



Effect of different extraction methods on yield and physico-chemical properties of garden cress (*Lepidium sativum* L.) oil

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Abstract

Effect of different extraction methods on yield and physico-chemical properties of garden cress oil was studied. Oil from the dried garden cress seeds were extracted by screw press, solvent extraction (without heating) and soxhlet extraction method. Physical properties studied were specific gravity, viscosity and colour, whereas, chemical properties analyzed were peroxide value, acid value and iodine value. Soxhlet extraction was found to give highest yield of oil. Non-significant difference was observed for specific gravity of oil extracted by all methods. Viscosity of the oil extracted with screw press was highest (53.7 mPa.s) than solvent extracted oil and soxhlet extracted oil (39.9 and 42.7 mPa.s, respectively). Oil extracted by soxhlet method was darker in colour than the solvent extracted. Peroxide value (2.53 meq.kg⁻¹) and acid value (0.74 mg KOH.g⁻¹) were higher for oil extracted with the soxhlet extraction method followed by solvent and screw press extraction method (1.27, 0.69 meq.kg⁻¹ and 0.83, 0.61 mg KOH.g⁻¹, respectively) whereas, iodine value was highest (134.2 g I₂.100g⁻¹) for solvent extraction method followed by soxhlet extraction and screw press method (131.7, 97.3 g I₂.100g⁻¹, respectively). Fatty acid profile and tocopherols content analysis of the oil extracted by soxhlet method showed that, garden cress seed oil contains 34.46 % of alfa linolenic acid and 971.6 ppm total tocopherols. Oil stored in polyvinyl terephthalate bottle for 90 days showed significant increase in peroxide (25.8meq.kg⁻¹).

Key words: Extraction, fatty acid profile, garden cress oil, storage, tocopherols, viscosity

Introduction

Garden cress (*Lepidium sativum* L.) an annual herb of Brassicaceae family it contain good amount of oil containing polyunsaturated fatty acids. Garden cress seeds contain 24% oil in which 32–34% is á linolenic acid (ALA). Garden cress oil (GCO) have very high amount of tocopherol (1699 mg.kg⁻¹), compared to other oils (Diwakar *et al.* 2010). 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 Akinoso and Oni (2012). It contains good amount of lignans (29.4 %) and antioxidants. 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.

There were several methods to extract oil from seeds viz., pressing, solvent extraction, soxhlet extraction and supercritical fluid extraction (Dasari and Goud, 2013). Some of the pretreatment prior to extraction has also been studied on different oil bearing materials. Selection of proper extraction method is important factor. Diwakar *et al.* (2010) extracted garden cress seed oil using hydraulic press, soxhlet extraction and supercritical fluid extraction. Significant difference in yield and physic-chemical properties of oil were reported. Total oil content of solvent extracted GCO was 21.5%, supercritical CO₂ extraction was 18.15% and cold expression was 12.6%. Maximum oil extraction was obtained by soxhlet method (21.5%). Therefore, the experiment was undertaken to study the effect of screw press, solvent and soxhlet extraction on the yield and physic-chemical properties of garden cress seed oil.

Material and Method

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

Extraction of oil

Oil was extracted by three extraction methods *viz.*, Screw press, Solvent extraction and soxhlet extraction. Batch of 10 kg whole seeds was used for extraction of oil by using screw press (Ghani, available in the local market). For solvent extraction and soxhlet extraction, seeds were ground using grinder. For solvent extraction, flour of the seed was soaked in the petroleum ether for 36 h with frequent shaking. A lab scale Soxhlet apparatus (Make: Borosil; Model: Hot extraction unit with 250 mL flat bottom flax) was used to extract oil from garden cress seeds. About 20 g of ground seed flour was used for the extraction with petroleum ether (Menkiti *et al.*, 2015).

Oil Yield

The extracted oil yield was expressed in percentage, which is defined as weight of oil extracted over weight of the sample taken. The percentage oil yield was calculated as follow (Menkiti *et al.*, 2015)

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

Physical properties of garden cress seed oil

Specific gravity

Specific gravity was measured as described by AOCS (1998). An empty pycnometer bottle (W_1), filled with water (W_2), and oil (W_3) weighed separately. The SG of the oil was calculated using mathematical relationship:

$$\text{Specific gravity (SG)} = \frac{W_3 - W_1}{W_2 - W_1} \quad \dots (2)$$

Colour

Colour of the fresh garden cress seed oil was observed by colour scanning machine (K-Mac Gardner Mumbai).

Viscosity

Viscosity of the oil was studied using rheometer (Make: Brookfield; Model: R/S SST coaxial rheometer). A cup and bob assembly was used for testing the viscosity. The sample of oil was filled to the mark in the cup and sheared between 0 to 400 s^{-1} . Viscosity at the end of test was reported.

Chemical properties of garden cress seed oil

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. A sample of 2.0 g oil was dissolved in 30 mL mixture of glacial acetic and chloroforms (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. 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 Kolanowski *et al.* (2004).

