Optimization of Inulin Extraction from Sisal Wastes Using Response Surface Methodology

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Abstract

Inulin, a natural prebiotic dietary fiber with several health benefits, is commonly extracted from Jerusalem and Chicory plants. Even though boles and sisal wastes contain a high amount of inulin, extraction of inulin from such materials has not been well explored or optimized. The conventional methods that are normally used in inulin extraction from other plant sources have created a foundation for the extraction of inulin from sisal wastes. However, the methods need to be optimized to improve yield and quality. The purpose of this study was to identify and optimize important factors that influence inulin extraction. The Response Surface Methodology (RSM) with Box–Behnken design involving three levels of three independent variables (i.e. extraction temperature, extraction time, and solvent composition) was used. A second-order polynomial equation was formulated to describe the relationship between the dependent and independent variables. The optimal extraction conditions, which had high a inulin yield (18.24%), were extraction temperature of 80°C, time of 90 min, and solvent composition of 40%. The analysis of variance (ANOVA) indicated that the model was highly significant, with a coefficient of determination (R^2) of 0.9205. The optimization of the extraction process of inulin from sisal waste resulted in a significant increase in the yield of the extracted inulin. Therefore, sisal waste can be a potential source of inulin, and the optimized extraction process can be useful in industrial applications.

Keywords: optimization, inulin extraction, sisal wastes, response surface methodology

Introduction

Inulin, a versatile dietary fiber with prebiotic properties and health benefits, has gained significant attention. The growing incidences of metabolic diseases, including diabetes and obesity, which are associated with changes in eating habits, especially increased intake of fat and decreased dietary fiber intake (Ahmed and Rashid, 2019) increase the economic burden, especially in developing nations. Intake of dietary fibers has received a lot of attention across the world as a management strategy for metabolic disorders (Korcz *et al.,* 2018). Inulin is among the dietary fiber/ polysaccharides that play vital roles in human health (Chawla and Patil, 2010). Inulin is recognized as a natural food ingredient by most countries (Ahmed and Rashid, 2019). Consumption of 3-30 g of inulin per day reduces serum triglycerides, cholesterol, and Low-Density Lipoprotein (LDL) cholesterol in the blood (Li and Xu, 2021). It reduces food intake due to satiety; thus, controlling body weight. Moreover, the inclusion of inulin in diets has been shown to be effective in relieving constipation (Lan *et al.,* 2020) and enhancing mineral absorption. Fermentation of inulin in the colon produces short-chain fatty acids which reduce luminal pH and are used by beneficial bacteria in the colon (Blaak *et al.,* 2020; Schaafsma and Slavin, 2015). Low pH prohibits the growth of potentially pathogenic bacteria (Schaafsma and Slavin, 2015). However, inulin is industrially produced from Chicory and Jerusalem plants (Canli and Tasar, 2023). Although sisal is a potentially rich source for extraction of inulin than most plants, it is not well exploited especially in Tanzania.

Sisal is mainly cultivated for its sturdy fibers, which is only 2% of the leaf; the rest of the leaf (98%) and the boles are wasted (Msuya *et al.,* 2018). The production of inulin from sisal wastes and boles creates an opportunity to maximize resource utilization and expand the scope of sisal products benefiting the stakeholders in the chain. Despite the potential of sisal boles to produce inulin that could be used to make various valuable materials for industrial and human consumption (as a food fortificant), their utilization to extract inulin is very restricted. Inulin from sisal has not been fully characterized and its quality and safety have not been assessed. Sisal processing companies in the country use sisal boles and wastes mainly for the generation of biogas or composite (Mshandete *et al.,* 2013). Extraction of inulin from sisal wastes and boles will add value to the plant and extends the economic value of the sisal plant beyond its traditional use as fiber. Inulin may be used as a fortificant or supplement for various products which are deficient in dietary fiber and reduce the overweight and obesity problems in Tanzania (Foschia *et al.,* 2013).

This study aims to explore the feasibility and potential of inulin production from sisal boles and sisal wastes. Extraction and purification of inulin, and evaluation of quality and quantity, will provide insights on the production process's economic viability and sustainability. Additionally, the purified inulin creates opportunities to be used as an ingredient in food formulations, dietary supplements, and other relevant products.

