Original Research Article

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Physicochemical characteristics and thermal stability of perilla seed oil of Indian origin

Abstract

There is increasing interest of food scientists in finding new alternatives of PUFA rich edible oil. Perilla seed oil (CPSO), an underutilized oilseed, can be used as an edible oil source. Oil extracted by the cold-pressed method from perilla seeds gives a yield of 36.50%. This study reports the physicochemical properties, oxidative and thermal stability of cold-pressed perilla seed oil. The viscosity, specific gravity, refractive index, and smoke point of CPSO were 28 m.Pa.s, 0.92, 1.43, and 241 ℃, respectively. The peroxide, acid, iodine, saponification value and unsaponifies matter of CPSO were 4.81 meq O2/kg oil, 1.61 g KOH/kg oil, 132 g KOH/kg oil, 180 g I2/kg oil and 0.64%, respectively. It consist of high α–-linolenic acid (55.80% of total oil) followed by oleic acid (20.54%). The extracted oil is analyzed for its thermal stability (p~~P~~eroxide value, f~~F~~ree Fatty a~~A~~cids, p-a~~A~~nisidine value, t~~T~~otox value and t~~T~~otal polar compounds) and storage stability of 120 days in two different storage condition (r~~R~~efrigerated and r~~R~~oom temperature). ~~d~~Despite of having high nutritional benefits, the oil stability i~~I~~ndex (0.50 h) of the perilla seed oil is low, limiting its utilization as frying oil. Therefore, perilla seed oil requires process optimization to increase its stability during heating.

Keywords- α-— linolenic fatty acid, o~~O~~xidative stability, p~~P~~erilla seed oil, t~~T~~hermal stability

1. INTRODUCTION

Nowadays, there is a significant focus on utilizing plant oils due to their healthy bioactive and polyunsaturated fatty acid constituents (PUFAs). The nutritional quality of fats and oils depends on the type of fatty acid, degree of unsaturation, and arrangement of fatty acid in triacylglycerol structure (Dorni *et al*.,2018). Several researchers proved a direct association of type and amount of oil consumption with cardiovascular diseases CVDs. Therefore, the focus of nutrition research has shifted to replacing saturated and trans fats with healthy PUFA rich oils (Ayerza, 2010). Most commonly consumed oils are rich in saturated and monounsaturated fatty acids but deficient in PUFA. α -Linolenic acid and linoleic acids are important types of PUFA and are classified as essential fatty acids. Numerous studies have proven the role of these essential fatty acids in normal growth and development and in preventing various diseases such as cardiovascular, dyslipidemia, hypertension, diabetes mellitus, obesity, inflammatory diseases (Heshmati, 2021). Fish oil is the richest animal source of essential fatty acid. However, it is not suitable for the vegetarian population. Thus, scientists and food manufacturers are interested in plant-based PUFA rich oils with the increasing demand for essential fatty acids.

Perilla plant (Perilla frutescence) is a member of the Lamiaceae family and is commonly called perilla. This annual crop is native to India, China, Korea and Japan.

The valuable parts of perilla plant are its seeds and leaves.The major constituents of perilla seeds include 42-45~~-~~% of oil and 25~~-~~% of protein (Sargi *et al*., 2013). Perilla seed oil is a golden yellow clear and transparent liquid with an pungent odor. The oil consists of 78% unsaturated fatty acids, 60% of which is α - linolenic acid. Other unsaturated fatty acids are o~~O~~leic acid and linoleic acid. This oil also contains about 6.7 -7% saturated fatty acids (Ciftci *et al*.,2012).

