

# Optimization of Crude Inulin Extraction from Garlic (*Allium sativum* L.) Agro-industrial Waste using the Response Surface Methodology.

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**Optimization of crude inulin extraction from garlic (*Allium sativum* L.)  
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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 waste

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was maximized, by applying the response surface methodology in a central composite design (CCD), in which different distilled-water (DW)-to-garlic-agro-industrial-waste (GAIW) ratios (3 and 5 mL/g) and different temperatures (60 and 80 °C) were evaluated. Optimal condition was obtained with a DW/GAIW 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  $8.17 \pm 0.89$  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 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 valorisation, 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 (Suleria et al. 2015; Ried 2016; Shang et al. 2019).

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 and 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 and Venkatesh 2016), polysaccharides (Hughes et al. 2017), polyphenols (Ichikawa et al. 2003), cellulose (Reddy and Rhim 2018), lignin, and to absorb the heavy metals (Liu et al. 2014; Chen et al. 2018). 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 wastes are especially attractive sources for waste valorisation 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 (Moldes et al. 2002; Otles and Kartal 2018; Galanakis 2021).

Through the valorisation of agro-industrial 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 (Hiloidhari et al. 2020; Galanakis 2021).

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 (Franck 2002, 2016; Apolinário et al. 2014; 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 (Panesar and Bali 2016; Shoaib et al. 2016; James et al. 2017; 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 and Tamashiro 2018; Ghaffari and Roshanravan 2020; Guarino et al. 2020), prevents the development of colon cancer (Pool-Zobel and 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 (Wang and Gibson 1993; Jung et al. 2015).

Inulin is soluble in water (Yanovsky and 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 ethanol precipitation has been used as a common method of obtaining commercial inulin from different natural sources (Niness 1999; Álvarez-Borroto et al. 2015). 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 industrial 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 wastes (GAIWs) are formed by damaged bulbs, husks and the garlic paste lumps formed after cooking.

GAIWs 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 GAIWs 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-GAIW ratios (3 and 5 mL of DW per gram of GAIW), combined with two temperature levels (70 and 90 °C).

Thirty grams (30 g) GAIWs was used for all treatments, and the extraction time was 45 min with constant agitation of 200 rpm (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<sub>3</sub> 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 (Park and Johnson 1949; Hizukuri et al. 1981).

### **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<sup>-1</sup> at 32 scans with a resolution of 4 cm<sup>-1</sup>. 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 wastes**

The central composite experimental design (CCD) of the response surface methodology (RSM) was executed (Myers et al. 2016; Yolmeh and Jafari 2017), to find the combination of the DW-to-mass of GAIW 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:

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

Where  $Y$  is the yield of crude inulin (g of crude inulin/100 g of GAIW or % (w/w));  $\beta_0$  is the average value of all effects in the model;  $\beta_{11}$  represents the effect of factor  $X_1$  ( $R$ , mL/g);  $\beta_{21}$  represents the effect of factor  $X_2$  ( $T$ , °C);  $\beta_{12}$  represents the quadratic



effect of factor  $X_1$ ;  $\beta_{22}$  represents the quadratic effect of factor  $X_2$ , and  $\beta_{33}$  is the effect of the interaction of factors  $X_1$  and  $X_2$ . The  $\varepsilon$  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 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  $Y_{max} = 8.169 \pm 0.815$  g/100 g of garlic 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 (Wang et al. 2015). The amount of inulin in garlic bulbs is between 9-16 g/100 g (Madrigal and Sangronis 2007; Lara-Fiallos et al. 2017). A value of  $9.80 \pm 0.03$  g/100 g for garlic bulbs and statistically similar values for onion (*Allium cepa* L.), leek (*Allium porrum* L.) and dandelion (*Taraxacum 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 GAIWs, 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  $3280\text{ cm}^{-1}$  (RM) and  $3254\text{ cm}^{-1}$  (PS) were assigned to the stretch (O-H) of  $(R)_2\text{-CH-OH}$  and  $R\text{-CH}_2\text{-OH}$  groups of alcohol chains. The peaks at  $2883\text{ cm}^{-1}$  (in both RM and PS) assigned to C-H stretching vibrations indicated the presence of alkanes type of  $(R)_3\text{-CH}$ , while the peaks at  $2930\text{ cm}^{-1}$  (RM) and  $2932\text{ cm}^{-1}$  (PS) corresponds with strong asymmetrical stretching vibrations of the group C-H of alkanes type  $R\text{-CH}_2\text{-R}$ .

