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Syrup of natural carob sugars and a process for its production using Response Surface Methodology

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Abstract
Experimental design was used to investigate the effect of three parameters (extraction temperature, extraction time and ratio of water to pulp) on syrups yields. The ranges of the factors investigated were 20–45°C for extraction temperature (X1), 1–3h for extraction time (X2) and 1–3 for extraction ratio of water to pulp (X3). The extracting parameters for syrups yields were optimized by using three-factor-three-level Box Behnken design (BBD) and response surface methodology based on the single-factor experiments. As results, the optimum conditions were extracting temperature 43.45°C, extracting time 2.40 h and the ratio of extraction solvent (water) to pulp (v/w) 2.27. Under these conditions, the experimental values were in close agreement with values predicted by the model and for wish. Predicted yield of syrup extracted is 39.51 %.
Keywords: Carob pods; pulp; Syrup yield; Optimization experiment; Box Behnken design.

1. Introduction

The carob tree (Ceratonia siliqua L.) belonging to the family Cesalpiniaceae sub family of the family Leguminoseae, is widely used in the Mediterranean regions (Batle & Tous 1997; Yousif & Alghzawi 2000) cultivated for ornamental and industrial purposes.

World production is estimated at about 315 000 tons per year, produced from about 200 000 hectares with very variable yields depending on the cultivar, region, and farming practices (Makris et al. 2004) and the main producers for (pulp, seeds) respectively are Spain (36%, 28%), Morocco (24%, 38%), Italy (10%, 8%), Portugal (10%, 8%), Greece (8%, 6%), Turkey (4%, 6%) and Cyprus (3%, 2%) of the world production (Ait Chitt et al. 2007).

Chemical composition of the carob pod depends on cultivar, origin and harvesting time (Albanell et al. 1991). The two main carob pod constituents are (by weight): pulp (90%) and seed (10%) (Tous et al. 1995).

The pulp of carob pods (fruit of Ceratonia siliqua L.) contains a high content of sugar is 40–60% sugar, predominantly sucrose, which constitutes about 30%. It is low in protein (3–4%) and lipids (0.4–0.8%) (Yousif & Alghzawi 2000; Santos et al. 2005). The pods also contain a high amount of dietary fiber and polyphenols (Marakis 1996). The pulp of carob pods is used extensively as a raw material for the production of syrups (Petit & Pinilla 1995; Özcan 2007 et al.) and crystallized sucrose for the food industry.

Today, carob fruits have a variety of uses. Carob fruits can be made into syrup, honey, bean meal powder, and alcohol. Carob alcohol is used in the pharmaceutical industry and in wine; carob honey is used in cakes and pastries and as a sweetener for compotes and jams; and carob powder is used in baby foods and baked goods (Shaouli & Fisher 1997). The objective of this present study was to optimize the process for production of the syrup from carob pulp, using response surface methodology (RSM), employing a three-factor-three-level Box–Behnken design (BBD) (Box & Behnken 1960).

2. Materials and methods

2.1 Selection and preparation of samples

The material collection was carried out during summer, in 2009; from Morocco (Beni-Mellal region) in here they grow naturally. 30 trees were randomly chosen for collection of composite samples.

For the production of syrup from the pods, the samples were separated from seeds and dried at 40 °C for 1 day. Then, 100 g of pulp, with an average size between 0.5 and 1.0 cm, was suspended in water (ratio of water to raw material, 1 to 3 in v/w) and stirred under experimental conditions (T =[20 – 45] ° C, t = [1 – 3] h). The sugars extract and solid phase were separated by centrifugation.

The juice obtained in the previous stage, has to be concentrated to the commercial levels of 66.5 °Brix .The concentration of sugar must approach but not quite reach the super-saturation point: the sugar concentration should be between 65 and 67% in weight. A lower percentage of sugar makes the syrup an excellent nutriment for yeast and other microorganisms. A sugar saturated syrup lead to crystallization of a part of the sugar under conditions of changing temperature.