Numerous studies have shown that the consumption of p~~P~~erilla seed oil associated with lower level of blood lipids and serum cholesterol. In o~~u~~ur previous review paper, we have compiled the pharmacological properties of perilla oil such as anti-asthmatic, antidiabetic, anti-diprrssent, anti cancer, anti-microbial, anti oxidant, cardioprotective and neuroprotective (Dhyani *et al*., 2019). Perilla seed oil has been recently used as a functional food due to its good health effects in various countries such as China, Japan, Korea and U.S.A. Japanese and chinease manufacturers have added perilla seed oil to various products for the production of new health food (Zhu & He, 2018). There are numerous researches on the nutritional composition and physicochemical characteristics of p~~P~~erilla seed oil varieties from different countries, e.g., India (Joshi et al., 2015), Iran (Ghaleshahi et al 2019)), China (Zhao et al., 2021), Japan (Bondioli et al.2020), Korea (Kim et al., 2019), Bangladesh (Mojamdar et al., 2021) considering its scope as an alternative PUFA n-3 rich vegetable oil source. The studies, as mentioned earlier, only reported about the nutritional and physicochemical properties of perilla seed oil. No previous study has been conducted so far on the oxidative and thermal stability of perilla seed oil, which are important parameters for the processing and utilization of oil. Therefore, this study aimed to investigate physicochemical characteristics, fatty acid profile, oxidative and thermal characteristics of perilla seed oil extracted by the cold-pressed method (CPSO).

2. MATERIALS AND METHODS

2.1.Materials

The perilla seeds were procured from the Forest Research Institute (F.R.I.), Dehradun, India. All reagents used were E. Merck or Sigma Aldrich.

2.2.Oil extraction - Perilla oil from perilla seed was extracted by cold-pressed method (Screw Press Model 85 mm) in two batch (1 kg in one batch) at 10 MPa for 20 min from a commercial mill (Mohan oil mill) in New Delhi, India. The extracted oil was filtered through muslin cloth and Whatman no.2 filter paper to remove the impurities and stored in a sealed dark amber bottle until further use (Mazaheri *et al*., 2019).

2.3.Determination of physical properties

Color was measured using the Lovibond tintometer (Model F) method; the color intensity was measured in 1" cell in the transmittance mode of white glass filter, yellow glass filter, and red glass color and expressed as 5R+Y Lovibond units, the method given by AOCS Method no. Cc 13e-92 (AOCS, 2000). Moisture content of oil was as a loss of weight from the intial weight on keeping the oil sample in hot air oven for 1 hour at 105 ± 1–°C (IS, 2010). Viscosity was measured using Brookfield viscometer at constant temperature (25 °C) and shear rate. Specific gravity was measured using a 10 ml pycnometer at 20ºC. Refractive index was determined by Abbe refractometer, the temperature of the refractometer was maintained at 25 ± 0.1ºC using a thermostatically controlled water bath. Smoke point of oil was measured according to the AOCS method, Cc 9a-48. First, 150 ml of oil was taken in a beaker and heated until it started producing smoke; a digital thermometer was used to measured this temperature.

2.4.Determination of chemical properties

The peroxide value (PV), acid value (AV), iodine value (IV), saponification value (SV) and unsaponifiable matter (USM) were measured by the standard methods of American Oil Chemist's Society (AOCS, 2000).

2.5.Determination of oxidative stability index (Rancimat)

Oxidative stability of CPSO can be achieved by conducting an accelerated oxidation test and measured by Induction period (IP, hour) using the method given by Anwar *et al*., 2007. Oxidation induction times were measured by a Rancimat model 743 using 3 g of oil, heated at 120 ℃ with 20L/h airflow. At the end of the process, volatile and secondary products are formed, absorbed by measuring a vessel containing deionized water, and then measured electrical conductivity.

2.6.Fatty Acid Composition

Fatty acid composition by gas chromatography as per method given by Chaudhary et al., 2015. Fatty acids of the oil sample were converted to fatty acid methyl esters (FAMEs) using the IUPAC standard method (IUPAC 1987). FAMEs were analyzed with a gas chromatograph (Agilent 7890 B), equipped with a flame ionization detector (FID) and FP 2560 cephalic column (100 mm X 0.25 µm X 0.2 µm) coated with CP-SIL 88 as the stationary phase. Temperature of the oven was ~~at~~ 200℃. The injector and FID temperature was 250℃. The FAMEs were expressed as relative area percentages.

~~Calculated Oxidisability (COX) value~~ The calculated oxidisability (COX) value of the Perilla seed oil was calculated by the formula given by Fatemi and Hammond~~,~~ (1980).

