Hindawi Publishing Corporation
Journal of Chemistry
Volume 2014, Article ID 627103, 13 pages
http://dx.doi.org/10.1155/2014/627103

Research Article

Optimization of Insoluble and Soluble Fibres Extraction from *Agave americana* L. Using Response Surface Methodology

Amine Bouaziz,[1] Manel Masmoudi,[1] Amel Kamoun,[2] and Souhail Besbes[1]

[1] *Université de Sfax, Laboratoire Analyses, Valorisation et Sécurité des Aliments, Ecole Nationale d'Ingénieurs de Sfax, Route de Soukra, 3038 Sfax, Tunisia*
[2] *Université de Sfax, Laboratoire de Chimie Industrielle, Ecole Nationale d'Ingénieurs de Sfax, Route de Soukra, 3038 Sfax, Tunisia*

Correspondence should be addressed to Manel Masmoudi; manel_masmoudi@yahoo.fr

Received 30 May 2013; Accepted 11 October 2013; Published 5 January 2014

Academic Editor: Christophe Blecker

Experimental design methodology was used to determine significant factors affecting the extraction yield of soluble and insoluble fibres from *Agave americana* L. and in second time to find optimum conditions leading to the highest yield. Results clearly indicated that the temperature, the powder to water (P/W) ratio, and the agitation speed were the most important factors influencing fibres extraction yield which increased with temperature, P/W ratio, and agitation speed. Ionic strength affected significantly soluble fibre extraction yield and was the most important factor among nonsignificant ones influencing insoluble fibres extraction yield. Then, a Box-Behnken design was carried out to maximise fibres extraction. Selected optimal conditions were temperature: 90°C; P/W ratio: 0.1625; agitation speed: 400 rpm; and ionic strength: 1.5 g/L. These conditions yielded 93.02% and 80.46% of insoluble and soluble fibres, respectively. Concentrates showed high fibres purity and good functional properties.

1. Introduction

The Agavaceae is a plant family with nine genera and about 293 species. *Agave*, a monocotyledonous and monocarpic plant, is the most important genus with about 166 species [1, 2]. It is a voluminous, herbaceous, and perennial plant with long, succulent spiny leaves growing directly out from the central stalk to form a dense rosette. Its floral stalk, sometimes termed the trunk, can reach 10 to 20 m of length [3, 4].

The most important diversity center is the Mexican territory, with species spread from southwestern United States through Central America, the Caribbean and into northern South America [1]. Plants were taken from there to Europe, Africa, and the Far-East by the Spanish and Portuguese, where they naturalized rapidly, especially in the high arid regions around the shores of the Mediterranean [5]. They can prosper there due to their shallow rooting system and succulent morphology, while traditional annual crops cannot [6].

In Tunisia, the *Agave americana* L. is the most abundant variety of *Agave* but it has never been exploited, while it is worldwide used for commercial (rope, paper, fibres, pectin, mezcal, aguamiel, pulque, and tequila), ornamental (yucca, century plant and mother-in-law's tongue) and medicinal applications (steroid extraction and antibacterial salves) [2–7].

Plant's leaves are characterized by the abundance of agrate bundles of short fibres that are good holders of water, which gives the *Agave*'s leaves their rigidity and succulence [4]. Like most natural fibres, *Agave* fibrous bundles are composed mainly of α-cellulose (64.8%), lignin (15.9%), and hemicelluloses (5.1%) and also can contain low content of waxes and ash [4].

In *Agave* species (CAM plants), fructans are reserve carbohydrates and they are synthesized and stored in the stems. The main function of these fructose polymers is storage before flowering and acts as osmoprotectants during drought [8].

Nowadays, fibres have a capital interest due to their physiological and nutritional roles. Besides, high-fibres diets are associated with the prevention and treatment of some

diseases such as constipation, colon cancer, coronary heart disease, diverticulosis and diabetes [9, 10]. Dietary fibres may be divided into two parts when they are dispersed in water: soluble and insoluble fractions. The latter one is related to both water absorption and intestinal regulation, whereas, soluble fibres are associated with the reduction of cholesterol in blood and the diminution in the intestinal absorption of glucose [9].

Incorporation of dietary fibres into a wide range of products will contribute to the development of value-added foods or functional foods that currently are in high demand [11]. In addition to the physiological benefits provided by high fibre foods, studies have shown that fibres components can give textural, gelling, thickening, emulsifying, and stabilizing properties to certain foods. By understanding functional properties of dietary fibres, one can increase their use in food applications and aid in developing food products with high consumer acceptance [10].

The aim of the present work is to look for the experimental conditions leading to the maximum extraction yield of soluble and insoluble fibres fractions from basal part of leaves of *Agave americana* L. using experimental design methodology. As many factors can influence the extraction yield, a step of screening was firstly applied in order to retain statistically significant factors. Then, a response surface methodology (RSM) was undertaken to fit and exploit a mathematical model representing relationship between the response (extraction yield) and variables retained in the first step of optimization.

2. Material and Methods

2.1. Samples. *Agave americana*'s leaves were procured from Essghar region (Sfax, Tunisia). After elimination of chlorophyllous cuticle, leaves were cut into regular pieces, rinsed with water, dried for 72 h at 50°C, milled twice to obtain a very fine powder and preserved at −20°C prior to analysis and extraction.

2.2. Dietary Fibre Extraction. Figure 1 shows the extraction process from *Agave*'s leaves powder to produce DF concentrates. Hot water was used to extract DF from milled leaves in a jar, homogenised using a mechanical stirrer 2021 (Heidolph rzr, Metrohm, USA) and maintained in a thermostatic bath (Raypa, Spain); operating conditions were fixed by the experimentation's matrix. After solubilisation of free sugars and fructans, insoluble DFs were recuperated by centrifugation (6500 g, 10 min) using a 4K15 centrifuge (Sigma, Osterode, Germany) [12]. Its concentration was carried out by a succession of five rinsings (water at 40°C) and of five centrifugations until the residue was free of sugars. In the other hand, soluble DFs (fructans) were settled overnight in ethanol 95% and then recuperated by centrifugation (6500 g, 10 min). Its concentration was carried out by rinsing three times with 50%, 70% and 100% ethanol [13]. The residues obtained were freeze-dried in a Benchtop 3 L freeze dryer (Virtis, Gardiner, NY) to give the DF concentrates and stored at 3°C for subsequent analyses.

2.3. Physicochemical Analyses of Raw Material and DF Concentrates. All values given were the mean of three replications and were expressed as the mean ± standard deviation ($\overline{x}$ ± SD).

2.3.1. Dry Matter. Dry matter was determined by oven-drying at 105°C to constant weight [14].

2.3.2. pH. The pH of 10% aquatic suspension of *Agave* powder and all other solutions was determined potentiometrically with a pH meter MP 220 (Mettler toledo, Barcelona, Spain) according to NF 05-108 [15].

2.3.3. Water Activity (aw). The water activity was determined at 25°C by a Novasina AW SPRINT TH-500 apparatus.

2.3.4. Ash and Mineral Content. Ash content was determined by incinerating sample at 550°C for 8 h. The total ash was expressed as the percent of dry weight [16]. The residue was digested with a nitric/perchloric acid (2 : 1, v/v) mixture and then adjusted to 50 mL with ultrapure water [12–17]. Mineral constituents (K, Ca, Na, Mg, Zn, and Cu) were analyzed separately using an atomic absorption spectrophotometer (analytik-jena ZEEnit 700, Germany) [14].

2.3.5. Protein. Total nitrogen was determined by the Kjeldahl method [15]. Protein was calculated using the general factor (6.25).

2.3.6. Total Fat. Crude fat was estimated by Soxhlet extraction with hexane over a period of 8 h [18].

2.3.7. Reducing Sugars. Reducing sugars were determined according to Bertrand and Thomas method. This latter is well described in detail by Blanchard [19].

