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Correlation between rheological properties and limonene release in pectin gels using an electronic nose

María Eugenia Monge[a], R. Martín Negri[a], Daniela Giacomazza[b], Donatella Bulone[b,*]

[a]*Instituto de Química Física de Materiales, Ambiente y Energía (INQUIMAE), Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, C1428EHA Buenos Aires, Argentina*
[b]*Istituto di BioFisica-Palermo, Consiglio Nazionale delle Ricerche (CNR), Via U. La Malfa 153, I-90146 Palermo, Italy*

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Abstract

Pectin gels at different polymer concentrations were used as matrices for the encapsulation of a volatile flavour (limonene). This provides a useful model system for studying the influence of matrix viscoelastic properties on flavour release towards the gel headspace. The electronic nose technique and principal component analysis, a multivariate data analysis, were used for detecting changes in the fingerprint of the released vapour as a function of pectin concentration. Samples with different compositions were also studied by rheological measurements in order to discriminate the effects of polymer concentration on the gelation kinetics from those due to the addition of limonene and the detergent used to dissolve it.

The combined approach, rheometry–electronic nose, allows obtaining a direct semi-quantitative correlation between the expected decrease of flavour release intensity and the increasing solid-like character of the matrix due to the trapping effect. In fact, the comparison of the viscoelastic properties for matrixes with and without the flavour, together with direct observation by optical microscopy, suggests that the release modulation is mainly due to interaction of the gelling pectin with the microemulsion of detergent and flavour.

Keywords: Pectin; Flavour release; E-nose; Rheological properties; Gelation

1. Introduction

Gel matrices based on biopolymers are widely used as encapsulation and release systems in food, agricultural or pharmaceutical applications. The requirement of matrices with structural properties suitable for a specific use has prompted a large amount of experimental work aimed at finding new control parameters of gel properties, such as texture, strength or porosity (Brinker & Scherer, 1990; Lynch & Dawson, 2003; Patras, Qiao, & Solomon, 2001; Takeoka & Watanabe, 2003; Zeller, Saleeb, & Ludescher, 1999). A central issue in this research field is to understand, on a physical chemical basis, how the release is modulated by the matrix properties.

One important topic in food technology is the release of aroma compounds from hydrocolloidal solutions or gels due to the wide use of hydrocolloids as thickeners or texturing agents. An experimental finding, consolidated from several studies, is that the intensity of flavour released from a liquid solution or gel decreases with increasing hydrocolloid concentration (Guichard, 2002; Renard, van de Velde, & Visschers, 2006; Roberts, Elmore, Langley, & Bakker, 1996). This has been explained either in terms of a hindered diffusion of the volatile compound entrapped in the matrix or as due to a direct binding between aroma and polymer chains. Actually, experimental studies of flavour release from gel matrices have shown that the release is reduced on increasing the gel hardness (Guinard & Marty, 1995; Hansson, Leufvén, Pehrson, & Stenlöf, 2002); other studies showed that the release is strongly controlled by the chemical affinity of the aroma compound and the polymer (Boland, Buhr, Giannuoli, & van Ruth, 2004; Boland,

*Corresponding author. Tel.: +39 09 1680 9320; fax: +39 09 1688 7692.
E-mail address: donatella.bulone@pa.ibf.cnr.it (D. Bulone).

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Delahunty, & van Ruth, 2006; Hansson, Leufvén, & van Ruth, 2003; Secouard, Malhiac, Grisel, & Decroix, 2003). How the matrix properties are affected by the presence of the compound to be released was also addressed (François, Rojas, Daraio, & Bernik, 2003; Lubbers & Decourcelle, 2004; Monge, Bulone, Giacomazza, Negri, & Bernik, 2004). This point can be of large practical interest in projecting and manufacturing matrices with appropriate structural properties. Indeed, interactions of gel-forming polymers with surfactants and aroma compounds (Paulsson & Edsam, 2002; Semenova, 2005), or even the addition of cosolutes to the solvent, can modulate the gelation mechanism that, in turn, determines the gel structure (Bulone, Emanuele, & San Biagio, 1999; San Biagio, Newman, Madonia, & Palma, 1989).

The aim of the present work is to study the relation between gel structure and flavour release by the combined use of rheological and electronic nose (E-nose) techniques. More classical techniques based on gas chromatography are generally used for analysing flavour release (Boland et al., 2004, 2006; Hansson et al., 2002, 2003; Lubbers & Decourcelle, 2004; Roberts et al., 1996; Secouard et al., 2003). For example, Secouard et al. analysed the release behaviour of limonene and macroscopic properties of different polysaccharide solutions by using a solid-phase microextraction technique combined with gas chromatography analysis. These techniques are based on separation, identification and quantification of the odour compounds. A different way of sensing flavours, avoiding molecular recognition, is by using E-noses. E-noses are devices composed of an array of non-specific chemical sensors coupled with multivariate data analysis methods such as principal component analysis (PCA), cluster analysis or artificial neural networks, and are used for discriminating multi-component systems through pattern recognition. This analysis is based on the comparison of fingerprints of different samples. The fingerprint of each sample is constructed with the set of electrical responses given by each sensor in the array. In the present work, we used an E-nose developed by one of the authors (Branca, Simonian, Ferrante, Novas, & Negri, 2003; O'Connell, Valdora, Peltzer, & Negri, 2001).

