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Palm olein and perilla seed oil blends for the improvement of nutritional and thermal stability

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SUMMARY: This study aimed to develop a healthy blended oil with a balanced fatty acid ratio, as well as high thermal and oxidative stability. The blending of highly saturated Palm olein (PO) with polyunsaturated fatty acid-rich (PUFA) Perilla seed oil (PeO) in two different proportions, 70:30 (B1) and 80:20 (B2) v/v was studied. The physicochemical parameters, fatty acid composition, and oxidative stability of cold-pressed perilla seed oil (PeO), palm olein, and their blends were analyzed. The blends presented higher oxidative stability (6.5 h) with enhanced α-linolenic acid content (18%) than pure oils. The fatty acid ratio in both blend (B1- 1:1.4:1 and B2- 1.5:1.5:1) was found close to the WHO recommended ratio i.e., 1:1-5:1. The evaluation of the thermal stability of the blended oils revealed that PeO oxidized quickly during heating (Peroxide value-15.16 meq O_2/kg); whereas thermal stability improved with blending (Peroxide value: B1-7.92 and B2- 7.69 meq O_2/kg).

***KEYWORDS*:** *α-Linolenic acid; Blending; Perilla seed oil; Thermal stability.*

RESUMEN: ***Mezclas de aceites de semilla de perilla y oleína de palma para mejorar la estabilidad nutricional y térmica.*** Este estudio tuvo como objetivo desarrollar una mezcla de aceites saludables con una proporción equilibrada de ácidos grasos, alta estabilidad térmica y oxidativa. Se ha estudiado la mezcla de oleína de palma (PO) altamente saturada con aceite de semilla de perilla (PeO) rico en ácidos grasos poliinsaturados (PUFA) en dos proporciones diferentes, 70:30 (B1) y 80:20 (B2) v/v. Se analizaron los parámetros fisicoquímicos, la composición de ácidos grasos y la estabilidad oxidativa del aceite de semilla de perilla prensado en frío (PeO), la oleína de palma y sus mezclas. Las mezclas presentaron mayor estabilidad oxidativa (6,5 h) con mayor contenido de ácido α-linolénico (18%) que los aceites puros. La proporción de ácidos grasos de ambas mezclas (B1- 1:1,4:1 y B2- 1,5:1,5:1) se encontró cerca de la proporción recomendada por la OMS, es decir, 1:1-5:1. La evaluación de la estabilidad térmica de los aceites mezclados reveló que el PeO se oxidaba rápidamente durante el calentamiento (Valor de peróxido: 15,16 meq O_2/kg), mientras que la estabilidad térmica mejoraba con la mezcla (Valor de peróxido: B1-7,92 y B2- 7,69 meq O_2/kg).

PALABRAS CLAVE: *Aceite de semilla de perilla; Ácido α-linolénico; Estabilidad térmica; Mezcla.*

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1. INTRODUCTION

Fats and oils are essential constituents of the daily diet and play a crucial role as a cooking medium. Due to the significant prevalence of Non-communicable diseases, people's focus is now shifted toward consuming healthy cooking oil. The nutritional quality of fats and oils is dependent on the type of fatty acid, degree of unsaturation, and arrangement of fatty acid in the triacylglycerol structure (Dorni *et al.*, 2018). The WHO has given a particular recommendation for healthy oil, i.e., the ratio of saturated, mono, and polyunsaturated fats should be 1:1.5:1; the balance of essential fatty acid, linoleic acid (n-6), and α-linolenic acid (n-3) should be 5-10:1 or less, with the presence of antioxidants (WHO, 2008). Most commonly-consumed oils are rich in saturated and monounsaturated fatty acids and deficient in polyunsaturated fatty acids, mainly omega-3 fatty acids (α-linolenic fatty acid). Omega-3 fatty acids are essential fatty acids, and their consumption prevents various non-communicable diseases such as coronary artery disease, diabetes, and cancer. As per the WHO reports, almost 61% of deaths globally are solely due to NCDs. But the major limitation of omega-3-rich oils is that they cannot be used for culinary purposes because they are highly susceptible to oxidation due to the presence of double bonds.

The vegetarian source of essential fatty acid (omega-3) is very limited. In recent years, the need for unconventional oils has increased due to their bioactive components and essential fatty acid composition (Al- Farga *et al*., 2020). Perilla frutescens is an underutilized herb of the mint family (Lamiaceae), mainly found in Asian countries like China, Japan, Thailand, and India. It has been listed as one of the edible medicinal plants by the Ministry of Health of the People's Republic of China (Yu *et al.*, 2017; Dhyani *et al*., 2019). This plant is receiving attention in western countries due to its medicinal importance. Perilla seeds contain 35-51% oil and play a significant role in the human diet and health due to their fatty acid composition. The foremost vital characteristic of PeO is that is one of the rich plant sources of omega-3 fatty acid. However, PeO, a rich source of n-3 PUFA, cannot be utilized for culinary purposes due to its high susceptibility to oxidation. Our previous study showed the poor oxidative stability of PeO (<1 h), measured by the Rancimat method (Dhyani *et al*., 2021).

