

Influence of Aroma Compounds on the Mechanical Properties of Pectin Gels

SAMUEL LUBBERS* AND NICOLAS DECOURCELLE

Unité Mixte de Recherche sur les Arômes ENESAD-INRA, Etablissement National d'Enseignement Supérieur Agronomique de Dijon, 21 Boulevard Docteur Petitjean, F-21000 Dijon, France

A detailed study of the rheological behavior of pectin gels, in the presence of aroma compounds in food concentration is reported. In high methoxylated pectin (HMP) gels, it has not yet been shown if aroma compounds can be responsible for modifications in rheology. Two rheological techniques were used to measure the impact of aroma substances on rheological properties of HMP-based systems. Maximum strain to fracture (σF) was compared between flavored and unflavored gels on stress-displacement curve, which was obtained with uniaxial compression until fracture. An oscillatory rheometer was applied to determine the gelation time (Tgel). It appeared that all the aroma compounds studied increased significantly σF . It is generally acknowledged that hydrophobic interactions are the main interactions leading to HMP gelation, and the more hydrophobic interactions there are, the higher the σF . It is assumed that esters might increase σF through the increase of hydrophobic interactions in HMP network. For solvents (ethanol, propylene glycol), a cosolute effect could explain the increase in firmness for HMP-based gels.

KEYWORDS: Pectin; gelation; aroma compounds; rheology; propylene glycol

INTRODUCTION

High methoxylated pectin (HMP) is one of the most used gelling agents in the food industry and has numerous applications in jelly and fruit based products. Pectin is mainly formed with D-galacturonic acid molecules which are linked to each other by $\alpha(1,4)$ -glycosidic bonds to become polygalacturonic acid. The carboxyl groups are partially esterified with methanol. The presence of neutral sugars such as galactose, arabinose, or xylose, which are linked as side chains to the pectin macromolecule, and the rupture of the main chain caused by rhamnose make pectin to a branched zigzag-shaped heteropolysaccharide.

Pectin has the ability to form gels that can influence the amount and intensity of aroma release. Lundgren et al. (1) increased high HMP concentrations in orange-flavored gels and demonstrated by sensory analysis that the more firm the gel, the less the panelists perceived aroma. In strawberry jams, investigations showed that HMP lead to a significant retention of seven aroma compounds (2). More recently, Hansson et al. (3), observed aroma retention in a flavored-soft-drink model with HMP and in pectin gels. In the absence of clear investigations on the molecular interactions between aroma molecules and pectin agents, previous authors have concluded that a physical entrapment of aroma compounds within the food matrix can explain the effect of pectin on the release of flavor. Pangborn and Szczesniak (4) were already aware of this hypothesis.

More recently, there has been an interest in the investigation of modifying rheological parameters of gelling products, induced

by flavoring. Oakenfull and Scott (5) observed that 1-butanol and ethanol had effects on the maximal force of HMP gels using uniaxial compression. They concluded that this was due to an increase in gel strength. The addition of isoamyl acetate in starch gels increased the apparent elasticity modulus proportionally to the amount of aroma (6). To highlight disturbances observed in the release of aroma compounds in jelly products, it also appears necessary to determine whether aroma compounds act on HMP network by way of rheological techniques. The aim of this investigation is to suggest a rheological approach, despite obtaining quantitative data, when there is a modification in the texture in flavored HMP gels. Uniaxial compression until fracture was applied to perform maximal stress to fracture σF (6–8). σF is representative of gel firmness and was observed when gels were disrupted. The apparent elasticity modulus, E , extracted from the graph of stress versus displacement was successfully used in various studies (5–6). HMP gel-forming ability and rates of gel-structure development (gel network state) can be performed by small dynamic oscillatory deformations. Elastic modulus (G') and viscous modulus (G'') measurements were used to explore the effect of volatiles on the formation of the structure of HMP network (9). The gelation point (Tgel) was observed to follow the effects of aroma compounds on the transition from viscoelastic liquid to viscoelastic solid. Tgel was obtained when $G' = f(t)$ and $G'' = f(t)$ curves crossed (9–12).

In the present study, we focused on the effects of some compounds, ethanol, propylene glycol, and esters, on the firmness and the apparent elasticity of pectin gel performed by uniaxial compression until fracture, and oscillatory rheometry.

* To whom correspondence should be addressed. Tel.: +33380693542. Fax: +33380693227. E-mail s.lubbers@enesad.fr.

