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ScienceDirect
Carbohydrate Polymers 74 (2008) 185–192
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology

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Received 14 October 2007; received in revised form 29 January 2008; accepted 13 February 2008
Available online 29 February 2008

Abstract

Response surface methodology was used to optimize pectin recovery from lemon by-product using an acidified date juice as extraction solution. When enriched in pectin, this latter can be useful for preparation of date-lemon jelly. The effects of three parameters namely temperature, pH and extraction time, on pectin extraction were studied. The fitted mathematical model allowed us to plot response surfaces as well as isoresponse curves and to determine optimal extraction conditions. Results clearly indicated that the temperature was the main factor influencing the pectin yield which increased with temperature and time or decreasing pH. The selected optimal conditions were: temperature 84.34 °C; extraction time 3 h 34 min and pH 2.8. These conditions yielded about 11.21% of pectin versus 10.89% for the predicted value.

Keywords: Pectin extraction; Lemon by-product; Acidified date juice; Response surface methodology

1. Introduction

Lemon (*Citrus limon* L.) and date (*Phoenix dactylifera* L.) have always played an important part in the economic and social lives in Tunisia. In fact, the production of lemon and limes reached nearly 27,000 metric tons and that of the dates was about 125,000 tons in 2005 (FAOSTAT, 2006).

A large quantity of dates (about 30% of the total production) is lost during picking, manufacture of some date products, or storage of second category dates which are generally discarded or partially integrated in animal feed (Besbes et al., 2006). Studies concerning the use of these by-products to develop new products are scarce and concern metabolites or biomass production (Abou Zeid, Abderrahman, & Baghlef, 1991; Besbes et al., 2006). Owing to their high content in sugar, dates were also used for the preparation of some food products, such as date juice, syrup, date preserves or date jellies (Besbes et al., 2006).

Likewise, during citrus juice processing, a considerable quantity of wastes or by-products is generated. Though some portion of these by-products is consumed as animal feed, the majority of the processing wastes are thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications for these by-products which have been revealed to be a source of many important natural compounds such as citric acid, flavonoïds and especially pectin (Kim, Lee, Lee, & Kim, 2004).

Pectins are complex polysaccharides from higher plants, composed mainly of α-1,4-linked D-galacturonic acid (Gal A) chains (so called homogalacturonan or smooth regions) in which the carboxyl groups of the Gal A can be free or

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doi:10.1016/j.carbpol.2008.02.003

methyl-esterified. Pectins are also constituted of hairy regions (also called rhamnogalacturonan I) with Gal A-rhamnose regions, where the rhamnose moieties can be substituted with neutral sugars (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Pectins are widely used in food industry as thickening and gelling agents for the preparation of jams and jellies (May, 1990).

Pectins may be obtained by extractions of the cell-wall material by hot dilute acid, by cold dilute sodium hydroxide or by solutions of chelating agents (Levigne, Ralet, & Thibault, 2002). Commercial pectins are extracted at high temperatures by hydrolyzing protopectin using acids such as sulfuric, phosphoric, nitric, hydrochloric or citric acid (May, 1990; Minkov, Minchev, & Paev 1996). After concentration, the pectin is precipitated by addition of alcohol, dried, ground and finally sieved (May, 1990). Many authors reported that pH, temperature, extraction time, particle size, agitation and solid to liquid ratio have effects on the yield and the quality of pectin (El-Nawawi & Shehata, 1987, 1988; Levigne et al., 2002; May, 1990). Pectin has been extracted from many plants, such as lemon, orange, peach pomace and sugar beet pulps and characterized (Attri & Maini, 1996; Kim et al., 2004; Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985; Pagan & Ibarz, 1999; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). Pagan and Ibarz (1999) extracted pectin from fresh peach pomace at different experimental conditions and found that the highest yields were obtained at the highest temperatures and at the lowest assayed pH. El-Nawawi and Shehata (1987) who studied the effect of experimental conditions on the yield of pectin extracted from Egyptian orange, found an optimum yield at 90 °C, a pH of 1.7 and 2 h of extraction. Fishman, Chau, Hoagland, and Hotchkiss (2006) and Wang et al. (2007) used microwave heating to extract pectin under different conditions from lemon peel and apple pomace, respectively, and studied their effect on the yield and characteristics of pectin.