We prepared gels based on natural high-methoxylated pectin, one of the most used gelling agents in the food industry. Pectin is a polysaccharide whose major constituent is a linear sequence of 1,4-linked α-D-galactopyranosyluronic with some of the carboxyl groups esterified with methanol. Interruptions with 1,2 linked L-rhamnose residues make the backbone irregular and provide kinks responsible for flexibility. Depending on the proportion of methyl ester groups, pectin is classified as high methoxyl (HM) having 50% or higher esterification degree, or low methoxyl (LM). HM pectin may form a gel at acid pH (lower than 3.5) only in the presence of large amounts of sugars or other cosolutes that are known to reduce water activity (Morris, 1986, chapter 3; Oakenfull & Scott, 1984; Reed, 1969). It is well established that pectin gels are stabilized by both hydrogen bonds and hydrophobic interactions (Oakenfull & Scott, 1984). The latter primarily involve the methyl ester groups (Walkinshaw & Arnott, 1981). Pectin gel structures characterized by transmission electron microscopy appear as a rather loose network of stiff strands with large pores (Löfgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005).

Limonene, a well-known non-polar flavouring compound, was encapsulated in different matrices containing HM pectin. Tween 80 was used as the emulsifying agent in order to achieve limonene encapsulation.

The viscoelastic properties of the system composed of the polymer + flavour + detergent (Tween 80) were compared with those of a system not containing the flavour, and with those of a system not containing either the flavour or the detergent. Pectin concentration was systematically changed in all cases, and the effect of the flavour and detergent on the gelation kinetics and viscoelastic properties of the materials were determined.

2. Materials and methods

2.1. Materials

Commercial citrus High Methoxylated Pectin (HMP) type 105 rapid set (origin Brazil; provided by Rosenfeld, Argentina) was used as purchased. Tween 80 (Sigma-Aldrich) and limonene (Givaudan Argentina) were used as purchased. Analytical grade sucrose, potassium citrate, citric acid, sodium benzoate and Milli Q water were used in the gel preparation.

2.2. Sample preparation

The first step of the preparation was to mix the pectin powder with an amount of sucrose equal to 10% (w/w) of the final sucrose content 60% (w/w). This dry mixture was dissolved in a 12 mM potassium citrate buffer (pH = 3.4) and heated while stirring at 100 °C for 20 min. The rest of the sucrose was added during the stirring (10 min after initiating the procedure). Then the sample was brought to 40 °C while stirring for 5 min. After that, Tween 80, limonene and a few microlitres of 50% citric acid solution (about 50 μL in 10 g of the solution) were added, and the mixture was stirred for a few seconds with a vortex. The addition of Tween 80 was necessary to achieve encapsulation of the non-polar flavour; however, we noticed that the stabilizing effect of Tween 80 was reduced in the solvent conditions necessary for the pectin gelation (acid pH and a large amount of sucrose). In fact, control experiments on samples without pectin revealed that the emulsion was not stable over a very long time and a macroscopic phase separation was observed after some hours. For the rheological measurements, the mixture was immediately poured into the rheometer's sample compartment that was previously thermostated at 33 °C. This temperature was

chosen to observe a faster gelation process (Lopes da Silva, Gonçalves, & Rao, 1995). For experiments with the E-nose, the mixture was separated into four equal volumes and stored at 33 °C (using a circulating water bath) in closed containers. The vapours in the headspace of these replicates were analysed with the E-nose after 180 min of storage. Systems with six different pectin concentrations ranging from 0.075% to 0.4% (w/w) were analysed. Flavour release experiments were done at two values of limonene concentrations (0.4% and 4% (w/w)). Samples without limonene and without both limonene and Tween 80, used in rheological experiments, were prepared following the same procedure and substituting the flavour or detergent with water in order to obtain the same value of pectin concentration in all cases.

2.3. Rheological measurements

Rheological measurements under low-amplitude oscillatory shear were performed on a controlled stress AR 1000 (TA Instruments, UK) rheometer. Both a titanium cone-plate geometry (angle 1°, radius 20 mm, gap 26 μm) and a double-cylinder geometry (rotor outer radius 21.96 mm, rotor inner radius 20.38 mm, stator outer radius 20.00 mm, cylinder immersed height 59.50 mm, gap 500 μm) were used. The hot solution was loaded into the rheometer that was previously thermostated at 33 °C. Temperature was controlled by a Peltier system in the cone-plate geometry and by a Haake DC 30 circulating bath in the double-cylinder geometry. The thin air–sample interface in the cone-plate geometry and the cylinder–cylinder upper gap in the other geometry were coated with silicon oil to avoid loss of solvent.

