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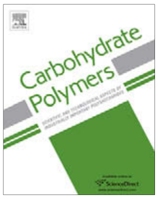

Structural characterization and surface activity of hydrophobically functionalized extracted pectins

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ABSTRACT

The technological properties of pectins are generally influenced by their chemical modification. Thus, amidated pectins are important derivatives with good emulsifying properties at low concentrations. The present article focuses on the comparative study of physicochemical properties of three modified pectin derivatives. Various amphiphilic derivatives in which pectin is associated with hydrophobic amines chains were prepared. The reaction was carried out in heterogeneous medium in methanol at 20 °C for 7 days and with 0.5 pectin/alkylamine mass ratio. The degrees of amidation (DA) of the derivatives were calculated based on the results of FTIR spectroscopy. The surface-active properties of the modified pectins were determined by surface tension (air/water) and interfacial tension (oil/water) measurements. The aminolysis of pectins appears to be an interesting way to produce pectin derivatives with new properties able to stabilize oil-in-water emulsions.

1. Introduction

Pectins are a complex family of heterogenous branched polysaccharides that arise from the primary cell walls and intercellular regions of higher plants (Ralet, Bonnin, & Thibault, 2002). Basically it is a biocompatible anionic polysaccharide of α-D-galacturonic acid with 1–4 linkages (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Tho, Kjoniksen, Nystrom, & Roots, 2003). Depending on the degree of methoxylation (DM), pectins are usually classified into high-methoxyl (HM) pectin (methoxyl content > 50%) and low-methoxyl (LM) pectin (methoxyl content < 50%). The degree of methoxylation governs the type of interactions in semidilute solutions and gelling effects of aqueous solutions of these systems. The main sources of commercial pectin are citrus peel (lemon, lime and grapefruit), apple pomace and sugar beet pulps. Pectins from different sources are widely used as gelling agents, thickeners, texturisers, emulsifiers and stabilisers in food, pharmaceutical, and many others industries (Levigne, Ralet, & Thibault, 2002; Schieber et al., 2003).This multifunctionality of pectin is due to their functional groups which can be enhanced or even changed by introducing new functional groups onto the pectin polymer backbone using either chemical or enzymatic methods (Pappas et al., 2004).

Chemical modifications of pectin can lead to new products with significant physicochemical and biological properties. An introduction of non-polar residues increases hydrophobic character of pectin macromolecules. The hydrophilicity–lipophilicity relationship of such polymers depends on the degree of substitution, i.e. the content of non-polar substituents attached to the original polar macromolecule (Synytsya et al., 2004). At small degrees of substitution, the polymer is soluble in water like the hydrophilic precursor. Slightly substituted polymers have surface-active properties and can be valorized in various applications. Synytsya et al. (2004) prepared a number of N-alkylamides of highly methylated pectin used as bioavailable sorbents and drug delivery systems.

The purpose of this paper was to study and evaluate the physicochemical and structural properties of hydrophobically functionalized pectin. The properties of pectins extracted from local beet and citrus were compared with those of commercial pectin. In an attempt to formulate emulsions, the ability of these modified polysaccharides to stabilize oil-in-water emulsions was determined. The comparison between modified and non modified pectins was also presented.

2. Materials and methods

2.1. Materials

High-methoxyl commercial pectin was provided by Degussa texturant Systems (France). Three N-alkylamines (N-dodecylamine (98%), N-octadecylamine (99%) and N-octylamine (99%)) were purchased from Sigma–Aldrich. All other chemicals used here were of reagent grade and were used as received.

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2.2. Extraction of pectins

High molecular-weight pectins were extracted from local dried citrus peel and sugar beet pulp by hydrolysis with hydrochloric acid at pH 1.2 for 3 h at 80 °C (Buchholt, Christensen, Fallesen, Ralet, & Thibault, 2004; Mesbahi, Jamalian, & Farahnaky, 2005), and were referred as P_1 and P_2, respectively. After purifying the slurries by filtration, the pectin samples were recovered from the slurry syrups by precipitation into ethanol. The products were then dried and ground. The commercial pectin was referred to as P_3.