Nowadays, the oil industry's primary challenge is finding the most economically feasible nutritional oil with desirable functionality and improved oxidative and thermal stability. Unfortunately, a single oil could not provide all these quality characteristics. The blending of oil is the most feasible and economical way to modify oil's physicochemical and nutritional properties and improve its oxidative stability or make use of the properties of different oils in a single oil; blending increases bioactive and antioxidant composition. Nowadays, oil blending has become common in various countries (Hashempour-Baltork, *et al*., 2016). Various studies have proven the nutritional and physicochemical benefits of oil blending. The cardiologist society of India defined "blending as a positive approach toward the enhancement of oxidative and thermal stability of oils" Also, it is recommended that replacing commonly-consumed cooking oil with blended oil is a feasible alternative to fulfill dietary recommendations and to reduce the risk of coronary heart diseases (CHD) (Manchanda and Passi, 2016).

Palm olein is mainly utilized as a frying oil due to its high smoke point and resistance to thermal degradation. It is mainly used to prepare fried food due to its high oxidative stability. Palm olein is a saturated fat but deficient in essential fatty acids (α-linolenic acid). Perilla seed oil is a major source of essential fatty acids (linoleic acid and α-linolenic acid). By blending PO and PeO, we could improve fatty acids and the n-6:n-3 ratio in the blend without compromising oxidative stability, thermal stability and long term storage stability. Various studies have proven that blending PUFA-rich oils with palm olein improved its nutritional as well as functional properties (Dhyani *et al*., 2022; Joshi *et al*., 2021; Siddique *et al*., 2010). To the best of our knowledge, a detailed study on the effect of temperature (thermal stability) and time (storage stability) on the stability of perilla oil, palm olein and their blends has not been reported to date. Thus, the present study investigates the effects of blending n-3 PUFA-rich PeO with PO on the Physico-chemical properties, fatty acid composition, oxidative stability, thermal stability, and storage stability of the blend. This study may provide a new oil alternative with balanced fatty acid composition and improved oxidative and thermal stability at an affordable price.

2. MATERIALS AND METHODS

2.1. Materials

Perilla seeds were procured from the Forest Research Institute (FRI), Dehradun, India. Refined cooking oil, such as Palm olein was procured from the local market (Delhi, India). All reagents and chemicals were acquired from E. Merck or Sigma Aldrich.

2.2. Methods

2.2.1. Oil extraction and blends formulation

Perilla seed oil was extracted according to the cold-pressed method (Screw-press Model, 40kg, capacity 8-10 kg/hour, Power 1.2 KW). The extracted oil was kept overnight at room temperature for sedimentation. The decanted oil was first filtered through a muslin cloth and then Whatman filter paper no. 2 to remove impurities. The filtered oil was stored in a sealed, dark amber bottle until further use.

PO oil blends with PeO were prepared in two ratios: B1 (PO:PeO 70:30 v/v) and B2 (PO:PeO 80:20 v/v) as recommended by FSSAI. The uniform blends were prepared using a magnetic stirrer at 180 rpm for 15 minutes. Then, all the oils and blends were stored in a dark amber glass bottle at 4 °C until further analysis.

2.2.2. Initial physicochemical characterization of the blends

Fatty acid composition. Blended oils were analyzed for their fatty acid composition by gas chromatography as per the method provided by Choudhary, Grover, and Kaur, 2015. The fatty acids in the oil samples were converted to fatty acid methyl esters (FAMEs) using the IUPAC standard method. 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. The temperature of the oven was at 200 °C. The injector and FID temperature was 250 °C. The FAMEs were expressed as relative area percentages.

Determination of other physicochemical parameters. The color was measured using the Lovibond tintometer (Model F) method. The color intensity was measured in 1" cells in the transmittance mode of a white glass filter, yellow glass filter, and red glass color and expressed as 5R+Y Lovibond units, according to the method described in AOCS (2017). The viscosity of the pure and blended oil was determined using a Brookfield viscometer at constant temperature (30 °C). The smoke point was determined as per the method described by Das *et al*. (2013).

The determinations of peroxide value (PV), acid value (AV), iodine value (IV), saponification value (SV) were performed according to the method provided by AOCS (2017).

2.2.3. Determination of oxidative stability by Rancimat method

The oxidative stability of oils can be determined by conducting an accelerated oxidation test and measuring the induction period (IP hour). Oxidation induction times were measured by a Rancimat model 743 using 3 g of oil, heated at 120 °C with a 20 L/h airflow. At the end of the process, volatile and secondary products were formed, absorbed by measuring a vessel containing deionized water, and then the electrical conductivity was measured (Ben Hammouda *et al.,* 2018).

2.2.4. Determination of thermal stability by heating cycle method

The heating procedure was conducted as per the method reported by Anwar and colleagues with slight modifications in time duration (Anwar *et al.*, 2007). In this method, freshly blended oils were heated at 180 °C in an electric fryer with temperature control (Inalsa Professional 2 fryer, 18/8 steel, 2 L, digital timer). Successive heating was conducted over 4 days for 6 hours each day, giving a total heating time of 24 hours. After completing the heating cycle, 100 mL of oil sample were drawn and stored in amber glass bottles at 4 °C for further analysis. The thermo-oxidative degradation level in the 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. The methodology for color, peroxide value, and free fatty acid is discussed in section 2.4.

Total polar compounds (TPC). The TPC of the frying oils was determined by the Column Chromatography technique, as per the Official Internation-

al Union of Pure and Applied Chemistry (IUPAC) method. It is based on separating polar compounds from non-polar components as provided by Arslan *et al*. (2017). The percentage of TPCs was calculated using the equation:

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

p-*Anisidine value (*p-*AV). The *p*-AV is a measurement of secondary oxidation products (aldehyde content) in the oil; it was determined according to the method provided by the AOCS (2017).