Measurements were done at a frequency of 0.5 Hz and a strain of 4×10^{-3} in the viscoelastic linear region. Elastic (G') and viscous (G'') moduli were monitored as functions of time. Experiments at varying pectin concentrations were replicated on three different types of samples in order to single out the effect of flavour and detergent on the rheological properties of the system. In one type of sample, neither limonene nor Tween 80 was added, in the second type, limonene was not added, while the third sample type was the same as that used in flavour release experiments. We will refer to these systems along the text as A, B and C, respectively. The G' and G'' values shown in the figures are the average of four different measurements (the standard deviation is about 10% for G' and 7% for G'' in all cases except for the system with the lowest pectin concentration, where the standard deviation was about 25% for G' and 15% for G'').

For the sample at 0.25% (w/w) pectin concentration and without both limonene and Tween 80, a control experiment was performed in order to verify the consistency of results obtained by using the cone-plate or double-cylinder geometries.

2.4. Electronic nose and flavour release determinations

An E-nose device developed at the laboratory of one of the authors was used to detect limonene in the headspace (O'Connell et al., 2001). The device consists of an array of non-specific gas sensors placed in a sample chamber with an aspiration pump and a regulating valve. In the present case, eight polycrystalline tin dioxide-based sensors were used, whose electrical conductivity increases in the presence of a reducing vapour such as limonene. The whole set of sensors' signals is referred to as a "fingerprint". We used the E-nose methodology in previous studies to obtain and to discriminate the fingerprints of complex mixtures under different conditions (Branca et al., 2003; Monge, Bulone, Giacomazza, Negri et al., 2004; Monge, Bulone, Giacomazza, Bernik, & Negri, 2004; O'Connell et al., 2001).

The gas phase present in the headspace of the samples was extracted by means of a controlled flux aspiration pump with a syringe injected through the septa of a flask containing the gel. The measurements were done 180 min after placing samples in the thermostatic water bath at 331 °C.

Each measurement consists of recording the sensors' signals for 10 min. After this time, the sensors' signals reached a steady-state situation described as a plateau. The values of the sensors' signals at the plateau were used for the analysis after subtraction of a baseline (Monge, Bulone, Giacomazza, Bernik, et al., 2004). The baseline was achieved by recording the sensors' response to pure air working at the same controlled flux. Therefore, a set of eight signals $\{S_1, \ldots, S_8\}$ was obtained in each measurement after subtracting the baseline. Each signal is indicative of the electrical conductance change in the respective sensor due to the presence of the flavour in the chamber.

Several controls were performed. E-nose measurements of pectin gels without the essence were performed in order to discard the possibility of a background signal due to the gel components. The sensors' signals thus obtained were significantly smaller than the signal obtained for the loaded gels, indicating that the contribution of the gel odour does not interfere with limonene detection. The signal patterns of replicated and control samples were recorded on different days. No differences were detected, showing that the drift of the sensors over several days is negligible in the situations described in the present work.

We will use the term "free" limonene to refer to non-encapsulated limonene that is placed in a closed flask and in equilibrium with its respective vapour at the same controlled temperature of 33 °C.

2.5. Data analysis: principal component analysis (PCA)

We analyse the differences in the samples by comparing both the changes of individual sensor signals and the changes of the fingerprints provided by the array. E-nose methodology uses multivariate data analysis in order to discriminate between groups of signals, and hence to

discriminate samples. In the present work, we used the method of PCA, an unsupervised method that is useful for classification prediction and data discrimination. This feature extraction method consists of projecting the N-dimensional data set (in this case N is the number of sensors) in a new base of the same dimension N, but now defined by the eigenvectors of the covariance or the correlation matrix of the data set. The components (projections) of the original data vectors on this new base are the so-called principal components, obtaining one set of principal components $\{PC_1,\ldots, PC_N\}$ for each data set $\{S_1,\ldots, S_N\}$. The important point is that, when analysing the new data set $\{PC_1, PC_2,\ldots, PC_N\}$, a large percentage of the total data variance is accumulated in a few of the principal components. For example, in most of the studies associated with E-noses, 95% of the total variance is accumulated into the three first principal components, representing a substantial reduction of the problem dimension and complexity. In those cases where an important percentage of the total data variance is contained in the first two or three principal components, the data points can be qualitatively discriminated by observing how they group in a 2D or 3D plot (*PCA map*) of the principal components.

PCA was performed with the group of data corresponding to the release of limonene from different pectin gels. Thus, for every sensor's data group $\{S_1,\ldots, S_8\}$ corresponding to each sample measurement, a set of principal components $\{PC_1, PC_2,\ldots, PC_8\}$ is obtained. The sensor's data groups were used without normalization. More than 99% of the total data variance was contained in the groups defined by the vectors $\{PC_1, PC_2\}$ after performing a PCA, indicating an important dimension reduction of the problem.

