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Release of limonene from polysaccharide matrices: viscosity and synergy effect

Sébastien Secouard, Catherine Malhiac, Michel Grisel*, Bernard Decroix

Unité de Recherche en Chimie Organique et Macromoléculaire, URCOM, EA 3221,
Université du Havre, 25 rue P. Lebon, BP 540, F-76058, Le Havre cedex, France

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Abstract

A detailed study of limonene release behaviour from water-based low-concentration polysaccharide solutions and weak gels is reported. On the one hand, rheological measurements made it possible to characterize xanthan (X) and galactomannan [guar gum (G) and carob gum (C)], alone or mixed together. On the other hand, release behaviour of limonene from the previous matrices was quantified using a solid phase micro extraction technique combined with gas chromatography analysis. All of the results indicated large differences in release behaviour, depending on both the matrix composition and the solution viscosity. Moreover, when synergistic interactions between the polysaccharides were developed in the media (X/C mixtures), the results clearly indicated that the release was greatly reduced, thus showing that the volatile release behaviour was not only governed by steric phenomena but also resulted from the occurrence of specific interactions that remain to be elucidated.

Keywords: Polysaccharides; Xanthan; Galactomananns; Interactions; Limonene; Flavour release; HS/SPME; Rheology

1. Introduction

Using hydrocolloids as thickeners leads to a reduction in flavour release as a result of two main mechanisms: diffusion decrease in the media and molecular interactions between flavour compounds and matrix constituents.

For the first mechanism, the Stokes–Einstein and Wilke–Chang equations predict the reduction of flavour molecule diffusion as the solution viscosity increases (Baines & Morris, 1987; Pangborn, Gibbs, & Tassan, 1978; Wilke & Chang, 1955).

For the second one, specific binding interactions of small molecules are often the consequence of adsorption, complexation leading to entrapment in micro-regions, encapsulation and hydrogen bonds (Cayot, Taisant, & Voilley, 1998; Godshall, 1997; Kinsella, 1989).

Many researchers have sought a better understanding of the effects that govern the flavour release from complex matrices as this represents an important target in many fields, such as the food industries (Guichard, 2000). Affinity chromatography can be used to estimate the molecular interactions directly, as described in the literature (Hummel & Dreyer, 1962). This method requires a column and an efficient detection system able to analyse both the macromolecules and the ligand, this last being difficult to detect as it is present at a very low concentration in the media. Other methods, such as gas chromatography coupled with static or dynamic head-space extraction, are particularly well adapted for the analysis of the volatiles and are currently used to quantify the flavour release (Roberts, Elmore, Langley, & Bakker, 1996).

The present study deals with these phenomena by using model matrices consisting of galactomannans combined with xanthan. Both polysaccharides are widely employed in numerous industrial applications and they are also likely to develop well-known synergistic properties, depending on experimental conditions (Bresolin, Sander, Reicher, Sierakowski, Rinaudo, & Ganter, 1997; Cairns, Miles, & Morris, 1986; Schorsh, Garnier, & Doublier, 1997). However, in spite of abundant literature, the mechanisms of interaction still remain controversial.

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* Corresponding author. Tel.: +33-232744400; fax: +33-232744391.
E-mail address: michel.grisel@univ-lehavre.fr (M. Grisel).

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In order to study the aroma release from the previous matrices, both limonene and menthol were chosen, due to their volatility/polarity differences.

For the present study, the volatile concentration always remains below its water solubility limit and the results reported mainly deal with limonene (the physicochemical characteristics of both aroma compounds are listed in Table 1).

Volatile release from the matrices was quantified by using solid phase micro extraction (SPME), a fairly efficient headspace extraction technique introduced by Pawliszyn (1997) and particularly well adapted for volatile flavour compound analysis. This cheap and easy-to-use technique allows analysis of volatile compounds at low concentrations with no other specific instrumentation. Very recently, a comparison of HS/SPME methods with classical head-space methods for the measurement of interaction between milk proteins and aroma compounds has underlined the importance of SPME for the quantification of flavour release (Fabre, Aubry, & Guichard, 2002).

In order to link the release behaviour of the volatile and the macroscopic properties of matrices, a rheological characterization was performed.

2. Materials and methods

2.1. Materials

2.1.1. Flavours

(R)-(+)-Limonene (97%) and (−)-Menthol (99%) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The flavour compound was first dissolved in ethanol at 1000 mg/l and this stock solutions was kept at 4 °C for 1 week.

