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Viscoelasticity of Oxidised Starch and Low Methoxy Pectin in the Presence of Glucose Syrup

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لزوجة ومرونة النشا المؤكسد والبكتين منخفض الميثوكسية في وجود مركز عصير الجلوكوز

الملخص: تمت دراسة الخواص البنائية لمخاليط البكتين والنشا المؤكسد وعصير الجلوكوز المركز باستخدام تذبذبات حركية تشويهية ضئيلة. وتم إختيار مكونات مركبات الخليط و درجة حموضتها ودرجة الحرارة أثناء التحليل لمحاكاة تطور منتجات الخليط. في غياب الكالسيوم المضاف ، تكون مركبات البكتين منخفض الميثوكسي مع مركز الجلوكوز محاليل لزجة تبقى خالية من البلورات عند درجة حرارة أقل من الصفر. أظهرت عينات النشا المؤكسد ومركز عصير الجلوكوز شكلا شبه صلب نسبة للطبيعة البلورية للوالب شبه الأميوزية . أظهر خليطا السكريات المتعددة مع المحاليل المرافقة تحولا عكسيا من حالة السائل إلى الحالات شبه الصلبة مع زيادة معدلات النشا في المركب . وانعكس هذا التحول في خصائص الملمس للعينات التي تراوحت من محاليل غليظة إلى جيلوتين صلب . ويمكن تعديل لزوجة ومرونة الخليط بصورة أكبر بإضافة البكتين عالي الميثوكسين . وبالتالي فإن المركبات التي تحوي البكتين عالي الميثوكسين ومركز عصير الجلوكوز تكون جيلاتينا مطاطيا تمر طبيعتها الاميوزية بحالة تحويل وزجاجية خلال فترة التبريد . كانت احتياجات الطاقة للتحول الى المطاط الطبيعي ، في حالة توافق مع المعلومات المتوفرة في البحوث المنشورة .

ABSTRACT: The structural properties of mixtures with pectin, oxidised starch and glucose syrup were investigated using small deformation dynamic oscillation. The composition of the mixture, pH and temperature course of analysis were designed to imitate the development of confectionery products. In the absence of added calcium, preparations of low methoxy pectin with glucose syrup form viscous solutions, which remain crystal-free at subzero temperatures. Samples of oxidised starch and glucose syrup, on the other hand, exhibit solid-like behaviour due to the crystalline nature of the amylose-like helices. Mixtures of the two polyssacharides with the co-solute show clearly the phase inversion from liquid to solid-like behaviour with increasing amounts of starch in the formulation. The transformation is reflected in the textural properties of samples which vary from thick solutions to firm gels. The viscoelasticity of the system can be modified further by the introduction of high methoxy pectin. Thus preparations of high methoxy pectin and glucose syrup form rubbery gels whose amorphous nature undergoes a glass transition during cooling. The energetic requirement for the formation of the biological rubber was in good agreement with corresponding data in the literature.

Pectin is a structural polysaccharide that is found in the cell wall of most higher plants, where in combination with cellulose is largely responsible for firmness of the plant tissue. The main source of commercial pectins is citrus fruits and apple pomace. It is extracted under mildly acidic conditions, precipitated and then ground to a fine powder of standardised properties (May, 1992). Since pectin is present in all fruits and vegetables, it has always been an integral part of the human diet and is an excellent "fibre provider". It has a low net calorific value because is hydrolised in the large intestine rather than the stomach (Rolin, 1994). Structurally, the molecule is a linear polysaccharide made up of about 200 to 1000 1,4-linked α-D-galacturonate residues. Some of the galacturonate residues are esterified at C6 and present as galacturonate acid methyl esters. The chain is interrupted periodically by the insertion of 1,2-linked rhamnose residues, with L-arabinose and D-galactose being covalently bound to rhamnose (Rolin, 1993).

The degree of esterification (DE) gives the ratio of esterified galacturonic acid units to total galacturonic acid units in the molecule. This categorises pectins in two broad classes - low methoxy (LM) with a DE <50% and a high methoxy (HM) with a DE >50%. LM pectin is obtained either enzymatically, *in vivo*, or by the controlled de-esterification of HM pectin in either acidic or alkaline conditions. Ammonia is sometimes used in the process, introducing some amide groups into the molecule, yielding an "amidated" pectin (Kratz, 1995). The pH of most biological and industrial conditions is higher than the pKa of pectin (~ 3.3). Pectin thus exists as a negatively charged, anionic polymer in solution, the size of the charge increasing as the pH is increased (Rinaudo, 1996).

Starch is the chief source of carbohydrate in the human diet. It is isolated from many sources, particularly cereals, where is laid down in the form of granules which are insoluble in the cell medium. Granules vary considerably in shape from spheres to rods, and size from a diameter of 2 μm in the pollen starch amylomaize to 175 μm for canna starch (Galliard, 1987). Starch is composed essentially of two high molecular weight components, amylose and amylopectin. Amylose is a linear polymer in which all anhydroglucose units are linked through 1,4-α glycosidic bonds. Amylopectin has a highly branched structure, consisting of an α-(1$\rightarrow$6)-linked branches. The molecular weight of starch molecules varies from 10^4 to 10^8 daltons. Amylose is normally the minor constituent by weight, accounting for an amount of the total starch between 15 and 30%, but some mutant varieties can contain almost 100% amylopectin (waxy starch). In the granule, the backbone of the amylopectin molecule remains in the amorphous state whereas the bundles of the side chains form double helical, crystalline structures (French, 1984). As to amylose, there is evidence that this fraction is located within the amorphous region of the granule (Zobel, 1992).