2.6. Optical microscopy

A Leitz DMRX optical microscope was used. Gels with and without the inclusion of a fluorescent probe were prepared and observed with the microscope for all pectin concentrations. The assays with Rhodamine C18, a fluorescent non-polar probe, were performed in order to understand the micro-heterogeneity of the systems containing pectin, sucrose, Tween 80 and limonene. The probe was dissolved either in Tween 80 or in limonene and was added to the system within the same procedure described in *gel preparation* in order to compare the results obtained with the different techniques used in this work. The photographs correspond to the surfaces of the systems at 180 min after storage at 33 °C and the images were obtained with a 10× lens. The photographs in black and white were taken with a CCD camera coupled to the microscope and connected to a personal computer, while the colour photographs were taken with a digital camera through the ocular of the microscope.

3. Results and discussion

3.1. Rheology results

Previous work (Monge, Bulone, Giacomazza, Negri et al., 2004; Monge, Bulone, Giacomazza, Bernik et al., 2004) showed that the addition of flavour can have a large effect on the gelation kinetics of pectin. It was also observed that even the detergent itself (necessary for dissolving hydrophobic compounds) can affect the viscoelastic behaviour. Therefore, in studying the relation between flavour release and structural properties of the matrix, it can be valuable to compare these properties with those relative to the "unperturbed system". For this reason, we studied the effect of pectin concentration on flavour release by rheological measurements on three different systems: one without both limonene and Tween 80 (system A), one without limonene (system B) and one containing both limonene and Tween 80 (system C). All samples have a constant sucrose content 60% (w/w) and are quenched at 33 °C. Fig. 1(a) shows the time course of the storage (G') and loss (G'') modulus at different pectin concentrations for the system without both limonene and Tween 80. At the lowest concentration, G' starts to grow after a lag time that is reduced by more than one order of magnitude by a fourfold increase of pectin concentration. Correspondingly,

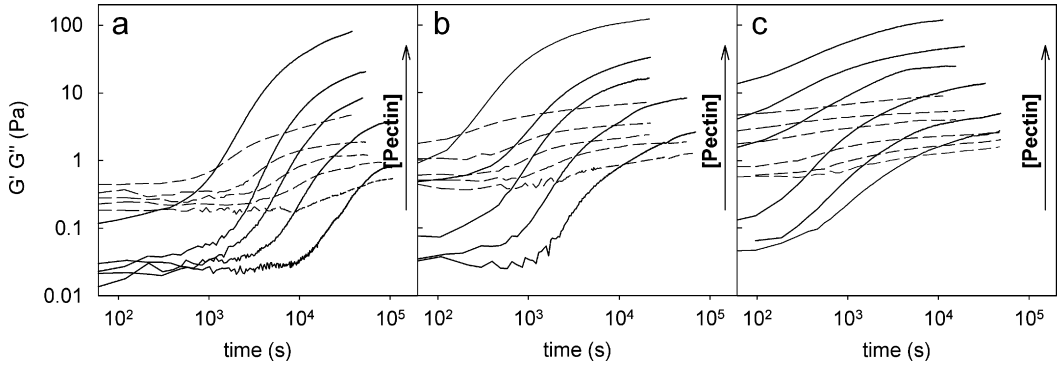

Fig. 1. G' (solid line) and G'' (dashed line) as a function of time at different pectin concentrations for the three different systems studied: (a) pectin without limonene and Tween 80; (b) pectin with Tween 80 added; (c) pectin with both Tween 80 and limonene added. Concentration values are 0.1%, 0.15%, 0.2%, 0.25%, 0.36% w/w for systems in (a) and (b); the system in (c) is studied at the same concentration values and also at 0.075% w/w (lighter curve).

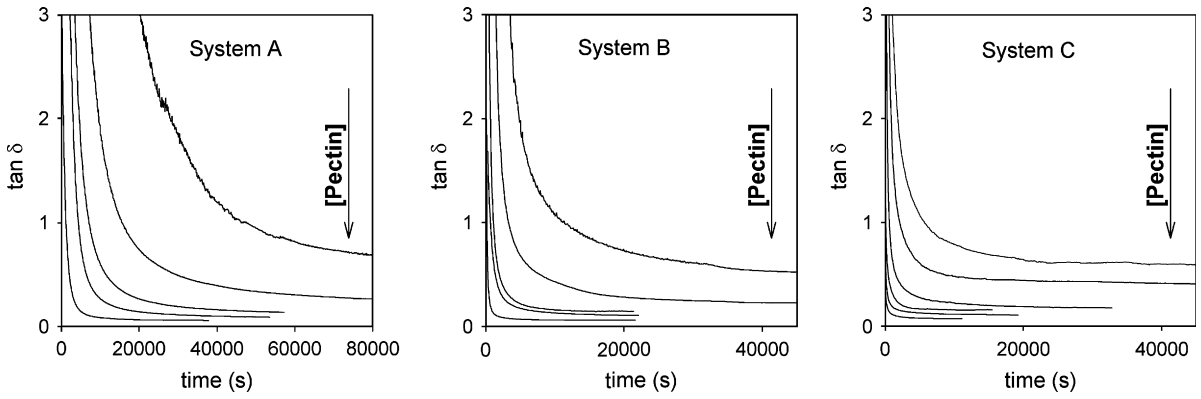

Fig. 2. Time course of tan δ for the three systems of Fig. 1.

