Indian Journal of Traditional Knowledge
Vol 22(3), July 2023, pp 594-603
DOI: 10.56042/ijtk.v22i3.5746

Blending of edible oils with Pomegranate seed oil- An approach to improve the quality by incorporating ω-5 fatty acid

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Received 25 November 2020; revised 23 February 2021; accepted 03 August 2023

Pomegranate seed oil (PSO) contains punicic acid (PA) also known as ω-5 fatty acid. PSO mainly contains polyunsaturated fatty acids (PUFA>88%) which exhibit benefits in wide range of diseases. Therefore, blends of PSO with other vegetable oils *viz.*, palm oil (PO), mustard oil (MO), and sunflower oil (SFO) were prepared in the ratio of 10:90 and 20:80. The prepared blends showed rationale increase of PUFA (11.96, 8.52 and 10.37), total tocopherols (740-885; 638-793; 501-679 mg/kg) in PO, MO and SFO, respectively. The blends also showed improved levels of radical scavenging activity (RSA). The storage studies of blends have showed slight decrease in PUFA, total tocopherols and RSA of the samples stored at 27°C and 37°C. The oxidative stability of PSO and other vegetable oils (PO, MO and SFO) upon storage (60 days) showed increase in the peroxide value in the order of PSO>SFO>MO>PO. Addition of PSO to other vegetable oils in the ratio of 10 and 20% showed decrease in the peroxide content and upon storage, slight increase was observed in the values. Thus, blending of PSO with other vegetable oils provides PUFA, oxidative stability and natural antioxidants to the blends with a greater radical scavenging activity.

Keywords: DPPH scavenging activity, Fatty acid composition, Oxidative stability, Pomegranate seed oil, Vegetable oil blends

IPC Code: Int Cl[23]: A23D 7/00, G01N 33/03

Pomegranate juice processing industries discard up to 50% byproduct after juice extraction, in which 22% are seeds[1,2]. Pomegranate seed oil (PSO) contains punicic acid (PA) which is a predominant fatty acid present in PSO to an extent 78-84%[3]. PA, a conjugated linolenic acid (CLnA), possess a number of health beneficial properties[4]. PSO has been commercialized in spite of its extremely high sensitivity to oxidation and high price.

Blending of vegetable oils having different fatty acid profiles is a general practice to advance their physical-chemical characteristics, resulting in new industrial application(s)[5], to improve nutritional value at affordable prices, and also being an alternative for traditional oils[6,7]. Edible vegetable fats/oils with different properties and its blending are the simplest method to create new products with preferred textural and oxidative properties and very precise composition which is aimed to a particular category of the population precise products. These blends would have a new set of physico-chemical characteristics. The physico-chemical properties of any pure vegetable oil may have low dietary properties and could also possess deprived oxidative stability. In case of low-cost oil, the low levels of vital fatty acids and high oxidative stability with high amounts of saturated fatty acids were observed in palm oil. Therefore, blending can be a feasible way to take benefit of the diverse attribute properties of each of the oil. In many countries, oil blending is till used as a widespread practice. According to World Health Organization[8], there are basically three parameters to produce healthy cooking oil *i.e.*, ratio of saturated/mono unsaturated/polyunsaturated fatty acid, ratio of essential fatty acids (Omega 6/Omega 3) and also presence of natural antioxidants. In order to obtain various benefits in blended oils in the presence of known ingredients that offer protection, like antioxidant effect and frying recyclability (Toliwal *et al*[9].) edible oils can be blended. Thus, in this study, the blend of PSO with more stable and accessible oil, such as Palm oil (PO), Sunflower oil (SFO) and Mustard oil (MO) was attempted which will contribute to the manufacture of more stable blends

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with extended shelf life. Mustard oil has been widely used in domestic cooking due to its monounsaturated and polyunsaturated fatty acids[10]. Though there are several reports on blending which result in efficient oil with vigorous n-6/n-3 ratio[11], but this is the first ever study on blending of PSO with ω-5 fatty acid with commonly used oils to develop blended oil products with health beneficial properties.

Materials & Methods

Materials

The pomegranate seeds were procured from M/S Royal Food Stuffs India Pvt. Ltd., Navi Mumbai. Pomegranate Seed Oil (PSO) was extracted from cleaned and dried seeds at pilot scale in the pilot plant of CSIR-CFTRI. Refined Mustard oil (MO), refined sunflower oil (SFO), and refined palm oil (PO), were procured from a local supermarket in Mysore. Tert-Butyl hydro quinine (TBHQ) was procured from Loba Chemie, Mumbai, India. 2,2-diphenyl-1-picryl hydrazyl (DPPH) was procured from Sigma Aldrich., St. Louis, USA. Standard FAME mix (Fatty acid Methyl Esters) C_{10} - C_{22} was procured from Sigma Aldrich., St. Louis, USA. The other chemicals and reagents used for analysis were of analytical reagent grade.