2.3.8. Total Sugars. Soluble sugars concentration was determined by the phenol-sulphuric acid method [20] after ethanol extraction. Insoluble sugars fraction was submitted to a hydrochloric acid digestion for 2 h at 60°C. Released sugars were also determined by the phenol-sulphuric acid method.

2.3.9. Dietary Fibres. Insoluble and soluble dietary fibres (DF) were determined according to the AOAC enzymatic-gravimetric method of Prosky et al. [21]. Briefly, samples were gelatinized in phosphate buffer with heat-stable alpha amylase (A-3306, Sigma-Aldrich Chemical Co., St. Louis, USA) (100°C, pH 6, 15 min) and then enzymatically digested with a protease (P-3910, Sigma-Aldrich Chemical Co., St. Louis, USA) (60°C, pH 7.5, 30 min), followed by incubation with amyloglucosidase (A-3042, Sigma-Aldrich Chemical Co., St. Louis, USA) (60°C, pH 4.5, 30 min) to remove protein and starch. Then, samples were filtered, washed (with water, 95% ethanol, and acetone), dried, and weighed to determine insoluble fibres content. Four volumes of 95% ethanol (preheated to 60°C) were added to the filtrate and to the water washings. Then, precipitates were filtered and

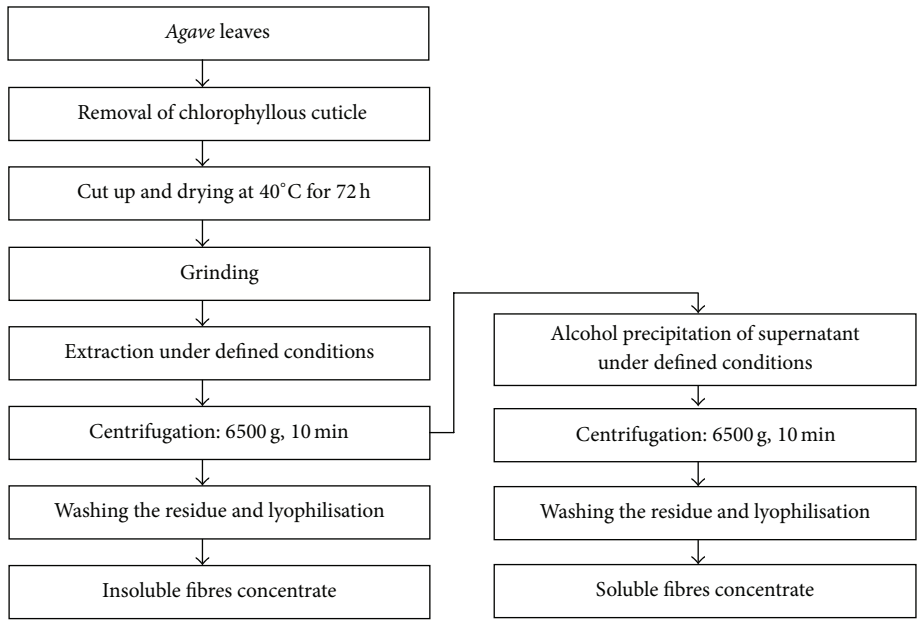

Figure 1: Elaboration process of insoluble and soluble fibres concentrates from *Agave americana* L.

washed with 78% ethanol, 95% ethanol, and acetone. After that, the residues (soluble DF) were dried and weighed. The obtained values were corrected for ash and protein. Total DF was determined by summing insoluble and soluble DF.

2.3.10. Microstructure Visualization. DF shape and the surface morphology at the micrometer scale were investigated with a scanning electron microscope (SEM), XL30 type ESEM-FEG (Philips/FEI) at 20 kV, using a working distance of 10.0 mm. DF concentrates were metalized with a gold/palladium coating of thickness $l = 4$ nm using a BALTEC MED 020 device prior to observation [7]. The detection system used was a back-scattered electron detector (BSE) [22, 23].

2.3.11. Water Holding Capacity. Water holding capacity (WHC) was determined using the method described by Macconnell et al. [24]. Hundred milligrams of DF concentrates was added to 10 mL of distilled water in a 50 mL centrifuge tube and stirred overnight at 4°C. Then the mixture was centrifuged at 14000 g for 20 min. The free water was decanted and absorbed water was then determined.

2.3.12. Oil Holding Capacity. Oil holding capacity (OHC) was measured using the method described by Caprez et al. [25]. Hundred milligrams of DF concentrates was added to 10 mL of corn oil in a 50 mL centrifuge tube. The content was stirred, and then the tubes were centrifuged at 1500 g for 30 min. The free oil was decanted and the absorbed oil was determined.

2.4. Experimental Methodology. When many factors affect a desired response, it can be an exhausting task to optimize a process [13]. Therefore, screening step seems to be indispensable to retain only significant factors influencing fibre extraction yield, while response surface methodology (RSM) can be an effective tool for optimizing the response. Screening allows the investigation of up to $N - 1$ variables with N experiments using a fractional factorial design such as Plackett-Burman design [26]. Response surface methodology is defined as a statistical method that uses quantitative data from appropriate experimental design to determine optimal conditions and predict the optimum response. Box-Behnken design (BBD), one of RSM, is more efficient and easier for arranging and interpreting experiments in comparison with others [27].

In the present study, experimental design software NEMROD-W [28] was used in order to select significant factors and look for the best experimental conditions leading to the highest fibres extraction yield.

2.4.1. Screening Step. Two Plackett-Burman designs were used to select significant factors, starting from six factors (U_1 to U_6) for the first design and eight factors (U_1 to U_8) for the second one, chosen to evaluate their effect on insoluble and soluble fibres extraction yield, respectively. Table 1 shows different factors and the two levels (+1) and (−1) of each variable used for screening.

2.4.2. Response Surface Methodology. The fibres extraction procedure was optimized using the RSM. This latter has been extensively utilized to optimize culture conditions and medium composition of fermentation process, conditions of enzyme reaction, and processing parameters in the production of food and drug [13–29]. A Box-Behnken design was chosen to look for the best experimental conditions.

Table 1: Experimental domain for screening step.

	Factor	Unit	Low level	High level
U_1	P/W ratio	g powder/mL water	0.0333	0.1
U_2	Temperature	°C	60	80
U_3	pH	—	4	9
U_4	Time	hour	0.5	2
U_5	Agitation speed	rpm	200	400
U_6	Ionic strength	g NaCl/l medium	1	2
U_7	M/E ratio	l/l	0.25	0.5
U_8	Precipitation temperature	°C	5	35

Table 2: Chemical composition of *Agave* leaves (g/100 g dry matter).

Components	Values
Dry matter (%)	16.42 ± 0.71
pH	5.03 ± 0.08
Protein	28.90 ± 1.46
Fat	5.46 ± 0.02
Ash	3.53 ± 0.33
Total carbohydrates	62.74 ± 1.38
Reducing sugars	20.06 ± 0.10
Soluble dietary fibres	7.72 ± 0.21
Insoluble dietary fibres	37.88 ± 0.49
Total dietary fibres	45.05 ± 0.96

Table 3: Mineral composition of *Agave* leaves.

Mineral	Values
K[a]	1.103 ± 0.017
Ca[a]	0.571 ± 0.016
Na[a]	0.037 ± 0.002
Mg[a]	0.086 ± 0.006
Zn[b]	1.299 ± 0.151
Cu[b]	0.307 ± 0.015

[a]g/100 g dry matter; [b]mg/100 g dry matter.

3. Results and Discussion

3.1. Chemical Composition. The main characteristics of the *Agave*'s leaves are given in Table 2. High moisture and protein and total carbohydrate contents were observed. However, low fat and ash contents were found. This plant exhibited a low pH value. This low pH could be explained by the presence of many organic acids such as malic, citric, and oxalic acids [30]. *Agave*'s leaves had high contents of soluble and insoluble fibres (7.72% and 37.88%, resp.). These values are comparable with those reported for *Opuntia ficus indica* f. *inermis* cladodes (8.78% and 30.36% of soluble and insoluble fibres, resp.) [17].