2.3. Preparation of N-alkylpectinamides

2.3.1. Conversion of pectin into acid form

Pectins (P_1, P_2, and P_3) were purified and converted into H-form by washing with hydrochloric acid (HCl) solution (0.1 mol.l^{-1}, prepared in ethanol–water mixture (1:1 v/v)). Then pectins were washed successively with ethanol–water mixture and ethanol (96%), and then filtered repeatable through a paper filter until the chloride reaction with silver nitrate was negative, and finally dried at 50 °C for 6 h.

2.3.2. Preparation of amidated pectins

The reactions of pectins (P_1, P_2, and P_3) with three N-alkylamines were carried out in heterogeneous system (methanol medium) as described by Sinitsya, Copikova, Prutyanov, Skoblya, and Machovic (2000) with slight modification. Amount of 4 g of each N-alkylamine was dissolved in 120 ml of methanol. Pectin powder (2 g) was suspended in 50 ml of methanol into a 250-ml flask. Then the solution was gradually added to the flask under stirring. The reaction was carried out in covered flasks under continuous mixing at 20 °C for 7 days. At the end of each reaction, the mixture was decanted in order to eliminate the liquid phase. The solid product was washed successively with methanol at appropriate temperature to remove the free amine and then washed with HCl solution (0.1 M, prepared in ethanol–water mixture (1:1 v/v)) to convert free carboxylic groups into the protonated form. Finally, the product was washed by 80% aqueous ethanol, filtered and dried at 60 °C.

2.4. Analysis of pectin samples

Moisture content of pectin was determined by oven-drying, using an air-circulated oven 105 °C for 24 h (Happi Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008). All values were determined on a dry-weight basis. Ash content was determined according to the method proposed by McCready (Iglesias & Lozano, 2004). Pectin sample (1 g) was incinerated in furnace at 600 °C for 4 h, cooled and stored in desiccator until weighting.

The determination of the degree of esterification (DE) of pectin was carried out by the titrimetric method of Food Chemical Codex (fcc, 1981) and USP 26 NF 21 (Singthong, Cuib, Ningsanonda, & Goff, 2004). Acetyl and methoxyl contents were determined according to the titrimetric method proposed by Virk and Sogi (2004). Pectin average molecular weight (M_v) was estimated by applying the Mark–Houwinks relationship, $[\eta] = KM_v^a$ ($k = 9.55 \times 10^{-2}$ ml/g, and $a = 0.73$), using a capillary viscometer method.

2.5. Characterisation of N-alkylpectinamides

2.5.1. Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra of natural pectins and their derivatives have been recorded for KBr discs using a 90:10 salt: sample proportion. FTIR spectra were collected at the absorbance mode in a Shimadzu-8400 spectrophotometer at 4 cm^{-1} resolution and a number of scanne of 128.

2.5.2. Degree of amidation (DA)

The degree of amidation was obtained by deconvolution of FTIR spectra (1800–1500 cm^{-1} of N-alkylpectinamides. This analysis was carried out using OPUS software. The degree of amidation was calculated as the ratio of the area of amide band (A_{amide}) on the sum of areas of all the bands of the carboxylic groups (A_{tot}) according to the following equation (Sinitsya et al., 2000):

$$\text{DA}\ (\%) = (A_{amide}/A_{tot}) \times 100 \tag{1}$$

2.5.3. Surfaces properties

Surface and interfacial tension measurements were performed at 25 °C using the Du Nouy ring method with a bidirectional tensiometer (CSC Du Nouy 70545). For surface tension (air/water) measurements, pectin solutions (10^{-4} to 5 g l^{-1}) were prepared by dissolving dried pectin in distilled water containing 0.02% (in wt.) of sodium azide as a bactericide, and then stirred for 12 h at room temperature and overnight at 4 °C, before measurements. Interfacial tension measurements were carried out at the oil/water interface using pectin solution prepared in distilled water by dissolving dried pectin in distilled water containing 0.02% of sodium azide and hexadecane as oil layer (Miralles-Houzelle, Hubert, & Dellacherie, 2001). Pectin solutions were prepared in triplicate and measurements were performed three times for each solution. Surface properties were measured for the original pectins and their N-dodecylpectinamides derivatives.

