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Determination of the physical functionality of galactomannans in kappa-carrageenan/galactomannan mixed systems by periodate oxidation

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Galactomannan samples of different origins (guar gum, tara gum, and locust-bean gums), with mannose-to-galactose (M/G) ratios from 1·7 to 4·8, have been characterized. They exhibited strong differences with respect to their molecular weight and their fine structure (M/G). The different locust-bean-gum samples differed significantly in this respect from each other. All galactomannans were submitted to periodate oxidation. The periodate oxidation of galactomannan samples was characterized by the formic acid liberated. As expected, the formic acid liberated was directly related to the M/G ratios of galactomannan samples. Thus galactomannan periodate oxidation was shown to be a simple and precise analytical method to predict the M/G ratio of galactomannan samples. It was demonstrated that galactomannan periodate oxidation, as a measure of the M/G ratio, can be directly related to the physical functionality of galactomannans in terms of synergistic interactions between the kappa-carrageenan and the galactomannans.

INTRODUCTION

Galactomannans are plant polysaccharides widely used in the food industry. These polysaccharides in aqueous media can form viscous solutions or gelling systems when mixed with other agents, such as xanthan or carrageenans (Dea & Morrison, 1975; Fernandes *et al.*, 1989). Galactomannans are plant-seed polysaccharides containing a backbone of (1->4)-β-D-mannopyrannosyl units with attached (1->6)-α-D-galactose units (Dey, 1978). The most common galactomannans are locust-bean-gum (LBG), tara gum (TG), and guar gum (GG). They differ in the mannose-to-galactose (M/G) ratio, which determines their structural characteristics. LBG presents an M/G ratio of about 4, TG has an M/G ratio of about 3, and GG presents an M/G ratio of about 2 (Hui & Neukom, 1964; Cairns *et al.*, 1986). The synergistic interactions observed between the galactomannans and other polysaccharides, such as xanthan gum or kappa-carrageenan, are ruled by the galactose-free regions of the mannose chain. Apparently, the increase in smooth regions (>M/G) improves the possibility of inter-molecular binding (Fernandes *et al.*, 1992; Mannion *et al.*, 1992). A rheological study of kappa-carrageenan/galactomannan mixed gels, made by using galactomannans of different origins and M/G ratios, has shown that the maximum synergistic effects were obtained for a mixing ratio of 4 : 1 (carrageenan:galactomannan), which was independent of the galactomannan used (Fernandes *et al.*, 1991). Furthermore, Fernandes *et al.* (1991) observed that the storage modulus, G', at the maximum of the synergist effects ($G'_{\max}$) was strongly dependent on the size or number of unsubstituted regions in the main chain of mannose (approximating to the M/G ratio) and the size of the galactomannan biopolymer, which is directly related to the intrinsic viscosity ($[\eta]$):

$$G'_{\max} \sim [\eta]\,(\mathrm{M/G})^2$$

Up to now, several techniques, such as GLC, HPLC, polarimetry, vacuum ultra-violet circular dichroism, RMN, and enzymology, have been used for the determination of the M/G ratio (Dea & Morrison, 1975; McCleary *et al.*, 1984; Patel & Hawes, 1986; Morris, 1990; Rochas *et al.*, 1991). However, all these techniques need expensive equipment or specific reagents. The periodate oxidation was used in order to establish the fine structure of galactomannans in terms of the galactose distribution on the mannose chain (Whistler *et al.*, 1948; Ishak & Painter, 1973; Painter *et al.*, 1979). Recently, Ikkala *et al.* (1988) detected adulteration

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of LBG with guar gum by an analytical method of galactomannan periodate oxidation.

The aim of this work is to determine the M/G ratio of galactomannans by periodate oxidation and consequently the physical functionality of each locust-bean-gum sample when mixed with kappa-carrageenan. It is described by a standard curve relating the M/G of galactomannans, ranging from 1·7 to 4·8, determined by a typical GLC analysis with the amount of liberated formic acid obtained from periodate oxidation of the galactomannan samples. This standard curve was related to the linear relation mentioned above, $G'_{\max} \sim [\eta](M/G)^2$, in order to predict the synergistic effects of each galactomannan sample when mixed with the kappa-carrageenan.

