



Rheological behavior of a pectic fraction from the pulp of cupuassu (*Theobroma grandiflorum*)

Lúcia C. Vriesmann, Joana L.M. Silveira, Carmen Lúcia de O. Petkowicz *

Universidade Federal do Paraná, Departamento de Bioquímica e Biologia Molecular, CP 19046, CEP 81531-990, Curitiba, PR, Brazil

ARTICLE INFO

Article history:

Received 30 December 2008

Received in revised form 28 July 2009

Accepted 6 August 2009

Available online 12 August 2009

Keywords:

Cupuassu

Theobroma grandiflorum

Pectin

Starch

Gel

Rheology

ABSTRACT

Rheological examination of water-soluble pectic fractions (W-1 and PW-1) isolated from the pulp of cupuassu (*Theobroma grandiflorum*) were evaluated to determine the influence of starch on the water-soluble pectin. Pectin gels (1–3%, w/w) were prepared at pH 3.0 in the presence of high sucrose concentrations (55–65%). Small deformation measurements of storage (G') and loss (G'') moduli at 25 °C showed that increasing the pectin and the sucrose concentrations of the gels both resulted in an increase of G' . In general, stronger gels were those obtained for fraction W-1, in terms of viscoelastic properties as well as thermal stability, suggesting that starch could be cooperating in the formation of water-soluble pectin gels.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Pectins are anionic polysaccharides extractible from plant cell walls. Due to their thickening and gelling properties, pectins are used in a variety of food products, including jams, jellies, confectioneries and dairy products. Pectins are stable at low pH values and can be applied in acidic food products (May, 1990; Rolin, 1993; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

Pectins are polysaccharides consisting of α -D-galacturonic acid residues with a low rhamnose content, the former being substituted by side chains containing other neutral sugars, such as arabinose and galactose. The galacturonic acid residues can be partly methyl-esterified at the C-6 carboxy group, and in OH-2 and OH-3 partly acetylated. The proportion of methyl-esterified compared to total carboxyl groups is expressed as degree of esterification (DE), which classifies pectins as being high-methoxyl (HM) with DE > 50%, or low-methoxyl (LM) with DE < 50% (Ridley, O'Neill, & Mohnem, 2001; Rolin, 1993; Voragen et al., 1995).

Gelation of pectins is affected by the temperature and several parameters related to pectin structure, including the degree of esterification (DE), molecular weight and charge distribution along their backbone. Depending on their DE, pectins can form gels when subjected to different conditions. High-methoxyl pectins (HM), with DE > 50%, require a relatively high concentration of soluble solids, as sucrose, at low pH, where electrostatic repulsions and

the activity of water are reduced. Whereas low-methoxyl (LM), with DE < 50%, only require the presence of divalent cations, particularly Ca^{2+} , over a wide range of pHs, and can be used in low calorie foods (Ridley, O'Neill, & Mohnem, 2001; Rolin, 1993).

In one case, gel formation is governed mainly by hydrophobic interactions and hydrogen bonding (Oakenfull, 1991), and in another, involves electrostatic interactions, where carboxy groups of different pectin chains are linked together by calcium ions (Axelos & Thibault, 1991).

Pectins have numerous applications and new sources are constantly sought. Although they commonly occur in most plant tissues, the number of sources commercially used is quite limited and commercial pectins are almost exclusively derived from either citrus peels or apple pomace (May, 1990; Simpson, Egyankor, & Martin, 1984).

Pectins are solubilized from the fruit during jam making and give the final product its characteristic gel texture (Candy, 1980). Cupuassu (*Theobroma grandiflorum*) is a Brazilian fruit from the Amazon, whose pulp is used in domestic jam and jelly production (Cavalcante, 1991), indicating the presence of gelling agents.

Cupuassu (*T. grandiflorum*) is a native Brazilian Amazon tree which belongs to the same genus as that of cocoa. Its fruits are large, round and have a hard, smooth shell that is dark brown. Its seeds are surrounded by a white pulp. When ripe, the cupuassu simply falls to the ground and does not need to be harvested from the tree. The fleshy pulp is removed, frozen and sold. The seeds are used to prepare a chocolate-like product known as cupulate (Ministério da Educação, 2007).

* Corresponding author. Tel.: +55 41 3361 1661; fax: +55 41 3266 2042.
E-mail address: clp@ufpr.br (C.L.O. Petkowicz).

Many types of delicacies are made from the pulp which is known by the strong flavour. Several companies in Amazon are exporting the frozen pulp to other countries. In the first half of 2005, Brazil exported 50 tons of pulp and the profits from exports reached US\$ 20 million in the same period (Ministério da Educação, 2007).

In a previous investigation, we described the extraction and characterization of a water-soluble pectin (W-1) from cupuassu pulp (Vriesmann & Petkowicz, 2009). We now study its rheological properties, being analyzed before and after enzymatic treatment to remove starch, in order to evaluate its contribution in gel behavior of the pectin.

