An Aqueous Solvent Extraction of Polyphenols from Jack Fruit Waste: Response Surface Modelling and Optimization

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ABSTRACT

The main aim of the study is to realize optimized extraction conditions for polyphenols extraction of by applying Box-Behnken response surface design. The non-edible portion of the *Artocaspus heterophyllus* Lam, such as rinds and rags which are generally discarded are said to contain decent amounts of phenolic content. An aqueous solvent extraction method was implemented to enhance the process variables such as temperature, time and feed solvent ratio by using response surface-methodology (RSM). The results obtained shows that the role of the quadratic model was significant for each and every one the responses ($p<0.05$). Second order statistical regression models were developed and were found to be fit well with observed data and 3D response surfaces were plotted from the mathematical model. Optimal values of the independent variable, obtained using Derringer's desired function methodology, were an extraction temperature of 37^0C, time of 9 hours, feed solvent ratio of 1:23 and ethanol water composition (75:25). Under these conditions, predicted yield of extraction 759.98 (µg of GAE/g DW), in close to that of actual values obtained using the optimal conditions. This process would there by support of phenolic compounds extraction from non-edible portion of *Artocaspus heterophyllu*s Lam and give dimension in using fruit waste for polyphenols extraction.

KEY WORDS: Aqueous Solvent extraction, Response Surface Methodology, Quadratic model, Polyphenols.

1. INTRODUCTION

For the past two decades, researchers and food manufacturers are interested in polyphenols extraction. The main presumption is due to the recognition of the antioxidant potential of polyphenols. In addition to antioxidant properties, polyphenols have several other definite biological actions that are less understood (Claudine, 2004). *Artocarpus heterophyllus* Lam, is one of the important and commonly found trees in the home gardens of South India. Jackfruit is a well-liked food and ranks third in total annual production after mango and banana in South India (Manjeshwar, 2011**)**. *Artocarpus heterophyllus* Lam rinds and rags are normally disposed as wastes by food industries and vendor's Proper utilisation of these wastes can increase economic value and reduce cost of waste disposal. The extraction methods for phenolic components from solid materials have been focused on using organic solvents (Patricia, 2010). Commonly used extraction solvents are alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate. However, very polar phenolic acids could not be take out completely with unadulterated organic solvents, and mixtures of alcohol–water or acetone–water are recommended (Constantine, 2007). A panoptical literature review indicated that several extraction process variables, i.e., mixture of solvents, type of solvent, solvent composition, feed-solvent ratio, temperature and extraction time have significantly influenced the extraction yield (Bucic, 2009).

Response Surface-Methodology (RSM) is a collection of statistical and mathematical techniques useful for emerging, improving, and optimising processes, in which a response of interest is stimulated by several factors (independent variables). RSM not only describes the properties of the independent variables, but also makes mathematical models, which describes the chemical or biochemical processes and give optimized condition for target yield of the process (Pompeu, 2009). Currently, RSM is used in the extraction of phenolic compounds from many plant materials (Kim, 2004; Liyana-Pathirana, 2005; Silva, 2007).

Response surface-methodology (RSM) examines the correlation between process variables and the responses with minimum number of experiments (Prakash Maran, 2013). The spherical, revolving Box–Behnken response surface design (BBD) consists of a set of points lying at the midpoint of each edge and the replicated center point of a multidimensional cube. RSM was successfully used to find out the optimal extraction conditions in several fields, especially in extraction process (Hayouni, 2007; Pompeu, 2009; Pinelo, 2005; Huang, 2009; Yang, 2010). Hence, in this work an effort was prepared to evaluate the individual and interactive effect and optimize the process parameters (extraction temperature, extraction time, and feed solvent ratio and ethanol water composition) in order to extract a maximum yield of polyphenols from *Artocarpus heterophyllus* Lam using Box–Behnken design.

2. MATERIALS AND METHODS

Raw Materials and Chemicals: *Artocarpus heterophyllus* Lam rinds and rags were collected from a Kolli Hills local market and samples were sun dried for one day and frozen at -20^0C, and by a freeze-drying at -4^0C and freeze dryer for 48 hours until obtaining 3% (dry basis) moisture, storing at 20^0C until analysis (Ruiz, 2015). Ethanol, Folin–Ciocalteu Reagent, sodium bicarbonate, gallic acid were purchased from Vijaya Scientific, Chennai.