2.5.4. Emulsifying properties

Emulsifying activity and emulsion stability were assessed according to the work of Yapo, Robert, Etienne, and Wathelet (2007) with slight modification. Briefly, oil-in-water (O/W) emulsions were prepared by adding 3 ml of hexadecane to 3 ml of N-dodecylpectinamide solutions (0.5% in wt.) in 15 ml graduated transparent centrifuge tubes. The whole system was then treated vigorously in vortex mixer set to a continuous mode at maximum speed, for 2 min at room temperature. The emulsions were finally centrifuged at 500 rpm, for 5 min and the whole volume (Wv) of the system and emulsified layer volume (ELv) were measured. Emulsifying activity (EA) was calculated as:

$$\text{EA}\ (\%) = (\text{ELv}/\text{Wv}) \times 100 \tag{2}$$

For the emulsions stability (ES), four emulsions tubes were prepared as above. Two from them were cooled to 4 °C, and centrifuged at 500 rpm, for 5 min after which the initial emulsified layer volumes (ELv_i) were measured, and stored at 4 °C. The other 2 tubes were treated in the same way as previously but at room temperature. After storage of 1 and 30 days, the remaining emulsified layer volumes (ELv_f) were measured after centrifugation and emulsion stability was calculated as:

$$\text{ES}\ (\%) = (\text{ELv}_\text{f}/\text{ELv}_\text{i}) \times 100 \tag{3}$$

Table 1

Chemical composition of pectins.

Pectin type	P_1	P_2	P_3
M_v ($\times 10^{-4}$) g/mol	5.92	5.10	6.18
DE (%)	69.00	44.40	68.40
Ash (%)	2.50	5.40	2.60
MeOH (%)	7.60	5.04	7.70
AcOH (%)	2.60	21.60	4.50
Moisture (%)	8.09	10.20	4.50

3. Results and discussion

3.1. Chemical composition of pectins

The chemical composition of the extracted and commercial pectins is shown in Table 1. The obtained results show that beet pectin contains more ash and acetyl contents than citrus pectin; these observations reflect the differences in the composition of starting raw materials. Beet pulp pectin and citrus pectin extracted in this study can be categorized as low and high methoxyl pectin respectively. This result is confirmed by esterification degree values which are 69% (>50%) for citrus pectin and 44.4% (<50%) for beet pulp pectin. These characteristics may well affect the conditions of gelation and viscosity of pectin solutions. The pectin molecular-weight values show that citrus pectin had higher average molecular weight than beet pectin. These values are lower than those reported by Mesbahi et al. (2005). This difference is due probably to the choice of extraction method and operational parameters.

3.2. Degree of amidation

FTIR spectra of citrus pectin P_1 (**1**) and their derivatives (**N1a, N1b, N1c**), beet pulp pectin P_2 (**2**) and their derivatives (**N2a, N2b, N2c**) and commercial pectin P_3 (**3**) and their derivatives (**N3a, N3b, N3c**) are presented in Figs. 1–3, respectively. In all cases, the spectral differences between the initial pectin and their derivatives were explained by the subsequent addition of N-alkylamide substituents. The carboxyl vibration region of 1900–1500 cm^{-1} is the most important for the analysis of FTIR spectra. FTIR spectra of all N-alkylpectinamides showed the appearance of new band at 1560 ± 5 cm^{-1} assigned to the amide groups CONHR.

The presence of this band and the reduction in the intensity of the carboxylic group bands indicates that the substituent is bound to the pectin chain by covalent amide bond. The samples of N-alkylpectinamides showed more intensive C-H absorption in the region of 2970–2920 cm^{-1} than the original pectin, which can be explained by the increased C-H bond content after substitution of