Methods

Extraction of oil

The pomegranate seeds were powdered and oil extraction was carried out by soaking powder in food grade solvent n-hexane overnight. Seed powder was twice re-extracted with n-hexane flushed with nitrogen. The total yield of the oil was measured gravimetrically and stored at different temperature conditions.

Preparation of blends

The basis of selection of three blends with PSO at 10 and 20% (v/v) levels with MO, PO and SFO was a combination of three factors: (1) presence of strong PSO aroma, (2) presence of adequate amounts of PUFA, and natural antioxidants, and (3) to incorporate PSO for consumption with commonly used oils. The binary oil blends were prepared as follows (v/v): 10PSO:90MO, 20PSO:80MO; 10PSO:90PO, 20PSO:80PO; 10PSO:90SFO, 20PSO:80SFO. A 100 g of PSO and other vegetable oil were placed in 250-mL beakers in duplicate for each blend and were mixed by using a mechanical stirrer at 180 rpm for 15 min at 65°C. Seventeen different blends having various proportions of PSO and other vegetable oils were prepared in this manner. Sampling for analysis was carried out once every 10 days for the 90 days of storage time as per method of Bhatnagar *et al*[12].

Physico-chemical characteristics of native and blended oils

Refractive index 1

Refractive index of native oil samples and PSO blended oils were determined as per AOCS (Cd 1-25, 1993)[13] by using RFM 340 refractometer (Bellingham Stanley Ltd. England) at 40°C.

Free fatty acid value 2

Free fatty acid (FFA) in PSO and various blends was determined by AOCS official method (2002)[14]. 5 g of PSO/blended oil was taken in conical flask; to this 30 mL of warm alcohol, neutralized with 0.1N NaOH was added and was heated at 40-50°C. Indicator (Phenolphthalein) was added and titrated against 0.1 N NaOH. The FFA (%) was expressed as percent oleic acid.

$$FFA\ (as\ \%\ oleicacid) = \frac{[Normality\ of\ NaOH \times 28.2]}{Wt.of the oil}$$

$$Acidvalue = FFA\ (asoleic) \times 1.99$$

Unsaponifiable matter 3

Unsaponifiable content in PSO and blended oils was measured according to the AOCS official method (2017)[15]. 5 g of sample was mixed with absolute ethanol (30 mL) and 60% aqueous KOH (5 mL) was refluxed for 1 h. Unsaponifiable matter was extracted using diethyl ether during reflux and refluxed diethyl ether fractions were evaporated to dryness and the residue was desiccated and weighed. It was expressed as percentage (%) of oil.

Fatty acid composition by gas chromatography

The FAME samples were prepared by mixing oil sample (100 mg) with 100 uL of 2 M methanolic KOH and was kept for 30 min, centrifuged at 3000 g for 15 min and dried with Na_2SO_4, according to AOCS Method No: Ce 1j-07 2017[16]. The ensuing FAME samples were analyzed by Shimadzu GC-2010 Plus-01 gas liquid chromatography system, equipped with FID (Flame ionization detector). The compounds were separated on 180X0.3 cm RTX-2330 Chromosorb (oven temperature 80°C, column inlet pressure 50 kPa) using flame ionization detector

(detector temperature 260°C, run time-30 min). The carrier gas used was nitrogen with a flow rate of 40 mL/min. The analyses were conducted isothermally at 210°C. Retention times of respective reference fatty acids were compared with the individual fatty acids of the experimental samples.

Oxidative stability measurement

Blends of PSO with SFO, PO and MO were separately prepared in the ratio of 10:90 and 20:80 (v/v), respectively, in 100 g × 2 batches as described under ‘‘Methods’’. Each blend (50 g × 2) along with individual samples (50 g × 2) of PSO, SFO, MO, and PO were placed in beakers and incubated at 37°C and 55% RH in an incubator to study the oxidative stability of the blends over a period of 90 days. Samples (4 g × 2) were withdrawn at weekly intervals and analyzed for their peroxide value as per AOCS Method No: Cd 8-53, (1997)[17].

Measurement of natural antioxidants in the oil blends

The total tocopherol content was determined by using AOAC method Ce 8-89 (1997)[18]. The sample (0.01 g) was dissolved in 10 mL of hexane and the absorbance was read at 314 nm in 1-cm cell (double beam UV–visible recording spectrophotometer model UV-1601, Shimadzu Corporation, Kyoto, Japan). The total tocopherol content was calculated by using the formula:

$$\left[\left(\frac{\mathrm{A}}{\mathrm{W}}\right)\times\frac{100}{248.1}\right]$$

Where, A is the absorbance of the sample, W is the weight of the sample in gram/100 mL, 248.1 (extinction coefficient) is $E_{1cm}^{1\%}$ for α- tocopherol (μmol/L).