The most variable region of the FTIR spectrum among inulin's of different origin is  $1500 - 800\text{ cm}^{-1}$  (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 ( $1450 - 900\text{ cm}^{-1}$ ) 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 industrial 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 GAIW. 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 waste. To add this product to company's portfolio further studies must demonstrated the economic feasibility production of crude inulin from GAIW.

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## **Abbreviations**

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

## **Authors’ contributions**

MVLF and EGS conceived and designed the experiments. LABD, DTMV, RCEV, and JNP performed experiments. APM, NSV, and HRC provided important technical

support for experiments. JMPC elaborated and reviewed the manuscript. All authors discussed the results, and all authors read and approved the final manuscript.

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#### **Availability of data and materials**

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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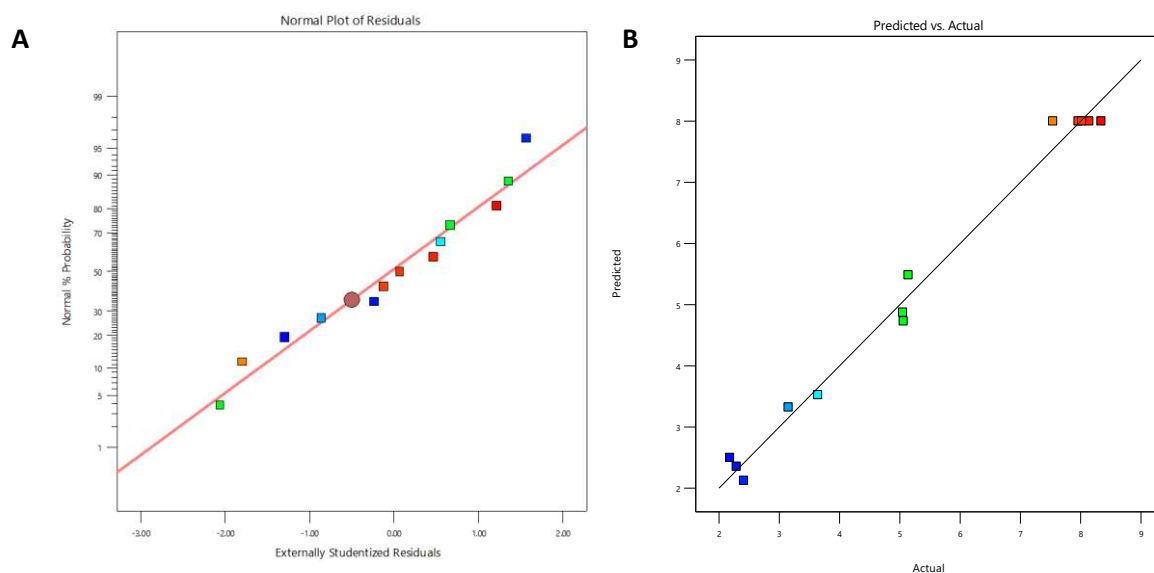
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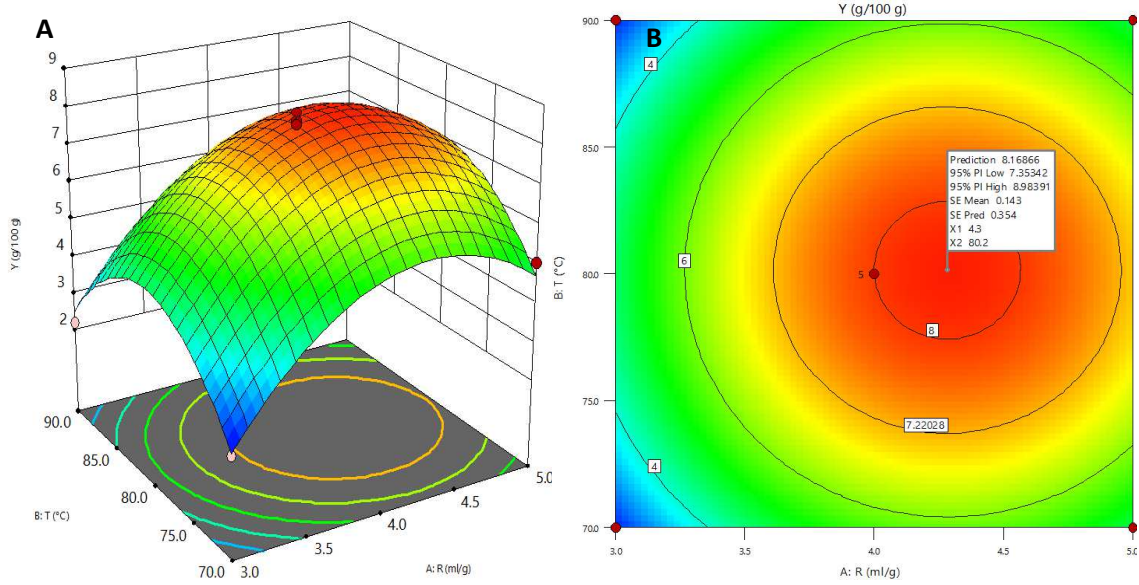
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## FIGURES



