EFFECT OF ENZYMIC MODIFICATION ON THE SOLUTION AND INTERACTION PROPERTIES OF GALACTOMANNANS

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ABSTRACT

Detailed studies have been performed on the solution and interaction properties of guar and carob galactomannans modified by treatment with highly purified α-D-galactosidase, *endo*-β-D-mannanase and *exo*-β-D-mannanase enzymes.

KEYWORDS

Galactomannans; enzymic modification; solution properties; interactions; α-D-galactosidase; *endo*-β-D-mannanase; *exo*-β-D-mannanase.

INTRODUCTION

The commercially available galactomannans are derived from the seed endosperms of carob (*Ceratonia siliqua*), guar (*Cyamopsis tetragonolobus*) and to a lesser extent tara (*Caesalpinia spinosa*) (Dea and Morrison, 1975; Neukom and Nittner, 1980). These galactomannans all give solutions of high viscosity at low polymer concentration, but each has its own unique characteristics. Guar gum hydrates rapidly in either hot or cold water, whereas complete dissolution of carob galactomannan requires cooking at elevated temperatures (Hui and Neukom, 1964). Carob galactomannan interacts strongly with a number of polysaccharides including agar, carrageenans and xanthan (Dea and Morrison, 1975), and these interactions are exploited commercially. The interesting and novel chemical and physical properties of other galactomannans such as those from *Caesalpinia spinosa* (tara gum) and *Leucaena leucocephala* seeds (McCleary, 1979a) are currently being realised.

The traditional source of galactomannan is from seeds of the carob tree which was cultivated many centuries before the Christian era. The ancient Egyptians prepared the strips with which they bound their mummies, using carob paste. Carob is a native of Southern Europe and the Near East and the best quality seeds come from Sicily, where the trees were probably planted in the 16th-17th century.

Guar emerged as a commercial source of galactomannan in response to the limited supply of carob to the U.S.A. during World War II. The guar plant is native to north-west India and Pakistan where it has been grown for thousands of years for use as cattle fodder and as a green vegetable. World demand for guar gum has

increased rapidly in recent years and is reaching a point where traditional suppliers such as India, Pakistan and the U.S.A. are hard pressed to meet the demand. Consequently, considerable effort is being expended to develop guar as an economic agricultural crop in other countries, including Australia.

Galactomannans are present in a wide range of legume seeds in amounts varying from 0.1 to about 35% of seed weight (Dea and Morrison, 1975). The viscosity and thickening properties of most of these galactomannans are similar to those of carob and guar galactomannans. With the current shortage and high price of carob seeds, the possibility of economically producing, from another seed source, a galactomannan with similar solution and interaction properties to carob galactomannan, becomes more attractive. To some extent tara gum will help fill this requirement. However, other legumes which produce a similar galactomannan may prove economically viable, e.g. *Crotalaria mucronata* and *Caesalpinia vesicaria*. *Caesalpinia vesicaria* produces copious quantities of seeds which contain 28.5% galactomannan (galactose/mannose = 28:72). This galactomannan (limiting viscosity number, 1330 mL/g) has similar solution properties to guar galactomannan, but interacts with xanthan to an extent intermediate between that of guar and tara galactomannans (McCleary, unpublished data). *Sesbania cannabina* seeds contain 17.5% of a high viscosity galactomannan (limiting viscosity number, 1440 mL/g) but the polymer has a high galactose content (39%) and the degree of interaction with xanthan is low.