Radical scavenging activity (RSA)

RSA of native oils and prepared oil blends was determined by reduction of DPPH radicals in toluene. According to the method of Ramadan *et al*[19]., a toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. The oil samples (50±1 mg) were placed in test tubes and DPPH solution was added. The total sample (4 mL) was vortexed for 10 seconds at room temperature. Pure toluene without DPPH solution was taken as blank. The decrease in the absorption at 515 nm was measured in a 1-cm quartz cell after 1, 30, and 60 min of mixing, using a UV-visible spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto, Japan). RSA was expressed as percentage inhibition using the following equation:

$$\%\,RSA = \frac{[Absorbance\ of\ control - Absorbance\ of\ test\ sample]}{Absorbance\ of control}\times 100$$

Statistical analysis

All the analyses was carried out in triplicate and the results were mentioned mean ± SD. Graphpad prism version 5.0 (San Diego, CA 92108) was used as a statistical software for experimental value analysis by mean, standard deviation, ANOVA (two tailed p value) and correlation coefficient.

Results

Physico-chemical characteristics

Refractive Index 1

The initial refractive index of PSO was 1.48 and other vegetable oils (SFO, MO and PO) were found to be 1.46, 1.47, and 1.48, respectively (Table 1). The mean change of refractive index for PSO blended oils was in the range of 1.46 - 1.44, which appeared to be insignificantly higher ($p>0.05$) than those of native oils. Any oil sample with refractive index of 1.5±0.10 is considered as high purity oil which is almost equal to refractive index of glass. Similar results were obtained in this study and have been shown within the standard range.

Acid value 2

The acid value of PSO was found to be 2.1% (expressed as oleic acid), while those of SFO and MO were found to be 4.30% and 4.10% (Oleic acid) respectively and PO was in the range of 3.8 of 4.47% (as palmitic acid). After blending of native oils with PSO the free fatty acid (FFA) values were in the standard range of 0.96% (as oleic acid) and 1.3 to 2.6 for blended oils (Table 1).

Saponification value 3

The initial saponification values (SV) recorded for PSO, SFO, MO and PO were in the range of 165.12, 188.03, 193.89 and 190.53 mg KOH/g of oil, respectively (Table 1). The SV values for SFO, MO and PO were found to be well within the range as defined by Codex standards[20] (SV for PO, SFO and MO, should be in the range between 194-1202, 186-195 and 181-194 mg KOH/g oil, respectively, as per standards). The blended oils did not show significant change in the saponification value after blending

Table 1 — Physico-chemical characteristics of PSO, other vegetable oils and blends

Oil samples	RI	Acid value	Saponification value	Unsaponifiable matter
PSO	1.4832±0.01	2.1±0.2	165.12±1	1.35±0.1
SFO	1.4619±0.01	4.3±0.3	188.03±3	1.25±0.3
MO	1.4728±0.00	4.1±0.1	193.89±3	2.8±0.1
PO	1.4830±0.00	3.8±0.4	190.53±2	1.3±0.2
PSO + SFO				
10 : 90	1.4712±0.001	4.2±0.2	193.43±1	1.32±0.3
20 : 80	1.4809±0.001	4.5±0.3	191.29±4	1.28±0.2
PSO + MO				
10 : 90	1.4613±0.002	4.1±0.1	192.08±3	2.32±0.1
20 : 80	1.4746±0.003	3.8±0.4	193.73±5	2.15±0.2
PSO + PO				
10 : 90	1.4872±0.005	3.2±0.5	189.13±2	1.3±0.3
20 : 80	1.4873±0.006	3.1±0.3	192.35±3	1.5±0.12

*Values are mean ± SD of three triplicate values. There is no significant difference between the Native and blended oils $p>0.05$, *MO mustard oil, PO palm oil, SFO sunflower oil, PSO pomegranate seed oil

with the PSO. Nevertheless, the SV values of blended oil sample were in the range of the values of the native oils.

Unsaponification value 4

The unsaponifiable content for PSO, SFO, MO and PO were initially found to be 1.35, 1.25, 2.8 and 1.3, respectively (Table 1). The value for blends of SFO+PSO was in the range 1.28-1.32%, for MO+PSO it was 2.15-2.32% and for PO+PSO, it was 1.31- 1.5%.

Fatty acids composition of PSO and other vegetable oils

An examination of the data on fatty acid composition of native and blended oils showed nine major fatty acids for pomegranate seed oil (Table 2). The other native oils showed a variation in the content of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). PSO is rich in PA (84%), followed by SFA (palmitic acid-2.67%) and MUFA (oleic acid-4.56%). On the other hand, PO is deficient in PUFA, while other vegetable oils are rich in MUFA (27%) and PUFA (60%).

