

Multiple Regression Analysis for Optimization of Inulin Production from Chicory Roots

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Article History:

Received: 28-09-2024

Revised: 16-10-2024

Accepted: 08-12-2024

Abstract:

Inulin is a polysaccharide of fructose produced from various plant sources and has wide applications in food industries. The present work aims to perform multiple regression analysis for optimization of inulin yield generated from the roots of chicory. Chicory roots were used to extract the main compound inulin using water as the extractant. Multiple regression analysis was carried out by investigating 3 independent variables, Volume of solvent (15-75 mL), time of extraction (10-30 min), and temperature of extraction (30-90 °C). From the analysis, a maximum inulin yield of 1.91% at optimum volume of solvent, extraction time and temperature of 50 mL, 20.5 min and 90 °C, respectively. Hence, multiple regression analysis could be effectively used to maximize inulin yield from chicory roots.

Keywords: Chicory roots, Inulin, Multiple regression, Optimization.

1. Introduction

Numerous plants, particularly Jerusalem artichokes, chicory, as well as asparagus, contain a soluble fiber called inulin. [1]. It is a naturally occurring polysaccharide, which means it is made up of multiple sugar molecules linked together. Inulin is not digested by the upper GI track particularly small intestine, so it moves through to the lower GI track means large intestine where it is going to ferment by different types of beneficial bacterium, producing short-chain fatty acids (SCFAs) such as butyrate[2]. Inulin has gained popularity as a prebiotic, a type of dietary fiber that promotes the growth of beneficial bacteria in the gut[3]. It has also been shown to have potential health benefits such as improving digestion, increasing calcium absorption, and reducing the risk of certain diseases such as colorectal cancer and type 2 diabetes. Inulin can be found as an ingredient in a variety of foods, such as yogurt, bars, and cereals, and is also available as a dietary supplement[4]. Inulin is a type of soluble dietary fiber that is found in many plants, such as chicory root, artichokes, and asparagus[5]. It is commonly used as a prebiotic and a sweetener in food and beverage applications.

Several methods are available for producing inulin, including extraction from plant sources such as chicory roots, by washing and grinding the roots to create a pulp[6]. The pulp is then mixed with water and heated to extract the inulin. The resulting solution is then filtered and purified. In enzymatic hydrolysis method, enzymes are used to break down the starch molecules in plant sources into smaller chains of fructose molecules, which can be further processed to produce inulin[7]. The fructose chains are then extracted and purified to obtain inulin. By microbial fermentation bacteria and yeasts have the ability to convert sugar into inulin through a process called microbial fermentation[8]. In this method, the plant source is first broken down into simple sugars, which are then fermented by the microbes to produce inulin. The resulting inulin can be further purified and used in food and beverage applications. Inulin can also be synthesized chemically from fructose or glucose molecules[9]. However, this method is not commonly used due to its high cost and the potential for undesirable by products. The selection of method is influenced by elements including production cost, output, and purity criteria. Each approach has pros and cons. Chicory roots contain inulin, a form of dietary fiber that may be found in many plants.

Chicory (*Cichorium intybus* L.) is considered to be a perennial herbaceous plant that is grown commercially for its roots, which contain high levels of inulin. Inulin has several health benefits, including improving gut health and regulating blood sugar levels. The process of inulin production from chicory roots involves several steps[10]. Harvesting of chicory roots happens in the autumn when they have reached maturity[11]. The roots are washed, chopped, and then extracted with hot water to remove the inulin. The inulin extract is then filtered to remove any impurities. The inulin extract is concentrated by removing the water through a process of evaporation. The concentrated inulin is purified using techniques such as chromatography to remove any remaining impurities. The purified inulin is dried to produce a fine powder that can be used in food products or dietary supplements. The resulting inulin powder can be used as a natural sweetener, a prebiotic, and a functional ingredient in various food products, including dairy, bakery, and meat products[12]. In addition, inulin is also used in dietary supplements and functional foods to improve gut health and regulate blood sugar levels.

