Structural and technological characterisation of pectin extracted with sodium citrate and nitric acid from sunflower heads

Nerea Muñoz-Almagro¹, Fabián Rico-Rodriguez², Peter J. Wilde³, Antonia Montilla^{1*} and Mar Villamiel¹

¹Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM) CEI (CSIC+UAM). Nicolás Cabrera, 9. Campus de la Universidad Autónoma de Madrid, 28049-Madrid (Spain).

²Departamento de Ingeniería Química y Ambiental, Facultad de Ingeniería. Universidad Nacional de Colombia – Sede Bogotá, Cr 30 N°45-03, Bogotá D.C. (Colombia)

³Quadram Institute Bioscience, Norwich Research Park, Norwich NR4 7UA, UK

*Author to whom correspondence should be addressed: Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC-UAM),

C/ Nicolás Cabrera 9, Campus de la Universidad Autónoma de Madrid,

E-28049 Madrid (Spain).

Tel: +34 910017951

E-mail: a.montilla@csic.es

Abbreviations

DM, methylesterification degree; GalA, galacturonic acid; LMP, low-methoxyl pectin; Mw, molecular weight; RSM, response surface methodology; SH, sunflower heads; SP-NA, sunflower pectin extracted with nitric acid; SPO-NA, sunflower pectin extracted with nitric acid at optimal conditions; SP-SC, sunflower pectin extracted with sodium citrate; SPO-SC, sunflower pectin extracted with sodium citrate at optimal conditions.

Key words: Emulsion / Gel / Extraction / Pectin / Sunflower

Abstract

An optimisation of temperature, time and extracting agent concentration of pectin extraction from sunflower heads using sodium citrate and nitric acid (SP-SC and SP-NA) was carried out. At optimal conditions, the yield of extraction with nitric acid (SPO-NA) was 2-fold greater than the corresponding with sodium citrate (SPO-SC) (14.3 vs 7.7%, respectively). Regarding pectin structure, the galacturonic acid (GalA) content in both, SPO-SC and SPO-NA, was similar (~85%). However, SPO-NA showed lower molecular weight (Mw) (88.9 kDa) and neutral sugar content (4%) than SPO-SC (464 kDa, 9%), indicating that nitric acid deeply degraded pectin structure. These differences derived into dissimilar behaviour in their technological functionality. SPO-SC showed higher viscosity and better emulsifying capacity than SPO-NA, although any of them were able to stabilise the oil/water emulsion. Both sunflower pectins formed gels with Ca^{2+} (75 mg/g of pectin) at pH 3.0. However, when sucrose was added, the gels formed by SP-SC and 20% sucrose presented the same hardness as those of SP-NA with 40% sucrose. These results suggest that the pectin extracted with sodium citrate, an eco-friendly agent, could be a promising ingredient, with good thickening and gelling properties.

1. Introduction

Pectin is a complex polysaccharide from higher plants, consisting mainly of a backbone of (1→4)-linked α -D-galacturonic acid (GalA) units, partly methylesterified or acetylated. Besides, this linear chain may be interrupted by (1→2)-linked α -L-rhamnopyranosyl units bearing neutral sugar side

chains mostly arabinans and/or arabinogalactans [1, 2]. In Medicine, pectin can be used to decrease the cholesterol, remove heavy metal and stabilising blood pressure, although the most important biological activity seems to be its antitumor properties [3]. However, pectin is most widely used in the food industry for the formulation of marmalades, jams, sauces, milk products and low calories jellies for its thickening, gelling and emulsifying properties. These applications have generated an increasing industrial demand, being 45,000 Tons approximately the annual pectin consumption [4-6]. The interest toward pectin has promoted the search of other pectin sources alternative to commercial ones (mainly apple pomace and citrus peel).

Sunflower is the third biggest oilseed crop worldwide, with 40.4 million Tons of seeds in 2015/2016 [7]. Nevertheless, after harvesting the seeds, most of the waste remains in the field constituting an environmental problem. Among the different sunflower by-products, the heads are an interesting source of low-methoxyl pectin (LMP) (15-25%). Thus, their use for pectin extraction is a good alternative not only from an economic point of view, but also from an environmental perspective [8, 9].

Numerous studies have reported that pectin is strongly associated to cellulose and hemicellulose in cell wall, which makes difficult its extraction. Industrially, is extracted employing long times (up to 5 h), high temperatures (up to 90 °C) and nitric, hydrochloric and sulphuric acids. The preference of the industry towards these acids is mainly due to their ability to generate pectin enriched in GalA as consequence of the hydrolysis of neutral sugars of side chains [10, 11]. Nonetheless, the main drawbacks of these procedures are the degradation of pectin structure, their well-known toxicity and the generation of environment unfriendly effluents, requiring special treatments. Hence, milder and more eco-friendly extracting agents are preferred.

Recent studies have shown that organic salts and acids, as sodium citrate and citric acid, are safe food additives causing low depolymerisation of pectin due to its lower hydrolysing capacity than mineral strong acids. Other factors that may affect the structural characteristics of pectin

(GalA, neutral sugars, degree of methylesterification (DM), molecular weight (Mw) distribution), and consequently to influence on its gelling and emulsifying properties, are the time, temperature and type of acid. Thereby, it is necessary to establish the optimal conditions of the extraction for keeping the structural and functional characteristics [4, 11- 13]. Response surface methodology (RSM) is a statistical tool reducing the number of assays, allowing the evaluation of multiple parameters and their interactions [14]. To the best of our knowledge, RSM has not been used to optimise the extraction of pectin from sunflower heads (SH) so far. In previous studies on sunflower pectin, Sahari et al. [15] compared different acid-washing treatments after extracting pectin with sodium hexametaphosphate. Iglesias et al. [8] and Wang et al. [9] obtained pectin with ammonium oxalate and investigated the gel formation capacity. More recently, Kang et al. [16] have studied the structural properties of pectin extracted with sodium citrate but no optimisation of conditions was done.