SOLUTION PROPERTIES OF GALACTOMANNANS

Carob and guar galactomannans have similar solution viscosities, but differ markedly in their ease of dissolution and in their interaction properties. The limiting viscosity numbers determined for dilute solutions of guar and carob galactomannans are alike i.e. 1330 and 1030 mL/g respectively (McCleary, Matheson and Small, 1976). However, unlike carob galactomannan, guar galactomannan has the ability to hydrate rapidly in cold water to produce highly viscous solutions. The viscosities of 1% solutions of guar and carob galactomannans, hydrated at 25°C, are 4200 and 100 cps, respectively (Whistler and Hymowitz, 1979). The difficulties experienced in the dissolution of carob galactomannan are believed to be due to interchain associations which restrict hydration. These associations occur at regions in the mannan backbone which are unsubstituted or lightly substituted with galactose (Morris and colleagues, 1977). More such regions are likely to be present in carob galactomannan than in guar galactomannan, due to the lower galactose content of the former polysaccharide i.e. the galactose/mannose ratio of carob galactomannan is 23:77 and that of guar galactomannan is 38:62 (McCleary, 1979a).

INTERACTION PROPERTIES OF GALACTOMANNANS

Carob galactomannan interacts strongly with a number of polysaccharides including agar, carrageenans and xanthan (Dea and Morrison, 1975). Gels are formed at polymer concentrations as low as 0.1% w/v. Guar galactomannan also interacts with these polysaccharides but to a much lesser extent than carob galactomannan, resulting only in viscosity enhancement.

Attempts to explain the marked difference in interaction of carob and guar galactomannans with other polysaccharides, have involved detailed investigations of the "fine-structures" of these galactomannans. Initial investigations employing X-ray diffraction (Palmer and Ballantyne, 1950), chemical (Baker and Whistler, 1975) and enzymic techniques (Courtois and Le Dizet, 1970) indicated that the galactose distribution along the D-mannan backbone of guar galactomannan was uniform i.e. a galactose substituent on every second mannosyl residue; and that in carob galacto-

mannan the galactose was distributed in a block-type pattern i.e. blocks of the D-mannan backbone that are totally (or almost totally) substituted with single galactopyranosyl groups, which are separated by regions of the D-mannan chain that are essentially unsubstituted with galactose (Fig. 1). However, recent results obtained using highly purified enzymes (McCleary, 1979a) and a range of chemical and physical techniques including periodate oxidation (Hoffman and colleagues, 1975), n.m.r. (Grasdalen and Painter, 1980) and X-ray diffraction (Marchessault and co-workers, 1979), indicate that, in fact, the galactosyl residues in both guar and carob galactomannans are distributed in an irregular to random pattern (Fig. 1).

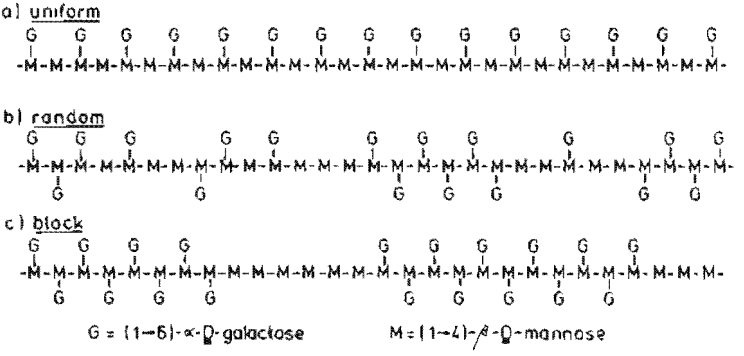

Fig. 1. Possible distribution patterns of galactosyl residues along the D-mannan backbone of galactomannans.

It is now generally accepted that agar, carrageenans and xanthan interact with galactomannans at regions in the D-mannan backbone which are either unsubstituted or lightly substituted with galactose. Recent research has also indicated that sections of the mannan backbone which are substituted on only one side with galactose, can also interact with xanthan (Fig. 2) (McCleary, 1979a).