Totox value. Totox value was calculated using *p*-AV and PV, and the formula used was TV = 2PV + *p*-AV. However, since PV is an important parameter affecting refined oil's stability, the *p*-AV, 2 was used as a factor for multiples of PV (Nayak, 2017).

2.2.5. Determination of storage stability of PeO, PO and their blends

The PeO, PO, and blend were subjected to long-term storage to study their storage stability. Storage was done in amber glass bottles at room temperature (25 °C±2) for 180 days. The stability was assessed periodically after every 45 days in order to measure PV, *p*-Anisidine, TOTOX, color, and fatty acid composition.

2.3. Statistical analysis

All the data are reported as means ± standard deviation (*SD*). The data were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS statistical software 20. In addition, for all thermo-degradative parameters, a two-way analysis of variance (ANOVA) followed by the Duncan's post-hoc test was employed to express the significant differences among the mean values at the 0.05 level ($p < 0.05$).

3. RESULTS

3.1. Initial characterization of oil and their blends

3.1.1. Fatty acid composition

Table 1 shows the fatty acid composition of PeO, PO, and their blends. Fatty acid composition plays a crucial role in deciding the nutritional value, functional value, oxidative stability, and industrial application of edible oils. PO's major primary fatty acids were palmitic, oleic, and stearic, accounting for 40.79%, 42.45%, and 4%, respectively. PO was found to be deficient in α-linolenic fatty acid (0.18%). PeO indicated the highest level of α- linolenic acid (55.92%), followed by oleic acid (20.58%) and linoleic acid (13.58%). The fatty acid profile of PeO was in agreement with results reported by other authors (Scapin *et al.*, 2017; Yu *et al.*, 2017).

The blended oil samples B1 and B2 showed different nutritional properties in terms of fatty acid

Table 1. Fatty acid composition (%) of perilla seed oil (PeO), palm olein (PO), and their blends.

Fatty acid composition (%)	Oil samples			
	PO	**PeO**	**B1**	**B2**
Palmitic acid (C16:0)	40.79±0.50^{A}	7.44±0.06^{D}	27.71±0.08^{C}	32.72±0.14^{B}
Palmitoleic acid (C16:1)	0.19±0.00^{B}	0.23±0.02^{A}	0.18±0.00^{B}	0.19±0.00^{B}
Stearic acid (C18:0)	3.99±0.21^{A}	2.24±0.19^{C}	3.49±0.23^{B}	3.35±0.03^{B}
Oleic acid (C18:1)	42.45±0.50^{A}	20.54±0.20^{D}	38±0.38^{B}	37.75±0.15^{B}
Linoleic acid (C18:2)	11±0.23^{C}	13.75±0.15^{A}	11.79±0.20^{B}	11.86±0.11^{B}
α-Linolenic acid (C18:3)	0.18±0.00^{D}	55.80±0.53^{A}	17.86±0.18^{B}	12.96±0.16^{C}
SFA	46.18±0.19^{A}	9.68±0.25^{D}	32.06±0.28^{C}	37.24±0.11^{B}
MUFA	42.64±0.41^{A}	20.77±0.19^{D}	38.18±0.38^{C}	37.94±0.15^{B}
PUFA	11.17±0.24^{D}	69.55±0.42^{A}	29.68±0.41^{B}	24.82±0.26^{C}
SFA: MUFA: PUFA	4.1:3.8:1	1:2.1:7.1	**1:1.3:1**	**1.5:1.5:1**

Values are taken in triplicate and expressed as Mean ±SD; $^{A-D}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$) according to one-way analysis of variance (Duncan's post-hoc test); SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, ND not detected, PeO Perilla seed oil; PO Palm olein; B1- PO: PeO (70:30), B2 - PO: PeO (80:20)

compared to pure PeO and PO. A peculiarity of PeO was its high level of omega -3 fatty acids (α-linolenic fatty acid) at a concentration of about 55.92%. On the other hand, PO had a minimal percentage of omega 3 acids (0.18%) and higher saturated fatty acid levels (46%). This study indicates that blending leads to balancing the fatty acid composition (Table 1). Thus, the addition of 30% PeO into PO (B1) showed a significant (< 0.05) increase in α-linolenic fatty acid content by 98.9% from 0.18 to 17.86 in B1 as compared to PO. Various studies have proven that blending omega -3 rich oil with other vegetable oil increases the α - linolenic acid content of the resultant mixture (Wang *et al.*, 2016; Hashempour-Baltork *et al.*, 2016).

It has been proven that a low level of PUFA:SFA in the diet is the leading cause of increased blood cholesterol levels (below 0.45). According to the results, the PUFA:SFA of PeO was very high (7.16), and with the blending process, the PUFA:SFA ratio of the final blends (B1 – 0.88 and B2- 0.67) of PeO and PO improved. Although, according to WHO, the recommended ratio of SFA:MUFA:PUFA is 1:1-5:1, both blends of PO and PeO were near the recommended value (B1- 1:1.4:1 and B2- 1.5:1.5:1) (WHO, 2008). Thus, blended oils presented an increase in omega-3 fatty acid (α-linolenic acid) content and decreased MUFA and SFA levels compared to pure oils.

