Optimization of inulin extraction from garlic (*Allium sativum* L.) waste using the response surface methodology.

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Abstract

Inulin is a polysaccharide with several applications within the chemical, pharmaceutical, and food industry. It is considered a dietary fibre that provides multiple health benefits. In this work, the yield of raw inulin obtained from garlic agro-industrial useless organic waste was maximized, by applying the response surface methodology in a central composite design (CCD), in which different distilled-water (DW)-to-garlic-agro-industrial-organic-waste (GAIOW) ratios (3 and 5 mL/g) and different temperatures (60 and 80 °C) were evaluated. Optimal condition was obtained with a DW/GAIOW ratio of 4.3 mL/g and a temperature of 80.2 °C. Under this condition, the quadratic model showed a maximum yield of crude inulin of 8.17 ± 0.89 g/100 g. Further, the CCD model obtained was validated with three additional experiments at the same optimal condition. The FTIR spectra of inulin obtained from garlic agro-industrial organic residues and chicory inulin showed similarities and differences, presumably related to the different degrees of polymerization of the fructans present.

Keywords: inulin, *Allium sativum* L., aqueous extraction, waste valorization, central composite experimental design, response surface methodology.

Introduction

Garlic (*Allium sativum* L.) is an aromatic crop native to Central Asia, although its cultivation has spread throughout the five continents, and has been widely used as a condiment for the preparation of numerous dishes in different countries (Charron et al., 2016). Additionally, the positive effects of garlic consumption on health are well known and documented (Ried, 2016; Shang et al., 2019; Suleria et al., 2015).

China is the world's leading producer (>20 million tons per year, about 80% of the worldwide production) and consumer of garlic. Among the first ten garlic producing countries are India, Bangladesh, Egypt, South Korea, Russia, and Ukraine, all with production levels above 100,000 tons per year (FAO, 2019).

By 2012, more than 3.7 million tons of garlic by-products were generated from the garlic processing industry (Kallel & Chaabouni, 2017), representing more than 15% of the whole worldwide production (FAO, 2019). The damages cloves, straw and husk can be utilized to extract bioactive compounds (Dietrich et al., 2016; El-Mashad et al., 2019) like dietary fiber (Chandrashekara & Venkatesh, 2016), polysaccharides (Hughes et al., 2017), polyphenols (Ichikawa et al., 2003), cellulose (Reddy & Rhim, 2018), lignin, and to absorb the heavy metals (Chen et al., 2018; Liu et al., 2014). Instead, it is dismissed and burned as a waste product contributing to global warming without any kind of benefits.

There are inadequacies in waste management due to aspects such as insufficient economic resources, technological capacity, and regulations that regulate and guarantee integral management of said waste from its generation to its final disposal (Bernache Pérez, 2015).

Plant-based agro-industrial organic wastes are especially attractive sources for waste valorization because of their content in chemical compounds (like sugars, pigments, food fiber, protein, polyphenols, lignin, etc.) and can be potentially useful when chemical or microbiological treatments transform them into products of high added value (Galanakis, 2021; Moldes et al., 2002; Otles & Kartal, 2018).

Through the valorization of agro-industrial organic waste, the portfolio of valuable products of agro-industrial companies could be increased, improving their competitiveness, and obtaining increasingly efficient and sustainable agro-industrial processes (Galanakis, 2021; Hiloidhari et al., 2020).

Inulin is a non-digestible fructan-type polysaccharide found in many plants as a storage carbohydrate, usually in vegetables, fruits, and cereals of important nutritional properties (Apolinário et al., 2014; A. Franck, 2002; Anne Franck, 2016; Shalini et al., 2017). Inulin can be used as an industrial food ingredient improving organoleptic characteristics, the stability of foams and emulsions, and as a fat substitute offering an advantage in taste and texture (James et al., 2017; Panesar & Bali, 2016; Shoaib et al., 2016; Singh et al., 2017).

Inulin acts as a dietary fibre providing health benefits, contributes to the decrease of lipid levels, blood glucose and pressure, and laxative action, due to its prebiotic effect (Choque Delgado & Tamashiro, 2018; Ghaffari & Roshanravan, 2020; Guarino et al., 2020), prevents the development of colon cancer (Pool-Zobel & Sauer, 2007). It has also been reported that the use of inulin produces an increase in the absorption of cations and magnesium, an increase in the excretion of sulfur, and a decrease in uremia (Jung et al., 2015; X. Wang & Gibson, 1993).