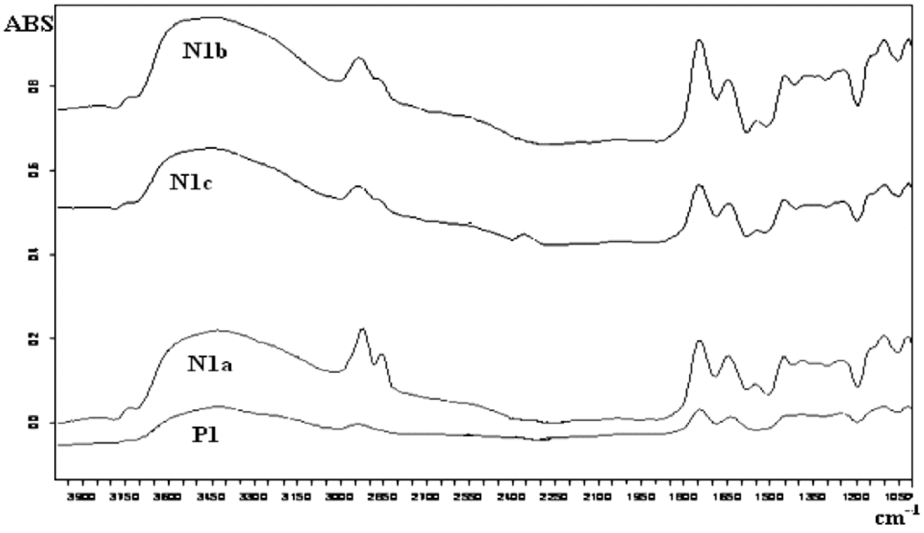

Fig. 1. FTIR spectra of citrus pectin P_1 and N-alkylpectinamides: N1a (N-octylpectinamide), N1b (N-dodecylpectinamide), N1c (N-octadecylpectinamide).

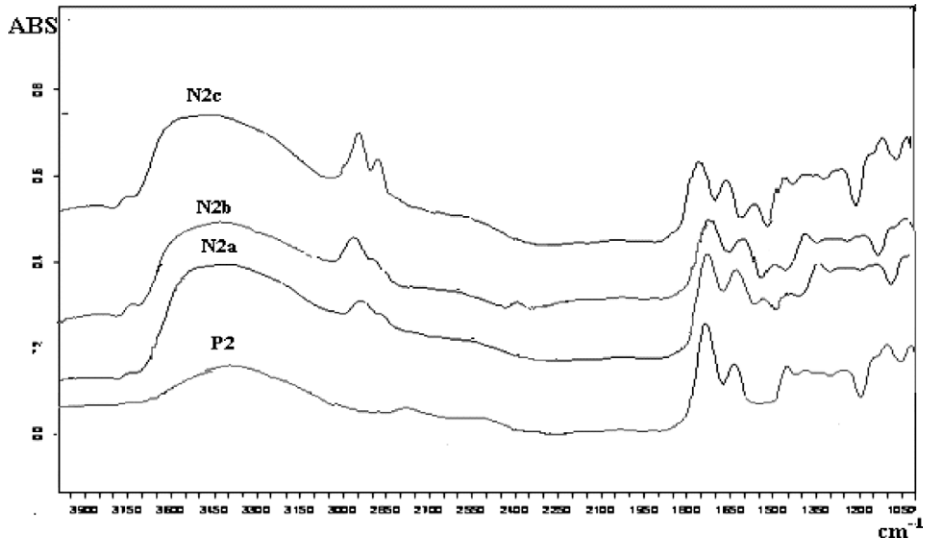

Fig. 2. FTIR spectra of beet pectin P_2 and N-alkylpectinamides: N2a, N2b, N2c.