Thus, the main objective of this work was to compare the structural characteristics (GalA, DM, Mw distribution) and techno-functional (rheological and emulsifying) properties of pectin obtained from SH under optimised extraction conditions with sodium citrate and nitric acid.

2. Materials and methods

2.1. Materials and chemicals

Sunflower heads (SH) were from Albacete (Spain) supplied by Syngenta (Madrid, Spain). Standard glucose, mannose, rhamnose, arabinose, galactose, GalA and xylose, Pullulan Standard Set (0.34-805 kDa), β -phenylglucoside, hexamethyldisilazane, trifluoroacetic acid, calcium chloride, sodium citrate tribasic dihydrate and citric acid monohydrate were purchased from Sigma (St. Louis, MO, USA). Sucrose was from Fisher Scientific UK (Loughborough, UK). Nitric acid and ammonium acetate were acquired from Panreac Applichem (Darmstadt, Germany) and ethanol 96% was obtained from VWR (Barcelona, Spain). Food grade sunflower oil was purchased from local supermarkets (Norwich, United Kingdom).

2.2. Initial characterisation of sunflower heads

SH samples were grinded with a mill (Letslab Delivering Solutions, S.L.U., Barcelona, Spain) and sieved (particle size $\leq 100 \mu\text{m}$) before characterisation.

The dry matter content was gravimetrically determined according to the Association of Official Analytical Chemists (AOAC method 950.01) [17]. Water activity (a_w) measurement was carried out in an AW Sprint TH-500 instrument (Novasina, Pfäffikon, Switzerland). The pH (2%, w/v) was measured using a pHmeter (Mettler Toledo GmbH, Schwerzenbach, Switzerland). Protein was determined by Kjeldhal method, using a conversion factor of 6.25. Fat content was determined gravimetrically using a Gerhardt soxtherm extractor (Germany).

2.3. Experimental design

Pectin was extracted from SH with sodium citrate and nitric acid by applying a 3^3 Centred Composite Design using “Design-Expert[®]10 trial version” (Stat-Ease Inc., Minneapolis, USA) with 3 central points, totalising 17 trials for each type of extraction (Table 1) at the 0.05 significance level. The effects of temperature (X_1 , 50-80 °C), time (X_2 , 80-240 min) and extracting agent concentration (X_3 , 0.3-1% for sodium citrate and 0.01-0.2% for nitric acid) were studied obtaining surface responses for pectin yield, GalA, xylose and arabinose, rhamnose, galactose, DM and Mw [5, 16]. RSM was used and a desirability function was applied to determine the best conditions of extraction. The experiments were performed randomly to avoid systematic errors.

The quadratic model for predicting the optimal point was expressed as follows:

$$Y_i = \beta_0 + \beta_i X_i + \beta_{ii} X_i^2 + \beta_{ij} X_i X_j \quad (\text{Equation 1})$$

where Y_i is the predicted response for each experience ($i=1-17$) and each experimental response (X_1 , X_2 , X_3), β_0 is the intercept, β_i , are linear coefficients, β_{ii} are squared coefficients, and β_{ij} are the

interaction coefficients for each independent variable. The quality of fit of the second-order model equation was expressed by the coefficient of determination R^2 . Also, R^2 -Adjusted (percentage of explained variance) and R^2 -Predicted (indicator of how well the regression model predicts responses for new observations) were considered to evaluate the model uniformity. Statistical significance was determined by P-value. The significance of the regression coefficients was tested by t-value.

2.4. Pectin extraction

20 g SH was washed with 800 mL distilled water for 30 min at room temperature to remove salts, pigments, proteins and monosaccharides. Afterward, the mixture was centrifuged at 3700 g (Heraeus Multifuge 3SR Plus, Thermo Scientific, Massachusetts, USA) for 10 min; the wet solid was extracted with 400 mL of extracting agents (sodium citrate (SP-SC) or nitric acid (SP-NA); original solid/liquid ratio 1:20) using conditions of the experimental design. The pH of the reaction with sodium citrate was adjusted to 3.3 using citric acid. Once the extraction time elapsed, the mixture was centrifuged at 3700 g for 10 min. The supernatant was precipitated with two volumes of 96% ethanol (0.2% HCl v/v) and kept overnight at 4 °C. The solution was centrifuged and the pellet washed with 96% ethanol (0.04% HCl v/v pellet/solvent ratio 1:1) and later with ethanol (96%) (ratio 1:1, v/v). Pectins were lyophilised and stored at -20 °C. The yield of extraction was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of dried extracted pectin (g)}}{\text{weight of dried powder (g)}} \times 100 \text{ (Equation 2)}$$

2.5. Structural characterisation

2.5.1. Estimation of molecular weight (Mw) distribution

The estimation of Mw distribution of pectin samples was determined according to the method of Muñoz-Almagro et al. [18]. Extracted pectins were dissolved in water (0.1% w/v) and filtered using

a 0.45 μm syringe filter (Symta, Madrid, Spain). Samples (50 μL) were eluted with 0.01 M NH_4Ac at a 0.5 mL/min for 50 min at 30 $^{\circ}\text{C}$.