Table 3 summarized the mineral composition of the *Agave*'s leaves. Potassium is the most abundant mineral (1.103 g/100 g of dry weight). It was as twice as calcium content. Magnesium and sodium contents were, respectively, 86 and 37 mg/100 g of dry weight. However copper and zinc were found at very low level. These values were lower than those observed by Ayadi et al. [17] for *Opuntia ficus* cladodes and those found by Femenia et al. [31] in Aloe Vera.

The chemical composition of the *Agave* leaves (high amount of fibres and proteins), as well as the low cost of exploitation, can justify the search of valorization of this product. Indeed, *Agave* leaves could be considered as a potential source of dietary fibres.

3.2. Experimental Approach

3.2.1. Screening Step. Twelve experiments were carried out to select significant factors from those cited in Table 1 according to the conditions indicated in Tables 4 and 5 for insoluble and soluble fibres extraction, respectively. Response values (fibres yields) are reported in the last column of each table. These observed responses were used to compute coefficients of each model. This allowed us to write the following models:

$$\begin{aligned}\widehat{y}_1 &= 63.009 + 5.193X_1 + 8.171X_2 + 0.136X_3 \\ &\quad + 0.516X_4 + 4.898X_5 + 3.336X_6, \\ \widehat{y}_2 &= 51.076 + 5.623X_1 + 8.193X_2 \\ &\quad + 0.583X_3 + 0.547X_4 + 3.576X_5 \\ &\quad + 3.576X_6 + 1.099X_7 - 1.433X_8,\end{aligned} \tag{1}$$

where $\widehat{y}_1$ is insoluble fibres extraction yield, $\widehat{y}_2$ is soluble fibres extraction yield, X_1 is powder to water ratio (P/W), X_2 is extraction temperature, X_3 is pH, X_4 is time, X_5 is agitation speed, X_6 is ionic strength, X_7 is medium to ethanol ratio (M/E), and X_8 is ethanol precipitation temperature.

The analysis of variance showed that the regression sum of squares was statistically significant at the level of 95% for both insoluble and soluble fibres extractions. Results of this analysis for each design are summarized in Table 6. Coefficients of determination, R^2, were 0.886 and 0.977 for insoluble and soluble fibres extraction yield respectively. Thus, the predicted models well represented the observed values.

The analysis of the contrast coefficient b_j as it can be seen in Figure 2(a) showed that three factors: temperature, *Agave* powder to water ratio, and agitation speed had pronounced influence on the insoluble fibres extraction yield. Temperature and powder to water ratio improve the hydrodynamic process by increasing the diffusion of hydrophilic biomolecules to liquid extraction which allows the best fibres

Table 4: Experimental conditions of Plackett-Burman design for insoluble fibres extraction and the corresponding experimental responses.

N° exp	P/W ratio	Temperature	pH	Time	Agitation speed	Ionic strength	IF extraction yield (%)
1	0.1	80	4	2	400	2	89.87
2	0.0333	80	9	0.5	400	2	74.10
3	0.1	60	9	2	200	2	63.03
4	0.0333	80	4	2	400	1	65.67
5	0.0333	60	9	0.5	400	2	57.24
6	0.0333	60	4	2	200	2	46.57
7	0.1	60	4	0.5	400	1	66.24
8	0.1	80	4	0.5	200	2	67.26
9	0.1	80	9	0.5	200	1	68.49
10	0.0333	80	9	2	200	1	61.69
11	0.1	60	9	2	400	1	54.32
12	0.0333	60	4	0.5	200	1	41.63

Table 5: Experimental conditions of Plackett-Burman design for soluble fibres extraction and the corresponding experimental responses.

N° exp	P/W ratio	Temperature	pH	Time	Agitation speed	Ionic strength	M/E ratio	Precipitation temperature	SF extraction yield (%)
1	0.1	80	4	2	400	2	0.25	5	73.03
2	0.0333	80	9	0.5	400	2	0.5	5	65.61
3	0.1	60	9	2	200	2	0.5	35	51.85
4	0.0333	80	4	2	400	1	0.5	35	52.89
5	0.0333	60	9	0.5	400	2	0.25	35	39.67
6	0.0333	60	4	2	200	2	0.5	5	37.21
7	0.1	60	4	0.5	400	1	0.5	35	47.43
8	0.1	80	4	0.5	200	2	0.25	35	60.54
9	0.1	80	9	0.5	200	1	0.5	5	58.06
10	0.0333	80	9	2	200	1	0.25	35	45.48
11	0.1	60	9	2	400	1	0.25	5	49.28
12	0.0333	60	4	0.5	200	1	0.25	5	31.86

extraction efficiency and the highest purity [32, 33]. As for agitation speed, it accelerates the diffusion rate of molecules and homogenizes extraction medium. The other factors were statistically not significant. However, ionic strength was the most important factor from nonsignificant ones ($b_6 = 3.34$). This fact can be explained by the precipitation of protein present abundantly in *Agave* leaves leading to obtain a more pure DF concentrate. On the other hand, Figure 2(b) showed that four factors: temperature, *Agave* powder to water ratio (P/W), agitation speed, and ionic strength had pronounced effect on the soluble fibres extraction yields. Thus, it seems evident to retain these factors in order to optimize soluble and insoluble fibres extraction using the RSM.

3.2.2. Optimization by RSM. A Box-Behnken design was chosen to look for the best experimental conditions of four independent factors kept after the screening study which are X_1: powder to water ratio; X_2: extraction temperature (°C); X_3: agitation speed (rpm); and X_4: ionic strength (g of NaCl/L). For each factor, the experimental range was chosen on the basis of results of screening experiments. On the one hand, powder to water ratio and temperature domains were extended due to their high significance detected in the screening step. On the other hand, extraction time was fixed at 30 minutes and pH at its natural value to reduce the cost of this process. In addition, the precipitation of soluble fibres was carried out in ambient temperature using a 0.5 ethanol to medium ratio for economic reasons. The relationship between the extraction yield and the four variables for each response was approximated by the following second order polynomial function:

$$\begin{aligned}\hat{y} = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 \\ & + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 \\ & + b_{23}X_2X_3 + b_{14}X_1X_4 + b_{24}X_2X_4 + b_{34}X_3X_4.\end{aligned} \quad (2)$$

Twenty-five experiments were carried out to estimate the 15 model coefficients for each response according to the conditions indicated in Table 7. In order to estimate the pure

Table 6: Analysis of variance for Plackett-Burman designs.

Source of variation	Sum of squares	Degrees of freedom	Mean square	Ratio	Significance
IF					
Regression	1549.47	6	258.245		
Residuals	198.51	5	39.702	6.5046	2.89*
Total	**1747.98**	**11**			
SF					
Regression	1538.42	8	192.303	15.8162	2.22*
Residuals	36.48	3	12.159		
Total	**1574.9**	**11**			

*Significant at the level of 95%.

Table 7: Experimental conditions of Box-Behnken design and the corresponding experimental responses.