the time at which the G' and G'' crossover is observed becomes shorter. Following the criterion used by many authors, this time can be defined as the apparent gelation time (t_g). Indeed, as already discussed in previous work (Monge, Bulone, Giacomazza, Bernik, et al., 2004), the determination of gel point or gel time is always difficult and ambiguous (Ross-Murphy, 1991a) and different criteria, largely dependent on the chosen time scale, have been proposed to define it (Ross-Murphy, 1991a; Kavanagh & Ross-Murphy, 1998). Here we adopt the above criterion by considering that the crossover between G' and G'' marks the appearance of a solid-like character in the sample, at least at the chosen strain and frequency value.

The effect of Tween 80 addition and of both Tween 80 and limonene addition on the gelation kinetics at the same pectin concentrations is shown in Fig. 1, panels b and c, respectively. When comparing panel 1b with respect to 1a, it can be observed that the detergent addition makes the kinetics faster and the initial G' and G'' values somewhat larger. A similar and much larger effect is observed in the gelation of samples containing both limonene and Tween 80. Indeed, for the highest pectin concentrations (0.25% and 0.36%), the first measured G' is higher than G'', indicating that these samples have a larger solid-like character from the very beginning of the kinetics. Although G' has a higher initial growth rate, when increasing the number of components in the system (from panels a to c), not much larger values are reached at the later stage of gelation kinetics.

We observe that the main effect of increasing pectin concentration in all the systems is to favour gelation by decreasing t_g. Accordingly, the relative change of viscous and elastic components, that is tan δ, decreases faster when increasing pectin concentration, as shown in Fig. 2 for the three systems studied here.

In order to better appreciate the effect of Tween 80 and flavour addition on the rate of network formation, the derivative with time of G', dG'/dt, is represented as a function of time in Fig. 3(a) for the three samples at one chosen pectin concentration. In the case of the sample without both Tween 80 and limonene, dG'/dt shows a rise to a maximum, followed by a slow decrease down to a steady small value. This behaviour has been interpreted by other authors in terms of a fast initial network growth, followed by a slower rearrangement of the polymer chains towards a full-stabilized structure (Lopes da Silva et al., 1995). A similar two-step mechanism, where the building

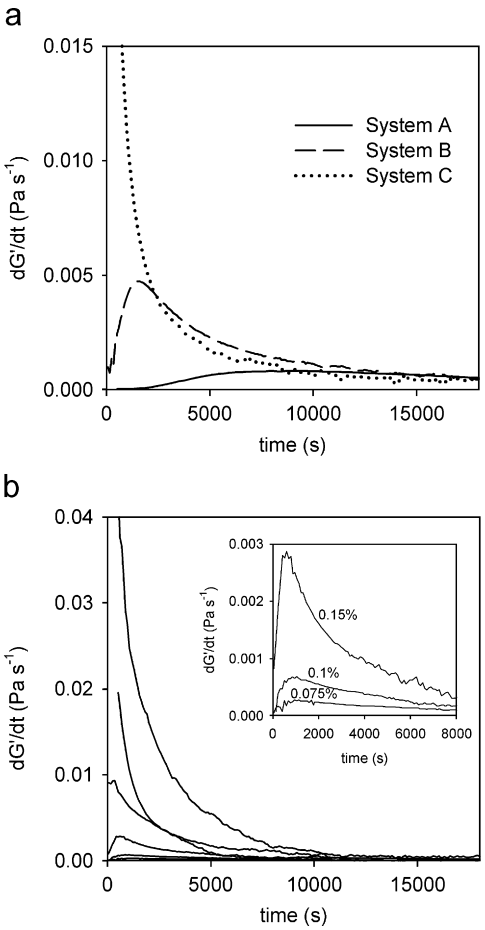

Fig. 3. Time course of the derivative dG'/dt, obtained by a numerical method followed by smoothing. Panel (a) shows data for the three systems of Fig. 1 at the same pectin concentration (0.25% w/w). Panel (b) shows data for the system with both Tween 80 and limonene at different pectin concentrations (concentration increasing from bottom to top); the inset in (b) shows data at the lowest pectin concentrations on expanded scale.

up of the network is followed by a strengthening and reinforcement stage, was also observed in the gelation kinetics of whey proteins (Verheul, Roefs, Mellema, & Kruif, 1998).