The availability and relatively low cost are major factors governing the use of starch in foods (Alexander and Zobel, 1988). To improve its resistance to degradation and enhance the functional properties, native starch can be modified by suitable treatments. They usually involve acid or enzymic hydrolysis, oxidation, esterification or etherification (Rapaille and Vanhemelrijck, 1992). This allows use of the polysaccharide as an extender, processing aid, thickener and stabiliser. In high-sugar foods, e.g. glazes and bakery fillings, oxidised starches are the best thickeners producing appealing textures of low or high rigidity depending on concentration. Pectin is also used in that type of foods, e.g. fruit-based jams and jellies. A distinct advantage that pectin has over other gelling agents and stabilisers, is that it is most stable to heat under acid conditions (Anon., 1984). It is thus an ideal choice of stabiliser in acidic food products. Despite the widespread use of low methoxy pectin and oxidised starch, little is known of their molecular interactions in high-sugar products. The aim of this work, therefore, is to explore the aforementioned chemical and conformational properties of the two components for the development of novel, appealing confectionaries. Since most practical applications involve acidic conditions, work focused primarily on pH 3.6 but a comparison was made with products at pH 6.0.

Materials and Methods

LOW METHOXY PECTIN: The low methoxy pectin sample was a gift (batch X-5006) from the Copenhagen Pectin Division of Hercules. It was made from lemon peel by progressive de-esterification with pectin esterase (*Aspergillus niger*). Compositional analysis was performed using standard procedures detailed elsewhere (Anon., 1996). This involves the content of pectin expressed as a percentage of the total weight of the sample, galacturonate residues (esterified and un-esterified) as a percentage of the total pectin, and methyl-esterified galacturonate (i.e. the DE) as a percentage of the total galacturonate. Then, a straight-forward calculation gives the effective formula weight per carboxyl group. For our sample these values were: content of pure pectin = 98.2%, of which 82.1% is galacturonate with DE 34.2, giving an effective formula weight per carboxyl group of 341. The degree of esterification is towards the low end of the commercial range (Christensen, 1986). The moisture content of the sample is about 8%.

OXIDISED STARCH: The oxidised starch used was C*Set 06598 (batch SH 1338) from Cerestar. It was prepared by a reaction run on an aqueous suspension of waxy maize starch using sodium hypochlorite under alkaline conditions as the converting agent. The oxidation process is a random reaction involving oxidation of hydroxyls to aldehydes, ketones and carboxyl groups as well as molecular scission. Thus the treatment yielded a material with a shorter chain length than those of natural starches. Its gelatinisation temperature was also lower (about 65°C), but it produced a solution of stable viscosity and good flow properties. Oxidation introduced approximately one carboxyl group per 35 glucose residues. Since carboxyls are substantially more bulky than hydroxyls, their introduction in the amylose fraction reduces its tendency to associate or retrograde. At low concentrations of the polysaccharide, this leads to the production of soft-bodied gels of high clarity. Thus it was developed primarily as an extender for gum arabic in confectionery gums and pastilles and, if needed, can be dissolved at concentrations up to 50% (Picout, 1998). Further, dilute solutions of the sample remain clear on prolonged storage, making them suitable for clear, canned soups.

Molecular weight estimations were performed chromatographically on Shodex columns S805 and S804 in series. The system was calibrated with a set of pullulan standards. Starch solutions were made in a 90/10 mixture of dimethyl sulphoxide (DMSO) and water. Elution was with 0.05 M NaOH at a flow rate of 1 ml/min. The GPC signal intensity *vs* retention times is depicted in Figure 1, which provides the following molecular distribution:

MW	Area (%)
$5x10^3$	1.81
$5x10^3$-$25x10^3$	20.98
$25x10^3$-$2x10^5$	60.19
$2x10^5$-10^6	16.76
10^6-$5x10^6$	0.26

Thus the number average molecular weight was found to be 15,050 and the mass average molecular weight was 97,710. The moisture content of the sample is 13%.

GLUCOSE SYRUP: The glucose syrup of this investigation was a Cerestar product (01136; Batch NX8472). Despite its name, the material is a non-sweet saccharide polymer. It is prepared by controlled hydrolysis (α-amylase) of starch down to glucose polymers linked by α-(1→4) glycosidic linkages. The functionality of the product is determined by the dextrose equivalent, which gives the content of reducing end groups relative to glucose as 100. Thus a dextrose equivalent of 25 corresponds to a (number average) degree of polymerisation of 4. The dextrose equivalent (d.e.) of the sample is 42; not to be confused with DE, the degree of esterification of pectin. The high d.e. of the sample imparts solubility, bulking and bodying characteristics which are desirable in confectionery products. The total level of solids is 81%. The water content of glucose syrup was taken into account in the calculation of the total level of solids for the preparation of the mixtures. Finally, GPC analysis provided the following relationship between degree of polymerisation (DP) and surface area (%) of the glucose syrup spectrum:

DP1	17.54
DP2	12.99
DP3	10.55
DP4	8.79
DP5	7.29
DP6	5.28
DP7	4.78
DP8	4.21
DP9	3.19
DP10	1.96
>DP10	23.40

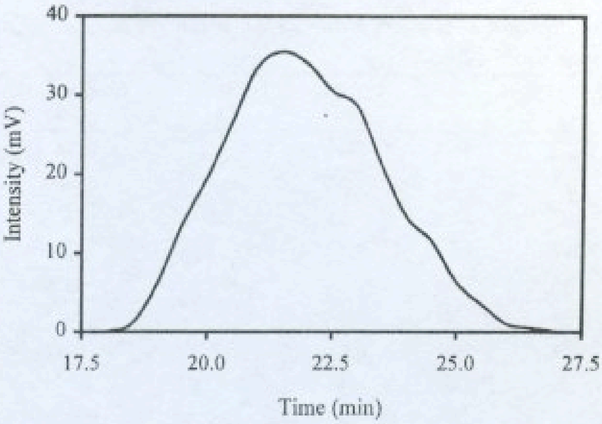

Figure 1. Typical experiment of gel permeation chromatography for oxidised starch used to calculate its molecular weight.