Materials and Methods

Sample collection

A representative sample of sisal boles was collected from sisal processing plants in Morogoro and Tanga regions. Adhering materials/impurities including stalks of dried leaves on the boles were removed by gently scrubbing followed by washing several times with water. The clean sisal boles were then stored at –18°C in sealed bags for further analysis.

Sample preparation and hot water extraction of inulin

Inulin was extracted from the sisal boles by an ultrasonic extractor (ROHS Digitals-DSA100-SK2 South), made in Korea. The ultrasound-assisted extraction experiments were carried out at different combinations of extraction temperatures, times, and solvent composition as indicated in the experimental design (Table 1). Sisal boles were chopped into small pieces, which were then placed into an Erlenmeyer flask. The Erlenmeyer flask containing the pieces of boles was placed into the ultrasonic extractor. After the extraction, the mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was then collected, filtered to remove insoluble residues using a paper filter of 0,45 µm (Thomas Scientific, NJ 08085; USA), and dried in an oven (Model DZF-6010, China) at 50°C to obtain the inulin powder. The percentage inulin yield was then calculated as follows;

$$Percentage\ Inulin\ yield(\% = \frac{Produced\ inulin\ powder\ weight(g)}{Sisal\ boles\ weight(g)} x100 \ldots(1)$$

Selection of factors and levels

Extraction temperature, extraction time, and solvent-to-solid ratio were selected as the key factors that affect inulin extraction. The range of levels for each factor to be investigated was determined. Three levels (-1, 0, +1) for each factor, representing low, medium, and high values, respectively were selected. Coded values were assigned and actual factor levels to coded values were converted using the following equation:

$$Coded\ value = \frac{Actual\ value - Center\ point\ value}{Range/2} \ldots..(2)$$

The center point value represents the midpoint of the factor range, and the range was calculated as the difference between the high and low levels.

Experimental Design

A central composite design (CCD) was constructed by the software Design Expert Version 13 (Stat-Ease Corporation, Minneapolis, MN, USA). A response surface methodology (RSM) was used to determine the effect of independent variables on yield (%). The independent variables for this were extraction temperature, time, and solvent composition encoded in three levels (Table 1). The yield of the extracted inulin was used as a response variable. A total of 20 experimental runs with five centers and various combinations of the independent variables (extraction temperatures, extraction times, and solvent composition) were created using Design Expert Software Version 13.

Table 1: Extraction factors and their levels from RSM

Run	Exp no	Extraction temp (C)	Extraction time (min)	Solvent composition (%)
10	1	80	90	40
4	2	80	120	20
16	3	60	90	40
20	4	60	90	40
8	5	80	120	60
18	6	60	90	40
12	7	60	120	40
11	8	60	60	40
7	9	40	120	60
17	10	60	90	40
15	11	60	90	40
2	12	80	60	20
6	13	80	60	60
13	14	60	90	20
14	15	60	90	60
1	16	40	60	20
19	17	60	90	40
5	18	40	60	60
9	19	40	90	40
3	20	40	120	20

optimization, contour, and 3D model graphs were utilized to show the ideal set of processing parameters for maximizing inulin yield. The statistical analysis's significance level was set at p=0.05 for each factor. The relationship between the dependent and independent variables was described by the following second-order polynomial equation:

Statistical analysis

Design-Expert software version 13 was used to fit a second-order polynomial model to the experimental data. Regression analysis was performed to estimate the coefficients of the model equation, including the main, interaction, and quadratic effects of the factors. Analysis of variance (ANOVA) was used to assess the significance of the model and to establish the importance of each component in relation to the response (yield). To visually represent

$$Y=b0+\text{þ}b1x1+\text{þ}b2x2+\text{þ}b3x3+\text{þ}b12x1x2+\text{þ}b13x1x3+b23x2x3+b11x^{2}1+b22x^{2}+b33x^{2}3 \qquad \text{...(3)}$$

where Y is the amount of inulin, b_0 is the intercept, b_1, b_2, and b_3 are constant coefficients, and x_1, x_2, and x_3 are the extraction temperature, extraction time, and solvent composition, respectively.