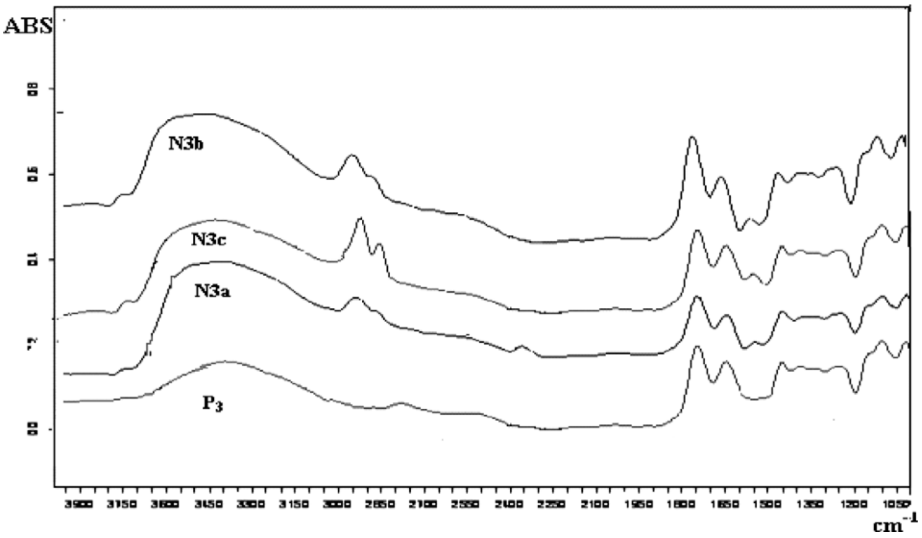

Fig. 3. FTIR spectra of commercial pectin P_3 and N-alkylpectinamides: N3a, N3b, N3c.

Table 2
DA values (%) of N-alkylpectinamides.

Amine reagent	Pectin type		
	P_1	P_2	P_3
N-octylamine	11.2	08.0	10.3
N-dodecylamine	14.6	09.5	11.4
N-octadecylamine	18.5	12.6	16.1

aliphatic amines with long chains. FTIR spectra of N-alkylpectinamides showed also that the intensity of C–H bands of 2970–2920 cm^{-1} is proportional to the length of amine alkyl chains.

Introducing the primary aliphatic amine to pectin leads to partially amidated pectin with secondary amide group. It results in changes of physical and chemical properties of pectin that depend on the number of amide groups formed and the amine radical structure. In this context, the analysis of the degree of substitution, as the degree of amidation (DA), is very important for the characterisation of amidated pectins. The DA values of N-alkylpectinamides are shown in Table 2. The found results showed that N-amine reagents with longer alkyls seem to be more effective amidation agents with DA values of 18.55, 12.6, and 16.09% for P_1, P_2 and P_3 respectively. This phenomenon could be explained by hydrophobic interactions between alkyls of amine molecules and N-alkylamide groups attached to the polysaccharide.

The results presented in Table 2 showed also that citrus pectin had higher DA values than beet pectin which can be explained by the differences in the chemical composition (different DE). However, the degree of amidation can be improved by optimizing the variation of different operational parameters (reaction time, temperature and pectin/amine mass ratio).

3.3. Surface and interfacial tensions

The variation of surface tension (γ) vs polymer concentration for the three pectins and their derivatives (N-P1: N-dodecylpectinamide of citrus pectin, N-P2: N-dodecylpectinamide of beet pectin,