Table 1. Chemical Characteristics of Aroma Compounds

aroma cpd	purity (%)	Log <i>P</i>	prov.	no. CAS
ethanol	99	-0.22	VWR	64-17-5
propylene glycol	96	-0.78	Aldrich	57-55-6
ethyl valerate	99	2.25	Aldrich	539-82-2
ethyl hexanoate	98	2.78	Aldrich	123-66-0
ethyl octanoate	96	3.96	Aldrich	106-32-1
ethyl decanoate	96	5.10	Aldrich	110-38-3

MATERIALS AND METHODS

Materials. UNIPECTINE MRS ND CITRUS from Degussa (France) is a high methoxylated pectin with 65–70% C6 groups methoxylated and extracted from citrus peel. To induce gelation, commercial sucrose (Erstein, France) was incorporated. **Table 1** shows chemical characteristics of the aroma compounds used. The logarithm of partition coefficient between water and octanol for aroma compounds ($\log P$) was estimated with Modeling Molecular Pro software.

Gels Manufacturing. HMP gelation needs both acidification and cosolutes as sucrose of more than 55% (w/w). A Citrate buffer was prepared according to literature (10, 13). The pH was rigidly controlled at 3.14 before each manufacturing. Pectin (3 g) was mixed with the citrate buffer (97 g) at pH 3.14 under magnetic stirring, at room temperature over a night (14–16). A 50-g sample of the above solution was placed into a jacked flask with hot circulating fluid. The system was placed on a magnetic stirrer. When the mix temperature reached 95 °C, sucrose was added (100 g). The sample was stirred until complete dissolution of sucrose (10 min). The hot circulating fluid was then stopped and the sample cooled until 70 °C under stirring. The flask was not closed thus solid content was controlled using refractive index apparatus. Water was added to obtain a 70–72% final Brix at 70 °C. Pure aroma compounds (500 μ L) were added and mixed slowly to prevent foam formation. This flavoring step preserves aroma compounds by reducing the heat treatment time. Flavors can be incorporated into the buffer at the beginning but would be heated from room temperature to 95 °C; as the aroma compounds would be heated for 20 min, it is assumed that chemical degradations would occur.

Dynamic Oscillatory Rheometry. At the end of the heating process, a sample (1 mL) of hot mixture was transferred to the rheometer plate with a syringe. Dynamic rheological measurements were performed using cone-and-plate geometry (diameter 40 mm; cone angle = 0.0194 rad; gap = 22.9 μ m) on a SR 5 rheometer (Universal Stress Rheometer, Rheometric Scientific, France). Linearity conditions were checked (7). Mechanical spectra of G' versus strain curve were obtained. G' remained constant with a linear increase in strain from 1 to 8.5% with a drastic decrease when strain is more than 8.5%. Following this result, SR 5 measurements were performed in a constant strain mode, with a low strain amplitude of 0.03 (3%). Measurements of G' and G'' during cooling (gelation period) were made at a fixed frequency of 0.5 Hz. A system put on the geometry axis was used to avoid evaporation of water during cooling.

As the temperature apparatus during measurements was constant (20 °C), the cooling stage was natural and not controlled (16). Rheological experiments were conducted in quadruplet, and their averages were computed.

Uniaxial Compression to Fracture. For uniaxial compression measurements, the pectin mixtures were molded into polyethylene boxes (40-mm diameter, 38-mm depth). The boxes were capped and cooled at a controlled temperature of 20 °C. After 24 h storage, the gels were removed from their boxes and were analyzed immediately by uniaxial compression at 20 °C. The rheology of pectin gel was characterized, using a texture analyzer TA-XT2 (Champlan, France) thermostated at 20 °C and equipped with a 5 kN sensor. Uniaxial compression measurements at constant rate of 1 mm \cdot s⁻¹ were made with a stainless steel plunger (diameter 100 mm). The product was compressed until macroscopic fracture. From stress-displacement curves (σ vs ϵ), the following parameters were extracted: the coordinates of the macroscopic fracture σF and the slope at the origin (apparent elasticity modulus) E .