2.2 Experimental design

On the basis of single-factor experiment for the syrup production, proper ranges of extraction temperature, extraction time and ratio of water to raw material were preliminarily determined. A three-level, three-variable BBD (software Design-Expert) was applied to determine the best combination of extraction variables for the production of syrup. Based on the investigations on single-factor experiment, the variables considered are extraction temperature, extraction time and ratio of water to raw material in this experimental design. Table 1 lists BBD matrix and the response values that were carried out for developing the model.
The whole design consisted of 17 experimental points carried out in random order. Five replicates (treatment 13–17) at the centre of the design were used to allow for estimation of a pure error sum of squares. The response value in each trial was average of duplicates.
Based on the experimental data, regression analysis was performed and was fitted into an empirical second-order polynomial model equation (1):

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^{2} + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j 1 \quad \textbf{(1)}$$

where Y is the response variable, A0, Ai, Aii, Aij are the regression coefficients of variables for intercept, linear, quadratic and interaction terms respectively, and Xi, Xj are independent variables (i – j). The coefficients of the second polynomial model and the responses obtained from each set of experimental design were subjected to multiple nonlinear regressions using software Design-Expert. The fitness of the polynomial model equation was expressed by the coefficient of determination R2, and its statistical significance was checked by F-test at a probability (P) of 0.001, 0.01 or 0.05. The significances of the regression coefficients were also tested by F-test.

Table 1: Independent variables and their levels used for Box Behnken design (BBD)

	-1	0	1
Extraction temperature °C	20	32.5	45
Extraction time (h)	1	2	3
Ratio of water to pulp	1	2	3

3. Results and discussion

3.1 Preliminary study

Single-factor experimental designs (extracting temperature, extracting times, and ratio of water to pulp) were carried out before RSM experiments, in order to determine the experimental fields.

3.1.1 Temperature

To study effect of different temperature on extraction yield of syrup, extraction process was carried out using the different extraction temperature of 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C when other extraction condition was as following: extraction time 2 h and ratio of water to pulp 2. The extraction yield of polysaccharides had been increasing when extraction temperature increased from 15 to 45 °C. As shown in Figure 1, the maximum yield (35.6%) of syrup was observed when extraction temperature was 40 °C.
syrup was observed when extraction temperature was 40 °C.

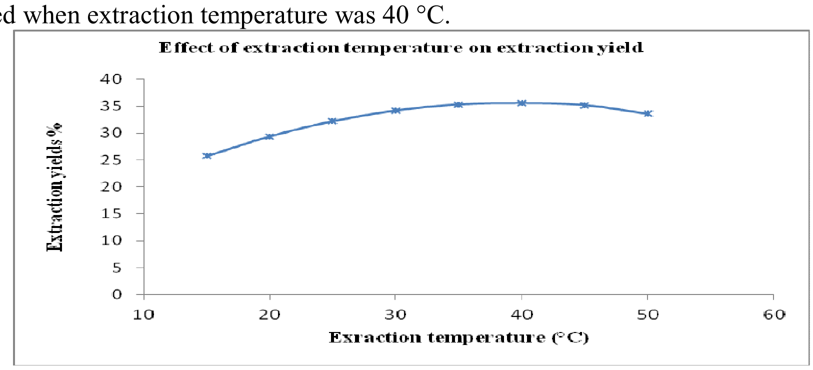

Figure 1. Effect of extraction temperature on extraction yield

3.1.2 Time

The effect of different time on extraction yield of syrup is shown in Figure 2. When extraction time varied from 0.5 to 2h, the variance of extraction yield was relatively rapid, and syrup yield reached a maximum at 2-3h, and then became stable as the extraction proceeded. This indicated that 3 h was sufficient to obtain maximum yield of syrup.

