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Effect of Water Treatment and Optimization of Extraction Applied to the Carob Pulp

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ABSTRACT-The objective of this study was initially to determine the effect of water treatment on the chemical composition of the carob pulp. After this treatment, the pulp is devoid of most of its sugar, which makes grinding without clogging or thermal decomposition, that's what we showed in our recently patented process. The powder thus obtained, called carob pulp powder, can be used in the food industry, especially in the chocolate industry, as they contain less fat than cocoa. The aqueous extract can be used for the preparation of fruit juices, honey or carob syrup, as well as for the production of bioethanol.

In a second step the entire process of extracting sugar from the pulp was studied in this work. Thus, the response surface methodology was used to optimize the parameters of extraction was affected by the temperature, time, the ratio (L/S) (liquid/solid) and the size of the particles forming the pulp. Indeed the best optimal conditions to extract as much sugar opted for a process involving a ratio (L/S) equal to 10 for a particle size of 500 microns and a heating temperature of 60°C for 180 min.

Keywords - Carob pulp, Extraction, Total sugar, Optimization.

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1. INTRODUCTION

Carob (Ceratonia Siliqua L) is a native plant of Mediterranean counties including Spain, Morocco, Italy, Portugal, Greece, Algeria and Tunisia, where is grown mostly on poor calcareous soils [1,2]. Carob belongs to the caeselpinacea subfamily of the family leguminous (syn Fabaceae) [1,3]. It is used for various purposes including industry, forestation, prevention of soil erosion, as ornamentals, for human nutrition and for animal feed [4,5].

Several products are produced from the seed and pod of carob, and the economic importance of the crop results from the use, by industry, of locust bean gum that is obtained from the seed. the pod of the carob has long been used as feed for livestock and human consumption, including sweets, biscuits, and traditional carob concentrate (karcacier et al 1995).when the fruits are ripe enough to be harvested .It has 91-92% total dry matter and 62-67% total soluble solids, which consists of 34 -42 % sucrose, 10-12% fructose and 7-10% glucose [6].

In Morocco wild carob trees are estimated at about 25000 Hectares and are found widely uncultivated in many areas, especially in the north, atlas and south of Morocco [7].

In the last years, an increase in carob plantations has been realized; such increase is attributed to the drought resistance characteristics of carob [8].

Carob is used in many Arab countries to make a popular drink which is consumed mainly in the month of Ramadan; carob is also used in preparation of special tradition types of Arabic confectionery.

The carob contains a high level of sugar and tannin for that it is difficult to prepare a milling by grinding pulp, so the objectives of this work are to make easy the milling of pulp after appropriate aqueous treatment in two forms and optimizing the carob water extraction using the response surface methodology which was introduced for the first time by Box and Wilson in 1951 [9].

We remember that response surface methodology is an experimental strategy for optimizing the best conditions for a multivariable system. This method had been successfully applied in the optimization of medium compositions [10], conditions of enzymatic hydrolysis [11] and fermentation processes [12].

Conditions of aqueous extraction and hydrolysis of sugar content in water extract was carried out on applying statically methods.

It should be noted that this work is a continuation of work that we have recently reported on the valuation of the carob pulp (patent), the determination of the chemical composition and morphological study of the carob pod and of optimizing the conditions of hydrolysis of the extract [13].

2. MATERIALS AND METHODS

The study was carried out using a chopped and deseeded carob from Chefchaouen location .

The sample were harvested during September from different regions of Chefchaouen, and carob pods were of the same physiological maturity (dark brown) and uniform shope and size.

The sampling was realized from each natural area, the elementary samples (100g per sample) were combined in order to provide the composition samples of 1kg.

Samples were stored at room temperature in the suitable conditions, the seeds were removed and the pulp were ground in different particle size.

2.1 Cold aqueous extraction

This extraction was carried out at ambient temperature in pulp to water ratio (L/S=4), during twenty four hours. Also, it can reveal about the interaction between variables, give the required information for design and process optimization and give multiple responses at the same time.

The aim of this work was to optimize Carob resulted after water extraction was sundried in open for six hours, to reduce the moisture content to 8-10 %.

