International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(2): 87-91

Received: 14-12-2020
Accepted: 23-01-2021

Iftikhar Alam
Department of Post-Harvest Process and Food Engineering, GBPUAT, Pantnagar, Uttarakhand, India

NC Shahi
Department of Post-Harvest Process and Food Engineering, GBPUAT, Pantnagar, Uttarakhand, India

UC Lohani
Department of Post-Harvest Process and Food Engineering, GBPUAT, Pantnagar, Uttarakhand, India

Anil Kumar
Department of Food Science and Technology, GBPUAT, Pantnagar, Uttarakhand, India

Om Prakash
Department of Chemistry, GBPUAT, Pantnagar, Uttarakhand, India

Corresponding Author:
Iftikhar Alam
Department of Post-Harvest Process and Food Engineering, GBPUAT, Pantnagar, Uttarakhand, India

Ultrasound assisted extraction of oil from black cumin (*Nigella sativa* L.)

Iftikhar Alam, NC Shahi, UC Lohani, Anil Kumar and Om Prakash

DOI: https://doi.org/10.22271/chemi.2021.v9.i2b.11698

Abstract
Black cumin (*Nigella sativa* L.) seed, also known as black seed, is an annual herb belongs to Ranunculaceae family. Black seed contains an ample amount of oil which can be extracted using various techniques and can be used in food and medicinal applications. In the present study, black cumin seed oil was acquired by ultrasound assisted extraction. The effects of different levels of ultrasonication power, extraction time, and solvent to solid ratio on the recovery of black seed oil were investigated. Black seed oil was extracted with different combinations of ultrasound power (150, 200, 250 W), treatment time (15, 30, 45 min) and solvent: solid ratio (10, 15, 20 ml/g). Box Behnken design of Response Surface Methodology was used to optimized the process parameters. As per the results, the highest oil recovery of 94.8% from black seed was attained at the ultrasound power of 200 W, ultrasound treatment time of 45 minutes and solvent to solid ratio of 1:20. Ultrasound assisted extraction enhanced the black seed oil recovery and reduced the extraction time as well.

Keywords: Black seed oil, ultrasound assisted extraction, solvent

Introduction

Oils and fats are necessary not only for their health benefits but also from a commercial point of view. They provide a major amount of energy needed by the body cells, the essential fatty acids required, as well as fat-soluble vitamins (Kebriti *et al.*, 2011) [3]. India is one of the major oil seeds grower as well importer of edible oils in the world. India stands fourth largest producer of vegetable oil after USA, China & Brazil. India is heavily dependent on imports to meet its edible oil supply and is the largest importer of vegetable oils in the world followed by China and USA (India MoA&FW, 2020) [2]. Oils and fats obtained from various plant species and animal sources may have different metabolic, physical, and chemical properties regarding their species (Zomorrodi *et al.*, 2003) [13]. In order to overcome the issues of oil requirement, need is to look for the alternatives of other possible sources which are safe to consume and provide nutritional benefit. Nigella sativa (black cumin) seeds is one such source for oil extraction which have been used traditionally as a spice and food preservative (Srinivasan, 2018) [8]. Black cumin seed oil is a good source of essential fatty acid, lipid soluble bioactive compounds, and linoleic acid, all of them makes it a nutritionally valuable product (Kiralan *et al.*, 2014) [4].

Vegetable oil is conventionally extracted using the filter press and other such traditional pressing equipments which is a time consuming and a low yielding process. The oil is also extracted by the solvent extraction method which is generally operated at 40-60°C and even at 70 °C for around 10-16 hours. This again is a time consuming process and with an added effect of high temperature, an alteration of minor constituent happened and quality of oil gets deteriorated (Cheikh-Rouhou *et al.*, 2007) [1]. To overcome this problem the ultrasound assisted extraction may be a good alternative. The positive role of ultrasound waves in extraction might be relevant to its effects on destruction of cell membrane and as a result its added impacts on extraction oil yield (Lingyun *et al.*, 2007) [6]. Ultrasound assisted solvent extraction is effective for the oil extraction as its mechanical impact takes toll on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation (Toma *et al.*, 2001) [9]. Ultrasound technique will thus enhance the rate of extraction, quality of the extracted oil, safety of the process, and reduce time of extraction (Virot *et al.*, 2010) [10].