Inulin is soluble in water (Yanovsky & Kingsbury, 1933), and fractions with a higher degree of polymerization can be precipitated with ethanol (Ku et al., 2003). For this reason, the extraction with hot water and its partial purification utilizing

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ethanol precipitation has been used as a common method of obtaining commercial inulin from different natural sources (Álvarez-Borroto et al., 2015; Niness, 1999). In the mass-transfer process of solid-liquid extraction of inulin from its natural plant sources to hot water, however, in addition to the hot water/solid ratio and the water temperature, other factors such as the extraction time, pH, and the agitation of the mixture could exert certain influence (Lingyun et al., 2007; Rubel et al., 2018).

The goal of the present work is to determine the optimal conditions that maximize the inulin yield in garlic agro-industrial organic wastes, through the selection of the best choice of the hot water/weight of garlic waste ratio and temperature, using a central composite design of experiments in the response surface methodology.

Materials and methods

Raw material and its preparation

Garlic agro-industrial waste was supplied by “Industrial Productos Moro scc” (Ibarra, Imbabura, Ecuador). Garlic agro-industrial organic wastes (GAIOWs) are formed by damaged bulbs, husks and the garlic paste lumps formed after cooking.

GAIOWs were washed and disinfected with a 1% (v/v) ethanol solution before use, then was washed with abundant distilled water, and dried overnight at 80 °C in an oven. Dried GAIOWs were chopped with the help of a crusher and a 4 mm of sieve mesh, to obtain a size of the homogeneous particle.

Experimental conditions

GAIWs were subjected to a solid-liquid extraction process, by using distilled water (DW) as a solvent with two different water-to-weight-of-GAIOW ratios (3 and 5 mL of DW per gram of GAIOW), combined with two temperature levels (70 and 90 °C).

Thirty grams (30 g) GAIOWs was used for all treatments, and the extraction time was 45 min with constant agitation of 200 rpm (Anne Franck, 2016). Subsequently, the first filtration was performed using 0.5 µm filter paper, and the clear obtained was adjusted to pH 10.2 with 0.1 M $CaCO_3$ at a temperature of 60 °C with constant stirring of 200 rpm, for 30 min. Then to remove different components of the waste, such as fats and proteins, the extract was adjusted to pH 8 with 0.1 N HCl and filtered again, to remove the sediments and impurities generated during the carbonation process inulin and in this way, the crude extract of inulin was obtained (Chacón-Villalobos, 2006; Escobar-Ledesma, 2017; Pinango Cuacango, 2019).

Inulin Determination

To determine the inulin content in the purified extracts, a UV-visible spectrometry. Inulin from chicory (I2255, Sigma-Aldrich) was used to elaborate a reference curve based on the Beer-Lambert’s law in which the absorbance at a wavelength of 715 nm was correlated against the knowing concentration of inulin following the procedure described elsewhere (Hizukuri et al., 1981; Park & Johnson, 1949).

Infrared Spectroscopy (IR) Characterization

The IR analysis of the reference material and purified samples were performed at room temperature on an IR Agilent Cary 630 FTIR model in a wavenumber range from 600 to 4000 cm^{-1} at 32 scans with a resolution of 4 cm^{-1}. An ATR sampling technique was used on a single bounce diamond crystal.

FTIR is a suitable technique to study the physicochemical properties of inulin, which constitutes a mixture of polysaccharides of different degrees of polymerization (Romano et al., 2018).

Statistical optimization of the extraction conditions of crude inulin from garlic agro-industrial organic wastes

The central composite experimental design (CCD) of the response surface methodology (RSM) was executed (Myers et al., 2016; Yolmeh & Jafari, 2017), to find the combination of the DW-to-mass of GAIOW ratio and the temperature that maximizes the raw inulin yield. All experiments were planned and analyzed using the Expert-Design 11.0.3.0 statistical package (Stat-Ease, Inc., Minneapolis, USA).

The response variable (crude (non-purified) inulin yield) was adjusted to a second-order statistical model described by the following equation:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_{i1} X_i + \sum_{i=1}^{2} \beta_{i2} {X_i}^2 + \sum_{i=1}^{1} \beta_{i+2,i+2} X_i X_{i+1} + \varepsilon$$

Where Y is the yield of crude inulin (g of crude inulin/100 g of GAIOW or % (w/w)); β_0 is the average value of all effects in the model; β_{11} represents the effect of factor X_1 (R, mL/g); β_{21} represents the effect of factor X_2 (T, °C); β_{12}

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represents the quadratic effect of factor X_1; β_{22} represents the quadratic effect of factor X_2, and β_{33} is the effect of the interaction of factors X_1 and X_2. The ε is the random model error caused by other sources of variability not considered in this model.