GALACTOMANNAN HYDROLYZING ENZYMES

A range of galactomannan degrading enzymes has been reported in the literature (Dey, 1978). These include α-D-galactosidase, *endo*-β-D-mannanase (β-D-mannanase), galactomannanase (Whistler and colleagues, 1950), *exo*-β-D-mannanase (Lee, 1965), β-D-mannosidase (Reese and Shibata, 1965), galactomannan depolymerase (Hylin and Sawai, 1964) and oligo-β-D-mannosyl-(1→4)-phosphorylase. Whistler and co-workers (1950) accurately predicted that the crude preparation of "galactomannanase" they extracted from germinating guar seeds contained two or more guaran hydrolyzing enzymes. Lee (1965) showed that enzyme preparations from guar seeds contained α-D-galactosidase, *endo*-β-D-mannanase and another enzyme described tentatively as *exo*-β-D-mannanase. The galactomannan-depolymerase reported by Hylin and Sawai (1964) from seeds of *Leucaena leucocephala* is thought to have consisted of a mixture of β-D-mannanase, α-D-galactosidase and β-D-mannosidase (or *exo*-β-D-mannanase).

In the current work, the effect of α-D-galactosidase, β-D-mannanase and *exo*-β-D-mannanase on the solution and interaction properties of galactomannans, was studied. Each of the enzymes used was highly purified and devoid of interfering

enzyme activities.

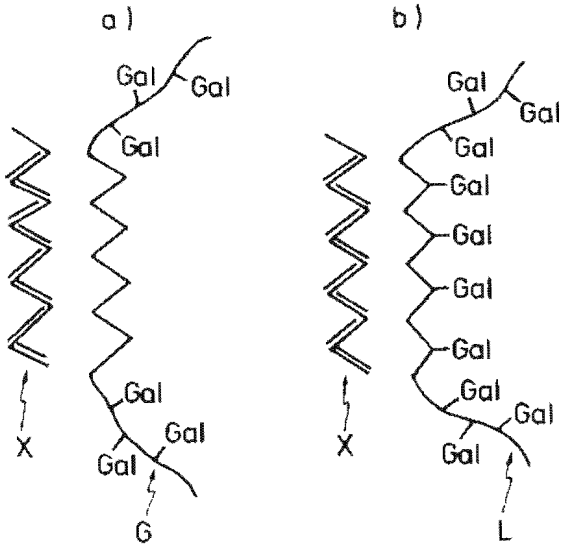

Fig. 2. Proposed structural requirements in galactomannans for interaction with xanthan: (a) A model proposed for the interaction between chains of xanthan (X) and galactomannan (G) (Morris and colleagues, 1977). (b) A model proposed for the interaction between chains of xanthan (X) and *Leucaena leucocephala* galactomannan (L) (McCleary, 1979a).

endo-β-D-Mannanase (β-D-Mannanase EC 3.2.1.78)

endo-β-D-Mannanase acts by random cleavage of the D-mannan chain, producing a series of manno- and galacto-manno-oligosaccharides and giving a rapid viscosity decrease (Dekker and Richards, 1976). Hydrolysis of galactomannans by this enzyme is affected by the degree of galactose substitution. Furthermore, β-D-mannanase enzymes from different sources have different abilities to cleave at points in the mannan backbone which are highly substituted with galactose (McCleary, 1979b).

Carob galactomannan interacts strongly with xanthan, resulting in gel formation at very low polymer concentrations. The sites of interaction in carob galactomannan are the same regions which are most susceptible to hydrolysis by β-D-mannanase i.e. those essentially unsubstituted with galactose. However, as shown in Fig. 3, the presence of xanthan has little effect in protecting carob galactomannan from hydrolysis by β-D-mannanase. This suggests that either only a very small proportion of the available unsubstituted sections of the D-mannan backbone of carob galactomannan are involved in interaction with xanthan at the "junction-zones", or alternatively that the interaction is dynamic, such that once the polysaccharide molecules separate the "galactose-poor" regions of carob galactomannan are susceptible to β-D-mannanase attack.

