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Microwave assisted extraction of bio-colorant from walnut hull

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Abstract

This study aimed at exploring the feasibility of using the walnut hull as a natural colorant source using microwave-assisted extraction. Extraction was carried out with different combinations of Microwave Power (160, 320, 480 W), treatment time (2, 3, 4 mins), solvent volume (20, 30, 40 ml/g) and particle size (150, 300, 450 µm). As a result, the highest yield of 44.99% was attained at the microwave power (160, 320, 480 W), microwave time (2, 3, 4 mins), Solvent (Water) volume (20, 30, 40 ml) and particle size (150, 300, 450 µm). In fact, application of microwave irradiation method proved to be a rapid and improved technique for natural colorant extraction and extensively reduced the extraction time.

Keywords: Microwave, bio-colorant, walnut hull

Introduction

Walnut (*Juglans regia*) is one of the most important nut crop of India. In India, it is mainly grown in Himalayan states namely, Jammu and Kashmir, Himachal Pradesh, Uttarakhand and Arunachal Pradesh. Jammu and Kashmir is the major walnut producer state which contributes 80.58% of total area and 91.16% total production of the country. In Uttarakhand, the major geographical regions of walnut production are Nainital, Dehradun, Pauri, Tehri, Chamoli, Almora and Pithoragarh, with overall state productivity of 1.10T/ha. The major importing countries of walnut from India are Spain, Egypt Arab Republic, Germany, Netherlands, United Kingdom, France, and Taiwan (APEDA, 2011-12) ^[1].

Walnut green husk is an agro-forest waste which is a source of natural compounds with antioxidant and antimicrobial properties. The aqueous extracts of walnut green husks have a strong antioxidant activity (assessed by three different assays: reducing power, scavenging activity on DPPH radicals and lipid peroxidation inhibition by beta carotene-linoleate system) and also inhibits the growth of different pathogenic gram positive bacteria (Oliveira *et al.*, 2008) ^[5].

In recent years, researchers worldwide have investigated the efficacy of different techniques for extraction of natural dyes from different plant parts. In the current study, extraction will be carried out by microwave assisted extraction. The microwave-assisted extraction is a novel method of extracting soluble products into a fluid from a wide range of materials using microwave energy (Paré *et al.*, 1994) ^[6]. Microwaves are electromagnetic fields in the frequency range from 300 MHz to 300 GHz. They are made up of two oscillating fields that are perpendicular such as electric field and magnetic field. The principle of heating using microwave is based upon its direct impacts on polar materials (Letellier and Budzinski, 1999) ^[3]. Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms.

The prime challenge in food processing research is to make use of agricultural waste, which can serve as a precursor molecule for various products with specific functional properties. Research work reporting extraction of colorant from walnut hulls is less. Extraction of colorant from walnut hull could be a good option for improving, extending and advertising its uses for future applications.

Material and Methods**Collection of raw materials**

Walnut was procured locally from market and the hull was peeled from the walnut.

The hull was kept in tray dryer for drying at 50 °C for 24 hours. Then the dried hull was grinded using grinder to make it in powder form. The hull powder was stored in desiccator for further processing.

Experimental Design

Box-Behnken Design (BBD) of response surface methodology is used for Ultrasound assisted extraction for extraction of colorant. The design consisted of 29 randomized runs (Table 1) with five replicates at the central point. For the designed experiments, four variables having 3 levels of each (microwave power, microwave time, solvent volume and particle size) for microwave assisted extraction were selected for the experiments. Table 2 and Table 3 represent the actual and coded independent variables for extraction.

