Assessment of industrial pectin and its application as edible coating prepared by ultrasound

Preparation of citrus pectin gels by power ultrasound and its application as edible coating in strawberries

Running title: Citrus pectin used as edible coating prepared by ultrasound

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Abstract

BACKGROUND: Pectin is heteropolysaccharide found in cell walls coming mainly from by-products as well as citrus peels, apple and sugar beet pulp and presenting biological and techno-functional properties. In this work a general proximal and structural characterisation of industrial citrus pectins was done together with a study of impact of power ultrasound (US) on their rheological properties with the aim of using them as edible coatings for fresh strawberries.

RESULTS: The results indicated that pure pectin showed a methylesterification degree greater than 50% and galacturonic acid content > 65%, indicating its consideration as additive E-440, conditions that were not achieved by pectin with sugar addition. Furthermore, in the rheological study, pectin gels showed a non-Newtonian flow and pseudoplastic behaviour and presented different viscosity ranges depending on the preparation methods, including power US. Gels were used as edible coating of fresh strawberries in order to improve their quality during storage over a period of 5 days, controlling quality characteristics such as humidity loss, acidity and colour parameters (L*, a*, b*, C, h°, ΔE).

CONCLUSION: The obtained results demonstrated that US treatments give rise to pectin gels that can improve the quality over the lifetime of strawberries.

Keywords: Citrus pectin, Pectin, characterisation, edible coating, strawberry, ultrasound, rheological properties
INTRODUCTION

Nowadays, the huge amount of wastes derived from the fruit and vegetable processing industry constitutes a serious environmental concern since, in most of the cases, they are wastes difficult to treat properly. These by-products are mainly formed by peels, seeds and pulps, having important nutritional properties and being potentially used to obtain functional ingredients.\(^1\) Within the fruit and vegetable sector, the by-products of citrus are some of the most valuable by its high content of bioactive components, including pectin and derivatives. Pectin is an heteropolysaccharide constituted by poly-\(\alpha\)-1-4-D-galacturonic acid (GalA) which form the most important and simple dominium called homogalacturonan (HG) (smooth regions) with lateral chains that can be attached to that structure with neutral sugars forming domains such as rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II) (hairy regions) and others as xylogalacturonan.\(^2\)

In recent years, there has been a renewed interest in pectin since it can exert beneficial effects in the gastrointestinal system as potential prebiotics, systemically as hypocholesterolemic, detoxifying agents and drug carrier, among others. In addition, new applications of pectin have emerged placing them as ingredients of choice in a range of fruit-based food, dairy and confectionery products, beverages and spreads.\(^3\) Nowadays, the use of polysaccharides as edible coatings to preserve fruits and vegetables has increased due to their advantages in terms of better control of the gases exchange and improvement of the organoleptic characteristics.\(^4\) In the literature, different applications of pectin in combination with other compounds have been reported.\(^5\)

Different extraction methods can be used to obtain pectin and consist of enzymatic, physical or chemical procedures being the latter the most used at the
industry. The chemical extractions are carried out mainly with strong acids at high temperatures for long periods, including afterwards purification steps with alcoholic precipitation. These conditions can modify the structure of pectin and, consequently, its functionality.\(^6,7\)

Among the different molecular characteristics of pectin, the degree of methylesterification (DM) and molecular weight (\(M_w\)) are the most relevant parameters which can affect its technological applications such as gelling, thickening and texturising agents. Considering the DM, pectin can be classified in high methoxyl pectins (HMP, > 50% of carboxyl groups are esterified) and low methoxyl pectins (LMP, < 50%).\(^7\) As it is known, the understanding of the rheology of carbohydrate solutions is important in the control of food processing, rheological properties being indicators of the product quality\(^3\) that can be affected by processing, storage and commercialisation stages.\(^8\)

Other aspect to take into account is the effect of power ultrasound (US) on the rheological properties of pectin. In general, US can decrease the strength and viscosity of pectin gels, depending on the sonication time as it has been shown in the case of pectin extraction studies.\(^9-11\) With respect to US treatment effect on pectin solutions, Seshadri et al.\(^12\) and Zhang et al.\(^13\) found that rheological properties of pectin gels decreased with increase of time and intensity, while optical properties improved. Tiwari et al.\(^14\) also found a significant reduction in apparent viscosity and a trend towards Newtonian flow behaviour of the pectin solutions depending on the US intensity. These results were related to the reduction of \(M_w\) due to cavitation and increase of temperature, among other effects.\(^15\) However, Muñoz-Almagro et al.\(^16\) reported only a limited reduction of \(M_w\) in citrus and apple pectin solutions (5-20 mg L\(^{-1}\)) treated by US. Despite
these antecedents, the possible use of such pectin gels prepared by US as an
edible coating has not been studied so far.

Thus, the aim of this investigation was to carry out an overall characterisation
of pectin from citrus by-products, together with a rheological study of the gels
prepared by physical methods including US in order to applicate them as edible
coatings in fresh strawberries.