Table 2. Effects of Aroma Compounds on Rheological Parameters (σF and E) of HMP Gels (72% Dry Content) Analyzed by Uniaxial Compression to Fracture (20 °C)^a

	σF		E		concn 10 ⁻⁵ mol/g of gel
	(N \cdot m ⁻²)	SD	(N \cdot m ⁻³)	SD	
control	21 399 ^b	3924	0.226 ^b	0.016	
ethanol	24 202 ^c	3120	0.276 ^d	0.011	3.22
PG	23 075 ^c	1350	0.265 ^c	0.011	4.21
ethyl valerate	26 121 ^c	1413	0.258 ^{bc}	0.015	0.24
ethyl hexanoate	27 908 ^{cd}	3553	0.256 ^c	0.027	0.28
ethyl octanoate	32 588 ^e	1957	0.298 ^d	0.033	0.19
ethyl decanoate	29 737 ^d	2206	0.306 ^d	0.028	0.20

^a Control, unflavored gel; PG, propylene glycol SD, standard deviation. ^{b–e} Values labeled with the same letter indicates that there are no significant differences between them at $P < 0.5$.

Aroma Compounds Quantification. Gel (1 g) was dissolved in deionized water (2 mL), with diethyl ether (5 mL) (solvent extract) and octanol solution (200 μ L, 500 mg/L) as an internal standard, being then added (each gel contained only one aroma compound). The mixture was homogenized (vortex 5 min), placed in an ultrasonic bath, and finally cooled for 1 h at -30 °C. The diethyl ether phase was sampled and analyzed. Extractions were carried out in triplicate. Results were expressed as mole of aroma compound per gram of gel.

Gas chromatographic analysis was performed using a Hewlett-Packard (HP 6890) gas chromatograph equipped with a D. B. WAX column (15-m, 0.25-mm i.d., 1- μ m, J & W). Extracts (1 μ L) were injected automatically in splitless mode. Oven temperature was held at 50 °C and programmed to rise at a rate 5 °C/min to 220 °C, injector and detector were at 250 °C, and the gas flow rates were as follows: gas carrier (N₂), 1 mL/min; air, 300 mL/min; and H₂, 30 mL/min.

Dry Matter. A 1-g sample of pectin gel was put into an oven at 105 °C initially, for 24 h, with the weight being controlled until a constant value was obtained; however, this took about 36 h to become constant. Measurements were carried out in triplicate.

Statistical Analysis. An ANOVA procedure was performed using Statgraphics software. If samples were significantly different, the LSD test was used at the 5% level to make comparisons between sample means (four replicates per sample).

RESULTS AND DISCUSSION

Dry Matter. The gels were prepared for uniaxial compression containing 72.9% \pm 1.7 solid (w/w), having a variation coefficient of 2.4%, and thus proving the preparation of the pectin gels to be reproducible. For the oscillatory rheometry experiments, the hot mixture was transferred into the apparatus and was allowed to cool naturally in isothermal conditions (20 °C). As recommended by Bradford and Pedersen (10), the amount of water in the gel formulation was increased to avoid any gelation that occurs in the syringe before transfer into the apparatus. Therefore, the dry matter had an overall content of 69.0% \pm 0.9 (w/w). Being aware of the differences between the two dry contents, we adapted the formulation to observe the gelation point for dynamic oscillatory testing.

Uniaxial Compression to Fracture. The stability of the pectin gels was observed after aging periods of 24 and 36 h. Statistical analysis showed no significant difference of σF between both aging times. A 24-h-cooling period was sufficient to obtain stable rheological parameters. Oscillatory rheometry attested this result. A frequency sweep testing between 0.1 and 20 Hz, was conducted on the aging gels between 5 and 24 h, with G' modulus presenting a constant value after 16 h of aging.

The effect of aroma compounds on macroscopic fracture (σF) and the apparent elasticity (E modulus) were studied, with the mean values of σF and E for each flavored gel being reported in **Table 2**. Statistical analyses indicated that σF and E were

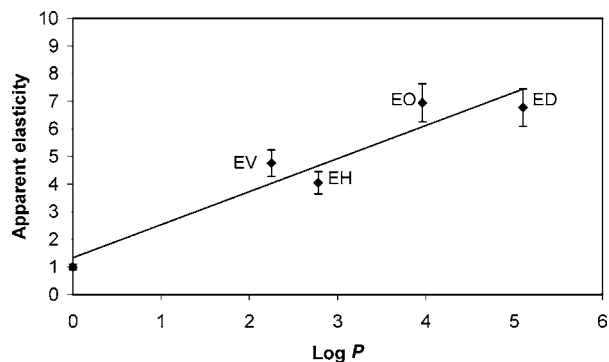


Figure 1. Evolution of apparent elasticity modulus E in function of hydrophobic constant of ethyl esters (EV, ethyl valerate; EH, ethyl hexanoate; EO, ethyl octanoate; ED, ethyl decanoate). Values were normalized (without unit).