MATERIALS AND METHODS

Materials

Two Portuguese locust-bean flours, canela and mulata, were obtained on the laboratory-scale according to Gonçalves *et al.* (1988). Two commercial-grade gums, guar gum and Lygomme 6 locust-bean gum, were kindly supplied by SBI (France). Two other commercial-grade gums, tara gum and locust-bean gum, were kindly provided by Marine Colloids (USA) and INDAL (Portugal), respectively. All the galactomannan samples except guar gum and Lygomme 6 had to be purified as described by Fernandes *et al.* (1991). The Indal gum was fractionated according to is solubility in water, and the procedure was carried out as previously described by Fernandes *et al.* (1991). The hot-water-soluble fraction of Indal LBG will be referred to as HWS. All chemicals used were Merck analytical-reagent grade or similar. Distilled deionized water was used throughout.

Methods

The moisture and ash content of the gums were obtained by the standard methods. The protein content was determined by the Kjeldhal method, a factor of 5·87 being used (Anderson, 1986).

The mannose/galactose ratios were determined according to the procedure of Blakeney *et al.* (1983). Polysaccharides were normally treated, at room temperature, with 72% sulfuric acid for 30 minutes under stirring, and then hydrolyzed at 100°C for 2 hours with dilute aqueous sulfuric acid. The resulting aldoses were reduced to alditols with sodium borohydride dissolved in dimethylsulfoxide (DMSO). Acetylation was effected with acetic anhydride. Dried alditol acetates were dissolved in dichloromethane and injected (1 μl) onto a GLC capillary column DB-225. The column was operated isothermally (220°C) by using a hydrogen-carrier flow rate of 0·8 ml/min. The injector and detector oven temperatures were maintained at 220°Cand 250°C, respectively. The M/G ratio of the HWS Indal aqueous fraction was determined directly from the solution without pre-treatment with 72% sulfuric acid. The solution was hydrolyzed at 100°C for 2 hours with diluted aqueous sulfuric acid, after which the procedure was identical to that described above.

The preparation of the solutions was carried out according to Fernandes *et al.* (1991). The galactomannan samples were first dispersed in water with vigorous stirring. The dispersion was stirred for 1 hour at room temperature and then heated at 90°C for 30 minutes while being stirred. The solutions were allowed to cool to room temperature overnight. The polysaccharide concentration was estimated by using the phenol-sulfuric method according to Dubois *et al.* (1956).

The analytical procedure of periodate oxidation of the galactomannan samples was carried out according to Ikkala *et al.* (1988). The volume of 90·0 ml of galactomannan solution at 0·10% (w/w) total polymer concentration was mixed with 10·0 ml of 30 mg/ml sodium metaperiodate ($NaIO_4$). The mixture was left for 3 days in the dark at 25°C. The liberated formic acid was then determined by titration with 0·010M sodium hydroxide with methyl red as indicator.

The viscosity of galactomannan dilute solutions was measured at 25°C. The intrinsic viscosity, $[\eta]$, was evaluated from classical Huggins and Kraemer plots as described by Fernandes *et al.* (1991).

RESULTS AND DISCUSSION

Determination of the mannose-to-galactose ratio of galactomannans

The chemical and macromolecular characteristics of the different galactomannan samples are presented in Table 1. Composition indicates good quality gums (Seaman, 1980). It maybe seen that M/G ratios varied to a large extent as expected, since galactomannans are of different origins. It is also noteworthy that the M/G ratio varies to a relatively large extent between the LBG samples. The intrinsic viscosity, which is an indirect measure of molecular weight (M_V: see Table 1), ranged between 9·40 and 16·0 dl/g (Fernandes *et al.*,

