parameters of yogurts from frequency sweeps

Sample code	G′, at 1Hz	G″, at 1 Hz	Loss tangent δ, at 1 Hz	Slope of log (G′) vs log (frequency)	Slope of log (G″) vs log (frequency)
NY	163.8±19.3^{b}	38.60±4.41^{b}	0.24±0.01^{a}	0.14±0.01^{b}	0.13±0.01^{b}
GY	139.2±16.4^{b}	33.84±4.26^{b}	0.24±0^{a}	0.15±0.01^{a}	0.16±0.01^{a}
WPI-X/L-Y	413.1±96.6^{a}	92.63±22.0^{a}	0.22±0.01^{b}	0.14±0.01^{ab}	0.13±0^{bc}

Means within a column with different letters are significantly different ($p < 0.05$). For definition of treatment codes see footnote to Table 3.

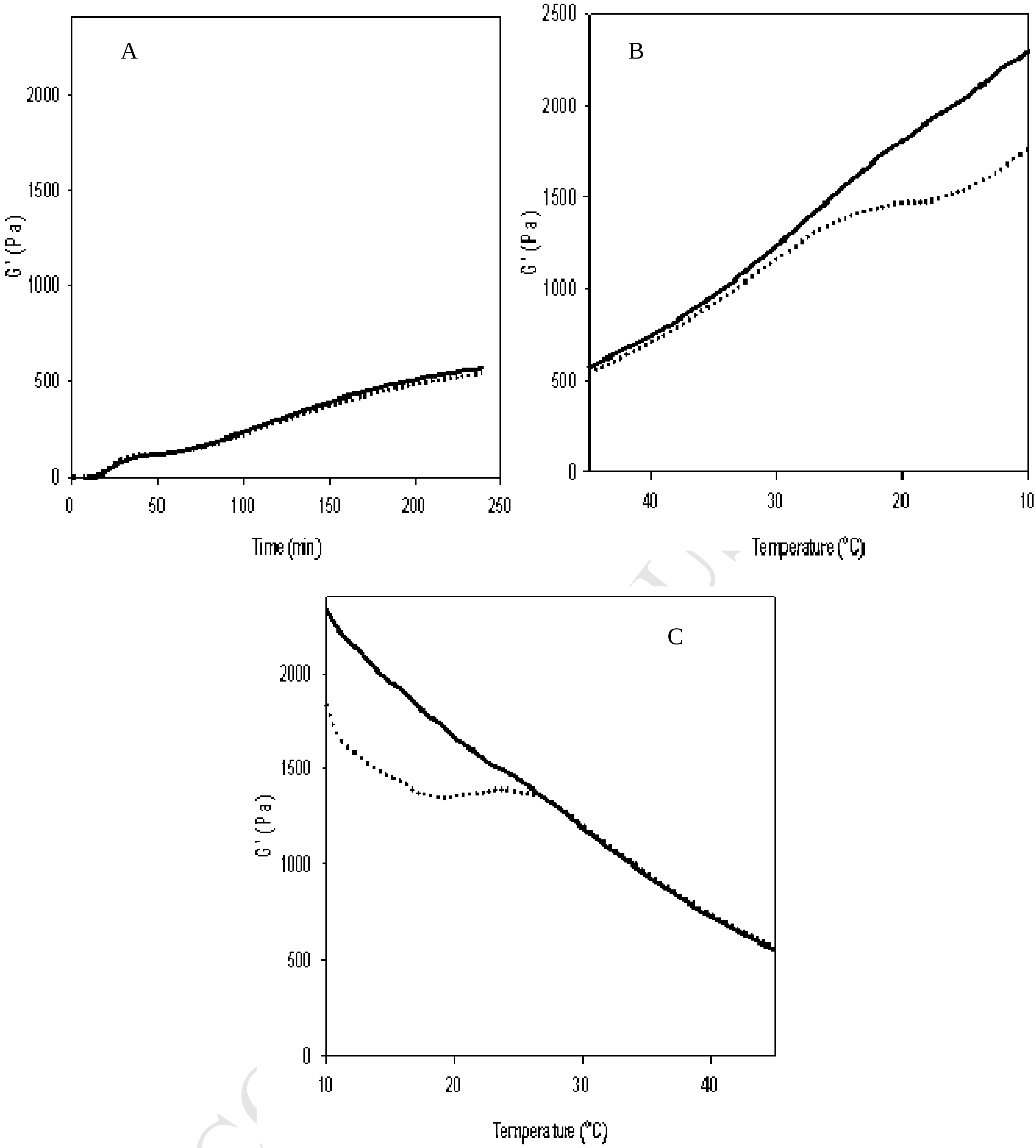
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