MATERIAL AND METHODS

Samples and gel preparation

Pectin-based industrial samples from lemon and lime peel (4400 and 4710 with sugars
added) were kindly provided by CEAMSA (Porriño, Spain) (Figure S1). The samples
were 4400 labelled as pectin and 4710 pectin, the latter with sugars added. Pectin
solutions were prepared (1, 3, 5 and 8% (w/v)) using magnetic stirrer (MS) or overhead
stirrer (OS), during 20 min until complete homogenisation at controlled temperature
(45-50 °C) in a hot plate. Afterwards, by visual examination of viscosity and
homogeneity, the concentration of 3% was chosen to study the pectin rheology and its
application as edible coating.

Pectin solutions at 3% (w/v) were divided into two aliquots and one of them was
treated with power ultrasound (US) (MSUS and OSUS) using an ultrasonic processor
operating at a frequency of 20 kHz, maximum power 400 W (Digital Sonifier, Branson
Ultrasonics Corporation, Danbury, CT, USA), 30 and 50% of amplitude, and pulsed
operating mode (2 s on/1 s off) for 30 min. A microtip horn of 3 mm diameter was
immersed 2 cm in depth with respect to the liquid surface into a beaker glass with 25
mL of pectin solutions. The temperature was controlled with an ice-bath to avoid greater
temperatures than 50 °C. The samples were prepared in duplicate.
The study of conservation to evaluate the potential of pectin as coating agents was done in strawberries (Fragaria x ananassa Duch.) purchased from a local market and harvested in Palos de la Frontera (Huelva, Spain).

Physical and chemical pectin characterisation

The pH determination was done in pectin-based dilutions (1% w/v) at 25 °C using a pH-meter (Mettler-Toledo, GmBH, Schwerzenbach, Switzerland). Water activity (a_w) was determined with a Novasina instrument (Aw Sprint Th 500, Pfäffikon, Switzerland) previously calibrated at controlled temperature. Humidity was gravimetrically determined in an oven (Thermo Scientific Heraeus, Madrid, Spain) at 102 °C for 3 days. Protein content was determined in a colorimetric analysis by the Bradford method (Bradford kit, Bio-Rad Laboratories Gmbh, Germany).

The analysis of 2-furoylmethyl-lysine (furosine) was determined by ion-pair Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) in a furosine-dedicated C8 column and an UV detector at 280 nm, based on the method of Gamboa-Santos et al.

To determine the degree of methylesterification (DM) of pectin samples, Fourier-transform infrared FTIR-spectroscopy (FT-IR) was made using a FTIR Bruker IFS66v. The spectra were recorded at absorbance mode from 7000 to 550 cm⁻¹ (mid infrared region) at 4 cm⁻¹ of resolution. The DM is defined as (esterified carboxylic groups / total carboxylic groups) x 100 and was determined by the method described by Muñoz-Almagro et al.

The estimation and distribution of M_w of pectin samples were determined by High Pressure Size Exclusion Chromatography attached to Evaporative Light Scattering Detector (HPSEC-ELSD). The analysis was performed on a LC Agilent Technologies
1220 Infinity and a detector ELSD 1260 Infinity (Agilent Technologies, Boeblingen, Germain), equipped with two consecutive TSK-GEL columns (G5000 PWXL, 7.8 x 300 mm, particle size 10 µm, G2500 PWXL, 7.8 x 300 mm, particle size 6 µm; Tosoh Bioscience, Stuttgart, Germany). Samples (50 µL) were eluted with 0.01 M NH₄Ac at a flow rate of 0.5 mL min⁻¹ for 50 min at 30 °C. Pullulans of Mw 788, 473, 212, 100, 1.3, 0.34 kDa were used as standards for calibration. All the Mw values specified were weight-average. Before analysis, all samples and standards were filtered through 0.45 µm Millipore membrane of PTFE.

Monomeric composition of samples was determined by Gas Chromatography – Flame Ionization Detector (GC-FID) in an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionisation detector after a previous acid hydrolysis. Samples (20 mg mL⁻¹ acid) were treated with trifluoroacetic acid (TFA) 2 M and N₂ were passed through to avoid the oxidation of the compounds liberated during hydrolysis and kept 4 h at 110 °C.

On the other hand, to determine GalA, an enzymatic hydrolysis was also carried out on samples. Pectin solutions (1% w/v) in 0.05 M sodium acetate buffer, pH 4.5, were hydrolysed with Viscozyme L (90 U mL⁻¹, 25 µL/mL), incubating at 50 °C for 24 h.

The mineral composition of the citrus pectin-based products was measured in an ICP-MS Elan 6000 Perkin-Elmer Sciex instrument (Waltham, MA, EEUU) from the Interdepartmental Investigation Service (SIdI-UAM) in Madrid. A semiquantitative and quantitative analysis of the elements of interest using the external calibration method and internal standards to correct instrumental drift were carried out.

Thermal behaviour was studied by differential scanning calorimetry (DSC Q100 TA Instruments, New Castle, DE, EEUU), performed by the service SIdI-UAM in...
Madrid according to the method used by Wang et al. Pectin-based samples (5-10 mg) were sealed into standard aluminium crucible and heated at 10 °C min\(^{-1}\) from 40 to 300 °C using nitrogen as purge gas.

