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# Optimization of soxhlet extraction of garden cress oil by response surface methodology

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**Abstract**
Present study was aimed to optimize the parameters *viz.,* particle size, extraction time and pre-heating temperature for soxhlet extraction of garden cress oil (GCO). Garden cress seeds were reduced to three size ranges (0.600, 0.425 and 0.300 mm) and heated in hot air oven for 20, 30 and 40 min at 100 °C. Extraction was carried out in soxhlet using petroleum ether for 2, 4 and 6 h. Effect of these parameters on yield and peroxide value of GCO was investigated. Experiment was designed according to face centered central composite design using design expert 10.0.0 software. Optimization of process parameters was done by response surface methodology. Results showed that, oil yield was higher from the smaller size of particles and increased with increasing heating time and extraction time. Peroxide value was also higher for oil extracted from the smaller size particles and increased with increasing heating and extraction time. Yield was ranged between 17.14 to 24.21% whereas PV ranged between 2.18 to 3.92 meq.kg$^{-1}$. Optimized condition was obtained for 0.425 mm size, heating for 30 min and extraction for 4 h giving yield 23.1% and PV 2.513 meq.kg$^{-1}$.

**Keywords:** Garden cress oil, soxhlet extraction, particle size, response surface methodolgy, optimization

## 1. Introduction
Garden cress (*Lepidium sativum* L.) an annual herb of Brassicaceae family have found to contain good amount of oil containing polyunsaturated fatty acids. Garden cress seeds contain 24% oil in which 32–34% is α-linolenic acid (ALA). Garden cress oil (GCO) has very high amount of tocopherols (1699 mg/kg), compared to other oils [1]. Vegetable oil demand has increased due to increasing domestic and industrial uses. Nutritionally, vegetable oil provides calories, vitamins, and EFA in the human diet in an easily digested form, and at relatively low cost [2]. Garden cress oil has Linoleic acid: Linolenic acid (LA: ALA) ratio in the range of 1:4–2:3, which could give it nutritional advantages over the currently available ALA-rich plant oils in altering the n- 6/n-3 ratio *in vivo*.

There are several methods to extract oil from seeds such as pressing, solvent extraction, soxhlet extraction and supercritical fluid extraction [3]. Some of the pretreatment prior to extraction are also been studied on different oil bearing materials. [1] Diwakar *et al*. (2010) extracted garden cress seed oil using hydraulic press, solvent extraction and supercritical fluid extraction. Significant difference in yield and physico-chemical properties of the oil were reported. Several researchers have investigated the effects of processing factors such as temperature and roasting time [2, 4], particle size [5], moisture content [6], pH and solid to water ratio [7], and applied pressure on oil yield from different oil seeds [8]. Heating leads to increased yield. It helps in killing those enzymes present in the plant tissue that have a deteriorating effect on oil quality. Heating temperature and time depend on the type of seed and extraction process [9].

Soxhlet (solvent) extraction is the standard technique where the fresh solvent contacts the sample frequently [10]. It is widely used technique because it is simple and easy to run. There are three major steps in solvent extraction i.e., oil seed preparation, oil seed extraction and desolventizing of the oil and meal [11]. The quality characteristics of crude oil obtained by solvent extraction methods are primarily dependent on extraction solvents, extraction temperature, and pretreatment of oil seeds [12]. Specific conditions of pre-treatments required vary from one seed to another [13, 14]. Such studies have been conducted on several oilseeds including cashew, locust bean, sesame, soybean, groundnut, conophor and flaxseed [15, 14, 16, 13, 17, 18, 19, 20].

It provides useful information for design or adaptation of existing machines for oil expression and performance evaluation of same. Therefore the selection of extraction method and suitable pretreatment is important factor. In order to improve the performance of a system, without increasing the cost, optimization process are important. Process optimization is a well-known efficient experimentation technique and applied in a broad range of fields such as food, automobile, drug and textile composites to produce high quality products and ensure more stable and reliable process [21]. One of the methodologies for obtaining the optimum process condition is response surface methodology (RSM). The approach is a collection of statistical and mathematical techniques useful for development, improving, and optimizing processes [22].