Results and discussion

The actual and coded values of the independent variables and the response obtained by both the quadratic model and the experimental values are presented in Table 1.

TABLE 1

The quadratic equations in terms of coded and real factors obtained were:

$$Y = 8.00 + 1.19 \cdot X_1 + 0.07 \cdot X_2 - 2.10 \cdot {X_1}^2 - 2.29 \cdot {X_2}^2$$

$$Y = -177.28 + 17.97 \cdot R + 3.67 \cdot T - 2.10 \cdot R^2 - 0.02 \cdot T^2$$

The analysis of variance (ANOVA) of the model is shown in Table 2. An F-value of 168.81 implies that the model is significant. All p-values were significant in the model ($p < 0.05$), except the one associated with X_2 that is included in the model to guarantee its hierarchy. Not-significant lack-of-fit relatives to pure error are useful for model and can be used to fit the experimental data.

TABLE 2

The suggested model can be used to find the maximum value of yield based on a combination of *R* and *T*, as shown, among others the R^2, R^2-adjusted and signal to noise ratio (adequate precision > 4) values (Table 3), and, by the graphs of the

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normal-plot of residues (Fig. 1A) and in the relationship between predicted vs. actual *Y*-values (Fig. 1B).

FIGURE 1

TABLE 3

3D-Graph of the crude inulin yield (*Y*, g/100 g) with the solvent/raw material ratio (*R*, mL/g) and the temperature (*T*, °C) shows the existence of an absolute maximum value inside of experimental surface for the yield (Fig. 2A), which was determined by a numerical algorithm by the Design-Expert software (Fig. 2B).

A unique optimal point was obtained, close to the central point, for *R** = 4.3 mL/g and *T** = 80.2 °C, which maximizes the model of crude inulin yield Y_{max} = 8.169 ± 0.815 g/100 g of garlic organic waste (Fig. 2B).

FIGURE 2

Three similar experiments were performed to validate the suggested model using the optimal point (*R** and *T**). The average result obtained, as well as the individual values, are within the range of values predicted by the model, which confirms the accuracy of the quadratic regression model for the crude inulin yield (Table 4).

TABLE 4

The results presented here are somewhat lower than the values reported in other reports (Y. Wang et al., 2015). The amount of inulin in garlic bulbs is between 9-16 g/100 g (Lara-Fiallos et al., 2017; Madrigal & Sangronis, 2007). A value of 9.80 ± 0.03 g/100 g for garlic bulbs and statistically similar values for onion (*Allium cepa* L.), leek (*Allium porrum* L.) and dandelion (*Taraxacum*

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officinale) have been reported elsewhere (Monroy-Rodríguez, 2010). These values are between 11.1-50% above the amounts reported in the present study. The difference is probably because, in the present investigation, the industrial residues of garlic were used, whereas, in the mentioned investigations, the whole bulbs of the commercial and edible parts of garlic are used.

Other factors that could influence the extraction yield of inulin from GAIOWs, like the extraction time and the power of stirring the mixture, are not considered in this investigation, not to extend the experimentation time (it would go from 13 for two variables to 30 experimental runs for four) and because the stirring power and the extraction time would be easily adjustable parameters of the process if it were decided to establish this process on a productive scale.

To validate the inulin-type nature of the purified sample obtained under the optimal extraction condition reached in this study, FTIR analysis of such a purified sample (PS) and the reference material (RM) from Sigma-Aldrich (inulin from chicory, I2255) were carried out (Fig. 3).

FIGURE 3

By using IR-analysis the main contributions of chemical groups of inulin were determined and summarized (Table 5).

TABLE 5

Among others, the peaks observed at 3280 cm^{-1} (RM) and 3254 cm^{-1} (PS) were assigned to the stretch (O-H) of $(R)_2$-CH-OH and R-CH_2-OH groups of alcohol chains. The peaks at 2883 cm^{-1} (in both RM and PS) assigned to C-H stretching vibrations indicated the presence of alkanes type of $(R)_3$-CH, while the peaks at

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2930 cm^{-1} (RM) and 2932 cm^{-1} (PS) corresponds with strong asymmetrical stretching vibrations of the group C-H of alkanes type R-CH_2-R.