Considering all the above points, Ultrasound-assisted extraction (UAE) with hexane as solvent could be used to obtain high-quality black cumin oil with greater efficiency than conventional methods. The overall objective of the research was to boost the effectiveness of oil extraction with respect to oil recovery from ground black cumin seed. The specific objective was thus to study the consequence of process parameters of ultrasound power, ultrasound time and solvent: solid ratio on the oil recovery from black cumin using ultrasound assisted extraction.

Materials and Methods

The main objective of this research study is to standardize the process parameters for extraction of oil from black cumin seed and then optimize these process parameters on the basis of oil recovery. Materials used and procedure adopted in this research is described as follows:

Raw Material, Chemicals and glassware

All the experiments were performed in the Quality Control Laboratory of the Department of Post-Harvest Process and Food Engineering, GBPUAT-Pantnagar, Uttarakhand, India. Seeds of black cumin was procured from the local market of Pantnagar. Hexane was utilized as the solvent for the extraction of oil from black cumin. Hexane, Glassware like beakers, conical flasks, measuring cylinders and funnels were collected from the department's store room.

Experimental setup

It consists of different equipment required to carry out experimental work which includes Sonicator, magnetic stirrer, refrigerator, weighing balance, grinder, and Soxhlet apparatus.

Experimental design

Box-Behnken Design (BBD) of response surface methodology was used for optimization of UAE for black cumin oil. This design consisted of 17 randomized runs (Table 2) with five replicates at the central point. For the designed experiments, three variables having 3 levels of each (ultrasound power, ultrasound treatment time and solid to solvent ratio) for UAE were selected for the experiments. Table 1 represent the actual and coded independent variables for extraction.

Table 1: Independent parameters for Box Behnken Design

Independent parameter	Coded value	Value of levels		
		-1	0	+1
Ultrasound power (W)	A	150	200	250
Ultrasound time (min)	B	15	30	45
Solvent: solid (ml/g)	C	10	15	20

Preparation of sample

Black cumin seed were cleaned to remove any foreign material. Clean black cumin seed were ground by means of a Cyclotec mill. A stainless steel screen with a mesh dimension of 1 mm was utilized to attain a constant particle dimension distribution of black cumin powder. Ground black cumin of size 1 mm were then packaged in air-tight glass jar until used.

Ultrasound treatment

Black cumin powder (10 g) was mixed with the selected quantity of solvent: solid (10, 15 and 20 ml/g) in a 500 ml glass beaker. The black cumin–solvent suspension was ultrasonicated for 15, 30 and 45 minutes using a 20 kHz ultrasonic Sonicator (Electrosonic Industries, India) with a diameter of 2.0 cm probe that was immersed in the suspension. Ultrasonic power used were 150, 200 and 250 W. Suspensions were endlessly stimulated at a continuous stirring at a rate of 550 rpm by a magnetic stirrer to avert heating of suspensions under the effect of high-intensity ultrasound. After extraction, oil-solvent mixture was filtered using Wattman filter paper no 1 from the ultrasound treated suspension. The filtrate from the filtration comprises of oil and solvent. The oil was separated from this mixture by means of a countercurrent distillation arrangement with the heating mantle as the heat source and water as the coolant. The solvent used was also recovered. Quantity of oil extracted was determined by weighing the oil obtained after solvent removal which was kept in properly washed and sterilized test tubes.