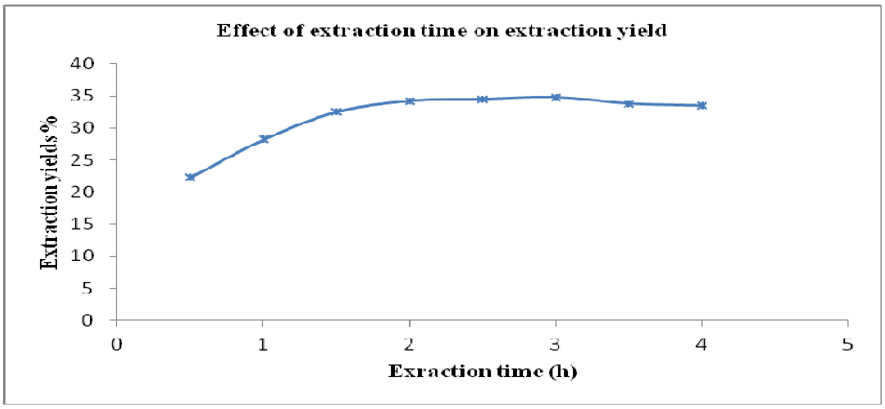

Figure 2. Effect of extraction time on extraction yield

3.1.3 Ratio of water to pulp

The effect of different ratio of water to pulp on extraction yield of syrup is shown in Figure 3. The extraction was carried out with ratios which vary between 1 and 4 under the following conditions of extraction: temperature = 32.5°C and Time = 2h**.**

Figure 3 shows that the syrup yield increased significantly from 28.03% to 37% as the ratio of water to the pulp increased from 1 to 4; this is due to the increase of the driving force for the mass transfer of polysaccharides (Bendahou et al. 2007). However, when the ratio continued to increase, the extraction yields no longer changed.

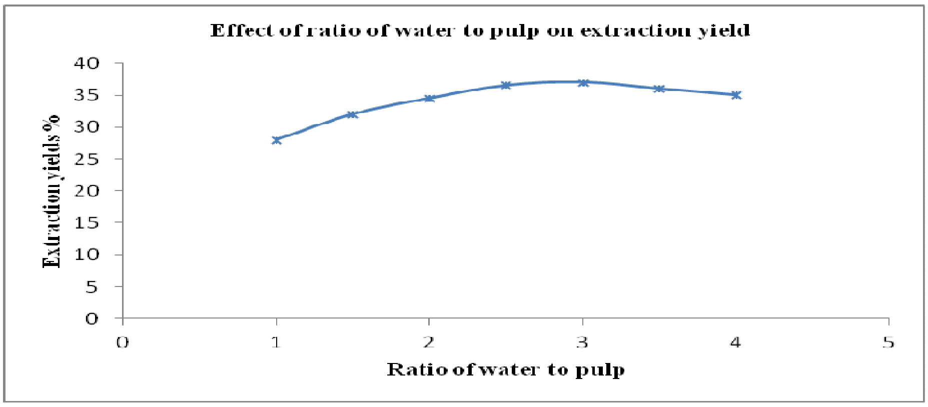

Figure 3. Effect of ratio of water to pulp on extraction yield

3.2 Predicted model and statistical analysis

Table 3 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized. The percentage yield ranged from 22.6% to 40.15%. The maximum value was found at the extraction temperature 45°C, extraction time 3h and ratio of water to pulp 2. The application of RSM offers, based on parameter estimates, an empirical relationship between the response variable (extraction yield of syrup) and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation (2):

$$Y=34.86 +2.81 * X_1 +3.13 * X_2 +4.44 * X_3 +5.55 * X_1 * X_2 +1.54 * X_1 * X_3 +1.68 * X * {}_2X_3 -2.65 * X_1 * X_1 - 3.51 * X_2 * X_2 - 2.39 * X_3 * X_3 \qquad (2)$$

Where X_1, X_2 and X_3 were the coded values of the test variables: extracting temperature (°C), extracting time (min) and ratio of water to pulp, respectively.

Table 2. The Box Behnken experimental design (in actual level of three variables) employed for production of syrup of carob

Run	Temperature	Time	Ratio of water to pulp	Syrup yield (%)	
	X1	X2	X3	Experimental	Predicted
1	-1	-1	0	28,36	28,31
2	1	-1	0	23,69	22,83
3	-1	1	0	22,6	23,45
4	1	1	0	40,15	40,19
5	-1	0	-1	24,42	24,1
6	1	0	-1	26,15	26,65
7	-1	0	1	30,42	29,91
8	1	0	1	38,3	38,61
9	0	-1	-1	22,72	23,07
10	0	1	-1	26,52	25,97
11	0	-1	1	28,06	28,6
12	0	1	1	38,56	38,2
13	0	0	0	35,1	34,86
14	0	0	0	34,25	34,86
15	0	0	0	35,26	34,86
16	0	0	0	35,21	34,86
17	0	0	0	34,5	34,86