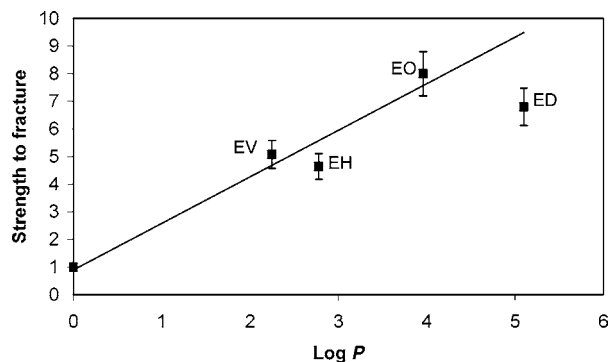


Figure 2. Evolution of maximal strength in function of hydrophobic constant of ethyl esters (EV, ethyl valerate; EH, ethyl hexanoate; EO, ethyl octanoate; ED, ethyl decanoate). Values were normalized (without unit).

significantly higher for gels flavored with alcohol components (solvents), than unflavored gels, (control). Ethanol and propylene glycol (PG) were tested as the solvents, which are very commonly used in the food industry for preparation in flavored blends. In the presence of ester compounds, the strength required to cause fracture of the gels and apparent elasticity increased.

The concentrations of the aroma compounds are in “molar units”, which are representative of molecules. Molar concentrations of ethyl octanoate and ethyl decanoate are lower than those of ethyl valerate and ethyl hexanoate concentrations because the flavoring process occurred at a high temperature; it was therefore difficult to obtain the same molar concentration for all of the aroma compounds. For ethyl decanoate, it is assumed that the limit of solubility in the gel had been reached. Some droplets were observed on the surface of the gel after cooling. However, the gels flavored with esters were significantly firmer than the gels without any aroma compounds, with a trend being found between the increase in the values of firmness, apparent elasticity, and the length of the carbon chain in the aroma compounds. Values of σF and E were normalized to authorize the comparison between the esters. A linear correlation ($r^2 = 0.903$) was observed between apparent elasticity (normalized values) and $\text{Log } P$ esters (**Figure 1**). For the strength to obtain fracture of the gels (normalized values), a linear correlation ($r^2 = 0.951$) was observed with the first three-ester compounds (**Figure 2**). The values of ethyl decanoate were not included in the correlations due to the low stability of its gel.

Oscillatory Rheometry. A more complete HMP based characterizing system has been developed by following G' during cooling to test the effects of the aroma compounds on the gelation process. When conditions are favorable to gelling,

Table 3. Time of Gelation (Tgel) for Pectin Gel without Aroma and in the Presence of Ethyl Esters

	Tgel (min)	lower value	higher value	variation coeff (%)
control	4.85	2.11	7.20	50
ethyl valerate	3.62	1.84	5.42	49
ethyl hexanoate	3.02	1.25	4.82	60

a progressive mechanism of chain aggregation takes place until they span the whole sample volume. The gelation point (Tgel), which characterizes the transition state between the solution and gel, can define the initiation of this phenomenon. Tgel was measured at the crossover-point ($G' = G''$) from the graphs $G' = f(t)$ and $G'' = f(t)$ (10, 15, 17, 18).

The presence of the aroma compounds in the gel seemed to modify the gelation point analyzed in the function of time (**Table 3**). Tgel of unflavored gels was determined at 4.85 min. In the presence of ethyl valerate and ethyl hexanoate, Tgel values were 3.62 and 3.02 min, respectively. However, unfortunately high variation coefficients were obtained, and it would be difficult to achieve better results with these high viscosity systems. According to da Silva et al. (16) high variations may be due to numerous operations taking place during manufacture of HMP-based systems.

In the present experiments, solvents such as ethanol and PG and ethyl esters were added to HMP gels to study their effects on gel texture. A dynamic oscillatory method was used to observe the HMP network state and the uniaxial compression until fracture (σF) for firmness modification. Flavor compounds or solvents used to obtain a blend of aromas are usually employed in small amounts in food. In the respect of food concentrations, with all the compounds studied, the firmness of the HMP gels increased significantly. Thus proving that small solutes such as aroma compounds can participate in the firmness of HMP gels. The impact of flavoring in the rheological properties of the products has been shown in few studies. For example: it has been observed that there are modifications in the texture of starch-based gels when in the presence of different aroma compounds (6).