Table 1. Composition and macromolecular characteristics of the galactomannans

Sample	Ash(%)	Protein(%)	Intrinsic viscosity (dl/g)	M_V[a] $\times 10^{-6}$	Mannose/ galactose ratio[b,c] Mean (S.D.)
Guar	0·10	0·22	12·0	1·67	1·68 (0·04)
Tara	0·20	0·45	9·40	0·97	3·10 (0·05)
Canela	0·33	0·40	13·8	1·58	3·46 (0·06)
Lyg.6	0·10	0·10	10·5	1·08	3·60 (0·05)
Mulata	0·40	0·47	14·3	1·66	3·80 (0·04)
Indal	0·31	0·50	15·4	1·83	4·04 (0·05)
HSW Indal	—	—	16·0	1·93	4·80 (0·06)

[a] Molecular weight as determined by Fernandes *et al.* (1991).
[b] As determined by GLC analysis.
[c] The values shown are the means of three determinations.

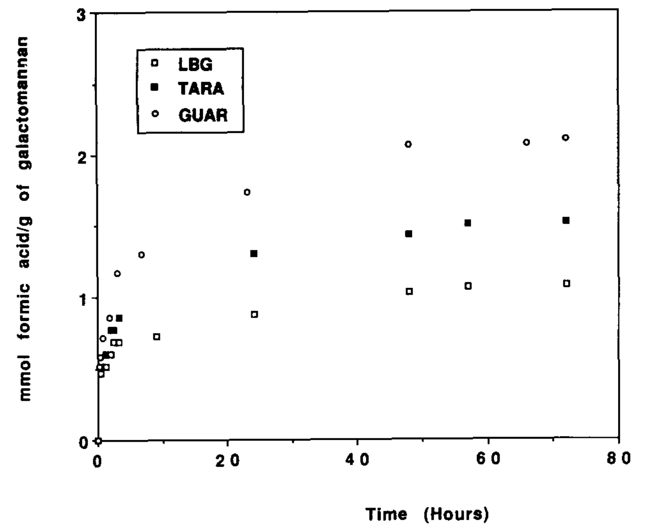

Fig. 1. Kinetics of periodate oxidation of guar-, tara-, and Indal-locust-bean-gum samples.

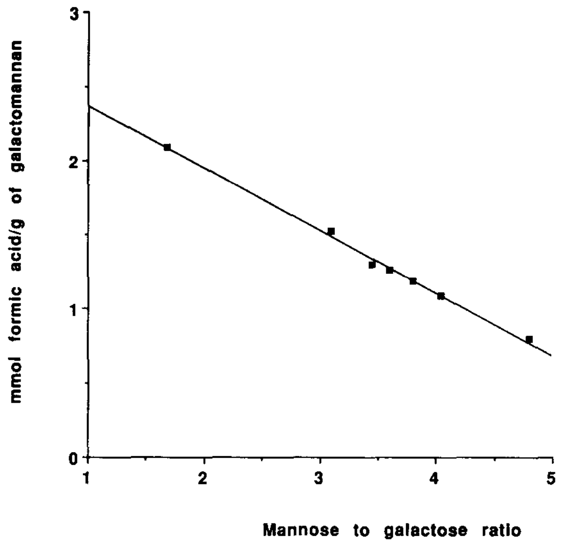

Fig. 2. Liberated formic acid/g of galactomannan as a function of mannose-to-galactose ratios of galactomannan samples. The values shown are the means of three determinations.

