Pectin extraction from quince (Cydonia oblonga) pomace applying alternative methods: Effect of process variables and preliminary optimization

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Article

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Abstract

The objectives of this study were to introduce alternative methods in the process of pectin extraction from quince pomace, to determine the effect of selected process variables (factors) on the obtained pectin, and to perform a preliminary optimization of the process. A fractional factorial experimental design was applied, where the factors considered were six: quince pomace pretreatment (washing vs blanching), drying method (hot air vs LPSSD), acid extraction condictions (pH, temperature, and time), and pectin extract concentration method (vacuum evaporation vs ultrafiltration). The effects of these factors and their interactions on pectin yield (Y: 0.2–34.2 mg/g), GalA content (44.5–76.2%), and DM (47.5–90.9%), were determined. For these three responses, extraction pH was the main effect, but it was involved in two and three factors interactions. Regarding alternative methods, LPSSD was required for maximum Y and GalA, and ultrafiltration for maximum GalA and DM. Response models were used to predict optimum process conditions (quince blanching, pomace drying by LPSSD, acid extraction at pH 2.20, 80 °C, 3 h, and concentration under vacuum) to simultaneously maximize Y (25.2 mg/g), GalA (66.3%), and DM (66.4%).

Keywords

Extraction systems, pectins, fruits, cell wall polysaccharides

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INTRODUCTION

Pectin is a polysaccharide present in the cell wall of all higher plants, and has been used as a gelling agent since the past two centuries (Oakenfull and Scott, 1984). The most important market application of pectin is in fruit jams, jellies, and marmalades, but it is also used in the pharmaceutical, dental, and cosmetic industries for its gelling, thickening, and stabilizing properties (May, 1990). Pectins are biopolymers consisting almost entirely of linearly connected α(1–4)D-galacturonic acid (GalA) units, occasionally interrupted by 1–2 linked rhamnose residues. Some of the carboxyl groups of the GalA residues are esterified with methanol (methyl-esterified). The degree of methyl esterification (also called degree of esterification or degree of methylation, DM) is the percentage of methyl-esterified GalA residues, and is a primary factor influencing the conditions and mechanism for gelling (Savary and Núñez, 2003). Pectins with DM higher than 50%, named high methoxyl pectins (HMPs), form gels after heating in sugar solutions at concentrations higher than 55% and pH values lower than 3.5. On the other hand, formation of gels with low methoxyl pectins (LMPs; DM < 50%) only requires the presence of calcium, extending the use of this gelling agent to a broader range of foods. Although LMP

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occurs naturally in some plants (Iglesias Cristobal and Lozano, 2004; Yapo and Koffi, 2006), they are usually manufactured by chemical or enzymatic treatment of HMP.

The main raw materials used to produce commercial pectins are apple pomace and citrus peels (May, 1990). Papers on the structure of citrus and apples and the quality of pectin extracted from these fruits are numerous (Constenla et al., 2002; Crandall et al., 1978; Fishman et al., 2006; Garna et al., 2007; Kurita et al., 2008; Masmoudi et al., 2008). However, there are other good sources of pectin, as sugar beet pulp (Levigne et al., 2002a; Michel et al., 1985; Phatak et al., 1988; Yapo et al., 2007), banana (Happi Emaga et al., 2008), peach (Pagán et al., 2001), mango (Koubala et al., 2008),chicory (Robert et al., 2006), cabbage (Westereng et al., 2008), sunflower (Iglesias Cristobal and Lozano, 2004), yellow passion fruit (Yapo and Koffi, 2006), and quince (Forni et al., 1994; Thomas and Thibault, 2002; Thomas et al., 2003). Quince is the fruit of a deciduous tree of *Rosaceae* family, *Cydonia oblonga Miller* (Silva et al., 2005, 2006; Sousa et al., 2007).

Pectin is present in the cell wall of plants in the form of protopectin, which is insoluble in cold water and is accompanied by cellulose, hemicelluloses, and smaller proportions of many other constituents (Wang et al., 2002). Pectin extraction is a multiple-stage physicochemical process in which the hydrolysis and extraction of pectin macromolecules from plant tissue (protopectins) and their solubilization take place under the influence of different factors, mainly temperature, pH, and time. Acid extraction is performed with dilute solutions of acids (Levigne et al., 2002a; Masmoudi et al., 2008; Michel et al., 1985), such as hydrochloric acid (Fishman et al., 2006; Iglesias Cristobal and Lozano, 2004; Koubala et al., 2008; Phatak et al., 1988; Wang et al., 2007), nitric acid (Constenla et al., 2002; Pagán et al., 2001; Yapo and Koffi, 2006), and sulfuric acid (Garna et al., 2007; Happi Emaga et al., 2008; Robert et al., 2006; Yapo et al., 2007). The cooked pomace is pressed, and the liquid is clarified and filtered. The extract obtained is frequently concentrated in vacuum evaporators; the extent of concentration is in the range of 4 : 1 to 5 : 1. Pectin may be precipitated from solution by the addition of salt, organic solvents (such as ethanol), or polyvalent ions (Kertesz, 1951). Concentration of the extract by a non-thermal method like ultrafiltration is an interesting and original alternative to vacuum evaporation, which might reduce pectin deterioration during this part of the process, as will be evaluated in this study.

A literature review of pectin acid extraction conditions showed the following ranges: solid/liquid ratio 1 : 20–1 : 80 w/v (g dry pomace/mL acid solution), pH 1.0–3.0, temperature 60–95 °C, and time 10–240 min (Constenla et al., 2002; Garna et al., 2007; Happi Emaga et al., 2008; Koubala et al., 2008; Levigne et al., 2002a; Masmoudi et al., 2008; Michel et al., 1985; Pagán et al., 2001; Robert et al., 2006; Yapo et al., 2007). Many studies have shown that the biochemical characteristics of pectins depend on the plant species, variety and maturity, and the extraction process (Koubala et al., 2008; Masmoudi et al., 2008).

The use of dry pomace for the manufacture of pectin offers several advantages over the use of fresh pomace. When dry pomace is used, pectin production is not limited to the period when fruits are available and may be carried on at any time of the year. Drying allows storage of pomace without spoilage. Furthermore, heating of pomace during drying causes some desirable changes in its constituents, making screening, and filtering operations easier (Kertesz, 1951). Most works used dried pomace or dried peels for pectin extraction (Happi Emaga et al., 2008; Koubala et al., 2008; Kurita et al., 2008; Masmoudi et al., 2008; Michel et al., 1985; Pagán et al., 2001; Phatak et al., 1988; Robert et al., 2006; Wang et al., 2007; Yapo et al., 2007). Temperature during apple pomace dehydration affected the degree of esterification and the degree of polymerization of extracted pectin (Constenla et al., 2002). In the past years, low-pressure superheated steam drying (LPSSD) has been applied successfully to many food products, providing less quality deterioration compared to hot air drying (HAD) due to reduction in oxygen content and steam blanching of the product (Elustondo et al., 2001; Leeratanarak et al., 2006; Nimmol et al., 2007). Consequently, drying of the pomace by LPSSD is an attractive alternative to the traditional HAD, which will be analyzed in this study.

Then, the first objective of this study was to obtain pectin from a non-traditionally commercial source like quince pomace, introducing alternative methods in some of the steps of the extraction process, namely: LPSSD drying of the pomace, and concentration of the pectin extract by ultrafiltration. The hypothesis is that LPSSD and ultrafiltration may improve pectin quality, compared to HAD and vacuum concentration, respectively. The second objective was to determine the effect of selected process variables (quince pretreatment; pomace drying method; pH, temperature, and time during acid extraction; and pectin extract concentration system) on pectin yield, purity (measured as GalA content, and DM). The third objective was to perform a preliminary optimization of the extraction process in order to meet the following goals, either individual or combined: (a) maximize pectin yield, (b) maximize GalA, and (c) achieve target values of DM.