Gel formation of high methoxylated pectin is described in two steps. In the first step, the heating of HMP, citrate buffer, and sucrose or cosolute mixtures involves HMP hydrogen bonds and HMP hydration. In the second step, hydrophobic interactions between HMP methyl ester groups occur (5, 15, 19). These hydrophobic interactions are responsible for the formation of the gel network, and its stability (5, 19). Oakenfull and Scott have shown that the increase of hydrophobic interactions coincides with the maximal strength to fracture in HMP gels (5). Therefore a change in the gel strength can be partially explained by variations of hydrophobic interactions between high methoxylated pectin chains. In our experiment, the firmness of the gel (σF) correlated with the hydrophobic index of ethyl esters up to 10 carbons. It is assumed that nonpolar compounds, like aroma compounds; strengthen the hydrophobic interactions in the gel network. By studying the gels at different temperatures, a more distinct effect of the aroma compounds on the rheological properties of the gels could be determined. Indeed by increasing the temperature, it has been shown to strengthen hydrophobic interactions.

Few studies have focused on the possibility of hydrophobic interactions between constituents of weak molecular weight (aspartame and hesperidine) and the HMP (22, 23). Where the methyl ester fragment of aspartame could join with the apolar

site in hydrocolloids (22). Ben-Shalom and Pinto (23) have stabilized solutions of hesperidine by using pectin, and described hydrophobic interactions between this compound and the HMP. However, these authors have not discussed the structural modifications of the products or their texture.

HMP junction zones are optimized by sugars or other cosolutes. The cosolutes increase the possibility of interacting between HMP chains by decreasing the free water in the media and by favoring hydrophobic interactions. Oakenfull and Scott have investigated the substitution of sucrose by ethanol in the firmness of HMP gels (5). They concluded that an increase in the strength was needed to obtain fracture of the gel in the presence of ethanol and proposed that a cosolute effect explains the role of ethanol. In accordance with these authors, with an addition of ethanol or propylene glycol, the firmness of the pectin gels increased. Furthermore, it was assumed that the amount of free water in the presence of ethanol and PG decreased, therefore blocking its plasticizing role.

The absence of results for the measurements of the gelation point with the oscillatory rheometry could be due to the accumulation of two phenomena: First, the Tgel parameter is very delicate to obtain and has a low reproducibility. Second, HMP gelation is complex and sensitive to variations of manufacture (15, 24). Further technical investigations are necessary to study the impact of aroma compounds on gel point parameters. For future experiments, we suggest working with pectin gels with lower sucrose concentrations that will induce a lower gelation temperature than that in the present study.

Small solutes such as solvents and aroma compounds have shown a significant effect on the macroscopic fracture and the apparent elastic modulus of HMP gels. Esters increase the maximum strain to fracture, σF , through the increase of hydrophobic interactions in HMP network, thus increasing the firmness of the gel flavored with these compounds. For solvents commonly used in flavor blends, a cosolute effect could explain the increase in firmness for HMP-based gels.

With aromatization, even if the compounds are in small quantities, they have an impact on the rheological properties of gelling product. The nature of the gel studied shows that low water content and the constraints of manufacturing partially explain the low sensitivity obtained in some measurements. Oscillatory rheometry measurements will have to be improved to determine precisely what effects small solutes have on the establishment of gel networks. Experiments at different temperatures would confirm the importance of the hydrophobic interactions between flavor compounds and pectin chains and their roles on the rheological properties. Sensory measurements are also necessary to confirm that the differences observed from measurements of firmness in our gels are detectable.

With these results, it is possible to understand the formulation of flavoring and the texture of confectionery products such as fruit pastilles.