The statistical significance of regression equation was checked by F-test, and the analysis of variance (ANOVA) for response surface quadratic polynomial model was done by software Design-Expert. The ANOVA of quadratic regression model demonstrated that the model was highly significant. And the Fisher's F-test had a very high model F-value (112.62) and a very low P-value ($P < 0.0001$). The value of R^2Adj (0.9843) for Eq. (3) is reasonably close to 1, and indicates a high degree of correlation between the observed and predicted values. A very low value of coefficient of the variation (C.V.) (2.41 %) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. The F-value (4.80) and P-value (0.0819) of lack-of-fit implied the lack-of-fit was not significant relative to the pure error. It indicates that the model equation is adequate for predicting the yield of syrup under any combination of values of the variables. The coefficient estimates of model equation, along with the corresponding P-values, were presented in Table 3. The P-values are used as a tool to check the significance of each coefficient, which also indicate the interaction strength between each independent variable. Smaller the P-value is, more significant the corresponding coefficient is (Muralidhar et al. 2001). When value of ''probability > F" is less than 0.05, the model terms is significant. It can be seen from Table 2 that all regression coefficients were highly significant, and X_1, X_2, X_3, $X_1 * X_2$, $X_1 * X_3$, $X_2 * X_3$, $X_1 * X_1$, $X_2 * X_2$, $X_3 * X_3$ were significant model terms.

Table 3. Test of significance for regression coefficients

Effect	Coefficient estimate	Standard error	F-value	P value
X_1	2,8112	0,2629	114,2882	< 0.0001
X_2	3,125	0,2629	141,2221	< 0.0001
X_3	4,4412	0,2629	285,2418	< 0.0001
X_1*X_1	5,555	0,3718	223,1212	< 0.0001
X_2*X_2	1,5375	0,3718	17,0923	0.0044
X_3*X_3	1,675	0,3718	20,2862	0.0028
X_1*X_2	-2,6532	0,3624	53,5803	0.0002
X_1*X_3	-3,5107	0,3624	93,8099	< 0.0001
X_2*X_3	-2,3882	0,3624	43,4118	0.0003

3.3 Analysis of response surfaces

The effects of the three factors as well as their interactive effects on the syrup yield are shown in Figure 4. Figure 4 denotes the three dimensional surfaces plots of effect of extraction temperature (X_1) and extraction time (X_2) on response. As can be seen, enhancing the extraction temperature (X_1) from15 to 45°C could increase the syrup yield. Enhancing the time (X_2) from 1 to 3h could also increase the syrup yield. It is concluded that a high syrup yield could be obtained by combining appropriate ratio of liquid to solid (X_2) and extraction time (X_1).

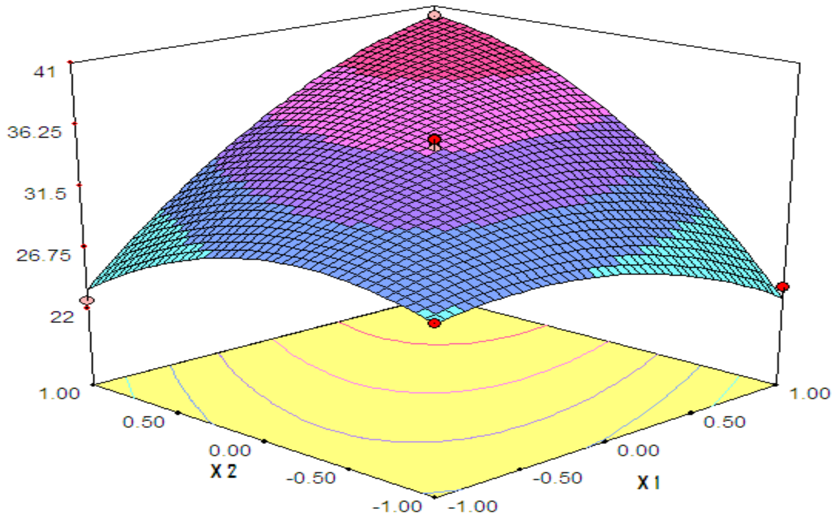

Figure 4. Response surface curve for syrup yield of carob showing the interaction between X_1 and X_2 at X_3 =0.