2.5.2. Neutral sugars and galacturonic acid analysis

Pectins were hydrolysed and derivatised forming their trimethylsilyl oximes before GC-FID analysis [19]. For quantitation, internal standard method was carried out using β -phenyl-glucoside. Analyses were performed in quadruplicate, obtaining relative standard deviations (RSD) below 10%.

2.5.3. Determination of the degree of methylesterification (DM)

Freeze-dried samples were analysed by FT-IR according to the method described by Muñoz-Almagro et al. [20]. The DM of pectin was determined as the average of the ratio of the peak area at 1747 cm^{-1} (COO-R) over the sum of the peak areas of 1747 cm^{-1} (COO-R) and 1632 cm^{-1} (COO $^{-}$).

2.6. Technological properties

The determination of these properties was carried out for sunflower pectins extracted at optimal conditions.

2.6.1. Emulsifying properties

2.6.1.1. Surface tension

Surface tension measurements were performed using the pendant drop technique with drop tensiometer FTA200 (First Ten Angstroms, Portsmouth, VA, USA). Sunflower oil was placed in a glass cuvette. The droplet of aqueous phase, formed at the tip of the needle, was immersed into the sunflower oil. Surface tension of pectin samples (0.01-1% w/v) was measured at 25 $^{\circ}\text{C}$ by triplicate, capturing an image every five seconds for 25 minutes.

2.6.1.2. Determination of droplet-size distribution in the emulsions

Oil/water (O/W) emulsions consisted of extracted pectins (1% w/v) and sunflower oil (1:9 v/v). The mixture was emulsified with a high-speed homogeniser Ultra-Turrax T25 (IKA[®] England LTD, Oxford, UK) at a speed of 10,000 rpm for 1 min.

The droplet size distribution in the emulsions was measured using a Coulter LS13 320 Laser Diffraction Particle Sizer (Beckman Coulter Inc., California) and the surface mean diameter $D_{3,2}$ was recorded. The emulsions were analysed in triplicate.

2.6.1.3. Light microscopy

To elucidate the microstructure of the O/W prepared emulsions, these were photographed, immediately after preparation, under bright field illumination with a 10X objective lens on an inverted microscope Olympus BX60 Microscope (Ibis Optics, Inc. Florida). Microphotographs were taken using a ProgRes[®]C10 camera (Jenoptik, Camberley, UK) coupled to the microscope.

2.6.2. Rheological properties

Rheological measurements were done using an AR 2000 rheometer (TA Instruments Ltd., Crawley, U.K.). Data were analysed with the software Rheology Advantage (TA Instruments, Waters Co., Ltd). Two different cones (40/60 mm diameter, 1° and 0° angle) were employed for viscosity and gel preparations (1% w/v) using a gap of 85 μm at 25 °C, respectively. For steady flow studies, the shear rate changed from 0.1 to 1200 s^{-1} . The pH was adjusted to 3 with citric acid before oscillation procedures. Afterwards, CaCl_2 was added to achieve specific Ca^{2+} concentration of 0, 25, 50, 75 mg/g of pectin. Besides, the effect of the addition of sucrose (0, 10, 20 and 40%) was also investigated. Rheological tests were performed by triplicate in the range 0.05-10 Hz (0.5% strain). Magnitudes G' , G'' were acquired.

3. Results and discussion

A global characterisation of initial sunflower by-product was carried out. This powder presented a very high dry matter content (90%), which, together with the low a_w and pH values (0.4 ± 0.01 and 4.81 ± 0.03 , respectively), guaranteed its stability against deterioration by microorganism and enzymatic and non-enzymatic reactions. Furthermore, the fat (3.8%) and protein (9.6%) contents were in agreement with those reported by Marechal et al. [21].

3.1 Pectin extraction

Seventeen experiments were done for each extracting agent (Table 1) and yield, GalA and neutral sugars content, DM and Mw, were determined.

3.1.1 Yield

The ANOVA for the yield of SP-SC and SP-NA (Table 1S) indicated a linear significance ($p < 0.05$) of the three parameters (temperature, time and concentration of extracting agent). The adj-R^2 ($> 94\%$) were similar to R^2 , indicating that both models were suitable for predicting the experimental data (Table 1). Unlike SP-NA, all parameters were significant for the recovery of SP-SC. The SP-SC yields ranged from 0.2 to 10.2% obtaining the highest value at 65 °C, 160 min and 0.65% of extracting agent whilst the yields of SP-NA reached values up to 15.6% at 90.2 °C, 160 min and 0.105% of nitric acid (Table 1). These differences might be attributed to the acid strength, which determines the pH. This was lower in the extraction of SP-NA than SP-SC (~ 2 vs 3.2, respectively), favouring the release of pectin together with other cell wall constituents as cellulose, hemicellulose and lignin. However, even when the same extracting conditions (pH, time and temperature) are used, it has been reported that higher pectin yield from cocoa extracted with nitric acid than with citric acid [22, 23].

The general trend (Table 1) seems to indicate that, although high temperatures together with long times and elevated extracting concentrations could contribute to increase the yields, it is necessary to consider equilibrium since elevated values of these parameters can cause the pectin breakdown [5].

In sunflower, Sahari et al. [15] and Iglesias et al. [8] used sodium hexametaphosphate and found 7.4-11.5% of pectin yield, similar to the values obtained in SP-SC and SP-NA.