Table 1: Experimental Design BBD for microwave assisted extraction

S. No.	Run	Particle Size (µm)	Microwave power (watt)	Microwave time (min)	Solvent Volume (ml/g)
1.	22	300	480	3	20
2.	18	450	320	2	30
3.	4	450	480	3	30
4.	23	300	160	3	40
5.	15	300	160	4	30
6.	14	300	480	2	30
7.	26	300	320	3	30
8.	21	300	160	3	20
9.	25	300	320	3	30
10.	8	300	320	4	40
11.	12	450	320	3	40
12.	24	300	480	3	40
13.	20	450	320	4	30
14.	10	450	320	3	20
15.	17	150	320	2	30
16.	16	300	480	4	30
17.	27	300	320	3	30
18.	11	150	320	3	40
19.	7	300	320	2	40
20.	9	150	320	3	20
21.	2	450	160	3	30
22.	6	300	320	4	20
23.	13	300	160	2	30
24.	3	150	480	3	30
25.	29	300	320	3	30
26.	5	300	320	2	20
27.	19	150	320	4	30
28.	1	150	160	3	30
29.	28	300	320	3	30

Table 2: Coded levels for Independent variables in MAE

Independent Variables		Coded Levels		
Name	Code	-1	0	1
		Actual Levels		
Particle Size (µm)	A	150	300	450
Microwave Power (W)	B	160	320	480
Microwave time (min)	C	2	3	4
Solvent Volume (ml/g)	D	20	30	40

Table 3: Constant Parameters for final experiments

S. No.	Parameter	Constant
1.	Solvent	Water
2.	Sample Size	15 g

Bio-colorant extraction

Bio-colorant was extracted with different combinations of

Microwave Power (160, 320, 480 W), treatment time (2, 3, 4 mins), solvent volume (20, 30, 40 ml/g) and particle size (150, 300, 450 µm). In every experimental run, walnut hull powder (15g) was mixed with water. It was then kept for 2, 3 and 4 minutes at the power 160, 320 and 480 W. After Microwave treatment, the mixture was kept at the room temperature for cooling after that the mixture was then centrifuged at 8000 rpm for 15 min. After that mixture was filtrate using filter paper (whatman no. 1) and the filtrate was kept in hot air oven at 70 °C for overnight. After that sample was stored at room temperature. The yield of bio colorant was calculated according to equation (1).

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \times 100 \quad \dots (1)$$

The model development was done using response surface methodology through use of Design expert 10.0.1 version. Complete second order model as given in equation was fitted to the data and the model adequacy was tested using R² (coefficient of multiple determination) and Fisher's F-test. The parametric effect on various responses was done through the interpretation of developed models. Regarding four independent variables, a second order response function has the following general formula:

$$Y = \beta_0 + [\beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4] + [\beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{44}X_{42}] + [\beta_{12}X_1X_2 + \beta_{23}X_2X_3 + \beta_{34}X_3X_4 + \beta_{41}X_4X_1] \quad \dots (2)$$

This test was performed as an analysis of variance (ANOVA) by calculating the F-ratio, which is the ratio between the regression mean square and the mean square error. The F-ratio, also called the variance ratio, is the ratio of variance due to the effect of a factor (in this case the model) and variance due to the error term. This F-ratio was used to calculate the p-value of the model which was finally used to measure the significance of the model under investigation. $p < 0.01$ corresponds to the fact that the variable is significant at 1% level of significance. Similarly $p < 0.05$ and $p < 0.10$ corresponds to the fact that the variable is significant at 5% and 10% level of significance, respectively.

Results and Discussion

This research was undertaken with an objective to interpret the outcome of process factors on yield of colorant from walnut hull using Microwave assisted extraction. The experimental design used for the study was Box-Behnken design having four variables and each variable having three levels. The variables with levels were microwave power (160, 320, 480W), microwave time (2, 3 and 4 mins), Solvent (Water) volume (20, 30, 40ml) and particle size (150, 300, 450µm). Response surface methodology was utilized to enhance the process parameters. A complete second order mathematical model was fitted in the response. The competence of the model was confirmed using coefficient of determination (R²) and Fisher's test. The results obtained on numerous characteristics of the study are discussed in detail.

Effect of Independent Parameters on extract Yield

Bio colorant from walnut hull powder was extracted using microwave assisted extraction. To enhance the extraction process, parameters studied were microwave power (160, 320, 480 W), microwave time (2, 3, 4 mins), Solvent (Water)

volume (20, 30, 40 ml) and particle size (150, 300, 450 μ m). The quantity of extract yield for all the 29 experiments is showed in Table 4.