2.1.2. Polysaccharides

Samples of xanthan, carob and guar gum powder were kindly given by Systems Bio Industries (SKW). The salt (NaCl) was provided by Merck (Merck 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).

2.2. Preparation of the solutions and mixtures

Xanthan gum powder was gently dispersed in water, in the presence of 0.01 M sodium chloride and sodium azide (400 ppm); it was mechanically stirred overnight at room temperature. These conditions allow xanthan to stay in its native form (Capron, Brigand, & Muller, 1997).

Galactomannan powder was dispersed at 85 °C for 3 h in pure water under mechanical stirring, and it was stopped after an additional 2 h at 25 °C. Then, the galactomannan solutions were centrifuged at 2800 rpm for 2 h. Dry extracts allowed the determination of the final polysaccharide concentration of supernatant. Finally, the appropriate amounts of salt and bactericide (400 ppm) were then added to obtain a 0.01 M salted solution.

These solutions were diluted by adding salt water (0.01 M) in order to obtain the final required concentration. After 2 h of stirring, they were stored overnight at 4 °C.

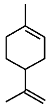
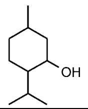
Blends of the xanthan with galactomannan at 0.1% total polysaccharide concentration were obtained by mixing appropriate amounts of xanthan and galactomannan solutions at 25 °C; the mixture was then stirred for 1 min at 1600 rpm by means of a vortex before storage at 4 °C.

2.3. Quantification of flavour release

2.3.1. Preparation of the matrix containing flavour compounds

As soon as the thickened samples were taken out of the refrigerator (4 °C±1 °C), a precise amount of the

Table 1
Physicochemical properties of aroma compounds

Name	Formula		MW (g/mol)	Boiling point (°C)	Vapour pressure[a] (Pa) 20 °C	Solubility in water at 25 °C[b]	$\log K_{OW}$[c]
Limonene	$C_{10}H_{16}$		136.24	176	213	10.6 mg/l 5.6 mg/l	4.38
Menthol	$C_{10}H_{18}O$		156.27	212	106	464 mg/l	3.40

[a] Vapour pressures were determined as by Fichan, Larroche, and Gros (1998).

[b] Menthol and limonene solubilities in water were measured as by Jouenne and Crouzet (2000) and also as by Li et al. (1998) for limonene.

[c] Log K octanol/water (hydrophobicity) were measured as by Griffin, Grant Wyllie, & Marklam (1999).

stock alcoholic solution of aroma was added to give a final aroma concentration of 1 ppm. The ethanol level present in the final sample was less than 0.1%, which would have no effect on the flavour volatility (Williams & Rosser, 1981).

Although the limonene solubility is moderately influenced by the temperature variation, the introduction of the aroma at low temperature allows its volatility to be greatly decreased (Li, Perdue, Pavlostathis, & Araujo, 1998), thus minimizing the aroma loss during its introduction into the matrix. The cap was then immediately screwed on the flask.

The solutions were stirred for 1 min ± 1 s at 1600 rpm by means of a vortex. A 10-ml vial (Supelco, Sigma-Aldrich, Saint Quentin Fallavier, France) was then filled with 5 g of this solution. An aluminium film was placed on the top of the vial to avoid adsorption of the aroma compound on the septum (Teflon® Faced Butyl Septa provided by Supelco) and the vial was quickly capped and thermostatically heated to 35 °C for 4 h before analysis.

A solution of limonene was prepared according to the same protocol but using water instead of polysaccharide solution as the reference solution.

2.3.2. Headspace/SPME operating conditions

A 100 μm polydimethylsiloxane fibber was inserted into the headspace sample using a manual holder for 5 min ± 1 s to trap the free aroma. The fibber was then directly desorbed at 250 °C in the injector of the gas chromatograph.

2.3.3. Gas chromatographic analytical conditions

Chromatographic analysis was performed by means of the Varian 3800 equipped with a flame ionization detector (FID). Separation was performed on a 15 m×0.25 mm i.d. column coated with a 0.25 μm film of dimethylpolysiloxane (SPB-1, Supelco). The GC conditions were 250 °C for the detector and injector temperatures with an isothermal oven temperature (120 °C). Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

The analysis of the flavour release from the different matrices was carried out 10 times, and the reference solution was analysed daily. Analysis of the standard variation (σ) was performed with Microsoft Excel® on the peak area. The significance of the calculated mean value (S_{moy}) and the standard variation was performed using a Student's *t*-test at 95% confidence interval for $N = 10$ replicates ($S_{\mathrm{moy}} \pm 2.262\ \sigma/\sqrt{10}$). The relative percent of the free aroma in the vapour phase corresponds to the ratio between the aroma quantity, measured in the presence of macromolecules, and the quantity measured with pure water (reference).