Regression analysis is used for relating independent and dependent variables for prediction and forecasting[13]. A single regression analysis relates one output factor to an input factor. A statistical method called multiple regression analysis can be used to examine the relationship between several variables. [14]. The regression models are either linear or non linear. An equation containing linear, factor interactive, quadratic, cubic and higher orders are considered as linear models[15]. An equation containing power, logarithmic, exponential, trigonometric and other functions are considered as non linear models. The fitting of experimental data to the model could be evaluated by error functions[16].

According to literature, various scientific publications have been published on the production of inulin from Jerusalem artichoke[17,18], garlic[19], raw asparagus[20], raw onion pulp[21], wheat[22], raw barley[23]. The previous work studied the optimization of inulin production from chicory roots using single regression analysis [24]. However, there is a paucity of studies on the multiple regression analysis on the manufacture of inulin from the roots of chicory. A survey of the literature revealed that limited research has been done on process optimization with the manufacture of inulin from the roots of chicory using multiple regression analysis. The present study aimed to optimize process parameters for the manufacture of inulin from the roots of chicory.

2. Methods and Materials

2.1. Materials and experimental methods:

The materials and methods used in the previous study [24] were followed. Chicory roots (*Cichorium intybus* L.) were bought in a Salalah, Oman, local market. It is necessary to peel and clean the tubers and roots as given in Fig.1. The fresh chicory inflorescences were manually separated from the internal, edible bracts of the core—also referred to as the receptacle, head, or capitulum—and the outside, inedible bracts and stalks, which constitute the by-product. The samples were all cut, dried, and processed when they could all fit through a 1 mm sieve and were sieved. The dehydrated samples were preserved in a dry, 0.2% moisture content container at room temperature and shielded from the sun. A commercial standard for inulin was created by Sigma-Aldrich Chemicals (Salalah, Oman). ORAFTEC Espana S.L. provided other commercial standards, such as oligofructose (Barcelona, Spain). The supplier of 3,5-dinitro salicylic acid (DNS) reagent 2 was Sigma-Aldrich, which also provided fructose (USA), stachyose, raffinose, sucrose, and glucose. Panreac supplied the anthrone reagent (Spain). The remaining reagents were of high analytical quality.

The extraction procedure was carried out in accordance with the experimental design below, which varies the temperature, duration, and solvent to solid ratio. Chicory powder inulin was extracted for this purpose by dissolving it in ethanol or water as given in Fig. 1. We refer to this as a solid-liquid extraction. The amount of glucose and inulin that can be produced, as well as the quantity of each, are estimated. One by one, a stock solution was created by adding fructose and glucose to water. A 50 mL glass vial containing one gramme of dry powdered materials was filled with 10–40 mL of water, which served as the extraction solvent. At 30 to 90°C, the process took place in 10 to 30 minutes. Following each extraction, the sample-filled tubes were allowed to come to room temperature before being spun for 15 minutes at 3300 x g to remove any remaining insoluble material.

Whatman cellulose filter paper (3 mm) is used to filter the supernatant. After filtering, the liquid was reduced to 25 mL by boiling it at 450 °C in a rotatory evaporator. Different proportions of chicory solution were created by varying the solvent quantity. The absorbance of the solutions was measured using an Ultra Violet (UV) spectrophotometer and Fourier-transform infrared spectroscopy (FTIR). The impact of the variation in the percentage of solute on the amount of inulin extracted can be determined by calculating the mass and recovery

percentage of the glucose and fructose. Until further testing was conducted, the remaining extracts were stored at 180 °C.