The fatty acid composition of PSO blended oils is presented in Table 3 and 4. Blending of selected vegetable oils with PSO non-significantly changed the type of key fatty acids in the blends. The major changes in the fatty acid were noted after blending of native oils with PSO in the contents of linolenic acid (C18:3). PSO had highest percentage of PUFA (88%) and the lowest MUFA. Blending of MO with PSO in the ratio of (80:20) showed significant increase in PUFA content by 8.52%. PO did not contain PUFA and it has more of SFA, whereas SFO had less

Table 2 — Fatty acid composition of native oils

Fatty acid	PSO	SFO	PO	MO
C14:0	2.67±1.10	ND	1.7±0.16	ND
C16:0	4.90±0.12	7.10±1.2	38.46±0.12	2.02±0.92
C18:0	5.47±1.5	3.04±1.5	4.8±1.3	1.38±0.8
C18:1	2.03±1.1	28.72±1.6	46.22±1.3	10.81±1.3
C18:2	1.84±1.9	49.11±1.1	7.36±0.6	25.41±0.9
C18:3	84.98±0.8	0.61±1.0	ND	5.08±0.7
C20:0	0.10±1.3	ND	ND	ND
C22:0	0.34±1.1	ND	ND	1.22±1.2
C22:1	0.33±1.4	ND	ND	50.60±1.4
Total	100	100	100	100
SFA %	13.07±1.9	10.14±1.9	43.26±0.7	3.40±1.8
MUFA %	2.03±1.8	28.72±1.8	46.22±1.2	62.10±1.3
PUFA %	84.98±1.7	49.11±1.7	7.36±1.5	27.13±1.9

*Values are mean ± SD of three triplicate values. There is no significant difference between the Native and blended oils $p>0.05$ The fatty acid compositions of PSO (pomegranate seed oil), PO (palm oil), SFO (sunflower oil). MO (mustard oil), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), ND (not detected)

linolenic acid. During 90 days storage at 27°C, the blends of PSO with MO, SFO and PO at two different concentrations (10:90) and (20:80) have showed slight decrease in the content of linolenic acid when compared with initial storage conditions (zero day). Storage studies at accelerated temperature (37°C) of PSO blends for 90 days showed decrease in the content of linolenic acid in three blends (PO, SFO and MO at 10:90 and 20: 80 ratio) to 12.10, 10.73, and 8.83%.

Oxidative stability

The previous reports on the oxidative stability of native oils and blended oils indicated that apart from intrinsic natural antioxidants in oil, PSO, by virtue of being rich in PUFA, could play an important role in

Table 3 — Fatty acid composition of blended oil (PSO 10:90 different vegetable oils) stored for 90 days at different storage conditions

Storage Temp.				27° C						37°C					
Study days	0 day			30 day			90 day			30 day			90 day		