MATERIALS AND METHODS

Material

Quince fruits (cv. *Cydonia oblonga*) were purchased in a local supermarket. The fruits were washed, and milled with a lab cutter (Mervisa Model 3, Brasil). Juice was extracted by pressing the pulp in a hydraulic rack and cloth press (5 ton) through 80–100 mesh filter (Bucher-Guyer Ag; Germany). Juice was discarded, and the pomace obtained was washed both in cold (ambient temperature) and hot (70 °C) water for 10 min, to reduce sugar content. The second treatment (with hot water) also had a blanching effect, inhibiting enzymatic activity. The blanched samples were cooled down in cold water. Excess liquid was removed by a second pressing, and 0.6 kg pomace batches were individually dried both in a conventional rotary HAD, and in a LPSSD (Figure 1). The ability of superheated steam to dry materials is due to the addition of sensible heat to raise its temperature above the corresponding saturation temperature at a given pressure. The moisture evaporated from the product becomes part of the drying medium, and the excess steam is released when the pressure increases beyond the set point (Pronyk et al., 2004). In both dryers, the speed of the rotary basket was set at 45 r/min. In the HAD dryer, air velocity was 1.0 m/s, and outlet air temperature was 60 °C. In the LPSSD dryer, gas temperature was set at 60 °C and total pressure was reduced to 100 mBar (saturation temperature 45 °C), such that vapor in the gas phase was superheated. Initial pomace moisture content (X_o) was approximately 82% w.b. (wet basis). A typical experimental drying cycle took 4–6 h to reach a final to initial water content ratio (X/X_o) < 0.07, where X is the water content.

Pectin extraction

Pectin acid extraction was performed by immersion of the dried quince pomace in a HNO_3 solution (85 g/L). Extraction was performed in a Büchi Rotovapor Model R-151 (Büchi Co., Switzerland), at two levels of pH (1.5 and 2.5), temperature (70 °C and 80 °C), and extraction time (1 and 3 h). Acid extraction conditions were selected within the range of previous works (Constenla et al., 2002; Garna et al., 2007; Happi Emaga et al., 2008; Koubala et al., 2008; Levigne et al., 2002a; Masmoudi et al., 2008; Michel et al., 1985; Pagán et al., 2001; Robert et al., 2006; Yapo et al., 2007). The hot acid extract was filtered through diatomaceous earth on Whatman N°4 paper. The filtrate (~2.5 l of pectin dispersion) was concentrated to a final volume of 0.5 l, by two different methods: (a) vacuum evaporation at 70 °C in a rotovapor (Büchi Co., Switzerland) and (b) ultrafiltration. The ultra/nano-filtration system consisted of a Prep/Scale Spiral Wound TFF-1, Module PTHK, 100 kDa cut-off, 0.1 m^2 membrane (Millipore; Billerica, MA), a hydraulic press to hold the cells tightly joined avoiding leaks, and peristaltic pump, pressure gage, and tubing for driving of retentate and permeate streams. In this process, concentration of the pectin extract (retentate stream) was achieved by removing water through the membrane (permeate stream).

Pectin was precipitated from the concentrated dispersion by addition of 0.5 l of 95% v/v ethanol at

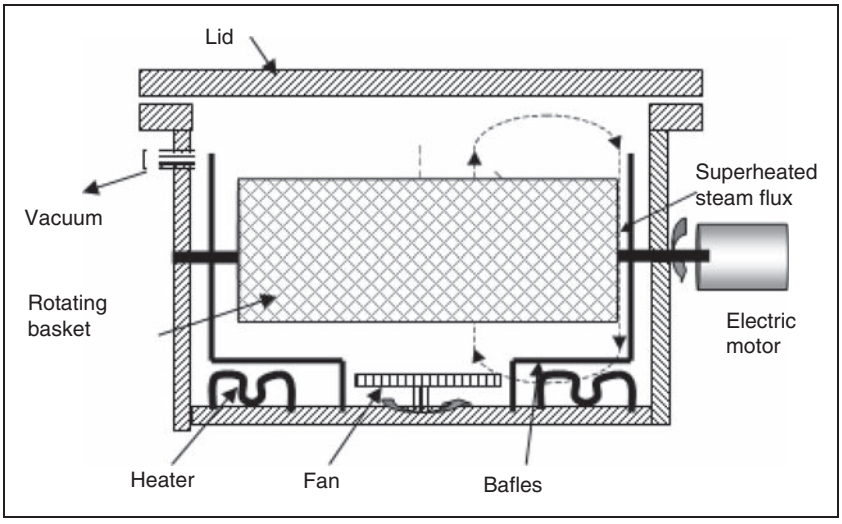

Figure 1. Schematic representation of the LPSSD.
LPSSD: low-pressure superheated steam dryer.

room temperature, and allowed to settle overnight at 4 °C in order to achieve a good separation. Coagulated pectin was washed three times with ethanol 75% v/v to remove mono and disaccharides. Washed pectin was pressed twice (the first time manually, the second time with a lab-scale pneumatic press) to remove excess ethanol through a filter cloth. Afterward the pectin floc was freeze-dried, and finally it was grinded to a particulate powder. Pectin yield was calculated as Y = mg pectin/g pomace (dry weight basis).

Determination of the GalA content (GalA%)

The GalA content (%) was determined photometrically from a standard curve of GalA according to the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). It is a simple and indirect method based upon the appearance of a chromogen when uronic acid heated to 100 °C in concentrated sulfuric acid/tetraborate is treated with *m*-hydroxydiphenyl.

The reagent was a 0.15% w/v solution of *m*-hydroxydiphenyl in 0.5% w/v NaOH. The 6 mL of sulfuric acid was added to 1 mL of the sample containing 140 µg uronic acids. The tubes were refrigerated in crushed ice. The mixture was shaken in a vortex mixer and the tubes heated in a water bath at 100 °C for 5 min. After cooling in a water–ice bath, 50 µL of *m*-hydroxydiphenyl reagent was added. The tubes were shaken and, after 5 min, absorbance measurements made at 520 nm in a UV/VIS Lambda 3 spectrophotometer (Perkin Elmer, Massachusetts, USA). As carbohydrates produce a pinkish chromogen with sulfuric acid at 100 °C, a blank sample was run without addition of the reagent, which was replaced by 20 µL of 0.5% NaOH. The absorbance of the blank sample was subtracted from the total absorbance. The calibration curve obtained was $Y = 1.12*10^{-2}*X$ ($R^2 = 0.994$), where Y is the absorbance at 520 nm, and X the GalA concentration of the solution (µg GalA/mL).

Determination of the DM

DM was determined by gas chromatography (Lee et al., 1975; Levigne et al., 2002b). A stock solution of pectin was prepared at a concentration of 8.33 mg/mL in distilled water. Pectin methyl esters were hydrolyzed as follows: 1 mL of NaOH 1 N was added to 12 mL of pectin solution. This solution was incubated during 30 min at room temperature, and finally diluted with distilled water to give 20 g of solution. Aliquots of the hydrolyzed pectin were analyzed for methanol content. Gas chromatography was run on a Varian 3700 (Varian Inc, Palo Alto, CA), with a capillary column BP-20 0.25 mm × 30 m × 0.25 µm (SGE Analytical Sci., Austin, TX). The following conditions were used: H_2 as carrier gas with a flow rate of 20 mL/min; column temperature was set at 80 °C for 4 min, and then programmed to 130 °C at rate of 30 °C/min; the frame ionization detector was set at 220 °C.