The aim of this study was to look for the experimental conditions leading to the maximum pectin extraction from lemon by-product using an acidified date juice as extraction solution. This latter, enriched with lemon pectin and flavour, has been selected as it will be used in a subsequent study for the formulation of date-lemon jelly. As many factors can influence the extraction yield, response surface methodology (RSM) was applied to fit and exploit a mathematical model representing the relationship between the response (extraction yield) and variables (temperature, extraction time and pH).

2. Material and methods

2.1. Experimental section

2.1.1. Lemon by-product

One batch of 25 kg of lemon by-product (*Citrus limon* L.) was supplied by a fruit beverage industry (Zina, Sfax, Tunisia) using mixed lemon varieties from Nabeul region (Tunisia). This by-product consists of the peel, the pulp and the pips. Upon its arrival, the pips were discarded and the remaining matter was lyophilised and milled. The obtained powder was sieved (60-mesh size screen) and stored at −20 °C until use.

2.1.2. Dates

Dates (*Phoenix dactylifera* L.) of "Deglet Nour" variety were provided by the National Institute of arid zone (Degach, Tunisia). We used a batch of 50 kg of dates of second category (hard texture) collected at the "Tamr" stage (full ripeness). Dates were directly pitted, washed in running tap water and dried 12 h in a drying oven at 45 °C. Then, the collected pulp was milled to obtain date paste.

2.1.3. Date juice preparation

Date juice was prepared by adding water to date paste at a ratio of 3:1 (v/w) as described by Youssif, Alshaawan, Mininah, and Eltaisan (1987) and Youssif, Abou Ali, and Abou Idreese (1990). The date paste–water mixture was boiled gently with continuous stirring for 5 min. The extract was filtered through fine-mesh cheesecloth. The obtained date juice was stored at −20 °C until analysis and use.

2.1.4. Physico-chemical analysis of the lemon by-product, dates and date juice

Dry matter was determined by drying samples at 106 °C to constant weights (AOAC, 1995). Ash content was determined at 550 °C using a muffle furnace (NABER, Germany) for 8 h. The total ash was expressed as percent of dry weight (AOAC, 1995).

Protein ($N \times 6.25$) was analysed according to the Kjeldhal procedure (AOAC, 1995). The determination of the cellulosic content was performed according to the Weende method (AOAC, 1995). Total carbohydrate was determined with the phenol-sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as a standard. Pectin content (galacturonic acids) was determined by the colorimetric method described by Englyst, Quigley, and Hudson (1994). The pH of the date juice was determined potentiometrically with a pH meter (Mettler Toledo, Switzerland).

2.1.5. Pectin extraction

A preliminary study showed that pH, temperature and time were the main parameters affecting the yield of pectin extraction (unpublished results). Extractions at different conditions were carried out in a glass flask immersed in a water bath as follows: 12 g of lyophilised lemon by-product were stirred at 250 rpm (Stirrer Heidolph RZR 20051 electronic, Germany) in 300 ml of the prepared date juice (solid–liquid ratio; 1:25; w/v). The pH of the mixtures (lemon by-product/date juice) was adjusted with citric acid.

The resulting slurries were allowed to cool to room temperature (25 °C) and filtered through a cheesecloth. The filtrate was centrifuged at 20 °C for 30 min at 7000 rpm to remove solid particles. The supernatant was filtered again through Whatman filter paper N. 1 (Kim et al., 2004).

For pectin precipitation, two volumes of 96% w/w ethanol were added to one volume of pectin extracts, while gently stirring to break up the gelatinous lumps. The obtained mixture was kept for 1 h at 4 °C. Then, pectin gels were centrifuged at 5000 rpm, for 20 min at 10 °C. To remove the mono and disaccharides, the pectin precipitate was washed three times with 50%, 75% and 100% ethanol and centrifuged at 5000 rpm for 10 min at 10 °C. Finally, the obtained pectin was dried at 50 °C to a constant weight, and ground in a mortar.

The pectin extraction yield, subject of this study, was calculated as follows:

Yield (%) = (weight of the total soluble pectin − weight of date juice pectin) × 100/weight of dried lemon by-product.

2.2. Experimental methodology

When many factors affect a desired response, it can be an exhausting task to optimize a process. Therefore, response surface methodology (RSM) can be an effective tool for optimizing the response (Box, Hunter, & Hunter, 1978; Carlson, 1992; Goupy, 1999; Montgomery, 1991). Response surface methodology is defined as statistical method that uses quantitative data from appropriate experimental design to determine optimal conditions (Giovanni, 1983).