When adding Tween 80, a larger value of dG'/dt maximum is reached in a much shorter time, and when limonene is added, the rise to a maximum is no longer visible. Instead, we can only observe the decrease of dG'/dt from a very large initial value down to a steady small value that does not differ much from that observed in the other two systems. This different behaviour is observed at each value of pectin concentration. As shown in Fig. 3(b), for samples containing both Tween 80 and limonene, the network formation is always so fast that the dG'/dt maximum can be observed only at the lowest concentrations studied. This indicates that the main effect of detergent and flavour addition is to accelerate the initial stage of network formation.

The dependence of the apparent gelation time, t_g, on pectin concentrations for the three systems is shown in Fig. 4 on log–log scale. In the case of the system with Tween 80 and limonene, the two points at higher concentration are obtained by extrapolation of the initial part of G' and G'' curves. Overall, there is a difference of about one order of magnitude in t_g for the three systems at each pectin concentration. Data of each set fit well to a power law with an exponent value that increases with the system complexity. For the system without Tween 80 and limonene, we found a value of 2.8 ± 0.2, which is between the values of 2 and 3.6 found for HM pectin by other authors (Lopes da Silva et al., 1995; Oakenfull & Scott, 1986), depending on the sample type and the procedure followed for gel preparation. In the systems with Tween 80 added or with both Tween 80 and limonene, the exponent is 3.2 and 3.9 (± 0.2), respectively. A power law dependence of t_g on concentration is predicted by the kinetic model for chemical gelation of Ross-Murphy (1991b). Although the exponent n has only a phenomenological meaning in the case of the formation of physical gels (Ross-Murphy, 2003), still its relative values in the systems studied indicate that the flavour and detergent addition favour crosslink formation.

The storage modulus G' at large time ($t \gg t_g$) also follows a power law dependence on pectin concentration (G'–C^n), as reported for other gelling systems (Clark & Ross-Murphy, 1987; Lopes da Silva et al., 1995; Normand, Muller, Ravey, & Parker, 2000; Verheul et al., 1998). The power law exponent is 3.3, 3, 2.5 (± 0.2) in order of increasing system complexity.

4. Electronic nose results

As discussed in detail in previous studies (Monge, Bulone, Giacomazza, Negri, et al., 2004; Monge, Bulone, Giacomazza, Bernik, et al., 2004), E-nose results can be conveniently represented as a radar plot in which each vertex corresponds to the signal of one sensor of the E-nose. Fig. 5 shows radar plots related to limonene release from different pectin gels at 180 min. We remark that at this time the rate of structure development reached a steady value, as shown in Fig. 3(b). Fig. 5(a) includes the radar plots of "free" limonene and of the release from a gel with 0.25% pectin. Fig. 5(b) compares the radar plots of "free" limonene with that of released limonene from a gel with 0.075% pectin. It seems from visual inspection of the plots that although the limonene concentration is the same, the three fingerprints have similar shapes but different intensities (Monge, Bulone, Giacomazza, Bernik, et al., 2004). This difference in pattern intensity between pectin gels reflects the different retention of limonene in the matrix.

A PCA, performed with all the E-nose measurements, confirms that conclusion. Fig. 6 shows the PCA map resulting from the analysis of limonene release measurements (after 180 min of being stored) from systems with different pectin and limonene concentrations. Each point represents a measurement and the different symbols are associated with different pectin concentrations. It is

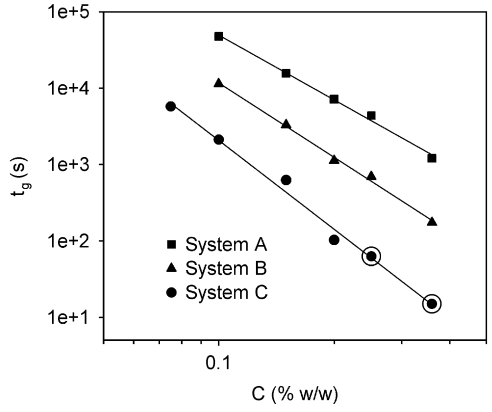

Fig. 4. Values of t_g as a function of pectin concentration on a log–log scale for the three systems.

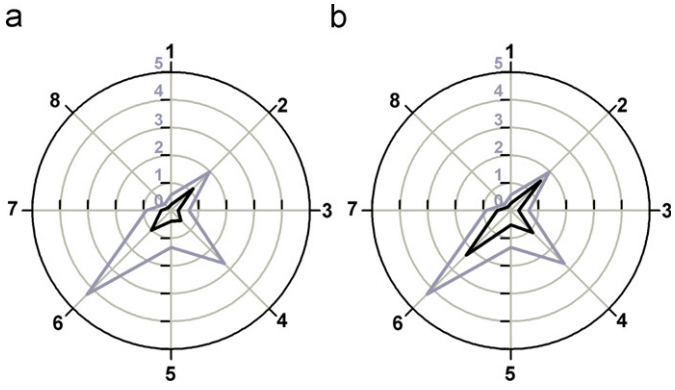

Fig. 5. Radar plots associated with limonene release measurements from gels (black lines) with 0.25% pectin (a) and 0.075% pectin (b) compared with free limonene fingerprint (grey lines).