~~Cox~~ COX = [1(C18:1%) +10.3 (C18:2%) +21.6 (C18:3%)]/100

2.7.Evaluation of thermal stability test

Heating Procedure: The heating procedure was performed according to the method given by Anwar *et al*. (2007) with slight modifications in time duration. In this method, fresh oils were heated at 180~~-~~°C in an electrical fryer with temperature control (Inalsa Professional 2 fryer, 18/8 steel, 2 L, digital timer). Successive heating was conducted for 4 days which took 6 hours of heating each day, giving total heating ~~for~~ of 24 hours. 100 mL of oil was taken at the end of each day and stored in amber glass bottles at 4°C until used for analysis. The thermo-oxidative degradation level in oils was assessed by measuring changes in color, peroxide value, free fatty acid, p-anisidine value, totox value, oxidative stability (Rancimat), and total polar components (TPC) after each cycle. Methodology of color, peroxide value, free fatty acid had discussed in section 2.3.

p-~~-~~Anisidine value (p-AV): The p-AV measures secondary oxidation products (aldehyde content) in the oil; it was determined according to the method given by the AOCS method number Cd 18-90.

Totox value: The TOTOX (i.e., total oxidation products) value was introduced to evaluate the stability of oils. The p-AV is often used in combination with PV to calculate the TOTOX value given formula as TV = 2PV + p-AV. The reason for the multiplication of PV by a factor 2 is that the PV has a additional obvious consequence on the stability of oil than the p–AV (Nayak *et al*., 2016).

Total polar compounds: The TPC of frying oils were determined by the Column Chromatography technique, as per Official International Union of Pure and Applied Chemistry (IUPAC) method. It is based on separating polar compounds from non-polar components and is given by Arslan et al. (2017). The percentage of TPCs was calculated using the equation:

$$\text{Total Polar compounds (\%)} = \frac{weight\ of\ oil\ sample - weight\ of\ non-polar\ fraction}{weight\ of\ oil\ samples} \times 100$$

2.8.Evaluation of oxidative stability

The cold pressed perilla seed oil was stored in amber bottles in two storage conditions, at room temperature 25 ± 2~~-~~°C and at 4~~-~~°C for 120 days. T~~t~~he oxidative stability of oil was evaluated by measuring peroxide and acid value after every 30 days interval.

3. RESULT AND DISCUSSION

This study summarises the physicochemical properties of perilla seed oil of Indian origin obtained by the cold-pressed method. The results are compared with other variety of perilla seed oil from different countries and also with the other PUFA rich oils such as chia seed oil and linseed oil. This paper also reports the oxidative and thermal stability of cold pressed perilla seed oil (CPSO) and co~~a~~mpared it with commercial refined palm olein (RPO)~~-~~. Palm olein is a very popular and common vegetable oil in india, which is used in many cooking application, hence the cold pressed perilla seed oil (CPSO) was co~~a~~mpared with commercialy available RPO for thermal and oxidative study .

3.1.Sample Procurement

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Perilla seeds were procured from the forest research institute (Dehradun. Uttarakhand, India). Seeds were cleaned to removed impurities and stored in dark at room temperature untill further use.

3.2.Oil extraction and yield

Perilla oil seed contains a substantial amount of oil, about 38-59%. Temperature and pressure employed during the extraction process govern the oil extraction yield. In present study, perilla oil was extracted using the cold-pressed method and contributed a yield of 36.50~~-~~%, while a recent study reported a similar value (34.40%) by cold pressing method (Zhao et al., 2021). Oil yield is a significant characteristic of oil seeds and pivot on different varieties and regions in which they are grown. Various studies have reported the different oil yield of perilla seed by employing different extraction techniques such as solvent extraction (39.61–%), mechanical expeller (38.4%), aqueous enzymatic extraction (31.28%) (Zhao et al., 2021; Li et al., 2015; Li et al., 2017). Several factors responsible for the variation in the oil yield during extraction are moisture content of seeds, solvent type, time, temperature and type of extraction (Li *et al.*, 2015). Furthermore, various other pretreatment of perilla seeds before extraction were tested to improve the oil yield, such as roasting of seeds, use of superheated steam treatment, use of freeze-thaw pretreatment, use of compressed fluids (CO_2 and L.P.G) (Zhao *et al*., 2012; Lee *et al*., 2021; Lee et al., 2021; Scapin et al., 2017).