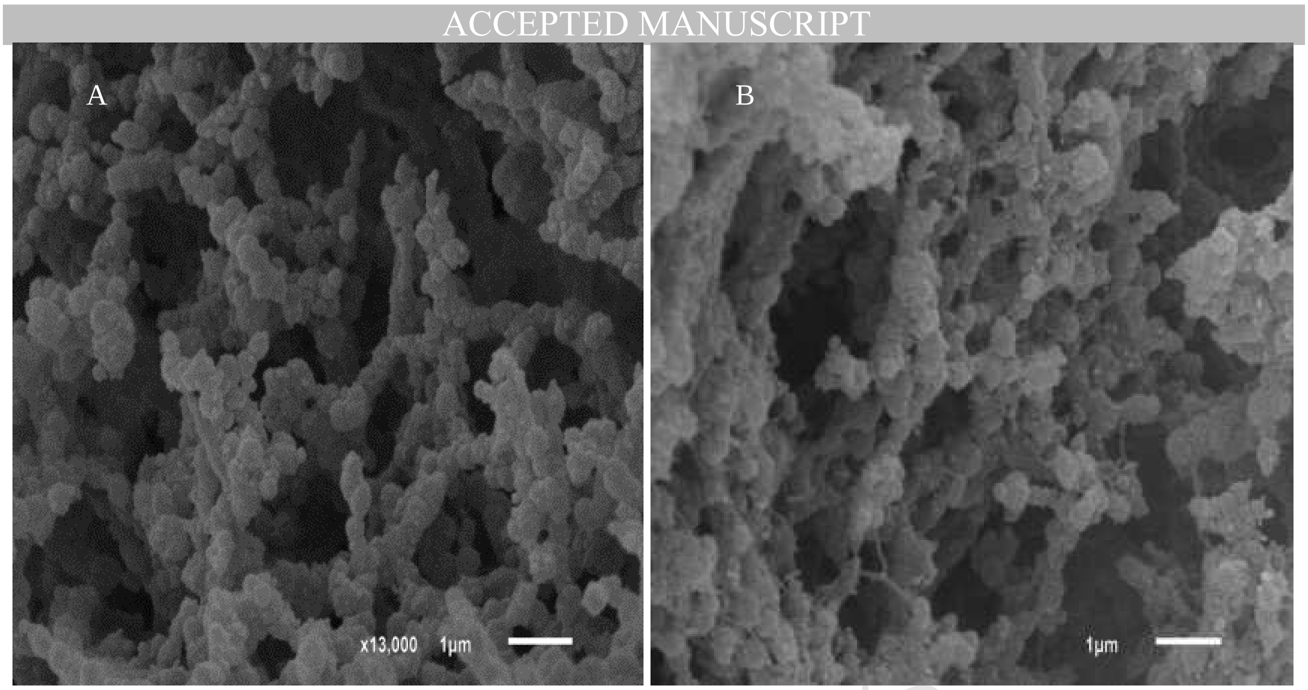
Figure 1



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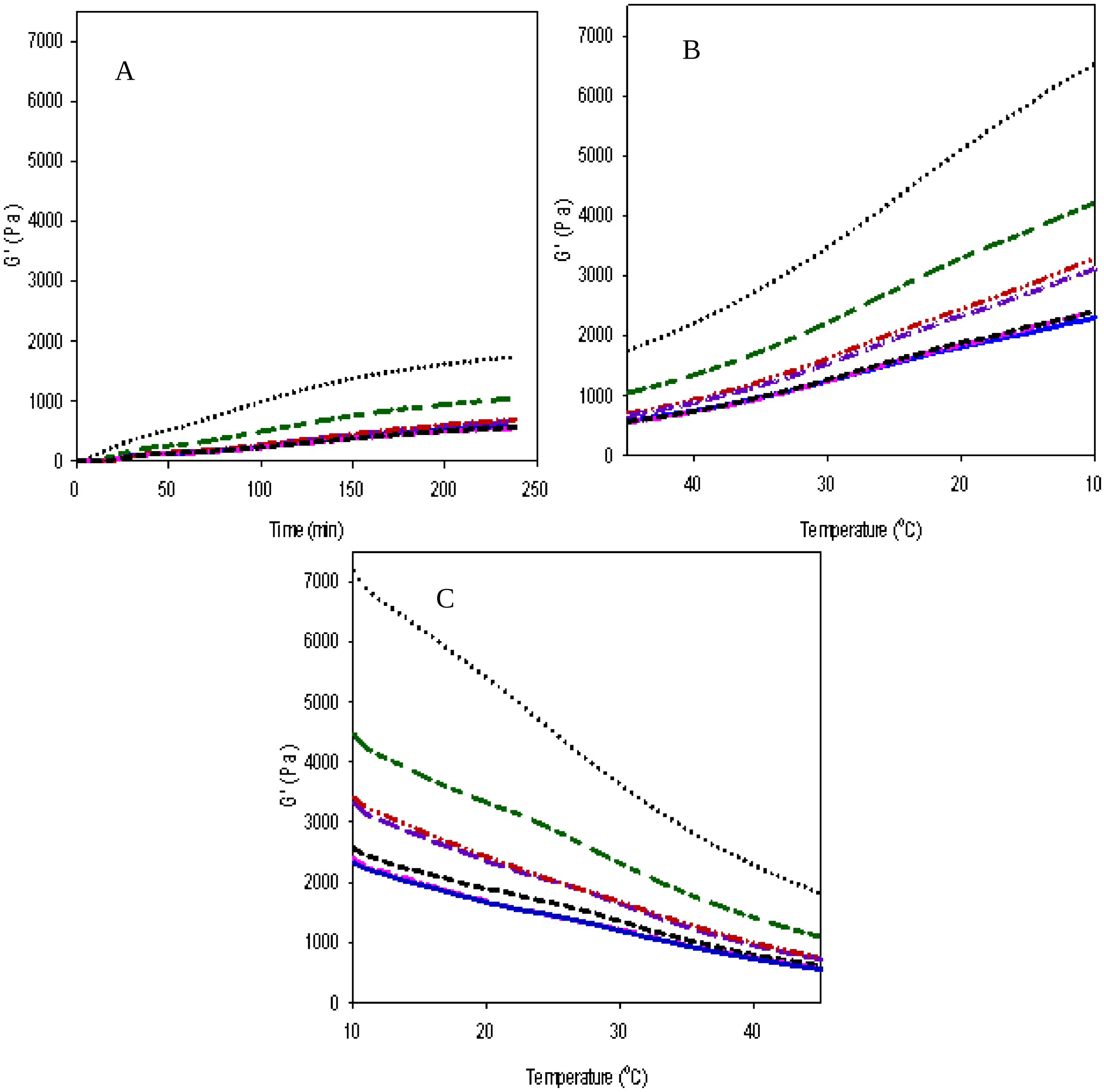
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