No work has been reported on the effect of particle size, pre-heating time and extraction time of soxhlet extraction on the extraction yield and peroxide value of garden cress oil. In order to study the effects of independent variables on oil extraction, face centered central composite design (FCCD) with response surface methodology (RSM) was used. FCCD has the advantage to predict responses based on few sets of experimental data in which all variables are varied within a chosen range. Multiple regression analysis on experimental data can determine optimal conditions.

## 2. Material and Methods

Garden cress seeds were procured from the local market of Rahuri. Seeds were cleaned prior to the extraction.

### 2.1 Experimental design

Effect of three process parameters such as particle size, pre-heating time and extraction time were investigated to optimize the operating conditions for achieving maximum yield and minimum peroxide value (PV). Experiments were designed according to face centered central composite design (FCCD) using Design expert 10.0.0 software.

### 2.2 Preparation of samples

Garden cress seeds were then ground using mixer. Flour obtained was then sieved using BSS 50, 40 and 30 number sieves having opening diameter of 0.600, 0.425 and 0.300 mm. Fraction of ground seeds retained on No. 50 sieve was considered as coarse, retained on sieve No. 40 was considered as medium and retained on 30 No. sieve was considered as fine. These fractions were then heated in hot air oven at 100 °C for 30, 40 and 50 min.

### 2.3 Extraction of oil

A lab scale Soxhlet apparatus was used to extract oil from garden cress seeds. About 20 g of ground seed flour was extracted for 2, 4 and 6 h with petroleum ether.

### 2.4 Yield of the oil

The extracted oil yield was expressed in percentage, which is defined as weight of oil extracted from weight of the sample taken. The percentage oil yield was calculated as follow:

$$\text{Oil yield, \%} = \frac{\text{Weight of oil obtained}}{\text{Weight of seeds used for extraction}} \times 100 \qquad (1)$$

### 2.5 Peroxide value

The peroxide value (PV) was determined by iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the fat sample, using [23] procedure with little modifications. A 2.0 g sample of oil was dissolved in 30 mL mixture of glacial acetic and chloroform (30:70 v/v). Then 0.5 mL saturated potassium iodide solution was added. After 1 min under darkness, 30 mL $H_2O$ purified was immediately added and titrated with 0.01 N sodium thiosulphate ($Na_2S_2O_3$). The liberated $I_2$ was titrated with 0.01 N $Na_2S_2O_3$ using a starch solution (1%) as an indicator, until the solution became colorless.

## 3. Results and Discussion

The experimental results are given in Table 1 and statistical analysis of the data is presented in Table 2.

**Table 1:** Effect of soxhlet extraction variables on oil yield and peroxide value of garden cress seed oil

| Run | Coded | | | Actual | | | Yield (%) | Peroxide value ($meq.kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | Particle size | Heating time | Extraction time | Particle size | Heating time | Extraction time | | |
| 1 | -1 | -1 | -1 | 30 | 20 | 2 | 17.14 | 2.18 |
| 2 | 1 | -1 | -1 | 50 | 20 | 2 | 18.93 | 2.37 |
| 3 | -1 | 1 | -1 | 30 | 40 | 2 | 21.36 | 2.50 |
| 4 | 1 | 1 | -1 | 50 | 40 | 2 | 21.84 | 2.51 |
| 5 | -1 | -1 | 1 | 30 | 20 | 6 | 22.45 | 3.03 |
| 6 | 1 | -1 | 1 | 50 | 20 | 6 | 23.94 | 3.12 |
| 7 | -1 | 1 | 1 | 30 | 40 | 6 | 24.20 | 3.79 |
| 8 | 1 | 1 | 1 | 50 | 40 | 6 | 24.21 | 3.92 |
| 9 | -1 | 0 | 0 | 30 | 30 | 4 | 21.88 | 2.53 |
| 10 | 1 | 0 | 0 | 50 | 30 | 4 | 23.96 | 2.85 |
| 11 | 0 | -1 | 0 | 40 | 20 | 4 | 22.72 | 2.50 |
| 12 | 0 | 1 | 0 | 40 | 40 | 4 | 24.08 | 2.98 |
| 13 | 0 | 0 | -1 | 40 | 30 | 2 | 20.48 | 2.42 |
| 14 | 0 | 0 | 1 | 40 | 30 | 6 | 24.21 | 3.22 |
| 15 | 0 | 0 | 0 | 40 | 30 | 4 | 23.27 | 2.22 |
| 16 | 0 | 0 | 0 | 40 | 30 | 4 | 24.14 | 2.64 |
| 17 | 0 | 0 | 0 | 40 | 30 | 4 | 23.93 | 2.92 |