**Rheological measurements**

The rheological properties were determined using a rotational rheometer (Haake MARS, Thermo, USA), with a circulating bath at 25 °C, equipped with a double cone-plate system (DC60/2°, Haake, Germany) and a solvent trap to avoid evaporation effects and programmed by software Rheo Win 4 Job Manager. The variation of both the shear stress and viscosity as a function of shear rate (flow and viscosity curves, respectively) were recorded in controlled rate mode up to a shear rate of 1000 s\(^{-1}\) using the following program: phase 1, increasing the shear rate between 0 and 1000 s\(^{-1}\) during 300 s; phase 2, constant shear rate (1000 s\(^{-1}\)) for 60 s; and phase 3, decreasing the shear rate from 1000 to 0 s\(^{-1}\) in 300 s.

**Storage study**

Strawberries were selected by the degree of ripeness, size and absence of physiological damage.

The coating forming solutions were based on pectin gels at 3% (w/v) prepared as it was previously described for the rheological analysis of samples (Samples and gel preparations section). Strawberries were coated by hand-immersion for 2 min and the excess was dripped off for 30 s\(^{21}\), and then the edible film was dried by convection using a computer controlled air tray dryer (SBANC, Edibon Seada Control and Data Acquisition Software Edibon Technical Teaching Units, Spain) with air rate control (AVE, 2-6 m s\(^{-1}\)) at room temperature during 30 min\(^{18}\). Control samples were not coated
in order to compare the effect of the coating on the fruit. All samples were stored in polystyrene boxes over a period of 5 days at 4 °C.

Throughout the storage period, 72 individual strawberries (control and 8 gels x 8 fruits) were used to determine quality parameters (pH, weight loss and colour analysis) being analysed just before storage (day 0), and after 3 and 5 days storage. pH was determined using fruit puree made adding the same weight of distilled water. Humidity loss was determined gravimetrically, by the difference between the initial weight (day 0, \( W_i \)) and the fruit weight at the end of storage (day 3 and 5, \( W_f \)), being the results expressed as the percentage of weight loss (\( %WL \)) through the following equation:

\[
%WL = \left( \frac{W_i - W_f}{W_i} \right) \times 100
\]

To measure the colour of the strawberries was used a Chroma Meter CM-508i (Minolta Co. LTD, Japan) with the CIELab scale (\( L^* \), \( a^* \) and \( b^* \)). The \( L^* \) represents the lightness (0 = black, 100 = white), the chromaticity \( a^* \) the redness ((- ) green to (+) red) and the chromaticity \( b^* \) the yellowness ((- ) blue to (+) yellow), which were used to determine:

- The colour saturation (Chroma) \( [C^* = (a^{*2} + b^{*2})^{0.5}] \)
- Hue angle \( [h^\circ = \arctan b^*/a^*] \)
- Total colour differences \( \Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \)

where \( \Delta L^* \), \( \Delta a^* \) and \( \Delta b^* \) represent the differences between the colour parameters of the strawberries coated and the control ones from the beginning of storage.

**Statistical analysis**

The storage study of the samples was processed in duplicate and analysed twice (\( n=4 \)), and the mean values ± Standard Deviation were reported. Analyses of variance...
(ANOVA, p < 0.05) and Tukey’s test to evaluate the differences were performed using SPSS Statistics 22.0 (BM SPSS Statistics 20.0 Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characterisation of pectin-based products

Physical and chemical overall characterisation

The results for physicochemical characteristics of pectin-based products are shown in Table 1, whose values of humidity, $a_w$ and pH can ensure the microbiological stability of the samples. Taking into account the specifications provided by the supplier, sample 4710 included pectin and sugar whereas the composition of sample 4400 was only pectin. According to this, the studied samples may present diverse applications being the sample 4400 specially indicated for the manufacture of confectionery candy, and the sample 4710 mostly used for jams and marmalades and pectin jellies. Regarding pH and proteins, both samples presented similar values to those reported in the literature.\textsuperscript{24,25}

Due to the adsorbent potential of heavy metals of pectins, it was necessary to verify the absence of toxic elements or elements that could catalyse deterioration reactions. Low concentrations of heavy metals (cadmium, chromium, copper, nickel, lead, zinc, arsenic, manganese) were detected but always at lower levels than those allowed in foods.\textsuperscript{26} The high concentrations of sodium could be attributed to the obtainment method which included a cation exchange step between calcium and sodium.

Other parameter analysed was the content in furosine, an indicator of the early steps of Maillard reaction, which can appear during the processing of foods at high temperatures and/or subsequent storage under inadequate conditions due to the interaction between the carbonyl groups of the reducing carbohydrates and the free
amino groups of amino acids, peptides or proteins. The amounts of furosine for both
samples are included in Table 1. No data have been previously reported on the presence
of furosine in pectins. Data of furosine in commercial pectin here obtained are similar to
those previously obtained in dehydrated fruits, including citrus. Considering $a_w$, pH and
protein contents, both samples are similar, however sample 4400 presented a higher
value of furosine probably due to the processing and storage conditions; further,
according to the manufacturer, maltodextrin was added to the sample 4710 contributing
to the results. As it has been demonstrated in other products from vegetal origin,
furosine could be an useful parameter of quality indicating if the processing and storage
of pectins has been adequate, in order to preserve their functional properties. 27