SAMPLE PREPARATION: Preparation of confectionery products involves hydration of biopolymers and sugars to form a hot liquor (70-80°C) at about 75% total level of solids. Then the pH is adjusted with HCl/NaOH and colour and flavouring ingredients are added. The liquor is deposited immediately into impressions made in the surface of a preconditioned starch tray. The tray is stoved at 50°C for 4-7 days in a stoving room. Products are then removed and stored in warehouses at temperatures varying from ambient to domestic freezing (-19°C). This study addresses the effect of composition, temperature and pH on the textural properties of the liquor, which will largely determine the quality of the finished product.

Low methoxy pectin was dissolved in distilled water at 90°C and then mixed with hot glucose syrup. Oxidised starch was dispersed in cold water and then mixed with glucose syrup. The dispersion was heated to 90°C until the starch was completely gelatinised leading to a clear solution. Liquors were made by mixing appropriate amounts of the hot stock solutions thus yielding ternary solutions of pectin/starch/glucose syrup. The pH of stock solutions and liquors was adjusted to 3.6 or 6.0. These values of pH reflected a situation either close to or away from the isoelectric point of pectin. As shown in Table 1, the two pH investigations were carried out at 75% total level of solids.

RHEOLOGICAL METHODOLOGY: Single and mixed preparations of pectin and starch were loaded onto the pre-heated plate (90°C) of a controlled stress Carri-Med CSL 500 rheometer. Temperature was controlled by a Haake circulating water batch and measured with a thermocouple attached to the bottom plate (moving element) of the instrument. The bottom plate was raised to a distance of 1 mm below the top plate

TABLE 1

Composition of liquors made of low methoxy pectin, oxidised starch and glucose syrup

Samples	pH	Pectin (%)	Starch (%)	Glucose Syrup (%)
A1	3.6	1.0	-	74
A2	3.6	0.7	8.3	66
A3	3.6	0.5	16.0	58.5
A4	3.6	0.2	21.7	53.1
A5	3.6	-	30.9	44.1
B1	6.0	0.8	-	74.2
B2	6.0	0.6	6.7	67.7
B3	6.0	0.4	13.7	60.9
B4	6.0	0.2	22.0	52.8
B5	6.0	-	31.2	43.8

stationary element). The sample was thus contained between the two plates with its exposed edges being covered with silicone fluid (50 cs) to prevent evaporation. Samples were left at 90°C for 10 min to equilibrate followed by a frequency sweep of their viscoelasticity. They were then cooled to -20°C at a scan rate of 1°C/min, a process that induced a spectacular increase in their rigidity from 10^0 to 10^7 Pa). To cope with such strong samples and to minimise the compliance of the machine, parallel plates with diameter 4, 2 and 0.8 cm were designed and constructed in-house thus extending the working range of the instrument to the upper values of rigidity. To prevent freezing of the circulating water at -20°C, a 50:50 mixture of ethylene glycol and water was used in the water batch. Another frequency sweep was taken at the end of the cooling run and then the sample was heated to 90°C at 1°C/min. The applied strain was 1%, i.e. a level of deformation well within the linear viscoelastic regime of our samples. Finally, the frequency of the cooling and heating runs was fixed at the experimentally convenient value of 1.6 Hz (approximately 10 rad/s).

Results and Discussion

RHEOLOGICAL CHARACTERISATION OF THE HIGH-SUGAR LOW METHOXY PECTIN SAMPLES: Small deformation dynamic oscillation affords a thorough investigation of the underlying structures and processes appearing in binary and ternary mixtures. Upon application of a time-dependent strain wave, a response was generated which could be resolved into the elastic (G') and viscous (G") properties of our sample. These are recorded as a function of the phase-angle difference (δ) between the applied and the generated sinusoidal waves:

$$G' = (\sigma_0/\gamma_0)\cos\delta$$
$$G'' = (\sigma_0/\gamma_0)\sin\delta \qquad (1)$$

where σ_0 and γ_0 are the maximum stress and strain amplitudes, respectively. Recording the elastic and viscous moduli of the stock solutions and liquor during controlled cooling and heating results in the profiles of Figure 2.

Mixing 1% low methoxy pectin (LMP) with 74% glucose syrup produces a solution across the temperature range of 60 to -20°C (sample A1 in Table 1). The cooling and heating scans are virtually identical with both moduli showing a gradual progression with temperature. The liquid-like nature of our sample is signified by the values of tan δ, the ratio of viscous to elastic modules (G"/G'), which are 3.54 and 11.37 at 60 and -20°C, respectively (see also Table 2). Therefore, the development of electrostatic repulsion between the negatively charged LMP chains prevents gelation of the polysaccharide; as mentioned in the introduction, the pKa of pectin (3.3) is lower than the working pH of 3.6.