### 3.1 Effect of extraction parameters on oil yield

Results shown in Table 1 revealed that, the oil yield varied from 17.14 to 24.21%. Oil yield was highly significant at probability level $p<0.05$ for first order terms of particle size, heating temperature and extraction time. The interaction term of heating time and extraction time was significant at probability level $p<0.05$. The following second-order polynomial equation in terms of coded units was generated to obtain the empirical relationship between the experimental results on the basis of central composite design.

$$Yield = 23.63 + 0.58 \times A + 1.05 \times B + 1.93 \times C - 0.35 \times A \times B - 0.096 \times A \times C - 0.64 \times B \times C - 0.60 \times A2 - 0.12 \times B2 - 1.18 \times C2 \quad \ldots (4.1)$$

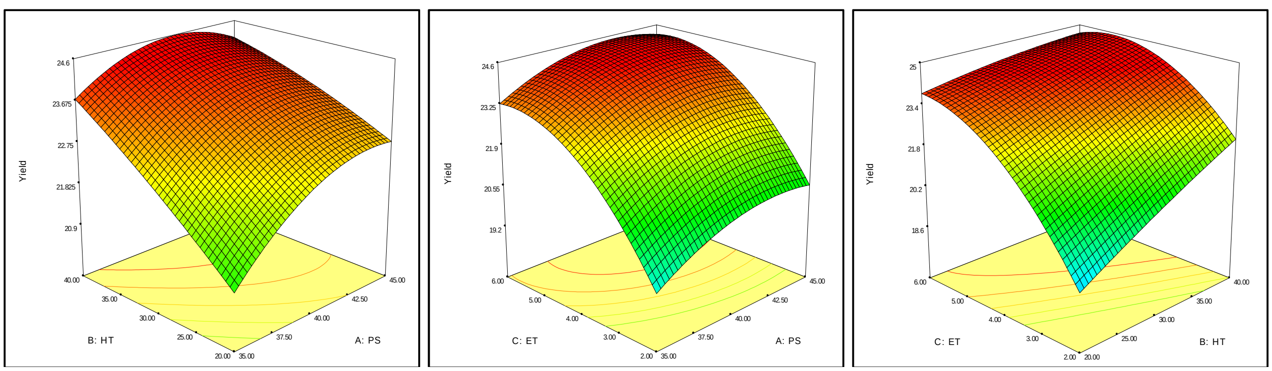
Where, A, B and C are the coded values of particle size, heating time and extraction time.

