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Using of Volatile Release Measurements to Understand Polysaccharide Molecular Interactions: An Example with Guar Gum Study

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Summary: It is well establishment that polysaccharides are commonly used in many fields of formulation such as food and cosmetic industries as viscosity modifiers or gelling agents. Among the different impacts on the mixture properties, these materials are also known to affect the aroma release or retention thus its sensory perception; the effect depends whether polysaccharide structure induces specific retention or release phenomena from the complex media. Present study deals with studying aroma compounds behaviour from dilute and semi-dilute aqueous polysaccharide solutions consisting of different guar gum samples.

Results allow stating that the whole polymer characteristics as determined by means of structural analysis, protein content determination, rheological and surface tension measurements play a major role on the polysaccharide/aroma compound interaction mechanisms. In particular, results evidence a strong Mannose to Galactose (M/G) ratio effect, thus allowing stating the occurrence of hydrophobic *intra* and *inter*-molecular mechanism. In addition to that, proteins are demonstrated to only slightly affect the volatile retention.

More generally, such an original study allows new highlights concerning macromolecular organisation and interactions in solution.

Keywords: aroma release; guar; molecular interactions; structural properties

Introduction

Thickeners and stabilizers are commonly used as formulation aids in food industry mainly for their ability to increase viscosity or to lead to gelled mixtures. Among the numerous polysaccharides employed in the food industry, galactomannans gums as extracted from plant seeds are some of the most commonly employed ones.

As visible below, the chemical structure of galactomannans consists of a linear backbone of β-D-mannose residues (M) attached by $(1 \rightarrow 4)$ linkages, this main backbone being substituted with α-D-galactose residues (G) attached through $(1 \rightarrow 6)$ linkages. Guar gums belong a low M/G ratio ($\sim$1.6) while locust bean gums are less substituted (M/G $\approx$ 3.5–4.0).

Due to the large industrial utilization of galactomannan polysaccharides, most of it have been extensively studied to elucidate first their chemical and structural characteristics but also to better understand their tendency to self associate in aqueous media through intra and/or intermolecular mechanisms;[1,2] unfortunately, in spite of numerous studies dealing with many experimental approaches, many questions still remain unanswered.

Otherwise, guar and locust bean gums, the most used galactomannans in food applications, have been demonstrated to markedly influence the aroma compounds

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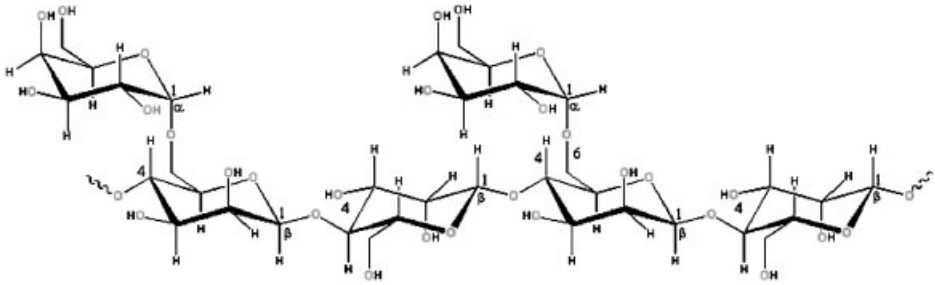

Figure 1.

retention or release: different authors established that the more hydrophobic the volatile compound the larger the retention.[3–5]

However, the exact mechanism involved in aroma retention by the polysaccharide solutions has not been completely understood. One of the reasons is that the chemical structure of such polysaccharides varies according to the source and/or the industrial process used to extract and purify it as ones more recently underlined.[6] As an example, the galactose content of galactomannans can vary from zero to one galactose on every mannose residues, depending on the botanical origin.[7]

The polysaccharide variability effect on properties such as rheology has been already established, but limited data concerning its impact on aroma retention are available.

The objective of this study is to clearly link the chemical structure of galactomannan of guar type to the mechanisms involved in aroma retention, thus bringing information for macromolecular mechanisms of interaction.

Materials

The aroma compounds used in the study were obtained from Sigma-Aldrich (France). Their physicochemical properties are given in Table 1. Different guar gum powders were kindly furnished by Danisco (Portugal), Degussa (France) and Alland & Robert (France). The salt (NaCl) was provided by Merck (Merk eurolab, France) and the bactericide (NaN_3) by Prolabo (Rhône-Poulenc France). Pure Water was obtained from a Easypure UV Compact ultrapure water system (Barnstead).