The first step of the pectin extraction procedure has been optimized using RSM. A central composite design was chosen to look for the best experimental conditions of three independent factors affecting the extraction process which are: X_1: extraction temperature (°C); X_2: pH of the mixture of lemon by-product in date juice; and X_3: time of extraction (h). For each factor, the experimental range was chosen on the basis of results of preliminary experiments.

In this work, the relationship between the extraction yield and the three selected quantitative variables was approximated by the following second order polynomial function:

$$\hat{y} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where $\hat{y}$ is the calculated response function, X_j are the coded variables with no dimension related to the natural variables U_j by the equation

$$X_j = (U_j - U_j(0))/\Delta u_j$$

where $U_j(0)$ is the level of the natural variable at the centre of the domain,ΔU_j is the increment of U_j corresponding to 1 U of X_j. b_0, b_j, b_{jk} and b_{jj} are the model coefficients.

The observed response y_i at the ith experiment is related to $\hat{y}_i$ by the equation

$$y_i = \hat{y}_i + e_i \quad (e_i : \text{error}).$$

2.2.1. Orthogonal central composite design

An orthogonal central composite design including 15 experiments was used to estimate the 10 model coefficients. This design consists of eight points of a factorial design, six axial points at a distance $\alpha = \pm 1.215$ from the centre, and a centre point, were used to estimate the model coefficients (Table 1). The distance α is calculated to have vectors of square variables X_k mutually near orthogonal (El Feki, Chaabouni, Ayedi, Heughebaert, & Vaillant, 1987). The experiments were conducted in double replications at each design point. In order to estimate pure error variance, four replications were performed at the centre point (Goupy, 1999; Myers & Montgomery, 1995).

The levels of the three retained variables (X_1: extraction temperature (°C); X_2: pH of the mixture lemon by-product/date juice; and X_3: time of extraction (h)) are indicated in Table 2.

The obtained response values were used to estimate the model coefficients b_j by the least square method using the experimental design software NEMROD-W (Mathieu, Nony, & Phan-Tan-Luu, 2000).

2.2.2. Validation of the model

The mathematical model must be validated before exploiting it. In the case of a composite design, the validation of the model is carried out by an appropriate analysis of variance (ANOVA). The method can be described as follows (Kamoun, Samet, Bouaziz, & Châabouni, 1999):

- The total sum of squares SS_T (with 17 degrees of freedom) is divided into the sum of squares SS_X due to regression (with 9 degrees of freedom) and the residual sum of squares SS_R (with 8 degrees of freedom):

  $$SS_T = SS_X + SS_R$$

- The residual sum of squares (SS_R) can be partitioned into two parts, the first part is due to pure experimental error (SS_E) and is computed as the sum of squared deviations in the centre point experiments calculated with 3

Table 1
Experimental matrix for the central composite orthogonal design

N_i	X_1	X_2	X_3
1	−1	−1	−1
2	1	−1	−1
3	−1	1	−1
4	1	1	−1
5	−1	−1	1
6	1	−1	1
7	−1	1	1
8	1	1	1
9	−1.215	0	0
10	1.215	0	0
11	0	−1.215	0
12	0	1.215	0
13	0	0	−1.215
14	0	0	1.215
15	0	0	0

Table 2
Experimental domain of the central orthogonal composite design

X_j	Factor levels				
	−1.215	−1	0	+1	+1.215
Temperature (°C)	35.70	40	60	80	84.30
pH	2.74	2.8	3.1	3.4	3.46
Time (h)	0.68	1	2.5	4	4.32

degrees of freedom (4 − 1), the second part (SS_L) corresponds to the lack of fit. It is used to assess the significance of the model. SS_L is determined with 5 degrees of freedom (8 − 3).

- The fitted model is considered adequate if the variance due to the lack of fit is not significantly different (*F*-test at the 95% level) from the pure error variance.

3. Results and discussions

3.1. Characteristics of the lemon by-product, dates and the date juice

The main characteristics of the lemon, dates and the prepared date juice are given in Table 3. The lemon by-product had high contents of pectin and cellulose (21.56% and 11.97%, respectively). These values are comparable with those reported for orange by-product (16.13% and 16.6% of pectin and cellulose, respectively) (Grigelmo-Miguel & Martin-Belloso, 1999). Citrus peels are a good source of pectin, which is well exploited by food industry for many preparations such as jellies, jams and acidic milk products (Kim, Kim, Lee, Kim, & Kim, 2000; Schols, Ros, Daas, Bakx, & Voragen, 1998).