3.2. Determination of other physico-chemical parameters of the blends

The quality of native oil and its blend before heating was analyzed by evaluating Physico-chemical properties (Table 2). Table 2 summarizes the physical properties of PO, PeO, and their blends. The oil color is one of the superior physical properties that influences consumer acceptance. Fresh PO had a lighter color at 11.43 ± 0.40. However, PeO had significantly ($P \leq 0.05$) the highest color value (34.83 ± 0.76). The dark color of PeO was attributed to its high level of pigments and polyphenolic compounds (Yu *et al.*, 2017). When PO was blended with PeO, a significant decrease in the golden color of the blended oil was observed due to dilution with a lighter color, thus improving the blended oil's color and acceptability. No significant difference ($P \leq 0.05$) was observed between the color value of blends (B1 and B2).

Viscosity is another important indicator of the quality and stability of edible oil. PO showed the highest viscosity (45mPa.s); whereas PeO displayed the lowest (28mPa.s). After blending, there was a slight decrease observed in the viscosity of blended oil compared to the Palm olein; whereas no significant difference ($P \leq 0.05$) was found in the viscosity blends B1 and B2. The result was in agreement with the findings of previous studies, where authors reported that the blending of oil with PUFA-rich oil leads to a decrease in oil viscosity as viscosity decreases with the increase in PUFA content (Debnath et al., 2012; Flores et al.,2021).

The temperature at which fats and oils produce continuous smoke during heating is known as the smoke point (Hashempour-Baltork *et al.*, 2016). The highest smoke point was reported for PO (241.5 °C ± 2.8) and lowest for PeO (201 °C ±3.2) (Table 2). However, the blends showed better smoke point than perilla seed oil and no significant differences ($P \leq 0.05$) between B1and B2 were observed. Therefore, the blended oil sample reported a smoke point to fulfill the recommended value for frying oils, i.e.,

Table 2. Physicochemical parameters of Palm olein (PO), Perilla oil (PeO), and their blends

Samples	Physicochemical Parameters						
	Color (5R+Y Lovibond units)	Viscosity (mPa.s)	Smoke point (°C)	Peroxide Value (meq O_2/kg)	Acid Value (mg KOH/g)	Iodine Value (g/100g)	Saponification value (mg KOH/g)
PeO	34.83±0.76^{a}	28±0.16^{c}	201±3.^{2}b	4.81 ± 0.40^{a}	1.61±0.02^{a}	192.33±1.25^{a}	180.66 ±1.25^{d}
PO	11.43±0.40^{c}	45±0.23^{a}	241.5±2.8^{a}	0.95±0.15^{d}	0.33±0.01^{b}	67.00±1.00^{d}	190.87±1.02^{a}
B1	25.83±0.76^{b}	43±0.30^{b}	232±2.51^{c}	2.49±0.13^{bc}	0.28±0.01^{c}	86.50±1.80^{b}	186.83±1.75^{b}
B2	25.16±0.28^{b}	43±0.40^{b}	232±2.35^{c}	2.20±0.17^{c}	0.26±0.02^{c}	77.00±0.50^{c}	184.44±0.68^{c}

Values are taken in triplicate and expressed as Mean ± SD**;** $^{a-d}$ Means within each column with different superscripts are significantly different ($P \leq 0.05$) according to one-way analysis of variance (Duncan's post-hoc test)**;** PeO Perilla seed oil; PO Palm olein; B1- PO: PeO (70:30), B2 - PO: PeO (80:20)

above 200 °C (AOCS, 2003). Blends of flaxseed oil with palm olein showed a higher smoke point than flaxseed oil. Therefore, the blending of oils helps to achieve a higher smoke point (Joshi et al., 2021).

The results of the chemical parameters such as peroxide, acid, iodine, and saponification value of the blended oils are presented in Table 2. Peroxide value is a measure of primary oxidation products (hydroperoxides) in oils, and it is correlated with the quality of the oil. The peroxide value (PV) of PeO was the highest (4.81 ± 0.40 meq O_2/kg) among the oils and the blends (Table 3). This may be due to the high PUFA content in PeO. A similar result was reported by Pan *et al.*, (2019). The addition of PO to cold-pressed PeO resulted in a significant decline in their PV, therefore enhancing the oxidative stability of blends. On the other hand, PO showed the lowest PV (0.95 meq O_2/kg), and the peroxide value of all the blended oil samples was within the permissible limit (10 meq O_2/kg) for edible oils.

The acid value measures triacylglycerol hydrolysis, and it is also a crucial quality parameter for edible oil. The acid value of PeO was found to be highest among all the blended oil samples, and it decreased with the increase in the volume of PO. A higher AV of any vegetable oil is due to lipase activity, and it can be reduced by employing certain pre-processing treatments such as roasting and microwaving. Furthermore, lipase enzyme activity is responsible for the hydrolysis of triacylglycerol molecules, leading

Table 3. Effect of heating cycle on chemical parameters of perilla seed oil (PeO), palm olein (PO), and their blends (180 ± 5 °C)