3.1.2 Composition of the extracted pectins

3.1.2.1. Galacturonic acid content

Regarding to GalA (Table 1), all samples presented values above 65%, which is the minimum value for pectin to be considered as food ingredient by FAO [24]. According to ANOVA (Table 1S), the insignificant lack-of-fit tests of 0.101 for SP-SC and 0.495 for SP-NA and the R^2 -predict (around 90%) indicated the suitability of both models. For SP-SC, the linear effect of temperature was the most relevant parameter, whilst for SP-NA, the temperature and time affected the GalA content.

The ranges of GalA content were very narrow (~80-92%) in both models being upper than those of Kang et al. [16] who found 49.4% in sunflower pectin with sodium citrate (0.6% w/v) at 85 °C during 3.5 h. However, Iglesias et al. [8] obtained pectin from same plant with high GalA content (77-85.5%) using sodium hexametaphosphate.

3.1.2.2. Neutral sugars

The main neutral sugars present were rhamnose, arabinose, xylose and galactose, suggesting that the structure could consist mainly of homogalacturonan backbone and a small part of rhamnogalacturonan with arabinan, and/or arabinogalactan-rich side chains. Although glucose and manose, were not considered in the model because they were derived from other polysaccharides bound to pectin [1, 16], it is noteworthy that they were more abundant in the extraction with nitric acid (3.3-10%) than with sodium citrate (0.5-5.5%). For both, glucose was the main contaminant.

Analysing the experimental design (Table 1S), the neutral sugars mainly depended on temperature ($p < 0.05$).

As depicted in Figure 1 (A and B) and Table 1, the homogalacturonan was the major structural constituent for both pectins and pectin obtained with sodium citrate was less affected in its composition of neutral sugars that pectin extracted with nitric acid, probably due to the strength of this acid. Based on the susceptibility of the glycosidic linkages in pectins to acid hydrolysis (GalA-GalA < GalA-neutral sugar < neutral sugar-neutral sugar), Yapo et al. [25] reported that at pH values ≤ 2 , a fast hydrolysis of pectin side chains to oligomers and monomers occurred.

3.1.2.3. Degree of methylesterification

Table 1 indicates that all extractions gave rise to low methoxyl pectin (DM < 50%). The DM corresponding to SP-NA varied in the range 15-44% whereas SP-SC pectin presented values between 34 and 49%.

Values of “Lack-of-Fit” and R^2 -predict (>75%) demonstrated that data fitted to model. Concentration had a high influence on DM in both extractions ($p < 0.05$) (Table 1S). Thereby, as indicated Table 1, the DM values increased (36 vs 47) at higher concentrations of sodium citrate, and the same temperature and time, probably due to the increase of carbonyl groups number from dicarboxylic acids generated by the glucose oxidation in medium conditions (65°C, 160 min, 1.2/0.06%) [26]. The same trend occurred in the obtainment of pectin by nitric acid (SP-NA).

Sahari et al. [15] found DM values of 35-39% in pectins extracted from three variety of sunflowers with hexametaphosphate. Other reported works showed lower results employing the same extracting agent and sodium citrate, respectively (11-27%) [8, 16].

3.1.2.4. Molecular weight

For Mw the extractant concentration and temperature were the most important factors and a second order empirical model for Mw was fitted ($R^2 > 81\%$). Mw of SP-NA was considerably smaller than

that of SP-SC, which suggested that the former was more deeply degraded during extraction due to the strong effect of nitric acid. The range of Mw values (Table 1) found in SP-SC was 285–591 kDa, 3-fold higher than the values corresponding to SP-NA (99 – 263 kDa). This difference could be explained, among other factors, by the low dissociation constant of the sodium citrate that causes a minor depolymerisation, as compared to the mineral acids [10]. In both cases, the highest Mw was obtained under mild conditions. Thus, the degradation of pectin could start in the neutral sugar sidechain and under stronger conditions the breaking of glycosidic bonds of rhamnogalacturonan-I could explain the greater depolymerisation that suffered SP-NA [16, 25].

3.1.3. Extraction under the optimal selected conditions

Taking into account the yield and GalA content as the most important parameters for pectin extraction, a desirability function was developed being the optimal conditions with sodium citrate (SPO-SC) 52 °C, 184 min and 0.7% of extracting agent and using nitric acid (SPO-NA) 79 °C, 240 min and 0.2% of extractant (Figure 1S). Both desirability functions had values higher than 0.79.

Table 2 shows that the experimental values were in agreement with the predicted counterparts for all the parameters evaluated after pectin extractions under the optimal conditions. Comparing both extractions, very different yield and neutral sugar content and similar values of GalA and DM were observed. Although with nitric acid the yield was 2-fold higher than with sodium citrate (16% vs 8%), it is clear that the former led to a more degraded sunflower pectin. All these structural changes have to be considered in the evaluation of the technological properties and, consequently, the choice of the extracting procedure is highly influenced by the application of pectin as ingredient.

3.2. Technological characteristics

3.2.1. Emulsifying properties

Given that pectins obtained at the optimal conditions (SPO-NA and SPO-SC) had very different structural characteristics and that pectins are widely used to stabilise numerous foods as emulsifiers, it was decided to study the emulsifying properties of both pectins.