The most variable region of the FTIR spectrum among inulin's of different origin is 1500 - 800 cm^{-1} (Fig. 3), which is presumably due to the difference between the degrees of polymerization of the mixture of polysaccharides that form the inulin between the reference material (obtained from chicory) and the studied sample (obtained from garlic waste). A similar area of the FTIR spectrum (1450 - 900 cm^{-1}) was reported in another study where the greatest differences observed between two inulin samples were found, formed by mixtures of oligosaccharides and polysaccharides, one of low and the other of the high degree of polymerization (Romano et al., 2018).

Conclusions

By using a CCD in the RSM, a quadratic model of the yield of crude (non-purified) inulin extraction from agro-industrial organic garlic wastes with two independent variables were obtained. This research showed an optimal yield of around 8 g of crude inulin per 100 g of GAIOW. Although this value is lower than inulin obtained from a whole bulb of commercial garlic, even so, maybe attractive enough to implement an industrial process to produce inulin from agro-industrial garlic useless organic waste. To add this product to company's portfolio further studies must demonstrated the economic feasibility production of crude inulin from GAIOW.

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Abbreviations

ANOVA: Analysis of variance; CCD: central composite design; DW: distilled water; FTIR: Fourier-transformed infra-red spectra; GAIOWs: garlic agro-industrial organic wastes; IR: infra-red spectra; PS: purified sample; RM: reference material; RSM: response surface methodology.

Authors' contributions

Conceptualization: Marco V. Lara-Fiallos, Erenio González Suárez; Methodology: Marco V. Lara-Fiallos, José M. Pais-Chanfrau, Erenio González Suárez; Formal analysis and investigation: Leiker A. Bastidas-Delgado, Dayana T. Montalvo-Villacreses, Rosario C. Espín-Valladares, Jimmy Núñez-Pérez, Amaury Pérez-Martínez, Nelson Santiago-Vispo, Hortensia Rodríguez-Cabrera; Writing - original draft preparation: Leiker A. Bastidas-Delgado; Writing - review and editing: José M. Pais-Chanfrau; All authors read and approved the final manuscript.

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All data obtained or analyzed during this study are included in this article and available from the corresponding author.

Ethics approval and consent to participate.

Not applicable.

Consent for publication

The publication of the paper has been agreed by the authors.

Competing Interests

The authors declare that they have no competing interests.

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FIGURES

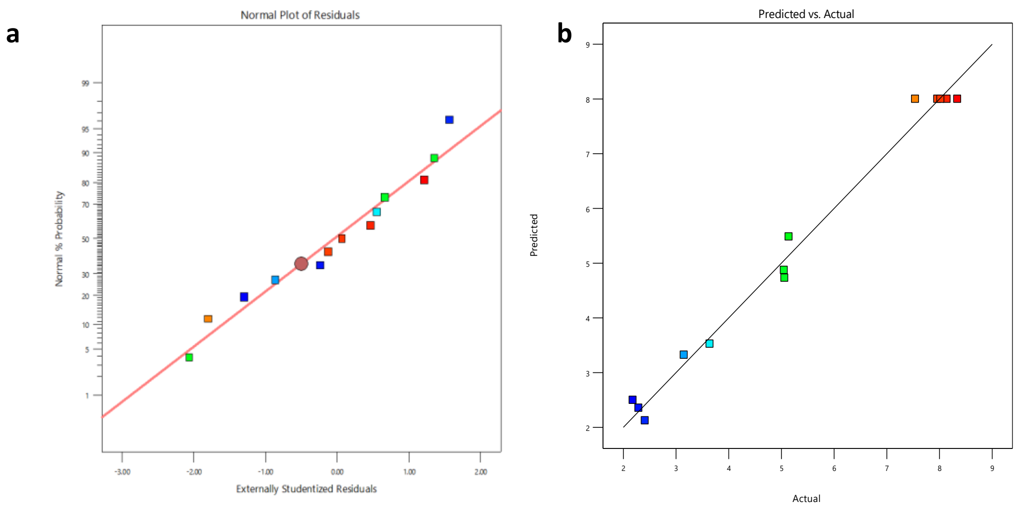

Fig. 1 (a) Normal plot of residuals and (b) predicted *vs*. actual values for the quadratic model of yield with a ration of solvent/mass of garlic organic waste and temperature of solvent extraction.