The sundried pulp was used further for chemicals analysis.

2.2 Warm aqueous extraction

This investigation was realized at 80°C for two hours in 1:4 dilution rate (carob: water ratio). The restituted of its major compounds, sundried in open for six hours in order to subtract its moisture content to low level.

2.3 General methods

Total ash: The ash content of was determined gravimetrically after dry mineralization using the International Standard method [**14**].

Total fat: Lipids from the carob (sample weight about 2g) were determined by using 150ml of hexane during six hours. Lipids were extracted in soxhlet apparatus, the extraction beakers (containing the extracted lipids) were dried in oven at 103°C before being weighed. The determination was carried out according to the AFNOR method [**15**]. NF V 03-905/ December 1973.

Protein: The crude protein (N.6.25) content was determined according to Kjeldahl method from 0.5g of carob. The protein amount evaluated by the International standard method [**16**]. ISO 8968-3: 2004.

Crude fiber: The crude fiber of the carob was carried out gravimetrically after a double treatments (acid and basic). This determination was carried out using the International method descript in regulation of European Unit [**1 7**].

HPLC analysis of sugar: The study was carried using grinded and deseeded carob pod sieved 0.25 µm . The sugar extraction was realized with cold water, weight sample was immersed in the adequate amount of water and mechanically shook in open flask at ambient temperature (20-25°C) until extraction equilibrium.

The standard was obtained from JANSSEN CHIMICA and LABOSI. The sugars are determined by the method as described in AOAC [**18**].

Total and reducing sugar: Sample weight is added with two agents of defecation, complete with distilled water until the gauge (100ml), after filter the solution through whatman filter paper n°2.

Reducing and total sugar were determined according to Luff-Shoorl as described [**19**].

Tannins: Tannin content was determined with gravimetric method using the cupper acetate such an agent of association with phenol compounds. The determination was carried out according to method [**20**].

Caffeine: The caffeine was extracted in a soxhlet apparatus with the chloroform; the residue is taken with the warm distilled water, and then filtered through Millipore filter 0.45µm before injecting in liquid chromatograph. The caffeine is determined by the method as described in analysis [**21**].

Theobromine: Two grams of grinding carob were immersed in the adequate amount of water. Shake vigorously for 30min in order to exhaust theobromine content from matrix; the slurry was analyzed by HPLC according to appropriate method [**22**].

2.4 Optimization of extraction and hydrolysis carob sugar

2.4.1 Aqueous extraction test

Sugar extraction from carob pods (S) were carried out with water (L) at different (L/S) ratio (4, 5, 7.5, 10 and 15) total reflux.

In this part of study, we test other parameters, which can affected total sugar yield during extraction such temperature, extraction time and particle size of milled carob. A weight sample of shopped and grinded carob pod was immersed in sufficient amount of distilled water at different temperature (60- 90°C) and varying a time of extraction from 30min to 150min, the particle size was tested ranging from 125µm to 1000µm .

The slurry was filtered and analyzed for its amount of total sugar and used for hydrolysis tests. The yield of total sugar in the extract is calculated by the following equation described and exploited by S. Sanchez et al,

Yield T.S (w/w) = (Total sugar in the solution / Total sugar amount of grinded carob pod). 100

2.4.2 Experimental design method

In order to find the optimal conditions for carob extraction and hydrolysis of sugar fraction, we test many parameters such time, particle size, temperature, dilution rate influencing the amount of total sugar. The key factors affecting the total sugar yield must be evaluated and subjected to different treatments. The principal factors were optimized using Response Surface Methodology design

3. RESULTS AND DISCUSSION

This study was composed from two parts; the first was axed on warm and cold aqueous extraction and its effect on carob chemical composition. The second part studied all parameters from extraction to hydrolysis in order to optimize the best total sugar yield.

3.1 Cold and warm extraction

Results of those special treatments are given in table1.

As can be shown in this table, there is a significant decrease in sugar fraction and tannin but crude fiber amount decrease slightly, this diminution of sugar content make easy the grinding of carob and to ovoid the pulp to be warped.

However, a significant difference can be at crude fiber and tannin amount which are important in treated in water cold extraction than warm aqueous form.