Oil recovery determination

The oil recovery oil was calculated using the following formula:

$$\text{Yield (\%)} = (We / Wt) \times 100$$

Where, We is the quantity of oil extracted from the sample (g) and Wt is the quantity of total oil in the sample (g). Wt was obtained by Soxhlet standard extraction mode.

Data analysis

The model development was done by means of response surface methodology through use of Design expert software version 10.0.1.0. Complete second order model as given in Equation-1 was fitted to the data and the model adequacy was tested using R^2 (coefficient of multiple determination) and fisher's F-test. The parametric effect on various responses was done through the analysis of developed models. Regarding three independent variables a second order response function has the following general form

$$Y = \beta_0 + [\beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4] + [\beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} + \beta_{44} X_{42}] + [\beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{34} X_3 X_4 + \beta_{41} X_4 X_1] \quad (1)$$

Where

β_0 is the intercept, β_1, β_2, β_3 and β_4 are coefficients of the linear terms, β_{11}, β_{22}, β_{33} and β_{44} are quadratic coefficients and β_{12}, β_{23}, β_{34} and β_{41}

Multiple regression analysis was used to examine the experimental data so as to develop response functions and obtain variable parameters optimized corresponding to best outputs. The values of model coefficients and connected statistics in relations of lack of fit and P-value were obtained through the program. The value of P represents the possibility of significance. A high F-value is an indication of the point that the model had a significant lack of fit and therefore is inadequate. A model with lower values of P is considered better. The models having P-value lower than 0.1 were acknowledged. Equation is solved by means of a statistical method called technique of least square which is a multiple regression method utilized to fit a mathematical model to a set of experiment data producing the lowest residual possible. The results of regression analysis are obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination (R^2), lack of fit, etc. These determine adequacy of predictive model and effect of independent parameters on the response.

Results and Discussion

The current study was taken with the aim of studying the outcome of process factors on oil yield from black cumin using Ultrasound assisted extraction and the optimization of process parameters for oil on the basis of its yield. The experimental design used in the current study was Box-Behnken design having three variables ultrasound power, ultrasound time and solvent: solid ratio with three levels of each (150, 200 and 250 W), (15, 30 and 40 min) and (10, 15 and 20 ml/g) respectively. Response surface methodology was utilized to improve the process parameters. A complete second order mathematical model was fitted in the response. The competence of the model was tested using coefficient of determination (R^2) and Fisher's test. The results obtained on numerous characteristics of the study are given below.

Table 2: Box Behnken Designs for ultrasound assisted extraction

run	Coded level			Actual level			Oil recovery (%)
	A	B	C	Ultrasound power (W)	Ultrasound time (min)	Solvent: solid (ml/g)	
1	0	-1	1	200	15	20	87.55
2	0	1	1	200	45	20	92.71
3	-1	1	0	150	45	15	89.52
4	1	-1	0	250	15	15	88.70
5	0	0	0	200	30	15	90.50
6	-1	0	-1	150	30	10	88.42
7	1	0	1	250	30	20	94.80
8	0	0	0	200	30	15	90.70
9	0	0	0	200	30	15	90.40
10	0	-1	-1	200	15	10	85.51
11	0	1	-1	200	45	10	89.67
12	1	1	0	250	45	15	92.86
13	1	0	-1	250	30	10	89.74
14	-1	-1	0	150	15	15	85.56
15	0	0	0	200	30	15	90.80
16	-1	0	1	150	30	20	89.44
17	0	0	0	200	30	15	90.60

Effect of independent parameters on oil recovery

Black cumin seed oil was extracted from ground black cumin using ultrasound assisted extraction while taking hexane as the solvent. To enhance the extraction process, parameters studies were ultrasound power (150, 200 and 250W), ultrasound time (15, 30 and 45 minutes), solvent to solid volume (10, 15 and 20 ml/g). The quantity of oil obtained during extraction is showed in table 2. The oil recovery ranged from 85.51% to 94.80% from sample amount of 10 g of sample over entire experimental conditions. The experimental data was studied to observe the noteworthy consequence of numerous process variables on the oil yield. The outcomes of study of variance for oil yield are given in Table 3. F-value was used to investigate the significance of linear, quadratic and interactive terms. Fcal value for model was superior to Ftab, which implies that model was significant ($p< 0.01$). The outcome of independent variables on oil yield at linear level was found highly significant at 1% level of significance (Fcal>Ftab), for quadratic and interactive terms it was also observed significant