3.3.Physical Parameters

Physical parameters of Perilla oil are listed in table 1. Color is an important physical parameter for consumer acceptance; perilla seed oil had a golden yellow appearance and its darkness is attributed to the presence of high content of polyphenols. The Lovibond Tintometer reading in the transmittance mode for perilla seed oil was in 35 Lovibond units. The value is 25 units lesser then the value reported by Mojumdar and colleagues in perilla seed oil by solvent extraction i.e., 60 Lovibond units. The lesser color value in cold pressed oil attributed to the fact that some of the pigments extracted along with the oil (Mojumdar et al., 2021). Moisture content is an important quality parameter for fats and oils; it is one of the deciding factor in the storage condition and processesing of oil-. It was reported 0.09-% in perilla seed oil, which is within the acceptable range i.e. 0.05-0.30 (Choo et al.,2007), high moisture content in oils increases the rate of hydrolytic breakdown leading to the formation of free fatty acid and rancidity. The viscosity of the oil indicated its stability; CPSO showed a

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viscosity of 23 m.Pa.s. S~~s~~pecific gravity and refractive index are important factors for determining quality of any vegetable oils.~~,~~ T~~t~~hey both parameters are essential for the optimaztion of any processesing technique. The specific gravity of CPSO was reported to 0.92 at 25℃, same value was reported by Mojumdar et al., (2021). Specific gravity is directly proportional to chain length but inversely related to degree of unsaturation. The specific gravity of CPSO was found to be 0.92 at 25℃,   The refractive index is a suitable and low cost method to measure the authenticity of oil; it measure the degree of unsaturation and presence of uncommon components in the oil. It increases with the increase in chain length and double bonds. The RI of perilla oil was found to be 1.48; T~~t~~he value is similar to the value reported by Scapin et al (2017), and value is within the accepted range of vegetable oils. However,~~.~~ chia seeds oil and Flaxseed oil also reported the same value (Timilsena et al., 2017. The smoke point is the temperature at which oil starts producing continuous smoke during heating (Hashempour-Baltork *et al*., 2016). In the present study, the smoke point of CPSO is 241℃, and the value falls in the range of the literature value reported by Xu *et al*. (2013), the CPSO is reported to fulfill the recommended value of frying oils, i.e., above 200℃.

3.4.Chemical Properties

Chemical properties provide information regarding the stability and freshness of fats oils. Peroxide value and acid value measures the hydrolytic and oxidative rancidity of vegetable oils. The peroxide value of Indian origin CPSO was found to be 4.81 ± 0.40, which was higher than the perilla oil of Iranian origin (Table-2). The peroxide value of CPSO falls within the acceptable limit (15 meq O2/kg~~)~~) given by FSSAI. As per the Codex standards, the acid value of cold-pressed oils should not be more than

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4.0 mg KOH/g fat or oil, and the acid value of CPSO is within the standard limit. This suggests that cold-pressed CPSO is suitable for edible purposes.

Iodine value is a measure of the degree of unsaturation. Chia seeds oil and linseed oil were reported to have 54% and 64.3% more degree of unsaturation, respectively, than CPSO oil (Timilsena *et al*., 2017; Badhe, 2013). Iranian Perilla oil has been reported to have a higher iodine value as compared to Indian CPSO. This might be due to the higher PUFA content found in the Iranian perilla seed oil (Ghaleshahi et al 2019). The iodine value and saponification value of Perilla oil reported by Joshi and colleagues in their study and it was 2.93% and 2.56% lower than the result obtained in this study (Joshi *et al*., 2015), this variation may be due to the different methods employed for oil extraction. The saponification value of perilla seed oil reported in this study is lesser than the commonly consumed oils such as soybean oil (189-195), sunflower oil (190-196) and vigin olive oil (190-195); thus it indicates the prese~~e~~nce of high molecular weight fatty acids present in the oil. The unsaponified content (%) in CPSO was in agreement with the previous findings (Bondioli et al., 2021). The high unsaponified value indicates the presence of lignans, crude fibre, protein and minerals.