The extract yield obtained ranged from 26.67% to 44.99%. Maximum extract yield of 44.99% for the hull sample was obtained at the experiment run 29 having experimental conditions of Microwave power at 320 W, Microwave time at 3 min, Solvent volume 1:20 and particles size was 150 μ m. On other hand, the minimum extraction yield of 26.67% was obtained at the experiment run no. 19 having independent variable conditions of Microwave power at 160 W, Microwave time at 2 min, Solvent volume 1:30 and particles size was 300 μ m.

The experimental data was studied to note the remarkable consequences of numerous process variables on extract yield. The outcomes of variance for extract yield are given in Table 5. F-value was used to observe the significance of linear, quadratic and interactive terms. F_{cal} value for model was superior to F_{tab} , which infers that model was significant ($p < 0.01$). The outcome of independent variables on yield at linear level was observed to be highly significant at 1% level of significance ($F_{cal} > F_{tab}$), for quadratic and interactive terms it was also observed significant.

A second order polynomial equation was used to fit the coded variables (A, B, C, D) for extract yield using multiple regression analysis. Table 4.5 shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the extract yield was found as 0.9446

which implies that the model could account for 94.46% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the extract yield. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2 " of 0.7188 was in reasonable agreement with the "Adj R^2 " of 0.8891, the adequate precision was found to be 17.361 and the C.V was 4.16% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

A second order polynomial equation (Eq. 4.2) was developed representing an empirical relationship between the response (Extract Yield) and the independent variables Particle Size (A), Microwave Power(B), Microwave Time (C) and Solvent volume (D). The equation for extract yield is given below:

$$\text{Extract Yield (\%)} = 38.98 - 2.14A + 2.71B + 1.20C - 1.83D - 0.15AB - 0.058AC + 0.50AD + 0.23BC - 0.13BD - 0.050CD - 0.26A^2 - 5.18B^2 - 4.72C^2 + 0.36D^2$$

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation (Eq. 4.2a) was regenerated, that describes only the effect of significant process variables on extract yield from walnut hull. The equation is as follows:

$$\text{Extract Yield (\%)} = 38.98 - 2.14A + 2.71B + 1.20C - 1.83D - 0.26A^2 - 5.18B^2 - 4.72C^2 \quad (4.2a)$$

Table 4: Experimental data on MAE of walnut hull extract

Std.	Run	Size (μ m)	Power (W)	Time (min)	Solvent volume (ml)	Yield (%)
21	1	300	160	3	20	32.41
14	2	300	480	2	30	31.46
8	3	300	320	4	40	33.07
7	4	300	320	2	40	30.99
22	5	300	480	3	20	38.21
1	6	150	160	3	30	32.03
28	7	300	320	3	30	39.12
6	8	300	320	4	20	36.95
26	9	300	320	3	30	37.11
29	10	300	320	3	30	39.46
16	11	300	480	4	30	34.81
19	12	150	320	4	30	36.52
25	13	300	320	3	30	39.99
2	14	450	160	3	30	28.18
5	15	300	320	2	20	34.67
12	16	450	320	3	40	37.01
20	17	450	320	4	30	32.21
11	18	150	320	3	40	40.50
13	19	300	160	2	30	26.67*
24	20	300	480	3	40	34.22
15	21	300	160	4	30	29.08
27	22	300	320	3	30	39.24
23	23	300	160	3	40	28.95
3	24	150	480	3	30	37.77
17	25	150	320	2	30	34.25
10	26	450	320	3	20	39.50
18	27	450	320	2	30	30.18
4	28	450	480	3	30	33.33
9	29	150	320	3	20	44.99**