Methanol/water solutions were prepared to obtain the standard curve, $Y = 1.57*10^{8}*X$ ($R^2 = 0.995$), where Y is the peak area, and X the methanol concentration of the solution (g MeOH/100 g sol). DM was calculated as the ratio of milliequivalents of esterified carboxylic groups (ECG) to total carboxilic groups (TCG), per gram of pectin

$$DM\% = \frac{meq_{ECG}/g_P}{meq_{TCG}/g_P} \times 100 \quad (1)$$

where $meq_{TCG}/g_P = meq_{GalA}/g_P$, 1 $meq_{GalA} = 194.139\ mg_{GalA}$, and g_{GalA}/g_P was determined in the previous section as GalA%, then

$$\frac{meq_{TCG}}{g_P} = \frac{GalA\%/100}{194.139/1000} \quad (2)$$

On the other hand, $meq_{ECG}/g_P = meq_{MetOH}/g_P$, 1 $meq_{MetOH} = 32\ mg_{MetOH}$, and mg_{MetOH}/g_P was determined by gas cromatography as MetOH%, then

$$\frac{meq_{ECG}}{g_P} = \frac{MetOH\%/100}{32/1000} \quad (3)$$

Combining equations (1) to (3), DM (%) may be calculated as the molar ratio of methanol to GalA

$$DM\% = \frac{MetOH\%/32}{GalA\%/194.139} \times 100 \quad (4)$$

Experimental design

In order to explore the effect of selected variables of the extraction process (factors) on pectin yield (Y), GalA%, and DM% (responses), a fractional factorial two-level experimental design was employed. This type of design is intended to screen the vital few from the many trivial factors; it saves on runs but it produces aliases. There are not enough runs to independently estimate all possible effects, but some effects will be aliased or confounded (which means that the calculated aliased effect is actually the combination of two or more true effects). However, if the design resolution is V or higher, the design is just about as good as a full factorial (it will estimate a minimum of all main effects and two-factor interactions), with great savings in the number of experiments to perform (Montgomery, 2005). This is particularly useful when the number of factors is high and the experiments are time-consuming, as in this study.

Table 1. Independent variables of the process (factors) and their corresponding levels

Independent variable		−1	+1
Pretreatment	A	Blanching	Washing
Drying method	B	HAD	LPSSD
pH	C	1.5	2.5
Temperature	D	70 °C	80 °C
Time	E	1 h	3 h
Concentration system	F	Vacuum	Ultrafiltration

HAD: hot air drying; LPSSD: low-pressure superheated steam dryer.

In this study, six factors ($k = 6$) were used in the design, at two levels each (Table 1). Three of these factors were numeric, corresponding to the acid extraction conditions: pH (C), temperature (D), and time (E), while the other three were categoric factors, namely: pretreatment (A), drying method (B), and concentration method (F). Then, the fractional factorial design consisted of $2^{k-1} = 32$ experimental runs, and had a resolution VI. Table 2 presents the coded levels of the factors, for each run. The data were processed using the Design-Expert 7.0 software. This program optimized the aliasing pattern using an identity generator $I = ABCDEF$, such that the main effects and the two-factor interactions were aliased with nothing, and the

Table 2. Fractional factorial experimental design {categorical variables}, and the results obtained for pectin yield (Y), GalA, and DM (values in parenthesis are SDs)

Factors responses								
A	B	C	D	E	F	Y (mg/g)	%GalA	%DM
{1}	{−1}	−1	−1	−1	{1}	5.96 (0.02)	73.87 (3.82)	55.00 (4.49)
{−1}	{1}	1	1	−1	{1}	0.58 (0.07)	70.21 (5.80)	67.35 (5.33)
{−1}	{1}	1	1	1	{−1}	20.0 (2.96)	61.42 (1.83)	72.10 (5.26)
{1}	{−1}	1	1	1	{−1}	17.2 (2.58)	67.58 (4.87)	66.06 (8.29)
{−1}	{−1}	−1	1	−1	{1}	14.4 (1.63)	60.31 (6.29)	64.71 (7.36)
{1}	{1}	1	1	1	{1}	12.7 (1.31)	76.24 (4.55)	55.35 (3.91)
{−1}	{−1}	−1	1	1	{−1}	31.1 (0.04)	73.10 (2.99)	50.59 (4.20)
{−1}	{−1}	1	1	1	{1}	3.58 (0.26)	68.44 (1.49)	70.23 (5.96)
{1}	{−1}	−1	1	−1	{−1}	10.4 (1.01)	64.41 (2.02)	56.97 (2.25)
{1}	{1}	−1	1	−1	{1}	9.12 (1.18)	71.26 (0.75)	59.01 (2.43)
{−1}	{1}	1	−1	1	{1}	4.94 (0.64)	69.48 (2.37)	58.36 (5.58)
{−1}	{−1}	−1	−1	−1	{−1}	13.0 (1.28)	65.60 (1.59)	52.76 (3.90)
{−1}	{1}	−1	1	−1	{−1}	28.4 (3.76)	68.28 (0.27)	54.68 (3.08)
{−1}	{1}	−1	−1	1	{−1}	32.1 (1.70)	67.36 (3.23)	47.51 (3.90)
{−1}	{−1}	1	1	−1	{−1}	2.70 (0.34)	63.86 (2.75)	55.36 (1.29)
{1}	{1}	−1	−1	1	{1}	16.7 (2.59)	67.42 (4.63)	57.64 (0.45)
{1}	{1}	1	1	−1	{−1}	3.69 (0.54)	69.46 (0.60)	59.26 (1.04)
{1}	{−1}	−1	1	1	{1}	14.8 (0.73)	58.51 (1.38)	58.79 (12.17)
{−1}	{−1}	1	−1	1	{−1}	6.06 (0.55)	65.83 (5.39)	65.37 (0.54)
{1}	{−1}	1	−1	1	{1}	3.40 (0.20)	57.82 (6.61)	58.42 (6.92)
{1}	{−1}	1	−1	−1	{−1}	1.77 (0.27)	61.83 (4.56)	48.54 (3.67)
{1}	{1}	1	−1	−1	{1}	0.19 (0.03)	44.50 (0.69)	53.37 (0.34)
{1}	{1}	−1	−1	−1	{−1}	11.6 (0.17)	60.51 (0.31)	59.55 (0.87)
{1}	{−1}	1	1	−1	{1}	1.55 (0.14)	49.99 (5.19)	90.89 (6.51)
{1}	{1}	1	−1	1	{−1}	4.84 (0.12)	51.11 (5.39)	72.36 (3.50)
{−1}	{1}	1	−1	−1	{−1}	1.07 (0.01)	54.16 (0.65)	65.45 (3.16)
{1}	{−1}	−1	−1	1	{−1}	14.0 (1.98)	67.24 (2.88)	52.92 (2.13)
{−1}	{1}	−1	−1	−1	{1}	11.4 (0.87)	66.68 (6.25)	63.33 (0.70)
{−1}	{1}	−1	1	1	{1}	24.7 (1.78)	74.43 (1.96)	57.52 (2.54)
{−1}	{−1}	1	−1	−1	{1}	0.16 (0.03)	47.59 (1.39)	56.74 (4.88)
{−1}	{−1}	−1	−1	1	{1}	20.15 (2.81)	69.06 (5.95)	49.62 (6.09)
{1}	{1}	−1	1	1	{−1}	34.21 (5.31)	60.58 (3.73)	60.97 (10.22)

GalA: galacturonic acid; DM: degree of methylation.

three-factor interactions were aliased among them. Each run was done in duplicate. The reason to duplicate each run instead of performing a full factorial design was the need to obtain enough amount of pectin from each experiment for complete pectin characterization, including future analysis of pectin gels.