**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 waste and temperature of solvent extraction.

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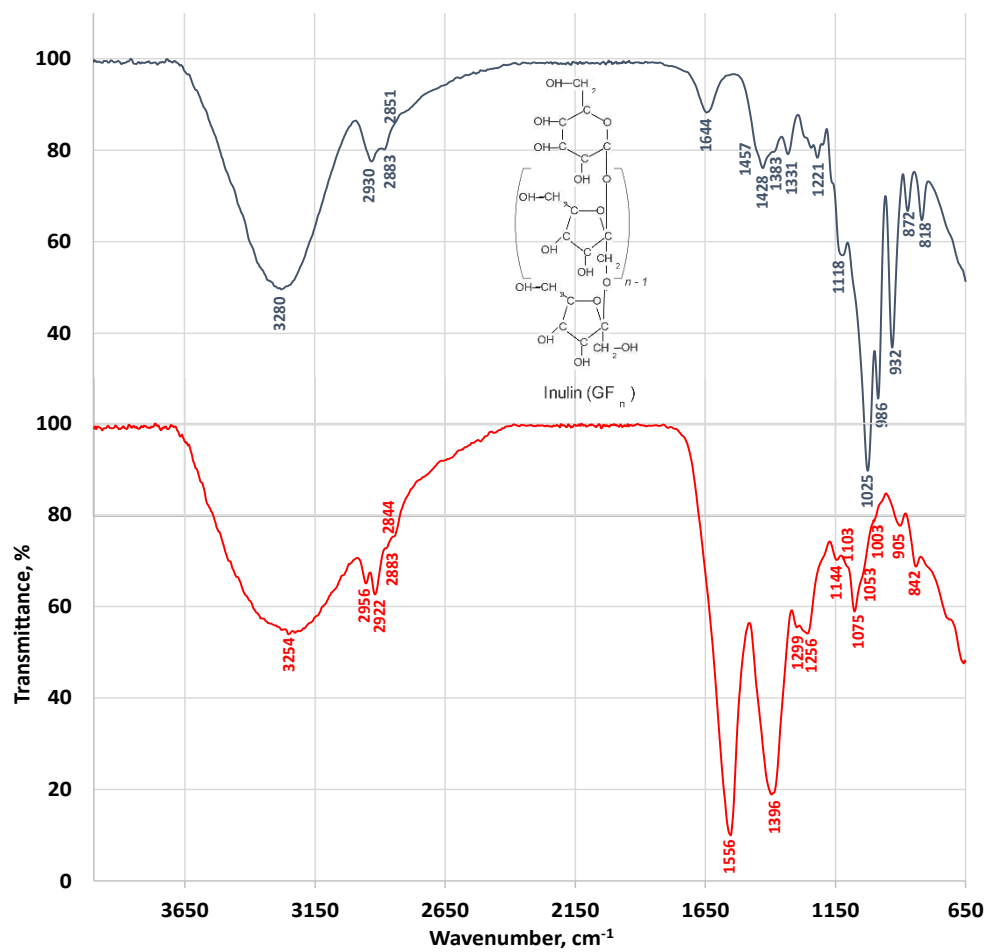
**Fig. 2** (A) 3D-graph representation of the quadratic model with experimental points, and

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(B) Contour representation of the model with the optimal value of  $Y_{\max} = 8.167$  g/100 g of

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garlic waste at  $R^* = 4.3$  mL/g and  $T^* = 80.2$  °C.