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Refining of edible oils is important to remove any undesirable elements (trace metals, pigments, waxes, and gums) present in crude oil. They might cause damage to human health and also act as a prooxidant during the storage of edible oil. Refining of perilla oil had reported a 72.5% decrease in peroxide and 82~~-~~% in a~~A~~cid values while a significant increase in saponification value (Pan et al 2019).

3.5.Oil Stability Index (OSI)

Oxidation stability is one of the crucial parameters for measuring the deteriortation in edible oils due to oxidation. Oxidative stability index (OSI) is defined as the time required for decomposition of primary oxidation products produced by oxidation in the oil; it also indicates the shelf life of the oil and measured through rancimat test. The rancimat test is a technique in which oil is exposed to higher temperature in the presence of excess air and temperature. Oils containing a high amount of unsaturated fatty acids are more prone to oxidation. The OSI of Perilla seed oil (0.50 hours) were less than those published for other commonly consumed vegetable oils (Reference ?) probably due to its high PUFA content. Various literature has reported a low oxidation stability index of perilla seed oil (≤ 1 h) (Torri *et al.*, 2019; Bondioli et al., 2020); however, Galeshahi and collegues reported high OSI of perilla seed oil (1.42 h) than basil seed oil and flaxseed oil (Galeshahi et al., 2019) . Inspite of having high PUFA content, Perilla seed oil have more OSI than chia seed oil (0.43 h) and flaxseed oil (0.37 h) (Jung *et al.*, 2021). This contradiction could be associated with the higher amounts of tocols and antioxidants in perilla seed oil. This suggests that perilla seed oil is prone to oxidative rancidity and requires innovative strategies for improving its stability.

3.6.Fatty acid composition

Table -3 summarizes the fatty acid profile of Perilla seed oil, and it was observed that amount of α-Linolenic acid (C18:3) is more in CPSO. Thus, α -Linolenic a~~A~~cid is the dominant fatty acid followed by oleic acid and linoleic acid in CPSO. Kim and colleagues reported similar composition of fatty acid of perilla seed (Kim *et al.*, 2019). The oil extracted from the Indian Perilla variety has the lowest PUFA content among two other varieties (Iranian & South Korean). Pan and colleges showed the fatty acids composition of perilla seed oil after every refining step, and reported no significant difference among and concluded that the refining process did not alter the fatty acid composition of CPSO them (Pan *et al.*, 2019).

3.7.Thermal Stability of Cold Pressed Perilla Seed Oil

Thermal stability is an essential measure in quantifying the heat-induced changes in fats and oils. Measuring the quality parameters (color, peroxide value, fatty acid %, p-anisidine value, totox value, and OSI) are the essential criteria to judge the thermal stability of the oil. The changes in color,~~.~~ peroxide value, free fatty acid, p- anisidine value, totox value and total polar compounds of CPSO in respond to four heating cycle (180°C) for total 24 hours are reported in table 4. Color is the initial parameter to judge frying oil quality; however, color darkness increases with heating time and temperature. For example, CPSO showed a color value of 34.83, and it gained 99.37 units to a value of 134.20 after the 4th day of heating, showing more than 4 fold increased while RPO showed 3 fold increased in color intensity. This may be due to the prolonged accumulation of non-volatile compounds in the heated oil. Generation of f~~F~~ree ~~F~~fatty acid is the key indicator in assessing oil stability as free fatty acids are more prone to oxidation than neutral fatty acids present in the oil. At the end of the heating cycle, i~~I~~t was observed that the amount of free fatty acid in CPSO is increased by 200% while RPO showed a FFA increase of 273 % after heating (Table 4).