N° Exp	P/W ratio	Temperature	Agitation velocity	Ionic strength	IF extraction yield (%)	SF extraction yield (%)
1	0.050	60	300	1.5	64.34	56.67
2	0.200	60	300	1.5	79.06	69.04
3	0.050	90	300	1.5	78.45	68.39
4	0.200	90	300	1.5	88.23	77.24
5	0.050	75	200	1.5	69.87	59.26
6	0.200	75	200	1.5	77.06	68.04
7	0.050	75	400	1.5	74.87	62.34
8	0.200	75	400	1.5	85.45	74.53
9	0.050	75	300	1.0	72.11	60.49
10	0.200	75	300	1.0	80.85	67.05
11	0.050	75	300	2.0	72.54	63.44
12	0.200	75	300	2.0	81.05	71.35
13	0.125	60	200	1.5	70.86	59.79
14	0.125	90	200	1.5	78.65	69.45
15	0.125	60	400	1.5	71.54	63.73
16	0.125	90	400	1.5	91.05	79.57
17	0.125	60	300	1.0	72.20	66.43
18	0.125	90	300	1.0	79.85	70.46
19	0.125	60	300	2.0	72.67	62.41
20	0.125	90	300	2.0	81.56	75.16
21	0.125	75	200	1.0	73.32	61.48
22	0.125	75	400	1.0	79.47	69.66
23	0.125	75	200	2.0	72.33	64.86
24	0.125	75	400	2.0	80.14	73.23
25	0.125	75	300	1.5	70.38	62.34
26	0.125	75	300	1.5	71.92	61.05
27	0.125	75	300	1.5	69.83	59.97
28	0.125	75	300	1.5	71.47	60.05
29	0.125	75	300	1.5	70.18	61.28
30	0.125	75	300	1.5	72.25	60.63

error variance, five replications were performed at the central point [13]. Response values (fibres yields) are reported in the two last columns of Table 7; these values were used to estimate the model coefficients b_j by the least square method which allowed us to write the following estimated models:

insoluble DF:

$$\begin{aligned}\widehat{y}_1 &= 71.005 + 4.960X_1 + 5.593X_2 \\ &\quad + 3.369X_3 + 3.003X_1^2 + 3.575X_2^2 \\ &\quad + 3.094X_3^2 + 2.279X_4^2 + 2.930X_2X_3,\end{aligned} \tag{3}$$

soluble DF:

$$\begin{aligned}\widehat{y}_2 &= 60.409 + 4.724X_1 + 5.144X_2 + 3.371X_3 \\ &\quad + 1.173X_4 + 2.282X_1^2 + 4.806X_2^2 + 3.298X_3^2 \\ &\quad + 3.222X_4^2 + 1.572X_2X_3 + 2.201X_2X_4.\end{aligned}$$

It can be seen from the regression equation of insoluble fibres extraction that only the linear coefficient corresponding to ionic strength was not significant. This result is in agreement with screening results. However, all quadric terms were highly significant; X_4^2 include all other quadric terms aliased with linear ones. Finally, a positive correlation between temperature and agitation velocity occurred. This result can be explained by the contribution of this interaction to break connections of fibres to other macromolecules which allows the increase of the purity of the fibre extract.

For the soluble fibres extraction, all linear and quadric terms were highly significant and had a positive effect on the response. Moreover, positive correlations occurred between temperature and agitation velocity on the one hand and temperature and ionic strength on the other hand. This latter interaction could be explained by the denaturation of protein leading to a higher purity.

The analysis of variance for fitted models showed that the regression sum of squares was statistically significant at the level of 99.99% for two responses and the lack of fit was not significant. Results of this analysis are summarized in Table 8. The coefficients of determination, R^2, for insoluble and soluble fibres extraction yields were 0.971 and 0.964, respectively. Thus, the predicted model well represented the observed values.

In order to validate the adequacy of model equations using point test method, five verification experiments were accomplished under various extracting conditions (within the experimental range). Table 9 presents design matrix along with the experimental results and theoretical values predicted by regression equations. Correlation coefficients (R^2) between experimental and predicted values for two responses were 0.967 and 0.964. There was no statistically significant difference at level of 95% between experimental and predicted values. Results clearly indicate that experimental values are in good agreement with predicted ones and also suggest that regression models are accurate and adequate for the extraction of both soluble and insoluble fibres which consolidate ANOVA results.

The relationship between responses and experimental variables can be illustrated graphically by contours plots (Figures 3 and 4). The topography of these responses is 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 studied domain and, consequently, in determining the optimal experimental conditions [34].

In Figure 3(a) the examination of the isoresponse contours plot showed that the yield increased when increasing temperature and/or increasing the *Agave* powder to water ratio. These effects were markedly shown for temperatures over 75°C and for P/W ratio higher than 0.145. This can be explained by the high solubility of fructans and simple sugars which increases with increasing temperature, giving a higher purity of extract. Similar results were obtained by Masmoudi et al. [13] for extracting pectin from lemon with acidified date juice. Qiao et al. [29] showed that the best yield of polysaccharide extracted from *Hyriopsis cumingii* occurs at a low ratio of water to raw material and a temperature of 80°C which is close to our results.

The positive effect of temperature above 75°C was also demonstrated in Figure 3(b). Thus, the increase of temperature and agitation speed improved the fibre extraction yield. This positive correlation can be explained by the thermodynamic effect on breaking connections of fibres to other biomolecules. In Figures 3(c) and 3(d), isoresponse curves are arranged in linear trajectory parallel to the vertical axis corresponding to the ionic strength. This fact appears as an evident, logical, and expected consequence which is in perfect harmony with the screening study.

Similar conclusions could be obtained from analyzing contours plots dressed in Figure 4. As previous discussion showed, all factors involved in the optimization study affect significantly fructans extraction. This fact is probably due to the thermodynamic and hydrodynamic phenomena. Ionic strength was significant as it intervenes by increasing purity while precipitating soluble protein.

Optimal extraction conditions: powder to water ratio of 0.1625, extracting temperature of 90°C, agitation speed of 400 rpm, and NaCl concentration of 1.5 g/L were determined using Nemrod W software. The suitability of these optimal extracting variables was also tested by executing the experiment under these conditions. Measured values belonged to the predicted intervals of both optimum insoluble and soluble fibres extraction yield (Table 10).

3.3. Characteristics of Insoluble Fibres Concentrate

3.3.1. Physical and Chemical Analysis of DF Concentrates. Analyses of lyophilised insoluble and soluble fibres are given in Table 11. Analysis showed high dry matter content (94.51% and 91.67%) and low water activity (0.225 and 0.337) for insoluble and soluble fibres, respectively, which allows a good microbiological stability and long preservation. The amount of fibres was of about 86 g/100 g and 90 g/100 g for insoluble and soluble fibres, respectively. These values are close to levels measured for DF preparations from date (ranging between

Table 8: Analysis of variance for Box-Behnken design.

Source of variation	Sum of squares	Degrees of freedom	Mean square	Ratio	Significance
IF					
Regression	1030.01	14	73.572	36.072	<0.01***
Residuals	30.594	15	2.039		
Validity	25.539	10	2.554	2.526	15.9
Error	5.055	5	1.011		
Total	**1060.6**	**29**			
SF					
Regression	999.31	14	71.379	29.567	<0.01***
Residuals	36.213	15	2.414		
Validity	32.313	10	3.231	4.143	6.5
Error	3.899	5	0.779		
Total	**1035.52**	**29**			

***Significant at the level of 99.99%.

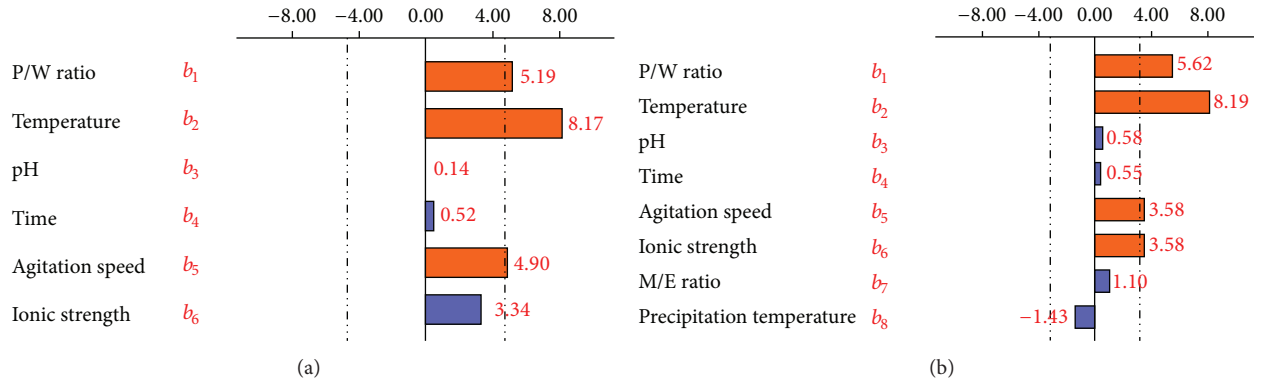

Figure 2: Graphical study of factor effects on soluble and insoluble fibres extraction (screening step): (a) insoluble fibres screening design and (b) soluble fibres screening design.