2.4. Rheological measurements

Experiments were performed using the AR2000 Advanced Rheometer *TA* Instruments, equipped with a cone-plate device (0.28° aluminium cone angle, 60 mm diameter or 1.58° acrylic cone angle, 60 mm). Temperature was controlled at 35 °C using a Peltier effect and a solvent trap was used to permit efficient temperature regulation and also to avoid solvent evaporation.

Measurements were performed after a 30 min ageing period at 35 °C. For the oscillatory experiments, the amplitude of deformation was chosen in order to remain within the linearity limits of viscoelasticity. Elastic modulus (G') and loss modulus (G'') were determined at 1 Hz, and the ratio $\tan\delta = G''/G'$ was defined (loss angle). For the more diluted solutions, a flow procedure was also performed and the zero shear-rate viscosity (η_0) was defined using a Cross model.

3. Results and discussion

3.1. Kinetic of release — process optimisation

The release of limonene as a function of time from aqueous solutions of xanthan (X) 0.5% (w/w), galactomannans (guar : G and carob : C), 0.5% (w/w), and xanthan/galactomannan (50/50) mixtures, 0.1% (w/w), was investigated (Fig. 1). Water was systematically used as the reference. The highest uncertainties were observed for the carob/limonene system (represented in the figure), but they were not represented for the other solutions in order to make the chart more readable.

Whatever the matrix, the release rate of limonene was at a maximum during the first hour after its introduction in the matrix. Significant decrease of limonene release rate in the following hours was observed and, after 4 h, further variation remained lower than 10%. Furthermore, uncertainties largely increased with time.

From these kinetic experiments, a 4-h-equilibration time for further experiments was chosen due to the time required to reach equilibrium and in order to limit both the uncertainties and the analysis times.

As shown in Fig. 1, limonene release from the different matrices was lower than that measured for pure water. Additionally, both pure xanthan solutions and X/C matrices showed marked limonene retention when compared to the limonene behaviour in other matrices. The present results are in good agreement with previous works about the release of flavour compounds in the presence of pure hydrocolloids (Roberts et al., 1996; Yven, Guichard, Giboreau, & Roberts, 1998), Roberts et al. (1996) demonstrated that the retention of an aroma compound, pinene for example, can be due not only to a mass transfer (guar gum as thickener) but can also result from both the viscosity of the matrix and specific interactions between the volatile compound and the matrix components (sucrose). In order to elucidate the factors responsible for limonene behaviour, the same protocol was applied to different model