Table 1: Composition before and after aqueous treatment

Determinations	Brut carob	Cold extraction	Warm extraction
Ash	3.0%	2.5%	2.1%
Crude fiber	6.9%	11.7%	9.3%
Reducing sugar	83.7mg/g	58mg/g	64mg/g
Total sugar	516.7mg/g	350mg/g	380mg/g
Fructose	7.18%	4.7%	5.2%
Glucose	2.2%	0.6%	2.0%
Sucrose	44.6%	21.0%	28.3%
Protein	2.74%	2.9%	3.0%
Tannins	6.7%	7.3%	3.3%

3.2. Optimization of extraction

Aqueous extraction optimization

a- Effect of particle size: Generally, the extraction is a contact equilibrium process and the specific surface increase when the particle size is low; the diffusion of soluble solids from into extract was favored from shopped pulp to 125µm particle size.

b- Effect of time on extraction: The optimal time of extraction is two hours in the present extraction conditions limited by temperature, dilution rate and particle size. The yields of extraction depends mainly on temperature, higher extract concentration is attaint with higher temperature.
When the temperature of the process was increased the extraction time decreases by Karkacier and al. [6]. Obviously, the diffusion of soluble solids from matrix was influenced by temperature Turhan and al. [4].

c- Effect of dilution rate: In order to analyze the effect of carob pod(S) to water (L) on the efficiency of the extraction process were carried with different ratio L/S ranging from 4 to 15 at 80°C .

It was also found that higher total sugar extraction yields were achieved using 10 L/S ratio. The result obtained was slightly over than found by Sanchez and al. [23].

It could be seen that the residual sugar concentration decreased with deceasing dilution rate. In contract, the amount of total sugar obtained was 82.3g/l.

d- Effect of temperature on extraction: When the temperature of extraction process increased, sugar concentration in the extract increased significantly during extraction.

When compared with 60°C and 90°C of extraction at 7.5 dilution rate and the same time 1 hour, more sugar concentration in the extract was obtained at 90°c (79.2 g/l) than 60°C (71.9g/l).

Obviously, the diffusion of soluble solids from matrix was affected by temperature [4].

It was deduced that temperature played a significant part in extraction of residual sugars from carob.

e- Optimization of parameters extraction: As we noted above, we have indicate that we applied the methodology response surface.

A total of 30 experiments with combinations of time, temperature, dilution rate and particle size were conducted.

A central composite design with five levels for all 4 factors: temperature (A); time (B); dilution rate (C) and particle size (D) were used for this study.

The range of variables is given in table 2. The experimental design and results obtained from experiments are shown in table 3. The results of these experiments were fitted with a second order polynomial equation. The values of regression coefficient were calculated, and the fitted equation (in terms of coded values) for predicting yield extraction (Y) was as given below:

Yield extraction = 124,635137 - 0,09059238 . **D** - 0,64183218 . **B** - 0,0189818 . **A** - 15,158674 . **C** + 0,883279 E^{-3} . **D . B** + 0,501092E^{-3} . **D . A** + 0,008194082 . **B . A** + 0,012161300 . **D . C** + 0,201029425 . **B . C** + 0,183901041 . **A . C** - 0,76707 E^{-5} . **D . B . A** - 0,16094 E^{-3} . **D . B . C** - 0,12584 E^{-3}. **D.A .C** - 0,00212368 . **B . A .C** + 0,163353 E^{-5}. **D.B.A.C** + 0,105716E^{-4} . $\mathbf{D}^2$ - 0,00122652 . $\mathbf{B}^2$ - 0,00422865 . $\mathbf{A}^2$ - 0,09869843 . $\mathbf{C}^2$

Table 2: Values of coded levels used for the experimental design

Factors	**Symbols**	**Actual levels of coded factors**		
		-1	0	1
Temperature	A	60	70	90
Time	B	60	90	150
Dilution rate	C	4	7,5	15
Particle size	D	125	500	1000