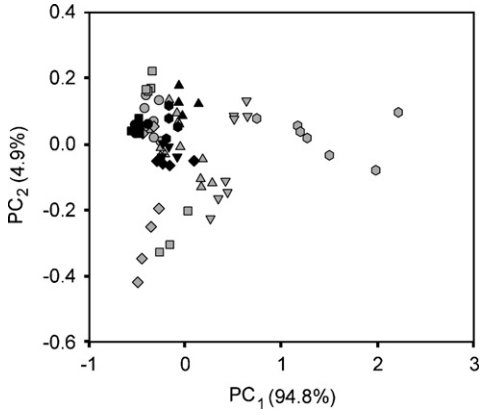

Fig. 6. PCA plot for limonene release from pectin gels at 180 min for different pectin and limonene concentrations. Grey symbols belong to 4% limonene and black symbols to 0.4% limonene. Pectin concentrations are 0.36% (●), 0.25% (■), 0.2% (▲), 0.15% (♦), 0.1% (▼) and 0.075% (⬢).

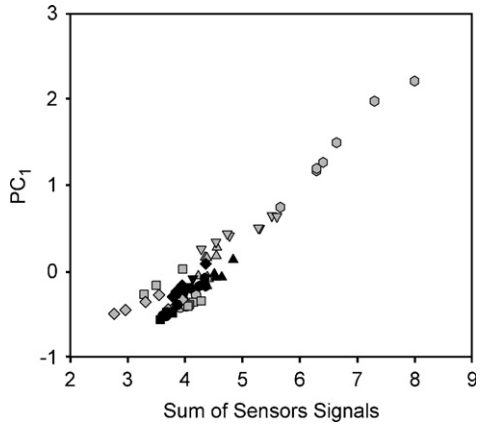

Fig. 7. PC_1 as a function of the sum of sensor signals for limonene release measurements from pectin gels at 180 min for different pectin and limonene concentrations. Grey symbols belong to limonene 4% and black symbols to limonene 0.4%. Pectin concentrations are 0.36% (●), 0.25% (■), 0.2% (▲), 0.15% (♦), 0.1% (▼) and 0.075% (⬢).

observed in the PCA map that the group of data points associated with 0.1% and 0.075% pectin and with the highest limonene concentration (4%) is well separated from the whole set of measurements. In order to link the PCA results with the radar plots, the first principal component PC_1, which contains 94.8% of the data variance, was plotted against the sum of the sensors' signals (see Fig. 7). The latter is related to the intensity of the fingerprints as the sum of each sensor signal grows with the radar areas. Since PC_1 increases when the total radar intensity increases, it is a good indicator of the flavour intensity. So, in order to see if the flavour release can be correlated with the viscoelastic properties of the matrix, PC_1 was plotted versus G''/G' (or $\tan\delta$) in Fig. 8. We recall that $\tan\delta$ can be taken as a measure of the ratio between liquid- and solid-like characters of the system. As different replicates

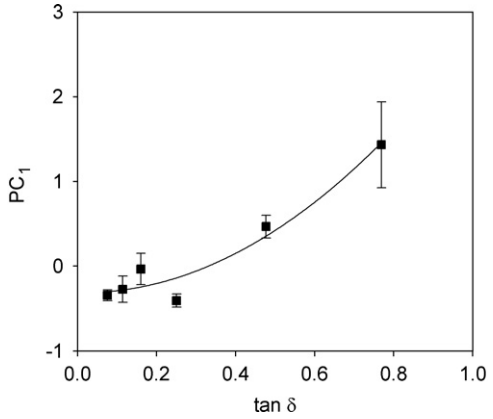

Fig. 8. PC_1 as a function of $\tan\delta$ obtained at 180 min for different pectin concentrations.

with the same pectin concentration were measured with the E-nose, average PC_1 values associated with their $\tan\delta$ values are presented in Fig. 8 (G''/G' was calculated using the average values of $G'$and G'' at 180 min). The figure shows that flavour release is largest at lower pectin concentrations, where the gel has a looser structure. As a consequence, the E-nose is able to discriminate between weak and strong gels.