Structural characterisation

Figure 1 illustrates the qualitative similar monomeric profile of both samples
determined by GC-FID after acid hydrolysis with TFA. Xylose, arabinose, rhamnose,
galactose, mannose and GalA were observed, as corresponds to the standard
composition of citrus pectin. Glucose was present in both, not only in the sample with
added maltodextrins, since during extraction process part of the cellulose or
hemicellulose can be released from the vegetal tissue. 16 Similarly, pectins do not
contain mannose in their structure; however, this monosaccharide may be present as a
component of mannans and galactomannans, precipitated together with pectins. 28 Table
2 shows the quantitative data of monomeric composition and the relation GalA/Rha
obtained after acid and enzymatic hydrolysis. In general, enzymatic hydrolysis was a
better method for quantification of monosaccharides, especially GalA and mannose. As
observed, the GalA content found in the case of acid hydrolysis was inconsistent with
the data obtained by enzymatic hydrolysis, highlighting the unsuitability of former
hydrolysis for a proper determination of this compound. In sample 4400, GalA was the main compound released, while in sample 4710 was glucose, probably due to the addition of other sugars (maltodextrin). Thus, the results obtained after the enzymatic hydrolysis indicated that sample 4400 had a content of GalA of 78.5% with respect to the total carbohydrates (63.2% respect to dry matter), being considered as food ingredient E-440 since this value was higher than 65% (27), while, sample 4710 presented lower value (56.8 and 53.7% with respect to the total carbohydrates and dry matter, respectively). Regarding neutral sugars derived from pectin, galactose and rhamnose were more abundant in sample 4400 than in 4710; while xylose+arabinose were present in similar amount in both pectins. With respect to glucose the sample 4710 had a very high content, probably due to the addition of other sugars (maltodextrin).

Taking into account the ratio Gal/Rha obtained in the case of the enzymatic method, both samples had HG as the main structure of pectin, in good agreement with the data previously reviewed by Babbar et al. (29) for pectins derived from citrus peels.

As observed, the GalA content found in the case of acid hydrolysis was inconsistent with the required values for citrus pectin, highlighting the unsuitability of the acid hydrolysis for a proper determination of monomeric composition of this type of samples.

On the other hand, to test if the samples had low molecular weight sugars, non-hydrolysed pectins were also analysed by GC-FID. Thus, it was possible to corroborate that sample 4710 showed a large amount of free glucose (134.1 mg g$^{-1}$ pectin), maltose (6.5 mg g$^{-1}$ pectin) and maltotriose (10.3 mg g$^{-1}$ pectin). These results were in agreement with those of HPSEC-ELSD determination, showed in Figure 2, where the HPSEC-ELSD profiles of both samples showed a minor peak at 19 min, corresponding to at a $M_w$ higher than 1000 kDa and a major peak at 25 min, compatible with the
presence of pectin. In sample 4400, this peak presented a concentration of 731 mg g\(^{-1}\)
and a \(M_w\) of 472 kDa, and for the sample 4710 a \(M_w\) of 553 kDa and lower
concentration (499 mg g\(^{-1}\)). This sample had two more peaks, one at 35 min, of \(M_n\) 0.95
kDa, corresponding probably to maltodextrin (73 mg g\(^{-1}\)), and other at 41 min probably
due to glucose (0.19 kDa, 146 mg g\(^{-1}\)).

The DM was calculated by FT-IR taking into account the bands 1750-1751 cm\(^{-1}\)
(corresponding to esterified carboxyl groups) and the bands 1634-1637 cm\(^{-1}\) and 1750-
1751 cm\(^{-1}\) (corresponding to the total number of carboxyl groups). The sample 4400
presented 70.7% of DM, while the sample 4710 has 64.4%, being both classified as
high methoxyl pectins, considered suitable to bakery, confectionery, beverages, thinned
food and others.\(^{30,31}\)

**Thermal analysis**

It is well-known that the thermal behaviour of polymers can provide specific and useful
information on structural modifications that can take place during their processing and
storage.\(^ {4,32}\) Figure 3 shows the DSC curves of both samples which consist of two mean
peaks, the first one endothermic and the second one exothermic. The endothermic peak,
corresponding to the melting temperature was 108.0 and 96.6 °C for the samples 4400
and 4710, respectively. According to the literature, the endothermic peak at 100 °C is
attributed to water evaporation corresponding to the hydrophilic nature of functional
groups. Moreover, it may be due to a fusion of the pectin and a possible demethylation,
hydroxylation and descarboxylation.\(^ {20,32–34}\) In the sample 4710 other peak of lower
intensity corresponding to melting point (157.1 °C) also appeared. This could be due to
the presence of other compounds such as maltodextrin present in its composition.\(^ {20,35}\)
The exothermic peak describes the thermal degradation of the sample and was similar in both (sample 4400, 249.0 °C; sample 4710, 252.0 °C) highlighting that these samples have a similar chemical profile. These values agree the results of by Einhorn-Stoll et al., with temperatures between 220 and 240 °C and Wang et al. between 237 and 243 °C for pectins from citrus.