Table 3: Central composite design for the experimental design and results

Particle size (µm)	**Time (min)**	**T °C**	**Dilution rate**	**Yield extraction %**	**Particle size (µm)**	**Time (min)**	**T °C**	**Dilution rate**	**Yield extraction %**
125	60	90	7,5	96,3	500	90	70	7,5	85,6
500	60	90	7,5	85,7	125	90	80	5	93,4
1000	60	90	7,5	76,4	250	60	90	8	90
500	150	70	4	78,5	250	90	80	5	89,9
125	150	90	4	82,6	1500	30	60	15	65
125	150	70	4	84,2	1500	120	60	10	74,9
500	60	90	7,5	83,9	250	60	70	4	87,5
1000	90	60	15	72,4	250	90	80	8	91,7
125	60	60	4	89,2	250	120	90	15	90,9
125	60	90	4	93,1	1500	60	60	4	70,9
500	90	90	7,5	85,4	1000	90	60	15	72,4
1000	90	90	4	75,9	125	30	60	4	89,2
500	150	60	4	79,3	500	150	60	5	82,7
500	60	60	4	80,9	500	60	60	4	80.9
500	150	90	7,5	82,4	500	30	90	8	80,9

Values, mean of three independent extractions and determinations (n=3)

The analysis of variance for the response surface quadratic model is shown in table 4. The latter allows to deduce the coefficients of the mathematical model of response listed in table 5 where the statistical significance of the regression model was checked by F-test.

The mathematic model was perfectly significant, as mentioned by the F-value and the probability value (P>F)= 0.000523. The regression represents the goodness of fit by coefficient (R^2), in this part of study, the R^2 is 0.95.

This result concerning the coefficient of regression indicated that the response model can explain about 95 % of the total variations. Generally, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation [**24**].

For the adjusted model, the coefficient (R^2_{adj} =0.84) was high enough to indicate the significance of the model.

Using the contour plot presentation (Figure 2) and the second order polynomial equation meaning the Excel program, the optimum of extraction parameters was A= 60°C, B= 180min, C= 10 and D = 500µm (Tableau 7).

This values represent an economic extraction process of sugar, because the temperature was the lowest and the particle size was the middle of test range which doesn't need a particularly preparation of carob.

The validity of mathematical model and the existence of the optimum point of extraction were confirmed by the results in table 6 and the plot in figure 1, whitch reflects the best agreement between predicted and experimental values. We have also recently applied this methodology to optimize hydrolysis parameters: T°C (72°C), t (24 min) et [H_2SO_4] (1,2 % v/v) [13c].

Then we report in this study a new parameter was introduced and tested in the range 125µm -1500µm and the results concerning extraction parameters found in this investigation are very economic than those published by Turhan [4] and Sanchez [23].

Table 4: Analysis of variance for response surface quadratic model obtained from experimental results

Effect	Degree of freedom	F (Fischer)	P (Probability)
Intercept	1	2,254856	0,164095
Particule size (D)	1	0,086055	0,775251
Time (B)	1	0,469808	0,508652
Temperature (A)	1	0,000124	0,991319
Ratio (C)	1	2,006924	0,186973
D . B	1	0,065876	0,802642
D . A	1	0,009871	0,922821
B . A	1	0,379976	0,551390
D . C	1	0,114317	0,742273
B . C	1	2,264290	0,163299
A . C	1	1,761377	0,213956
D . B . A	1	0,020019	0,890293
D . B . C	1	0,182424	0,678348
D . A . C	1	0,045046	0,836183
B . A . C	1	1,643656	0,228749
D . B . A . C	1	0,074515	0,790427
D^2	1	4,075405	0,071127
B^2	1	2,038408	0,183845
A^2	1	0,204603	0,660684
C^2	1	1,248630	0,289941

Table 5: Coefficients of mathematical model of the response

Dependant Variable	Multiple R2	Adjusted R2	SS	df	MS	SS	df	F	p
Extraction	0,945519	0,842006	1828,690	19	96,24684	105,3686	10	9,134299	0,000523

Table 6: Analysis of variance of the quadratic response surface from the experimental results

Observed, Predicted, and Residual Values (Spreadsheet1)

Sigma-restricted parameterization(Analysis sample)