Dates contained 2.27% of pectin. This amount is relatively higher than that reported by Mayhara, Taylor, Slominski, and Al-Bulushi (1998) who found 1.55% and 1.10% of uronic acids, respectively, in Fard and Khalas varieties. The dates used in this study had a high content in total sugars (87.71%) and a low content in proteins (2.84%) and ash (2.51%). This composition was comparable with that of dates of good quality (Barreveld, 1993; Vandercook, Hasegawa, & Maier, 1979). Cellulose content of dates was lower than that of lemon by-product which was approximately 2% versus 12%.

Table 3
Composition of the dried lemon by-product, dates and the date juice (g/100 g dry matter)

Component	Lemon by-product	Dates	Date juice
Dry matter (%)	88.53 ± 0.26	74.90 ± 0.75	17.63 ± 0.10
Ash	3.24 ± 0.07	2.51 ± 0.13	2.68 ± 0.02
Protein	7.88 ± 0.70	2.84 ± 0.13	1.79 ± 0.10
Total carbohydrate	13.77 ± 0.03	79.66 ± 0.06	77.72 ± 0.04
Cellulose	11.97 ± 0.90	1.98 ± 0.22	—
Pectin	21.56 ± 0.94	2.27 ± 0.06	0.96 ± 0.05
pH	—	—	5.62 ± 0.01

Given values are means of three determinations.

Table 4
Experimental conditions of the orthogonal central composite design and the corresponding experimental responses

N_i	X_1	X_2	X_3	Yield (%)
1	40	2.8	1	2.23
2	80	2.8	1	5.18
3	40	3.4	1	1.46
4	80	3.4	1	2.66
5	40	2.8	4	5.29
6	80	2.8	4	10.34
7	40	3.4	4	2.63
8	80	3.4	4	5.92
9	35.70	3.1	2.5	0.57
10	84.30	3.1	2.5	7.66
11	60	2.74	2.5	4.92
12	60	3.46	2.5	1.83
13	60	3.1	0.68	1.47
14	60	3.1	4.32	3.72
15	60	3.1	2.5	2.11
16	60	3.1	2.5	2.13
17	60	3.1	2.5	2.66
18	60	3.1	2.5	2.90

Like the date pulp, date juice presented high sugar and low pectin contents (85.62% and 0.96%, respectively). Mineral and protein contents were, respectively, 2.68% and 1.79%. The chemical composition of the date juice (high amount of sugars), as well as the low cost of dates can justify the search of valorization of this product. Indeed, date juice could be considered as a good substrate for date jelly production. However, it must be enriched with pectin to ensure its gelation. As a solution, pectin could be extracted from lemon by-product using acidified date juice.

3.2. Response measurements

Eighteen experiments were carried out according to the conditions indicated in Table 4. Response values (pectin yields) are reported in the last column of this table.

Similar result was found by Pagan and Ibarz (1999) who extracted 5.35% of pectin from peach pomace at 80 °C and a pH of 2.53 after 1 h of extraction.

However, Attri and Maini (1996) who used a strong inorganic acid (HCl 0.1 N) found a maximum pectin yield of 20.80% extracted from Galgal (an indigenous variety of lemon). Kar and Arslan (1999) reported that the extraction process and medium, as well as the fruit variety and maturity can affect the quantity and quality of extracted pectin.

3.3. Estimated model

The observed responses were used to compute the model coefficients using the least square method. This allowed us to write the following estimated model:

$$Y = 2.412 + 1.927X_1 - 1.290X_2 + 1.405X_3 - 0.439X_1X_2 + 0.524X_1X_3 - 0.474X_2X_3 + 1.188X_1^2 + 0.687X_2^2 + 0.159X_3^2$$

3.4. Statistical analysis and validation of the model

The analysis of variance for the fitted model showed that the regression sum of squares was statistically significant at the level 99.9% and the lack of fit is not significant. Results of this analysis are summarized in Table 5. The coefficient of determination, R^2, for pectin extraction yield was 0.949. Thus, the predicted model well represented the observed values.