2. Materials and methods

2.1. Pectin sample

After enzyme-inactivation with methanol–H₂O (4:1, v/v) under reflux for 20 min, the grounded pulp fruit was defatted with *p*-toluene–ethanol (2:1, v/v) in a Soxhlet and dried. The residue was submitted to sequential extractions with water, citric acid solutions and aqueous NaOH. Fraction W-1 was obtained by aqueous extraction at 25 °C for 90 min according to Vriesmann and Petkowicz (2009). The extract was concentrated and treated with ethanol (2:1, v/v) in order to obtain precipitated polysaccharide, which was then washed three times with ethanol and dried under vacuum.

The fraction contained a low proportion of starch, and was then treated with amylase lot no. 064K8806 (Sigma–Aldrich Co., St. Louis, MO, USA) and amyloglucosidase lot no. 50907 (Megazyme International Ireland Ltd., Wicklow, Ireland), according to the manufacturer's recommendation. The aqueous solution was boiled for 15 min for enzyme-inactivation and after centrifugation, the supernatant was dialyzed and added to two volumes of ethanol, resulting in precipitated starch-free PW-1.

The uronic acid was estimated by the sulfamate/3-phenylphenol colorimetric method (Filisetti-Cozzi & Carpita, 1991), using galacturonic acid as standard and the moisture content was calculated as the weight loss after drying at 105 °C.

Polysaccharides were hydrolyzed with 2 M TFA (5 h, 100 °C), and the products reduced with NaBH₄ (Wolfrom & Thompson, 1963b) to give alditols, which were acetylated with pyridine–acetic anhydride (1:1, v/v, 16 h at 25 °C) (Wolfrom & Thompson, 1963a). The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) using a model 5890 S II Hewlett–Packard gas chromatograph at 220 °C (flame ionization detector and injector temperature, 250 °C) with a DB-210 capillary column (0.25 mm internal diameter × 30 m), film thickness 0.25 µm, the carrier gas being nitrogen (2.0 ml/min).

2.2. FT-IR spectroscopy

Fourier transform-infrared (FT-IR) spectra were collected at the absorbance mode in the frequency range of 4000–400 cm^{−1} (Mid infrared region) using a Bomem MB-100 Spectrophotometer (Hartman & Braun, Canada), at 4 cm^{−1} resolution, employing powdered samples. Spectroscopic grade KBr powder (Merck, Darmstadt, Germany) was used and discs were prepared using a 90:10 salt:sample proportion.

2.3. Preparation of samples for rheological analysis

Samples of W-1 and PW-1 were prepared with concentrations of 1%, 2% and 3% (w/w) and to each, sucrose was added to obtain to a 65% (w/w) concentration. For the intermediate polysaccharide

concentration (2%), 55% and 60% sucrose (w/w) were also employed. Weighed amounts of W-1 and PW-1 were dissolved in de-ionized water with agitation over 16 h at 25 °C. Sucrose in the adequate proportion was then added. After solubilization, the mixtures were heated at 92 °C with continuous stirring for 15 min and cooled to room temperature. Their pH was then adjusted to 3.0 with a saturated solution of citric acid. The samples were stored under refrigeration for 16 h and then analyzed.

2.4. Rheological measurements

Rheological analysis were carried out using a model RS 75 Haake Rheometer, coupled with a DC5 heating circulator, with a C60/2° sensor (cone and plate geometry) or PP20/2° sensor (plate and plate geometry). Mechanical responses of gels were determined by subjecting them to a frequency sweep (0.1–10 Hz) at 25 °C with strain between 0.04% and 4%. These values refer to the viscoelastic-linear region, where the gel structure was preserved. The temperature of all analyses was controlled with a Peltier system, and in experiments with variation of temperature, the heating (5–95 °C) and the cooling (95–5 °C) gradients were performed at a rate of 1 °C/min, at a frequency of 1 Hz. The exposed sample edge was covered with a thin layer of low viscosity mineral oil to prevent evaporation of water during measurements.

3. Results and discussion

3.1. Characterization of starch-free PW-1

W-1 fraction contained 65.4% galacturonic acid and its degree of esterification (DE) was 53.1% (Vriesmann & Petkowicz, 2009). It had a low proportion of starch (7 mol%), and was then treated with amylase and amyloglucosidase, resulting in the starch-free fraction, PW-1 (purified W-1). The monosaccharide composition of PW-1 and W-1 (moisture contents of 9.0 ± 0.2 and 7.6 ± 0.4, respectively) is shown in Table 1. Fraction PW-1 contained mainly galacturonic acid (81.9 mol%), a value greater than that found by Koubala et al. (2008) for water-soluble pectins from ambarella peels (72.7%) and lime (49.3%).