Oil sample/blends	Heating Periods (days) (180 ± 5 °C)				
	Peroxide value (meq O_2/kg)				
	day 0	1st day	2nd day	3rd day	4th day
PeO	4.81±0.40^{aE}	8.05±0.35^{aD}	10.98±0.35^{dC}	14.04±0.93^{aB}	15.16±0.23^{aA}
PO	0.95±0.15^{cE}	1.72±0.25^{cD}	2.47±0.06^{eC}	4.32±0.20^{cB}	4.92±0.27^{cA}
B1	2.6±0.17^{bD}	4.24±0.23^{bC}	5.75±0.13^{bB}	7.69±0.16^{bA}	7.92±0.09^{bA}
B2	2.2±0.14^{bE}	3.8±0.34^{bD}	5.18±0.20^{aC}	6.98±0.37^{bB}	7.69±0.16^{bA}
	Free Fatty acid (% oleic acid)				
PeO	0.93±0.05^{aE}	2.12±0.02^{aD}	2.34±0.05^{aC}	2..51±0.02^{aB}	2.78±0.07^{aA}
PO	0.15±0.0^{bC}	0.15±0.0^{eC}	0.19±0.02^{dB}	0.51±0.01^{cA}	0.56±0.05^{dA}
B1	0.17±.01^{bD}	0.18±0.05^{bC}	0.28±0.00^{eB}	0.76±0.05^{bB}	1.01±0.15^{bA}
B2	0.14±0.01^{bE}	0.18±0.03^{bD}	0.36±0.05^{eC}	0.71±0.02^{bB}	0.83±0.05^{cA}
	p-Anisidine value				
PeO	2.56±0.39^{aE}	7.12±0.07^{aD}	15.89±0.80^{aC}	20.54±0.72^{aB}	25.64±0.41^{aA}
PO	0.41±0.15^{cE}	3.67±0.24^{bD}	7.4±0.42^{bC}	12.09±0.14^{bB}	15.05±0.60^{cA}
B1	1.22±0.19^{bE}	2.27±0.20^{eD}	7.7±0.25^{bC}	12.06±0.17^{bB}	20.10±0.06^{bA}
B2	0.76±0.17^{bcE}	1.62±0.45^{dD}	5.16±0.31^{eC}	8.65±0.48^{cB}	11.22±0.21^{dA}
	Totox value (2PV+ *p*-AV)				
PeO	12.18±1.01^{aE}	23.23±0.64^{aD}	37.86±1.42^{aC}	48.62±0.92^{aB}	55.96±0.88^{aA}
PO	2.31±0.39^{dE}	4.71±0.30^{dD}	10.35±0.32^{eC}	20.73±0.49^{dB}	24.86±1.15^{dA}
B1	6.42±0.15^{bE}	10.73±0.64^{bD}	18.07±0.42^{bC}	27.47±0.12^{bB}	35.95±0.16^{bA}
B2	5.16±0.52^{cE}	9.24±0.94^{cD}	16.66±0.12^{bC}	22.61±0.77^{cB}	26.60±0.54^{cA}
	Total Polar compounds				
PeO	5.25±0.25^{aE}	17.37±0.80^{aD}	20.71±0.84^{aC}	25.87±1.36^{aB}	34.63±0.78^{aA}
PO	2.44±0.20^{dD}	6.50±0.05^{dC}	7.79±0.69^{dB}	8.79±0.67^{dB}	10.87±0.79^{dA}
B1	4.65±0.30^{bE}	11.77±0.11^{bD}	14.21±0.39^{bC}	16.57±0.16^{bB}	19.80±0.62^{bA}
B2	4.00±0.35^{cE}	10.57±0.51^{cD}	11.65±0.30^{eC}	14.64±0.20^{cB}	16.83±0.72^{cA}

Values are taken in triplicate and expressed as Mean ± SE**;** $^{a-d}$ Means within each column with different superscripts are significantly different ($P \leq 0.05$); $^{A-E}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$) according to two-way analysis of variance (Duncan's post-hoc test); PeO Perilla seed oil; PO Palm olein; B1- PO: PeO (70:30), B2 - PO: PeO (80:20)

to free fatty acid formation in edible oils (Mazaheri *et al.*, 2019).

Iodine value is the indicator for the degree of unsaturation in fats and oils and is one of the important quality parameters of edible oils. The iodine value of PO and PeO was 67 and 192 g/ 100 g, respectively. The higher iodine value of PeO confirms the high degree of unsaturation. There was a significant ($P \leq 0.05$) reduction observed in the iodine value of blended oils compared to PeO. This reduction was observed due to the decrease in the predominance of polyunsaturated fatty acids in the blended oils. A study reported that the proportion of Palm olein caused a decrease in IV in Palm olein and olive oil blends, thus improving their stability against oxidative rancidity (Naghshineh *et al.*, 2010).

The saponification value represents the length of the carbon chain of the acid moiety of the lipid molecule. A greater amount of short-chain acids in fats and oil leads to a higher saponification value. The results for the SV of the blended oil were within the range, as reported in another study by Pan *et al.*, (2019).

3.3. Oxidative stability of oil and their blends

Oxidative stability is a vital quality parameter of edible vegetable oils. The stability of oils depends on various factors such as their fatty acid composition, free fatty acid, storage conditions, refining method, and processing methods. The oxidative stability index (OSI) of PUFA-rich fats and oils is low and could be improved by blending it with saturated fats and MUFA-rich oils. It is determined by the rancimat method, in which the induction time is measured, which is directly proportional to the stability of oils.

The results for the oxidative stability of PO, PeO, and their blends are presented in figure 3. The PO showed a remarkably high OSI value (12.29 h > 0.50 h) for induction time. As expected, the higher concentration of PUFA content in PeO is the reason for the low OSI value, i.e., 0.50 h (Torri *et al.*, 2019). The blends B1 and B2 showed intermediate values, i.e., 5.76 and 6.67. Hence, the increased PO levels in blended oil markedly correlated with increased oxidative stability. Therefore, the chemical composition and oxidative stability of the developed blends showed improved oxidative and nutritional stability.