Pectin gelation at this regime can be achieved by the addition of calcium and has been widely accepted to be based on the "egg-box" model (Rees, 1972). This stipulates that pairs of polygalacturonan chains with at least 14 residues and with a two-fold ("zig-zag") conformation are packed together forming cavities which are filled by calcium ions, like eggs in an egg-box. In the absence of calcium, LMP gels at pH values around 2 due to non-specific electrostatic interactions between the carboxyl oxygen atoms of the polysaccharide and a cloud of surrounding protons (Gidley *et al.*, 1980). Under the confectionery-related conditions of our investigation, however, the LMP chains remain in the disordered conformation and "dissolve" in the continuous phase of glucose syrup.

TABLE 2

Characteristic viscoelastic parameters of the liquors made of low methoxy pectin, oxidised starch and glucose syrup

Samples	60°C			-20°C		
	G' (Pa)	G" (Pa)	Tanδ	G' (kPa)	G" (kPa)	Tanδ
A1	1.3	4.6	3.54	0.98	11.12	11.37
A2	1.8	7.3	4.06	10.45	35.01	3.35
A3	9.8	54.1	5.52	144.4	186.9	1.29
A4	26100	18510	0.71	12400	9932	0.80
A5	573600	96570	0.17	11700	7883	0.67
B1	2.3	10.4	4.52	41.49	86.78	2.09
B2	4.7	23.3	4.96	47.92	120.2	2.51
B3	3420	3861	1.13	1576	2689	1.71
B4	32980	9865	0.30	2409	1969	0.82
B5	178400	24930	0.14	6909	4445	0.64

Experimental frequency is 1.6 Hz (approximately 10 rad/s)

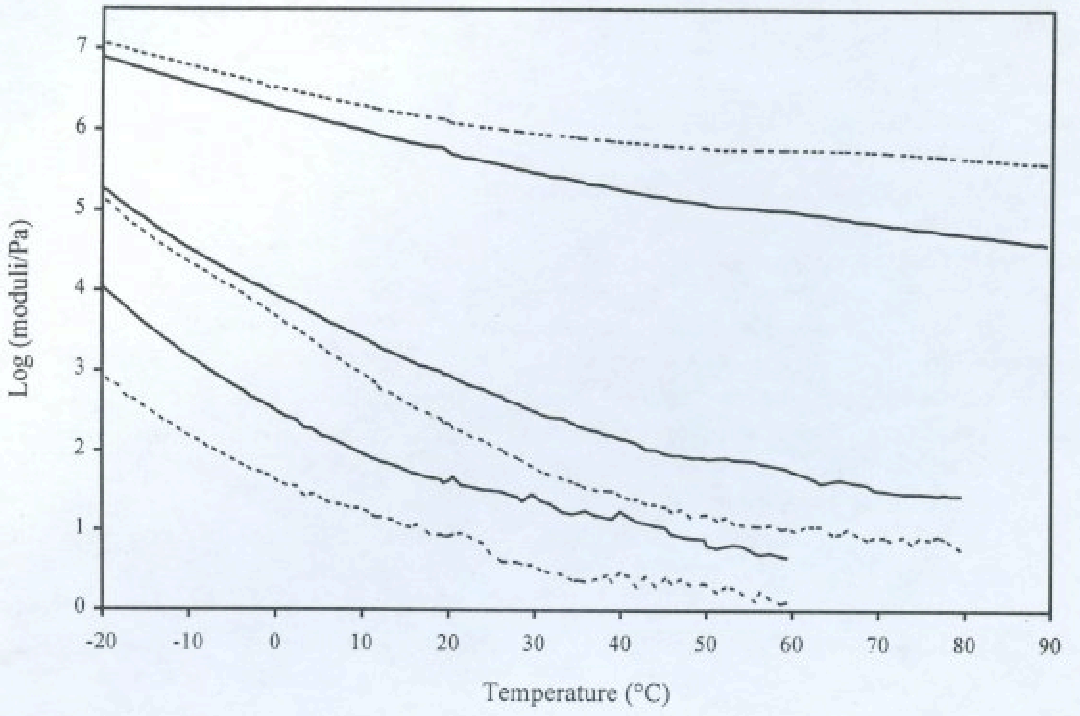

Figure 2. Temperature variation of G' (dashed line) and G" (solid line) for samples A1 (bottom), A3 (middle) and A5 (top). Scan rate 1°C/min; Frequency 1.6 Hz; Strain 1%.

Mechanical spectra of the aforementioned sample at -20°C produce the response of Figure 3. This was taken within the frequency window of 0.01 and 10 Hz, which covers the frequency of the temperature runs (1.6 Hz). Both moduli rise steeply covering four orders of magnitude from about 10^1 to almost 10^5 Pa. The mechanical response is predominantly liquid-like, with G" remaining above G' over the accessible frequency range. Further, the two traces run alongside each other thus averaging out a tan δ value of about 10. At a given frequency of oscillation (ω), the values of storage and loss modulus can be combined in the following formula to yield the complex dynamic viscosity (Richardson and Kasapis, 1998):

$$\eta^* = (G'^2 + G''^2)^{1/2}/\omega \qquad (2)$$

The frequency of oscillation of the strain wave, ω, can be regarded as the oscillatory analogue of shear rate. In Figure 3 the complex dynamic viscosity of sample A1 decreases linearly with frequency thus creating a shear thinning solution. 1% low methoxy pectin forms a concentrated solution where the disordered coils overlap and entangle. Therefore, the observed shear thinning should be ascribed to the disturbance of entanglements which are unable to reform within the period of oscillation.