Acid value

The free fatty acid in oil was estimated by titrating it against KOH in presence of phenolphthalein indicator. The acid number is defined as mg of KOH required to neutralize the free fatty acids present in 1 g of sample. Acid values of garden cress seed oil was determined by titration method (AOAC, 1990).

Iodine value

The iodine value is a measure of the degree of unsaturation of fatty acids and is used to characterize oils and fats. The garden cress seed oil contains both saturated and unsaturated fatty acids. Halogens add across the double bonds of unsaturated fatty acids to form additional compounds. Iodine monochloride (ICL) is allowed to react with the fat in the dark. Iodine gets incorporated into the fatty acids chain wherever the double bond exists. The amount of iodine consumed is then determined by titrating the iodine released (after adding KI) with standard thiosulphate and comparing with a blank in which the fat is omitted. Hence, the measure of iodine absorbed by an oil or fat gives the degree of unsaturation. Iodine value of

garden cress seed oil was determined by titration method (AOAC, 1990).

Total tocopherols

Total tocopherols content of garden cress seed oil extracted by soxhlet extraction method was analyzed using HPLC. Garden cress seed oil (1.0 g) was saponified with 4 ml of 5% ethanolic pyrogallol (w/v), 1 ml KOH (100%) and boiled in a water bath for 3 min. Samples were then cooled, 30 ml distilled water was added and the mixture was extracted three times with diethyl ether. The combined extracts were washed with water to neutralize and remove fatty acid soaps. The extract was dried with anhydrous sodium sulphate and evaporated to dryness under a vacuum at 40 °C. The residue was dissolved in 1.0 ml ethanol and 4.0 ml of benzene and dried under a stream of nitrogen. The residue was dissolved in 1.0 ml of ethanol and used for identification and quantification of tocopherol by an HPLC method (Hatman and Kayden, 1979) using a Agilent 1100 Series HPLC system equipped with a fluorescence detector and Phenominix C18, column (250 × 4.60 mm, 5 µm particle size). The excitation wavelength used was 290 nm and the emission wavelength was 330 nm. An isocratic elution program was employed using a mobile phase containing methanol. The flow rate was 1.0 ml/min. Tocopherol peak was identified by comparison to the retention time of reference standards.

Fatty acid profile

Fatty acid composition of the garden cress seed oil was determined using Gas chromatography of FAMES (Fatty Acid Methyl Esters) with Flame Ionization Detector. The oil (20-25 mg) was treated with 0.55 sodium methoxide solution (5ml) in a glass

stopper flask. The content was heated to 50°C for 10-15 min and 0.1ml glacial acetic acid was added followed by water 5-10 ml. The organic phase was extracted with hexane 15-20 ml and washed with water till neutral pH. The hexane extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure to get methyl esters. An Agilent GC fitted with a DB225 polar column (30m, 0.322mm, 0.25µ) and FID was used. The temperature of the oven, injectors, detector blocks were maintained at 210, 230, 250°C respectively. Nitrogen gas was used as the carrier gas. Peaks were identified by comparison with relative retention times of the standard FAMES. Concentration of each fatty acid was recorded by normalization of peak area using GC post run analysis software, manual integration and reported as percent of the particular fatty acid.

Storage stability

Garden cress seed oil extracted with soxhlet extraction method was stored in transparent polyvinyl terephthalate bottle for 90 days at room temperature. Peroxide value of stored oil was accessed at an interval of 30 days.

Results and Discussion

Yield and physical properties

Effect of extraction methods on the physical and chemical properties of the extracted oil was studied and results are given in Table 1 and 2. Yield of the oil extracted with different methods was found significantly different. Maximum yield was obtained for with Soxhlet extraction. The total oil content in GC seeds was is comparatively less than other edible oil seeds such as mustard (25–40%), rapeseed (40–45%) and camelina or false flax (40–45%) of

Table 1: Effect of extraction methods on yield and physical properties of garden cress oil

| Properties | Screw press | Solvent extraction | Soxhlet extraction | C.D. @ 5% | SE | C.V. | |
|-------------------|-------------|--------------------|--------------------|------------|-------|-------|-------|
| Yield (%) | 9.33±0.33 | 14.73±0.43 | 24.76±0.55 | 0.621 | 0.282 | 2.741 | |
| Viscosity (mPa.s) | 53.67±1.07 | 39.94±0.75 | 42.67±0.67 | 1.183 | 0.537 | 1.868 | |
| Specific gravity | 0.91±0.04 | 0.90±0.03 | 0.90±0.02 | NS | 0.021 | 3.644 | |
| Colour | L* | 28.92±0.53 | 27.47±0.40 | 29.59±0.41 | 0.628 | 0.285 | 1.574 |
| | a* | 2.47±0.14 | 1.78±0.08 | 3.59±0.33 | 0.292 | 0.133 | 8.029 |
| | b* | 8.97±0.76 | 5.84±0.37 | 10.41±0.57 | 0.822 | 0.373 | 7.016 |