Table 3: Regression analysis for extract recovery

Source	Sum of Squares	Df	Mean Square	Value	Prob > F
Model	90.46325	9	10.05147	171.6105	<0.0001**
A	21.6482	1	21.6482	369.6034	<0.0001**
B	38.0192	1	38.0192	649.1083	<0.0001**
C	15.5682	1	15.5682	265.7985	<0.0001**
AB	0.01	1	0.01	0.170732	0.691826
AC	4.0804	1	4.0804	69.66537	<0.0001**
BC	0.25	1	0.25	4.268293	0.077676
A^2	0.094737	1	0.094737	1.617458	0.244067
B^2	10.64463	1	10.64463	181.7376	<0.0001
C^2	0.094737	1	0.094737	1.617458	0.244067
Residual	0.41	7	0.058571		
Lack of Fit	0.31	3	0.103333	4.133333	0.102011
Pure Error	0.1	4	0.025		
Cor Total	90.87325	16			
R^2	0.995488				
Adj R^2	0.989687				
Pred R^2	0.943699				
Adeq Precision	49.91453				
C.V. %	0.26935				

**Significant at 1% level of significance, *Significant at 5% level of significance

Multiple regression analysis was performed to fit the response parameter, oil recovery. Regression coefficients for the second order model polynomial equations for the linear, quadratic and interaction terms are shown in Table 4.

Statistical analysis shows that the projected model was sufficient, having significant fit and with very reasonable values of R^2 for drying time. The R^2 value for the oil yield was 99.54%, which implies that the model could account for 99.54% data. Insignificant value of lack of fit showed that the developed model is valid. Second order polynomial equation was developed which represents response, oil recovery as a functions of ultrasound power, ultrasound time and solvent: solid ratio an empirical relationship among the responses and input variables in coded form can be expressed by the following equation:

$$\text{Oil recovery} = 90.60 + 1.645A + 2.180B + 1.395C + 0.049AB + 1.01AC + 0.25BC + 0.15A^2 - 1.59B^2 - 0.15C^2 \quad (2)$$

Where, Y is oil yield, A, B and C are the coded values of ultrasound power, ultrasound time and solvent: solid ratio respectively.