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

## 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<sub>1</sub>: R (mL/g) and X<sub>2</sub>: T (°C)).**

Run	<i>X</i> <sub>1</sub> : <i>R</i>		<i>X</i> <sub>2</sub> : <i>T</i>		Response ( <i>Y</i> , g/100 g)	
	coded	actual ( <i>R</i> , mL/g)	coded	actual ( <i>T</i> , °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

**Table 2 ANOVA of the performance of the quadratic model of the yield of crude inulin with the solvent-to-weight of garlic 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	<i>significant</i>
$X_1$ - $R$	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	<i>not significant</i>
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 wastes with solvent-to-weight of garlic wastes ratio and temperature of solvent extraction.**

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

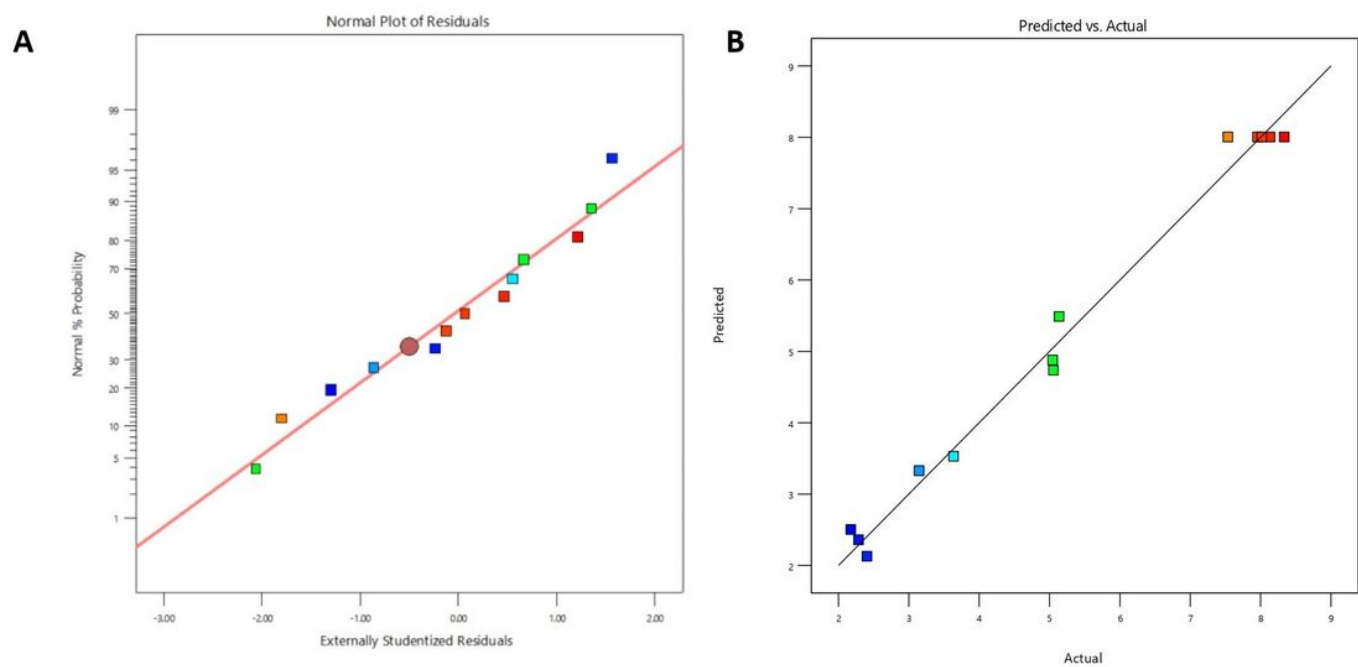
**Table 4 Results of validation experiments.**