This type of design provides a mathematical model to predict each response in terms of the selected factors. The model should consist of the effects (factors and interactions) that are significant, plus any terms that are needed to maintain hierarchy (Montgomery, 2005). For each response, significant effects for the model and their relative magnitudes were obtained from a Pareto chart (not shown). These models were used to search and find in the design space, factor settings that meet defined goals for individual or combined responses. These values can only be used as a first step in the optimization process, because two-level designs cannot fit curved surfaces. Additional experimental (center and axial) points are required to reach the final optimum value. Meanwhile, a preliminary optimization was performed using the models obtained with the current design. The values obtained are a first approximation to the optimum pectin extraction conditions required to obtain a specific product, maximizing yield and purity.

RESULTS AND DISCUSSION

Results of pectin yield (Y mg/g), GalA content (GalA%), and DM% obtained for each run are presented in Table 2, as average ± SD.

Pectin yield

Yield (Y) ranged from 0.16 ± 0.03 to 34.21 ± 5.31 [mg pectin/g dry pomace]. Maximum experimental yield was obtained at the following conditions: quince washing, pomace drying by LPSSD, extraction at pH 1.5, 80 °C, during 3 h, and concentration under vacuum. Statistical analysis of the data indicated that a transformation was required for normality and homocedasticity. The transformation recommended by the Box-Cox test, square root, was successfully applied to the data. Selected effects for the model to predict pectin yield (Y) are presented in Table 3. The analysis of variance (ANOVA) indicated that the model was significant, lack of fit was non-significant, and the following effects significantly affected pectin yield(in decreasing order of importance): pH (C) > time (E) > concentration method (F) > temperature (D) > AC > pomace drying method (B) > quince pretreatment (A) > DF > BCF > CE > ABC > BE > BC > CDE > CD > ACD > DE > AF > BD > AD. Some non-significant effects ($p > 0.05$) were included in the model to maintain its hierarchy (Table 3). Three-factor interactions rarely occur in factorials with only numeric factors, but are more likely to be significant when the experimental design contains categoric factors, as in this study. It should be remembered that in this design the three-factor interactions were aliased among them. Final model equation in terms of coded factors was ($R^2 = 0.9887$, adj $R^2 = 0.9821$, and pred $R^2 = 0.9710$)

$$\begin{aligned}\sqrt{Y} = {} & 3.06 - 0.17 * A + 0.21 * B - 1.09 * C \\ & + 0.39 * D + 0.75 * E - 0.41 * F - 0.047 * AB \\ & + 0.28 * AC + 0.067 * AD + 0.080 * AF \\ & - 0.10 * BC + 0.075 * BD + 0.11 * BE \\ & - 0.052 * BF + 0.091 * CD + 0.12 * CE \\ & - 0.014 * CF + 0.084 * DE - 0.15 * DF \\ & - 0.12 * ABC + 0.085 * ACD + 0.15 * BCF \\ & + 0.10 * CDE \end{aligned} \quad (5)$$

Figure 2(a) and (b) shows the response surface plot for the predicted effect of acid extraction conditions (pH, temperature, and time) on pectin yield, setting the categoric factors at favorable levels. The maximum yield predicted by the model (45.9 mg/g) was a bit higher than the maximum experimental value (34.2 mg/g), and was obtained at the same process conditions, except pretreatment: quince blanching, pomace drying by LPSSD, extraction at 80 °C, pH 1.5, during 3 h, and vacuum concentration (Table 6). According to the predictive models obtained in the following sections (equations (6) and (7)), pectins obtained under these conditions are expected to have a good purity (GalA = 74.7%) but a low DM = 48.8%.

It is worth noting that one of the process conditions required to obtain the maximum yield was drying by LPSSD. This may be explained by the fact that LPSSD is expected to render a very porous material (Elustondo et al., 2001), which probably benefited the subsequent acid extraction process. It should also be noted that pectin yield was higher when the pectin extract was concentrated by vacuum concentration, compared to ultrafiltration. This was attributed to the fact that some fraction of the low molecular weight pectin chains (<100 kDa) were not retained by the ultrafiltration membrane, resulting in a pectin loss through the permeate stream.

It can be observed (Figure 2(a) and (b), equation (5)) that pectin yield increased at decreasing pH, increasing temperature, and increasing time during acid extraction, provided the other process variables were fixed at favorable conditions. In other words, yield increased at increasing severity of the acid extraction, within the experimental range studied in this study. This increase

Table 3. Pectin yield (Y) ANOVA

	Sum of square	DF	Mean square	Valor *F*	*p* Valor
Model	151.97	23	6.61	151.60	<0.0001
A−pretratment	1.82	1	1.82	41.66	<0.0001
B−drying method	2.72	1	2.72	62.49	<0.0001
C−pH	75.94	1	75.94	1742.37	<0.0001
D−temperature	9.78	1	9.78	224.34	<0.0001
E−time	36.12	1	36.12	828.65	<0.0001
F−concentration method	10.91	1	10.91	250.34	<0.0001
AB	0.10	1	0.10	2.42	0.1271
AC	5.06	1	5.06	116.04	<0.0001
AD	0.28	1	0.28	6.51	0.0146
AF	0.41	1	0.41	9.42	0.0038
BC	0.68	1	0.68	15.65	0.0003
BD	0.36	1	0.36	8.23	0.0066
BE	0.73	1	0.73	16.79	0.0002
BF	0.17	1	0.17	3.92	0.0546
CD	0.52	1	0.52	12.06	0.0013
CE	0.99	1	0.99	22.91	<0.0001
CF	0.012	1	0.012	0.27	0.6052
DE	0.46	1	0.46	10.48	0.0024
DF	1.50	1	1.50	33.89	<0.0001
ACD	0.46	1	0.46	10.51	0.0024
CDE	0.65	1	0.65	14.95	0.0004
ABC	0.87	1	0.87	20.1	<0.0001
BCF	1.43	1	1.43	32.73	<0.0001
Residual	1.74	40	0.04		
Lack of fit	0.59	8	0.074	2.05	0.0717
Pure error	1.15	32	0.04		
Cor total	153.71	63			

ANOVA: analysis of variance.
p-Value < 0.05 indicates that the model and its terms are significant.

in the extraction rate at increasing temperatures has been attributed to the increase of both the solubility of the extracted pectin and the diffusion coefficient. Regarding the effect of pH, it has been reported that acidic conditions contribute to hydrolize the insoluble pectic constituents into soluble pectin, which increases the pectin recovery (Masmoudi et al., 2008).

However, one should be careful when trying to extrapolate these results to more severe conditions, because higher pectin yields might be obtained at the expense of lower pectin quality. As it is known, the basic principle of acidic extraction is the hydrolysis of protopectin. If the hydrolysis is excesive, some of the recovered pectin is degraded into low molecular weight components, which are undesirable. Thus, the success of the extraction process relies on achieving maximun solubilization and dissolution of the pectin, without further degradation.

The results obtained in this study on the effect of extraction pH, temperature, and time on pectin yield are in agreement with previous works (Garna et al., 2007; Happi Emaga et al., 2008; Levigne et al., 2002a; Masmoudi et al., 2008; Michel et al., 1985; Robert et al., 2006; Yapo et al., 2007). However, Robert et al. (2006) claimed that only the effect of temperature was significant ($p = 0.10$), Garna et al. (2007) reported that only the effect of pH was significant ($p = 0.05$), and Yapo et al. (2007) found that pH and time were the most influential effects, while temperature was non-significant. On the other hand, Pathak et al. (1988) found the same effects of pH and time as in this study, but claimed that higher temperatures had a negative effect on pectin yield of some extractions.