Preparation of the Polysaccharide Solutions

Galactomannan powders (0.5% w/w) were first dispersed at 85 °C for 3 hours in pure water under mechanical stirring, and then kept under stirring for 2 additional hours at room temperature. Then, the galactomannan solutions were centrifuged at 12 000 rpm for 30 min to remove insoluble species; at this stage, dry extract allowed determination of total polysaccharide concentration in the supernatant.

Table 1.
Physicochemical properties of aroma compounds.

Aroma compounds	Solubility in water $g \cdot L^{-1}$ (25 °C)	Saturated pressure mmHg (25 °C)	Log P
Ethyl butanoate	5.60	17.3	1.7
Ethyl hexanoate	0.63	1.56	2.8
Ethyl octanoate	0.07	0.21	3.8
Ethyl decanoate	0.016	0.03	4.8

All values from Syracuse Research Corporation (2006)://esc.syrres.com/interkow/physdemo.htm.

Finally, an appropriate amount sodium chloride was added to get 0.01 $mol \cdot L^{-1}$ salt content and 400 ppm of bactericide (NaN_3) was added to prevent polymer bacterial degradation.

Polysaccharide solutions were diluted with salted water to obtain a final concentration of 0.1% (w/w) for retention experiments. When needed for analytical experiments, further dilutions were obtained using salted water.

All analysis and measurements as described below were performed at $(35 \pm 0.1)\,^{\circ}C$.

Protein Determination

Protein content (% w/w) was determined by means of the Bradford method.[8] For galactomannans solutions, the protein determination was performed on purified solutions as obtained by centrifugation.

Viscometric Characteristics

Viscometric parameters of polysaccharide aqueous solutions were measured by means of an Ubbelohde capillary tube at 35 °C as fixed using an immersion controlled bath. All measurements were done at least in duplicate.

Intrinsic viscosity [η] and Huggins coefficient k' were determined using the so-called Huggins representation method. Critical overlapping concentration C_{Cr} was calculated by means of the Mark-Houwink relationship.[9]

Structural Characterisation

M/G ratios were estimated by measuring the optical rotation properties by means of polarimetric measurements, and using the relationship (1) proposed by Morris,[10] method recently modified by Doyle and co-workers.[11]

$$M/G = (235 - [\alpha]_D)/(50 + [\alpha]_D) \qquad (1)$$

With:

- $[\alpha]_D$ the specific rotation measured at 589 nm, and defined as $[\alpha]_D = \alpha/(c \times l)$;
- α is the solution optical rotation in degrees;
- c is total polymer concentration expressed in $g \cdot mL^{-1}$;
- l is path length in dm.

Optical rotation measurements were made using the emission lines from a mercury lamp at 365; 436; 546; 578 nm and a sodium lamp at 589 nm. The values of optical rotation at 589 nm were then derived from linear Drude plots ($1/\alpha$ versus λ^2). To improve the precision of the $[\alpha]_D$ values, measurements were performed for three concentrations.

Apparatus was Perkin Elmer 241 Polarimeter, using a cell of path length of 0.1 m, and experiments were performed at 20 °C.

Surface Tension Measurements

A 3S Tensiometer (GBX, France) with 10^{-4} g accuracy was used to measure the surface tension using Wilhelmy dynamic plate method at 35 °C in a humidity saturation chamber. Platinum plate was systematically carefully cleaned using successively purified water and acetone and finally by red flame heating. Results were computed with GBX software.

Aroma Retention/Retention Study

PRV (Phase Ratio Variation) method was used to measure partition coefficients K of aroma compounds, in each polysaccharide solution, considering salted water (NaCl 0.01 $mol \cdot L^{-1}$) in the presence of NaN_3 (400 ppm) as the reference as already described in the literature.[12] This method is based on the relationship between the phase ratio β (ratio of gas phase volume to liquid phase volume) and K. Details of corresponding theory is fully described elsewhere,[13] and description of experimental material and methods is fully described in the cited references.