Table 9: Models validation experiments.

Exp.	IF		SF	
	yi	$\hat{y}$i	yi	$\hat{y}$i
31	67.180	67.295	56.430	57.183
32	73.350	73.081	61.790	62.495
33	72.230	75.082	63.430	64.944
34	74.370	74.747	63.910	64.478
35	71.450	72.291	61.170	63.292

yi: experimental value
$\hat{y}$i: predicted value.

Table 10: Suitability of selected optimal conditions to the measured values.

IF	Measured values: yi (%)	93.02
	Predicted value: $\hat{y}$i (%)	92.56 ± 2.43
SF	Measured values: yi (%)	80.46
	Predicted value: $\hat{y}$i (%)	81.53 ± 2.55

88 and 92% depending on the variety used for the process) and apple (89.8%), but notably higher than those of other

Table 11: Chemical composition of insoluble and soluble fibres concentrates (g/100 g dry matter).

	IF	SF
Dry matter (%)	94.51 ± 0.14	91.67 ± 0.27
a_w	0.225 ± 0.002	0.337 ± 0.002
pH	6.35 ± 0.04	5.59 ± 0.04
Total fibres	86.23 ± 1.32	89.84 ± 0.88
Ash	8.01 ± 0.05	3.25 ± 0.17
proteins	2.32 ± 0.09	1.44 ± 0.11
Fat	1.24 ± 0.04	0.097 ± 0.003
Soluble sugars	1.58 ± 0.21	4.42 ± 0.54

fruits DF concentrates reported for grapefruit, lemon, orange and mango (28–78.2%), citrus peel (57%), and mango peel (~71%) [12]. Table 10 reveals the presence of ash as a major contaminant of insoluble DF concentrate (~8%) followed by protein, soluble sugars, and fat; this may be due to the use of sodium chloride in the extraction process. For fructans, soluble sugars and ash are the main contaminant of the extract. The low level of protein obtained could be due to the repeating washings after extraction leading to the

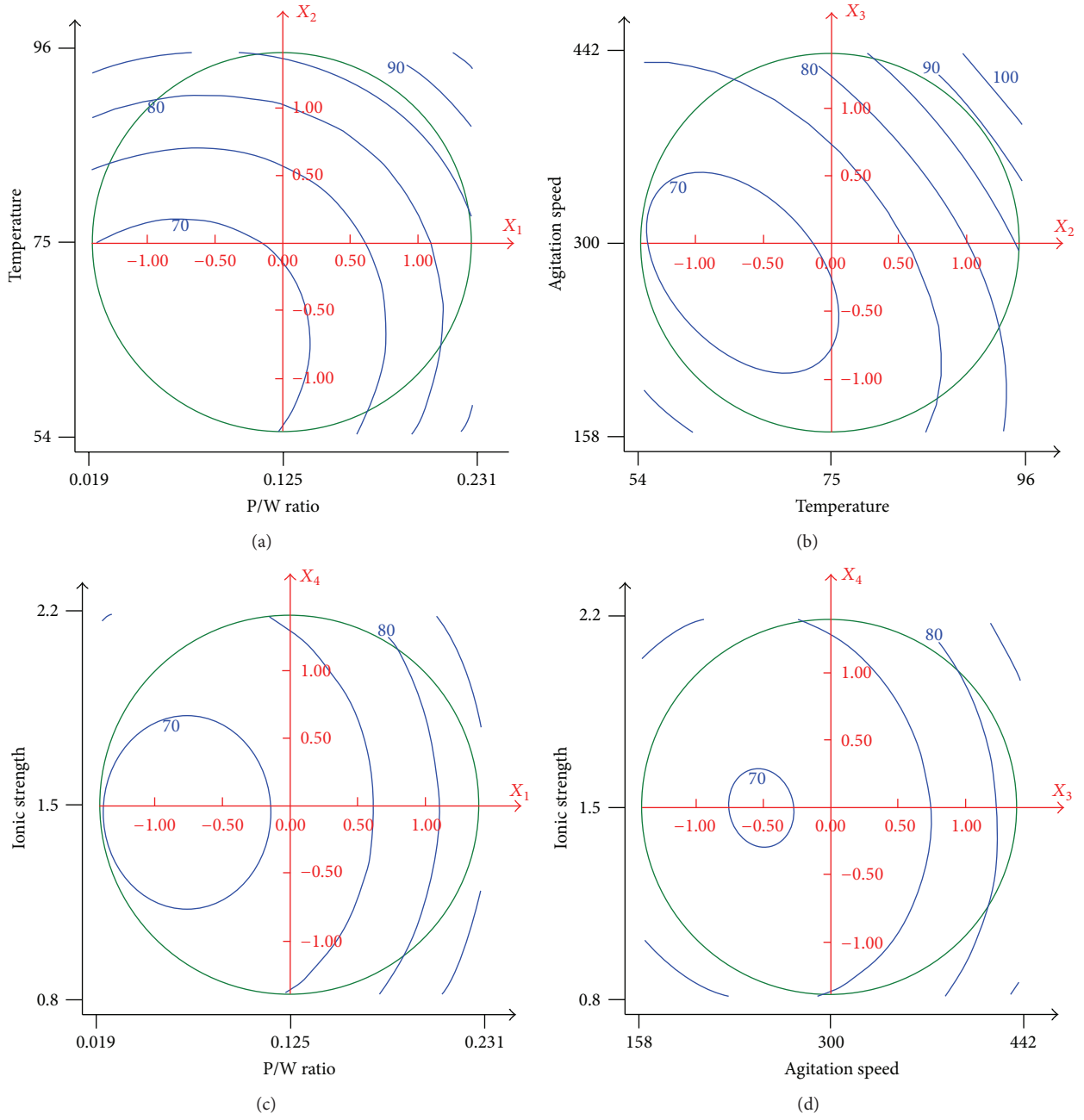

Figure 3: Contour plots illustrating the effect of (a) temperature and P/W ratio at constant agitation speed (300 rpm) and ionic strength (1.5 g/L), (b) temperature and agitation speed at constant P/W ratio (0.125) and ionic strength (1.5 g/L), (c) ionic strength and P/W ratio at constant agitation speed (300 rpm) and temperature (75°C), and (d) ionic strength and agitation speed at constant P/W ratio (0.125) and temperature (75°C) on insoluble fibres yield extracted from dried *Agave* leaves' powder.

elimination of amino acids issued from protein hydrolysis at high temperatures.

3.3.2. Water and Oil Holding Capacities (WHC and OHC). Insoluble fibres concentrate showed a high WHC (8.66 g water/g sample) (Table 12). This value is higher than those reported for other fruit fibre concentrates, such as citrus, apple, oat bran, and pear DF (3.6 to 6.8 g water/g sample) [9]. The high WHC of *Agave* insoluble fibres concentrate suggested that this material could be used as a functional ingredient in food to avoid syneresis of formulated products

Table 12: Water and oil holding capacities of *Agave* fibres concentrate (g/g of fibres concentrate).