An Aqueous Solvent Extraction: Polyphenol compounds were extracted from *Artocarpus heterophyllus* Lam waste, using a temperature controlled shaker for 12 hours at room temperatures (Umesh, 2010). Phenolic compounds were extracted with ethanol (solvent) -water solution with various feed solvent ratios (1:10–1:30 g/ml), different temperatures (30–40^0C) and times (4–12 hours) and ethanol water composition (60:40 – 80:20 ml). After anticipated extraction time, the extracts were cleaned and centrifuged at 4500 rpm for 15 min. The centrifuged extracts was collected and utilized for the experimental analysis.

Determination of Total Phenolic Content: The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent according to the method of Silva, 2006 with mild modification using gallic acid as a standard. Ethanol extracts (2 mL) was mixed with the Folin–Ciocalteu reagent (2.5 mL) and aqueous sodium carbonate 20% (5 mL), in a 50 mL-volumetric flask .This mixture was kept for 45 min in dark at room temperature .The mixture absorbance was measured at 760 nm. The extraction yields were represented as μg of GAE/g DW of dry fruit.

Experimental Design: Response surface-methodology (RSM) was utilized to get the optimized condition for the extraction of phenolic compounds through jack fruit waste. In this study, Box-Behnken Design (BBD) was applied to investigate the extraction process on the yield of phenolic compounds by using ethanol-water as a solvent. Extraction temperature (30–40^0C), time (4–12 hours), Feed solvent ratio (1:10–1:30 g: ml) and Ethanol water composition (60:40 to 80:20) are the input variables. The input variables and their levels of the BBD are given in Table.1. In total, 29 experiments were carried out in this process, the influence of the independent variables on the yield of phenolic content. A second-order polynomial equation was utilized to appropriate the experimental data. The constants of determination R^2, R^2 adj, and R^2 Pre were used for the attainment of suitability of the established mathematical polynomial equation.

Statistical Analysis: Design Expert Statistical Software package 7.0.0. Was used for statistical analysis of the experimental data. Multiple regression analysis was also used to investigate the fitness of the developed mathematical models and significance of the independent variables. Factors significance was computed using the statistical tool of Pareto analysis of variance (ANOVA) at 95% confidence level ($p<0.05$).

3. RESULTS AND DISCUSSION

Box-Behnken Design Analysis: The three factors; lower, middle and upper strategy points for RSM in coded and natural uncoded values are displayed in Table.1. In RSM, natural variables are distorted into coded variables that have distinct as dimensionless with a mean zero and the similar spread or standard deviation (Myers and Montgomery, 2002). Several regression equations were generated relating response variable to coded levels of the independent variables. Multiple regression coefficients were resolute by employing least squares method (Myers and Montgomery, 2002) to predict quadratic polynomial models for total Phenolic content of extracts.

Table.1. Level of variables considered for the extraction of Polyphenols from using Box-Behnken Design (BBD)

S.No	Variables	Independent Variables	Variable Levels		
			-1	0	1
1	Temperature (◦C)	X_1	30	35	40
2	Feed solvent ratio(g/ml)	X_2	1:10	1:20	1:30
3	Time (Hours)	X_3	4	8	12
4	Ethanol water composition (%)	X_4	60:40	70:30	80:20

Table.2. Box-Behnken design setting in the coded form of the independent variables (X_1, X_2, X_3, X_4) and experimental results and predicted results of Total Phenolic Content