Oil Blends	MO:PSO	SFO:PSO	PO:PSO	MO:PSO	SFO:PSO	PO:PSO	MO:PSO	SFO:PSO	PO:PSO	MO:PSO	SFO:PSO	PO:PSO	MO:PSO	SFO:PSO	PO:PSO
C14:0	1.5 ± 0.9	0.25 ± 1.10	0.86 ±0.3	1.4 ± 2.03	0.25 ± 0.19	0.73 ± 0.96	1.10 ± 0.02	0.14 ± 0.58	0.41 ± 0.38	1.25 ± 0.46	0.20 ± 0.36	0.70 ± 0.13	0.98 ± 0.03	0.10 ± 0.54	0.57 ± 0.03
C16:0	2.12 ±0.12	5.86 ± 0.12	34.85 ± 0.3	2.10 ± 1.52	5.75 ± 1.39	34.60 ± 1.13	1.62 ± 0.32	5.18 ± 0.18	33.81 ± 0.19	2.01 ± 1.73	5.63 ± 0.54	34.57 ± 1.93	1.91 ± 0.54	5.31 ± 0.87	33.57 ± 3.17
C18:0	1.15 ± 1.2	3.21 ± 1.5	9.44 ± 0.6	1.09 ± 0.73	3.19 ± 0.91	9.36 ± 2.59	0.82 ± 0.39	2.75 ± 1.35	9.07 ± 0.69	1. 03 ± 0.36	3.13 ± 0.67	9.30 ± 1.38	0.73 ± 0.43	2.86 ± 0.13	8.49 ± 0.37
C18:1	10.77 ±1.3	29.50 ± 1.1	40.01 ± 1.12	10.10 ± 2.36	29.10 ± 0.13	39.88 ± 1.69	9.56 ± 0.12	28.49 ± 0.73	39.12 ± 2.98	10.24 ± 1.62	29.03 ± 1.72	39.80 ± 1.34	9.15 ± 1.73	28.14 ± 1.18	39.01 ± 1.38
C18:2	24.51 ±0.6	51.03 ± 1.9	5.34 ± 1.9	24.08 ± 3.63	50.86 ± 1.19	5.34 ± 1.23	23.54 ± 0.36	50.10 ± 3.16	5.07 ± 0.85	24.01 ± 1.62	50.75 ± 2.38	5.30 ± 1.47	23.17 ± 1.37	50.07 ± 1.47	4.96 ± 0.98
C18:3	5.35 ± 1.16	8.72 ± 0.8	8.16 ± 1.6	5.14 ± 0.36	8.63 ± 1.03	8.10 ± 3.21	4.03 ± 1.39	7.35 ± 2.58	7.63 ± 0.82	5.05 ± 1.32	8.02 ± 1.68	8.03 ± 1.38	4.17 ± 0.13	7.04 ± 1.08	7.37 ± 1.37
C20:0	1.26 ± 1.09	0.26 ± 1.3	nd	1.18 ± 094	0.20 ± 0.32	nd	0.84 ± 0.23	0.07 ± 0.9	nd	1.11 ± 0.29	0.14 ± 0.87	nd	0.84 ± 0.18	0.05 ± 0.04	nd
C22:0	1.04 ± 1.89	0.21 ± 1.1	nd	0.97 ±1.14	0.18 ± 0.63	nd	0.65 ± 0.23	0.09 ± 0.07	nd	0.96 ± 0.69	0.15 ± 0.38	nd	0.64 ± 0.87	0.07 ± 0.01	nd
C22:1	44.30 ± 1.35	0.58 ± 1.4	0.31 ± 1.15	44.12 ± 2.47	0.50 ± 0.34	0.28 ± 0.31	43.16 ± 4.68	0.32 ± 1.87	0.15 ± 0.39	44.06 ± 1.67	0.48 ± 0.79	0.25 ± 0.40	43.27 ± 1.08	0.32 ± 0.17	0.10 ± 0.05
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
SFA%	4.77 ± 0.7	9.32 ± 1.9	45.15 ± 2.1	4.64 ± 1.39	9.24 ± 2.32	45.02 ± 0.56	4.09 ± 1.48	8.41 ± 1.38	44.04 ± 0.36	3.90 ± 0.36	9.20 ± 1.10	44.86 ± 1.28	4.01 ± 1.03	8.68 ± 2.34	43.68 ± 1.76
MUFA %	10.77 ± 1.2	29.50 ± 1.8	40.01 ±1.12	10.54 ± 2.12	29.15 ± 3.43	40.19 ± 1.18	9.06 ± 1.58	28.15 ± 1.39	39.45 ± 0.69	10.50 ± 1.92	29.01 ± 0.73	39.62 ± 1.67	9.57 ± 1.87	28.09 ± 3.08	38.05 ± 1.37
PUFA%	74.16 ± 1.5[a]	60.75 ± 1.7[a]	13.50 ± 0.95[a]	74.06 ± 3.52[a]	60.50 ± 4.16[a]	13.30 ± 1.69[a]	70.58 ± 2.35[a]	59.59 ± 2.68[a]	12.43 ± 1.59[a]	73.89 ± 2.18[a]	60.45 ± 2.19[a]	13.15 ± 2.03[a]	72.59 ± 0.14[a]	59.67 ± 2.88[a]	12.05 ± 2.09[a]