Response	Pred. Mean	Std. Dev.	n	SE Pred.	95% PI low	Data Mean	95% PI high
<i>Y</i> (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).**

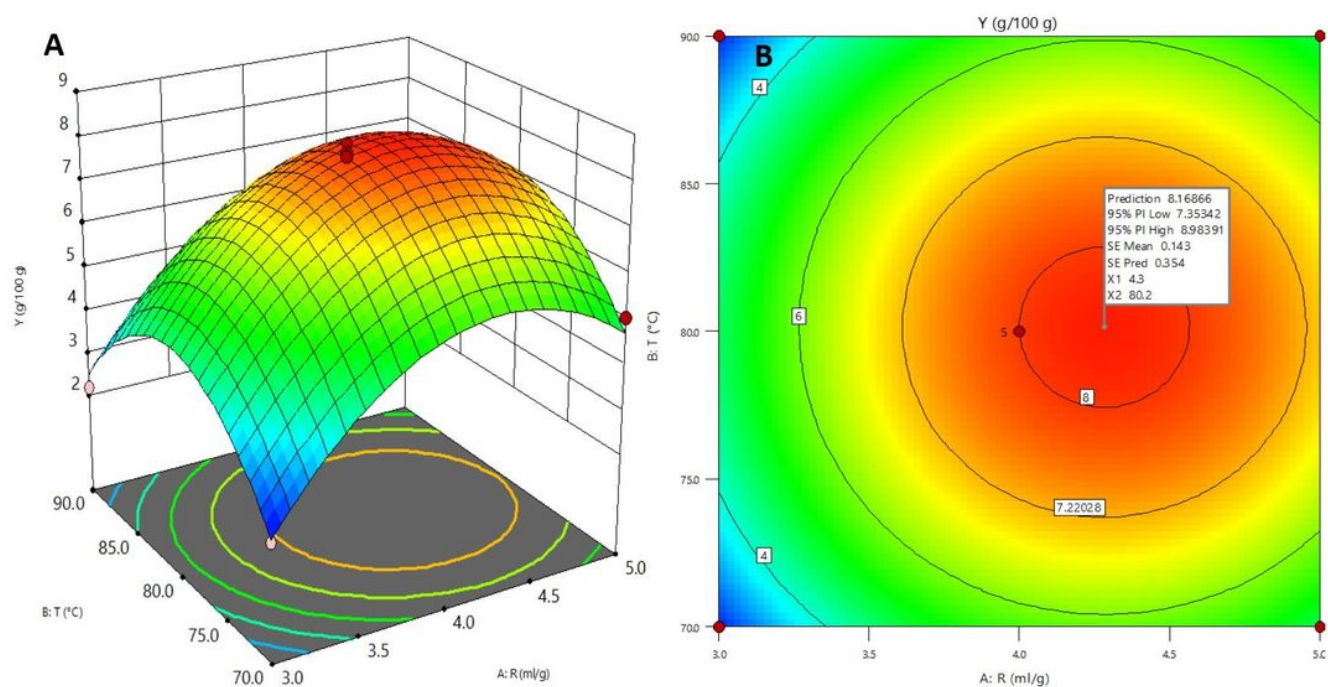
Classifications	Group	Bond	Intensity	Wavelength peak, cm <sup>-1</sup>	
				Range	Actual
Alcohols	(R) <sub>2</sub> -CH-OH	O-H	variable	3400-3200	RM: 3280; PS: 3254
		O-H	strong	1350-1260	RM: 1331; PS: 1299
		C-O	strong	1125-1090	RM: 1118; PS: 1103
Alcohols	R-CH <sub>2</sub> -OH	O-H	variable	3400-3200	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) <sub>3</sub> -CH	C-H	weak	2900-2880	RM: 2883; PS: 2883
		C-H	weak	1350-1320	RM: 1331; PS: overlapping
Alkanes	R-CH <sub>2</sub> -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

# Figures



**Figure 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 waste and temperature of solvent extraction.



**Figure 2**

(A) 3D-graph representation of the quadratic model with experimental points, and (B) Contour representation of the model with the optimal value of Ymax = 8.167 g/100 g of garlic waste at R\*= 4.3 mL/g and T\* = 80.2 °C.