3.2.1.1. Surface tension

The surface tension is one of the most important factors for determining the ability to form and stabilise emulsions [27]. SPO-NA and SPO-SC samples were studied at different concentrations to obtain a better understanding of their surface characteristics at sunflower oil/water interfaces (O/W). For all samples, the surface tension decreased with increasing concentration, indicating that they adsorbed to the water-sunflower oil interface (Figure 2S). The two pectins showed appreciable differences in their ability to decrease the surface tension. However, they are only weakly surface active, thus, at the highest concentration (1%), the surface tension of SPO-SC decreased up to 19 mN/m, whereas in SPO-NA the lowest value of this parameter was 15 mN/m. Surface active proteins can reduce the interfacial tension down to around 5 mN/m. Considering this factor, SPO-SC, with higher protein content (7.4%) than SPO-NA (5.1%), may have lower surface tension. However, it is likely that the Mw of the pectins restricts the packing density at the interface, resulting in a lower surface activity at higher Mw.

3.2.1.2. Light microscopy and determination of particle size

The micrographs for O/W emulsions prepared with SPO-NA and SPO-SC are presented in Figure 2. The oil droplets were highly flocculated and coalesced in both samples, the emulsion formed with SPO-NA (Figure 2B) showing larger droplet size than that formed with SPO-SC (Figure 2A) ($D_{3,2}$, 22.7 μm vs 7.6 μm , respectively). Droplet size defines the emulsifying activity and can be indicative of the stability of the emulsion [28].

Difference in the droplet size could be also due to the higher viscosity of SPO-SC (3.3 Pa s) compared to SPO-NA (0.03 Pa s). A higher viscosity of the continuous phase can induce greater

shear during homogenisation and hence result in smaller droplets. A higher viscosity can also restrict droplet movement and reduce coalescence. In addition, the formation of larger oil droplets at the expense of small droplets may be attributed to an unfavourable thermodynamic effect associated with interfacial curvature [29]. The higher protein content of SPO-SC in comparison to SPO-NA, as indicated above, could favour the emulsion stability [28-30].

3.2.2. Rheological measurements and pectin gels

Figure 3S illustrates the viscosity evolution with the change in the shear rate, showing that both samples had typical non-Newtonian, pseudoplastic flow behaviour (viscosity decreased with increasing shear rate) at 1% of concentration. As previously mentioned, the viscosity of SPO-SC was higher than that of SPO-NA regardless of shear rate, probably due to its higher Mw (464.3 vs 88.9 kDa) that contributes to increase the hydrodynamic volume [31]. Hua et al. (2015) extracted pectin from SH with ammonium oxalate and obtained similar values of viscosity to those of SPO-NA.

Sunflower pectin needs the presence of divalent ions such as calcium to form gel [8, 32]. However, several studies have reported that the addition of small amounts of sucrose further improves the rheological properties and avoids syneresis [32].

Gels were formed with pectin, SPO-SC and SPO-NA at pH 3.5 and concentration 1%, and the effect of calcium (Figure 3 A-D) and sucrose concentrations (Figure 3 E-H) was studied. The strength of gels is reflected by the storage (G') and loss (G'') modulus. When G' is higher than G'' , the pectin solution forms gel. G'' was higher than G' (Figure 3 A-C) when Ca^{2+} was added up to 50 mg/g. Therefore, the pectin solutions did not form gels. But, a transition from liquid to solid occurred at Ca^{2+} concentration of 75 mg/g of pectin in both samples being G' larger than G'' , indicating the adequate amount to form the gel (Figure 3D). An increase of calcium concentration can allow additional cross-linking of non-methylated GalA, generating a denser and more elastic gel

network [32, 33]. Han et al. [34] suggested that near the pectin isoelectric point (pH 3.5), the increase of Ca^{2+} level enhances the storage modulus (G') and gel strength by formation of Ca-bridges at dissociated carboxyl groups, and higher concentration of pectin provides more hydrophobic interactions and crosslinking junctions between the pectin chains.

The addition of sucrose (Figure 3 (E-H)) improved the firmness of all gels as compared to control with only Ca^{2+} . For SPO-SC (Figure 3 (G-H)), the strongest gels were reached by addition of 20 and 40% of sucrose, with no significant ($p>0.05$) differences between them, being the former preferred from a health point of view. For SPO-NA, although with sucrose rendered gels, at all concentrations their stability was worse than SPO-SC gels. When sugar was added, the stability of both gels (SPO-SC and SPO-NA) increased providing more hydroxyl groups to form hydrogen bonds to immobilise free water [34].

4. Conclusions

This study shows a wide range of experimental conditions for the extraction of pectin from sunflower heads and the technological properties of pectins extracted under the optimal conditions. The use of nitric acid appears to be more effective than the sodium citrate leading to a higher recovery of pectin from sunflower heads. However, nitric acid degraded the pectin structure in a greater extent than sodium citrate. Regarding emulsifying properties, SPO-SC showed a better ability to form emulsions than SPO-NA, although both showed poor stabilising activity. Furthermore, SPO-SC had higher viscosity and required the half amount of sucrose than SPO-NA to form a gel with similar strength. Thus, it can be inferred that the sodium citrate pectins presented better techno-functional properties, probably ascribed to the severe structural modifications provoked by nitric acid extraction. To the best of our knowledge, this is the first study that describes a comparison of the optimisation of pectin extraction with nitric acid and sodium citrate, showing the effect on emulsifying and gelling properties. Hence, the results here obtained underline the

importance of the choice of the extracting agent and conditions for pectin obtainment from agro-food by-products depending on its application.