Rheological measurements of pectin gels

Once the gels were prepared, their rheological properties were evaluated in order to choose the most adequate method for strawberry coating assays. Figure 4 illustrates the effect of shear rate on the shear stress of the gels prepared with the samples 4400 and 4710 by means of magnetic stirrer (MS) or overhead stirrer (OS), with and without the assistance of US. All the samples presented a very low hysteresis (area enclosed between increasing and decreasing ramps of the flow curves), which indicates a high stability of the samples subjected to shear forces. Figure 5 shows the corresponding viscosity curves of 4400 and 4710 samples prepared with overhead and magnetic stirrer. The viscosity decreased with the increase of shear rate, typical behaviour of non-Newtonian, pseudoplastic fluids, although this effect was minor when gels were treated with US at 30% amplitude. The same behaviour was observed in gels of pectins from fruits such as apple, cacao and banana MusaABB. The samples prepared with overhead stirrer without US and with US 30% showed a flow curve starting from the origin, thus meaning that there is no yield point, that is, a minimum shear stress that must be achieved to start the flow. However, those samples prepared with US 50% present a yield point with a Bingham type behaviour. In opposition, in samples prepared by magnetic stirring the samples prepared with US 30% had a more fluid behaviour and no yield point, while for both 4400 and 4710 samples there is a high apparent yield.
point with the curves of samples without US above those treated with pulsed sonication.

From the viscosity curves, it is possible to know the viscosity value reached at low
shear rate regime \( (10 \text{ s}^{-1}) \) and high shear rate regime \( (1000 \text{ s}^{-1}) \) as exposed in Table 3.

As observed, a wide variety of types of pectin can be obtained depending on the
preparation method used, diversifying their potential applications. In general, sample
4400 presented higher values of viscosity than sample 4710 with the exception of OS
preparation. With respect to the effect of US treatment, the lowest values were observed
in the samples treated with US at 30% of amplitude. With the increase of amplitude
(50%) no decrease in the viscosity was found. According to Donghong Liu et al.\(^{42}\) and
Seshadri et al.,\(^{12}\) the more time and intensity of sonication, the lower viscosity,
however, the effect of US increase with the increase of intensity but up to a certain
value, since a large amount of cavitation bubbles could act as a barrier to energy
transmission in the system, decreasing consequently the effect of US.\(^{43}\) In addition, the
temperature reached during treatments is related with the efficiency of US since, when
the temperature increases the bubbles can collapse earlier, producing less energy and,
therefore, decreasing the efficiency of US. In the case of treatments at 30 and 50% of
amplitude the values of temperatures reached were 40 and 50 °C, respectively.

The thixotropic index values of the samples were calculated as the area enclosed
between the up-curve and the down-curve in the viscosity curves and varied depending
on the preparation method (Table 3). The thixotropy is a rheological phenomenon
produced when the viscosity of a fluid undergoes shear stress, decreases with time when
flow is applied and the sample recovers its previous structure once the force ceases.

Although the time dependency values are in general low and must be taken with
cautions, it can be noted that for OS samples the US 30% preparation leads to negative
values for both 4400 and 4710 samples, i.e. the down-ramp curve goes above the up
curve, thus suggesting the formation of a light rheopectic structure, whereas the other samples have a small thixotropy. In the case of MS preparation the values are always negative, excepting for the sample 4710 prepared with US at amplitude 50, which has the lowest time dependency among all studied samples in agreement with those obtained for citrus pectins.\textsuperscript{44,45}

Storage of strawberries with gels of pectin

Both pectins (4400 and 4710 samples) presented similar results in the general characterisation as it was described previously, excepting when US was applied. Thus, gels prepared without US and with assistance of US at amplitude 30\%, which present values of viscosity lower than gels obtained with US at amplitude 50\%, were used as coating agents of fresh strawberries and their evolution during the storage at 4 °C for 5 days was assessed by different quality indicators. The coated strawberries presented brilliant appeal, even better than the uncoated samples (Figure S2).

All the samples had a considerable loss of weight throughout the storage (Figure 6), due to the loss of humidity, being the greatest loss in the control strawberries and in the strawberries covered with sample 4710 OS and 4710 OSUS. The lowest humidity loss was found in strawberry samples 4400 OSUS and 4710 MSUS, being significantly different with respect to the control sample (p < 0.05). Previous results with different coatings based on pectins or alginate, demonstrated a limited weight loss due to their behaviour as semipermeable barrier reducing water loss and respiration, contributing to the preservation of strawberry samples.\textsuperscript{46}

Total acidity due to organic acids can be also modified during conservation of strawberries. The total acidity can increase during the senescence of the strawberries due to the production of malic acid until it reaches a big-green stage, after this the
acidity decreases to a minimum value due to a decrease in malic acid.$^{7,47,48}$ In general, after 3 days of storage most of the samples (6 samples vs. 3 samples) presented a decrease of pH, including the control; however, from the third to the fifth day, the control had the highest increase of pH ($\Delta$ 0.13). Velickova et al.$^7$ for strawberries coated with chitosan-beeswax coating found a decrease of acidity during storage indicating maturity development and fruit senescence.

Data of colour changes (parameters $a^*$, $b^*$, $L^*$, $\Delta E$ and $C^*$) suffered during the storage of strawberries are shown in Table 4. In general, the $L^*$ value of strawberries decreased significantly during storage probably ascribed to the darkening of the skin produced for oxidation reactions and the loss of humidity.$^{49}$ The chromatic parameter $a^*$ and $b^*$ did not show significant differences between the treatments ($p > 0.05$). Only the control sample showed a significant difference in $a^*$ ($p < 0.05$), whose values decreased during the storage period probably due to browning reactions, affecting the quality of the fruit.$^{22}$

The Chroma ($C^*$) relates the chromatic parameters $a^*$, $b^*$ and $L^*$, and reflects the enzymatic activity being greater whenever that value decreases. As shown in Table 4, $C^*$ values did not change significantly throughout the days being the control sample the only one that suffered the highest and significant decrease. Samples 4400 OSUS, 4710 MS and 4710 MSUS showed the highest data, indicating the probably minor enzymatic activity, in good concordance with the above indicated results of humidity loss for these US treated samples.