3.5. Interpretation of the response surface model

The relationship between the responses and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figs. 1–3). The vertical axes show the pectin extraction yield (%), and each of the two horizontal axes represents any two of the three independent variables. In every plot, the factor not represented by the two horizontal axes was fixed at its 0 coded level. The topography of these response surfaces are also illustrated by isoresponse contours representing lines of constant response in a two variable plane. Such plots are helpful in studying the effects of the variation of the factors in the domain studied and consequently, in determining the optimal experimental conditions (Kamoun et al., 1999).

In Fig. 1, the examination of the isoresponse contours and three-dimensional plots showed that the yield increased when increasing temperature and/or decreasing the pH. These effects were markedly shown for temperatures over 70 °C and low pH levels. This can be explained by the solubility of the extracted pectin which increases with increasing temperature, giving a higher rate of extraction. Also, the diffusion coefficient will normally increase and so that improves the rate (Coulson & Richardson, 1978; Treybal, 1980). With regard to the effect of pH, El-Nawawi and Shehata (1987) reported that acidic conditions contribute to hydrolyze the insoluble pectic constituents into soluble pectin which increases the pectin recovery. At temperatures below 60 °C, the yield was relatively low (less than 6%), even if the pH was fixed at its lowest values (~2.6). This could be attributed to the fact that under these conditions, the temperature value was too low to permit the hydrolysis of protopectin (the insoluble form of pectin) (El-Nawawi & Shehata, 1987). Similar results were obtained by Graham and Shepherd (1953) who found that the amount of pectin extracted from Citrus peels in the hot dilute acid solution increased with the increase of temperature and acidity. Also, Kim et al. (2004) found that temperature was the main parameter influencing the pectin recovery from mandarin, using temperatures between 30 and 90 °C. However, Yapo et al. (2007) reported that the effect of temperature did not actually affect on the pectin yield from sugar beet pulp. This is probably due to the fact that these authors used a narrow temperature range (between 80 and 90 °C).

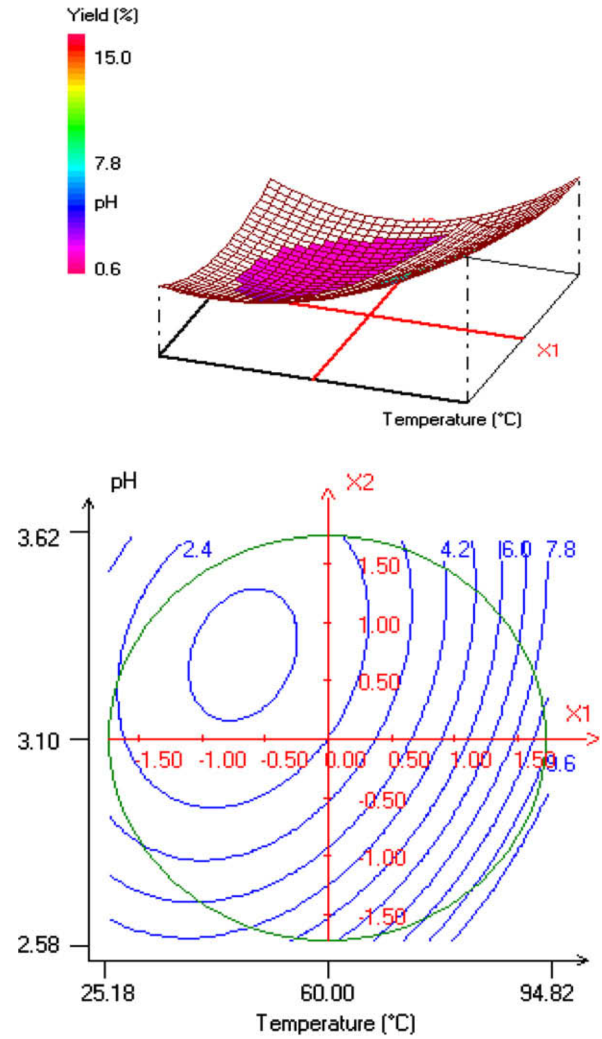

Fig. 1. Three-dimensional response surface and contour plots for the effect of pH and temperature at constant extraction time (2.5 h) on pectin yield extracted from dried lemon by-product in the date juice.