PSO (pomegranate seed oil), PO (palm oil), SFO (sunflower oil), MO (mustard oil), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), ND (not detected), *Values are mean ± SD of three triplicate values. [a]p value< 0.0001 (two tailed) value indicated a significant improvement in PUFA after the blending with PSO even after storage at 37°C in comparison to 100% PSO.

Table 4 — Fatty acid composition of blended oil (PSO 20:80 different vegetable oils) stored for 90 days at different storage conditions

Storage Temp.	27° C									37° C					
Study Days	0 day			30 day			60 day			30 day			60 day		
Oil Blends	MO:PSO	PO:PSO	SFO:PSO	MO:PSO	PO:PSO	SFO:PSO	MO:PSO	PO:PSO	SFO:PSO	MO:PSO	PO:PSO	SFO:PSO	MO:PSO	PO:PSO	SFO:PSO
C14:0	0.13 ± 0.8	0.78 ± 0.15	0.13 ± 0.05	0.10 ± 0.03	0.74 ± 0.13	0.12 ± 0.14	0.09 ± 0.10	0.67 ± 0.86	0.10 ± 0.01	0.07 ± 0.16	0.71 ± 0.96	0.10 ± 0.06	0.05 ± 0.01	0.65 ± 0.43	0.08 ± 0.02
C16:0	2.02 ± 0.92	32.07 ± 0.83	7.10 ± 1.2	1.89 ± 0.12	32.0 ± 1.2	7.04 ± 0.09	1.4 ± 0.46	30.96 ± 0.03	6.65 ± 0.13	1.63 ± 0.19	31.54 ± 0.31	7.10 ± 1.13	1.55 ± 0.79	31.04 ± 1.18	6.84 ± 0.16
C18:0	1.13 ± 0.8	9.32 ± 0.65	3.04 ± 1.5	1.06 ± 0.02	9.26 ± 0.04	2.98 ± 0.26	1.45 ± 0.08	8.58 ± 0.25	2.55 ± 0.34	1.03 ± 0.54	9.18 ± 0.48	2.73 ± 0.37	0.91 ± 0.12	8.94 ± 0.23	2.50 ± 0.28
C18:1	9.60 ± 1.3	38.72 ± 1.15	28.72 ± 1.6	9.13 ± 0.32	38.56 ± 0.56	28.81 ± 0.69	8.1 ± 0.18	36.65 ± 0.12	27.12 ± 0.15	9.03 ± 0.12	38.3 ± 0.52	28.49 ± 0.15	8.43 ± 1.15	37.45 ± 0.36	28.0 ± 0.94
C18:2	21.04 ± 0.9	2.56 ± 1.62	49.11 ± 1.02	21.04 ± 1.12	2.49 ± 0.32	48.98 ± 0.36	20.14 ± 0.14	1.73 ± 0.74	48.09 ± 0.11	21.05 ± 0.72	2.41 ± 0.21	48.36 ± 0.31	20.53 ± 0.17	2.30 ± 0.17	48.03 ± 1.18
C18:3	13.68 ± 0.7	11.96 ± 0.63	10.37 ± 1.0	13.12 ± 0.02	11.64 ± 0.13	10.11 ± 0.14	12.75 ± 0.76	10.72 ± 0.31	9.10 ± 0.04	13.09 ± 0.29	11.1 ± 0.27	10.09 ± 0.13	12.10 ± 1.98	10.73 ± 0.84	8.83 ± 0.57
C20:0	1.04 ± 0.12	nd	0.61 ± 1.15	1.0 ± 0.05	nd	0.58 ± 0.13	0.8 ± 0.09	nd	0.55 ± 0.43	0.97 ± 0.38	nd	0.51 ± 0.08	0.84 ± 0.34	nd	0.46 ± 0.09
C22:0	1.69 ± 1.2	nd	0.15 ± 1.3	1.52 ± 0.12	nd	0.14 ± 0.23	1.2 ± 0.96	nd	0.12 ± 0.31	1.50 ± 0.74	nd	0.13 ± 0.07	1.40 ± 0.78	nd	0.10 ± 0.04
C22:1	39.67 ± 1.4	0.68 ± 0.03	0.31 ± 1.6	39.12 ± 0.12	0.49 ± 0.33	0.30 ± 0.20	38.84 ± 0.17	0.86 ± 0.17	0.28 ± 0.19	39.09 ± 0.43	0.40 ± 0.53	0.29 ± 0.17	38.56 ± 2.10	0.35 ± 0.17	0.26 ± 1.13
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
SFA%	3.28 ± 1.8	42.17 ± 1.16	10.27 ± 1.9	3.15 ± 0.02	43.08 ± 0.23	10.12 ± 0.72	2.54 ± 0.31	43.83 ± 0.31	2.76 ± 0.69	3.01 ± 0.41	42.89 ± 1.43	10.07 ± 0.96	9.50 ± 0.14	42.30 ± 1.16	9.49 ± 1.67
MUFA %	9.60 ± 1.3	38.72 ± 1.15	28.72 ± 1.8	9.20 ± 0.42	38.49 ± 0.73	28.56 ± 0.14	8.40 ± 0.41	36.85 ± 0.49	8.42 ± 0.08	9.16 ± 0.34	38.1 ± 1.59	28.14 ± 0.34	8.58 ± 0.06	37.76 ± 1.19	27.44 ± 0.83
PUFA%	75.43 ± 1.9[a]	15.2 ± 0.12[a]	59.48 ± 1.7[a]	75.14 ± 0.12[a]	15.09 ± 0.11[a]	58.95 ± 0.97[a]	73.59 ± 1.15[a]	14.02 ± 0.83[a]	58.12 ± 0.13[a]	75.09 ± 0.86[a]	14.83 ± 0.34[a]	58.63 ± 1.59[a]	74.54 ± 2.43[a]	14.07 ± 1.94[a]	56.73 ± 2.59[a]

PSO (pomegranate seed oil), PO (palm oil), SFO (sunflower oil), MO (mustard oil), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), ND (not detected)

*Values are mean ± SD of three triplicate values. [a]p value< 0.003 (two tailed) value indicated a significant improvement in PUFA after the blending with PSO, even after storage at 37°C in comparison to 100% PSO.

influencing oxidative stability. The PUFA content of PSO blended vegetable oils have increased along with increase in SFA and MUFA content. Similarly, the oxidative stability of the blends could increase/ decrease and an attempt has been made by incorporation of PSO to other vegetable oils to study the pattern of oxidative stability in blends. Figure 1a,b exemplify the oxidative stability of the PSO and blends. PSO and SFO were more prone to peroxidation and had a greater rate of peroxide formation, followed by MO and PO. During 60 days oxidation test at ambient and accelerated temperatures, the peroxide value of PSO increased significantly (59.23 fold and 69.50, respectively), followed by SFO (58.03-66.08, respectively)> MO (45.03-65.03, respectively)> PO (38.12-35.36, respectively).

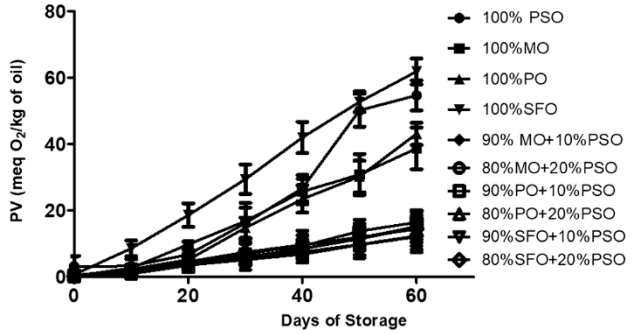

Fig. 1a — Peroxide values of native oils stored at 27°C showed significant increase in peroxide formation. The blends of SFO, PO and MO at 80:20 ratio showed Two-tailed P value < 0.0702; 0.0956; 0.0754 indicated a significant reduction in peroxide formation after blending with PSO, in comparison to control (100% SFO, PO and MO).