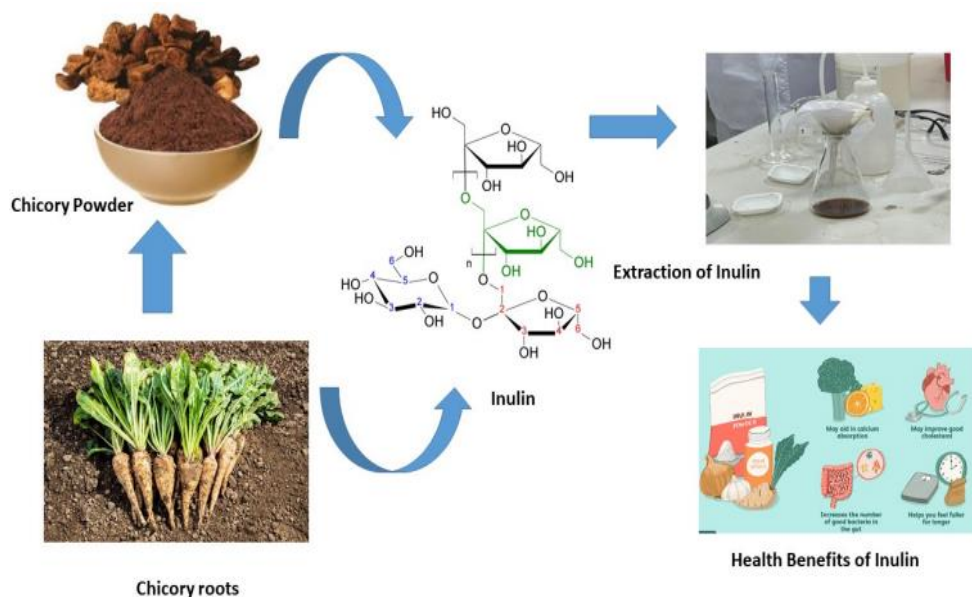


Figure 1. Extraction of inulin from chicory roots

2.2. Multiple regression analysis:

In the context of inulin production from chicory, multiple regression analysis can be used to identify the key factors that influence the yield of inulin from chicory roots [24]. To perform multiple regression analysis for inulin production, the following steps can be taken. The variables that may influence inulin production can include factors such as the temperature and duration of extraction, pH of the extraction medium, and the concentration of inulin in the raw material[25]. Data can be collected by conducting experiments to measure the yield of inulin under different conditions. Multiple regression analysis can be performed using statistical software to identify the significant factors that affect inulin yield[26][27][28]. The analysis will produce a regression equation that can be used to predict inulin yield based on the values of the input variables. The regression equation and statistical analysis can be used to determine which variables have the most significant impact on inulin yield[29]. This information can be used to optimize the extraction process to maximize inulin production. Overall, multiple regression analysis can be a useful tool for optimizing inulin production from chicory roots[30]. By identifying the key factors that influence inulin yield, it can help researchers and manufacturers to optimize the extraction process and maximize the production of this valuable dietary fiber[31]. In the present study extraction time, temperature and volume of solvent (water) at constant pH were investigated on inulin yield from chicory roots. The models considered in this study are given in Table 1.

Table 1. Types of models used in the present study for optimization of inulin from chicory roots

Model type	Equation
Linear with intercept	$y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C$
Linear without intercept	$y = \alpha_1 A + \alpha_2 B + \alpha_3 C$
2FI with intercept	$y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 BC + \alpha_6 CA$
2FI without intercept	$y = \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 BC + \alpha_6 CA$
Quadratic with intercept	$y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 BC + \alpha_6 CA + \alpha_7 A^2 + \alpha_8 B^2 + \alpha_9 C^2$
Quadratic without intercept	$y = \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 AB + \alpha_5 BC + \alpha_6 CA + \alpha_7 A^2 + \alpha_8 B^2 + \alpha_9 C^2$
Power with intercept	$y = \beta_0 + \beta_1 A^{n_1} + \beta_2 B^{n_2} + \beta_3 C^{n_3} + \beta_4 A^2 + \beta_5 B^2 + \beta_6 C^2$

A multiple regression analysis starts with fitting experimental data to basic linear models with and without intercept. A higher order linear and non linear models were further attempted if the linear model exhibits poor fit.