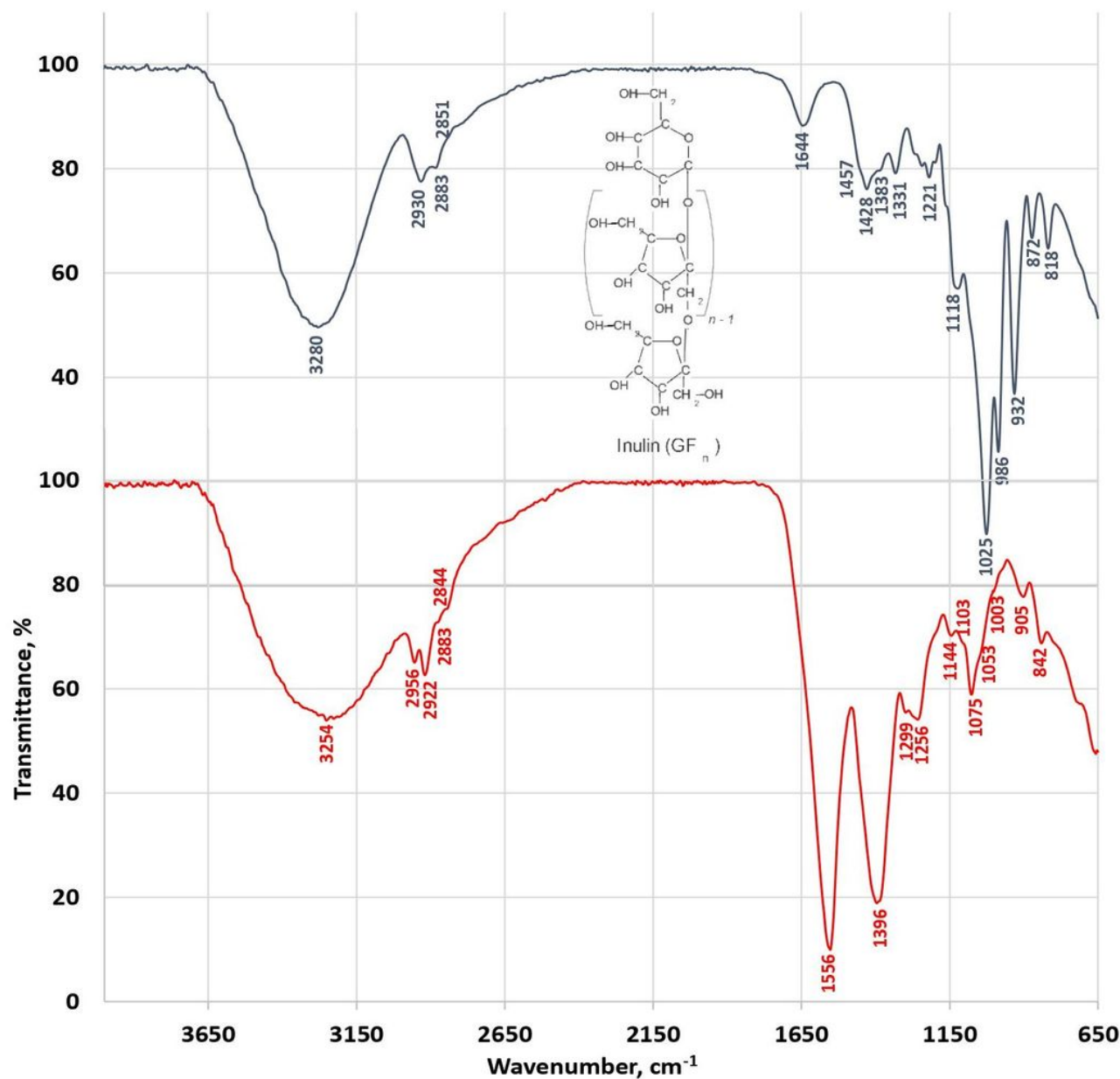


Figure 3

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

## Supplementary Files

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