Yield extraction	Yield extraction PREDICT	Yield extraction RESIDUAL
96,3	95,26854	1,03146
85,7	85,02760	0,67240
76,4	75,99808	0,40192
78,5	79,04068	-0,54068
82,6	82,82200	-0,22200
84,2	83,35503	0,84497
83,9	85,02760	-1,12760
72,4	72,44524	-0,04524
89,2	90,62453	-1,42453
93,1	92,28889	0,81111
85,4	85,93189	-0,53189
75,9	75,67164	0,22836
79,3	78,68644	0,61356
80,9	81,17582	-0,27582
82,4	81,11728	1,28272
85,6	86,48277	-0,88277
93,4	93,15339	0,24661
90.0	91,52453	-1,52453
89,9	89,97589	-0,07589
65.0	65,01235	-0,01235
74,9	74,83833	0,06167
87,5	88,45704	-0,95704
91,7	92,38729	-0,68729
90,9	90,92852	-0,02852
70,9	70,51522	0,38478
72,4	72,44524	-0,04524
89,2	88,96625	0,23375
82,7	82,30067	0,39933
80,9	81,17582	-0,27582
81,6	81,91556	-0,31556

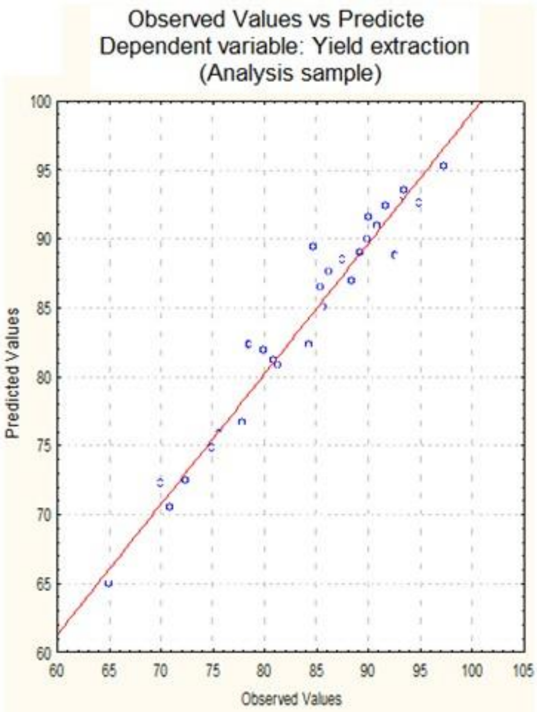
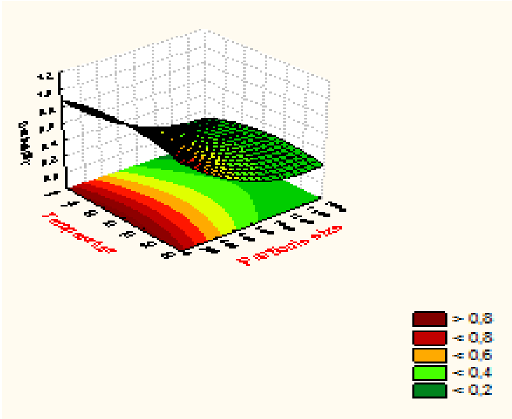


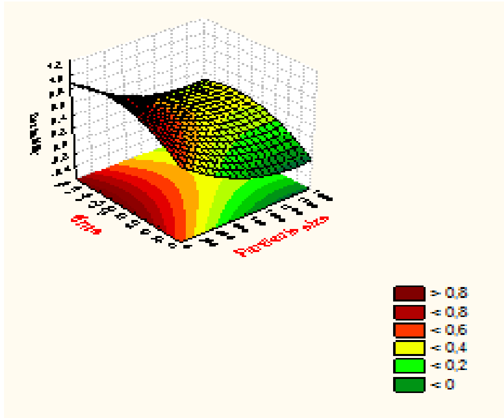
Figure 1: Predicted values = f (Observed values)