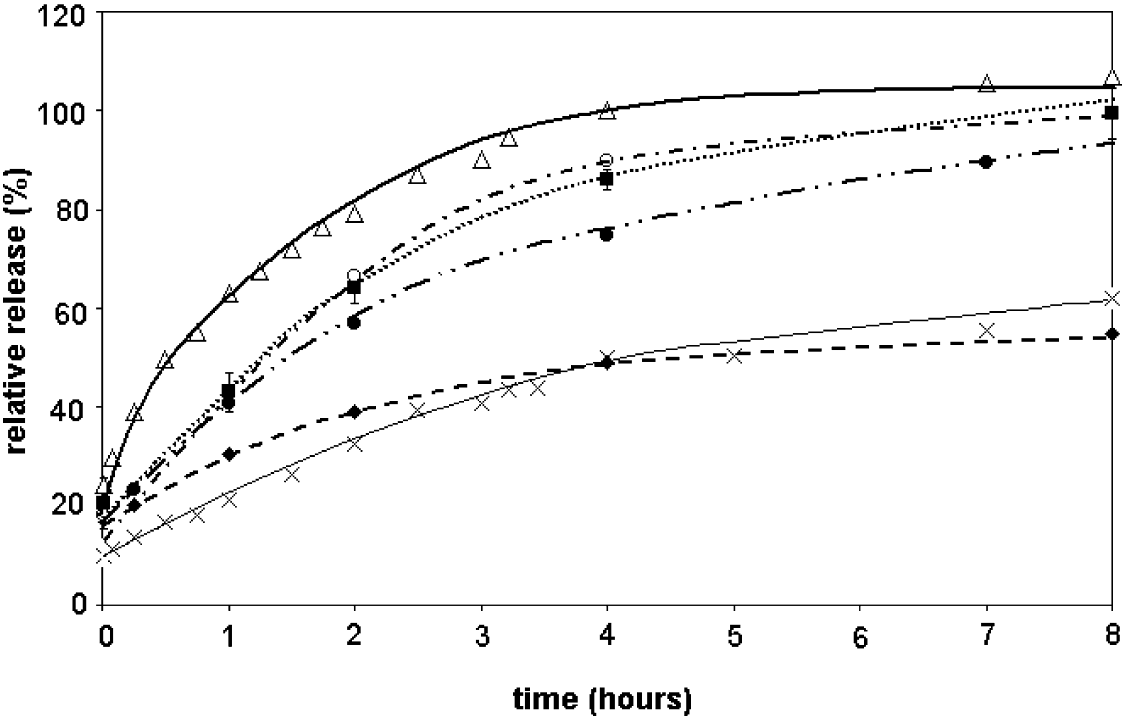


Fig. 1. Kinetics of limonene release from different matrices (35 °C–0.01 M NaCl). Water at a 4-h equilibration time is taken as the reference. △, Water; ○, X/G = 50/50 0.1%; ■, carob 0.5%; ●, guar 0.5%; ◆, X/C = 50/50 0.1%; ×, xanthan 0.5%.

matrices by varying the concentration and/or nature of the polysaccharides.

3.2. Release from xanthan solutions

Fig. 2 shows limonene release (at 4 h equilibration time) as a function of the xanthan concentration. It indicates that limonene release largely depends on the xanthan concentration regime (Cuvelier & Launay, 1986). Firstly, inasmuch as the xanthan concentration remains lower than the critical overlapping concentration (Region A), there was no significant difference between limonene release from xanthan-containing matrices and that of pure water. The explanation may be that, in the dilute regime, wherein macromolecules were isolated from each other, limonene behaviour remained constant when interactions between the polymer species remained negligible. Secondly, at higher polysaccharide concentrations, corresponding to the semi-dilute regime wherein the polymer chains begin to overlap (Region B), limonene release significantly decreased [−23% for 0.1% (w/w) of xanthan added], thus indicating that limonene was mainly retained by the xanthan solution through steric phenomena. Finally, when the concentrated regime was reached (Region C), the release decreased less and less and tended to a constant value with a limit corresponding to the maximum level of limonene retention in the media (about 50% of relative release in the present case).

All these matrices were characterised by means of rheology, and a good correlation between the inverse of the zero-rate-viscosity ($1/\eta_0$) of these matrices and the % of limonene release from these same matrices could be observed, thus confirming the dominating role of viscosity in the retention of limonene. Actually, a linear increase of the limonene retention, as a function of the xanthan concentration, would have tipped the balance for a retention due to flavour-xanthan specific interactions.

3.3. Release from xanthan- galactomannans mixtures

Limonene release was next studied as a function of the xanthan (X)/galactomannan (carob : C, guar : G) ratio, with a total polysaccharide concentration of 0.1% (w/w) in the matrix (Fig. 3). The total polysaccharide concentration was made high enough to achieve a significant limonene release from the mixtures when compared to its behaviour in pure water. However, the total polymeric concentration in the mixtures was limited to 0.1% to avoid gel formation; gel structure impedes efficient stirring, necessary to homogenize the mixture in the presence of aroma. Otherwise, bubbles, resulting from vigorous stirring, are incompatible with rheological characterization, as well as aroma release study.

In order to elucidate the influence of each parameter upon the limonene release behaviour, both a rheological characterization of the different matrices used and a SPME/GC study of aroma, present in headspace, were performed.