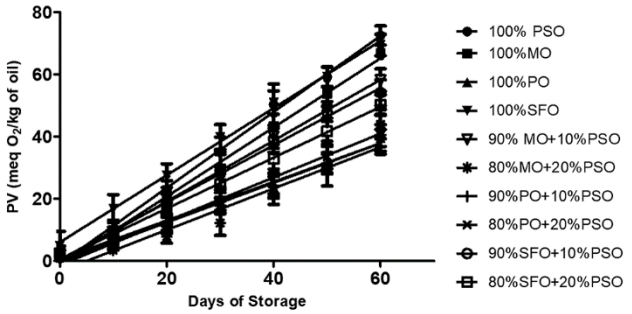

Fig. 1b — Peroxide values of native oils stored at 37°C showed significant increase in peroxide formation. The blends of SFO, PO and MO at 80:20 ratio showed Two-tailed P value < 0.0122; 0.0474: 0.0408 indicated a significant reduction in peroxide formation after blending with PSO, in comparison to control (100% SFO, PO and MO). *Values are mean ± SD of three values.* [*]*MO mustard oil, PO palm oil, SFO sunflower oil, PSO pomegranate seed oil.*

The peroxide values of PSO blends in the ratio of 90:10 and 80:20 with different vegetable oils at both the storage temperatures is represented in Figure 1a,b, respectively. The peroxide values of blends of SFO and PSO for both the combinations at both storage temperatures (27°C and 37°C) showed slight decrease. However, during the storage studies of oil blends, the formation of peroxides was less when compared with individual vegetable oil samples at both the storage temperatures.

Natural antioxidants and RSA

The total tocopherol content of PSO, other vegetable oils and blended oils is represented in Table 5. The total tocopherol content in blends was increased significantly (p value<0.05), this may be due to the presence of high content of phytosterols in PSO[21]. The tocopherol content in blends of PSO with SFO in the ratio of 20:80 showed the increase from 501 to 679 mg/kg. Similarly, the other two blends of PSO with MO and PO showed significant increase in tocopherol content from 638 to793, and 740 to 885 mg/kg, respectively. There was a significant change (p value<0.03) in the DPPH radical scavenging activity of the blends by 2 fold (Fig. 2a,b), wherein the addition of PSO to the vegetable oils increased the content of tocopherol and it is known from the literature that the PSO is a good source of phytosterols. The PSO, other vegetable oils and its blends, stored at ambient and accelerated conditions were analyzed for total tocopherol content. The native oils and blended oils showed slight decrease in the tocopherol content after 90 days storage at ambient temperature (27°C). Similar results were observed when these samples were stored at accelerated temperature (37°C).

The DPPH activity of native sample and oil blends were also studied at both the temperatures, with increase in storage duration, there was a slight decrease in the content of DPPH RSA in the samples stored at 27°C, whereas in case of samples stored at 37°C, there was a significant decrease in DPPH RSA values of native oils but due to the presence of antioxidants in PSO, the blended oils did not show significant change when compared with the native oils for 60 days storage.

Discussion

Acid values for the blended oils were shown to be within the prescribed limit described by FSSAI[22]. The saponifiable matter possibly will be attributed to

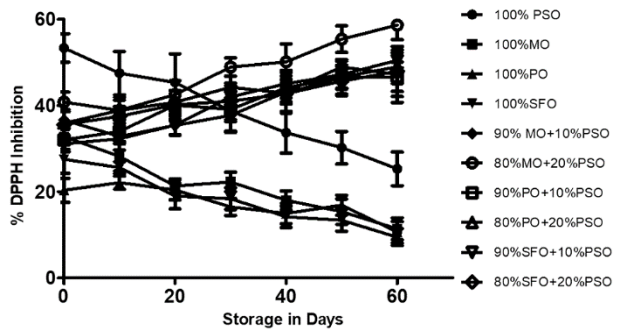

Fig. 2a — DPPH scavenging activity of PSO (20): 80 MO; 80 PO; 80 SFO blends at 27° C showed two-tailed P value < 0.0071; 0.0162; 0.0234 indicated a significant improvement in DPPH activity after blending with PSO, in comparison to control (100% PSO).