Table 7: Values of parameters and yield of extraction

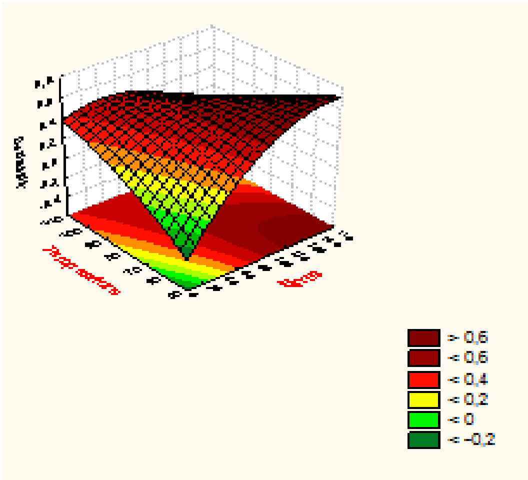
Optimisation extraction				
A	**B**	**C**	**D**	**Yield extraction prediction**
60	120	7.5	125	98,54
60	130	6	150	93,33
60	140	7.5	170	98,74
60	160	7	190	96,06
90	180	9	200	81,98
80	180	10	250	92,27
70	120	6	300	89,91
90	160	7	350	81,34
80	140	7.5	400	87,88
60	180	10	500	99,75



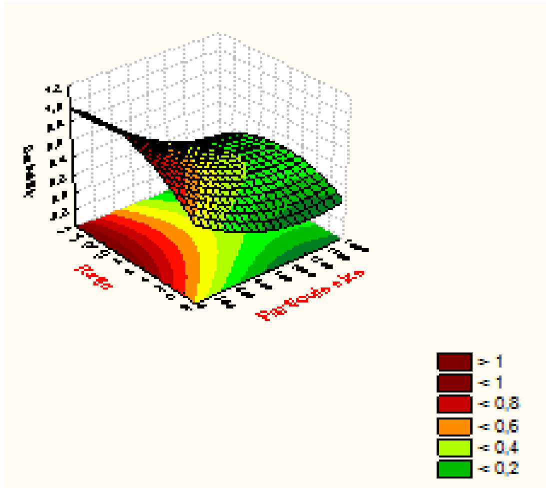
Response surface plot T(°C)vs.Particle size on Total sugars extractionYield



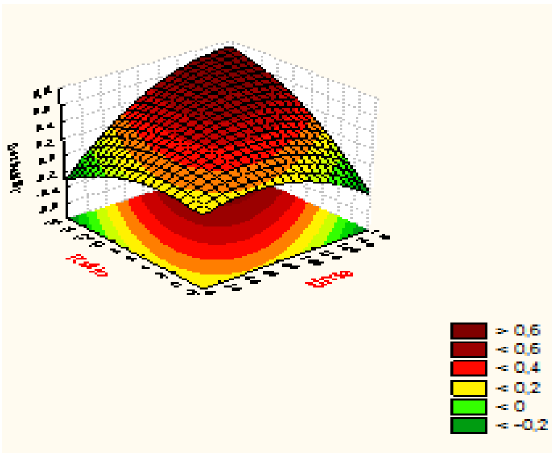
Response surface plot time(mn)vs.Particle size on Total sugars extractionYield



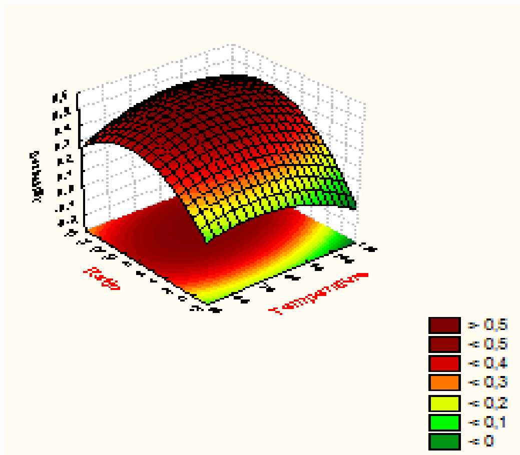
Response surface plot T(°C)vs.time on Total sugars extractionYield