Some of the problems experienced in the use of guar gum may be due to the presence of trace quantities of β-D-mannanase in the commercial flour or in the material with which the flour is mixed. β-D-mannanase contamination of guar flour can occur by microbial infection of the seed or by *de novo* synthesis of the enzyme in the seed. In seed production areas where high rainfall and humidity are experienced

in the pre-harvest period, seed blackening occurs. A wide range of fungi, including *Aspergillus* sp., *Fusarium* sp. and *Alternaria* sp. which occur on, or in guar seed (Jain and Patel, 1969) have been shown to be associated with severe cases of seed blackening. Each of these fungi produces a wide range of hemicellulases, including β-D-mannanase. Under the environmental conditions just described, β-D-mannanase may also be synthesized by the seed.

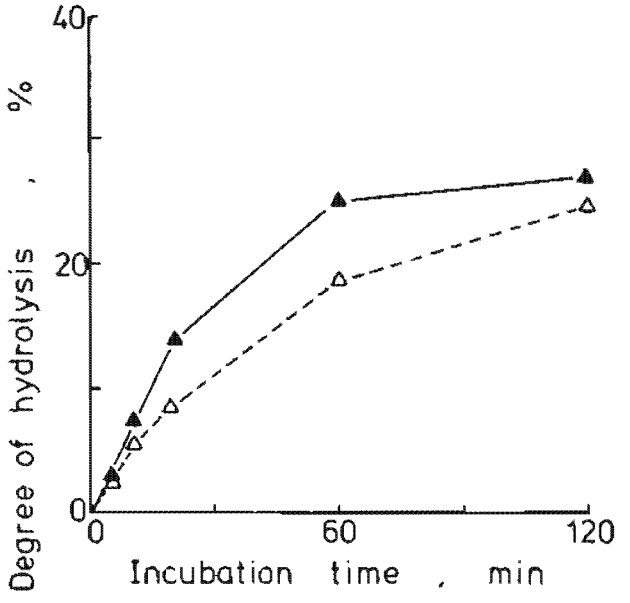

Fig. 3. Effect of the presence of xanthan on the hydrolysis of carob galactomannan by β-D-mannanase. Samples of carob galactomannan (0.2 mL, 0.1%) were mixed with xanthan (△, 0.2 mL, 0.1%) or water (▲) at 80°, stored at 4° and then equilibrated to 15° before addition of β-D-mannanase.

In certain applications, the galactomannan viscosity-destroying properties of β-D-mannanase can be used to advantage. In the production of guar gum, a high protein meal (guar meal) is obtained as a by-product (Whistler and Hymowitz, 1979). However, this meal contains significant amounts of guar gum due to incomplete separation of the seed endosperm from the proteinaceous cotyledonary material during the milling process. If untreated guar meal is fed to monogastric animals (pigs and poultry) the galactomannan "gels" in the intestinal tract resulting in poor absorption of nutrients as well as either diarrhoea or sticky excrement. Feeding trials with chickens have shown that this problem can be overcome by supplementing guar meal with commercial enzyme preparations containing β-D-mannanase. To minimize the cost of enzyme supplementation further research is needed to optimize the levels of enzyme required, and to identify the best source of a β-D-mannanase with the desired stability, pH activity and hydrolytic properties.

exo-β-D-Mannanase (EC 3.2.1.25)

This enzyme acts in an *exo*-fashion, removing single D-mannosyl residues from the non-reducing end of manno-polysaccharides and manno-oligosaccharides. β-D-Manno-oligosaccharides of a degree of polymerization greater than four are the preferred substrates for the *exo*-β-D-mannanase from guar seeds (McCleary, unpublished data).

Like most other *exo*-polysaccharases, this enzyme is unable to cleave glycosidic linkages beyond a branch point and thus it has negligible action on native galacto-mannans. Incubation of high levels of this enzyme (20 nkat on mannopentaitol) with carob galactomannan for two hours gave no detectable decrease in viscosity nor increase in reducing sugar level.

α-D-Galactosidase (EC 3.2.1.22)

α-D-Galactosidase catalyses the random cleavage of (1→6)-α-D-linked galactosyl residues from the D-mannan backbone of galactomannans (McCleary, 1979a). In current research it has been found that α-D-galactosidases from lucerne and guar seeds can remove essentially all of the D-galactosyl residues from a range of galactomannans including those from guar, carob and lucerne seeds.