Run Order	Temperature (X_1)	Feed solvent ratio (X_2)	Time (X_3)	Ethanol water Composition (X_4)	Actual GAE (μg/g)	Predicted GAE (μg/g)	% Error
1	1	1	0	0	285.98	284.54	1.44
2	0	0	-1	1	230.86	231.32	0.46
3	0	1	0	-1	69.82	69.96	0.14
4	0	0	0	0	756.32	756.32	0.00
5	0	0	-1	-1	50.92	51.44	0.52
6	0	0	1	1	124.11	124.65	0.54
7	-1	-1	0	0	123.89	122.94	0.05
8	0	-1	-1	0	08.34	06.77	1.57
9	0	0	1	-1	134.76	134.02	0.74
10	0	1	-1	0	187.91	188.43	0.52
11	-1	0	0	-1	194.37	195.67	1.30
12	1	0	0	-1	329.44	329.58	0.14

13	0	-1	0	-1	8.29	06.11	2.18
14	1	0	1	0	47.83	47.29	0.54
15	0	-1	1	0	05.71	06.03	0.32
16	1	0	0	1	261.53	261.07	0.46
17	0	0	0	0	756.32	756.32	0.00
18	1	0	-1	0	530.64	529.93	0.71
19	-1	0	1	0	535.86	534.29	1.57
20	0	1	0	1	271.88	272.16	0.28
21	0	0	0	0	756.32	756.32	0.00
22	-1	1	0	0	380.14	380.74	0.60
23	0	-1	0	1	10.79	12.35	1.56
24	-1	0	-1	0	76.56	77.03	0.59
25	0	1	1	0	140.95	140.16	0.79
26	-1	0	0	1	431.61	430.73	0.88
27	1	-1	0	0	182.27	184.9	0.63
28	0	0	0	0	756.32	756.32	0.00
29	0	0	0	0	756.32	756.32	0.00

There were total of 29 runs for optimizing the four individual parameters in the current BBD and results are listed in table.2. Various mathematical models were fitted to the observed data in order to attain the regression models and the results are exhibited in Table.2. From the Table.2, it was found that the quadratic model showed a maximum determination coefficient with highly significant p values ($p<0.0001$). Therefore, further analysis was done on the quadratic model.

Table.3. Adequacy of Model Tested

	Sum of		**Mean**	**F**	**p-value**	**Remarks**
Source	Squares	DF	Square	Value	Prob > F	
Mean vs Total	2436615	1	2436615			
Linear vs Mean	109168	4	27292.1	0.36577	0.8305	
2FI vs Linear	270518	6	45086.3	0.53383	0.7756	
Quadratic vs 2FI	1519469	4	379867	6890.66	< 0.0001	Suggested
Cubic vs Quadratic	614.695	8	76.8368	2.93466	0.1033	Aliased
Residual	157.095	6	26.1826			
Total	4336542	29	149536			

Second-Order Polynomial Model Building and Analysis of Variance: An empirical connection between the phenolic content yield and independent process variables has uttered by a second-order polynomial equation with interaction terms was fitted amid the experimental results attained on the basis of BBD, which will help to predict the extraction efficiency of dissimilar sets of combinations of four process variables on the responses (Prakash Maran et al 2015). The final mathematical model (second-order polynomial equation) as determined from the experimental results in terms of coded factors is given as:

$$Y = 756.32 - 8.72X_1 + 83.11X_2 - 8.0X_3 + 45.27X_4 - 38.14X_1X_2 - 235.53X_1X_3 - 76.29X_1X_4 - 11.08X_2X_3 + 49.89X_2X_4 - 47.65X_3X_4 - 148.33 X_1^2 - 361.35X_2^2 - 311.54X_3^2 - 306.05X_4^2$$

The ANOVA of the quadratic regression model showed that the values of determination coefficient (R^2) and the adjusted determination coefficient (Adj. R^2) were 0.9981 and 0.9916, respectively, which recommended that a high degree of association between the observed and expected values. Moreover, a low value of coefficient of the variation (CV=2.56 %) indicated a high degree of precision and a decent transaction of reliability of the experimental values **(**Xue-Lian, 2010). The p values are used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables. The coefficient estimation for the parameter optimization recommended that the independent variables (X_1, X_2, X_3 and X_4) and quadratic terms (X_1X_1, X_2X_2, X_3X_3 and X_4X_4) suggestively pretentious the polyphenols yield ($p<0.05$).