Response surface plot ratio vs. Particle size on Total sugars extractionYield



Response surface plot Ratio vs. Time (mn) on Total sugars extractionYield



Response surface plot Ratio vs. T (°C) on Total sugars extractionYield

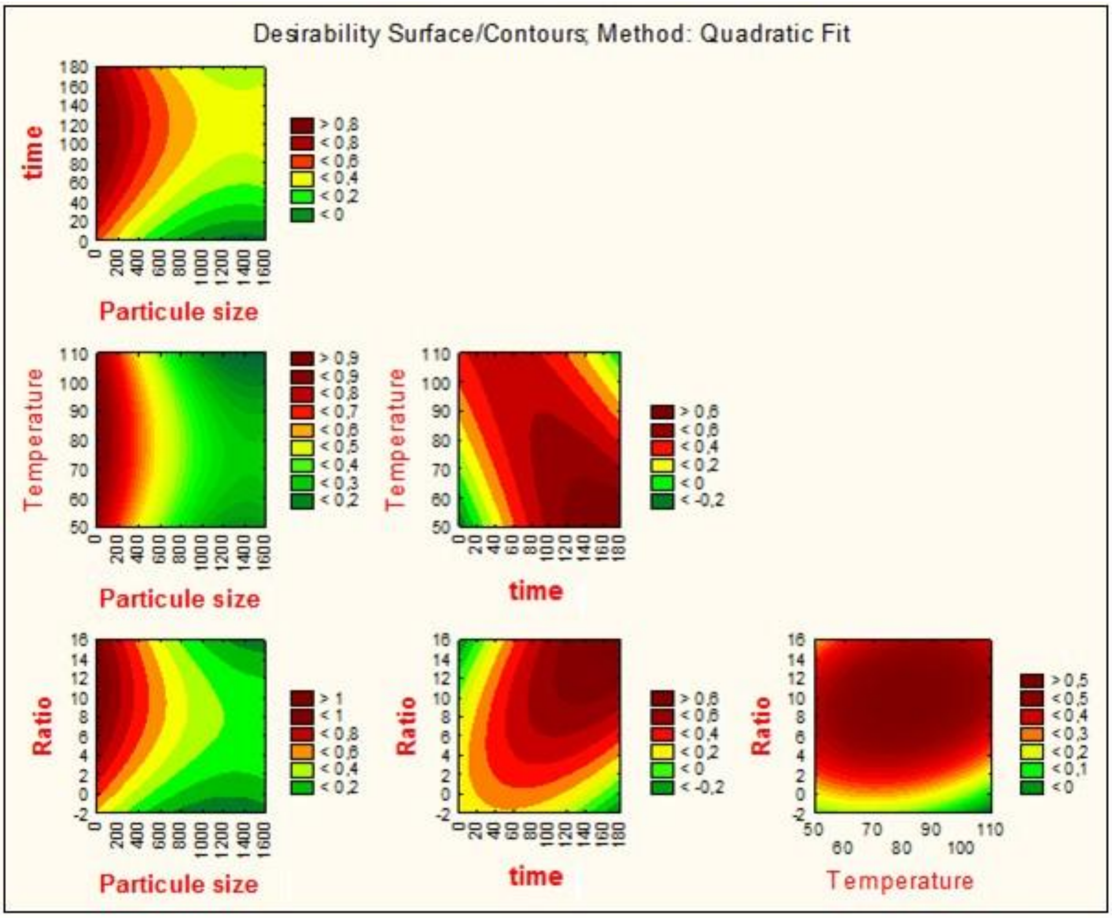


Figure 2: "Contour plot" presentations

4. CONCLUSION

Statistical optimization of extraction medium could overcome the limitations of classical methods and it was justified to be an efficiency tool for the optimization of total sugar production that the literature showed that carob pod can be a good feedstock for aqueous sugar extraction because it contains about sugar 53%.

Response surface methodology was suggested to study the combined effects of extraction parameters.

From data, the interactions between the independent variables with the responses (extraction yield) was appreciated. Therefore the optimum aqueous extraction parameters are as follows: temperature 60°C, time 180 min, dilution rate 10 and particle size 500µm.

The accuracy of models was verified by the statistic parameters deduced for experiments results and the residual values showed the important correlation between the experimental and predicted values.

5. ACKNOWLEDGMENT

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