Hardly any change was detected in the hue angle ($h^\circ$). Although without significant differences, the strawberries coated with the gels 4400 OSUS and 4710 MSUS showed the most suitable tonalities during storage. This result could be related to
a delay in the maturation of the fruits,\textsuperscript{48,50} in a similar way that it has been shown in the other parameters here studied.

As summary, Figure S3 shows the colour variation (\(\Delta E\)) of the different treatments during storage. In general, the pectin coatings presented a better behaviour in the variation of colour with respect to the control; being the sample prepared with the gel samples 4400 MSUS, 4400 OSUS and 4710 MSUS the best response. These results indicate the positive effect of the pectin coatings (without other additive) as selective barrier, preventing the exhibition of the fruit to the environmental oxygen and inhibiting possible oxidation reactions.\textsuperscript{22} These results point out the no influence of sugars present in sample 4710 when it is used as edible coating. Overall, both pectins (4400 and 4710 samples) presented similar results in the characterisation as it was described previously, excepting when US was applied and modify the viscosity.

**CONCLUSIONS**

At the sight of the obtained results it can be concluded that, in addition to the overall parameters of characterisation, furosine is a very interesting indicator of the quality of pectins providing complementary information of the processing conditions. The study of the rheological characteristics underlined the usefulness of pectin gels as ingredients in the food industry, particularly in the case of those prepared with different methods in a magnetic stirrer or overhead stirrer together with the application of US, pointed out the since the obtained diversification of viscosity of pectin gels, could increase the range of potential applications as ingredient in the food industry. Coatings with better results for preservation of strawberries were those corresponding to sample 4400 prepared with overhead stirrer and US and sample 4710 prepared with magnetic stirrer and US, resulting in a better quality than control samples according to the humidity loss and
colour parameters. Although more research is needed, these results indicate the suitability of pectin gel as coating prepared with the assistance of US to improve the quality over the lifetime of strawberries.

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REFERENCES


Legends of the tables

Table 1. Results obtained for the physico-chemical overall characterisation of samples 4400 and 4710.

Table 2. Monomeric composition (mg g$^{-1}$ of pectin-based products) of samples after acid hydrolysis with TFA 2 M for 4 h and enzymatic hydrolysis with Viscozyme (90 U mL$^{-1}$, 50 °C, 24 h).

Table 3. Average viscosity at low and high-shear and thixotropy values of pectin-based samples 4400 and 4710 with the different treatments.

Table 4. Quality parameters of strawberries coated with samples pectin-based 4400 and 4710 during storage at 4 °C.

Legends of the figures

Figure 1. Chromatographic profile obtained by GC-FID of TMS oximes of monosaccharides of pectin-based samples (4400 in blue; 4710 in red), after hydrolysis with 2N TFA at 110 °C for 4 h. 1: Xylose 1; 2: Xylose 2 + Arabinose; 3 and 4: Rhamnose; 5: Galactose 1; 6: Mannose 1; 7: Glucose 1; 8: Galactose 2 + Mannose 2 + Glucose 2; 9 and 10: Galacturonic acid; 11: Internal Standard.

Figure 2. Chromatographic profile obtained by HPSEC-ELSD of pectin-based samples, (a) 4400 and (b) 4710.

Figure 3. DSC thermograms of pectin-based samples 4400 and 4710 using heating rates...
of 10 °C min⁻¹ from 40 to 300 °C.

Figure 4. Flow curves at different shear rate of pectin-based samples 4400 and 4710 prepared with overhead stirrer (OS, color) and overhead stirrer plus ultrasound at 30% (OS+US 30%-30, color) and 50% (OS+US 50%-50, color) of amplitude and heating magnetic stirrer (MS) and heating magnetic stirrer with ultrasound at 30% (MS+US 30%, color) and 50% (MS+US 50%-50, color) of amplitude (MS+US).

Figure 5. Viscosity curves at different shear rate of pectin-based samples 4400 and 4710 prepared with overhead stirrer (OS, color) and overhead stirrer plus ultrasound at 30% (OS+US 30%-30, color) and 50% (OS+US 50%-50, color) of amplitude and heating magnetic stirrer (MS) and heating magnetic stirrer with ultrasound at 30% (MS+US 30%-30, color) and 50% (MS+US 50%-50, color) of amplitude (MS+US).

Figure 6. Weight loss of strawberry samples without and with edible coating during storage at 4 °C after 3 and 5 days (standard deviation < 0.01) (US 30%).

Legends of the figures of supplementary material

Figure S1. Flowchart of the extraction process of both pectins (4400 and 4710) provided by CEAMSA.

Figure S2. Visual aspect of strawberries before (A) and after (B) coating with pectin 4400.