Peroxide value and p-anisidine value helps in the determination of primary and secondary oxidation products of fats and oils. As the duration of heating increased, corresponding increased was obsevered in CPSO and RPO. Whereas, at a certain point the value did not show a significant increased. This is due to the formation of secondary oxidation products. The p-anisdine value of fresh CPSO was 2.56 units higher than the Iranian variety of Perilla oil. This difference might be due to the presence of the high amount of antioxidant in Iranian perilla oil which help in preventing oxidation (Ghaleshahi et al., 2019). However, the p- anisidine value of CPSO was slightly higher than the accepted p-anisidine value for good quality oil i.e.,

< 2 (Subramanian et al. 2000). This study reported that the p-anisidine, peroxide, and free fatty acids show increment with the increase in heating time. For example, after heating for 24 hours at 180℃, p-anisidine value of CPSO and RPO shows a spontaneous increase from 2.56 to 25.64 and from 0.41 to 11.22, respectively. Estimation of Totox value helps quantify the combined effect of primary and secondary oxidation products generated during oil heating. It was observed that the totox value increases significantly during the entire heating process of CPSO and RPO. The heating caused a significant and rapid increase in the TPC value of oil (Table 4). At the end of the heating cycle (4th day), the TPC level of CPSO and RPO were 34.63% and 10.87%, respectively. The recommended minimum standard limit for TPC varies from country to country and mainly ranges from 23-29% (Stier, 2013); the TPC value of CPSO were within the acceptable range till 12 hours of heating.

The OSI of the oil sample is inversely proportional to the degree of unsaturation. Therefore, the OSI of oil decreases with the heating due to a higher rate of deterioration during heating; in CPSO, OSI decrease at the rate of 93%; while the decrease in RPO was 47% (Figure 1). This higher rate of decrement of OSI in CPSO is due to the high content of unsaturated fatty acids in perilla seed oil.

3.8.Oxidative stability of cold pressed perilla seed oil

The storage stability of cold pressed perilla seed oil stored at different conditions (Room temperature 25 ± 2~~–~~℃; Refrigerated temperature (4~~–~~℃) was assessed by measuring PV and AV for 120 days at a frequent interval of 30 days and presented in figure 2 and 3. A high rate of increment in PV (233%) and AV (513 %) was reported in oil stored in room temperature. However, in ref~~e~~riger~~t~~ated condition the PV (128 %) and AV (210 %) was maintained at low levels compared to room temperature. In refrigerated condition, percent increase of PV and AV is 2 times lower than the room temperature. Thus, it indicates referigertaed condition provide more shelf life to CPSO than room temperature.

4. CONCLUSION

Perilla frutescence oilseeds is one of the novel seed traditionally used in India's Himalayan region and north-eastern area. This seed has started gaining recognition by scientists and the food industry due to its high oil yield and PUFA content. The physicochemical analysis reported in this study reports that this oil could be a good alternative to other PUFA rich oil. However, the oil stability index (OSI) value depicts that prolonged storage and heating or frying decreases stability. Therefore, further detailed studies need to be carried out to optimize the extraction procedure and storage conditions to increase its stability to mark profitability and use for industrial purposes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Physical parameters of cold pressed perilla seed oil (CPSO)

Physical parameters	**Results**
Color (5R+Y Lovibond units)	34.83 ± 0.76
Viscosity (m.Pa.s)	28 ± 0.16
Specific gravity	0.92 ± 0.01
Refractive index	1.47 ± 0.00
Smoke point (°C)	241 ± 3.20

Results are expressed as mean ± standard deviation of three measurements

Table 2: Chemical parameters of cold pressed perilla seed oil (CPSO)

Chemical parameters	**Perilla Seed oil** (Present Study)	Iran origin (Ghaleshahi et al 2019)	Japan Origin (Bondioli et al., 2020)	Bangladesh origin (Mojumdar et al.,2021)	China origin (Zhao et al.,2021)
Peroxide Value (meq O_2/kg)	4.81 ± 0.40	0.35	5.4	9.09	1.60
Acid Value (mg KOH/g)	1.61 ± 0.02	2.12	0.74	-	0.57
Iodine Value (g/100g)	132.00 ± 1.25	195	-	187	197.6
Saponification value (mg KOH/g)	180.00 ± 1.25	182	-	175	189.6
Unsaponified matter (%)	0.64 ± 0.25	1.49	0.60	0.20	0.57