1991). In Fig. 1, the kinetics of periodate oxidation for GG, TG, and LBG are shown. The evolution of galactomannan periodate oxidation in terms of curves of liberated formic acid against time is similar for the three galactomannan samples. However, their different magnitudes clearly reflect the galactose content of each galactomannan sample. Whatever the galactomannan used, steady values of liberated formic acid/g of galactomannan were attained between 50 and 60 hours. These results are in close agreement with those reported by Ishak & Painter (1973). In Fig. 2 is shown the concentration of liberated formic acid/g of galactomannan plotted against the M/G ratio of the galactomannan samples. The values of liberated formic acid/g of galactomannan (FA/g) are presented in Table 2. As expected, a direct relationship was found between the galactose content of galactomannans and the liberated formic acid from the periodate oxidation, which can be described by the equation:

$$\text{mmol FA/g} = 2{\cdot}79 - 0{\cdot}423\,(\text{M/G}) \qquad (1)$$

with a correlation coefficient of 0·998. The form of the curve (Fig. 2) and the standard deviation of the values of liberated formic acid (Table 2) indicate the acceptability of this analytical method. These data are consistent with those of Ikkala *et al.* (1988). On taking into account the galactomannan M_V values (Table 1), it is clear that the different molecular weights of the several galactomannan samples appear not to interfere as expected in their periodate oxidation. It is important to note that, in galactomannan periodate oxidation, the galactose units and the mannose end residues are both converted into formic acid and dialdehyde, whereas the mannose backbone residues produce only dialdeyde. In this way, it is also expected that there will be a contribution from the mannose end residues to the total liberated formic acid. However, the mannose end groups of the galactomannan chain represent only a very small part of the molecules, and the main amount of the liberated formic acid must certainly originate from galactose residues. In order to quantify the correlation between the values of the M/G ratio calculated from eqn (1) (Table 2) and those obtained by the GLC analysis (Table 1), the equation relating the M/G ratio calculated from eqn (1) to the M/G ratio from GLC analysis was determined and was found to be:

$$\text{M/G ratio from eqn (1)} = 0{\cdot}006 + 0{\cdot}999\,[\text{M/G ratio from GLC analysis}]$$

with a correlation coefficient of 0·998. Clearly, there is a good agreement between these last two parameters, which shows that the total amount of liberated formic acid can be a measure of the galactose content of galactomannan samples. It is important to note that, among the galactomannan gums used, two categories of samples can be distinguished: (*a*) guar, tara, Lygomme 6, and Indal of commercial origin and (*b*) canela and mulata prepared in the laboratory (Gonçalves *et al.* 1988). The periodate oxidation of galactomannans seems to be independent of the method of gum preparation.

Table 2. Parameters related to the physical functionality of galactomannans

Sample	mmol FA/g[a] of galact. Mean(S.D.)	M/G from eqn(1) Mean(S.D.)	G'_{max}[b] from eqn(3) Mean(S.D.)	G'_{max}[b,c] experimental
Guar	2·090 (0·006)	1·655 (0·014)	—	31·6
Tara	1·521 (0·006)	3·000 (0·014)	336·4 (1·7)	329
Canela	1·282 (0·005)	3·565 (0·012)	522·5 (2·4)	471
Lyg.6	1·260 (0·007)	3·617 (0·017)	444·6 (2·6)	477
Mulata	1·179 (0·006)	3·809 (0·014)	588·2 (3·2)	596
Indal	1·081 (0·006)	4·040 (0·014)	678·3 (3·6)	637
Indal HWS	0·789 (0·006)	4·731 (0·014)	897·0 (4·4)	907

[a] The values shown are the means of three determinations.
[b] In Pa.
[c] As determined by Fernandes *et al.* (1991).

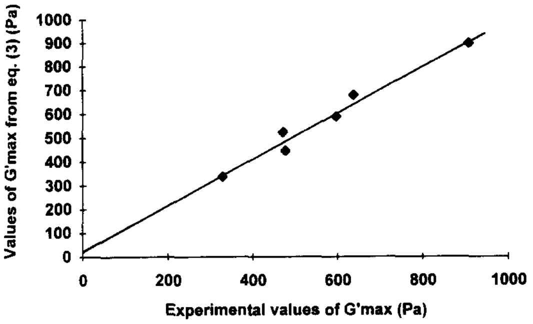

Fig. 3. Values of $G'_{\max}$ calculated from eqn (3) as a function of the experimental values of $G'_{\max}$ of Fernandes *et al.* (1991).