Table 5
Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean square	Ratio	Significance
Regression	99.8622	9	11.0958	16.4870	0.0298[a]
Residuals	5.3840	8	0.6730		
Validity	4.9194	5	0.9839	6.3531	7.9
Error	0.4646	3	0.1549		
Total	105.2462	17			

[a] Significant at the level 99.9%.

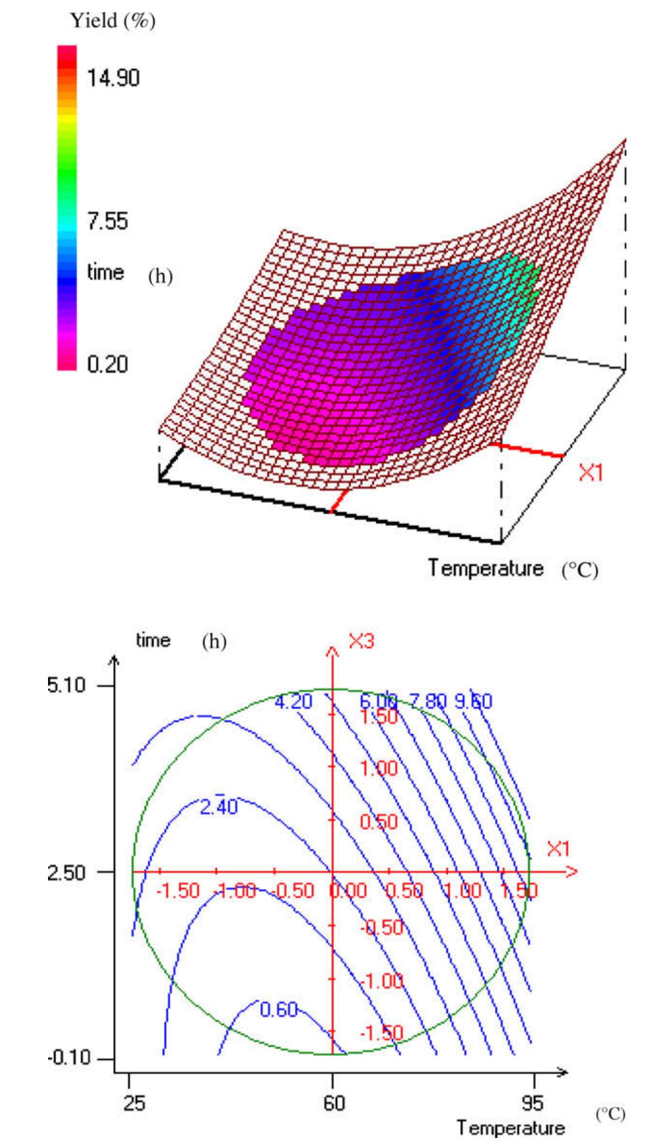

Fig. 2. Three-dimensional response surface and contour plots for the effect of extraction time and temperature at constant pH (3.1) on pectin yield extracted from dried lemon by-product in the date juice.

The positive effect of temperature was also demonstrated in Fig. 2. When the pH was fixed at 3.1, the increase of temperature and time improved the pectin extraction from lemon by-product. This result was close to that already published by Yapo et al. (2007) which studied the extraction of pectin from sugar beet pulp.

The effect of time and pH was given in Fig. 3. Once again, the isoresponse curves showed that a high time and a low pH of extraction led to higher yields. At 60 °C, the maximum yields (between 6 and 8.7) were reached at a pH in the range of 2.6–3 and extraction time upper than 2 h.

3.6. Determination of optimum conditions

In order to select optimal conditions, we fixed the pH value at 2.8 and we plot time versus temperature (Fig. 4).