Table 2: Effect of extraction methods on chemical properties of garden cress oil

| Properties | Screw press | Solvent extraction | Soxhlet extraction | C.D. @ 5% | SE | C.V. |
|----------------|-------------|--------------------|--------------------|-----------|-------|-------|
| Peroxide value | 0.83±0.04 | 1.27±0.04 | 2.53±0.05 | 0.060 | 0.027 | 2.784 |
| Acid value | 0.61±0.02 | 0.69±0.03 | 0.74±0.02 | 0.033 | 0.015 | 3.439 |
| Iodine value | 97.27±1.95 | 134.20±2.5 | 131.70±2.0 | 3.055 | 1.387 | 1.811 |

Cruciferae family (Budin *et al.*, 1995). Specific gravity of oil extracted with different extraction methods was non-significant.

Colour of the oil extracted with different method is significantly different. Solvent extracted oil showed light yellowish colour followed by cold press extracted whereas, soxhlet extracted oil was dark yellow in colour. Diwakar *et al.* (2010) also observed that, the soxhlet extracted oil had dark yellow colour.

Chemical properties

Peroxide value (PV) of the soxhlet extracted oil was higher than solvent extracted and screw press oil. The high PV in soxhlet extracted oil could be due to the exposure of the oil to high temperature during extraction (Diwakar *et al.*, 2010). Similarly FFA value of soxhlet extracted oil was high. The iodine value (IV) of solvent extracted oil was relatively higher than screw pressed and soxhlet extracted GCO.

Total tocopherols

The garden cress seed oil showed 971.6 ppm of total tocopherols. The result showed that garden cress seed oil is rich source of natural antioxidant tocopherol which can help to protect the oil from oxidation. Gunstone (2004) reported the high amount of tocopherols (877 to 1077 ppm) in garden cress seed oil and Moser (2009) reported 1422 ppm tocopherols in garden cress seed oil.

Fatty acid profile

Fatty acid profile of garden cress seed oil extracted by soxhlet method and petroleum ether as solvent is presented in Table 3. Alpha linolenic acid was found to be highest among all fatty acids (34.5 %) followed by oleic acid (18.6 %) and linoleic acid (12.9 %). Total saturated, monounsaturated and polyunsaturated fatty acid was found to be 16.78,

Table 3: Fatty acid profile of fresh garden cress seed oil

| Component name | Amount (%) |
|-----------------------------------|------------|
| Myristic acid | 0.11 |
| Palmitic acid | 9.49 |
| Palmitoleic acid | 0.17 |
| Heptadecanoic acid | 0.05 |
| Stearic acid | 2.92 |
| Oleic acid | 18.57 |
| Linoleic acid | 12.90 |
| Arachidic acid | 3.10 |
| Gamma linolenic acid | 0.38 |
| Eicosenoic acid | 11.07 |
| Alpha linolenic acid | 34.46 |
| cis 11,14-Ecosadienoic acid | 0.71 |
| Behenic acid | 0.70 |
| Erucic acid | 4.33 |
| cis-11,14,17-Eicodsatrienoic acid | 0.62 |
| Lignoceric acid | 0.41 |
| Total Area | 100 |
| Σ Saturated (%) | 16.78 |
| Σ Monounsaturated (%) | 30.43 |
| Σ Polyunsaturated (%) | 52.78 |
| n6/n3 | 0.33 |

34.1 and 49.1 % respectively giving the ratio of

SFA:MUFA:PUFA 1:2.03:2.92. Ratio of n-6 to n-3 was found to be 0.37. These results were in conformity with Diwakar *et al.* (2010) and Umesha *et al.* (2013).

Storage stability

Steady increase in the peroxide value of the garden cress seed oil during 90 days storage was observed. Since the garden cress seed oil contains more amount of unsaturated fatty acid, it is susceptible to oxidation. Similar results were reported by Diwakar *et al.* (2010).

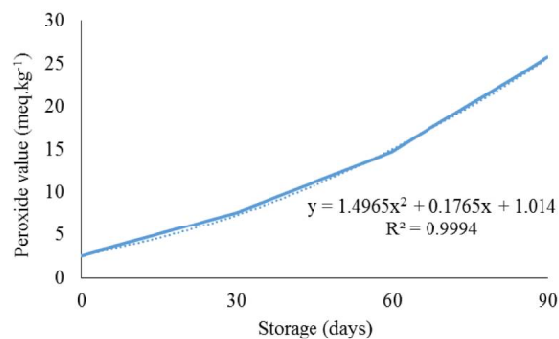


Fig.1: Effect of storage on peroxide value (PV) and acid value (AV) of garden cress oil

Conclusions

Soxhlet extraction method was found to give maximum yield (24.76 %) of the oil. Soxhlet extracted oil was dark brown in colour and viscosity of (42.67±0.67 mPa.s) Oil was found to contain better amount of unsaturated fatty acid. Alfa linolenic acid and total tocopherols content of the oil was 34.46 % and 971.6 ppm, respectively. Oil was also found to be unstable at room temperature.

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