Figure. 5 depicts the effect of extraction time (X_1) and extraction temperature (X_3) on the syrup yield. It was observed that syrup yield increased with the increase in extraction time (X_1). Also, extraction rate increased with the increase in extraction temperature (X_3).

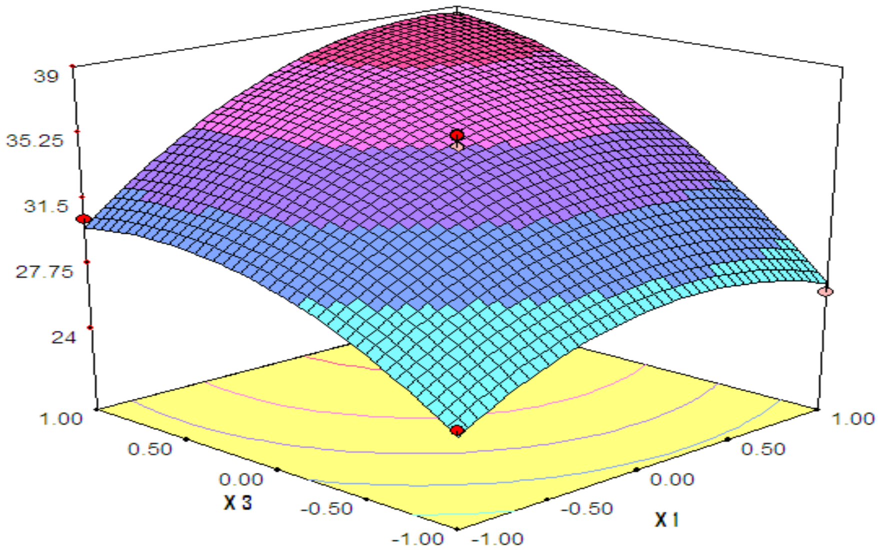

Figure 5. Response surface curve for syrup yield of carob showing the interaction between X_1 and X_3 at X_2 =0.

Figure 6 shows the effect of ratio of liquid to solid (X_3) and extraction time (X_2) on the syrup yield. It was observed that syrup yield increased with the increase in ratio of liquid to solid (X_3). Also, extraction rate increased with the increase in extraction time (X_2).

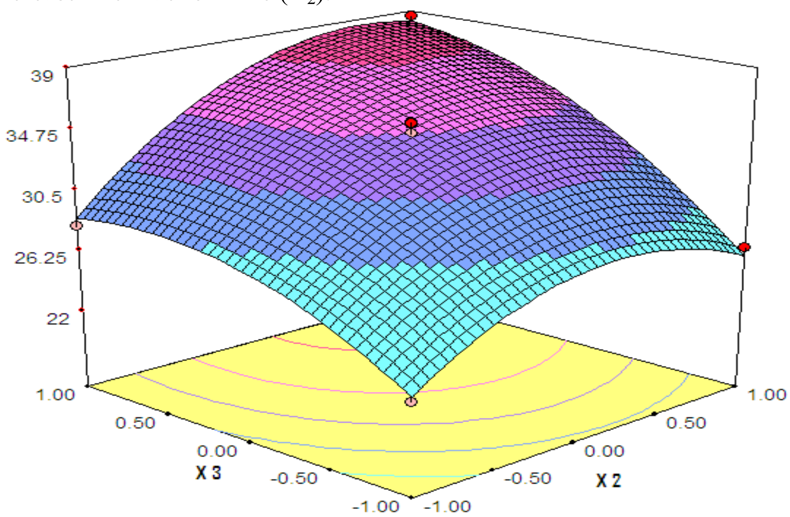

Figure 6. Response surface curve for syrup yield of carob showing the interaction between X_2 and X_3 at X_1 =0.

3.4 Verification of predictive model

To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 38.73 ± 1.72 (N = 3), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization

4. Conclusion

The performance of the production of the syrup from carob pulp was studied with a statistical method based on the response surface methodology in order to identify and quantify the variables which may maximize the yield of syrup. The three variables chosen, namely extraction temperature, extraction time, and ratio of water to pulp all have a positive influence on the yield of syrup using the extraction method.

Through optimization, the optimal conditions for the production of syrup are as the following: extracting temperature 43.45°C, extracting time 2.40 h and the ratio of water to pulp 2.27. Under these conditions, the experimental yield of syrup was 38.73%.

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