Pure esters were added to polysaccharide solution to obtain a final aroma concentration of 25 ppm, except for ethyl decanoate that was first dissolved in ethanol ($5\,g \cdot L^{-1}$) and then introduced in each solution to get a final concentration of 9 ppm, this being the consequence of its

low solubility in aqueous media when compared to the other esters used.

Increasing volumes (1, 2, 3 and 4 mL) of mixtures were then transferred in 20.5 mL vials in order to get different phase ratios β (19.5, 9.25, 5.83 and 4.125) and then vials were hermetically sealed. Each phase ratio was tested duplicate. All samples were then left 6 hours at 35 °C until equilibrium phase repartition is reached. Finally, a 1 mL volume of the gas phase was automatically picked up from the vial by means of a syringe and then introduced in the chromatographic column for quantification. K was measured in triplicate for each vial, and finally the retention was determined by means of Equation (2):

$$R(\%) = (1 - (K_{poly}/K_{water}) \times 100 \qquad (2)$$

K_{poly} is the partition coefficient of aroma compounds in polysaccharide solutions and K_{water} the partition coefficient of aroma compounds in water.

As the retention measurements were performed at equilibrium, the viscosity effect often involved in aroma retention by diffusion process was negligible and present results allow highlighting physicochemical interactions between aroma compounds and polysaccharides. Retention of ethyl hexanoate and ethyl decanoate was particularly investigated as allowing a better understanding of the nature of interactions taking place in such polysaccharidic systems.

Chromatographic analysis were performed using a Varian 3800 equipped with a Combipal CTCanalytics: FID 250 °C, injector: 250 °C, capillary column (SPB1, Supelco) (length: 15 m; internal diameter: 0.25 mm; film thickness: 0.25 μm), helium: 1 $mL \cdot min^{-1}$, oven temperature programme: 150 °C during 3 min.

Results and Discussion

Guar Gum Structural Characterization and Solution Properties

Table 2 reports the polysaccharide properties for 4 different guar gum samples as determined by using the analytical methods explained above.

Firstly, the protein content appears quite similar from one sample to another, and is related to the presence of proteins species strongly linked to galactomannan backbone. Different authors link the presence of proteins together with the surface tension reduction of corresponding galactomannan solutions,[14,15] this resulting in specific binding of low polar compounds such as aromas.

Moreover, Mannose to Galactose ratio (M/G) varies from 1.23 to 1.83 as the consequence of variability of plant seeds used to extract the polysaccharide samples.

The intrinsic viscosity $[\eta]$ is almost identical for the different specimen except for Guar B, thus indicating low average molecular weight for this last sample when compared to the 3 others. As a direct consequence, critical overlapping concentration is higher for guar B, and 0.1% (w/w) concentrations as used for aroma release experiments correspond to semi-dilute regime for A, C and D guar while B gum still remains in dilute conditions. The knowledge of the concentration regime is actually of primary importance as gum addition may have an effect on aroma volatility only at levels above C^* as indicated by several authors.[16,17]

Table 2.
Chemical composition and physicochemical properties of guar samples.

Sample	M/G	Proteins % (w/w)	[η] (dL/g)	k′	C_{Cr} (% w/w)	Surface Tension
Guar A	1.45	1.33	13.2	0.82	0.06	58
Guar B	1.83	0.9	4.0	0.83	0.19	53
Guar C	1.61	0.61	16.2	1.17	0.05	55
Guar D	1.23	0.85	14.2	0.97	0.05	64

The Huggins's coefficients k′ allows to evidence solute-solvent level interactions and macromolecular intra and/or intermolecular aggregation. The better the solvent quality the lower k′ parameter, while the higher the aggregation tendency the higher the k′ becomes. In the present case, k′ appears quite similar from one sample to another thus indicating a same fairly low aggregation tendency in solution at 0.1% (w/w).

Aroma Release Study from Guar Solutions

Figures 2 and 3 illustrate the behaviour of aroma compounds in 0.1% (w/w) guar solutions for ethyl decanoate and ethyl hexanoate respectively.

From a general point of view, the protein content being roughly identical from one guar sample to another, results clearly demonstrate that polymer-volatile interactions are not simply governed by specific interactions of volatile with protein species.