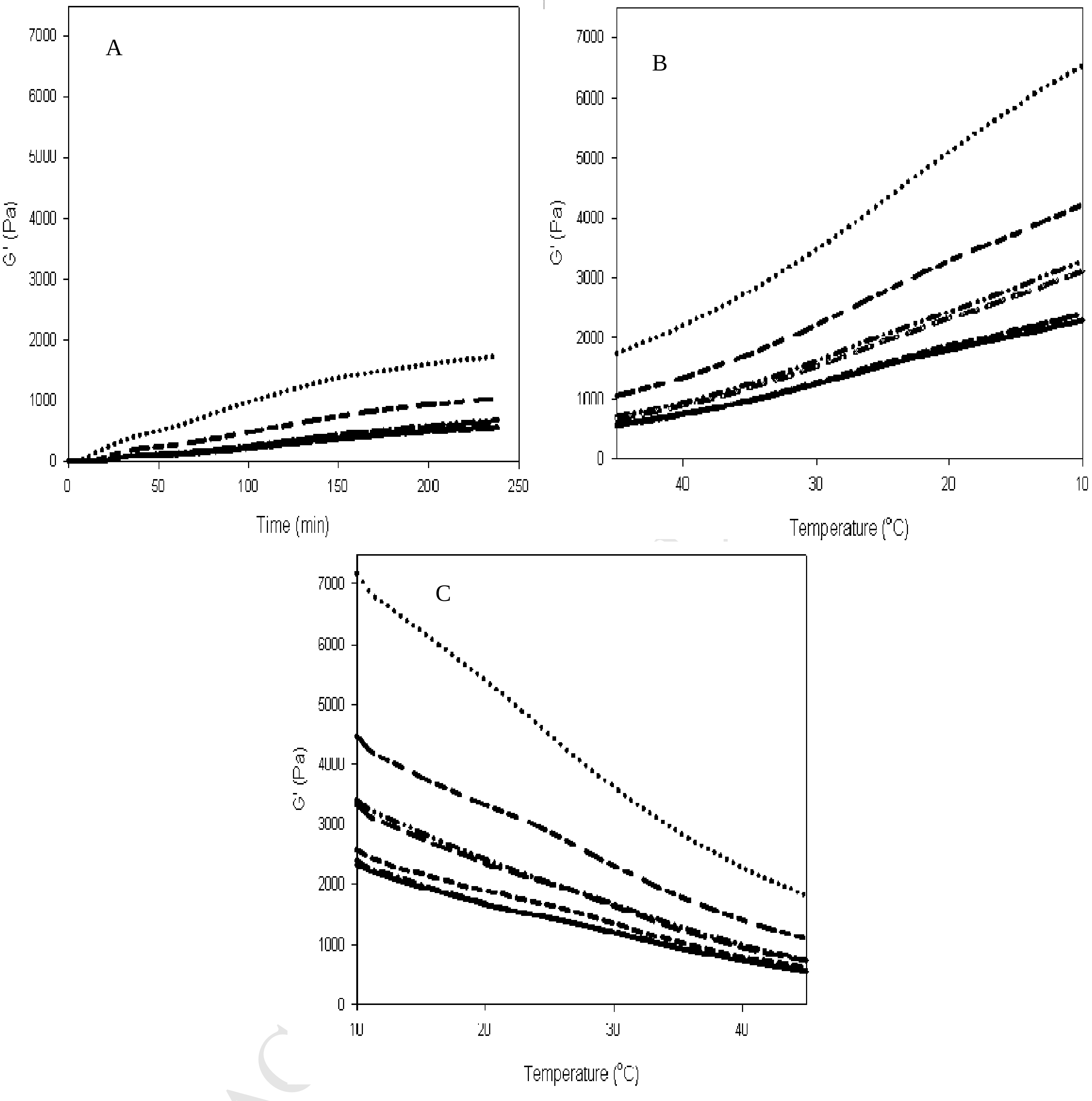
Figure 3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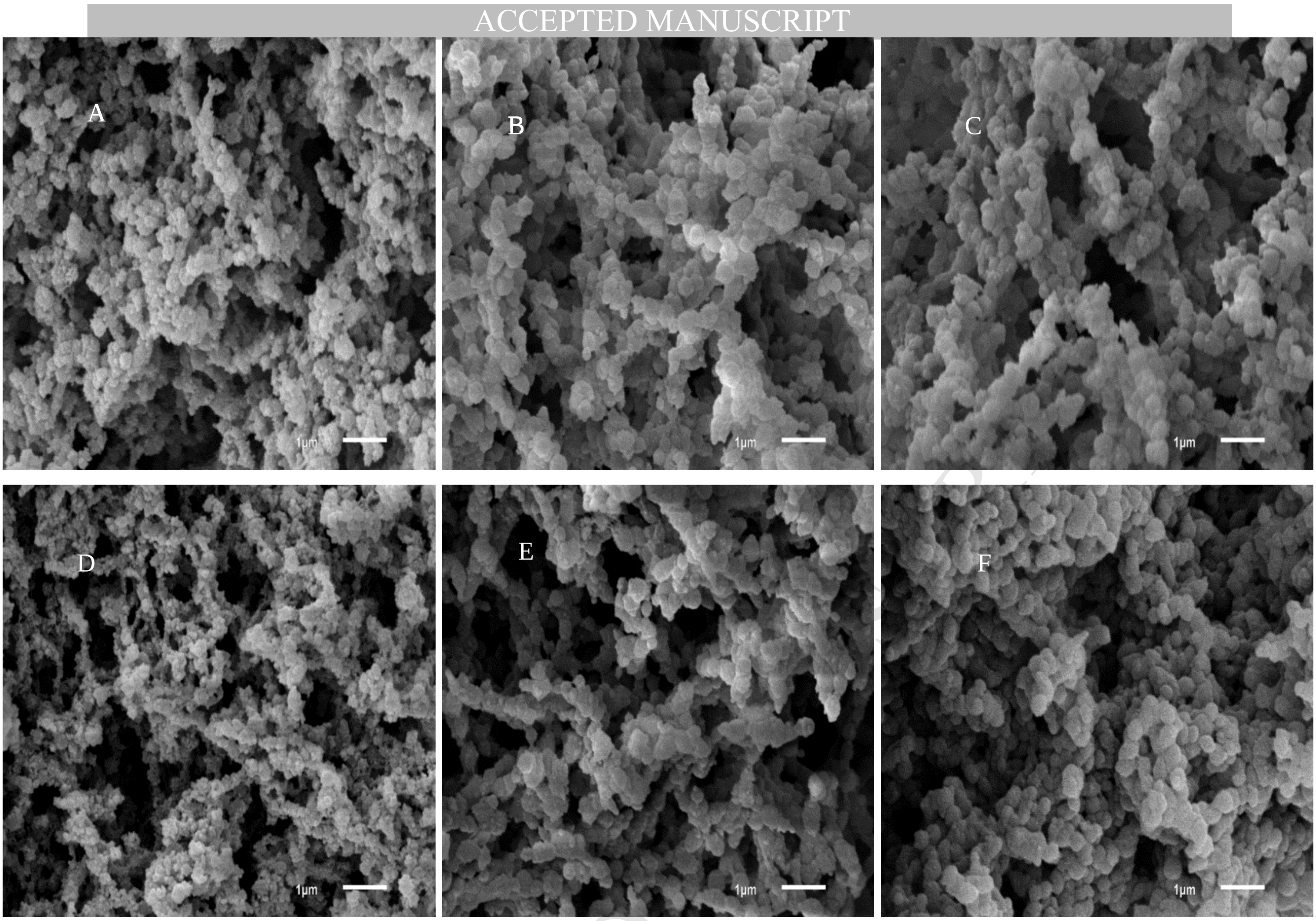

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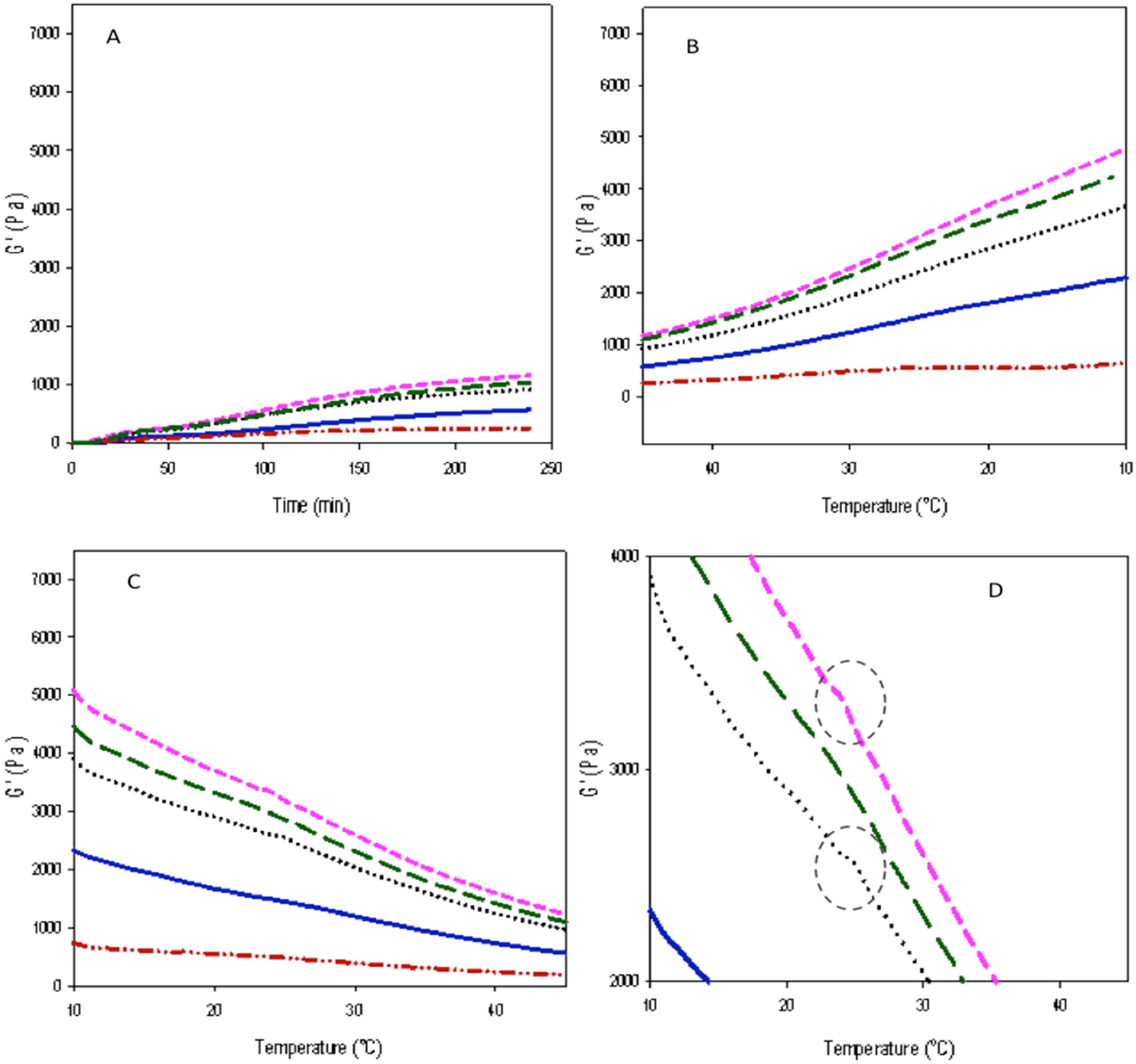