**Table 2:** Analysis of variance and statistical parameters of the model

| Source | Sum of Squares | |
|---|---|---|
| | Oil yield (OY) | Peroxide value (PV) |
| Model | 67.095** | 3.73** |
| A-Size | 3.418 ** | 0.053 |
| B-Heating time | 11.050 ** | 0.63** |
| C-Extraction time | 37.099 ** | 2.62** |
| AB | 0.972 | 2.63E-03 |
| AC | 0.073 | 6.61E-05 |
| BC | 3.264 ** | 0.15 |
| A^2 | 0.970 | 4.62E-03 |
| B^2 | 0.039 | 0.024 |
| C^2 | 3.739 | 0.079 |
| Lack of Fit | 1.025 | 0.06 |
| C.V. % | 2.009 | 7.51 |
| R-Squared | 0.979 | 0.923 |
| Adj R-Squared | 0.952 | 0.824 |

The coefficient of determination value for above eqation was 0.979 as shown in Table 2. It indicated that the model explained 97.9% of the variability of the oil yield during extraction process. F-value for lack of fit of this model was 1.025 with probability level of $p > 0.05$. The model coefficient of the regression equation explained that the model was highly significant (0.001). Positive linear terms of the independent parameters indicated that while increasing these parameters, oil yield was increased. Among the interaction terms, combinatio of heating time and extraction time showed significant negative effect.

Fig 1 (a), (b) and (c) represents three dimensional plots demonstrating the oil yield with respect to any two parameters when other parameter kept constant at center point. Fig 1 (a) explains the significant increase in oil yield with increasing the heating time while the oil yield increased with decreasing particle up to certain level and then became constant when extraction time was kept constant at center point (30, 40 and 50 are the BSS test sieve numbers, which indicates smaller particle sizes with increasing sieve number). From Fig 1 (b), it is clear that the oil yield increased significantly with increasing the extraction time and became constant at longer extraction time. Similar trend was observed when particle size was kept constant at center point, as shown in Fig. 1 (c).




(a) (b) (c)

**Fig 1:** Effect of extraction parameters on garden cress oil yield

### 3.2 Effect of extraction parameters on peroxide value of garden cress seed oil

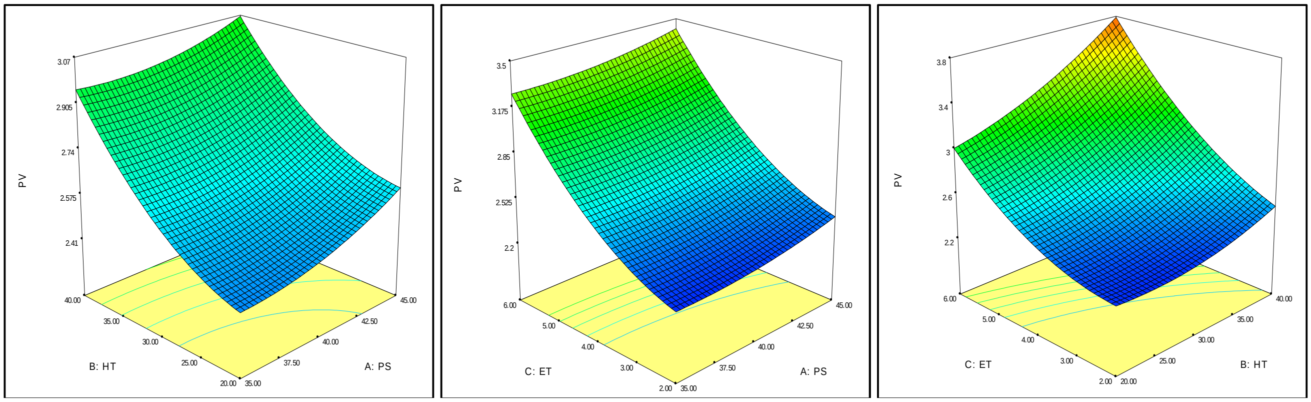
Experimental results are shown in Table 1. The peroxide value of oil extracted at different experimental conditions varied from 2.18 to 3.92 meq.kg$^{-1}$ oil for various experimental points. Peroxide value of garden cress seed oil extracted was significantly affected by the heating time and extraction time at probability level of $p<0.001$ whereas effect of particle size was non-significant. Effect of interaction terms was also non-significant. The regression equation of the model showing the net effect of independent parameters on peroxide value, in coded level of the parameters, is given as:

$$PV = 2.62 + 0.073 \times A + 0.25 \times B + 0.51 \times C - 0.018 \times A \times B + 2.88 \times 10^{-3} \times A \times C + 0.14 \times B \times C + 0.042 \times A^2 + 0.095 \times B^2 + 0.17 \times C^2 \quad \ldots (4.2)$$

Where, A, B and C are the coded values of particle size, heating time and extraction time.