The significant reduction of Glc content in PW-1 indicates that most of the starch present in fraction W-1 was removed. O'Donoghue and Somerfield (2008) combined extraction of pectin from buttercup squash with starch digestion, using amylase, amyloglucosidase and pullulanase, but even so, residual starch remained in the pectins.

Fig. 1 shows the FT-IR spectrum of fraction PW-1, the bands centered at 1749 and 1630 cm^{−1} having been used to determine the degree of esterification (DE) in PW-1 according to Vriesmann and Petkowicz (2009). The band centered at 1749 cm^{−1} arose from the ester carbonyl groups and that at 1630 cm^{−1}, from the carboxylate ion stretching band. The relative intensity of these two bands shows a DE of 51.3%, similar to that previously determined for the native fraction, W-1 (DE 53.1%). This slight difference could be due to the processing during purification since the enzyme-inactivation was complete after boiling.

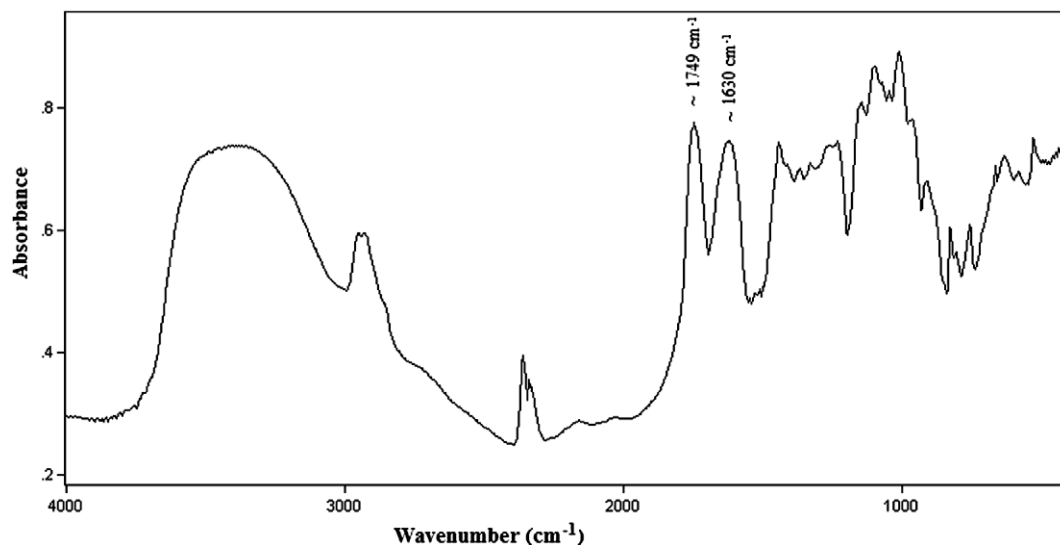
3.2. Rheological analysis

Fractions W-1 and PW-1 are composed mainly of HM pectins. Their gel formation occurs in the presence of high concentrations of co-solutes and acid pH.

Low pH reduces the negative charges on the carboxy groups, thus minimizing electrostatic chain repulsion, whereas a high sucrose concentration creates low activity of water, where chain to chain interactions are greater when compared with those of chain

Table 1Monosaccharide composition^a of fractions PW-1 and W-1.

Fraction	Rha (mol%)	Fuc (mol%)	Ara (mol%)	Xyl (mol%)	Man (mol%)	Gal (mol%)	Glc (mol%)	GalA ^b (mol%)
PW-1	1.9	–	3.9	0.8	0.9	9.8	0.8	81.9
W-1 ^c	3.3	0.6	5.9	3.9	1.0	12.8	7.1	65.4

^a Determined by GLC.^b Determined by colorimetric method.^c From Vriesmann and Petkowicz (2009).**Fig. 1.** FT-IR spectrum of PW-1.

to water. In this way, hydrophobic methoxyl group interactions and hydrogen bonds between pectin chains are facilitated (Voragen et al., 1995).