As indicated by ANOVA results (Table 3), eventhough extraction pH was the major effect on pectin yield, it was involved in a significant interaction with pomace pretreatment. This is illustrated in Figure 3, which shows that at pH = 1.5 pectin yield was higher when pomace was blanched; however when extraction pH was 2.5 there was not singificant

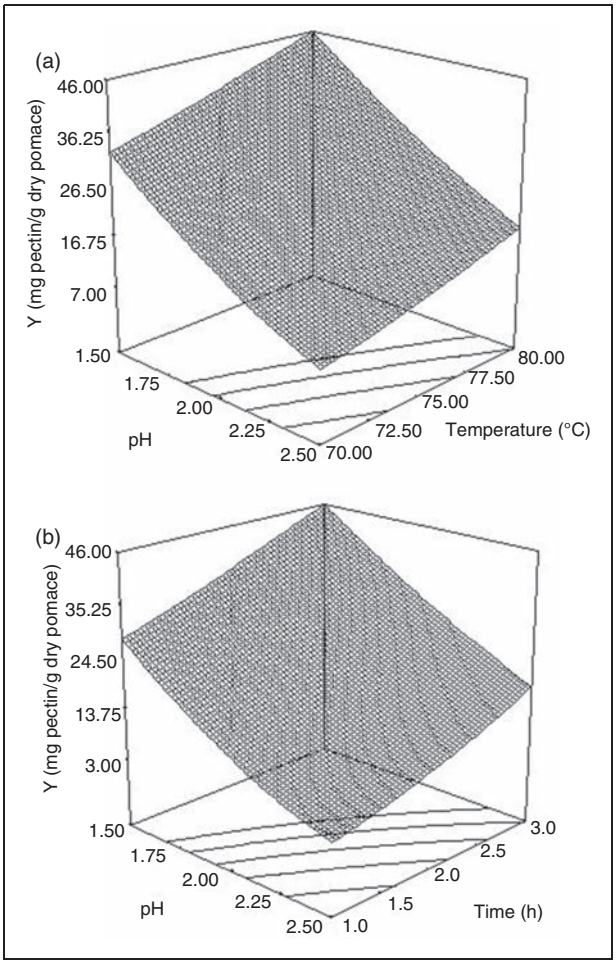

Figure 2. Response surface plots for the effect of pH and temperature at $t = 3$ h (a), and the effect of pH and time at $T = 80\,^{\circ}$C (b), on pectin yield (Y%).
Categoric factors were set at: blanching pretreatment, LPSSD drying, and vacuum concentration.
LPSSD: low-pressure superheated steam dryer.

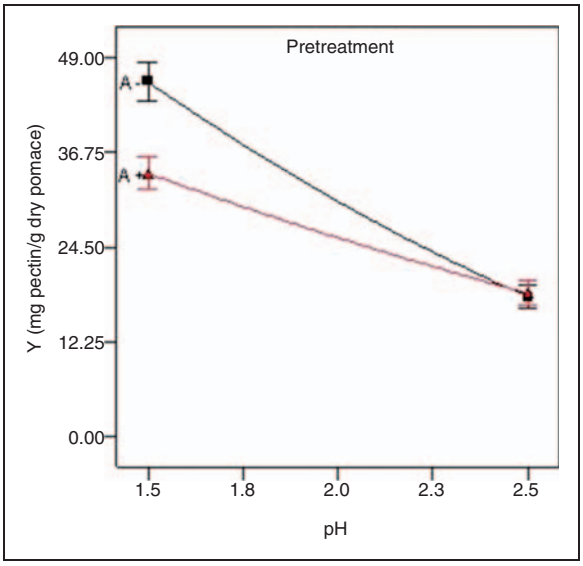

Figure 3. Effect of pH–pretreatment (A− = blanching; A+ = washing) interaction on pectin yield (Y%).
The other process variables were fixed at: LPSSD drying, $T = 80\,^{\circ}$C, $t = 3$ h, and vacuum concentration.
LPSSD: low-pressure superheated steam dryer.

difference due to pretreatment, provided the other variables were fixed. Consequently, the effect of pH cannot be interpreted alone, since it was found to be sensitive to the pomace pretreatment. This may explain why the maximum experimental and predicted yields were obtained at different pomace pretreatments.

GalA content

One of the most important properties of pectin is the content of GalA, as it is the fundamental unit of the polysaccharide chain and defines its purity. Industrial pectins contain at least 65% GalA (Robert et al., 2006). GalA contents obtained in this study ranged from $44.5 \pm 0.7\%$ to $76.2 \pm 4.6\%$, in agreement with other works (Happi Emaga et al., 2008; Yapo et al., 2007). Maximum experimental GalA content was obtained at the following conditions: quince washing, pomace drying by LPSSD, acid extraction at pH 2.5, 80 °C, during 3 h, and concentration by ultrafiltration. Selected effects for the model to predict GalA content were listed in Table 4. The ANOVA indicated that the model was significant, lack of fit was non-significant, and GalA content was significantly affected by the following effects (in decreasing order of importance): C > BF > CFE > CD > D > DB > E > CE > CAF > F-E > A > CDA > AE > CAE > CBF > CDF > CF. Some non-significant effects ($p > 0.05$) were included in the model to maintain its hierarchy (Table 4). As in the case of pectin yield, extraction pH produced the major effect on GalA content, but this effect cannot be interpreted alone because it was involved in two- and three-factor interactions. Final model equation in terms of coded factors was ($R^2 = 0.8795$, adj $R^2 = 0.8054$, and pred $R^2 = 0.6756$)

$$\begin{aligned}\text{GalA}\ (\%) = {} & 64.12 - 1.45 * \text{A} + 0.44 * \text{B} - 2.91 * \text{C} \\ & + 2.25 * \text{D} + 1.88 * \text{E} + 0.23 * \text{F} \\ & + 0.043 * \text{AC} - 0.11 * \text{AD} - 1.19 * \text{AE} \\ & - 0.39 * \text{AF} + 0.41 * \text{BC} + 2.17 * \text{BD} \\ & + 2.73 * \text{BF} + 2.44 * \text{CD} + 1.63 * \text{CE} \\ & - 0.91 * \text{CF} + 0.055 * \text{DF} + 1.50 * \text{EF} \\ & + 1.43 * \text{ACD} + 1.04 * \text{ACE} \\ & - 1.60 * \text{ACF} + 0.99 * \text{BCF} \\ & + 0.95 * \text{CDF} + 2.44 * \text{CEF} \end{aligned} \quad (6)$$

Table 4. GalA content (GalA%) ANOVA

	Sum of square	DF	Mean square	Valor *F*	*p* Valor
Model	3742.9	24	155.95	11.86	<0.0001
A–pretratament	133.92	1	133.92	10.19	0.0028
B–drying method	12.66	1	12.66	0.96	0.3325
C–pH	540.14	1	540.14	41.09	<0.0001
D–temperature	322.63	1	322.63	24.55	<0.0001
E–time	227.22	1	227.22	17.29	0.0002
F–concentration method	3.35	1	3.35	0.25	0.6166
AE	89.93	1	89.93	6.84	0.0126
AF	9.77	1	9.77	0.74	0.3938
BF	476.83	1	476.83	36.28	<0.0001
CA	0.12	1	0.12	9.147 E-003	0.9243
CB	10.64	1	10.64	0.81	0.3738
CD	379.52	1	379.52	28.87	<0.0001
CE	170.96	1	170.96	13.01	0.0009
CF	53.55	1	53.55	4.07	0.0505
DA	0.72	1	0.72	0.055	0.8166
DB	301.47	1	301.47	22.94	<0.0001
DF	0.19	1	9.19	0.015	0.9047
FE	144.53	1	144.53	11.00	0.0020
CDA	130.21	1	130.21	9.91	0.0032
CDF	57.92	1	57.92	4.41	0.0423
CAF	164.16	1	164.16	12.49	0.0011
CAE	68.70	1	68.70	5.23	0.0278
CBF	62.97	1	62.97	4.79	0.0347
CFE	380.79	1	380.79	28.97	<0.0001
Residual	512.64	39	13.14		
Lack of fit	97	7	13.86	1.07	0.4068
Pure error	415.64	32	12.99		
Cor total	4255.54	63			

GalA: galacturonic acid; ANOVA: analysis of variance.
p-Value < 0.05 indicates that the model and its terms are significant.