Prediction of the physical functionality of galactomannans

The linear relation, $G'_{\max} \sim [\eta](M/G)^2$, reported by Fernandes *et al.* (1991) for 1% 4 : 1 kappa-carrageenan/galactomannan mixed gels can be described according to the equation:

$$G'_{\max} = 163 + 2{\cdot}05\,[\eta](M/G)^2 \tag{2}$$

with a correlation coefficient of 0·987 (15 points). It seems clear that the synergistic effects of a galactomannan sample when mixed with kappa-carrageenan, taken as the $G'_{\max}$ value, are strongly dependent on the macromolecular characteristics of the galactomannan, namely of M/G ratio. Thus it is possible to use this parameter (M/G) in order to predict the physical functionality of galactomannans in kappa-carrageenan/galactomannan mixed gels. As demonstrated above, galactomannan periodate oxidation seems to be a good analytical method to determine the M/G ratio of galactomannans. We thus replaced the M/G-ratio factor in eqn (2) by its equivalent in eqn. (1) and obtained the following equation:

$$G'_{\max} = 163 + 2{\cdot}05\,[\eta]> ((2{\cdot}79 - \text{mmol FA/g})/0{\cdot}423)^2 \tag{3}$$

which directly relates the number of mmol of liberated formic acid from the galactomannan periodate oxidation with the $G'_{\max}$ value of 1% 4 : 1 kappa-carrageenan/galactomannan mixed gels. In order to study the application of eqn. (3) in the prediction of the galactomannan physical functionality, the mmol FA/g values of the several galactomannans were used to calculate $G'_{\max}$. The values of $G'_{\max}$ obtained from eqn. (3) are presented in Table 2. In this table, the experimental values of $G'_{\max}$, as reported by Fernandes *et al.* (1991), are also shown for comparison. The guar-gum sample was not considered in the above calculation because this galactomannan does not present synergistic effects when mixed with kappa-carrageenan. It should be noted that, in the applicability of eqn (3), the value of the intrinsic viscosity of the galactomannan sample is needed since this parameter has to be taken into account in the synergistic effects between the kappa-carrageenan and galactomannans. However, everyone should by now be aware of the benefits that accrue by using the master curve of zero-shear-rate specific viscosity, η_{spo}, against reduced concentration, $c[\eta]$, that was established for LBG samples (Doublier & Launay, 1981; Fernandes *et al.*, 1989). The intrinsic viscosity can be rapidly determined as described by Fernandes *et al.* (1991). In Fig. 3 is plotted the $G'_{\max}$ values calculated from eqn (3) against the $G'_{\max}$ experimental values. As can be seen, a linear relation between these two parameters was found, which can be described by the following equation:

$$G' \text{ from eqn (3)} = 21{\cdot}9 + 0{\cdot}976\ G'_{\mathrm{exp.}}$$

with a correlation factor of 0·987. In fact, there is good agreement between the $G'_{\max}$ obtained from eqn (3) and the experimental values of $G'_{\max}$ reported by Fernandes *et al.* (1991). Thus, it seems possible to determine accurately the physical functionality of galactomannans in kappa-carrageenan/galactomannan mixed gels by a simple galactomannan periodate oxidation.

CONCLUSION

The use of the analytical method of galactomannan periodate oxidation to determine the M/G ratio of the polysaccharide is a highly attractive possibility because the results obtained have shown that the procedure is simple to operate, precise, and less expensive than all the other methods used. Furthermore, it is possible to distinguish the M/G ratio among several galactomannans (tara and locust-bean gums), which, in fact, can be directly related to the synergistic interactions observed in galactomannan/kappa-carrageenan systems (Fernandes *et al.*, 1991, 1992), and thus to predict the physical functionality of each LBG sample. It is noteworthy that the relationship between galactomannan periodate oxidation and M/G-ratio values showed: (*a*) that it did not depend on the type of gum used (commercial or prepared at the laboratory) and (*b*) that it was valid for a very large range of M/G-ratio values (from 1·7 up to 4·8).

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