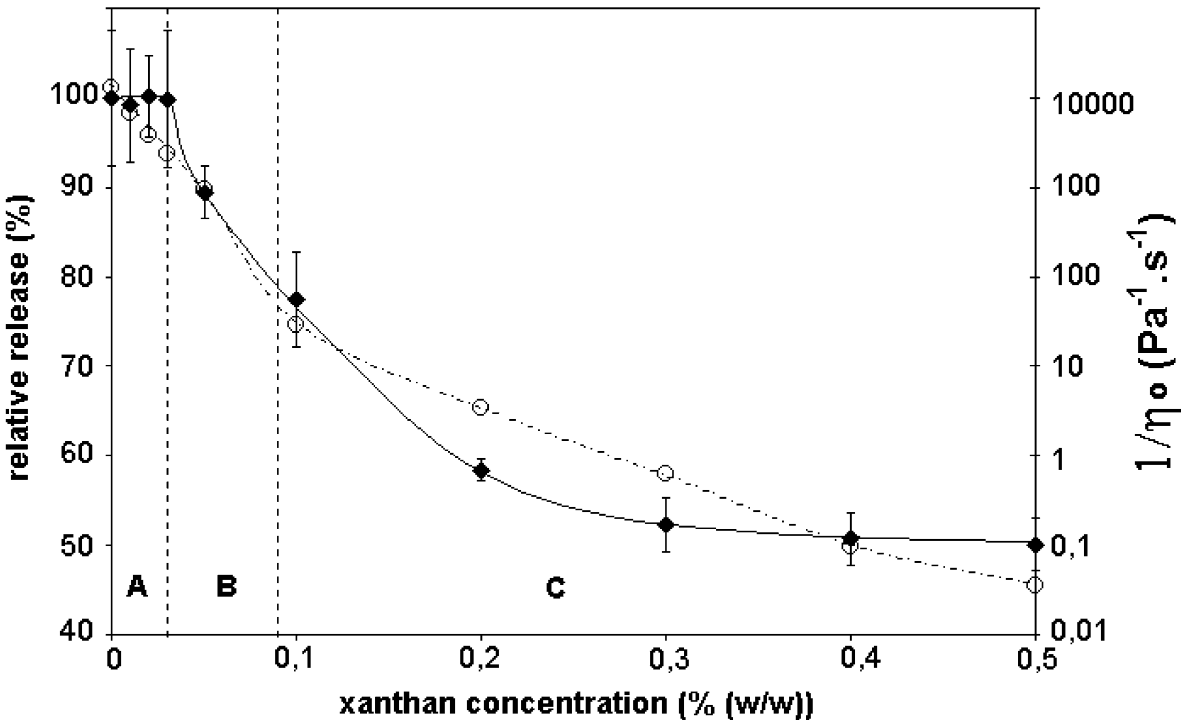


Fig. 2. Limonene release ◆ and inverse zero shear-rate-viscosity ○ as a function of xanthan concentration (35 °C–0.01 M NaCl). Region A: dilute regime; Region B: semi-dilute regime; Region C; concentrated regime.