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Figure 5

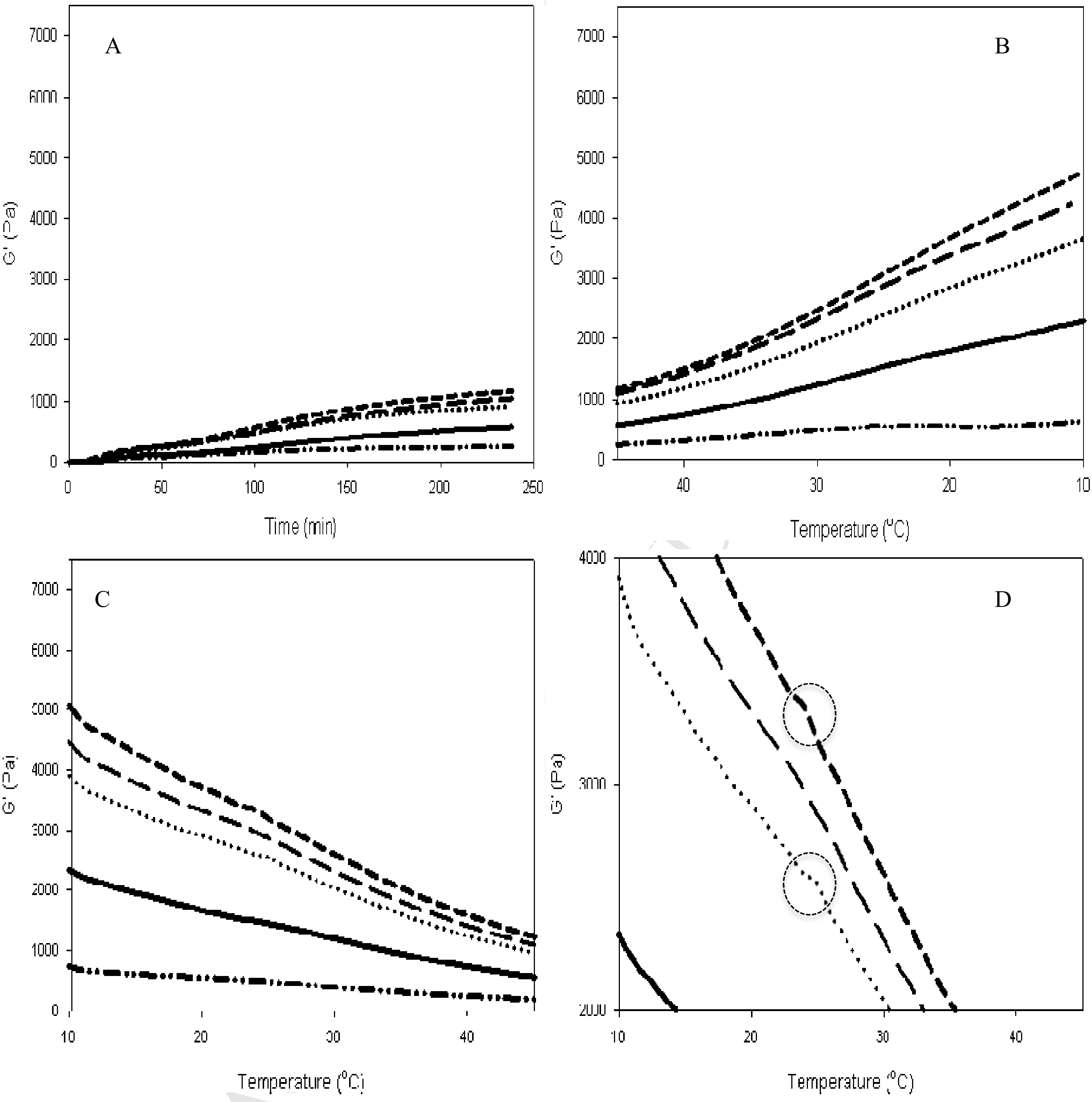
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Figure 5