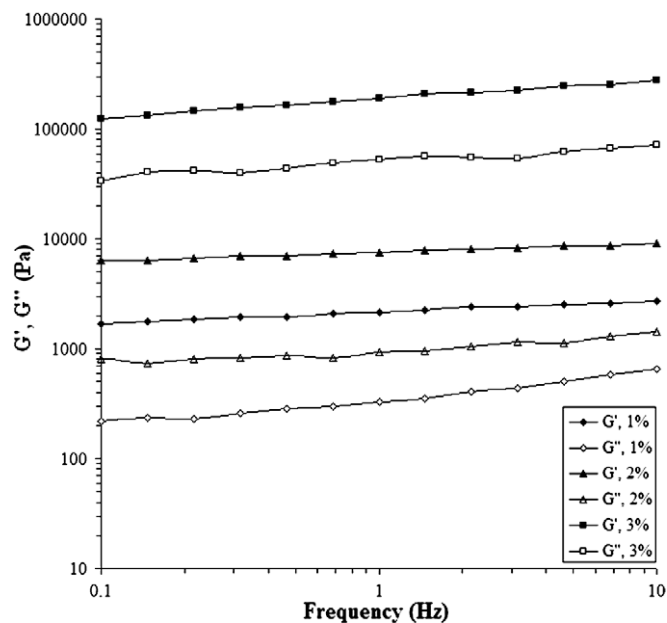
With reduction of repulsion and less water available to solvate HM pectin, the chain to chain approach, facilitating the formation of junction zones. These are stabilized by hydrogen bonding between undissociated carboxyl and secondary alcohol groups, and by hydrophobic interactions between methoxyl groups (Thakur, Singh, & Handa, 1997; Voragen et al., 1995; Walkinshaw & Arnott, 1981).

Although other co-solutes, such as xylitol, sorbitol, fructose and glucose, can be employed in HM pectin gels, Tsoga, Richardson, and Morris (2004) showed that sucrose addition produced better results for commercial HM pectins. Accordingly, samples of W-1 and PW-1 were prepared in the presence of a high sucrose concentration at pH 3.0.

Initially, samples were prepared with a fixed sucrose content (65%, w/w) varying pectin concentration from 1% to 3% (w/w) at pH 3.0 in order to verify their influence on rheological behavior. Afterwards, samples were prepared at a fixed pectin concentration (2%, w/w) varying sucrose content (55–65%, w/w) at pH 3.0 in order to verify the influence of sucrose concentration on rheological behavior. The comparative results obtained for native W-1 and starch-free PW-1 are shown and discussed as follows.

3.2.1. Effect of pectin concentration on viscoelastic behavior

Figs. 2 and 3 show the variation of storage (G') and loss moduli (G'') with frequency at 25 °C for W-1 and PW-1 samples at different respective concentrations. The samples were prepared at pH 3.0, using 65% (w/v) sucrose. For all samples, a typical gel-like behavior was observed, where G' is greater than G'' along the complete frequency scale and G' is less dependent on frequency than G'' , indicating the predominance of a solid-like character, as would be

**Fig. 2.** Frequency sweeps at 25 °C of fraction W-1 at 1%, 2% and 3% with 65% sucrose in strains of 0.4%, 0.12% and 0.04%, respectively.

expected for an elastic network (Clark & Ross-Murphy, 1987; Endress, Döschl-Volle, & Dengler, 1996; Morris, 1995).

Furthermore, an increase in pectin concentration resulted in a large increase in the G' values, presumably due to an increase in the number of cross-linked junction zones between the pectin chains. According to Glicksman (1984), the resistance of HM pectin gels increases, when the polysaccharide concentrations are raised. When the polysaccharide concentration is reduced, there is an

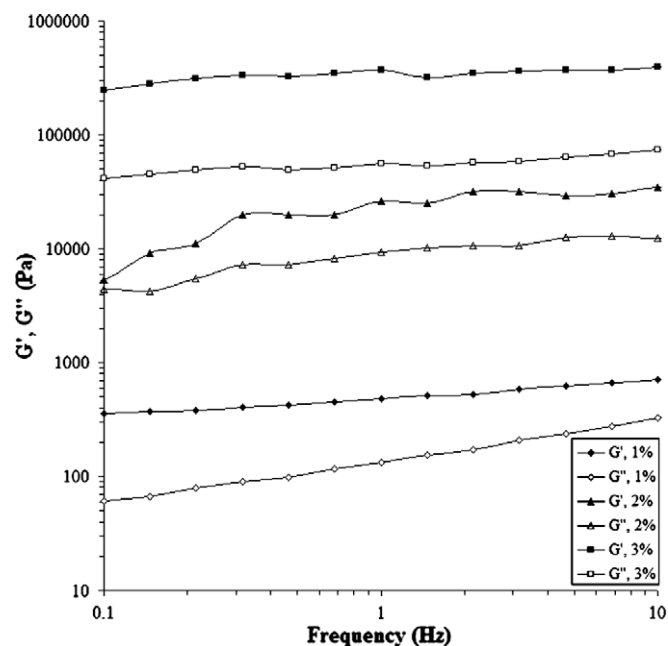


Fig. 3. Frequency sweeps at 25 °C of fraction PW-1 at 1%, 2% and 3% with 65% sucrose in strains of 2.0%, 0.3% and 0.22%, respectively.

increasing probability of intramolecular junctions being formed, which do not contribute to intermolecular cross-linking (Morris, 1995).

When gels of W-1 and PW-1 are compared under the same conditions, it can be seen that PW-1 gels have a greater dependence of G' and G'' on frequency than those of W-1, especially with G' and mainly in samples with lower pectin concentrations.