Statisticle parameters of analysis are shown in Table 2. The model coefficient of the regression equation explained that the model was highly significant ($p<0.001$). Positive linear terms of the independent parameters indicated that while increasing these parameters, peroxide value was increased. Among the interaction terms, significant combinatio of heating time and extraction time showed negative effect.

Fig 2 (a), (b) and (c) represents three dimensional plots demonstrating the peroxide value with respect to any two parameters when other parameter kept constant at center point. Fig 2 (a) explains the significant increase in peroxide value with increasing the heating decreasing. Effect of heating time was highly significant as compared to particle size. At smaller particle size (higher sieve number) peroxide value was higher at all heating time. From Fig 2 (b), it is clear that the peroxide value increased significantly with increasing the extraction time. Similar trend was observed from Fig 2 (c) that, when particle size was kept constant at center point, peroxide value increased with increasing heating time as well as extraction time. This could be due to exposer to temperature.




(a) (b) (c)

**Fig 2:** Effect of extraction parameters on peroxide value (PV) of garden cress oil.

### 3.3 Optimization and validation

The optimization of process parameters was carried out using State-Ease Design Expert 10.0.0 software trial version. Simultaneous optimization of the multiple responses were carried out using numerical optimization technique of the Design-Expert software. The numerical optimization evaluates a point that maximizes the desirability function. In this case, oil yield was maximized whereas peroxide value was minimized. Solution having the maximum desirability value (0.83) was selected as optimum condition for extraction of oil from garden cress seeds. Optimum condition obtained was, extracting oil from the garden cress ground particles of size 40 oven heating pre-treatment for 30 min and extracting for a period of 4 h using soxhlet extraction. Optimized results derived from design expert software are given in Table 3. Yield obtained was 23.10 per cent and the peroxide value of the extracted oil was 2.513 meq.kg$^{-1}$, which closely related with the predicted value of 23.451 per cent and 2.576 meq.kg$^{-1}$ for yield and peroxide value respectively.

**Table 3:** Optimized condition for extraction of garden cress seed oil by Soxhlet

| Name | Goal | Lower limit | Upper Limit | Impor-tance | Predicted | Actual | Variation (%) |
|---|---|---|---|---|---|---|---|
| Particle size | is in range | 30 | 50 | 3 | 41.64 | 40 | |
| Heating time | is in range | 20 | 40 | 3 | 29.88 | 30 | |
| Extraction time | is in range | 2 | 6 | 3 | 3.76 | 4 | |
| Yield | maximize | 17.14 | 24.21 | 3 | 23.451 | 23.1 | 1.50 |
| PV | minimize | 2.18 | 3.918 | 3 | 2.576 | 2.513 | 2.45 |

### 4. Conclusion

Particle size, heating time and extraction time affected oil extraction from garden cress seed significantly in terms of the yield and PV. Polynomial relationship exists between the variables and responses under study, thus response surface quadratic models were adequate in expressing the relationship. Results showed that, oil yield was higher from the smaller size of particles and increased with increasing heating time and extraction time. Peroxide value was also higher for oil extracted from the smaller size particles and increased with increasing heating and extraction time. Yield was ranged between 17.14 to 24.21% whereas PV ranged between 2.18 to 3.92 meq.kg$^{-1}$. Optimized condition was obtained for 0.425 mm size, heating for 30 min and extraction for 4 h giving yield 23.1% and PV 2.513 meq.kg$^{-1}$.

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