The effect of pure α-D-galactosidase and β-D-mannanase on the solution viscosity of carob galactomannan is shown in Fig. 4. Over the incubation period used (2 hours), α-D-galactosidase (3.3 nkat on this substrate) removed 35% of the D-galactosyl residues from carob galactomannan with no concomitant decrease in viscosity. However, even 0.0004 nkat of β-D-mannanase caused a significant decrease in viscosity over the same incubation period.

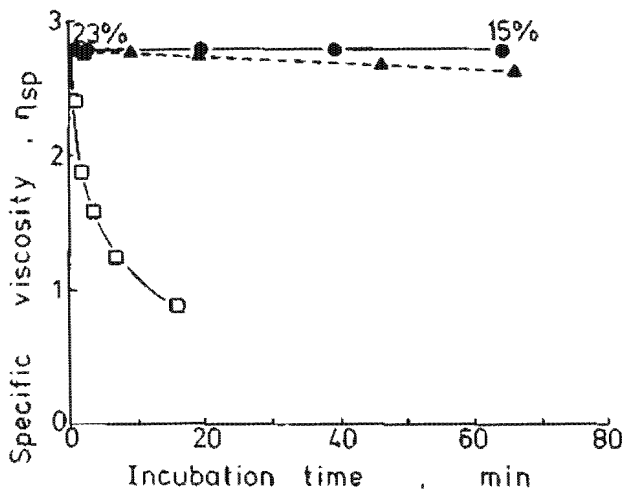

Fig. 4. Effect of α-D-galactosidase and β-D-mannanase on the solution viscosity of carob galactomannan (McCleary and colleagues, 1981). Carob galactomannan (15 mL, 0.1%) in 0.1 M acetate buffer (pH 4.5) was incubated at 30° in an Ubbelohde suspended-level viscometer with either α-D-galactosidase (●) 3.3 nkat (on this substrate), or β-D-mannanase, (□) 0.4 nkat or (▲) 0.0004 nkat. During 65 min incubation, the galactose content of carob galactomannan was diminished from 23 to 15% by 3.3 nkat of α-D-galactosidase.

The hydrolysis of guar and carob galactomannans by α-D-galactosidase A from lucerne seed is shown in Fig. 5. In this experiment essentially all the D-galactosyl residues were removed after a two hour incubation period, but there was only a slight decrease in solution viscosity. The large viscosity decrease experienced over the next four hours was due to an alignment of β-D-mannan-type polymers which eventually formed particles large enough to be visible. The solution became distinctly turbid as a β-D-mannan precipitate formed and settled from solution. The

time required for the formation of this β-D-mannan precipitate was directly related to the concentration of polysaccharide.

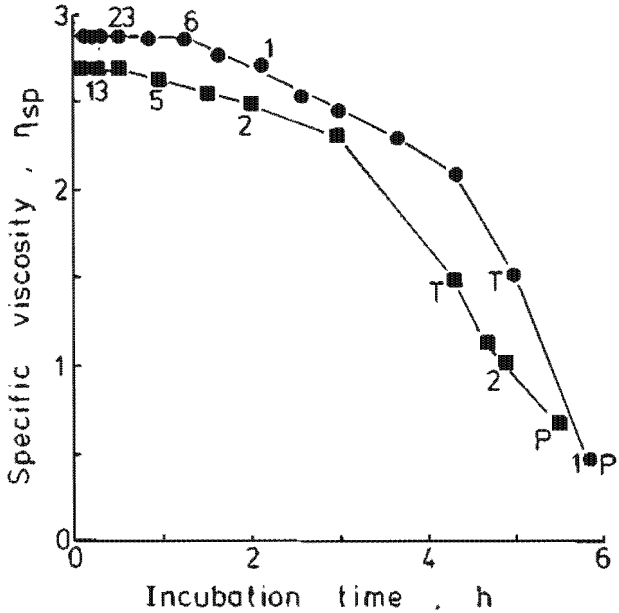

Fig. 5. Effect of galactose removal on the solution viscosity and solubility of galactomannans from guar (●), and carob (■). Galactomannan solution (17 mL, 0.1%) in 0.1 M acetate buffer (pH 4.5) was incubated with lucerne α-D-galactosidase A (6.2 nkat) in an Ubbelohde suspended-level viscometer at 40°. Numbers represent the galactose content of the remaining polysaccharide. At point T, the solution was very turbid; at point P, a precipitate had formed.