For the 1% PW-1 gel, G' and G'' moduli values and the difference between them are less than those for fraction W-1, being more dependent on frequency. For the 2% PW-1 gel, although both moduli values are greater than for fraction W-1, lower difference occurred with a greater oscillation during the analysis. For the 3% PW-1 gel, both the difference between the moduli and the magnitude of their values were greater than those for 3% W-1, especially at lower frequencies.

3.2.2. Effect of sucrose concentration on viscoelastic behavior

The intermediate concentration of sample of 2% was chosen to check the effect of sucrose concentration on rheological behavior.

Figs. 4 and 5 show the results obtained at 25 °C with 2% W-1 and PW-1 fraction samples, respectively, at different sucrose concentrations. Frequency sweeps show that G' is greater than G'' throughout the experimental frequency range (0.1–10 Hz) for both fractions at all sucrose concentrations, showing that the samples had a predominantly solid-like response, characterizing strong gels (Morris, 1995).

Furthermore, magnitudes of G' were dependent on sucrose concentration, similar to that occurring on increase of concentration. At a given pectin concentration, G' values increased with the sucrose content, which reduces the water activity, presumably due to an increase in junction zones and chain entanglements.

Sharma, Liptay, and Le Maguer (1998) showed that the gel strength of HM pectin from tomato fruit pulp, at a constant pH, increased with sucrose concentration from 50% to 70%, as did BeMiller (1986), probably due an increased size and number of junction zones during gelation obtained by higher pectin concentration.

Fu and Rao (2001) suggested that G' increase in LM pectin gels with those of sucrose content indicates that the co-solute may pro-

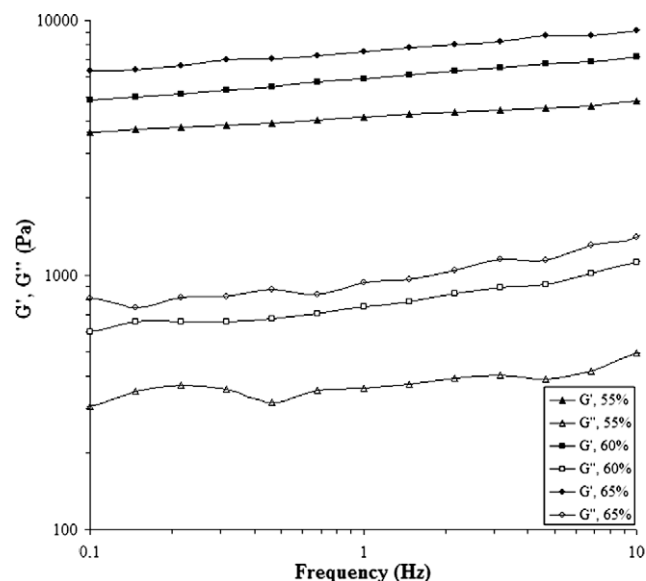


Fig. 4. Frequency sweeps at 25 °C of fraction W-1 at 2% with 55%, 60% and 65% of sucrose in strains of 0.27%, 0.7% and 0.12%, respectively.

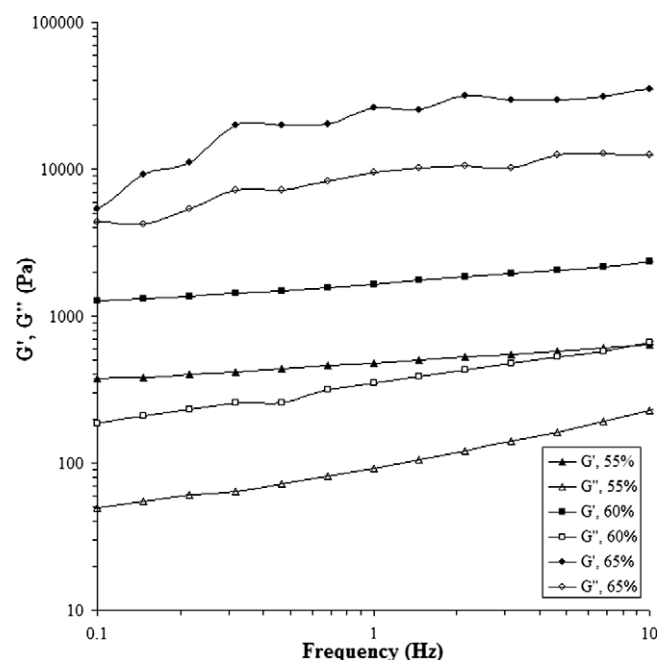


Fig. 5. Frequency sweeps at 25 °C of fraction PW-1 at 2% with 55%, 60% and 65% of sucrose in strains of 4.0%, 0.4% and 0.3%, respectively.

vide additional hydroxyl groups to stabilize the structure of junction zones and promote hydrogen bonds to immobilize water molecules, thus facilitating gel formation.