Effect of Process Variables on Phenolic Content: Edible portion of jack fruit contains significant amount of phenolic compounds. (Soong and Barlow, 2004; Jagtap, 2010; Akshatha, 2015). In this study, the phenolic content yield ranged from 5.71 (µg GAE/g DW) to 756.32 (µg GAE/g DW) whereas organic solvent ethanol used for traditional convection technique from waste portion of jack fruits. The amount of extraction of polyphenols mainly based on the complexity of these compounds and method of extraction, using extraction organic solvents and analysis (Kalt, 2001).

Two variables within the actual range were portrayed in 3-D surface plots, while the two other variable were kept constant at zero level shown in figure.1. Four extraction factors such as temperature (X_1), feed solvent ratio

(X_2), time (X_3) and solvent composition (X_4) had a positive impact on the polyphenols extraction. There was an increased in the yield of the polyphenols with an increased in four factors up to threshold level. Beyond this level the polyphenols yield slightly decreased. Extraction temperature and feed solvent ratio significantly ($p<0.0001$) influenced the total phenolic yield. Extraction temperature and extraction time also markedly ($p<0.0001$) governed in total phenolic content. Figures.1b, 1c shows that the phenolic yield was increased with respect to increasing extraction temperature.

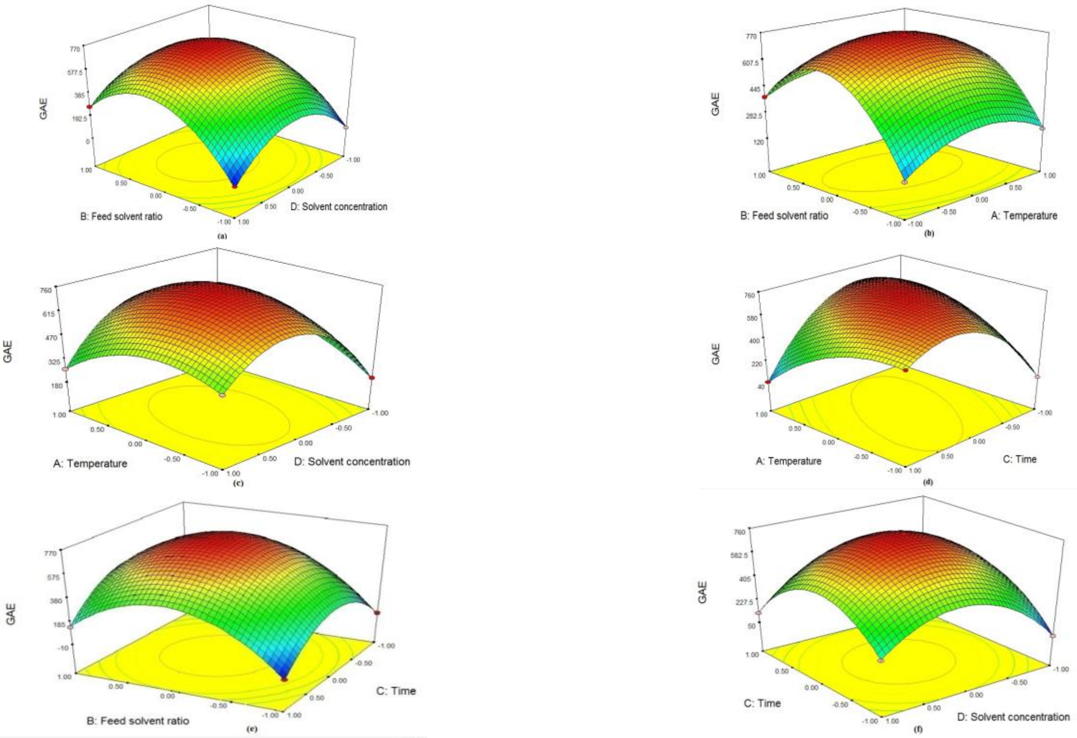

Figure.1. Three –dimensional (3D) response surface and contour plot for effect process variables; a) Feed solvent ratio and Solvent concentration, b) Feed solvent ratio and Temperature, c) Temperature and Solvent concentration, d) Temperature and Time, e) Feed solvent ratio and Time, f) Solvent concentration and Time