THE FUNCTIONAL PROPERTIES OF LIQUOR IN THE PRESENCE OF STARCH: Addition of starch transforms gradually the structural properties of the liquor. This is accompanied by a drop in the concentration of LMP, but overall the viscoelasticity is enhanced (Tables 1 and 2). For example, the values of G' at -20°C increase from about 1 to 10.5 kPa for the samples A1 and A2, respectively. Next we doubled the amount of starch to 16% and reduced that of LMP to 0.5% (liquor A3). As illustrated in Figure 2, a dominant viscous response is

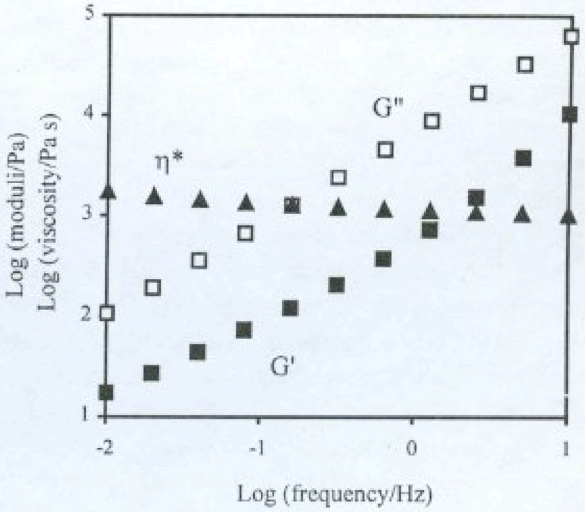

Figure 3. Frequency sweep of G', G" and η* at -20°C for sample A1. The strain is 1%.

obtained at the upper range of temperature with the tanδ at 60°C being 5.52. Cooling, however, induces a disproportionate development of the elastic component which converges upon the loss modulus. Thus at -20°C the tan δ values drop to 1.29.

The reinforcement of G' should be attributed to the gelatinisation and subsequent retrogradation of starch. When the slurry of starch is heated at 90°C, the granules swell uniformly to many times their original size. Next amylose starts to leak out into the solution. Eventually, the granules will reach their maximum hydration and then begin to rupture and collapse (end of gelatinisation in Morris, 1990). Waxy maize starch is, of course, devoid of amylose (see *Introduction*), but the hypochlorite treatment of our sample results in molecular scission and the release of amylose-like segments which are capable of retrogradation. The depression of amylose-like molecules and granule fragments imparts mainly viscosity to the system, as opposed to the build up of a gel. Thus the tanδ values of preparations A2 and A3 at 60°C remain high at 4.06 and 5.52 in Table 2. Cooling of the liquor, however, allows starch to retrograde, i.e. to return to the crystalline state. Amylose-like segments retrograde first leading to the formation of double helices between the linear chains which aggregate into crystallites. The process should be checked to some extent by the carboxyl groups which act as wedges between the polymeric segments. Amylopectin-like sequences retrograde at a considerable lower rate (Ring *et al.*, 1987). The increased order of the system is reflected in lower tan δ values at -20°C, i.e. from 11.37 and 3.35 to 1.29 for the top three samples of Table 2. Nevertheless, the sample remains liquid-like with the continuous phase of glucose syrup suspending the filler particles of oxidised starch.

At sufficiently high concentrations, starch will form a paste or gel even at high temperatures, where the swollen granules and starch fragments are embedded in a continuous matrix of entangled amylose-like molecules. This is seen in sample A4 comprising 21.7% starch, 0.2% LMP and 53.1% glucose syrup. The progressive change from solution-like to gel-like response is recorded in the tan δ which passes through 1.0 (G' = G"), the frequency-dependent point of critical crosslinking, and achieves values of 0.71 and 0.8 at 60 and -20°C, respectively (Table 2). Therefore, a starch matrix has been formed which supports the bulky inclusions of glucose syrup and pectin. The formation of starch network is also evident in Figure 2 where the temperature profile of 30.9% starch plus 44.1% glucose syrup is reproduced (sample A5). Clearly, a three-dimensional structure has been established at the top of the temperature range, yielding a tan δ value of 0.17 at 60°C. As the temperature is lowered, there is a monotonic increase in viscoelasticity with the solid-like response prevailing throughout. Mechanical spectra at -20°C unveil a gelling system with G'>G", some frequency dependence in both moduli and the slope of shear thinning viscosity being close to the theoretical maximum value of -1.0 (Figure 4). This profile, of course, is entirely different from the response of the sugar/pectin mixture where the loss modules dominates across the frequency range studied (Figure 3).

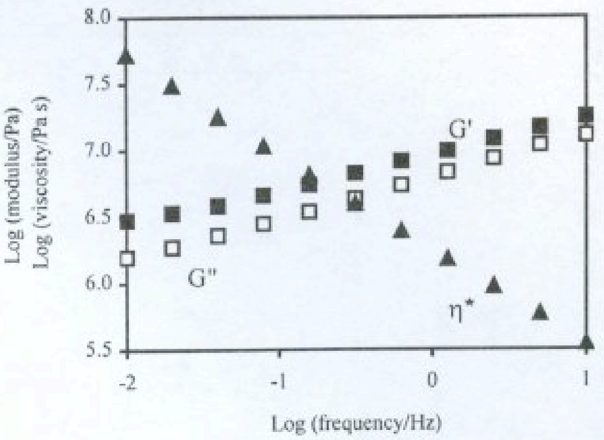

Figure 4. Mechanical spectrum of G', G" and η* at -20°C for sample A5. The strain is 1%.