	IF	SF
Water holding capacity	8.66 ± 0.02	2.28 ± 0.03
Oil holding capacity	5.62 ± 0.03	3.37 ± 0.01

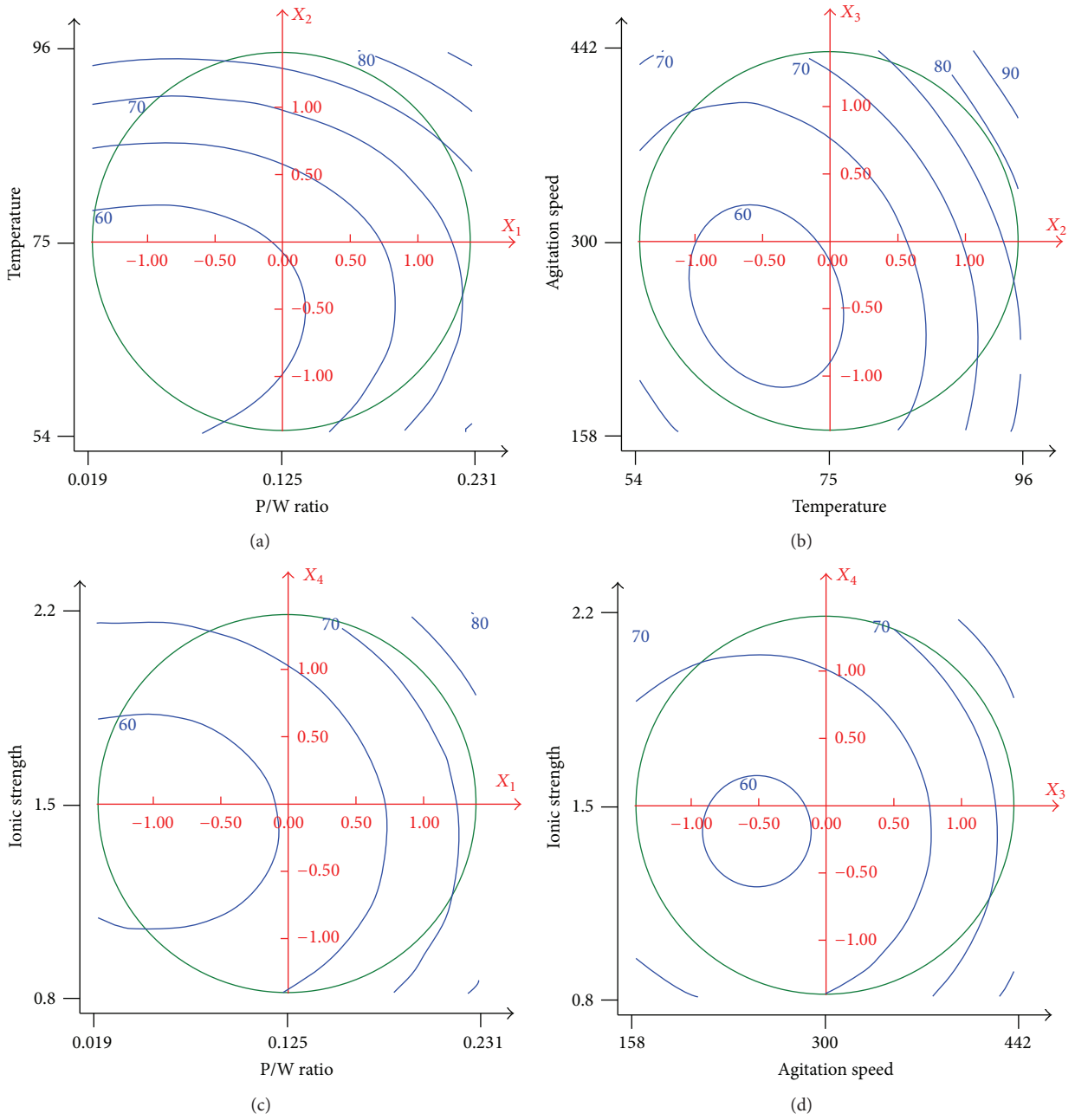

Figure 4: Contour plots illustrating the effect of (a) temperature and P/W ratio at constant agitation speed (300 rpm) and ionic strength (1.5 g/L), (b) temperature and agitation speed at constant P/W ratio (0.125) and ionic strength (1.5 g/L), (c) ionic strength and P/W ratio at constant agitation speed (300 rpm) and temperature (75°C), and (d) ionic strength and agitation speed at constant P/W ratio (0.125) and temperature (75°C) on soluble fibres yield extracted from dried *Agave* leaves' powder.

[12]. This concentrate is also characterised by a high OHC (5.6 g oil/g sample) (Table 12), superior to some agricultural byproducts and DF concentrates cited by Abdul-Hamid et al. [9, 10], for peach DF concentrate and rice bran (1.11 and 4.54 g oil/g sample). The high OHC of *Agave* insoluble DF concentrate suggested that this material could be used as an ingredient to stabilize foods with a high percentage of fat and emulsion [12]. Fructans concentrate has not good water and oil holding capacities; however, it showed a high solubility at 25°C of 86.52 ± 3.47 g/L which is comparable with commercial chicory inulin (113.68 ± 4.14 g/L).

3.3.3. Microstructure Visualization. As it can be well seen in Figure 5, *Agave* insoluble fibres have two distinct morphologies. In micrograph of Figure 5(e), hemicelluloses filaments overlap to form an amorphous nodular network and play the role of gathering and supporting elements of principal