The viscosity curves and limiting viscosity numbers of α-D-galactosidase treated guar galactomannan samples, are shown in Fig. 6. The removal of galactose from guar galactomannan resulted in galactomannans which had higher limiting viscosity numbers and steeper viscosity curves. But, if the viscosities were plotted against the concentration of the "mannan-backbone" in these polymers, a single curve was obtained, independent of the galactose content. This indicates that the solution viscosity of such galactomannans is totally dependent on the nature of the mannan backbone. The galactose side groups play a very important role in determining the ease with which galactomannans can be dissolved, but they do not affect the degree of interaction between galactomannan molecules in dilute solutions (assuming of course that the galactose content is sufficient to maintain solubility). Furthermore, the galactose side groups have no apparent effect on the conformation of galactomannan molecules in dilute solutions.

The solution properties of the α-D-galactosidase modified galactomannans are summarised in Table 1. At concentrations of 0.4% w/v, solutions of galactomannans with D-galactose contents ranging from 25 to 38% showed no tendency towards gel formation or retrogradation on storage at 4°. However, those containing between 15 and 20% D-galactose formed gels on storage at 4° for 15 days. On storage for a further 45 days the gels began to retrograde and became quite opaque. Solutions of galactomannans which contained less than 10% D-galactose were quite unstable at 30° or 4° and an insoluble mannan-type precipitate rapidly formed. Gel formation

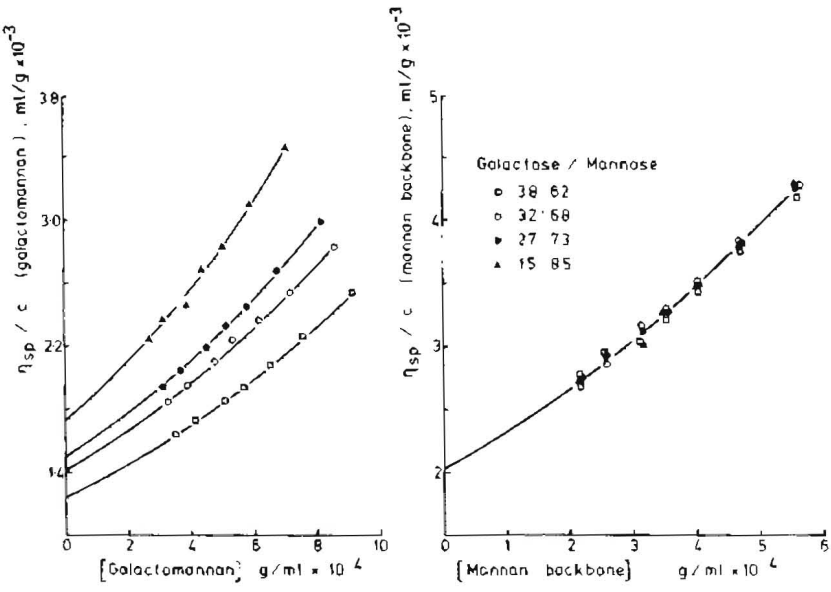

Fig. 6. Effect of galactose removal from guar galactomannan on limiting viscosity number. The galactose/mannose ratios of the polysaccharides are 38:62 (□), 32:68 (○), 27:73 (●), and 15: 85 (▲).