LITERATURE CITED

- (1) Lundgren, B.; Pangborn, R. M.; Daget, N.; Yoshida, M.; Laing, D. G.; McBride, R. L.; Griffiths, N.; Hyvönen, L.; Sauvageot, F.; Paulus, K.; Barylko-Pikielna, N. An interlaboratory study of firmness, aroma, and taste of pectin gels. *Lebensm.-Wiss. Technol.* **1986**, *19*, 87–88.
- (2) Guichard, E.; Issanchou, S.; Descourvieres, A.; Etievant, P. Pectin concentration, molecular weight, and degree of esterification: influence on volatile composition and sensory characteristics of strawberry jam. *J. Food Sci.* **1991**, *56*, 1621–1627.
- (3) Hansson, A.; Andersson, J.; Leufén, A. The effect of sugars and pectin on flavour release from a soft drink-related model system. *Food Chem.* **2001**, *72*, 363–368.
- (4) Pangborn, R. M.; Szczesniak, A. S. Effect of hydrocolloids and viscosity on flavor and odor intensities of aromatic flavor compounds. *J. Text. Stud.* **1974**, *4*, 467–482.
- (5) Oakenfull, D. G.; Scott, A. G. Gelation of high methoxyl pectin. *Food Technol. Aust.* **1985**, *37*, 158–158.
- (6) Cayot, N.; Lafarge, C.; Arvisenet, G.; Taisant, C. Influence of aroma compounds on the mechanical properties of starch-based food systems. *J. Text. Stud.* **2000**, *31*, 297–313.
- (7) Kavanagh, G. M.; Ross-Murphy, S. B. Rheological characterization of polymer gels. *Prog. Polym. Sci.* **1998**, *23*, 533–562.
- (8) Abd Karim, A.; Norziah, M. H.; Seow, C. C. Methods for the study of starch retrogradation. *Food Chem.* **2000**, *71*, 9–36.
- (9) Arenaz, M. F.; Lozano, J. E. Measurement of gel point temperature and modulus of pectin gels. *J. Food Sci.* **1998**, *63*, 979–982.
- (10) Barford, N. M.; Pedersen, K. S. Determining the setting temperature of high-methoxyl pectin Gels. *Food Technol.* **1990**, 139–141.
- (11) Dahme, A. Gel point measurements on high-methoxyl pectin gels by different techniques. *J. Text. Stud.* **1992**, *23*, 1–11.
- (12) Yoshimura, K.; Terashima, M.; Hozan, D.; Ebato, T.; Nomura, Y.; Ishii, Y.; Shirai, K. Physical properties of shark gelatin compared with pig gelatin. *J. Agric. Food Chem.* **2000**, *48*, 2023–2027.
- (13) Evageliou, V.; Richardson, R. K.; Morris, E. R. Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels. *Carbohydr. Polym.* **2000**, *42*, 245–259.
- (14) Michel, F.; Thibault, J.-F.; Doublier, J.-L. Viscometric and potentiometric study of high-methoxyl pectins in the presence of sucrose. *Carbohydr. Polym.* **1984**, *4*, 283–297.
- (15) da Silva, J. A.-L.; Gonçalves, M. P. Rheological study into the aging process of high methoxyl pectin/sucrose aqueous gel. *Carbohydr. Polym.* **1994**, *24*, 235–245.
- (16) da Silva, J. A.-L.; Gonçalves, M. P.; Rao, M. A. Kinetics and thermal behaviour of the structure formation process in HMP/sucrose gelation. *Int. J. Bio. Macromol.* **1995**, *17*, 25–32.
- (17) Doublier, J.-L.; Launay, B.; Cuvelier, G. Viscoelastic Properties of Food Gels. In *Viscoelastic Properties of Foods*, E. A. F. S. Series; Elsevier Applied Science Publishers: London, 1992; pp 378–401.
- (18) Ross-Murphy, S. B. Rheological characterisation of gels. *J. Text. Stud.* **1995**, *26*, 391–400.
- (19) Oakenfull, D.; Scott, A. Hydrophobic interaction in the gelation of high methoxyl pectins. *J. Food Sci.* **1984**, *49*, 1093–1098.
- (20) Wafwoyo, W.; Hansen, P. M. T.; Chism, G. W. Interaction of aspartame with selected hydrocolloids: solubility of aspartame. *Food hydrocolloids* **1999**, *13*, 299–302.
- (21) Ben-Shalom, N.; Pinto, R. Natural colloidal particles: the mechanism of the specific interaction between hesperidin and pectin. *Carbohydr. Polym.* **1999**, *38*, 179–182.
- (22) Norsker, M.; Friis, A.; Bregnhøj, D. Gelation properties of pectin after heat treatment. *Pol. J. Food Nutr. Sci.* **1998**, *48* (7), 148–152.

Received for review June 25, 2003. Revised manuscript received January 9, 2004. Accepted January 17, 2004.

JF034680B