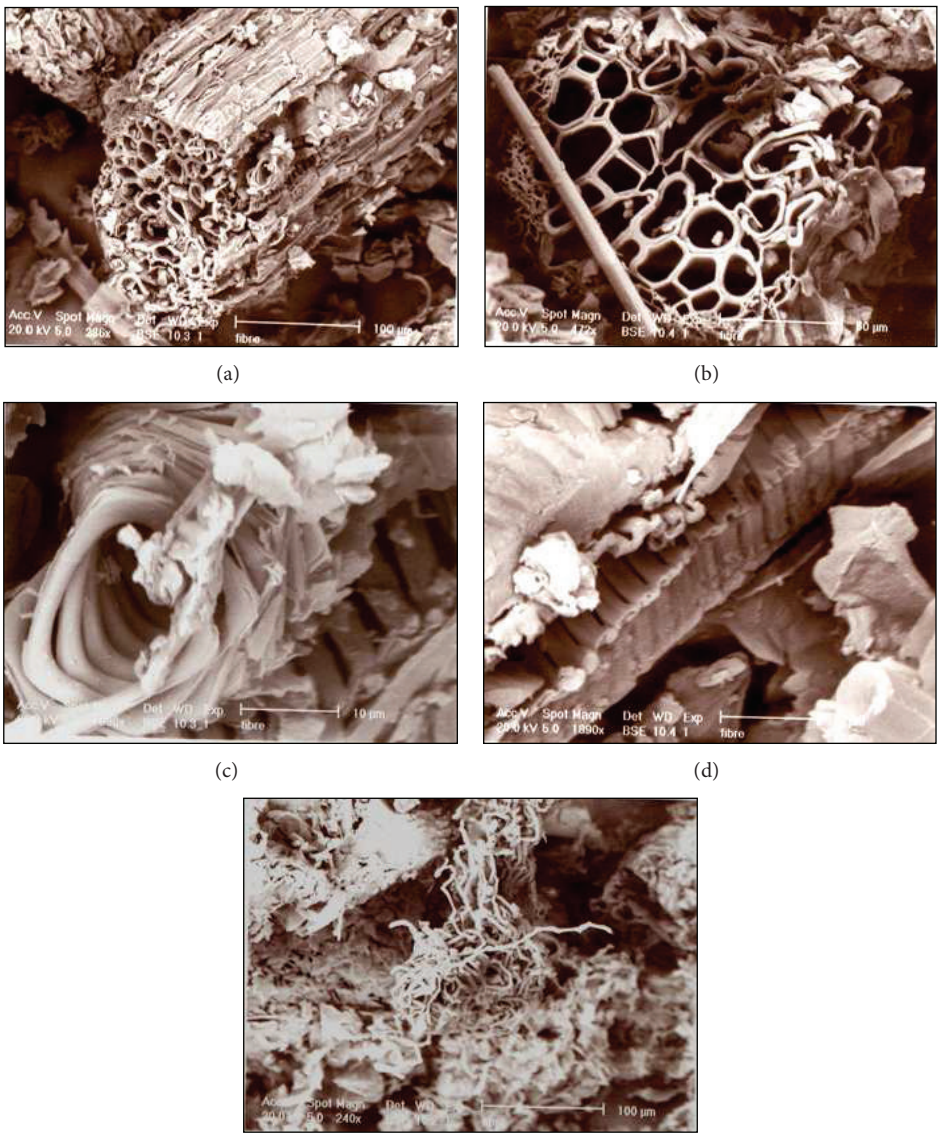

Figure 5: Scanning electron microscopy images of insoluble fibres concentrate from *Agave americana* L.; scale bar for (a) and (e), 100 μm; scale bar for (b), 50 μm; scale bar for (c) and (d), 10 μm.

structure [7]. This latter, of 150 to 200 μm of diameter (micrographs of Figures 5(a) and 5(b)), has a cylindrical shape associating helicoidal microfibrils called tracheids (micrographs of Figures 5(c) and 5(d)). These voluminous structures have a low density and show a lot of vacuum which illustrate and justify the high water and oil holding capacities. Izydorczyk and Dexter [35] revealed similar structures for barley fibres and found a high WHC when they incorporate this product in bread formulation.

Agave fructans morphology was also visualized using scanning electron microscopy and compared to commercial chicory inulin in order to have additional information concerning impact of botanic source and extraction conditions on fructans structures (Figure 6). Micrographs of Figures 6(a), 6(b), and 6(c) show irregular structure of commercial product. Nonuniform particles sized from 50 to 70 μm are squeezed with small spherical particles sized under 10 μm. However, micrographs of Figures 6(d), 6(e), and 6(f) show a heterogenic structure illustrating pronounced morphological and dimensional variability. Indeed, commercial product is finely milled and microfluidized; this fact explains the observed homogeneity of structure. This result is in accordance with the discussion of other works which illustrated that microfluidized inulin particles have a uniform shape and were interspaced by voids when nonmicrofluidized inulin was arranged as a superposition of lamella or on amorphous shape [23–36].

4. Conclusion

This work has revealed that after screening step, the response surface methodology was a useful tool to determine the

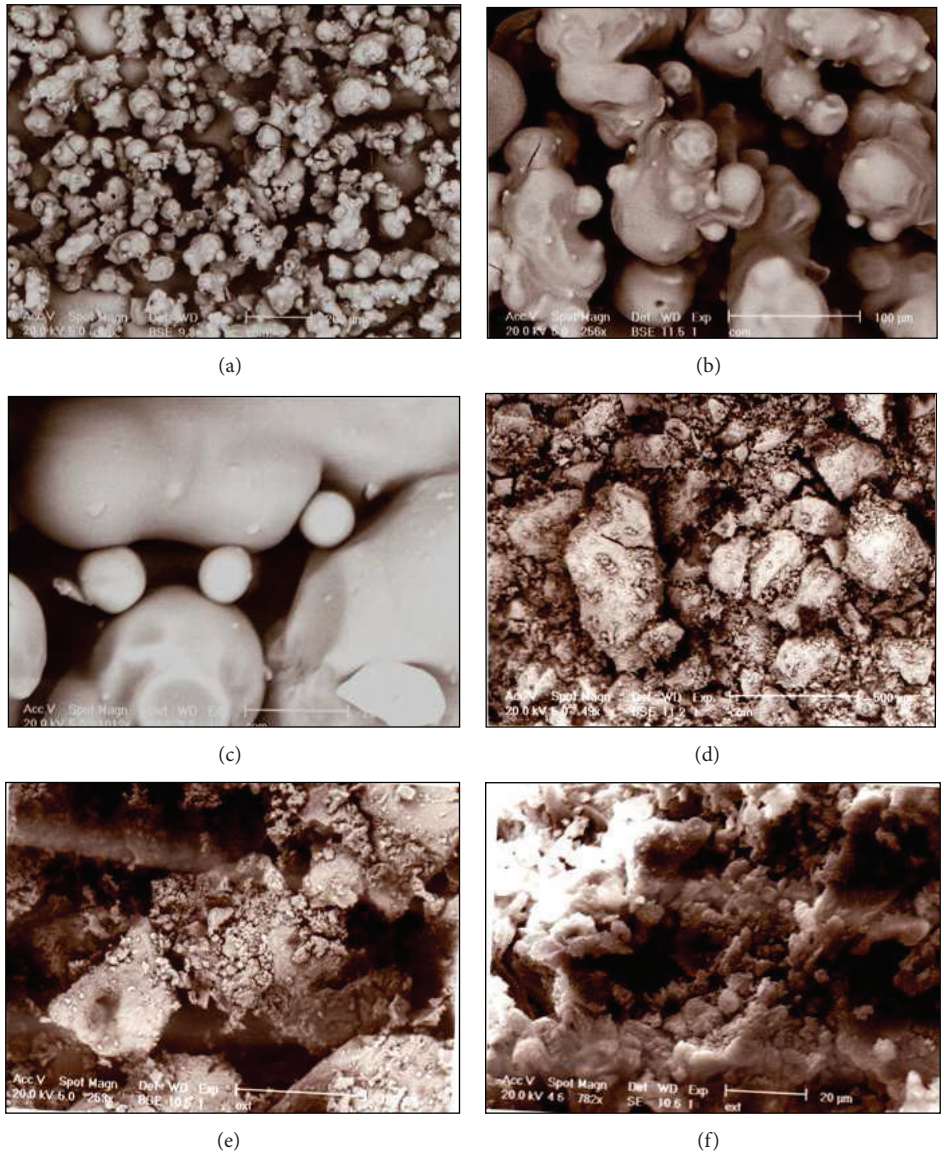

Figure 6: Scanning electron microscopy images of commercial inulin (a, b, and c) and soluble fibres concentrate of *Agave Americana* L. (d, e, and f); scale bar for (d), 500 μm; scale bar for (a) and (e), 200 μm; scale bar for (b), 100 μm; scale bar for (c) and (f), 20 μm.

optimal experimental conditions of soluble and insoluble fibres extraction from *Agave americana* leaves. The extraction yield of insoluble fibres increased significantly with increasing temperature, *Agave* powder to water ratio, and agitation speed, while ionic strength has no effect on the studied response. However, fructans extraction yield depended on all investigated factors. Under selected optimal conditions (temperature: 90°C; P/W ratio: 0.1625; agitation speed: 400 rpm; and ionic strength: 1.5 g/L), insoluble and soluble DF extraction yields reached 92.56±2.43% and 81.53±2.55%, respectively. These concentrates showed a high purity and excellent functional properties. Thus, it is promising to focus on the possibility to incorporate these fibres concentrates in food applications. In a future study, the extraction and the characterisation of protein should be studied in order to add value to this unexploited plant.

Acknowledgment

The authors are very grateful to Pr. Moncef Chaabouni (Industrial Chemistry Laboratory, Sfax, Tunisia) for his technical assistance.

References

[1] B. Rodriguez-Garay, J. A. Lomeli-Sencion, E. Tapia-Campos et al., "Morphological and molecular diversity of *Agave tequilana* Weber var. Azul and *Agave angustifolia* Haw. var. Lineno," *Industrial Crops and Products*, vol. 29, no. 1, pp. 220–228, 2009.