3.4. Effect of heating cycle method on physicochemical parameters of blends

Color is the characteristic parameter used by the food industry for the rapid screening of frying oil quality. The color value of oil increases with increasing heating temperature and time. The color value increases due to the oxidation and polymerization of unsaturated fatty acids in the oil and the solubilization of other non-polar compounds in the oil (Wang *et al.*, 2016). The results in Figures 1 and 2 show that the color value was changed from the beginning to subsequent heating days. A significant ($P \leq 0.05$) increase in the color value of all the blended oils was observed during 24 hours of the heating process. The PeO showed the highest initial color value (34.83), which increased (134.20) by 74.04% after the 4th

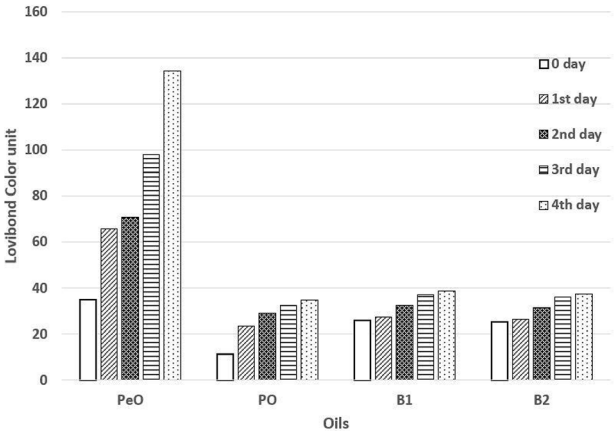

Figure 1. Change in Lovibond color unit of oil during the heating cycle (180 ± 5 °C). Values are taken in triplicate and expressed as Mean ± SE; Perilla seed oil (PeO), Palm olein (PO), B1- PO: PeO (70:30), B2 - PO: PeO (80:20)

day of heating; while PO showed the lowest initial color value (11.43). On the other hand, the blending of PeO with PO showed a significant decrease ($P \leq 0.05$) in the color value of blended oils, due to the lower level of degradation in blended oil. The color values for both the blends PO:PeO (70:30) and PO:PeO (80:20) significantly increased by ($P \leq 0.05$) 33.04 and 29.87%, respectively, during the 4th day of heating. These results indicate that blended oil was more stable against color change during frying than pure PeO. The darkening of color is related to the formation of hydroperoxides, aldehydes, ketones, and hydroxides during heating. However, various studies reported that blending slows down the rate of increased color value or darkening of oil due to

Figure 2. Changes in color of oil during the heating cycle (180 ± 5 °C). Palm olein (PO), Perilla seed oil (PeO), B1- PO: PeO (70:30), B2 - PO: PeO (80:20). 1,2,3, and 4 represent the subsequent days of heating.

heating or frying (Wang *et al.*, 2016; Ben Hammouda *et al.*, 2018).

The changes in peroxide value (PV) of the pure and blended oil during the heating process at 180 °C are shown in Table 3. The peroxide value of all the oil samples increased significantly at the end of the 4-day (24 hours) heating process. The peroxide value of PeO was the highest due to the high content of α-linolenic acid, which is more prone to oxidation. In contrast, the slower increment in the rate of PV was analyzed in PO and blended oil (B1 and B2). However, there was a significant ($P \leq 0.05$) increase in the PV in PO, PeO, and their blends at the end of each heating cycle, but PV was less than 10 meq O_2/kg. As expected, the blending of PO with PeO resulted in a stable blend with respect to PV. Thus, according to the codex, these blended oil samples were in the acceptable range of fresh oil; whereas the PV of PeO was higher than 10 meq O_2/kg at the end of the heating cycle and thus cannot be used in domestic cooking. In a study, Arslan *et al.*, (2017) compared cottonseed and Palm olein's frying and oxidative

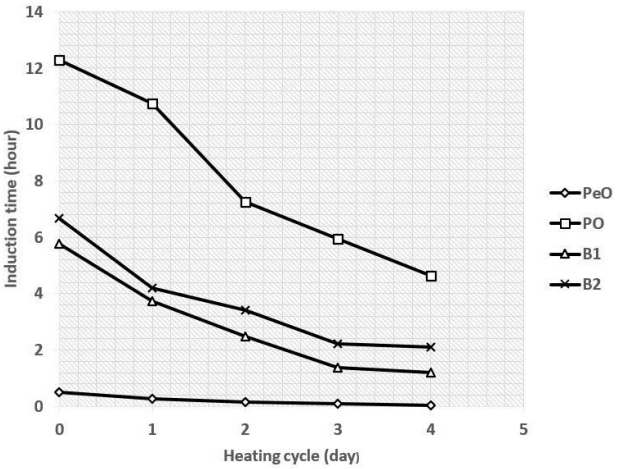

Figure 3. Oxidative stability index (OSI) of Perilla, Palm, and their blends during the heating cycle (180 ± 5 °C). Values are taken in triplicate and expressed as Mean ± SE; PeO Perilla seed oil; PO Palm olein; B1- PO: PeO (70:30), B2 - PO: PeO (80:20)

stability with their blends. It was reported that the PV of cottonseed and Palm olein blends lies within the acceptable range for fresh oil even after 10-hours of frying. However, peroxide value alone cannot be

used as a measure of stability because peroxides and hydroperoxides are volatile compounds and further decompose into aldehydes and ketones, thus reducing the peroxide value. From the data for PV in Table 3, it was found that the rate of increment in PV is significantly ($P \leq 0.05$) decreased in the third and fourth heating cycles compared to initial heating cycles. This finding could be explained by the fact that peroxides are volatile, and upon heating, they further decompose into secondary oxidation products.