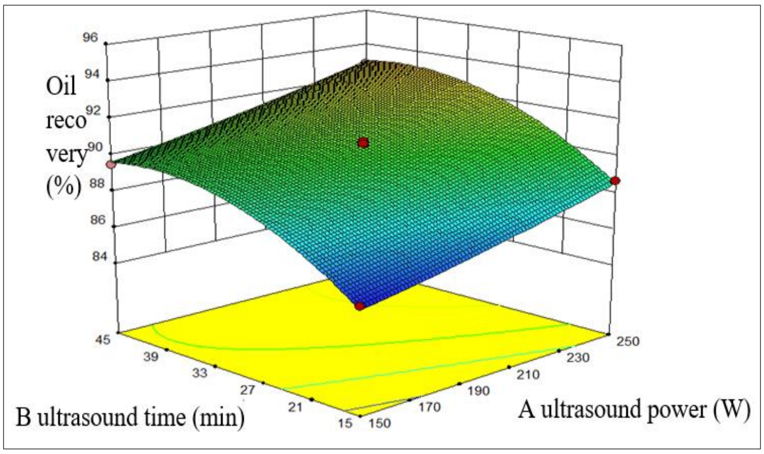

Fig 1A: Effect of ultrasound power and ultrasound time on oil recovery

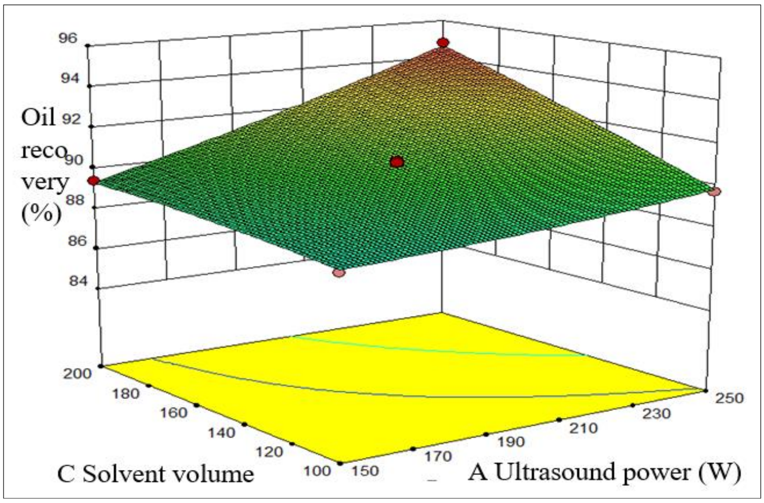

Fig 1B: Effect of ultrasound power and solvent volume on oil recovery

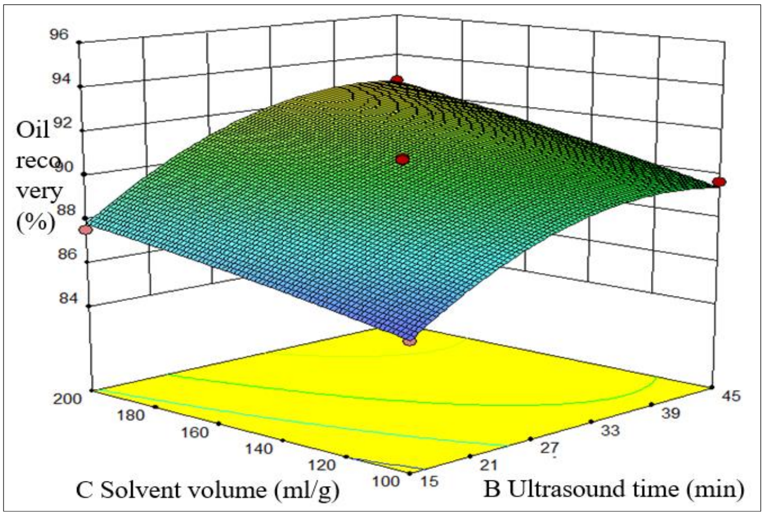

Fig 1C: Effect of solvent volume and ultrasound time on oil recovery

Fig 1(A-C): Effect of independent variable on oil recovery of black cumin seed

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 effect of ultrasonic power, ultrasound time and solvent: solid ratio on the oil recovery of black cumin seed oil is shown in Figure 1A-C. Figure 1A shows the effect of experimental duration for extraction on the oil recovery of black cumin. It was observed that the oil recovery increases from 84 to 95% with the upsurge in extraction time from 15 to 45 minutes. This process shows that the consequence of ultrasound is more effective at 30 to 40 minutes. It may be that ultrasonic wave possibly will disrupt the cell walls, so greater interaction region between solvent and material was generated and additional oil was released. Similar results were obtained by (Li *et al.,* 2004) [5] and (Sivakumar *et al.,* 2007) [7],

The effect of ultrasonic power on the oil recovery of black cumin oil is shown in Figure 1B. It can be seen that the oil recovery of black cumin oil increases almost linearly from 84 to 95% with increasing ultrasonic power from 150 to 250 W. Since the temperature and pressure were extremely great inside the bubbles and the breakdown of bubbles followed over very petite time, the violent shock wave and high speed jet were produced which could boost the permeation of the solvent into the cell tissues and speed up the intracellular product discharge into the solvent by distracting the cell walls. Similar results were obtained by (Yousuf *et al.,* 2018) [11]. As shown in Figure 1C, the solvent volume had significant result on the oil recovery of black cumin. The oil recovery was found to increase with the rise in solvent volume from 100 ml to 200 ml. The higher liquid (solvent) to solid ratio signifies a higher concentration difference which aids mass transfer. Similar result has also been shown by (Zhao *et al.,* 2007) [12].