Results and Discussion

Model Analysis

Based on results obtained from factorial design, response surface methodology (RSM) was used, followed by the central composite design (CCD). The first-order model used to fit the results of the two-level design is represented by Equation 4:

Inulin content=3.43273+0.293155*A + 0.19697 *B+-0.00486364*C+0.000166667*AB +0.00025*AC+-0.00075*BC+-0.00182045* A2+-0.00099798*B2 + 0.000254545*C2 .....(4)

The Model F-value of 12.87 implies that the model is significant (Table 3). There is only a 0.02% chance that an F-value this large could occur due to noise. While p-values less than 0.05 indicate model terms are significant. In this case, A, and C are significant model terms. On the other hand, the Lack of Fit F-value of 1.72 implies the Lack of Fit is not significant relative to the pure error and there is a 28.27% chance that a Lack of Fit F-value this large could occur due to noise. Therefore, a non-significant lack of fit is good since we want the model to fit.

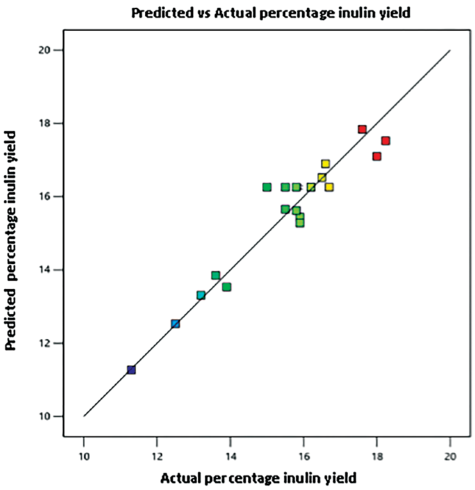

Figure 1: Variation between actual and predicted percentage inulin yield

The Predicted R^2 of 0.7220 is in reasonable agreement with the Adjusted R^2 of 0.8490; i.e., the difference is less than 0.2. Moreover, according to Table 4, adequate precision has a ratio of 13.270 greater than 4 which indicates an adequate signal that stipulates the signal-to-noise ratio. A ratio greater than 4 is desirable; therefore, this model can be used to navigate the design space.

Inulin yield

The results obtained are summarized in Table 2. The percentage yield of extracted inulin ranged from 11.3% to 18.24%. The maximum percentage yield (18.24%) was obtained at the following optimal conditions; extraction temperature of 80°C, extraction time of 90 min, and solvent composition of 40%. The percentage inulin yield from sisal boles depended on extraction temperature, time, and solvent composition. However, temperature and solvent composition showed a significant effect at p=0.05 on inulin yield with p values of 0.0001 and 0.0075 (Table 3), respectively compared to extraction time (0.7253). These results are in agreement with previous studies which identified temperature as a critical factor for inulin extraction (Kaufmann and Christen, 2002).

Effect of extraction temperature, extraction time, and solvent concentration on inulin yield

Inulin yield was significantly impacted by the extraction temperature and solvent composition as seen in Table 3 and Figures 1 and 2. The highest inulin yield obtained was 18.24% and the lowest was 11.3% (Table 2). This is consistent with earlier research that showed substantial inulin recovery at 80°C (Lopes *et al.,* 2015; Zhu *et al.,* 2012; Toneli *et al.,* 2007). However, the time spent in this study was less (90 min) than the 120 min documented in most studies (Kim *et al.,* 2021; Terkmane et al., 2016), this might be attributed to loosened inulin contained matrix of the sisal boles (Apolinário *et al.,* 2017).

The majority of factors had an impact on inulin yield, with linear and quadratic effects. However, the interaction effects were not significant (Table 3) for all factors. A significant difference was observed in extraction time and solvent concentration. The model was significant ($p \leq 0.05$, Table 3), suggesting that it can predict variation patterns, making it useful for design