The phase inversion from a glucose syrup to a starch-continuous system is also tangible in the textural properties of the products. Thus glucose syrup-dominated samples were found to be cohesive solutions with a chewy mouthfeel and a stringy appearance upon pulling. The starchy counterparts, on the other hand, form hard, sticky gels with a short mouthfeel which fragment easily.

TEXTURAL ASPECTS OF THE LIQUOR AT NEAR NEUTRAL CONDITIONS: Taste panelling also exposes the main difference in the behaviour of samples with the change in pH. Thus increasing the pH from acidic (3.6) to near neutral (6.0) leads to pectin decomposition. This is a β-eliminative reaction splitting the glycosidic bond to O-4 of an esterified galacturonic acid subunit and creating a double bond between C-4 and C-5 of the same residue (Rolin, 1993). At low concentrations of starch, samples B1-B3 are dominated by the liquid-like properties of glucose syrup/depolymerised pectin, as noted for the acidic counterparts (Table 2). Similarly, phase inversion to starch-based systems generates gel-like characteristics in samples B4 and B5. The high values of storage modulus observed for the starch alone sample at pH 3.6 (e.g. 0.57 MPa at 60°C in A5) could be attributed to the acidic environment which neutralizes the carboxyl groups and allows extensive intermolecular associations. By contrast, at near neutral pH the corresponding values of the negatively charged chains do not exceed 0.18 MPa at 60°C (gel B5 in Table 2).

Pectin depolymerisation is accompanied by a change in colour which turns brown. Today, there is a tendency to develop a brownish appearance in mincemeat and certain jams made from some fresh or frozen fruits, particularly strawberry (Cross, 1986). Products made from sulphited fruits lose some of the colour, since not all of the original colour returns when the sulphur dioxide is removed. Lack or loss of colour during processing of high-solid products can be compensated by the addition of artificial colouring maters (e.g. caramel) or, on a healthy streak, with low methoxy pectin at pH between 5 and 7. The high methoxy counterpart should not be used because it does not gel at slightly acidic conditions.

CONTRAST BETWEEN CRYSTALLINE AND AMORPHOUS BEHAVIOUR IN HIGH SUGAR PECTIN/STARCH MIXTURES: Starch-rich networks are capable of turning into rigid materials at the lowest experimental temperature. Thus the storage modulus of sample A5 achieves values in excess of 10^7 Pa at the frequency of 10 Hz (Figure 4). However, the tan δ values of starch-continuous mixtures argue for a disproportionate development of the viscous element with cooling (Table 2). For example, the viscoelastic ratio of sample B4 increases from 0.3 to 0.82 at 60 and -20°C, respectively. The reinforcement of loss modulus at subzero temperatures has been associated with the vitrification of the amorphous parts of a material (Evagelou *et al.*, 1998). Glucose syrup is a polydisperse material (see *Experimental Section*), which should readily vitrify alongside the densely packed aggregates of oxidised starch. Overall, a starch-continuous system can be considered as a composite of crystalline and amorphous phases with the former being predominant (tan δ remains below 1).

The preceding paragraph argued that the process of vitrification in our mixtures has been arrested by the undue crystallinity of the starch network. It would be expected from this proposal that vitrification might be allowed to proceed in the absence of starch. Therefore, we replaced starch with glucose syrup in the samples in order to verify the truth of this statement. Further, glass transitions develop rapidly upon cooling a rubbery network (Papageorgiou *et al.*, 1994), a requirement that can not be met by the high sugar/LMP system since it remains in solution within the accessible temperature range (sample A1 in Figure 2). Addition of calcium would not help either, since the aggressive interactions between the counterion and the galacturonate residues lead to a highly aggregated structure (Rolin and De Vries, 1990). It appears, however, that a rubbery network can be engineered by introducing methyl esters in the pectin molecule. These are hydrophobic groups which tend to form clusters in order to minimise the contacting area with water (Morris *et al.*, 1980). The arrangement involves an entropy loss and assists in the stabilisation of the network of high methoxy pectin.