When W-1 and PW-1 gels were compared, using the same analysis conditions, it is notable that PW-1 gels had lower values of G' and G'' and they are more sensitive to frequency variations. This indicates that their viscoelastic structure is more fragile than those of W-1 gels.

Whereas W-1 samples had both G' e G'' moduli that were essentially independent over the entire frequency range, PW-1 samples containing lower sucrose concentrations of 55% and 60% showed a greater dependence of G' and G'' on frequency, especially found with G'' . In spite of the 65% sucrose gel having greater values of G' than the equivalent gel of W-1, it can be seen that the difference

between the moduli is less than with W-1 gels and that oscillations also occurred during the experiment (Fig. 5).

In general, for water-soluble pectin from cupuassu fruit pulp, starch-free PW-1 gels had a lesser elastic component and were more sensitive to frequency increases than with native W-1 gels, both on variation of the pectin and sucrose contents. These results suggest that starch probably cooperates in forming the structural network of the W-1 pectin gel.

Khondkar, Tester, Hudson, Karkalas, and Morrow (2007) characterized rheologically binary systems of uncross-linked and cross-linked waxy maize starch with low-methoxyl pectin and found that the elastic component of all starch gels increased after cross-linking, suggesting that cross-linking between starch and pectin molecules can give rise to novel rheological properties. Their results and those now obtained indicate that starch and pectin probably have synergism in mixed polymer systems, but the rheological responses were dependent of the quantity, processing and nature of these polysaccharides.

3.2.3. Effect of temperature on G' and G''

To evaluate their thermal stability, the gels were submitted to temperature variations, from 5 to 95 °C at 1 °C/min, and vice versa.

Fig. 6 shows the behavior of G' during heating of gels containing 65% sucrose and different W-1 contents (1%, 2% and 3%, w/w). For all W-1 concentrations, G' values decreased during heating, although they were greater than those of G'' during the entire process (data not shown), consistent with predominance of the solid character of the gels.

Elastic (G') and viscous (G'') moduli had dependence on temperature, which can be attributed to different thermal behavior of intermolecular interactions that do not stabilizes any permanent cross-linking of the HM pectin gel network (Lopes da Silva & Gonçalves, 1994).

During the heating-cooling cycle, 1%, 2% and 3% W-1 and PW-1 gels with 65% sucrose each had a similar behavior, where G' and G'' values decreased with temperature increase, and on cooling did not return to their initial values. However, PW-1 showed lower thermal stability than the respective W-1 samples (data not shown).

Our samples with varying sucrose concentrations were submitted to temperature gradients under identical conditions as explained for samples with varying pectin content. Fig. 7 shows a slight reduction of G' for 2% (w/w) W-1 samples (pH 3.0) in the presence of 55%, 60% and 65% sucrose (w/w) during heating from 5 to 95 °C. Those containing 55% and 60% sucrose were the more thermally stable.

A behavior different from that of W-1 type gels was encountered, during heating of HM pectin gels by Evageliou, Richardson,

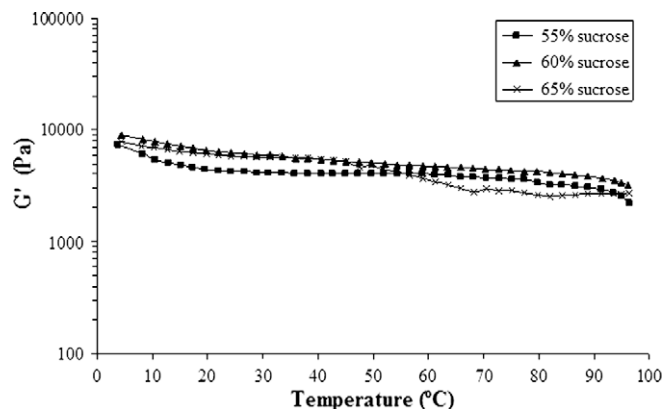


Fig. 7. Effect of increasing temperature on G' of W-1 pectin gels containing different sucrose contents at 1 Hz.

and Morris (2000) for those containing sucrose as co-solute, and by Tsoga et al. (2004) for gels with xylitol and other co-solutes. They observed that there was an initial reduction of their G' values with a subsequent increase at higher temperatures, which was attributed to an increase in the degree of cross-linking of the hydrophobic interactions between methyl ester groups.

After heating followed by cooling to 5 °C, 2% W-1 gel (w/w) with 55% and 60% sucrose (w/w) showed a slight increase in the moduli values when compared with initial ones (data not shown). The data obtained for 55% and 60% sucrose W-1 gels suggest that the total thermal stability could arise from simple preservation and stabilization of structures formed during cooling after preparation of the gel, with no indication of any significant intermolecular associations occurring during heating.