Table 2. Design matrix generated by expert software

Run	Exp no	Extraction temp (C)	Extraction time (min)	Solvent composition (%)	Inulin yield (%)
10	1	80	90	40	18.24
4	2	80	120	20	17.6
16	3	60	90	40	15
20	4	60	90	40	16.2
8	5	80	120	60	15.5
18	6	60	90	40	16.2
12	7	60	120	40	15.9
11	8	60	60	40	15.9
7	9	40	120	60	11.3
17	10	60	90	40	16.7
15	11	60	90	40	15.5
2	12	80	60	20	16.6
6	13	80	60	60	16.5
13	14	60	90	20	18
14	15	60	90	60	15.8
1	16	40	60	20	13.2
19	17	60	90	40	15.8
5	18	40	60	60	12.5
9	19	40	90	40	13.9
3	20	40	120	20	13.6

Table 3: ANOVA results for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	56.75	9	6.31	12.87	0.0002	significant
A-Temperature	39.76	1	39.76	81.15	< 0.0001	
B-Extraction time	0.0640	1	0.0640	0.1306	0.7253	
C-Solvent conc	5.48	1	5.48	11.18	0.0075	
AB	0.0800	1	0.0800	0.1633	0.6947	
AC	0.0800	1	0.0800	0.1633	0.6947	
BC	1.62	1	1.62	3.31	0.0990	
A^2	1.46	1	1.46	2.98	0.1152	
B^2	2.22	1	2.22	4.53	0.0592	
C^2	0.0285	1	0.0285	0.0582	0.8143	
Residual	4.90	10	0.4900			
Lack of Fit	3.10	5	0.6199	1.72	0.2827	not significant
Pure Error	1.80	5	0.3600			
Cor Total	**61.65**	**19**				

navigation. The lack of fit was insignificant (p = 0.6199> 0.05, Table 3) indicating that the model is well suited to the real data.

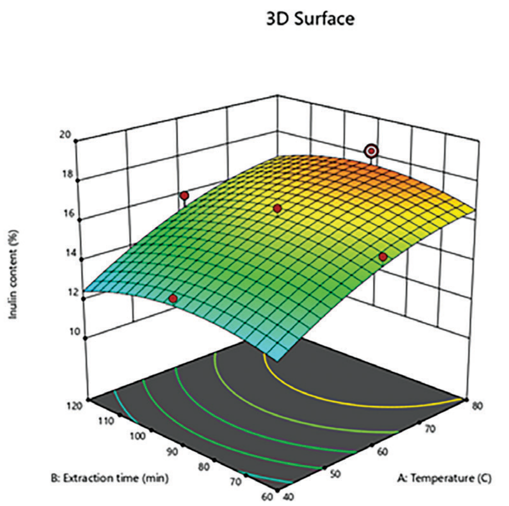

Figure 2: Graphical presentation of 3D surface to show the desirable region for optimal compositions of extraction time and extraction temperature in the final Inulin.

Note: *The colors from blue to red show the increase in the inulin yield towards the optimal inulin yield; red color is where the optimum inulin yield is found; red dots show the inulin yield at different extraction time and temperature.*

Effect of extraction temperature on inulin yield

The extraction temperature is an important parameter that can significantly affect the yield of inulin during the extraction process. The yield of inulin increases with an increase in extraction temperature up to a certain point, beyond which any further increase in temperature decreases the yield (Fig. 2). This might be due to thermal degradation of inulin to sugar components (Maumela *et al.,* 2020). Several studies have investigated the effect of extraction temperature on inulin yield from various sources, including chicory. A study in Brazil on the effect of extraction temperature on inulin yield from sisal waste revealed the inulin yield to increase with increasing extraction temperature up to 70°C, beyond which the yield decreased due to thermal degradation (Apolinário *et al.,* 2017). Another study on inulin yield from Jerusalem artichoke tubers found that the optimal extraction temperature for maximum yield was 85°C (Zhu, 2014). A study by Elisante and Msemwa (2010) which extracted inulin from sisal wastes and boles by drying method reported an optimal extraction temperature of 120°C. These indicate that the optimal extraction temperature for inulin extraction varies depending on the source of inulin and the extraction method. It is important to optimize the extraction temperature to achieve maximum yield while minimizing thermal degradation of inulin (Redondo-Cuenca *et al.,* 2021; Apolinário *et al.,* 2014).