5. Optical microscopy

The photographs of gel surfaces shown in Fig. 9 indicate that the systems consist of a gelled microemulsion (Monge, Bulone, Giacomazza, Negri, et al., 2004). On closer inspection it can be seen that there is a distribution of drop size, which is dependent on pectin concentration. That is, the sizes of the drops on the gel's surface are bigger at lower pectin concentrations. At the lowest pectin concentration, several small drops are clearly visible inside the bigger ones. In order to identify the nature of such heterogeneity, we performed assays with Rhodamine C18 dissolved in Tween 80 or limonene. Fig. 9(f), relative to the sample at the lowest pectin concentration, shows that the big drops consist of non-polar sites in the gel surface as they are coloured with the fluorescent probe, so indicating that limonene should be present there. We recall that the limonene emulsion in acid and sucrose-rich solvent, and without pectin, is not kinetically stabilized by Tween 80 over a long time, as already described under Section 2. All pectin gelled samples are instead macroscopically homogeneous. Therefore, results indicate that when pectin is present, the gelation process interferes with the coalescence of small drops into larger domains so avoiding the macroscopic phase separation. The gelation rate that increases with pectin concentration controls the size growth of limonene-rich domains. As a consequence, a lower degree of structural heterogeneity is observed at a higher pectin concentration where the reversion of the

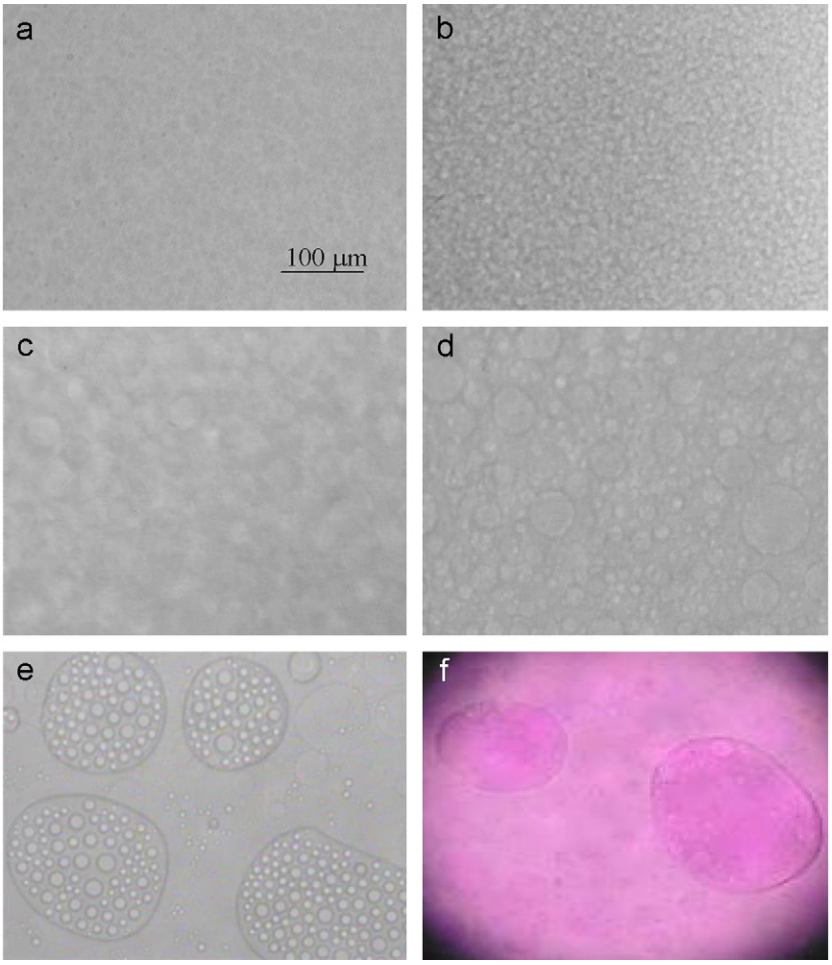

Fig. 9. Photographs taken with optical microscopy corresponding to the surface of gels with the following pectin concentrations: 0.36% (a), 0.25% (b), 0.2% (c), 0.1% (d) and 0.075% (e). Panel (f) in the figure is relative to the same system as (e) but marked with Rhodamine C18.

microemulsion into the stable state of two separated phases gets arrested earlier.

6. Conclusions

The main result is perhaps the demonstrated graphic correlation between the changes of $\tan\delta$ and PC_1, showing that the modulation of the release by the encapsulation matrix can be effectively followed by the E-nose methodology in a simple manner. The parallel study of the viscoelastic properties (by rheometry) and flavour release (by E-nose) is the central point, which allowed us to obtain a direct relation between the decrease of flavour release and the increased solid-like character of the matrix. This result is very promising concerning the uses of E-nose in flavour release studies from encapsulation matrixes, a relevant issue in food technology, cosmetic chemistry and pharmacology.

In addition, the effects of flavour and detergent on the gelation kinetics were also studied. We found that although the presence of limonene and Tween 80 increases the initial rate of network formation, the viscoelastic properties of the final gels are not very different. Optical microscopy shows that the gel with the lower pectin concentration presents the largest size (and possibly the largest size dispersion also) of limonene-rich domains.

To sum up, the correlation E-nose–rheology together with optical microscopy results are consistent with a larger surface exposure of limonene in weak gels. The pectin concentration determinates the degree of structural microheterogeneity through the change of the gelation rate. This makes pectin an effective stabilizing agent of the flavour compound.

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