The free fatty acid (FFA) content in the blended oil samples during the heating process is presented in Table 3. Free fatty acid is an essential parameter in the degradation of frying or heating oil, and FFA increases with heating due to hydrolytic degradation. However, free fatty acid cannot be taken alone as a parameter of oil deterioration during frying or heating. It should be considered with other methods such as peroxide value and *p*-Anisidine (Abdulkarim *et al.*, 2007). The free fatty acid content in PeO was the highest and the lowest was in PO; whereas no significant difference ($P \leq 0.05$) was observed in their blends. There was a significant($P \leq 0.05$) increase in the FFA of all the oil samples after each heating cycle, and similar increments were observed upon frying in various studies (Wang *et al.*, 2016). The FFA content in Palm olein during the heating cycle increases by 2.7 times; whereas in the case of PeO, it increased approximately by 3 times. The higher free fatty acid content in oil indicates volatile and non-volatiles degradation products which deteriorate the quality of the oil.

The *p*-Anisidine value (*p*-AV) is always used with the peroxide value to measure the oxidative rancidity of oil at high frying temperatures. However, *p*-AV is a more accurate test than PV as it reflects the presence of secondary oxidation products such as 2-alkenals and 2, 4-alkadienals, which are more stable during frying than peroxides. The results for the *p*-AV of PeO, PO, and their blends are shown in Table 3. There was a rapid increase in the value of *p*-AV after each heating cycle regardless of the type of oil sample. This was observed due to the further decomposition of primary oxidation products (hydroperoxides) into secondary oxidation products (aldehydes), which leads to the development of off-flavor and odor. At the end of the 4-day heating period (24 hours), the *p*-AV of PeO was the highest; whereas it was recorded lowest for PO. Therefore, the significantly higher *p*-AV of PeO compared to PO and their blends indicated extensive degradation of oxidized PUFAs.

The addition of PO into the PeO significantly lowered the *p*-AV of blended oils compared to PeO, which is due to a decrease in the percent of polyunsaturated acids. They are the chief targets of thermal-oxidative reactions. These results indicated that blended oils were more resistant to oxidative stability during frying than pure PeO. Similar results were obtained in various studies (Mishra and Sharma, 2014; Wang *et al.*, 2016).

The results of the totox value are reported in Table 4. The totox value for PO is significantly ($P \leq 0.05$) lower than PeO and their blends because of its higher saturated fatty acid content. The higher totox value of PeO indicates a higher susceptibility of PeO to oxidative rancidity than other oils due to the presence of high PUFA content. The results indicated that with the addition of PO in PeO, the PUFA content decreases, thus improving the oxidative stability of blended oils.

The results of the heating cycle of the oxidative stability index (OSI) for blended oil are reported in Figure 4. A higher degree of unsaturation is directly proportional to lower OSI. Therefore, the OSI of the oil sample decreases with heating time due to an increase in the deterioration of oil resulting from the heating process. To compare the rates of OSI increment between the native and blended oils during frying, the raised percentages were calculated for the initial and the end of frying sessions (day 4). Therefore, the fastest increment was found for pure PeO (about 92%) from 0.5h to 6.67h; whereas the lowest increment was found for B1 (about 38%), from 0.02h to 2.21h.

Total polar compound (TPC) is the most accurate measurement of oil deterioration due to its higher accuracy and reproducibility. It includes all the degraded products formed in oils due to thermal oxidation reactions or hydrolytic breakdown of triglyceride molecules during heating or frying. All vegetable oils consist of some parts of polar compounds, but the rate of formation of TPC increases with the application of frying or heating. The quality and shelf life of fried food depend upon frying oil quality. Henceforth, various countries have formulated and reported many quality standards for frying oils. The recommended minimum standard limit for TPC varies from country to country and mainly ranges from 23-29% (Stier,

2013). Table 3 shows the TPC of PO, PeO, and their blends at different heating cycles. The initial TPC of fresh oils was very low, reflecting their sound quality. As we can see, the heating caused a significant and rapid increase in the TPC value of pure and blended oils after every heating cycle. This change in the TPC value was linearly associated with heating temperature and heating time. At the end of the frying cycle (day 4 day), the TPC level of PeO was higher (34.63%) for PeO as compared to PO (10.87%). When PO was added to PeO the TPC level was significantly ($P \leq 0.05$) reduced in the blended oils. The low value of TPC in blended oil compared to PeO was due to an increase in the amount of SFA in blends. Similar findings were also reported by Ben Hammouda *et al* (2018). They suggested that blending PUFA-rich oil with SFA-rich oil results in low TPC during the frying cycle and is less prone to thermo-oxidation.

In the case of PeO, the TPC content reached the rejection range during the frying process, while its blends did not. Assuming a TPC content limit of 23-29%, the number of frying sessions necessary to reach this limit was used to measure frying stability. The frying stability of pure PeO was much lower than that of its blends. As a result, when compared to pure PeO, the blended oil performed well and was of good quality.

3.5. Storage stability of PeO, PO and their blends

The shelf life data (PV, AV, *p*-AV, and Totox value) for the PeO, PO, and their blends is shown in Table 4. The PV of the PeO, PO, B1, and B2 increased significantly throughout the study period. After 180 days of storage at 25 ±2 °C, only PeO exceeded the upper limit of PV (10.0 meqO_2/kg) as given by Codex Alimentarius. The legal upper limit of PeO was reached at between 45 and 90 days of storage. The PV of B1 and B2 after 180 days of storage was 10.32 ± 0.14 meqO_2/kg and 10.05 ± 0.07 meqO_2/kg, respectively. The change in the PV value during storage suggests the formation of primary oxidation compounds such as hydroperoxide. It was also found that there is less formation of hydroperoxide in B1 and B2 than in pure PeO as the PeO had higher PUFA, which results in higher oxidation.