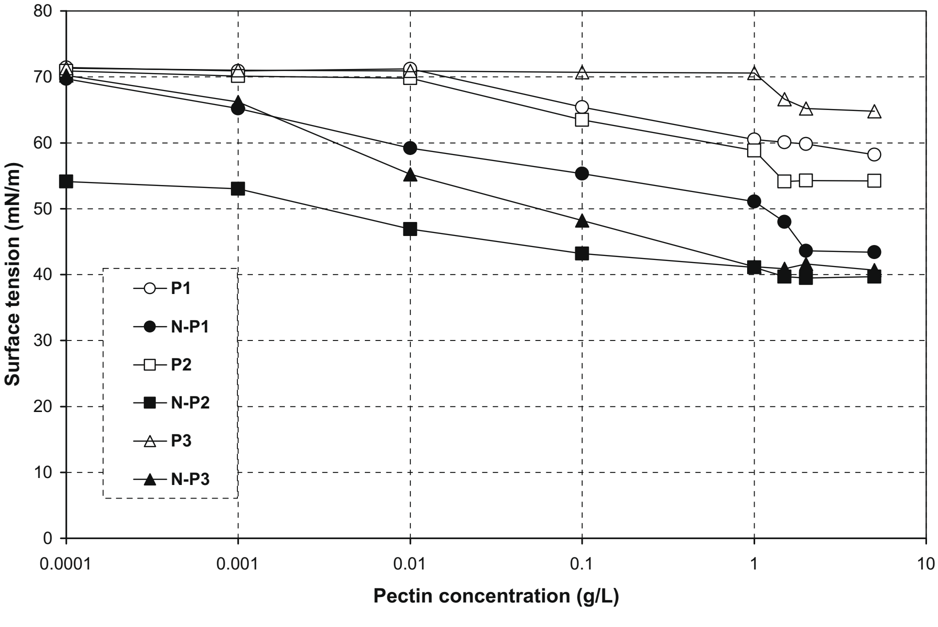

Fig. 4. Evolution of the surface tension according to the concentration of native pectins and their derivatives.

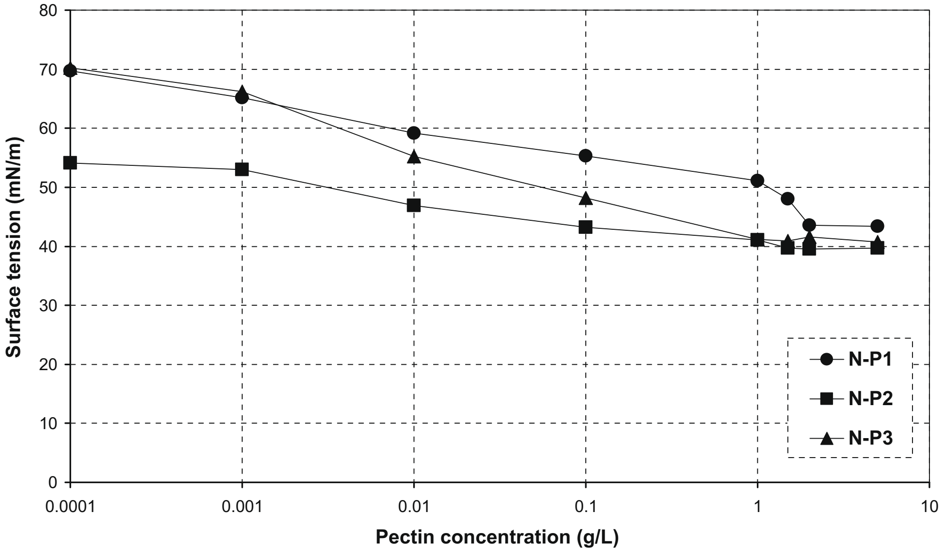

Fig. 5. Evolution of the surface tension according to the pectin concentration of modified citrus pectin (N-P1), modified sugar beet pectin (N-P2) and modified commercial pectin (N-P3).

Table 3
Interfacial tension, emulsifying properties and emulsion stability of hexadecane/05% N-dodecylpectinamide solutions.

Storage time	Interfacial tension (mN m^{-1})	Emulsifying activity (%)	Emulsion stability (%)			
			1 day		30 day	
Temperature	25 °C	25 °C	4 °C	25 °C	4 °C	25 °C
N-P$_1$	16.1	66.0	86.2	76.9	85.8	75.9
N-P$_2$	14.5	66.6	91.1	88.5	89.7	88.2
N-P$_3$	15.6	64.5	89.6	78.8	89.4	78.2

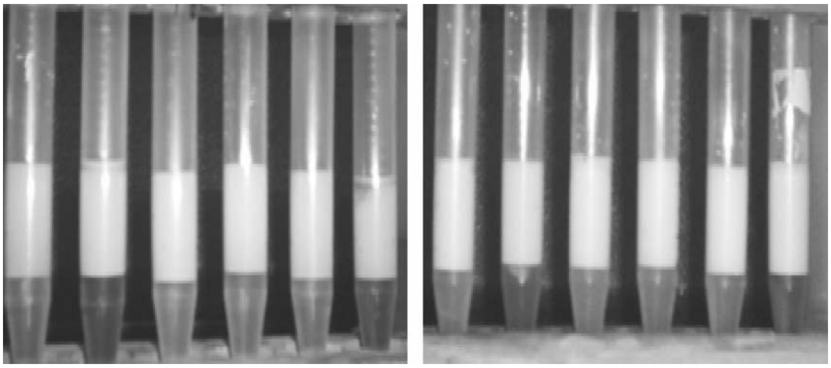

Fig. 6. Emulsion stability of N-dodecylpectinamides after 30 days of storage; A: 4 °C, B: 23 °C.