TABLE 1 Effect of Galactose Content on the Solution Properties of α-D-Galactosidase-Treated Guar Galactomannan[a]

Galactose: mannose ratio	Storage time (days)	Storage temperature		Freezing and thawing
		30°	4°	
38:62 to 25:75	1	S	S	S
20:80 to 15:85	1	S	S	G
	15	S	G	G
	60	-	G/P	G
Less than 10: 90	1	P	P	P

[a]Galactomannan solutions (0.4%, salt free) were stored under the described conditions and defined as totally soluble (S) if there was no evidence of either gel formation (G) or precipitate formation (P).

and retrogradation are due to ordered, non-covalent associations between sections of the galactomannans which are essentially unsubstituted with D-galactose. The extent of this interaction is dependent on the D-galactose content of the galactomannans, so that those samples containing 25-38% D-galactose have very few regions which can enter into these associations, whereas those with 15 to 20% D-galactose interact to form a three-dimensional gel network. Gel formation is due to an aggregation of the essentially unsubstituted mannan regions in a regular ribbon-like conformation, with the non-interacting "galactose-rich" regions serving to

solubilise the network (Dea and Morrison, 1975). Galactomannans with less than 10% D-galactose have a large proportion of the mannan chain available for interaction but have insufficient "galactose rich" regions to solubilise the network so an insoluble precipitate forms. Interchain associations are induced by a freeze-thaw treatment of galactomannan solutions. Ice crystal formation progressively raises the effective polymer concentration in the residual unfrozen solution and thus promotes association (Dea and Morrison, 1975). Thus, although solutions of treated guar galactomannan of D-galactose content of 15-20% are quite stable on storage at 4° for 24 hours, a freeze-thaw cycle results in gel-particle formation. Carob galactomannan (23% galactose) behaves in a very similar way.

The effect of galactose removal from guar galactomannan on its degree of interaction with xanthan polysaccharide is shown in Fig. 7. As the D-galactose content decreases the degree of interaction increases. Treated guar galactomannan with a

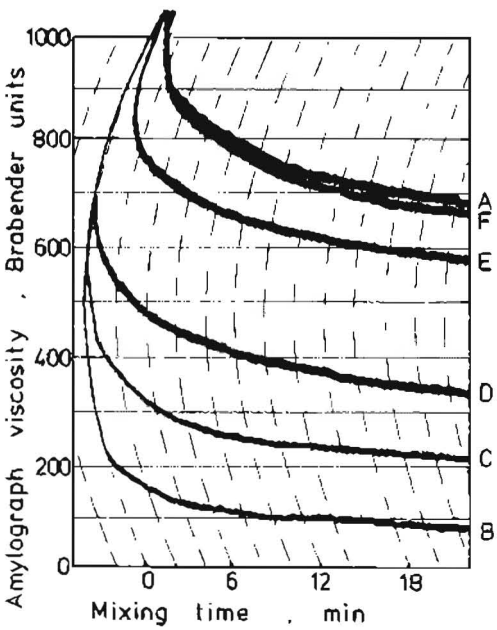

Fig. 7. Gelling interactions of galactomannans with xanthan as determined with a Brabender (Duisburg) Amylograph. Sample A is xanthan (0.1% w/v) plus carob galactomannan (0.1% w/v). Samples B-F are xanthan (0.1% w/v) plus α-D-galactosidase-treated guar galactomannan (0.1% w/v) with galactose/mannose ratios of B, 38:62 (native); C, 34:66; D, 29:71; E, 25:75 and F, 19:81.

D-galactose content of 19% interacts with xanthan to essentially the same degree as carob galactomannan (23% D-galactose content), and the galactose distribution in both these galactomannans has been shown to be irregular to random. Consequently, it would appear that the profound difference in the interaction of native guar and carob galactomannans with xanthan can be explained simply in terms of their different galactose/mannose ratios. A major difference in the fine-structures (i.e. the distribution of galactosyl residues along the mannan backbone) of these two galactomannans, as proposed by other workers, is not necessary to explain this phenomenon.

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