Acknowledgements

This work was funded by MICINN (Spain), Project AGL2014-53445- R and FPI fellowship of NM-A. Thanks to Mike Ridout and Visitación Calvo for technical assistance.

5. References

- [1] Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., Liu, D. *Food Hydrocoll.* 2016, 61, 730–739.
- [2] Hua, X., Wang, K., Yang, R., Kang, J., Zhang, J. *Food Hydrocoll.* 2015, 44, 122–128.
- [3] L.F. Zhang, X.Z. Zhang, D.H. Liu, T. Ding, X.Q. Ye. *LWT-Food Sci. Technol.* 2015, 61, 630–637.
- [4] Raji, Z., Khodaiyan, F., Rezaei, K., Kiani, H., Hosseini, S. S. *Int. J. Biol. Macromol.* 2017, 98, 709–716.
- [5] Abid, M., Renard, C. M. G. C., Watrelot, A. A., Fendri, I., Attia, H., Ayadi, M. A. *Int. J. Biol. Macromol.* 2016, 93, 186-194.
- [6] Maran, J. P., Swathi, K., Jeevitha, P., Jayalakshmi, J., Ashvini, G. *Carbohydr. Polym.* 2015, 123, 67–71.
- [7] United States Department of Agriculture (USDA) 2017
<https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf>
- [8] Iglesias, M. T., & Lozano, J. E. *J. Food Eng.* 2004, 62, 215–223.
- [9] Wang, X., Chen, Q., Lu, X. *Food Hydrocoll.* 2014, 38, 129–137.
- [10] Pereira, P. H. F., Oliveira, T. I. S., Rosa, M. F., Cavalcante, F. L., Moates, G. K., Wellner, N., Azeredo, H. M. C. *Int. J. Biol. Macromol.* 2016, 88, 373–379.
- [11] Minjares-Fuentes, R., Femenia, A., Garau, M. C., Meza-Velazquez, L. A., Simal, S., Rossello,

C. Carbohydr. Polym. 2014, 106, 179–189.

- [12] Jafari, F., Khodaiyan, F., Kiani, H., Hosseini, S. S. *Carbohydr. Polym.* 2017, 157, 1315–1322.
- [13] Oliveira, T. I. S., Rosa, M. F., Cavalcante, F. L., Pereira, P. H. F., Moates, G. K., Wellner, N., Azeredo, H. M. C. *Food Chem.* 2016, 198, 113–118.
- [14] Li, D., Jia, X., Wei, Z., Liu, Z. *Carbohydr. Polym.* 2012, 88, 342–346.
- [15] Sahari, M.A., Akbarian, M.A., Manuchehr, H. *Food Chem.* 2003, 83, 43-47.
- [16] Kang, J. Q., Hua, X., Yang, R. J., Chen, Y., & Yang, H. *Food Chem.* 2015, 180, 98–105.
- [17] AOAC, Official Methods of Analysis, 15th edition., Association of Official Analytical Chemists, Washington, DC, USA, 1995.
- [18] Muñoz-Almagro, N., Rico-Rodríguez, F., Villamiel, M., Montilla, A. *Food Chem.* 2018, 252, 271-276.
- [19] Muñoz-Labrador, A. M., Moreno, R., Villamiel, M., Montilla, A. J. *Sci. Food Agric.* 2018 (In Press)
- [20] Muñoz-Almagro, N., Montilla, A., Moreno, F.J., Villamiel, M. *Ultrason. Sonochem.* 2017, 38, 807-819.
- [21] Marechal, V., Rigal, L. *Ind. Crops Prod.* 1999, 10, 185-200.
- [22] Vriesmann, L.C. Teófilo, R.F., Petkowicz, C.L.O. *Carbohydr. Polym.* 2011, 84, 1230-1236.
- [23] Vriesmann, L.C. Teófilo, R.F., Petkowicz, C.L.O. *LWT-Food Sci. Technol.* 2012, 49, 108-116.
- [24] Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., Mohtashamy, M. *Chem. Eng. Process.* 2011, 50, 1237–1243
- [25] Yapo, B.M., Robert, C., Etienne, I., Wathelet, B., Paquot, M. *Food Chem.* 2007, 100, 1356-1364.
- [26] Vasudevan, D.M., Screekumani, S., Vaidyanathan, K, Biochemistry, Jaypee Highlights Medical Publishers, Inc., Panamá 2011.
- [27] Bai, L., Liu, F., Xu, X., Huan, S., & McClements, D. J. *J. Food Eng.* 2017, 207, 35-45.

- [28] Z.J. Kermani, A. Shpigelman, H.T.T. Pham, A.M. Van Loey, M.E. Hendrickx. *Food Hydrocoll.* 2015, 44, 424–434.
- [29] McClements, D. J., Gumus, C. E. *Adv. Colloid Interface Sci.* 2016, 234, 3-26.
- [30] X.F. Guo, W.T. Zhao, X.L. Pang, X.J. Liao, X.S. Hua, J.H. Wu. *Food Hydrocoll.* 2014, 35, 217–225.
- [31] Hua, X., Wang, K., Yang, R., Kang, J., Zhang, J. *Food Hydrocoll.* 2015, 44, 122-128.
- [32] Kim, W.J., Sosulski, F., Campbell, S.J. *J. Food Sci.* 1978, 43, 746-749.
- [33] K.C. Chang, N. Dhurandhar, X. You, A. Miyamoto. *J. Food Sci.* 1994, 59, 1207–1210.
- [34] Han, W., Meng, Y., Hu, C., Dong, G., Qu, Y., Deng, H., Guo, Y. *Food Hydrocoll.* 2017, 66, 37-48.