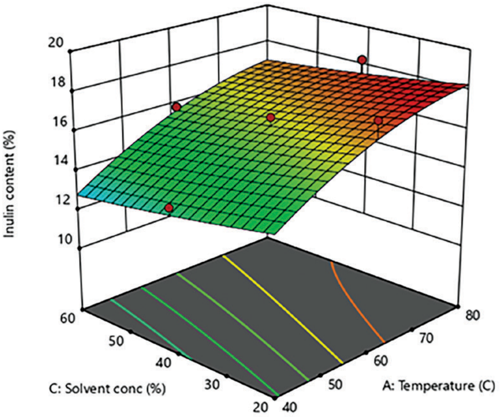

Figure 3: Graphical presentation of 3D surface to show the desirable region for optimal compositions of extraction temperature and solvent composition in the final Inulin

Note: *The color from blue to red shows the increase in the inulin yield towards the optimal inulin yield; the red color is where the optimum inulin yield is found; the red dots show the inulin yield at extraction temperature and solvent composition.*

Effect of extraction time on inulin yield

In the present study, each response surface presents the effect of any two factors on the inulin yield while the other two factors are held at the middle level. Figure 2 shows the interaction effect of time and temperature on inulin yield while acetone composition is kept constant. This response surface plot indicates that longer extraction times and higher temperatures favor inulin extraction. The maximum yield (18.24%) was obtained at an extraction time of 90 min and a temperature above 80°C. However, a further increase in temperature showed a decrease in inulin yield. This phenomenon of increase in yield with an increase in extraction time could be due to the enhancement in the extraction rates with long extraction time (Zia *et al.,* 2020; Eh and Teoh, 2012). Ultrasound-assisted extraction introduces cavitation bubbles which expand under high ultrasound intensity as a result it provides a larger surface area, increases accessibility of the solvent, and destroys the cell walls (Kumar *et al.,* 2021; Cravotto *et al.,* 2006). Similar effects of time and solvent concentration on inulin yield can also be observed in Figure 4. Higher inulin yields occurred when the extraction time was extended to 90 minutes and decreased beyond that time. However, the interaction between time and solvent concentration was not statistically significant ($p \geq 0.05$).

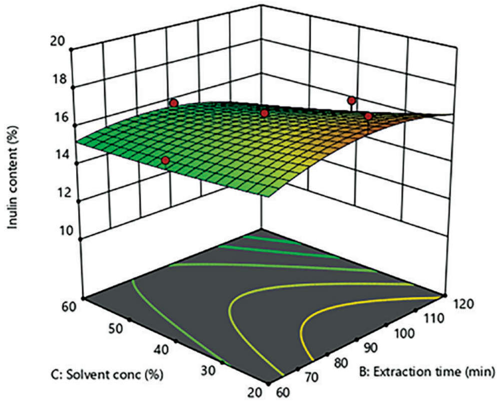

Figure 4: Graphical presentation of 3D surface to show the desirable region for optimal compositions of extraction time and solvent composition in the final Inulin

Note: *The colors from green to red show the increase in the inulin yield towards the optimal inulin yield; the red color is where the optimum inulin yield is found; the red dots show the inulin yield at extraction time and solvent composition.*

Effect of solvent concentration on inulin yield

The solvent concentration is another parameter that can affect inulin yield during the extraction process (Rubel *et al.,* 2018). The solvent concentration influences the solubility of inulin and other non-inulin components, which affects extraction efficiency. Although higher solvent concentrations can increase inulin yields, they may also increase the extraction of non-inulin components and lower inulin purity. High yields of inulin (18.24%) were attained when the solvent concentration ranged from 30% to 60%. It was noticed that the change of extraction yield with acetone had an increased trend; this is partly because a similar polar solvent dissolved a similar polar solute. Moreover, high yields are attained when the polarity of the fluid matches the polarity of the compound to be extracted. The high yields (18.24%) of inulin were obtained at 40% acetone concentration and an extraction time of 90 min. However, the interaction between extraction time and acetone concentration was not statistically significant ($p > 0.05$) for inulin yield.