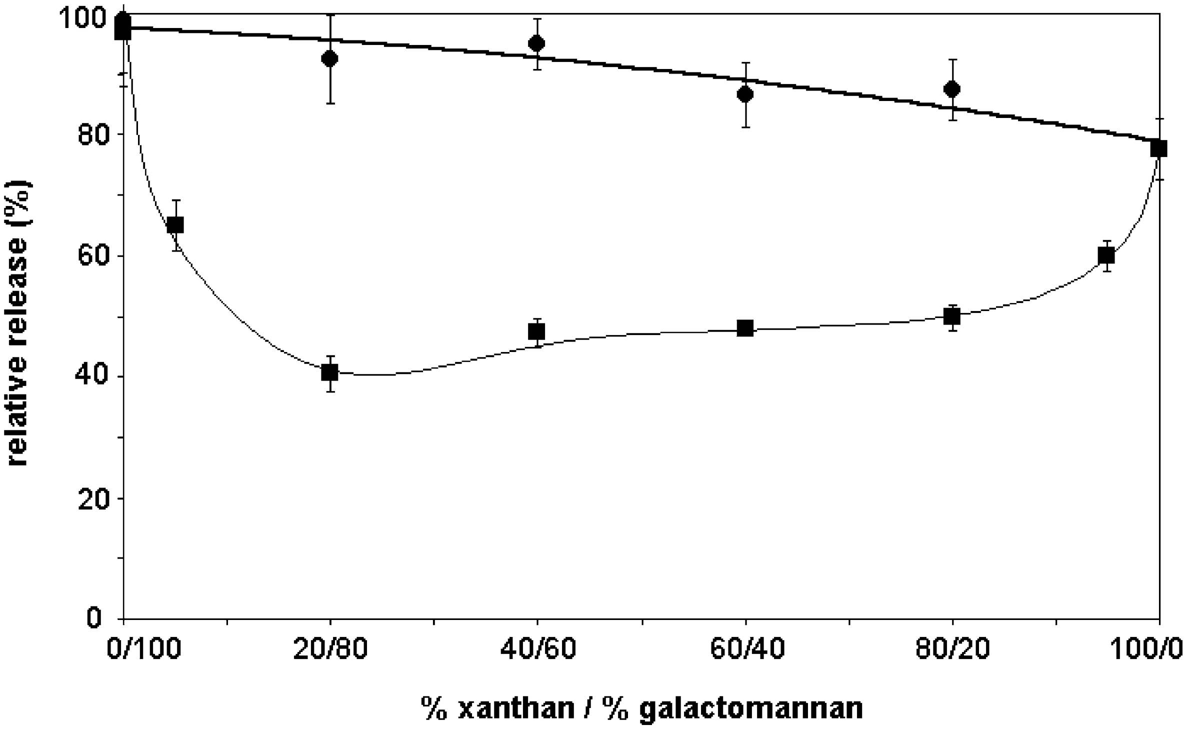


Fig. 3. Limonene release from xanthan/galactomannan matrices (35 °C–0.01 M NaCl). Total polymeric concentration was 0.1% (w/w). ●, Xanthan/guar; ■, xanthan/carob.

In spite of the low polymer concentration regime explored, the limonene behaviour was largely affected as its release became lower in polysaccharide (X/G or X/C)-containing solutions when compared with pure water media. Furthermore, the whole behaviour of X/G and X/C mixture appeared to be quite different, with a much more marked retention effect in the case of the carob gum.

As a consequence, the polysaccharide concentration in the matrix and the galactomannan structure played a major role in the volatile compound behaviour.

As shown in Fig. 4, the lower the inverse of the zero shear-rate viscosity ($1/\eta_0$) of the X/G mixtures, the lower was the limonene release. Such behaviour indicates that the limonene retention in the X/G system is mainly influenced by the viscosity of the media.

In the case of X/C mixtures (Fig. 5), an accurate zero shear-rate viscosity is difficult to establish as a result of polymer–polymer interactions; consequently, another rheological parameter was defined (tan δ), using oscillatory experiments. The lower the tan δ value, the higher the organisation of the system.

For all the X/C mixtures, the retention became much higher than those of pure water, polysaccharides taken alone or X/G mixtures. Moreover, the retention remained nearly the same whatever the X/C ratio.

It is interesting to note that there is a good correlation between the limonene release and the tan δ characterizing the X/C mixtures. Moreover, no difference in the limonene release (or retention) was noted for the different X/C mixtures, showing that a maximum amount of limonene, present in the media, is entrapped as soon as X and C are both introduced to the aqueous media.

However, in order to elucidate whether the retention is related to steric effects or specific interactions, release behaviour of limonene, from matrices with the same viscosity, was explored. To this end, the 80/20 X/C mixture flow behaviour made it possible to determine a reasonable uncertainty using the Cross model.

Fig. 6 draws a comparison of the limonene release from different matrices with the same zero shear-rate viscosity ($\eta_0 = 37 \pm 3$ mPa s), showing that the phenomena responsible for retention in the X/C mixtures are not only due to the viscosity of the media, but also result from specific limonene–matrix interactions that still remain to be clearly elucidated.

Similar experiments were carried out with menthol, but no significant differences of retention between the different matrices (water, simple matrices or mixtures) could be detected. These results could be attributed to the difference of molecular characteristics between menthol and limonene. The significant difference between relative hydrophobicities (Table 1, log K_{OW}) of these two aroma compounds may particularly explain the limonene retention phenomena. This interpretation agrees with the conclusions of Roberts et al. (1996) attributing the retention behaviour of apolar compounds in polysaccharide-containing aqueous matrices to hydrophobic inclusion complexes. Among all the mechanisms proposed for such hydrophobic zone formation, the most probable remains the hydrogen bond mechanism between water and hydroxyl groups of polysaccharides (Franks, 1983). In the case of xanthan/carob mixtures, such an explanation may allow better understanding of the nature of the xanthan-carob interactions that still remain controversial (Mannion et al., 1992; Schorch et al., 1997; Bresolin et al., 1997; Chandrasekaran & Radha, 1997).