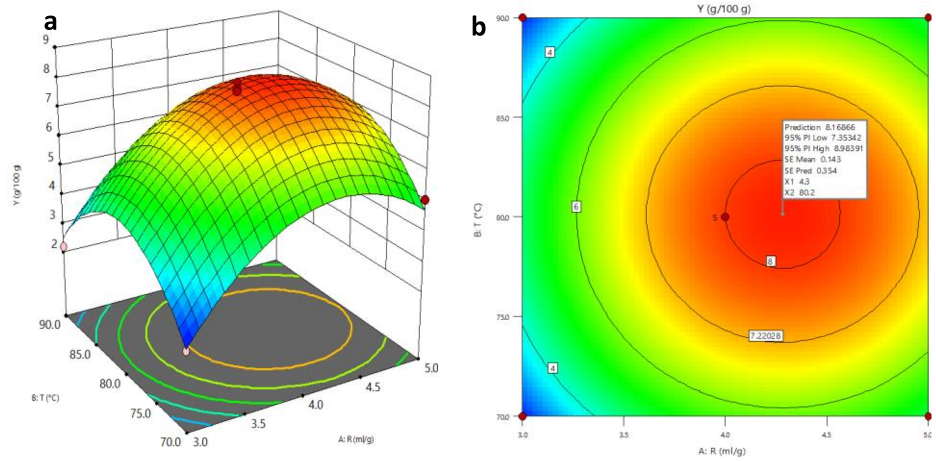

Fig. 2 (a) 3D-graph representation of the quadratic model with experimental points, and (b) Contour representation of the model with the optimal value of Y_{max} = 8.167 g/100 g of garlic waste at R*= 4.3 mL/g and T* = 80.2 °C.

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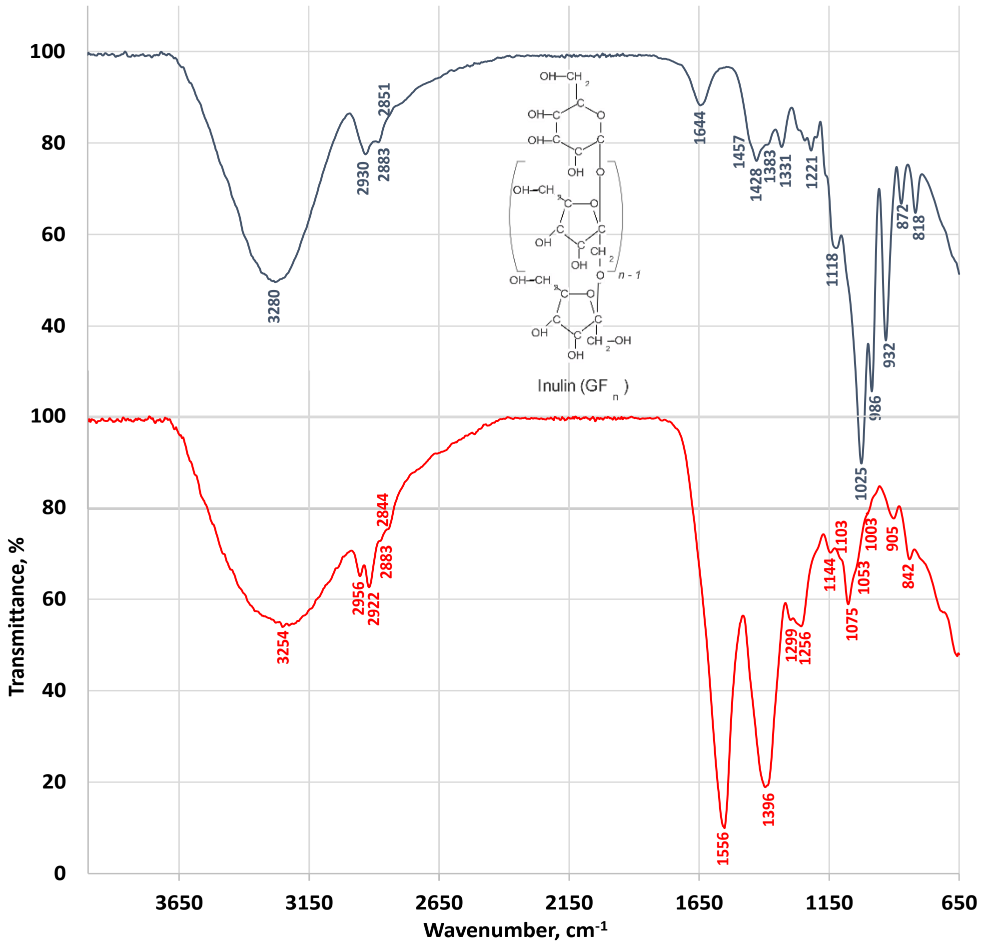

Fig. 3 FTIR spectrum of reference material from Sigma-Aldrich (RM, upper) and purified sample (PS, lower).