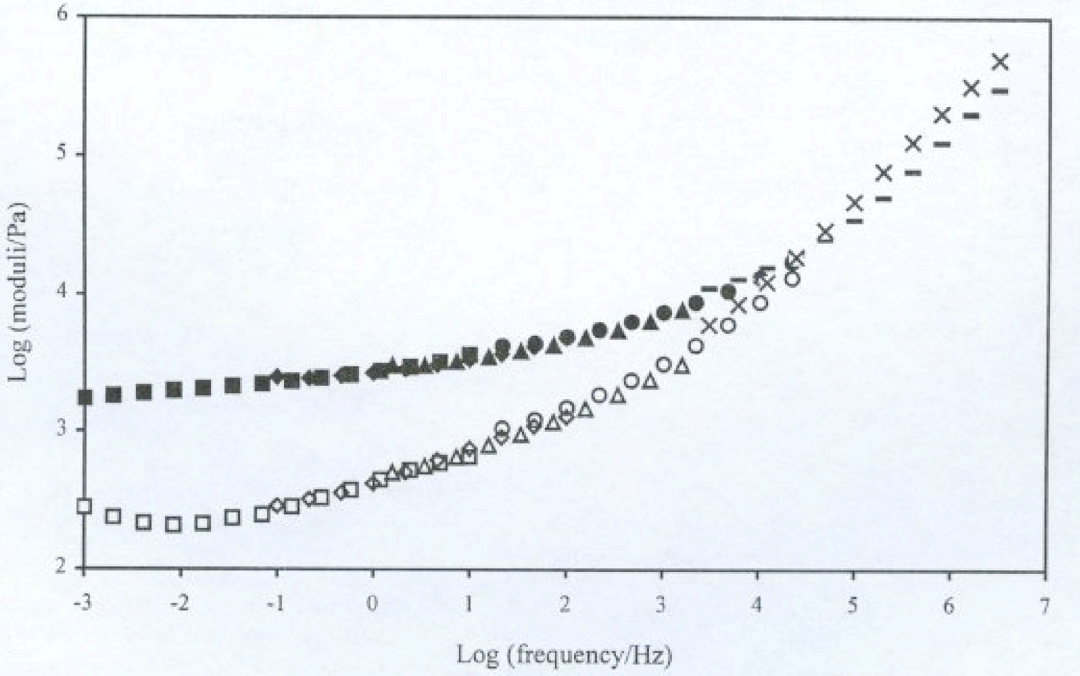

Figure 5. Master curve for the sample of 1% high methoxy pectin plus 77% glucose syrup comprising frequency sweeps at 90 [G'(■); G" (□)], 70 [G' (◆); G" (◇)], 50 [G'(▲); G" (Δ)], 30 [G' (•); G" (o)] and 5°C [G' (—); G" (x)]. Reference temperature is 90°C.

Entropic viscoelasticity is, of course, the underlying force behind the formation of a rubbery network (Ferry, 1980).

Figure 5 depicts the mechanical spectrum of 1% high methoxy pectin (HMP) with a galacturonate content (esterified and non-esterified) of 83% and a high degree of esterification (70%). Glucose syrup was also added to bring the total level of solids to 78%. The pH was adjusted to 3.6 in line with samples A1 to A5 in Table 1. The frequency window stretches over ten orders of magnitude although a single experimental run can not extend to more than three or at best four of them (see for example Figure 3). Clearly, the profile of Figure 5 combines several frequency sweeps thus forming a "master curve". Its construction is the result of recording a series of frequency sweeps within the range of 001 to 10 Hz at 90, 70, 50, 30 and 5°C (cooling rate between the isothermal runs was 1°C/min). Then, the mechanical spectrum at 90°C was chosen arbitrarily as a reference, and the remaining spectra were shifted horizontally along the log frequency axis until they fell into a single response.

The shift factors obtained from the superposition of the frequency sweeps are plotted against the reverse of temperature in Figure 6. Clearly, a linear fit is obtained which complies with the Arrhenius equation in the following form (Arridge, 1975):

$$\log a_T = \log G(T)/G(T_0) = (E_a/2.303R)[(1/T) - (1/T_0)] \quad (3)$$

where a_T is the shift factor, E_a the energy of activation and T_0 the reference temperature. A valid implementation of the approach requires superposition of both moduli with the same set of shift factors. It implies that viscoelastic data obtained at two temperatures refer to a single process once the frequency scale is multiplied by the appropriate shift factor (time-temperature superposition in Tobolsky, 1956).

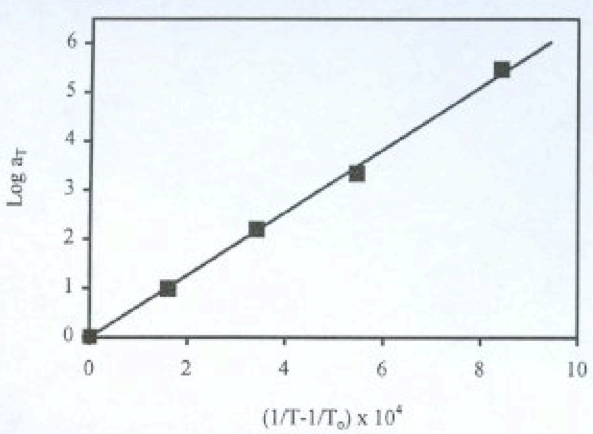

Figure 6. Log shift factor versus $1/\Delta T$ for the sample of Figure 5. The straight line reflects the quality of Arrhenius fit.

In our case, the transposition of the five mechanical spectra unveiled a trend in viscoelasticity well beyond the frequency range of the instrument at 90°C (Figure 5). The analysis also argues for an overriding mechanism of structure formation with a constant energy of activation of 122.4 kJ mol^{-1}. This value is of similar magnitude to the predictions for the high sugar/κ-carrageenan rubber (from 97.8 to 140.2 kJ mol^{-1} with increasing levels of solids) and further verifies the validity of the approach (Evageliou and Kasapis, 1997). In the absence of additional processes, e.g. in the form of crystallisation, a rubbery plateau is obtained with a flat G' trace and a characteristic dip in the profile of G" (lower frequency range in Figure 5). Further increase in the frequency of oscillation sees the onset of the glass transition with both moduli soaring and the viscous component becoming dominant.

Since only the onset of vitrification of the system has been seen in the frequency sweep taken at 5°C, the Arrhenius fit reflects the relaxation processes of the dominant rubbery state at the frequency sweeps of 90, 70, 50 and 30°C (Figure 5). Further inroads into the area of glass transition at lower temperatures (<5°C) would have supplanted the Arrhenius fit with the WLF kinetics (Ferry, 1980). The transformation from solid (G' > G") to liquid-like (G" > G') consistency documents the amorphous nature of the system and stands in direct contrast with the temperature profile of the starch only system (top profile in Figure 2 where G' > G" throughout). Therefore, the disparate molecular functions of starch, as a crystalliser, and pectin, as a vitrifier, in a high sugar environment have been documented.