N-P3: N-dodecylpectinamide of commercial pectin) are shown in Fig. 4. Non modified pectins do not exhibit significant surface activity. The decrease of γ in the concentration range under concern is very weak (~5 mN m^{-1} for commercial pectin and ~10 mN m^{-1}, for extracted pectins). However for N-alkylpectinamides, the surface tension decreases with polymer concentration to a certain concentration of the polymer, and thereafter remains constant. This concentration corresponds to the critical concentration (CC) above which no further decrease in surface tension was observed.

The noticeable decrease in surface tension obtained with modified pectins indicates that the N-alkylpectinamides have higher surface activity than the original samples ranged from 39.7 to 45 mN m^{-1}. The variation of surface tension according to polymer concentration for the three N-alkylpectinamides (Fig. 5) indicates that the highest surface tension reduction was observed with the sugar beet pectin (P$_2$). This is probably caused by the presence of acetyl groups (21.5%). It is clear that the decrease in surface tension is much more pronounced when the level of pectin concentration increases while there is little change in the lower inflexion point concentration, which corresponds to the critical concentration (CC). For the three N-alkylpectinamides the CC values were found to be of the order of 1–2 g l^{-1}. These results are in agreement with previous surface tensions measurements for hydrophobically modified Dextrans (Rouzes, Durand, Leonard, & Dellacherie, 2002) and for modified pectins (Miralles-Houzelle et al., 2001).

The interfacial tension measurements at the hexadecanel/N-dodecylpectinamide interface (Table 3) were significantly influenced by the type of modified pectin. Interfacial tension values are near 14.5 mN m^{-1}; this value is much lower than obtained for the same interface with non modified pectin (37.2 mN m^{-1}). This decrease in interfacial tension indicates the effectiveness of N-alkylpectinamides samples to act as surface-active hydrocolloids.

3.4. Emulsifying properties

The emulsifying activity and emulsion stability of N-dodecylpectinamide samples were studied with emulsions prepared with 0.5% (in wt.) N-dodecylpectinamide solutions. After centrifuging the emulsions, three phases were observed, a very small oil phase on the top, a N-dodecylpectinamide dispersed aqueous phase at the bottom, and between the two phases, an emulsified layer phase (Fig. 6). The emulsifying activity values of the emulsions prepared with N-dodecylpectinamides were ranged from 64.4% to 66.6% (Table 3). These values were higher than that found (47.3%) by Yapo et al. (2007) for non modified pectins in similar conditions. Moreover, the results of emulsion stability of N-dodecylpectinamide (Table 3) showed high stability levels of emulsions under two different storage temperatures, well obviously, with slight differences between the three kinds of pectin which explain the differences in the composition of starting raw materials. In comparison with published data, these values were higher than that found (80%) in the literature for non modified pectins (Yapo et al., 2007). From these results, it could be inferred that pectin's hydrophobization could produce and stabilize efficiently oil-in-water emulsions with low concentrations compared to emulsions prepared with high concentrations of non modified pectins.

4. Conclusion

The chemical and physicochemical characteristics and surface properties of acid-extracted pectins were significantly influenced by the extraction conditions and the differences in the composition of starting raw materials. Modification of pectin with primary amine leads to an interesting anionic water-soluble polymeric surfactant. The surface tension depends on the degree of amidation. The emulsifying activity and the emulsion stability of N-dodecylpectinamide showed suitable results. As pectin is a biocompatible polymer and can be easily derivatized. Oil-in-water emulsions or particles stabilized by the hydrophobized polysaccharide are promising systems. In addition, bioactive molecules as proteins or drugs can be immobilized on the pectin surface via amidation. Finally, the N-alylpectinamide chemistry could be of interest in the design of new materials that could be used as biosorbent for removing of non-polar compounds (Environmental application). The detailed results of such study will be published in a forthcoming paper.

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