Table 5: Regression analysis for extract yield for MAE

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	503.4374	14	35.95981	17.03964	2.05E-06
A	54.88246	1	54.88246	26.00619	0.000162
B	87.87031	1	87.87031	41.63756	1.52E-05
C	17.30401	1	17.30401	8.199546	0.012513
D	40.31427	1	40.31427	19.10301	0.00064
AB	0.087616	1	0.087616	0.041517	0.841475
AC	0.01334	1	0.01334	0.006321	0.937755
AD	0.994009	1	0.994009	0.471014	0.50373
BC	0.22043	1	0.22043	0.104451	0.751329
BD	0.069485	1	0.069485	0.032926	0.858612
CD	0.01	1	0.01	0.004739	0.946093
A ²	0.450913	1	0.450913	0.213666	0.001121
B ²	174.2368	1	174.2368	82.56251	3.02E-07
C ²	144.281	1	144.281	68.36792	9.31E-07
D ²	0.844467	1	0.844467	0.400153	0.537206
Residual	29.54506	14	2.110362		
Lack of Fit	24.71054	10	2.471054	2.044509	0.255921
Pure Error	4.83452	4	1.20863		
Cor Total	532.9824	28			
R ²	0.9446				
Adj R ²	0.8891				
Pred R ²	0.7188				
Adeq. Precision	17.361				
C.V. %	4.16				

Graphical Analysis

Graphical analysis was done for understanding the trend of various responses with respect to levels of significant process variables. To determine the operating range for the best result, graphs were drawn using software Design expert 10.0.1.

The overall extract yield from walnut hull is dependent on several variables. The current study is aimed at observing graphical trends of dependent variables with changes in independent variables under defined constant variables. The graphical analysis is based on linear trends observed in extract yield category.

In Fig. 1a, at linear level the extract yield varies with particle size at optimum conditions of Microwave power 383.307W, Time 3.002min, Solvent volume 21.716 for achieving maximum extract yield. The graph shows extract yield decreases with the increase in particle size in range from 150 μm to 450 μm . Smaller the particle size greater interfacial surface are between the solid and solvent, hence higher the transfer rate of material from sample to solvent. This is due to easier accessibility of solvent over the smaller sized solute/substrate surface. Thus results in overall increase in solvent extraction of dye in the microwave processing conditions. The readings are supported by (Wang *et al.*, 2011) [7].

In Fig. 1b, at linear level the extract yield varies with microwave power at optimum conditions of particle size 383.307 μm , Time 3.002min, Solvent volume 21.716 for achieving maximum extract yield. The graph shows extract yield increases with the increase in microwave power then

after subsequent increase in power overall yield decreases in range from 160W to 480W. It was observed that the extraction power was found to have positive influence on extraction yield (Lucchesi *et al.*, 2007) [4]. However prolonged exposure to microwave power may lead to degradation of the extracted colorant due to overheating of solute and solvent system.

In Fig. 1c, at linear level the extract yield varies with time at optimum conditions of Microwave power 383.307W, Particle size 218.135 μm , Solid to solvent ratio 21.716 for achieving maximum extract yield. The graph shows extract yield increases with the increase in time and in later stages decreases with increase in time, ranges from 2 min to 4 min. The extract yield was found optimum at the central level. In later stages of time intervals, resulted in decrease in microwave radiation per particle with the increase in solvent volume which led to relatively low dielectric heating effect thus reduced effectiveness of microwave radiation (Wang *et al.*, 2011) [7].

In Fig. 1d, at linear level the extract yield varies with solid to solvent ratio at optimum conditions of Microwave power 383.307W, Time 3.002min, Particle size 218.135 μm for achieving maximum extract yield. The graph shows extract yield decreases with the increase in solid to solvent ratio in range from 20 to 40. This is due to the enhancement of solubility of solute particles for a prolonged time interval which decreases the viscosity of extracting solvent and thus accelerating the release and dissolution of these compounds (Jawad and Langrish, 2012) [2].

Fig 1: Effect of independent variables on Extract yield

Conclusion

Bio colorant is an important naturally which can be widely utilized in the food industry. In this research, varied extraction conditions were studied for bio colorant extraction from walnut hull using microwave assistance. The Box–Behnken design was utilized to correlate the effect of microwave time, power, solvent volume and particle size on the yield. The outcome presents that the peak yield of biocolrant extracted at the ideal conditions of Microwave power at 320 W, Microwave time at 3 min, Solvent volume 1:20 and particles size was 150 μ m.

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