Figure 4(a) and (b) shows the response surface plots for the predicted effect of acid extraction conditions (pH, temperature, and time) on GalA content, setting the categoric factors at favorable levels. The highest GalA content predicted by the model (82.6%) was in agreement with the experimental one (76.2 %) and was obtained at the same process conditions, except pretreatment: quince blanching, pomace drying by LPSSD, acid extraction at pH 2.5, 80 °C, 3 h, and concentration by ultrafiltration (Table 6). According to the predictive models obtained in the other sections (equations (5) and (7)), pectins obtained under these conditions are expected to have a very low yield (Y = 9.8 mg/g), and a DM of a HMP (DM = 61.0%).

Figure 4(a) and (b) shows that GalA content increased at increasing values of acid extraction temperature and time, provided the categoric factors were fixed at favorable levels. ANOVA results (Table 4) showed that there were significant interactions between the acid extraction variables pH–temperature and pH–time. These interactions twist the planes of the response surfaces in Figure 4(a) and (b). At $T = 70\,^{\circ}C$, the GalA content was not significantly affected by pH, while at $T = 80\,^{\circ}C$ GalA increased with pH (Figure 4(a)). On the other hand, at $t = 1$ h GalA decreased at increasing pH values, but showed the opposite trend at $t = 3$ h (Figure 4(b)). Garna et al. (2007) found an increase of the GalA content with the extraction temperature and with an increase of the pH, with no significant effect of time. Happi Emaga et al. (2008) and Yapo et al. (2007) found that GalA content was not influenced by extraction time or temperature, while only pH had a significant effect. However, Happi Emaga et al. (2008) found that GalA content increased at increasing pH, while Yapo et al. (2007) reported the opposite trend. In our study, it was not possible to establish a general conclusion

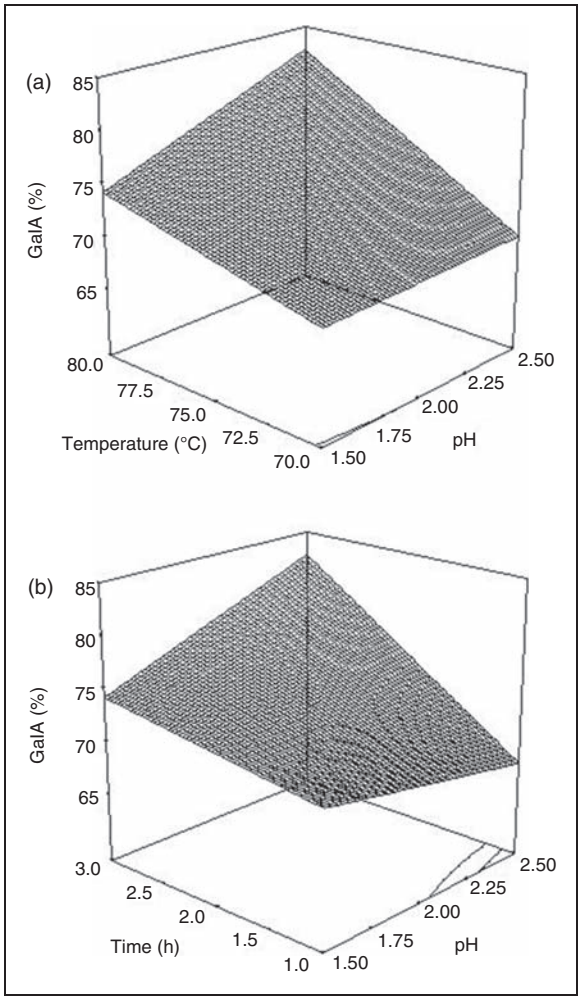

Figure 4. Response surface plots for the effect of pH and temperature at $t = 3$ h (a), and the effect of pH and time at $T = 80\,^{\circ}$C (b), on the GalA content (GalA%).
Categoric factors were set at: blanching pretreatment, LPSSD drying, and ultrafiltration concentration.
GalA: galacturonic acid; LPSSD: low-pressure superheated steam dryer.

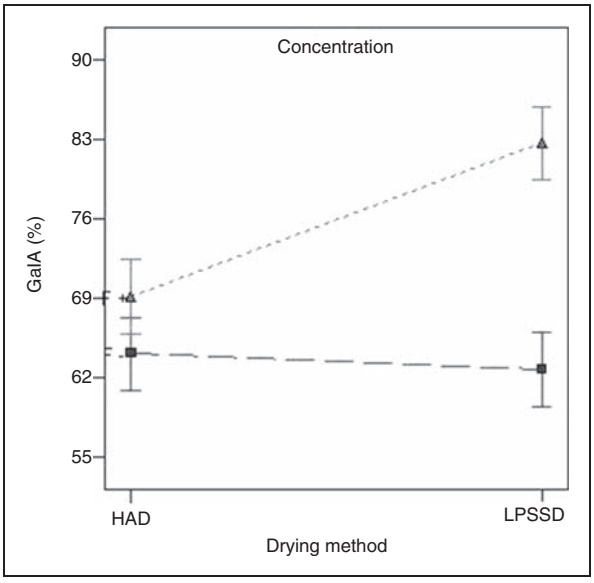

Figure 5. Effect of drying–concentration (F− = vacuum; F+ = ultrafiltration) interaction on GalA content (GalA%). The other process variables were fixed at: blanching pretreatment, pH 2.5, $T = 80\,^{\circ}$C, and $t = 3$ h.
GalA: galacturonic acid.

about the effect of extraction pH on GalA content, because it was involved in significant interactions with temperature and time. Michel et al. (1985) claimed that GalA content increased with severity of acid extraction (no statistical analysis provided). Levigne et al. (2002a) reported only a moderate effect of pH, pH^2, temperature, and interaction pH–temperature. Robert et al. (2006) found that only temperature had a significant positive effect.

As indicated by ANOVA results (Table 4), although the effect of the type of drying and the type of concentration were not significant by themselves, their interaction (BF) produced the second highest effect on the GalA content. This is illustrated in Figure 5, were it can be observed that the concentration method had little effect on GalA content when the pomace was dried by HAD, but there was a significant difference when it was dried by LPSSD, provided the other variables are fixed. It seems that the treatment with LPSSD drying combined with concentration by ultrafiltration was less severe, reducing the depolymerization of pectin.

Degree of methylation

DM experimental values ranged from $47.5 \pm 3.9\%$ to $90.9 \pm 6.5\%$, in agreement with other works (Happi Emaga et al., 2008; Levigne et al., 2002a). The highest DM was obtained at the following conditions: quince washing, pomace drying by HAD, acid extraction at pH 2.5, 80 °C, during 1 h, and concentration by ultrafiltration. The lowest DM was obtained at: quince blanching, pomace drying by LPSSD, acid extraction at pH 1.5, 70 °C, during 3 h, and concentration under vacuum.