However, 65% sucrose W-1 gel had a different behavior after the thermal cycle, with an accentuated decrease of G' when returned to 5 °C (data not shown).

Tsoga et al. (2004) studied rheological changes in G' and G'' on cooling, heating and re-cooling with 1.0% HM pectin (pH 3.0) in the presence of 50%, 55%, 60% and 65% xylitol, sorbitol, glucose, fructose and sucrose. They showed that depletion in gel strength on heating and re-cooling became more pronounced when the concentration of co-solute was raised to 65%. This observation could explain our results obtained with the 65% sucrose W-1 gel.

W-1 and PW-1 samples (2%) at pH 3.0, with different sucrose concentrations, were also submitted to temperature variations (data not shown) and PW-1 gels were more sensitive to temperature changes than the respective samples of W-1.

To summarize, for all samples, including those with variations in pectin and sucrose contents, G' values were greater than those of G'' throughout the heating and cooling process, characterizing the predominance of elastic behavior, for both W-1 and PW-1 gels. PW-1 gels showed lower thermal stability with higher reductions in G' and G'' during heating, and after cooling did not return to their initial values.

From our results, it can be suggested that starch has a synergistic effect in the gel formation of water-soluble pectin from cupuassu pulp, mainly in factors related to thermal stability. Starch could be assisting in formation and stabilization of interactions between pectin molecules. It is possible that the starch stabilizes certain pectin chain conformations, which are more favorable to intermolecular linking.

3.2.4. Effect of calcium addition to PW-1

Since fraction PW-1 contained a degree of methyl esterification slightly higher than 50%, it was also tested for gel formation in the presence of calcium ions. Initially, samples were prepared at 1%

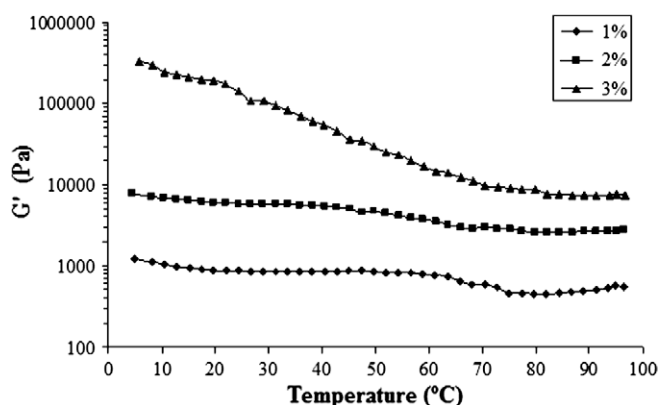


Fig. 6. Effect of increasing temperature on G' of 1%, 2% and 3% W-1 pectin gels containing 65% sucrose contents at 1 Hz.

with 0.1% calcium and 20% sucrose at pH 3.0. After successive heating, cooling and resting under refrigeration, gel formation did not occur. Tests with increasing pH, calcium and sucrose contents were carried out and again the gel formation did not take place. According to Voragen et al. (1995), a modification of calcium affinity towards randomly charged pectins occurs at a DE of about 50% and above this value, the behavior of the pectins in relation to calcium-induced gelation is roughly that predicted by theory. Thus, HM pectins will form gel at acidic pHs in the presence of high co-solute concentrations, but do not need calcium ions.

4. Conclusions

Rheological analysis of water-soluble native pectic fraction (W-1) and starch-free water-soluble pectic fraction (PW-1) from cupuassu pulp showed that W-1 gels are influenced by increases in pectin and sucrose concentrations, and are relatively thermally stable. PW-1 gels had the same behavior pattern, although their thermal stability and viscoelastic structure appeared to be more fragile than those of W-1 gels. The results indicate that starch presumably gave rise to a synergistic effect in gel formation of the water-soluble pectin from cupuassu pulp.

Acknowledgements

The authors thank the Brazilian agencies, CNPq and Fundação Araucária-PRONEX for financial support. Special thanks are due to Dr. Philip A.J. Gorin for providing suggestions to improve the English in this paper.