Table.4. Analysis of variance for the response surface quadratic model for total Phenolic content

Source	Sum of Squares	DF	Mean Square	F Value	p-value Prob > F	Remarks
Model	1899155	14	135653.9	2460.714	< 0.0001	significant
X_1-Temperature	914.2056	1	914.2056	16.58337	<0.0001	
X_2-Feed solvent ratio	82898.9	1	82898.9	1503.757	0.0037	
X_3-Time	768.16	1	768.16	13.93415	<0.0001	
X_4-Solvent composition	24587.04	1	24587.04	446.0003	0.0021	
X_1X_2	5817.113	1	5817.113	105.5204	0.0492	
X_1X_3	221892.8	1	221892.8	4025.058	0.0074	
X_1X_4	23279.13	1	23279.13	422.2753	< 0.0001	
X_2X_3	491.2872	1	491.2872	8.911778	0.0098	
X_2X_4	9956.048	1	9956.048	180.5992	< 0.0001	
X_3X_4	9081.137	1	9081.137	164.7286	0.0609	
X_1^2	142715.9	1	142715.9	2588.817	0.0715	
X_2^2	846963.4	1	846963.4	15363.62	0.0286	
X_3^2	629558.3	1	629558.3	11419.97	< 0.0001	
X_4^2	607560.5	1	607560.5	11020.93	0.0892	
Residual	771.79	14	55.12786			
Lack of Fit	771.79	10	77.179			
Pure Error	0	4	0			
Cor Total	1899927	28				

Temperature leads to soften the plant tissues and elevates the extraction rate by diffusion of solvent into the plant matrix and hence increase the phenolic yield whereas increase on mass transfer rate of phenolic content from sample to solvent (Shi, 2003). Table.4, clearly portrayed that phenolic content depends on three process variables such as temperature, feed solvent ratio and extraction time. There is a positive interactive effect between the temperature and feed solvent ratio at $p<0.001$. Phenolic content was decreased significantly with increasing temperature and feed solvent ratio, which is due to the deprivation of phenolic compounds as well as decreased in the polarity of the solvent (Yap, 2009; Al-farsi, 2008; Durling, 2007).

The elevated extraction time with higher temperature increased the loss of solvent by vaporization and decreased the yield of extraction (Lu, 1999). Meanwhile, increasing solid–liquid ratio can easily permeate in to the solid matrix and increased the diffusivity and mass transfer rate (Zhang, 2007). The solvent gets saturated with the solute at higher feed solvent ratio, which negatively affected the mass transfer rate and barricaded the penetration of the phenolics into the solvent and decreased the extraction yield. Fig.1e, indicated that extraction time and feed solvent ratio considerably affected the phenolic content yield (X_2 X_4 $p<0.0001$). Shorter extraction time with minimum feed solvent ratio not enough energy for solvent to penetrate into sample solid matrix. Increasing time which leads contact time between plant matrix and solvent gets increased to yield higher amount of phenolic content.

Validation of the optimized conditions: The Derringer's desirability function method was used to optimize the process variables. This function searches for a combination of factor levels that mutually optimize a set of responses by gratifying the requirements for each and every response in the design. The optimized conditions were obtained as extraction temperature of 37◦ C, time of 9 hours, feed solvent ratio of 1:23 and ethanol water composition (75:25) with 759.98 (µg of GAE /g DW). The actual values coincides well with predicted values.

4. CONCLUSION

Box-Behnken design was used to examine and optimize the process parameters that yield polyphenolic contents from non-edible portion of jack fruit by ethanol solvent extraction method. Second order polynomials were developed from the ascertained data. According to ANOVA, the effect of extraction temperature, extraction time, Feed solvent ratio and ethanol water composition were significant. Optimal values of the independent variable, obtained using Derringer's desired function methodology, were an extraction temperature of 37^0C, time of 9 hours, feed solvent ratio of 1:23 and ethanol water composition (75:25). Under these conditions, predicted extraction yield of 759.98 (µg of GAE /g DW) in close accord with actual values obtained using the optimal conditions.

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