Selected effects for the model to predict DM were listed in Table 5. The ANOVA indicated that the model was significant, lack of fit was non-significant, and the DM was significantly affected by the following effects (in decreasing order of importance): C > D > FE > CBF > BF > CFE > DB > DF > CDE > CE > CDF > CAE > CAB > CAF. Some non-significant effects ($p > 0.05$) were included in the model to maintain its hierarchy (Table 5). As in the case of pectin yield and GalA content, extraction pH produced the major effect on DM, but this effect cannot be interpreted alone

Table 5. DM% ANOVA

	Sum of square	DF	Mean square	Valor *F*	*p* Valor
Model	4495.69	26	172.91	7.74	<0.0001
A−pretratament	10.59	1	10.59	0.47	0.4954
B−drying method	6.84	1	6.84	0.31	0.5835
C−pH	812.81	1	812.81	36.38	<0.0001
D−temperature	425.22	1	425.22	19.03	<0.0001
E−time	5.71	1	5.71	0.26	0.6162
F−concentration method	82.16	1	82.16	3.68	0.0629
AB	61.66	1	61.66	2.76	0.1051
AC	43.76	1	43.76	1.96	0.1700
AE	4.63	1	4.63	0.21	0.6518
AF	8.68	1	8.68	0.39	0.5369
BC	43.81	1	43.81	1.96	0.1698
BF	355.27	1	355.27	15.90	0.0003
CD	70.10	1	70.10	3.14	0.0848
CE	169.99	1	169.99	7.61	0.0090
CF	35.67	1	35.67	1.60	0.2143
DB	271.91	1	271.91	12.17	0.0013
DE	37.47	1	37.47	1.68	0.2033
DF	226.74	1	226.74	10.15	0.0029
EF	393.99	1	393.99	17.63	0.0002
ABC	137.43	1	137.43	6.15	0.0178
ACE	160.30	1	160.30	7.17	0.0110
ACF	135.87	1	135.87	6.08	0.0184
BCF	360.95	1	360.95	16.15	0.003
CDE	186.25	1	186.25	8.34	0.0065
CDF	165.84	1	165.84	7.42	0.0098
CEF	282.07	1	282.07	12.62	0.011
Residual	826.72	37	22.34		
Lack of fit	119.12	5	23.82	1.08	0.3914
Pure error	707.60	32	22.11		
Cor total	5322.42	63			

DM: degree of methylation; ANOVA: analysis of variance.
p-Value < 0.05 indicates that the model and its terms are significant.

because it was involved in two- and three-factor interactions. Final model equation in terms of coded factors was ($R^2 = 0.8447$, adj $R^2 = 0.7355$, and pred $R^2 = 0.5353$)

$$\begin{aligned}\text{DM}\ (\%) = {} & +59.89 + 0.41 * \text{A} + 0.33 * \text{B} + 3.56 * \text{C} \\ & + 2.58 * \text{D} - 0.30 * \text{E} + 1.13 * \text{F} \\ & - 0.98 * \text{AB} - 0.83 * \text{AC} + 0.27 * \text{AE} \\ & - 0.37 * \text{AF} - 0.83 * \text{BC} - 2.06 * \text{BD} \\ & - 2.36 * \text{BF} + 1.05 * \text{CD} + 1.63 * \text{CE} \\ & - 0.75 * \text{CF} - 0.77 * \text{DE} + 1.88 * \text{DF} \\ & - 2.48 * \text{EF} - 1.47 * \text{ABC} - 1.58 * \text{ACE} \\ & + 1.46 * \text{ACF} - 2.37 * \text{BCF} - 2.10 * \text{CEF} \\ & - 1.71 * \text{CDE} + 1.61 * \text{CDF} \end{aligned} \quad (7)$$

Figure 6(a) and (b) shows the response surface plots for the predicted effect of acid extraction conditions (pH, temperature, and time) on DM, setting the categoric factors at favorable levels. Based on DM typical values of commercial HMPs, lower and upper limits for DM optimization were set at 40% and 90%, respectively. Within these limits, the highest DM predicted by the model (88.4%) was in agreement with the experimental value (90.9%), and was obtained at the same conditions: quince washing, pomace drying by HAD, acid extraction at pH 2.5, 80 °C for 1 h, and concentration by ultrafiltration (Table 6). According to the predictive models obtained in the other sections (equations (5) and (6)), pectins obtained under these conditions are expected to have a very low yield (Y = 1.3 mg/g), and very low purity (GalA = 50.1%). The lowest DM content predicted by the model (43.1%) was obtained at

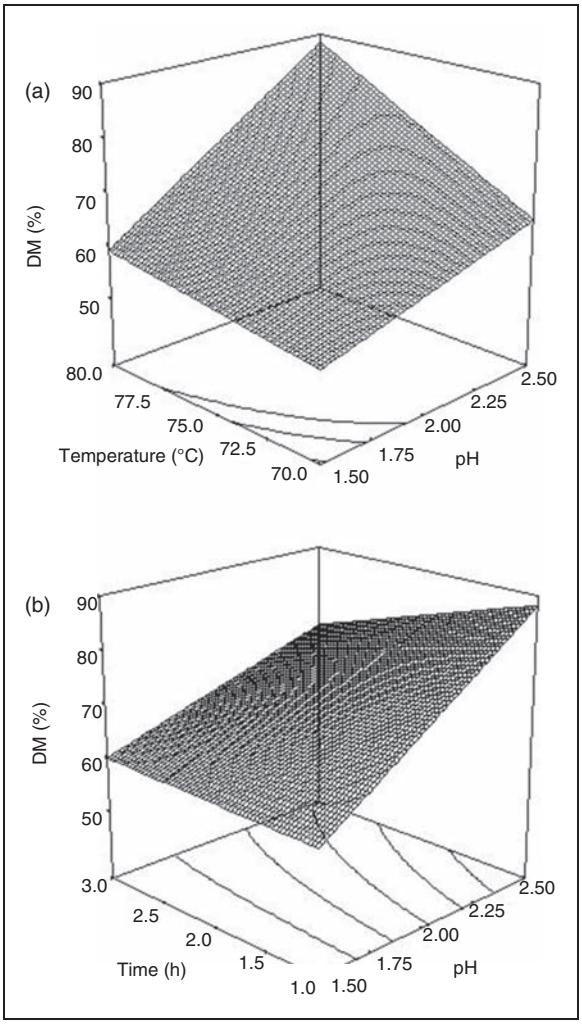

Figure 6. Response surface plots for the effect of pH and temperature at $t = 1$ h (a), and the effect of pH and time at $T = 80\,^{\circ}C$ (b), on the DM%.
Categoric factors were set at: washing pretreatment, HAD drying, and ultrafiltration concentration.
HAD: hot air drying; DM: degree of methylation.

the following conditions: quince blanching, pomace drying by HAD, acid extraction at pH 1.5, 70 °C, for 3 h, and vacuum concentration (not shown).

If one regards the point of maximum GalA and the point of maximum DM, it can be observed that in both cases the concentration method required was ultrafiltration. One possible explanation is that, compared to vacuum evaporation, ultrafiltration is a non-thermal treatment, which probably prevented some pectin deterioration during the concentration process. However, as previously mentioned, ultrafiltration had a negative effect on pectin yield.

It can be observed (Figure 6(a) and (b)) that DM decreased at decreasing pH values, provided the categoric variables were fixed at favorable conditions. This decrease of the methoxyl content was attributed to the hydrolysis of the methyl ester groups in an acidic environment, resulting in a deesterification of the polygalacturonic chain (Yapo et al., 2007). ANOVA results (Table 5) showed that even though extraction pH and temperature produced the two highest effects on DM, their interaction (CD) was not significant (at $p = 0.05$). On the other hand, the interaction pH–time was significant, which can be observed as a little twist in the plane of Figure 6(b). Several authors (Happi Emaga et al., 2008; Michel et al., 1985; Robert et al., 2006; Yapo et al., 2007) claimed that DM decreased with severity of extraction conditions. Levigne et al. (2002a) reported that DM increased at increasing pH, and found a significant interaction pH–time, in agreement with our results. Garna et al. (2007) found that DM decreased at increasing extraction times, but this effect was not significant, as well as pH and temperature. Yapo et al. (2007) reported that the effect of pH was more marked than the effect of temperature.

As indicated by ANOVA results (Table 5), even though extraction time and concentration method were non-significant by themselves, their interaction (EF) produced the third largest effect on DM. This is illustrated in Figure 7, where it can be observed that when the pectin extract was concentrated by ultrafiltration, DM decreased at increasing acid extraction times, following the opposite trend when it was vacuum concentrated.