References

- Axelos, M. A. V., & Thibault, J. F. (1991). The chemistry of low-methoxyl pectin gelation. In R. H. Walter (Ed.), *The chemistry and technology of pectin* (pp. 109–118). New York: Academic Press.
- BeMiller, J. N. (1986). An introduction to pectin: Structure and properties. In M. L. Fishman & J. J. Jen (Eds.), *Chemistry & function of pectins* (pp. 2–12). Washington, DC: ACS Symposium Series 310, American Chemical Society.
- Candy, D. J. (1980). *Biological functions of carbohydrates* (pp. 139–143). Glasgow: Blackie.
- Cavalcante, P. B. (1991). *Frutas Comestíveis da Amazônia* (5th ed., pp. 90–91). Belém: Edições CEJUP, 1991: CNPq: Museu Paraense Emílio Goeldi.
- Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymers gels. *Advanced Polymer Science*, 83, 57–192.
- Endress, H. U., Döschl-Voll, C., & Dengler, K. (1996). Rheological methods to characterize pectins in solutions and gels. In J. Visser & A. G. J. Voragen (Eds.), *Progress in biotechnology: Pectins and pectinases* (vol. 14, pp. 407–423). Amsterdam: Elsevier.
- Evageliou, V., Richardson, R. K., & Morris, E. R. (2000). Effect of pH, sugar type and thermal annealing on high methoxyl pectin gels. *Carbohydrate Polymers*, 42, 245–259.
- Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, 197, 157–162.
- Fu, J.-T., & Rao, M. A. (2001). Rheology and structure development during gelation of low-methoxyl pectin gels: The effect of sucrose. *Food Hydrocolloids*, 15, 93–100.
- Glicksman, M. (1984). *Food hydrocolloids* (Vol. III, pp. 205–229). Florida: CRS Press.
- Khondkar, D., Tester, R. F., Hudson, N., Karkalas, J., & Morrow, J. (2007). Rheological behavior of uncross-linked and cross-linked gelatinised waxy maize starch with pectin gels. *Food Hydrocolloids*, 21, 1296–1301.
- Koubala, B. B., Mbome, L. I., Kansci, G., Tchouanguep Mbiapo, F., Crepeau, M.-J., Thibault, J.-F., et al. (2008). Physicochemical properties of pectins from ambarella peels (*Spondia cytherea*) obtained using different extraction conditions. *Food Chemistry*, 106, 1202–1207.
- Lopes da Silva, J. A., & Gonçalves, M. P. (1994). Rheological study into the ageing process of high methoxyl pectin/sucrose aqueous gels. *Carbohydrate Polymers*, 24, 235–245.
- May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers*, 12, 79–99.
- Ministério da Educação, Secretaria de Educação Profissional e Tecnológica (2007). Cupuaçu. *Thematic Pamphlets*, Brasília, 28p. Available from http://portal.mec.gov.br/setec/arquivos/pdf3/publica_setec_cupuacu.pdf.
- Morris, E. R. (1995). Polysaccharide rheology and in-mouth perception. In A. M. Stephen (Ed.), *Food polysaccharides and their application* (pp. 517–546). New York: Marcel Dekker.
- Oakenfull, D. G. (1991). The chemistry of high-methoxyl pectins. In R. H. Walter (Ed.), *The Chemistry and Technology of Pectin* (pp. 88). New York: Academic Press.
- O'Donoghue, E. M., & Somerfield, S. D. (2008). Biochemical and rheological properties of gelling pectic isolates from buttercup squash fruit. *Food Hydrocolloids*, 22, 1326–1336.
- Ridley, B. L., O'Neill, M. A., & Mohnem, D. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, 57, 929–967.
- Rolin, C. (1993). Pectins (3rd ed., pp. 257–293). In R. L. Whistler & J. N. BeMiller (Eds.), *Industrial gums: Polysaccharides and their derivatives*. San Diego: Academic Press.
- Sharma, S. K., Liptay, A., & Le Maguer, M. (1998). Molecular characterization, physico-chemical and functional properties of tomato fruit pectin. *Food Research International*, 30, 543–547.
- Simpson, B. K., Egyankor, K. B., & Martin, A. M. J. (1984). Extraction, purification and determination of pectin in tropical fruits. *Journal of Food Processing and Preservation*, 8(2), 63–72.
- Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin – a review. *Critical Reviews in Food Science and Nutrition*, 37, 47–73.
- Tsoga, A., Richardson, R. K., & Morris, E. R. (2004). Role of cosolutes in gelation of high-methoxyl pectin. Part 1: Comparison of sugars and polyols. *Food Hydrocolloids*, 18, 907–919.
- Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 287–340). New York: Marcel Dekker.
- Vriesmann, L. C., & Petkowicz, C. L. O. (2009). Polysaccharides from the pulp of cupuassu (*Theobroma grandiflorum*): Structural characterization of a pectic fraction. *Carbohydrate Polymers*, 77, 72–79.
- Walkinshaw, M. D., & Arnott, S. (1981). Conformations and interactions of pectins. II: Models for junction zones in pectinic acid and calcium pectate gels. *Journal of Molecular Biology*, 153, 1075–1085.
- Wolf from, M. L., & Thompson, A. (1963a). Acetylation. *Methods in Carbohydrate Chemistry*, 2, 21.
- Wolf from, M. L., & Thompson, A. (1963b). Reduction with sodium borohydride. *Methods in Carbohydrate Chemistry*, 2, 65.