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TABLES

Table 1 Actual and predicted results for the response variable (Y: inulin yield (g/100 g)) obtained by the quadratic model based on the independent variables (X_1: R (mL/g) and X_2: T (°C)).

	X_1: R		X_2: T		Response (Y, g/100 g)	
Run	coded	actual (R, mL/g)	coded	actual (T, °C)	model	actual
1	+1.41	5.4	0.00	80.0	5.55	5.14
2	+1.00	5.0	-1.00	70.0	4.73	5.06
3	0.00	4.0	0.00	80.0	8.00	8.34
4	-1.41	2.6	0.00	80.0	2.23	2.41
5	0.00	4.0	0.00	80.0	8.00	8.02
6	-1.00	3.0	+1.00	90.0	2.50	2.18
7	+1.00	5.0	+1.00	90.0	4.87	5.05
8	0.00	4.0	+1.41	94.1	3.55	3.64
9	0.00	4.0	0.00	80.0	8.00	7.54
10	0.00	4.0	-1.41	65.9	3.35	3.15
11	-1.00	3.0	-1.00	70.0	2.36	2.29
12	0.00	4.0	0.00	80.0	8.00	7.96
13	0.00	4.0	0.00	80.0	8.00	8.14

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Table 2 ANOVA of the performance of the quadratic model of the yield of crude inulin with the solvent-to-weight of garlic organic wastes ratio and temperature of solvent extraction.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	70.63	4	17.66	168.81	< 0.0001	*significant*
X_1-R	11.28	1	11.28	107.87	< 0.0001	
X_2-T	0.041	1	0.041	0.3923	0.5485	
$X_1{}^2$	30.61	1	30.61	292.59	< 0.0001	
$X_2{}^2$	36.40	1	36.40	348.00	< 0.0001	
Residual	0.8368	8	0.1046			
Lack of Fit	0.4880	4	0.1220	1.4	0.3764	*not significant*
Pure Error	0.3488	4	0.0872			
Cor Total	71.47	12				

Table 3 Fit statistic values of the quadratic model for the yield of crude inulin from garlic organic wastes with solvent-to-weight of garlic wastes ratio and temperature of solvent extraction.

Std. Dev.	0.3234	R^2	0.9883
Mean	5.3015	Adjusted R^2	0.9824
C.V. %	6.1005	Predicted R^2	0.9594
		Adequate Precision	29.2881

Table 4 **Results of validation experiments.**

Response	Pred. Mean	Std. Dev.	n	SE Pred.	95% PI low	Data Mean	95% PI high
Y (g/100 g)	8.169	0.323	3	0.235	7.627	8.023	8.711

Table 5 Main peaks of the FTIR spectrum of inulin and the reference material (RM) and purified sample (PS).

Chem. Classif.	Group	Bond	Intensity	Wavelength peak, cm^{-1}	
				Range	Actual
Alcohols	$(R)_2$-CH-OH	O-H	variable	3400-3200	RM: 3280; PS: 3254
		O-H	strong	1350-1260	RM: 1331; PS: 1299
		C-O	strong	1125-1090	RM: 1118; PS: 1103
Alcohols	R-CH_2-OH	O-H	variable	3400-3200	RM: 3280; PS: 3254
		O-H	medium	1480-1410	RM: 1428,1457; PS: overlapping
		C-O	strong	1075-1000	RM: 1025; PS: 1003,1053,1075
Alkanes	$(R)_3$-CH	C-H	weak	2900-2880	RM: 2883; PS: 2883
		C-H	weak	1350-1320	RM: 1331; PS: overlapping
Alkanes	R-CH_2-R	C-H	strong	2940-2915	RM: 2930; PS: 2922
		C-H	strong	2863-2843	RM: 2851; PS: 2844
		C-H	medium	1485-1445	RM: 1457; PS: overlapping
Ethers	5-ring-ethers	C-O-C	strong	1080-1060	RM: overlapping; PS: 1075
		C-O-C	medium	920-905	RM: 932; PS: 905
Ethers	6-ring-ethers	C-O-C	strong	1110-1090	RM: overlapping; PS: 1103
		C-O-C	medium	820-805	RM: 818; PS: overlapping