Figure S3. Color variation (ΔE) of strawberry samples without and with edible pectin coating during storage at 4 °C after 3 and 5 days.
Table 1. Results obtained for the physico-chemical overall characterization of samples 4400 and 4710.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample 4400</th>
<th>Sample 4710</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity (%)</td>
<td>9.8 ± 0.6</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>(a_w)</td>
<td>0.22</td>
<td>0.20</td>
</tr>
<tr>
<td>pH*</td>
<td>3.04 ± 0.01</td>
<td>3.07 ± 0.01</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>0.67 ± 0.03</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>Minerals (mg g(^{-1})):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>2.36</td>
<td>1.91</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.32</td>
<td>0.16</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.60</td>
<td>0.33</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.15</td>
<td>1.14</td>
</tr>
<tr>
<td>Furosine ((\text{mg 100 g}^{-1} \text{ protein}))</td>
<td>782.1 ± 5.2</td>
<td>350.4 ± 6.3</td>
</tr>
<tr>
<td>(M_w) (kDa)</td>
<td>472</td>
<td>553</td>
</tr>
<tr>
<td>DEM (%)</td>
<td>70.7</td>
<td>64.4</td>
</tr>
</tbody>
</table>

* Pectin-based solution 1% (\(\text{pm/v}\))
<table>
<thead>
<tr>
<th>Samples</th>
<th>Xyl+Ara</th>
<th>Rha</th>
<th>Gal</th>
<th>Man</th>
<th>Glu</th>
<th>GalA</th>
<th>Total</th>
<th>GalA/Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid 4400</td>
<td>30.1 ± 6.1</td>
<td>20.0 ± 0.4</td>
<td>54.7 ± 3.6</td>
<td>1.7 ± 0.1</td>
<td>5.7 ± 0.2</td>
<td>168.4 ± 51.1</td>
<td>280.6 ± 8.4</td>
<td></td>
</tr>
<tr>
<td>Acid 4710</td>
<td>55.3 ± 0.7</td>
<td>12.4 ± 0.4</td>
<td>21.5 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>244.6 ± 1.1</td>
<td>194.6 ± 1.7</td>
<td>529.9 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>Enzymatic 4400</td>
<td>6.8 ± 0.9</td>
<td>21.9 ± 0.6</td>
<td>68.7 ± 1.3</td>
<td>57.7 ± 13.7</td>
<td>6.2 ± 5.4</td>
<td>610.8 ± 57.7</td>
<td>772.1 ± 27.9</td>
<td></td>
</tr>
<tr>
<td>Enzymatic 4710</td>
<td>29.6 ± 0.4</td>
<td>15.0 ± 1.1</td>
<td>64.0 ± 20.0</td>
<td>250.7 ± 1.3</td>
<td>495.7 ± 37.1</td>
<td>868.9 ± 33.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Average viscosity at low and high-shear and thixotropy values of pectin-based samples 4400 and 4710 with the different treatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>Viscosity (Pa.s)</th>
<th>Thixotropy (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low-shear (at 10 s(^{-1}))</td>
<td>High-shear (at 1000 s(^{-1}))</td>
</tr>
<tr>
<td>4400</td>
<td>OS</td>
<td>0.169</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>OS30</td>
<td>0.186</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>OS50</td>
<td>5.43</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>OS50</td>
<td>0.303</td>
<td>0.111</td>
</tr>
<tr>
<td>4710</td>
<td>OS30</td>
<td>0.095</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>OS50</td>
<td>1.86</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>OS50</td>
<td>5.55</td>
<td>0.207</td>
</tr>
<tr>
<td>4400</td>
<td>MS</td>
<td>0.161</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>MS30</td>
<td>0.101</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>MS50</td>
<td>6.10</td>
<td>0.116</td>
</tr>
<tr>
<td>4710</td>
<td>MS30</td>
<td>0.111</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>MS50</td>
<td>6.10</td>
<td>0.116</td>
</tr>
</tbody>
</table>