The coefficients in linear models are evaluated using principle of least squares which states that sum of square of errors should be minimum. Error is the difference between experimental and predicted data. The equations in matrix form is written in (1) as

$$(X^T X)\alpha = X^T Y \quad (1)$$

The solution of Equation (1) is given in equation (2) as

$$\alpha = (X^T X)^{-1} \cdot X^T Y \quad (2)$$

The coefficients in power model cannot be evaluated by the principle of least squares as it is not possible to represented in matrix form. Hence, Solver, an add-in program of Microsoft Excel, is used to evaluate the constants in power model. In Solver, sum of square of error is given as objective function and coefficients are initialized with appropriate values. The target is to minimize the sum of square of error and the coefficients are evaluated using generalized reduced gradient (GRG) algorithm. The goodness of fit of an appropriate model was evaluated using R^2 as given in Equation (3).

$$R^2 = 1 - \frac{SSE}{SST} \quad (3)$$

where SSE and SST are sum of square of errors and total sum of square, respectively.

3. Results and Discussion:

Table 2 represents the Independent factors and the range variables used for inulin extraction from chicory.

Table 2. Independent factors and levels used for inulin extraction from chicory

Independent factor	Symbol	Unit	Levels	
			Low	High
Volume of solvent	A	ml	15	75
Extraction time	B	min	10	20
Temperature	C	°C	30	90

Table 3 represents models with estimated coefficients and corresponding R^2 values. As evident from the equation (3), as error approaches zero, R^2 approaches one. Since total sum of squares is sum of squares of model and error, as sum of square of model approaches zero, R^2 approaches zero. The good fitness of model could be revealed from the R^2 value of greater than 0.81. An empirical modelling between volume of solvent (A), extraction time (B) and temperature (C) as independent variables and inulin yield (Y) as dependent variable exhibits that linear models do not fit well to the experimental data with R^2 less than 0.81. The power model was found to be a good fit with R^2 of greater than 0.81.

Table 3. Experimental design showing volume of solvent (A), extraction time (B) and temperature (C) on experimental inulin yield (Y_e) and predicted yield (Y_p)

A (mL)	B (min)	C (°C)	Y_e (%)	Predicted Inulin yield Y_p (%)						
				L,i	L,o	2FI, i	2FI,o	Q,i	Q,o	P
15	20	90	0.206	0.70851	0.6490	14.218	-2.920	-28.827	-38.393	0.1071
25	20	90	0.483	0.91483	0.8768	25.781	-6.273	-32.536	-51.132	0.8146
37.5	20	90	1.812	1.1727	1.1617	40.234	-10.465	-37.531	-67.056	1.4239
50	20	90	1.804	1.4306	1.4465	54.687	-14.657	-42.925	-82.980	1.6952
62.5	20	90	1.55	1.6885	1.7314	69.140	-18.848	-48.717	-98.904	1.6099
75	20	90	1.2	1.9464	2.0162	83.593	-23.040	-54.908	-114.82	1.1567
50	10	90	1.43276	1.4645	1.3715	15.507	-12.352	-43.770	-87.359	1.7733
50	20	90	1.85771	1.4306	1.4465	54.687	-14.657	-42.925	-82.980	1.6952
50	30	90	1.36493	1.3967	1.5216	93.867	-16.961	-42.907	-78.601	1.5650

50	20	30	1.428	1.4758	1.3418	31.718	-12.906	-33.965	-59.345	1.4355
50	20	60	1.549	1.4532	1.3942	43.203	-13.781	-38.516	-71.162	1.5350
50	20	90	1.826	1.4306	1.4465	54.687	-14.657	-42.925	-82.980	1.6952

L,i: Linear with intercept; L,o: Linear without intercept; 2FI, i: 2 Factor interactive with intercept; 2FI,o: 2 Factor interactive without intercept; Q,i: Quadratic with intercept; Q,o: Quadratic without intercept; P: Power.