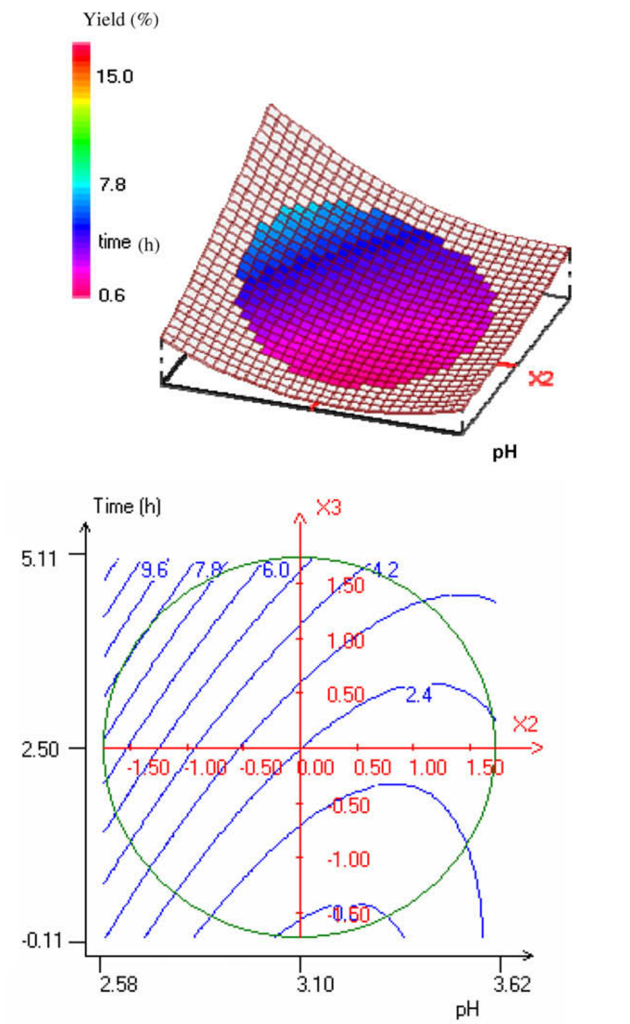

Fig. 3. Three-dimensional response surface and contour plots for the effect of extraction time and pH at constant temperature (60 °C) on pectin yield extracted from dried lemon by-product in the date juice.

This pH corresponds to that of the medium (pectin enriched date juice) which will be used for the preparation of date-lemon jelly. Indeed, the pH of commercial jellies must be comprised between 2.8 and 3.5 (CODEX STAN 79, 1981).

Optimal conditions selected by the software NEMROD-W were: temperature: 84.34 °C; pH: 2.8 and extraction time 3 h 34 min. Under these conditions, the expected value of the pectin yield was $\hat{y}_{op} = 10.89\% \pm 0.75$.

A supplementary experiment was carried out under the selected optimal conditions. It led to an experimental yield of pectin equal to 11.21% which is even more than the expected value (10.89%).

4. Conclusion

This work has revealed that the response surface methodology was a useful tool to determine the optimal experimental conditions of extraction of pectin from lemon by-product with an acidified date juice. The extraction yield

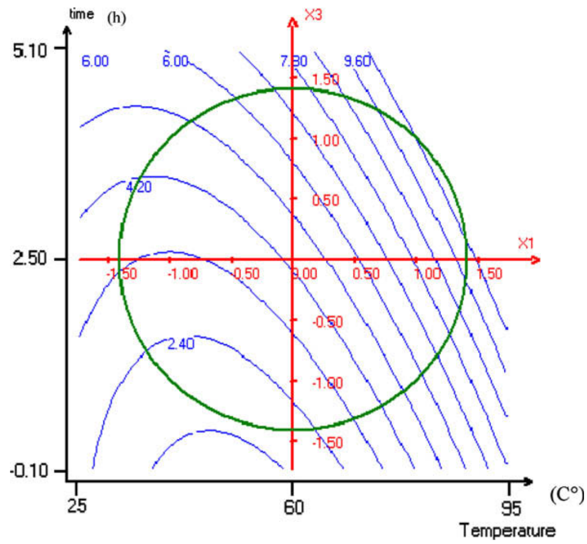

Fig. 4. Contour plots for the effect of extraction time and temperature at pH 2.8 on pectin yield extracted from dried lemon by-product in the date juice.

increased significantly with increasing temperature and time and decreasing pH. The selected optimal conditions (temperature: 84.34 °C; pH: 2.8 and extraction time: 3 h 34 min) have been checked and confirmed by a supplementary experiment. The experimental yield of pectin was found to be in good agreement with the predicted one (11.21% versus 10.89%, respectively).

However, extraction at high temperatures caused the dissolved pectin to degrade while it slightly improved the yield of pectin (Cho & Hwang, 2000). Furthermore, longer extraction time generally decreased the degree of methylation. That's why extraction conditions must take into accounts both recovery yield and characteristics of extracted pectin (Kim et al., 2004). Thus, a future study will focus on the influence of the experimental extraction conditions on the physical and chemical characteristics of the extracted pectins.

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