The result showed that the acid value increased with storage. The PeO showed significant ($P \leq 0.05$) changes in acid value. This could be due to the higher

TABLE 4. Effect on storage stability on chemical parameters in Perilla seed oil (PeO), Palm olein (PO), and their blends.

Parameters	Oil/blends	Storage Days				
		0 day	45 days	90 days	135 days	180 days
Peroxide value (meq O_2/kg)	PeO	4.81±0.40^{aE}	8.40±0.22^{aD}	14.11±0.16^{aC}	16.16±0.22^{aB}	20.12±0.17^{aA}
	PO	0.95±0.15^{cC}	1.48±0.12^{dC}	2.38±0.09^{dB}	4.36±0.43^{dA}	4.93±0.09^{cA}
	B1	2.6±0.17^{bE}	4.82±0.11^{bD}	7.88±0.16^{bC}	9.23±0.30^{bB}	10.32±0.14^{bA}
	B2	2.2±0.14^{bE}	3.93±0.09^{cD}	6.78±0.33^{cC}	8.32±0.17^{cB}	10.05±0.07^{bA}
Acid value	PeO	1.61±0.02^{aD}	1.72±0.39^{aD}	2.11±0.16^{aC}	4.64±0.19^{aB}	6.11±0.16^{aA}
	PO	0.33±0.01^{cD}	0.98±0.02^{cC}	1.05±0.15^{dBC}	1.15±0.14^{dB}	1.40±0.11^{dA}
	B1	0.38±0.01^{bE}	1.08±0.02^{bD}	1.72±0.14^{bC}	2.92±0.14^{bB}	3.32±0.06^{bA}
	B2	0.36±0.01^{bE}	1.06±0.01^{bD}	1.64±0.16^{bC}	2.76±0.10^{bB}	3.27±0.08^{bA}
p-Anisidine value	PeO	2.56±0.39^{aE}	5.48±0.11^{aD}	6.60±0.14^{aC}	8.72±0.31^{aB}	11.04±0.05^{aA}
	PO	0.41±0.15^{cD}	0.54±0.01^{cD}	0.98±0.02^{dC}	1.47±0.08^{dB}	2.33±0.31^{dA}
	B1	1.02±0.19^{bD}	1.19±0.08^{bD}	2.27±0.07^{bC}	5.72±0.08^{bB}	7.06±0.08^{bA}
	B2	0.76±0.17^{bcE}	1.03±0.07^{bD}	2.08±0.11^{cC}	3.55±0.07^{cB}	5.47±0.22^{cA}
Totox value	PeO	12.18±1.01^{aE}	22.28±0.56^{aD}	34.83±0.46^{aC}	40.82±1.08^{aB}	50.79±1.11^{aA}
	PO	2.31±0.39^{dE}	3.51±0.22^{dD}	5.74±0.21^{dC}	10.19±0.96^{dB}	12.20±1.13^{dA}
	B1	6.42±0.15^{bE}	10.83±0.31^{bD}	18.03±0.26^{bC}	24.19±0.52^{bB}	27.70±0.24^{bA}
	B2	5.16±0.52^{cE}	8.89±0.26^{cD}	15.64±0.77^{cC}	20.19±0.98^{cB}	25.57±0.07^{cA}

Values are taken in triplicate and expressed as Mean ± SE; $^{a-d}$ Means within each column with different superscripts are significantly different ($P \leq 0.05$); $^{A-E}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$) according to two-way analysis of variance (Duncan's post-hoc test); PeO Perilla seed oil; PO Palm olein; B1- PO:PeO (70:30), B2 - PO:PeO (80:20)

PUFA content in PeO. It was observed that there is no significant difference ($P \leq 0.05$) in the AV of either of the blended oils during storage. Perilla seed oil showed a higher *p*-AV at the initial stage of storage (t = 0) when compared to PO. On the contrary, in the case of blended oil, *p*-AV was reduced due to the blending of PO with PeO. The results indicated that the high content of PUFA in PeO leads to the formation of a high number of secondary oxidation products during storage. However, blending PeO with PO results in low oxidation product formation, leading to higher oxidative stability.

4. CONCLUSIONS

The result demonstrated that the objective of balancing the fatty acid ratio in oil blends was accomplished by selecting oil with low PUFA content such as PO and blending with PeO, which is rich in PUFA content, mainly ALA, so as to get a balanced fatty acid and LA/ALA ratio below 5:1.

The physico-chemical charaterization of the newly developed blends showed its suitability as good cooking oil. The determination of chemical parameters (peroxide value, free fatty acid, *p*-anisidine value, totox value, OSI, total polar compounds) confirmed that these blends are thermally stable up to a certain time during heating. Furthermore, the study showed that the blends exhibited higher thermal stability after a 4-day heating cycle than PeO. As a result, blended oil has a better SMP ratio than pure PeO or PO, with good oxidative stability which fluctuates between 6 and 7 hours at 120 °C and thermal stability of 24 hours at 180 °C.

Finally, the six-month storage stability study indicated that the addition of PO could provide oxidative stability to the blend containing oxidatively vulnerable PeO. Based on our findings, the formulation of perilla seed oil blends is crucial to the food industry, which is presently interested in nonconventional oils and functional foods to improve health and human nutrition.

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