*Treatments: MS = magnetic stirrer; MSUS = magnetic stirrer + US (30%); OS = overhead stirrer; OSUS = overhead stirrer + US (30%)
Table 4. Quality parameters of strawberries coated with samples pectin-based 4400 and 4710 during storage at 4 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>C*</th>
<th>h°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Day 0</td>
<td>37.3 ± 0.5 a</td>
<td>37.9 ± 1.5 a</td>
<td>20.8 ± 2.5 a</td>
<td>43.2 ± 2.2 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>30.1 ± 1.9 b</td>
<td>33.8 ± 0.8 ab</td>
<td>16.7 ± 0.5 a</td>
<td>37.7 ± 0.5 ab</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>31.6 ± 2.1 b</td>
<td>30.6 ± 2.6 b</td>
<td>15.2 ± 4.0 a</td>
<td>34.2 ± 4.2 b</td>
</tr>
<tr>
<td>4400 MS</td>
<td>Day 0</td>
<td>37.8 ± 1.0 a</td>
<td>35.2 ± 1.7 a</td>
<td>21.7 ± 1.6 a</td>
<td>41.4 ± 2.0 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>32.6 ± 0.1 b</td>
<td>34.1 ± 3.0 a</td>
<td>18.6 ± 2.5 a</td>
<td>38.9 ± 3.8 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>31.8 ± 0.9 b</td>
<td>33.7 ± 1.5 a</td>
<td>17.3 ± 1.8 a</td>
<td>34.2 ± 2.2 a</td>
</tr>
<tr>
<td>4400 MSUS</td>
<td>Day 0</td>
<td>33.1 ± 0.8 a</td>
<td>30.3 ± 4.4 a</td>
<td>13.3 ± 3.6 a</td>
<td>33.1 ± 5.5 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>29.3 ± 0.1 b</td>
<td>32.1 ± 2.8 a</td>
<td>14.1 ± 2.2 a</td>
<td>35.0 ± 3.5 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>29.3 ± 0.6 b</td>
<td>31.7 ± 3.3 a</td>
<td>14.5 ± 1.8 a</td>
<td>34.8 ± 3.8 a</td>
</tr>
<tr>
<td>4400 OS</td>
<td>Day 0</td>
<td>36.7 ± 1.4 a</td>
<td>36.1 ± 3.2 a</td>
<td>20.3 ± 1.8 a</td>
<td>41.4 ± 3.3 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>32.4 ± 2.1 b</td>
<td>35.1 ± 3.7 a</td>
<td>19.5 ± 3.0 a</td>
<td>40.2 ± 4.4 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>31.6 ± 1.5 b</td>
<td>30.0 ± 3.6 a</td>
<td>15.4 ± 3.1 a</td>
<td>33.8 ± 4.5 a</td>
</tr>
<tr>
<td>4400 OSUS</td>
<td>Day 0</td>
<td>37.4 ± 0.6 a</td>
<td>33.4 ± 2.3 a</td>
<td>19.6 ± 3.4 a</td>
<td>38.8 ± 3.7 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>31.4 ± 0.9 b</td>
<td>33.8 ± 0.9 a</td>
<td>19.7 ± 0.3 a</td>
<td>39.1 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>32.6 ± 1.2 b</td>
<td>32.5 ± 1.8 a</td>
<td>17.4 ± 2.3 a</td>
<td>36.9 ± 2.7 a</td>
</tr>
<tr>
<td>4710 MS</td>
<td>Day 0</td>
<td>36.1 ± 0.4 a</td>
<td>32.5 ± 2.4 a</td>
<td>16.3 ± 3.9 a</td>
<td>36.4 ± 3.6 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>33.5 ± 2.9 a</td>
<td>34.4 ± 3.1 a</td>
<td>19.0 ± 4.9 a</td>
<td>39.4 ± 4.9 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>34.6 ± 0.4 a</td>
<td>36.6 ± 0.8 a</td>
<td>20.0 ± 6.0 a</td>
<td>41.9 ± 3.4 a</td>
</tr>
<tr>
<td>4710 MSUS</td>
<td>Day 0</td>
<td>36.3 ± 1.5 a</td>
<td>32.6 ± 0.2 a</td>
<td>19.1 ± 2.6 a</td>
<td>37.8 ± 1.4 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>33.2 ± 1.1 a</td>
<td>34.9 ± 2.5 a</td>
<td>20.2 ± 4.9 a</td>
<td>40.4 ± 4.6 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>32.0 ± 3.7 a</td>
<td>32.5 ± 1.2 a</td>
<td>18.2 ± 3.8 a</td>
<td>37.4 ± 2.1 a</td>
</tr>
<tr>
<td>4710 OS</td>
<td>Day 0</td>
<td>34.0 ± 0.6 a</td>
<td>30.4 ± 1.8 a</td>
<td>13.7 ± 1.3 a</td>
<td>33.4 ± 2.2 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>29.1 ± 0.5 b</td>
<td>31.0 ± 0.9 a</td>
<td>14.6 ± 1.5 a</td>
<td>34.3 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>28.3 ± 0.7 b</td>
<td>31.0 ± 3.8 a</td>
<td>14.5 ± 2.2 a</td>
<td>34.2 ± 4.3 a</td>
</tr>
<tr>
<td>4710 OSUS</td>
<td>Day 0</td>
<td>35.8 ± 1.9 a</td>
<td>33.6 ± 2.1 a</td>
<td>16.9 ± 2.5 a</td>
<td>37.6 ± 3.0 a</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>29.5 ± 2.3 b</td>
<td>32.0 ± 3.1 a</td>
<td>15.3 ± 3.4 a</td>
<td>35.5 ± 4.2 a</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>27.7 ± 2.6 b</td>
<td>30.9 ± 4.7 a</td>
<td>14.4 ± 4.1 a</td>
<td>34.1 ± 6.0 a</td>
</tr>
</tbody>
</table>

For each parameter (column) different letters correspond at significant differences (P<0.05)
Figure 1. Chromatographic profile obtained by GC-FID of TMS oxymes of monosaccharides of pectin-based samples (4400 in blue; 4710 in red), after hydrolysis with 2N TFA at 110 °C for 4 h. 1: Xylose 1; 2: Xylose 2 + Arabinose; 3 and 4: Rhamnose; 5: Galactose 1; 6: Mannose 1; 7: Glucose 1; 8: Galactose 2 + Mannose 2 + Glucose 2; 9 and 10: Galacturonic acid; 11: Internal Standard.
Figure 2. Chromatographic profile obtained by HPSEC-ELSD of pectin-based samples, (A) 4400 and (B) 4710.
Figure 3. DSC thermograms of pectin-based samples 4400 and 4710 using heating rates of 10 °C min\(^{-1}\) from 40 to 300 °C.
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Figure 6. Weight loss of strawberry samples without and with edible coating during storage at 4 °C after 3 and 5 days (standard deviation < 0.01) (US 30%).