[2] R. I. Ortiz-Basurto, P. Williams, M. P. Belleville et al., "Presence of rhamnogalacturonan II in the juices produced by enzymatic liquefaction of *Agave pulquero* stem (*Agave mapisaga*)," *Carbohydrate Polymers*, vol. 77, no. 4, pp. 870–875, 2009.

[3] H. S. Gentry, Ed., *Agaves of Continental North America*, University of Arizona, Tucson, Ariz, USA, 1982.

[4] G. Iniguez-Covarrubias, R. Díaz-Teres, R. Sanjuan-Dueñas, J. Anzaldo-Hernández, and R. M. Rowell, "Utilization of by-products from the tequila industry. Part 2: potential value of *Agave tequilana* Weber *azul* leaves," *Bioresource Technology*, vol. 77, no. 2, pp. 101–108, 2001.

[5] A. Guendo, *Flore Analytique et Synoptique de La Tunisie*, Office de l'expérimentation et de la Vulgarisation Agricole de Tunisie, Tunis, Tunisia, 1954.

[6] A. Gobeille, J. Yavitt, P. Stalcup, and A. Valenzuela, "Effects of soil management practices on soil fertility measurements on *Agave tequilana* plantations in Western Central Mexico," *Soil and Tillage Research*, vol. 87, no. 1, pp. 80–88, 2006.

[7] A. Bessadok, S. Marais, S. Roudesli, C. Lixon, and M. Métayer, "Influence of chemical modifications on water-sorption and mechanical properties of Agave fibres," *Composites A*, vol. 39, no. 1, pp. 29–45, 2008.

[8] J. Arrizon, S. Morel, A. Gschaedler, and P. Monsan, "Comparison of the water-soluble carbohydrate composition and fructan structures of *Agave tequilana* plants of different ages," *Food Chemistry*, vol. 122, no. 1, pp. 123–130, 2010.

[9] N. Grigelmo-Miguel, S. Gorinstein, and O. Martin-Belloso, "Characterisation of peach dietary fibre concentrate as a food ingredient," *Food Chemistry*, vol. 65, no. 2, pp. 175–181, 1999.

[10] A. Abdul-Hamid and Y. S. Luan, "Functional properties of dietary fibre prepared from defatted rice bran," *Food Chemistry*, vol. 68, no. 1, pp. 15–19, 2000.

[11] R. B. Salah, B. Jaouadi, A. Bouaziz et al., "Fermentation of date palm juice by curdlan gum production from *Rhizobium radiobacter* ATCC 6466: purification, rheological and physico-chemical characterization," *LWT—Food Science and Technology*, vol. 44, no. 4, pp. 1026–1034, 2011.

[12] M. Elleuch, S. Besbes, O. Roiseux et al., "Date flesh: chemical composition and characteristics of the dietary fibre," *Food Chemistry*, vol. 111, no. 3, pp. 676–682, 2008.

[13] M. Masmoudi, S. Besbes, M. Chaabouni et al., "Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology," *Carbohydrate Polymers*, vol. 74, no. 2, pp. 185–192, 2008.

[14] AOAC, *Official Methods of Analysis, Association of Official Analytical Chemists*, Washington, DC, USA, 15th edition, 1990.

[15] AFNOR, "Produits dérives des fruits et légumes," Tech. Rep. NF 05-109, Association Française de Normalisation, Paris, France, 1970.

[16] AFNOR, "Jus de fruits et jus de légumes," Tech. Rep. NF 76-101, Association Française de Normalisation, Paris, France, 1976.

[17] M. A. Ayadi, W. Abdelmaksoud, M. Ennouri, and H. Attia, "Cladodes from *opuntia ficus indica* as a source of dietary fiber: effect on dough characteristics and cake making," *Industrial Crops and Products*, vol. 30, no. 1, pp. 40–47, 2009.

[18] N. R. Galla and G. R. Dubasi, "Chemical and functional characterization of Gum karaya (*Sterculia urens* L.) seed meal," *Food Hydrocolloids*, vol. 24, no. 5, pp. 479–485, 2010.

[19] L. Blanchard, "Détermination réductométrique du saccharose dans les laits concentrés sucrés," *Dairy Science and Technology*, vol. 31, no. 309-310, pp. 609–612, 1951.

[20] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350–356, 1956.

[21] L. Prosky, N. G. Asp, T. F. Schweizer, J. W. Devries, and I. Furda, "Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study," *Journal of the Association of Official Analytical Chemists*, vol. 71, no. 5, pp. 1017–1023, 1988.

[22] H. Attia, M. Bennasar, A. Lagaude et al., "Ultrafiltration with a microfiltration membrane of acid skimmed and fat-enriched milk coagula: hydrodynamic, microscopic and rheological approaches," *Journal of Dairy Science*, vol. 60, pp. 161–174, 1993.

[23] S. N. Ronkart, M. Paquot, C. S. Blecker et al., "Impact of the crystallinity on the physical properties of inulin during water sorption," *Food Biophysics*, vol. 4, no. 1, pp. 49–58, 2009.

[24] A. A. Mcconnell, M. A. Eastwood, and W. D. Mitchell, "Physical characteristics of vegetable foodstuffs that could influence bowel function," *Journal of the Science of Food and Agriculture*, vol. 25, no. 12, pp. 1457–1464, 1974.

[25] A. Caprez, E. Arrigoni, R. Amado et al., "Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran," *Journal of Cereal Science*, vol. 4, no. 3, pp. 233–239, 1986.

[26] R. L. Plackett and J. P. Burman, "The design of optimum multifactorial experiments," *Biometrika*, vol. 33, no. 4, pp. 305–325, 1946.

[27] G. E. P. Box and D. W. Behnken, "Some new three level designs for the study of quantitative variables," *Technometrics*, vol. 2, no. 4, pp. 455–475, 1960.

[28] D. Mathieu, J. Nony, and R. Phan-Tan-Luu, *NEMROD-W Software*, Société LPRAI, Marseille, France, 2000.

[29] D. Qiao, B. Hu, D. Gan, Y. Sun, H. Ye, and X. Zeng, "Extraction optimized by using response surface methodology, purification and preliminary characterization of polysaccharides from *Hyriopsis cumingii*," *Carbohydrate Polymers*, vol. 76, no. 3, pp. 422–429, 2009.

[30] F. C. Stintzing and R. Carle, "Cactus stems (Opuntia spp.): a review on their chemistry, technology, and uses," *Molecular Nutrition and Food Research*, vol. 49, no. 2, pp. 175–194, 2005.

[31] A. Femenia, E. S. Sanchez, S. Simal, and C. Rosselló, "Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues," *Carbohydrate Polymers*, vol. 39, no. 2, pp. 109–117, 1999.

[32] J. Liu, S. Miao, X. Wen, and Y. Sun, "Optimization of polysaccharides (ABP) extraction from the fruiting bodies of *Agaricus blazei* Murill using response surface methodology (RSM)," *Carbohydrate Polymers*, vol. 78, no. 4, pp. 704–709, 2009.

[33] X. Guo, X. Zou, and M. Sun, "Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from *Phellinus igniarius*," *Carbohydrate Polymers*, vol. 80, no. 2, pp. 344–349, 2010.

[34] A. Kamoun, B. Samet, J. Bouaziz, and M. Châabouni, "Application of a rotatable orthogonal central composite design to the optimization of the formulation and utilization of an useful plasticizer for cement," *Analusis*, vol. 27, no. 1, pp. 91–96, 1999.

[35] M. S. Izydorczyk and J. E. Dexter, "Barley β-glucans and arabinoxylans: molecular structure, physicochemical properties, and uses in food products—a review," *Food Research International*, vol. 41, no. 9, pp. 850–868, 2008.

[36] S. N. Ronkart, M. Paquot, C. Deroanne, C. Fougnies, S. Besbes, and C. S. Blecker, "Development of gelling properties of inulin by microfluidization," *Food Hydrocolloids*, vol. 24, no. 4, pp. 318–324, 2010.