Table 4 represents models with estimated coefficients and corresponding R^2 values. As evident from the equation (3), as error approaches zero, R^2 approaches one. Since total sum of squares is sum of squares of model and error, as sum of square of model approaches zero, R^2 approaches zero. The good fitness of model could be revealed from the R^2 value of greater than 0.81. An empirical modelling between volume of solvent (A), extraction time (B) and temperature (C) as independent variables and inulin yield (Y) as dependent variable exhibits that linear models do not fit well to the experimental data with R^2 less than 0.81. The power model was found to be a good fit with R^2 of greater than 0.81.

Table 4. Models with coefficients and R^2 value for optimization of inulin from chicory roots

Model type	Equation	R^2
Linear with intercept	$y = 0.5347 + 0.0206A - 0.0034B - 0.0008C$	0.4200
Linear without intercept	$y = 0.0227A + 0.0075B + 0.0017C$	0.7030
2FI with intercept	$y = -8.000 + 1.000A + 3.000B - 0.1250C$ $- 0.00977AB - 0.0156BC + 0.0039CA$	0.4200
2FI without intercept	$y = -0.2187A + 0.1406B + 0.0312C - 0.0039AB$ $+ 0.0019BC + 0.0004CA$	0.7140
Quadratic with intercept	$y = 43.420 - 1.6171A - 1.0340 - 0.407C + 0.0336AB$ $- 0.0049BC + 0.0069CA - 0.0012A^2$ $- 0.0041B^2 + 7.9 \times 10^{-5}C^2$	0.7948
Quadratic without intercept	$y = 1.4607A - 1.3430A - 0.4639C - 0.0228AB$ $- 0.0274BC - 0.0052CA - 0.0012A^2$ $- 0.0041B^2 + 7.9 \times 10^{-5}C^2$	0.7844
Power with intercept	$y = -0.0054 + 0.0710A^{1.1432} + 78.49B^{-40.41}$ $- 1.368C^{-0.0055} - 0.0013A^2 - 0.0002B^2$ $- 3.49 \times 10^{-5}C^2$	0.8413

The principle of minima and maxima reveals that a maximum inulin yield of 1.91% was achieved at optimum volume of solvent, extraction time and temperature of 50 mL, 20.5 min and 90 °C, respectively. The obtained maximum inulin yield was further validated by performing experiments under optimal conditions in triplicate and standard deviation was found to be less than 5%. The material-related aspects of inulin production from chicory roots is valuable, as materials play a crucial role in the efficiency, sustainability, and quality of the process. Inulin is a valuable polysaccharide found in chicory roots, and its extraction and production involve several material considerations. A few key material-related aspects to consider are raw material selection, extraction process, choice of solvents, and purification methods. The present research helps for industries to improve the yield of inulin at optimum conditions.

Collinearity reveals where two or more predictor variables in a multiple regression model are highly correlated. This leads to exploring deeper insights in statistical modeling and interpretation. Multicollinearity occurs where two or more predictor variables are highly correlated but not perfectly correlated. Hence, from Table 4, the data is multicollinear as p-value is not equal to zero.

Table 4. ANOVA for power model for optimization of inulin from chicory roots

Source of Variation	SS	df	MS	F	p-value	F crit
Model	44733.54	3	14911.18	95.67643	2.34E-16	2.891564
Error	5143.053	33	155.8501			
Total	51447.03	47				

Conclusion:

The present work focusses on optimization of inulin yield from chicory root by varying volume of solvent, extraction time and temperature using multiple regression analysis. Multiple regression analysis was employed by selecting linear, 2 factor interactive and quadratic models and non linear power model. And the suitable model was evaluated by R^2 (>0.81). The analysis of models infers that power equation is suitable for empirical modelling of inulin production from chicory roots. From the analysis, a maximum inulin yield of 1.91% at optimum volume of solvent, extraction time and temperature of 50 mL, 20.5 min and 90 °C, respectively. Hence, multiple regression analysis could be effectively used to maximize inulin yield from chicory roots.

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