Figure 1. Relation of the monosacharides of the pectin extracted with sodium citrate (SP-SC) and nitric acid (SP-NA). A) Galacturonic acid (GalA) *versus* rhamnose (Rha); B) Xylose, arabinose and galactose (Xyl, Ara, Gal) percentages *versus* rhamnose.

Figure 2. Micrographs (10x) of the O/W emulsions (1%) prepared with pectins obtained by using different extractants (A, sodium citrate; B, nitric acid).

A

B

Figure 3. Study of strength of gels of sunflower pectin at optimal conditions using sodium citrate (SPO-SC) and nitric acid (SPO-NA) (pH=3.5, pectin concentration 1%). 1) Influence of different $[Ca^{2+}]$. (A) 0 mg/g, (B) 25 mg/g, (C) 50 mg/g, (D) 75 mg/g; Influence of different amount of sucrose at 75 mg Ca^{2+} . (E) 0%, (F) 10%, (G) 20%, (H) 40%.

Table 1. Coded level and actual values of independent variables, temperature (°C, X_1), time (min, X_2) and extracting agent concentration (% , X_3) used in centered composite design with seven responses (in %, yield, GalA, Mw, DM, Xyl+Ara, Rha, Gal).

Sample	Independent variables			Experimental responses						
	Temperature (°C)	Time (min)	Extractant concentration (%)	Yield (%)	GalA (%)	Xyl+Ara (%)	Rha (%)	Gal (%)	DM (%)	Mw (kDa)
SP-SC	39.8	160	0.65	4.5	90.8	3.0	2.0	3.3	43	516
	50	80	1	2.1	90.1	3.7	2.0	4.6	46	438
	50	240	0.3	3.1	89.7	4.4	1.9	3.0	38	561
	50	80	0.3	1.8	89.3	4.9	2.3	3.0	48	591
	50	240	1	4.4	89.1	4.2	2.1	3.6	49	389
	65	294.5	0.65	6.7	88.0	5.7	2.1	3.3	43	554
	65	160	1.2	3.6	87.8	5.0	2.2	2.1	47	285
	65	160	0.65	9.8	86.8	5.0	2.3	2.4	39	422
	65	160	0.65	10.2	87.1	5.3	2.1	2.2	39	437
	65	160	0.65	10.1	86.7	6.0	2.2	2.5	38	441
	65	25.5	0.65	2.6	86.5	6.3	1.9	3.0	48	560
	65	160	0.06	0.2	86.2	5.9	2.6	2.6	36	505
	80	80	1	4.2	85.8	5.6	2.1	1.0	47	387
	80	240	0.3	4.8	84.8	5.0	2.6	4.9	34	565
	80	80	0.3	1.5	84.7	6.5	2.6	3.2	40	511
	80	240	1	8.6	84.5	6.4	2.7	1.6	49	397
	90.2	160	0.65	7.7	83.5	5.4	3.1	2.3	36	415
SP-NA	39.8	160	0.105	9.2	86.8	1.4	2.3	1.1	39	224
	50	80	0.2	2.2	79.7	2.1	1.8	1.3	44	175
	50	240	0.01	0.6	91.0	1.6	2.3	1.5	25	187
	50	80	0.01	7.0	88.4	2.1	2.3	1.5	30	252
	50	240	0.2	9.6	89.7	0.7	1.5	0.4	38	182
	65	294.5	0.105	10.7	91.6	1.9	1.7	1.5	40	155
	65	160	0.26	5.7	86.3	0.7	1.3	0.8	44	144
	65	160	0.105	12.8	88.1	1.3	1.7	1.2	40	226
	65	160	0.105	11.3	89.1	1.4	1.9	1.1	37	222
	65	160	0.105	11.4	88.2	1.3	2.0	1.2	36	227
	65	25.5	0.105	5.0	86.3	1.0	2.1	0.9	44	248
	65	160	0	3.6	90.7	1.8	2.0	1.3	15	263
	80	80	0.2	1.9	89.0	0.7	1.2	1.3	41	120
	80	240	0.01	7.9	86.1	2.9	1.4	1.8	18	142
	80	80	0.01	8.1	90.1	0.8	1.7	0.6	21	237
	80	240	0.2	15.6	92.6	1.6	0.9	1.6	37	99
	90.2	160	0.105	15.6	90.0	1.1	1.2	1.1	26	105

Table 2. Observed and predicted response values at optimal conditions for extraction of pectin with sodium citrate (52°C, 184 min, 0.7%) and nitric acid (79°C, 240 min, 0.2%).

Response	SPO-SC (%)		SPO-NA (%)	
	Observed	Predicted	Observed	Predicted
Yield (%)	7.7	8.5	14.3	16
Monosaccharides composition (%)				
GalA	85.4	88.9	88.3	92.3
Xyl+Ara	4.8	4.5	1.6	1.5
Rha	2.1	2.0	0.9	1.0
Gal	2.5	2.8	1.6	1.5
DM (%)	36.8	40.6	40.1	38.2
Mw (kDa)	464	445	89	99

Pectin extracted with sodium citrate at the optimal conditions (POSC); Pectin extracted with nitric acid (PONA) at the optimal conditions.