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References

Alexander, R.J. and H.F. Zobel. 1988. *Developments in Carbohydrate Chemistry*. The American Association of Cereal Chemists, St. Paul, Minnesota.

Anon. 1984. GENU Worldwide - Handbook for the Fruit and Processing Industry. Hercules Inc., The Copenhagen Pectin Factory Ltd.

Anon. 1996. Pectin. In: *Food Chemicals Codex Fourth Edition*, Committee on Food Chemicals Codex of the Institute of Medicine (Editors) 284-285. National Academy Press, Washington, D.C.

Arridge, R.G.C. 1975. The glass transition. In: *Mechanics of Polymers*, 24-50. Clarendon Press, Oxford.

Christensen, S.H. 1986. Pectins. In: *Food Hydrocolloids Vol III*, M. Glicksman (Editor) 205-230. CRC Press, Boca Raton, Florida.

Cross, D. 1986. Preserves. In: *Food Industries Manual-21st Edition*, M.D. Ranken (Editor) 266-285. Leonard Hill, Glasgow.

Evageliou, V. and S. Kasapis. 1997. The use of Arrhenius and WLF kinetics to rationalise the rubber-to-glass transition in high sugar κ-carrageenan systems. *MFLRBER* 62(4a): 1163-1498.

Evageliou, V., S. Kasapis, and G. Sworn. 1998. The transformation from enthalpic gels to entropic rubbery and glass-like states in high sugar biopolymer systems. In*: Gums and Stabilisers for the Food Industry 9*, G.O. Phillips, P.A. Williams, and D.J. Wedlock (Editors) 333-344. The Royal Society of Chemistry, Cambridge.

Ferry, J.D. 1980. Dependence of viscoelastic behavior on temperature and pressure. In: *Viscoelastic Properties of Polymers*, 264-365. John Wiley & Sons, New York.

French, D. 1984. Organisation of starch granules. In: *Starch: Chemistry and Technology*, R.L. Whistler, J.N. BeMiller and E.F. Paschall (Editors) 183-247. Academic Press, Orlando.

Galliard, T. 1987. Morphology and composition of starch. In: *Critical Reports on Applied Chemistry 1. Vol 13. Starch: Properties and Potential*, T. Galliard (Editor) 55-78. John Wiley and Sons, New York.

Gidley, M.J., E.R. Morris, E.J. Murray, D.A. Powell, and D.A. Rees. 1980. Evidence for two mechanisms of interchain association in calcium pectate gels. *Int. J. Biol. Macromol.* 2: 332-334.

Kratz, R. 1995. Recent developments in pectin technology: Instant-Pectins. In*: Food Technology International Europe*, A. Turner (Editor) 35-38. The Penrose Press, South Africa.

May, C.D. 1992. Pectins. In: *Thickening and Gelling Agents for Food*, A. Imeson (Editor) 124-152. Blackie Academic & Professional, London.

Morris, V.J. 1990. Starch gelation and retrogradation. *Trends in Food Science & Technology*. July: 2-6.

Morris, E.R., M.J. Gidley, E.J. Murray, D.A. Powell, and D.A. Rees. 1980. Characterisation of pectin gelation under conditions of low water activity by circular dichroism, competitive inhibition and mechanical properties. *Int. J. Biol. Macromol.* 2: 327-330.

Papageorgiou, M., S. Kasapis, and R.K. Richardson. 1994. Glassy-state phenomena in gellan-sucrose-corn syrup mixtures. *Carbohydrate Polymers* 25:101-109.

Picout, D.R. 1998. Segregation of calcium pectate in co-gelation with maltodextrin or oxidised starch. *PhD Thesis*. Silsoe College, Cranfield University.

Rapaille, A. and J. Vanhemelrijck. 1992. Modified starches. In: *Thickening and Gelling Agents for Food*, A. Imeson (Editor) 171-201. Blackie Academic & Professional, London.

Rees, D.A. 1972. Shapely polysaccharides. *Biochem. J.* 126: 257-273.

Richardson, R.K. and S. Kasapis. 1998. Rheological methods in the characterisation of food biopolymers. In: *Instrumental Methods in Food and Beverage Analysis*, D.L.B. Wetzel and G.Charalambous (Editors) 1-48. Elsevier, Amsterdam.

Rinaudo, M. 1996. Physicochemical properties of pectin in solution and gel states. In*: Pectins and Pectinases*, J. Visser and A.G.J. Voragen (Editors) 21-33. Elsevier, Amsterdam.

Ring, S.G., P. Colonna, K.J. I'Anson, M.T. Kalichevsky, M.J. Miles, V.J. Morris, and P.D. Orford. 1987. The gelation and crystallisation of amylopectin. *Carbohydrate Research* 162: 277-293.

Rolin, C. 1993. Pectin. In: *Industrial Gums*, R.L. Whistler and J.N. BeMiller (Editors) 257-293. Academic Press, San Diego.

Rolin, C. 1994. Pectin - label friendly, consistent, versatile. *The European Food and Drink Review*, Autumn: 61-65.

Rolin, C. and J. De Vries. 1990. Pectin. In: *Food Gels*, P. Harris (Editor) 401-434. Elsevier, London.

Tobolsky, A.V. 1956. Stress relaxation studies of the viscoelastic properties of polymers. *J. Appl. Phys*. 27: 673-685.

Zobel, H.F. 1992. Starch granule structure. In: *Developments in Carbohydrate Chemistry*, R.J. Alexander and H.F. Zobel (Editors) 1-36. The American Association of Cereal Chemists, St. Paul, Minnesota.