Optimization of combined responses

In the previous sections, the predictive models (equations (5) to (7)) were used to calculate the process conditions required to meet specific goals for each response, namely: maximum Y, maximum GalA, maximum DM, and minimum DM. It was observed that when one of the responses was maximized, the other two had low or very low values. Consequently, the objective of this section was to optimize combinations of two or three responses at the same time. Myers and Montgomery (2002) described a method to optimize multiple responses simultaneously. The method makes use of an objective function called desirability (D), which is defined as the geometric average of the desirabilities of the various responses (d_i)

$$D = \left(\prod d_i\right)^{\frac{1}{n}} \tag{8}$$

Each rating d_i ranges from zero to one, for the minimum and maximum desirable values of each response, respectively. Lower and upper desirability limits used for the responses studied in this study were 0–100 mg/g for yield, 40–85% for GalA, and 40–90% for DM. The optimization procedure was conducted under these

Table 6. Optimum values of factors for different goals, and the numerical solutions with the predicted values of the responses

Goal	Pretreatment	Drying	pH	Time (h)	Temperature (°C)	Concentration	Solution	D
Maximum Y	Blanching	LPSSD	1.5	3	80	Vacuum	**Y: 45.9** GalA: 74.7 DM: 48.8	0.678
Maximum GalA	Blanching	LPSSD	2.5	3	80	Ultrafiltration	Y: 9.8 **GalA: 82.7** DM: 61.0	0.948
Maximum DM	Washing	HAD	2.5	1	80	Ultrafiltration	Y: 1.3 GalA: 50.1 **DM: 88.4**	0.968
Maximum GalA Maximum DM	Blanching	LPSSD	2.5	2.58	80	Ultrafiltration	Y: 7.0 **GalA: 79.5** **DM: 62.9**	0.633
Maximum Y Maximum GalA	Blanching	LPSSD	1.5	3	80	Vacuum	**Y: 45.9** **GalA: 74.7** DM: 48.8	0.723
Maximum Y Maximum GalA Maximum DM	Blanching	LPSSD	2.20	3	80	Vacuum	**Y: 25.2** **GalA: 66.3** **DM: 66.4**	0.537
Maximum GalA DM 60%	Blanching	LPSSD	2.29	3	80	Ultrafiltration	Y: 12.5 GalA: 81.0 **DM: 60.0**	0.959
Maximum GalA DM 70%	Blanching	HAD	2.44	3	80	Ultrafiltration	Y: 4.9 GalA: 69.0 **DM: 70.0**	0.823
Maximum GalA DM 75%	Washing	HAD	2.5	2.90	80	Ultrafiltration	Y: 8.8 GalA: 64.0 **DM: 75.0**	0.790

HAD: hot air drying; LPSSD: low-pressure superheated steam dryer; DM: degree of methylation; GalA: galacturonic acid.
Note: Values in bold phase indicate the optimized response.

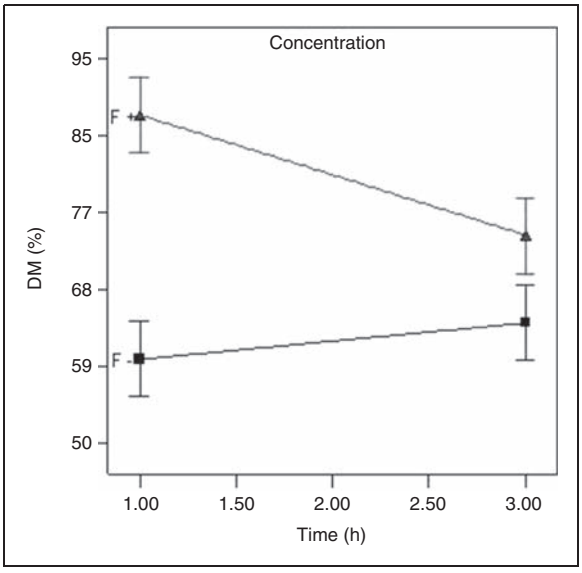

Figure 7. Effect of time–concentration (F− = vacuum; F+ = ultrafiltration) interaction on the DM%.
The other process variables were fixed at: washing pretreatment, HAD drying, pH 2.5, and $T = 80\,^{\circ}C$.
HAD: hot air drying; DM: degree of methylation.

restrictions. Optimum values of the factors obtained for different goals are presented in Table 6. In first place, optimum conditions to obtain individual maximum values of yield, GalA and DM were obtained (Table 6), as described in previous sections. Then, optimization of combined responses was performed. It should be reminded that the optimization (of individual or combined responses) performed with a fractional factorial design is a preliminary step, and more experimental points are needed to obtain a final optimum value, that has to be confirmed. This will be the aim of future works. Nevertheless, preliminary optimum values obtained in this study are a useful guide to indicate the location of the final optimum values.

First, optimum process conditions to maximize GalA and DM simultaneously were obtained, namely: quince blanching, pomace drying by LPSSD, extraction at pH 2.5, 80 °C, 2.58 h, and concentration by ultrafiltration. Under these conditions, good values of GalA = 79.5% and DM = 62.9% were predicted, although pectin yield was not so good (Y = 7.0 mg/g).

Second, optimum process conditions to maximize GalA and yield simultaneously were obtained, namely: quince blanching, pomace drying by LPSSD, extraction at pH 1.5, 80 °C, 3 h, and concentration by vacuum. Under these conditions, very good values of pectin yield Y = 45.9 mg/g and GalA = 74.7% were predicted, although the degree of methoxylation corresponds to a LMP (DM < 50%).

In third place, the three responses (Y, GalA, and DM) were maximized simultaneously, and the optimum process conditions obtained were: quince blanching, pomace drying by LPSSD, extraction at pH 2.20, 80 °C, 3 h, and concentration by vacuum. Under these conditions, acceptable values of Y = 25.2 mg/g, GalA = 66.3% and DM = 66.4%, were obtained.

Finally, we set target values of DM (60%, 70%, and 75%), keeping maximum GalA content (Table 6). The objective was to obtain HMPs with standard values of DM, and at the same time with the best possible purity (it should be mentioned that it was very difficult to reach targets with DM > 75% and maximum GalA). It can be observed that as the desired DM was increased, values of GalA decreased, and consequently the desirability decreased as well. For the three cases, the predicted pectin yield was quite low (12.5, 4.9, and 8.8 mg/g, respectively). It should be noted that for all the goals proposed in this study, the highest level of acid extraction temperature (80 °C) was always the most convenient (Table 6).

CONCLUSIONS

It was possible to obtain pectins from quince pomace, with properties (yield, purity, and DM) similar to those obtained from commercial sources and processes. It was shown that extraction conditions had significant effects on those properties. Furthermore, alternative methods employed in the extraction process had positive effects on some of those properties, like LPSSD on pectin yield and GalA content, and ultrafiltration on GalA and DM (Table 6). For the three responses studied, significant interactions were found between the selected variables of the process, and consequently it was not possible to deduce general conclusions about the effect of each variable on each response. For example, extraction pH was the major effect of the three responses, but in all cases it was involved in two- and three-factor interactions. Maximum and/or target values of individual and combined responses (goals) were used to estimate preliminary optimum extraction conditions required to obtain pectins with specific characteristics. Regarding the alternative methods proposed in this study, LPSSD was required to obtain maximum Y and maximum GalA, while ultrafiltration was required to obtain maximum GalA and maximum DM. However, ultrafiltration had a negative effect on pectin yield. The maximum of the three combined responses (Y = 25.2 mg/g, GalA = 66.3%, and DM = 66.4%) was obtained at: quince blanching, pomace drying by LPSSD, extraction at pH 2.20, 80 °C, 3 h, and concentration by vacuum.

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