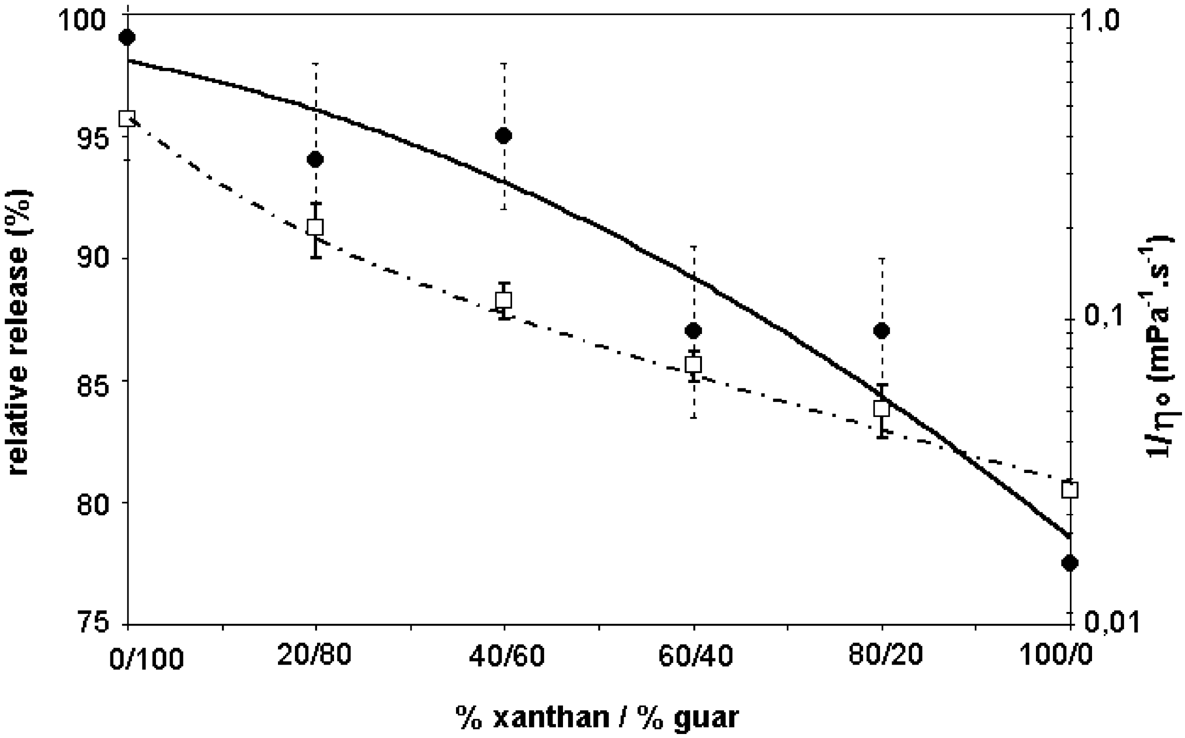


Fig. 4. Influence of the X/G ratio on limonene release ● and the inverse viscosity □ of the solutions (35 °C–0.01 M NaCl).

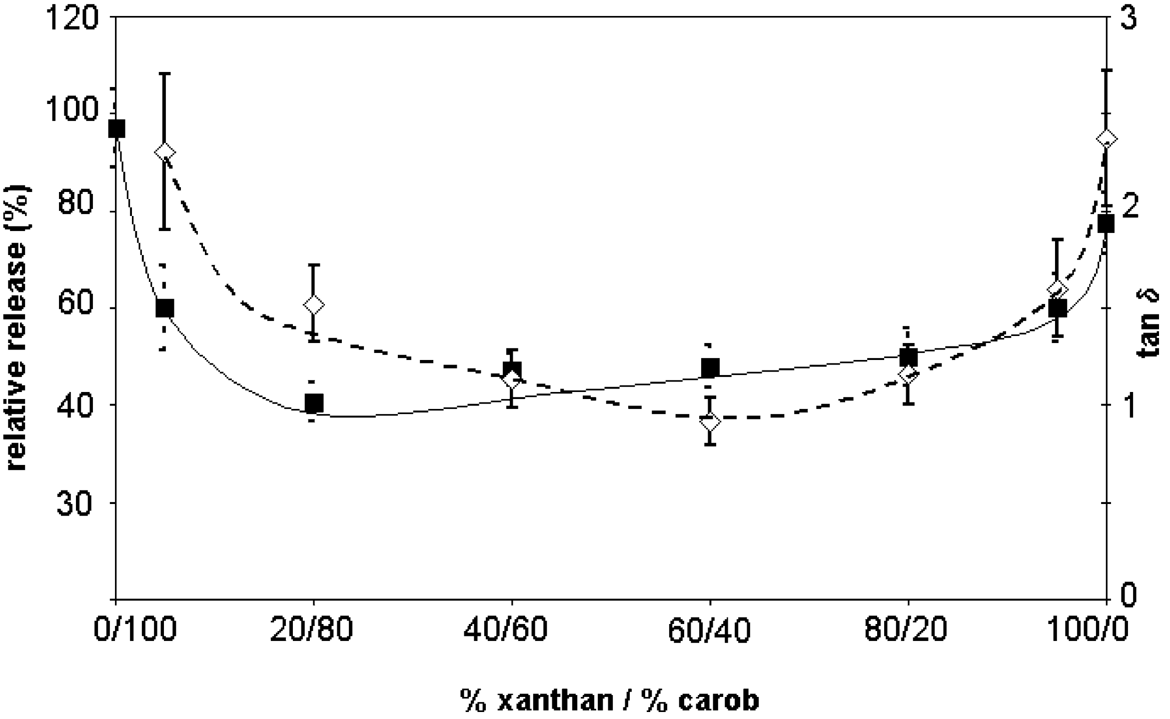


Fig. 5. Influence release of the X/C ratio on limonene release ■ and tan $\delta(\nu = 1$ Hz) ◇ of the solutions (35 °C–0.01 M NaCl).