Table 1S. Regression coefficients, R^2 , adjusted R^2 , probability values, and the significance of effect of each independent variable for pectins obtained by different extracting agents.

Samples	Source	Yield (%)		GalA (%)		Mw (kDa)		DM (%)		Xyl+Ara (%)		Rha (%)		Gal (%)	
		Coded coefficient	P-Value	Coded coefficient	P-Value	Coded coefficient	P-Value	Coded coefficient	P-Value	Coded coefficient	P-Value	Coded coefficient	P-Value	Coded coefficient	P-Value
SP-SC	Model		0.000		0.000		0.000		0.000		0.002		0.002		0.001
	Independent	10.04		87.86		433.01		38.51		6.44		2.17		2.35	
	Temperature (A)	0.95	0.000	-2.25	0.000	-21.14	0.001	-1.66	0.003	0.76	0.000	0.26	0.000	-0.38	0.003
	Time (B)	1.34	0.000	0.05	0.730	-1.77	0.676	-1.38	0.007	-0.13	0.240	0.05	0.209	0.13	0.164
	[Sodium Citrate] (C)	1.02	0.000	0.27	0.109	-72.29	0.000	3.65	0.000	-0.18	0.112	-0.09	0.038	-0.30	0.009
	A*A	-1.40	0.000	0.12	0.475	12.49	0.027	0.72	0.123	-0.46	0.004	0.13	0.011	0.22	0.047
	B*B	-1.92	0.000	0.16	0.363	44.77	0.000	2.64	0.000	0.18	0.147	-0.07	0.111	0.35	0.007
	C*C	-2.89	0.000	0.07	0.681	-12.43	0.027	1.32	0.014	-0.02	0.858	0.07	0.086	0.07	0.507
	A*B	0.52	0.000	-0.08	0.708	17.85	0.012	0.26	0.604	-0.09	0.514	0.11	0.038	0.41	0.008
	A*C	0.62	0.000	0.08	0.708	4.15	0.460	1.51	0.017	0.24	0.104	-0.04	0.422	-0.96	0.000
	B*C	0.26	0.005	-0.35	0.112	-7.85	0.183	2.56	0.001	0.41	0.014	0.14	0.017	-0.26	0.050
	Lack-of-Fit		0.6980		0.101		0.288		0.156		0.887		0.756		0.160
	R^2	99.86%		95.24%		98.60%		96.82%		93.91%		93.96%		92.45%	
	Adj- R^2	99.69%		94.14%		96.81%		92.74%		86.07%		86.19%		89.52%	
	Pred- R^2	99.24%		91.30%		90.23%		75.93%		70.45%		67.94%		66.93%	
SP-NA	Model		0.000		0.000		0.000		0.000		0.001		0.001		0.002
	Independent	11.61		88.03		207.88		40.96		1.18		1.73		1.10	
	Temperature (A)	1.66	0.002	1.97	0.000	-33.76	0.000	-2.39	0.023	-0.10	0.146	-0.32	0.000	0.15	0.010
	Time (B)	3.40	0.000	2.43	0.000	-15.68	0.006	-1.85	0.059	0.07	0.324	-0.13	0.017	0.01	0.801
	[Nitric Acid] (C)	-1.07	0.002	-0.48	0.008	-22.32	0.000	2.68	0.002	-0.15	0.009	-0.19	0.000	-0.07	0.043
	A*A	-0.13	0.710	-0.08	0.691	-24.31	0.000	-2.46	0.015	0.03	0.681	-0.07	0.132	0.01	0.872
	B*B	-1.74	0.001	0.12	0.564	-11.17	0.022	1.06	0.211	0.10	0.148	-0.02	0.711	0.04	0.331
	C*C	-0.79	0.000	-0.01	0.826	-2.93	0.028	-0.99	0.002	0.01	0.681	-0.03	0.036	0.00	0.793
	A*B	1.56	0.005	-1.63	0.000	-7.34	0.150	0.33	0.733	0.61	0.000	-0.04	0.458	0.30	0.000
	A*C	-0.18	0.414	1.01	0.000	-5.13	0.069	0.74	0.170	-0.03	0.403	0.02	0.458	0.12	0.002
	B*C	1.82	0.000	0.99	0.000	9.52	0.005	-0.08	0.874	-0.14	0.007	-0.02	0.458	-0.12	0.002
	Lack-of-Fit		0.365		0.495		0.034		0.44		0.059		0.68		0.125
	R^2	97.45%		97.95%		97.48%		96.78%		95.24%		95.59%		93.91%	
	Adj- R^2	94.18%		95.32%		94.25%		92.65%		89.13%		89.91%		86.07%	
	Pred- R^2	83.03%		87.07%		81.14%		79.22%		64.36%		74.58%		55.54%	

Sunflower pectins extracted with sodium citrate (SP-SC); Sunflower pectin extracted with nitric acid (SP-NA).

1
2
3
4

B

5

6

7 **Figure 1S.** Desirability function of the extraction of pectins using different extractants
8 A) Sodium citrate (SPO-SC), B) Nitric acid (SPO-NA).

9

Figure 2S. Variations of surface tension with the logarithm of the concentration of pectin solutions (0.01-1%, w/v) extracted with sodium citrate (SPO-SC) and nitric acid (SPO-NA) at the optimal conditions.

18
19
20

21

22 **Figure 3S.** The effect of shear rate on the apparent viscosity of pectins extracted at
23 optimal conditions with sodium citrate (SPO-SC) and nitric acid (SPO-NA).

24