In addition, effects of M/G ratio are clearly evidenced: firstly retention of ethyl hexanoate is negative in the case of low M/G ratio, then becomes positive for M/G = 1.5 and sharply increases for M/G = 1.6 and M/G = 1.8. At the same time, it is interesting to note that guar D and guar C samples, having similar molecular weight as related by calculation using viscometric data, induce an opposite behaviour of ethyl hexanoate. Among other polymer characteristics, this appears to be mainly the consequence of guar gum galactose content.

The whole experimental results allow to state that aroma behaviour is strongly related to its hydrophobicity: in all cases the retention become higher as the volatile compound becomes more hydrophobic; this result can be explained by the existence of low polar (or non-polar) hydrophobic interactions between low polar esters (ethyl octanoate and ethyl decanoate) and polymer chains.

The case of ethyl hexanoate in the presence of guar D solution is quite amazing because it actually shows an excess of release in the gas phase (corresponding to a ''negative retention''). This effect is currently attributed to a salting-out phenomenon revealing a competition between ethyl hexanoate and the macromolecules to bind water molecules. The same behaviour was already reported for ethyl hexanoate in soft drink model systems containing sugars,[18] for 2-butanone and 1-hexanol in maltodextrin solutions[12] and was also evidenced for ethyl acetate and ethyl isobutanoate in stirred yoghurt models.[19] In all such real food systems, the macromolecules may organize and link together with the water molecules, thus leaving less free water to interact with volatile compounds.

The better solubility of guar gum when compared to that of locust bean gum appeared directly related to the increased H-bonding density provided by the galactose side groups.[20] In the present study, water molecules may preferably interact with galactose units thus leading to a higher release of ethyl hexanoate.

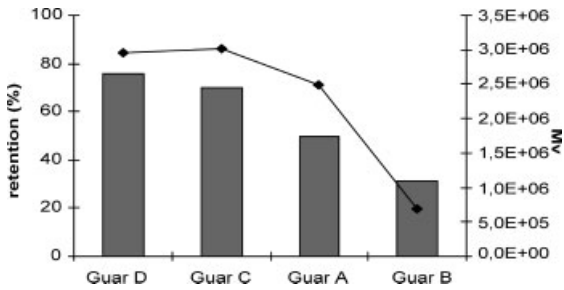

Figure 2.
Retention behaviour of ethyl decanoate by different 0.1% (w/w) guar solutions (NaCl, 0.01M) at 35 °C.

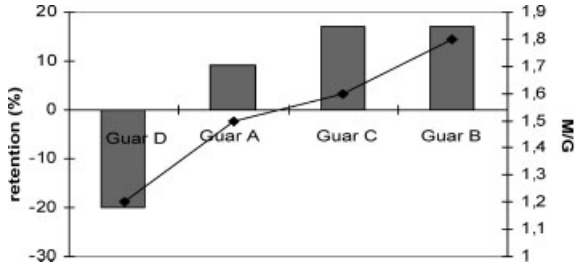

Figure 3.
Retention behaviour of ethyl hexanoate by different 0.1% (w/w) guar solutions (NaCl, 0.01M) at 35 °C.

As described elsewhere, the molecular weight of polysaccharides is another parameter which can markedly influence the aroma retention.[21] As an illustration, ethyl decanoate retention is closely dependant on the molecular weight of guar samples. Hydrophobic domains may then be favoured by the overlapping of high polymer chains, thus explaining the good retention of ethyl decanoate through hydrophobic interactions. Such an hypothesis was already proposed to explain the decrease of low-polar compounds volatility in guar, but also in the case of carboxymethylcellulose solutions.[3] In the present study, intermolecular aggregations suspected in guar D and guar C solutions could be assimilated to hydrophobic zones thus favouring ethyl decanoate retention. This last consideration is consistent the fairly low retention of ethyl decanoate observed in guar B, thus clearly confirming the critical role played by polymer chain overlapping to create zones with volatile compounds.

Conclusion

The aim of this paper was to demonstrate how a small modification in the chemical structure of functional additives such as guar gums may have an effect on aroma retention. The present multidisciplinary approach clearly indicates strong potential for volatile release or retention quantification studies to better understand molecular interactions in solution. Depending on the volatile compound properties, results allow pointing out the impact of H-bonds or the establishment of hydrophobic interactions as related to the molecular weight of the polysaccharide.

Such an approach can interestingly be envisaged for understanding molecular interactions of polysaccharides and may find application in formulation science.

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