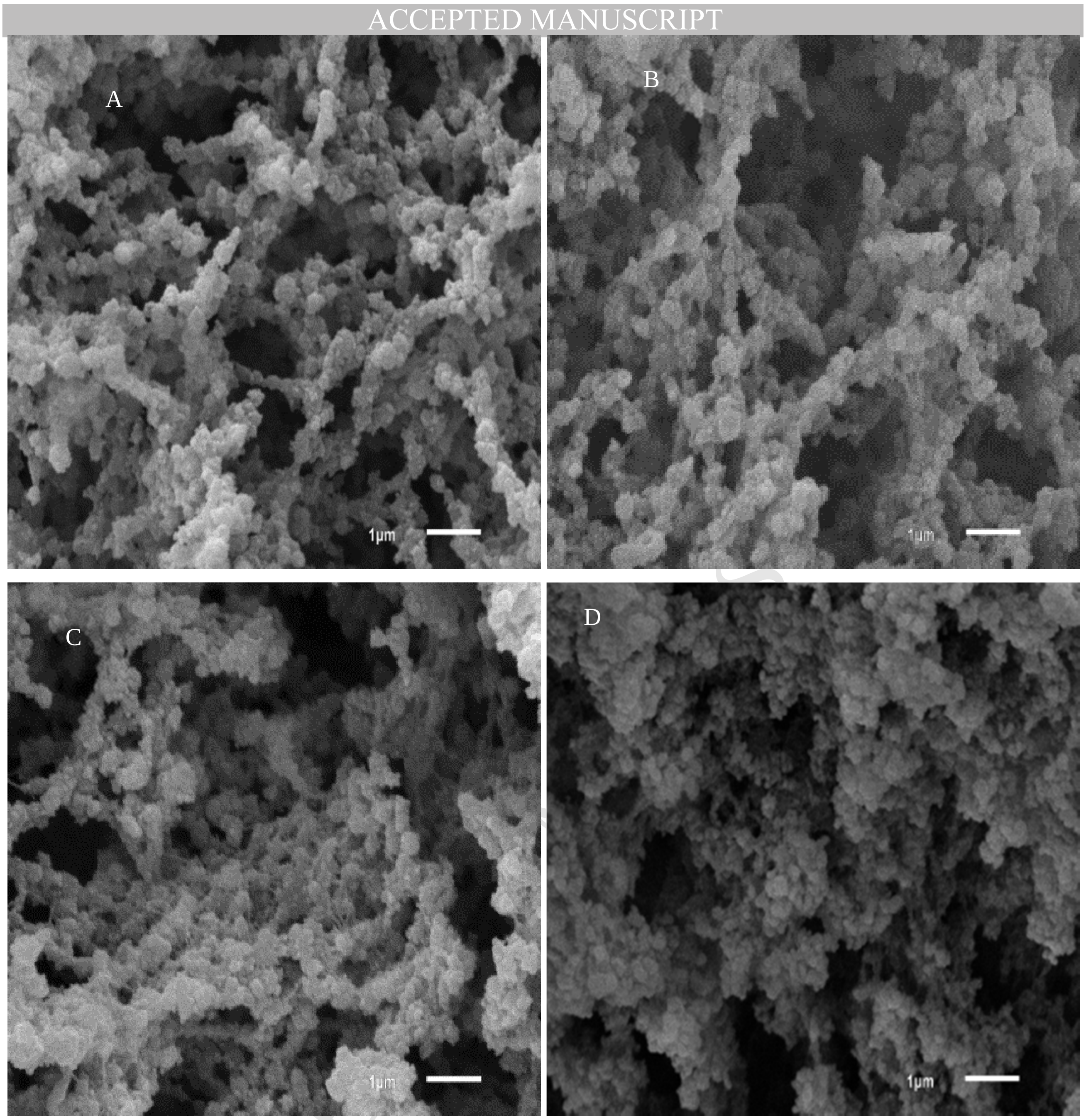

Figure 6

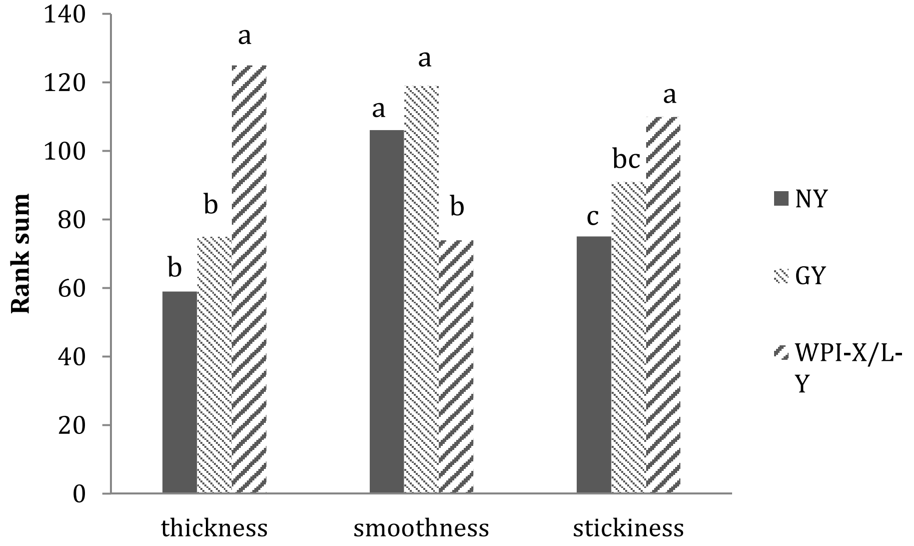
140
120
100
80
60
40
20
0
Rank sum
a
b
b
a
a
b
a
bc
c
thickness
smoothness
stickiness
NY
GY
WPI-X/L-Y

Figure 7

Highlights

1. Xanthan/locust bean gum (X/L) showed gelling properties, like gelatin
2. WPI enhanced water holding capacity of milk gels effectively
3. Combination of WPI and X/L showed promise as a replacer for gelatin in yogurt