Several studies have investigated the effect of solvent concentration on inulin yield from various sources like Chicory and Jerusalem artichoke (Kanakasabai *et al.,* 2023; Esmaeili *et al.,* 2021; Yudhistira *et al.,* 2020; Rubel *et al.,* 2018; Lingyun *et al.,* 2007). These studies reported the optimum inulin extraction of 1.83-1.86% at the solvent concentration ranging from 15-50%, which is relatively lower than what was observed in this study. A study in Belgium on the effect of solvent concentration on inulin yield from chicory roots found that the optimal solvent concentration was 50-60% ethanol for maximum inulin yield (Khuenpet *et al.,* 2017). Another study in Egypt found that the optimal solvent concentration for maximum inulin yield from Artichoke was 70% ethanol (El-Hadidy *et al.,* 2022). The variations in inulin yield might be attributed to the type of solvents used, physiological processes (Saengthongpinit *et al.,* 2005), plant cultivar, cultural practices, geographic location, harvest and storage time (Apolinário *et al.,* 2014).

Table 4 shows only 8% of the overall variation was not described by the projected model, according to the predicted model's coefficient of determination (R^2) of 0.9205 (Ross 1996; Baranyi *et al.,* 1999). Suggesting, that the model fits the data set and explains well the changes in the dependent variable (Hayes, 2021).

Table 4: Model summary table for Fitting General Linear Model

Std. Dev.	0.7000	R^2	0.9205
Mean	15.50	Adjusted R^2	0.8490
C.V. %	4.52	Predicted R^2	0.7220
		Adeq Precision	13.2698

Process optimization

The optimization of the extraction process of inulin from sisal waste using RSM resulted in a significant increase in inulin yield. The optimal extraction conditions are similar to what has been reported in previous studies (Lingyun *et al.,* 2007). It was observed that extraction temperature significantly ($p \leq 0.05$) affected inulin yield (Table 2) and yield increased with an increase in temperature (Fig. 2), this could have been attributed to better solubility of inulin at high temperatures. The optimal extraction (18.24%) conditions were at the extraction temperature of 80°C, an extraction time of 90 min, and a solvent concentration of 40%. The high coefficient of determination (R^2) of 0.9205 (Table 4) from the analysis of variance, suggests potential utilization of the optimized values design navigated at the industrial level.

Table 5: Variation between actual values of inulin yield in four runs in the experiment and the corresponding values predicted by the model described by Equation 3

Experiment no.	Actual yield (%)	Predicted yield (%)	Difference
6	16.50	16.51	0.01
18	16.20	16.25	0.05
5	12.50	12.53	0.03
20	16.20	16.25	0.05

Validation of the extraction model

Validation of an extraction model for inulin from sisal boles is an important step in ensuring the accuracy and reliability of the model and its potential for use in industrial applications. In addition to experimental validation, it is also important to validate the model by comparing it with other existing models or methods for inulin extraction from sisal waste. This can involve comparing the accuracy, precision, and computational efficiency of the model with other models or methods, as well as evaluating the scalability and feasibility of the model for potential industrial applications.

This validation process in inulin yield from sisal boles was done by using the model to predict the yield of inulin under various extraction conditions and then comparing these predictions to the actual experimental results obtained using those conditions. A series of experiments were conducted using different extraction conditions and the results inulin yield obtained were compared with the predicted values obtained from the model. The slight difference between experimental values and the predicted values (Table 5 and Figure 1) confirms the validity and adequacy of the predicted model.

Conclusion

The optimization of the extraction process of inulin from sisal waste has been successfully achieved in this study. The use of Response Surface Methodology (RSM) helped in identifying the optimal extraction conditions for maximum inulin yield. The results showed that the optimum extraction conditions were 80°C, 90 min of extraction time, and a solvent concentration of 40%, which resulted in a maximum inulin yield of 18.24%. Moreover, sisal waste is an abundant and low-cost agricultural waste, and the extraction of inulin from sisal waste can provide a sustainable and eco-friendly approach to utilizing this waste. Inulin extracted from sisal waste can also serve as an alternative source of this important dietary fiber, which is regarded as prebiotic. In conclusion, the optimization of the extraction process of inulin from sisal waste using RSM has improved yields of inulin with desirable physicochemical properties for various industrial applications. The use of sisal wastes as an alternative source of inulin is an innovative and sustainable approach to waste valorization, which reduces emission of greenhouse gases.

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