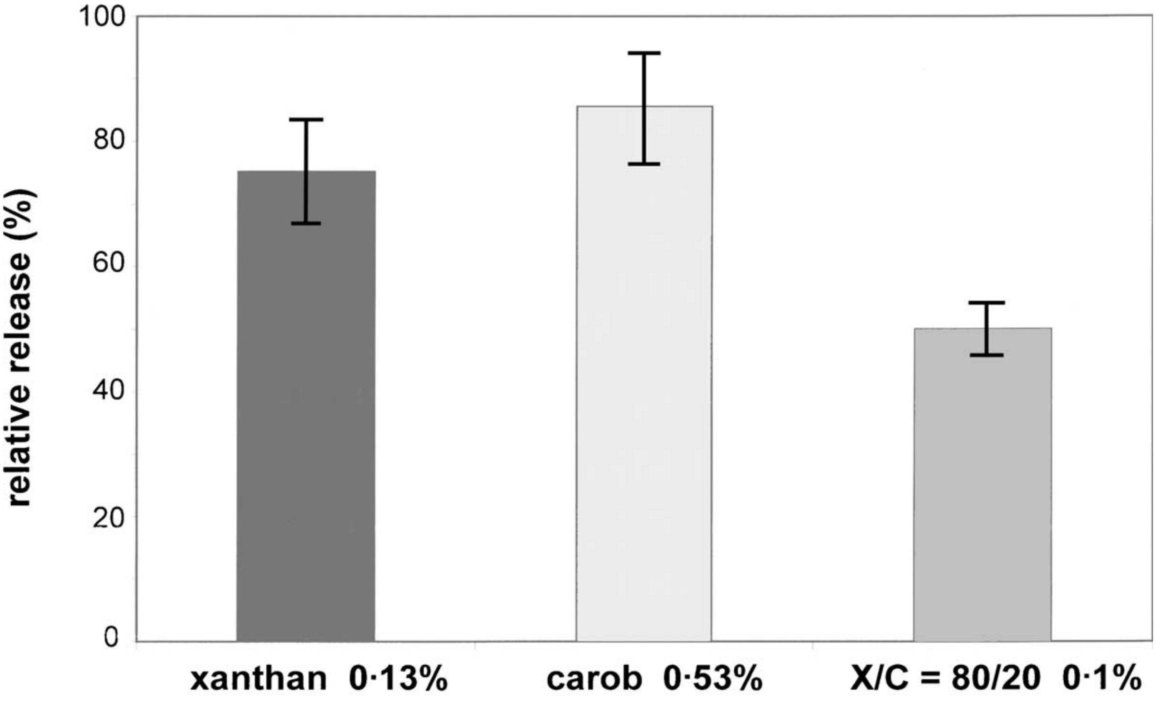


Fig. 6. Limonene release from matrices of identical viscosity (35 °C–0.01 M NaCl). $\eta_0 = 37 \pm 3$ mPa s.

4. Conclusion

The present paper illuminates the main reasons for aroma compound retention; as currently described in the literature, both the viscosity of the media and the aroma–matrix interactions have to be considered. Moreover, it demonstrates the major role of synergistic interactions between xanthan gum and carob gum, even at very low polymer content. The aroma behaviour is greatly influenced by synergistic interactions (X/C) but no spectacular changes of retention can be measured in X/G mixtures.

Otherwise, the aroma compound characteristics, and particularly hydrophobicity, play major roles in the retention/release behaviour of the matrices. Hydrophobic inclusion complexes, through hydrogen bonds involving water molecules and hydroxyl groups of polysaccharides, appear to constitute the most probable mechanism.

In the case of xanthan/carob mixtures, the nature of the interactions responsible for such a change in retention behaviour needs to be elucidated; to this end, other volatiles of different nature are now being investigated. All results may be useful as they may provide a better understanding of the nature of xanthan–galactomannan interactions.

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