Conclusion

Black cumin oil is widely utilized in the food industry. In this research, varied extraction conditions were studied for oil extraction from black cumin using ultrasound assisted extraction. The Box–Behnken design was utilized to correlate the effect of Ultrasound time, power and solvent to solid ratio. As per the results of the study the highest recovery of black cumin oil was achieved at the ultrasound power of 250 W, time taken was 30 min and the solvent to solid ratio was 20:1 ml/g. In contrast, the lowest recovery of black cumin oil was achieved at the ultrasound power of 250 W, time taken was 30 min and the solvent to solid ratio was 20:1 ml/g. As per the optimization of the data which was done using design expert, the best optimized result of 88.24% recovered black cumin oil was obtained at the ultrasound power of 150 W, ultrasound time 27.69 min and the solvent to solid ratio of 10 ml/g.

References

1. Cheikh-Rouhou S, Besbes S, Hentati B, Blecker C, Deroanne C, Atti H. *Nigella sativa* L.: chemical composition and physicochemical characteristics of lipid fraction. Food Chem 2007;101:673-681.
2. India. Ministry of Agriculture and farmers Welfare. Department of Agriculture, Cooperation and Farmers Welfare. Annual Report. New Delhi 2020, 1-134.
3. Kebriti M, Hoseini Mazhari SZ, Gerami A, Ghiassi B, Esfandyari C. Survey on the rate of losses and wastes in oil seeds processing plants in Tehran Province. J Food Technol Nutr 2011;8(1):42-50.
4. Kiralan M, Özkan G, Bayrak A, Ramadan MF. Physicochemical properties and stability of black cumin (Nigella sativa) seed oil as affected by different extraction methods. Industrial Crops and Products 2014;57:52-58.
5. Li H, Pordesimo L, Weiss J. High intensity ultrasound-assisted extraction of oil from soybeans. Food research international 2004;37(7):731-738.
6. Lingyun W, Jianhua W, Xiaodong Z, Da T, Yalin Y, Chenggang C *et al.*, Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. Journal of Food engineering 2007;79(3):1087-1093.
7. Sivakumar V, Verma VR, Rao PG, Swaminathan G. Studies on the use of power ultrasound in solid-liquid myrobalan extraction process. Journal of Cleaner Production 2007;15(18):1813-1818.
8. Srinivasan K. Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: traditional uses, chemical constituents, and nutraceutical effects. Food quality and safety 2018;2(1):1-16.
9. Toma M, Vinatoru M, Paniwnyk L, Mason TJ. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics sonochemistry* 2001;8(2):137-142.
10. Virot M, Tomao V, Le Bourvellec C, Renard CM, Chemat F. Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction. *Ultrasonics sonochemistry* 2010;17(6):1066-1074.
11. Yousuf O, Gaibimei P, Singh A. Ultrasound assisted extraction of oil from soybean. Int. J Current Microbiol. Appl. Sci 2018;7:843-852.
12. Zhao S, Kwok KC, Liang H. Investigation on ultrasound assisted extraction of saikosaponins from Radix Bupleuri. Separation and Purification Technology 2007;55(3):307-312.
13. Zomorrodi S, Shokrani R, Shahedi M, Dokhani S. Interesterification of mixtures of soybean oil with fully hydrogenated oil for production of shortenings. Iran J Agric Sci 2003;34(3):587-95.