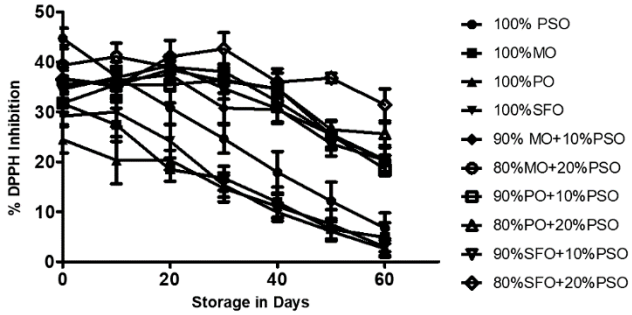

Fig. 2b — DPPH scavenging activity of PSO (20): 80 MO; 80 PO; 80 SFO blends at 37° C showed two-tailed P value < 0.048; 0.0474; 0.0122 indicated a significant improvement in DPPH activity after blending with PSO, in comparison to control (100% PSO). *Values are mean ± SD of three values.* MO mustard oil, PO palm oil, SFO sunflower oil, PSO pomegranate seed oil.*

change of fatty acids to carbonyl compound which lowers the FFA content in the oils and hence reduces the saponification value which helps in reduction of auto-oxidation of oils and also improves stability of the oils[23]. The unsaponifiable results are in concurrence with the previous studies on blended oil by Kumar *et al*[23]. If the unsaponifiable matter is (> 2%) in any of the oil sample, it shows enhancement in the oxidative stability. In the present study after blending with PSO, there was an improvement (lowering) in unsaponifiable contents of the blended oils of MO, PO and SFO.

Blending of PSO (20%) at higher concentration with PO and SFO had showed a significant change in the content of linolenic acid (11.96% and 10.37%), respectively, at initial storage conditions. Ramadan & Wahdan[24] reported that blending of corn oil with two spice oils (cumin seed oil and coriander seed oil) possibly changed the fatty acid profile of the blends. Similarly, the MUFA and SFA content of blended oils also decreased with increase in storage days and under both the storage temperatures (27°C, 37°C). These results are in agreement with Sliva-James *et al*[25] who studied blending of PSO with soyabean oil and showed increase in functional properties like antioxidant content which protects lipids against oxidative rancidity. In our studies also, blending of oils with PSO resulted in an increased amount of natural antioxidants which could help in lowering the cholesterol, apart from rendering the benefits towards the improvement of bone health by Bachagoal *et al*.[26] the virtue of the presence of PA in all the blends.

Oxidative stability of any PUFA rich oil can be modified after blending with the MUFA rich oil[27]. The similar trend was observed by Cao *et al*[28]. on the oxidative stability of different vegetable oils which showed PUFA as the predominant factors in the oxidation of vegetable oils, and tocopherols were the predominant factors in the antioxidants of oils.

The peroxide values of blended oils showed decline with storage. In line with our results, Bhatnagar *et al*.[12] and Ngassapa *et al*.[29] also reported a decrease in the peroxide value of high PUFA (rice bran oil, SFO) oils when it was blended with MUFA (MO) and SFA (coconut oil) rich oils and at different storage conditions and also when exposed to higher temperatures.

The phytosterols and tocopherol content of PSO was very high (Amri *et al*[30]) when compared with the native oils, which might have contributed to the blended oils, however the contribution of tocopherol and phytosterols in DPPH activity to the extent of 20% is still to be explored. These results are in concurrence with the previous studies conducted by Silva-James *et al*.[25] on PSO blends with soyabean oil and other vegetable oils where they showed an increase in the DPPH scavenging activity by 2.5 fold due to the addition of PSO in other vegetable oils. RSA values of native oils was decreased with increase in storage days, but the blended oils showed significant increase due to the presence of antioxidants in PSO, the blended oils did not show significant change when compared with the native oils for 60 days storage at 37°C.

Conclusions

Blending of PSO with SFO, MO and PO led to an improvement in the oxidative stability of the blends as well the nutrients. The physico-chemical characteristics, like refractive index, acid value,

saponification value and unsaponifiable matter for PSO and blended oils were found to be within the range specified by codex standards. The linolenic acid content after blending of PSO (10 and 20%) with MO, SFO and PO was in the range between 8.52, 10.37and 11.96%, whereas, these values decreased (1.5-3%) with storage days and also decreased at different storage conditions. The increase in linolenic acid content in the blends could help an individual to take required recommended daily allowance of ω (3 and 6) fatty acids to improve the bone health. The results of oxidative stability for 60 days at ambient and accelerated temperatures revealed that the peroxide value of PSO increased significantly, followed by SFO > MO > PO. Oxidative stability of blends of PSO with SFO, MO and PO had showed decrease in the peroxide content. The total tocopherol content in the PSO was very high (4200 mg/kg) which was contributed due to SFO, MO and PO after blending PSO at 20% level, which also rendered increase in DPPH RSA content. If the total tocopherol content decreases there is a decrease in the DPPH and this shows indirect effect on oxidative stability due to the loss of antioxidants in oil samples. With this successful formulation and also with increase in nutritional properties, this product could be helpful in the development and maintenance of bone health for all age groups and to reduce the risk of cardiovascular diseases. More importantly the studies conducted pave the way for utilization of PSP which is produced as waste form pomegranate processing industries.

Acknowledgement

The authors thank Director, CSIR-CFTRI, and Head of the Department of Biochemistry, CSIR-CFTRI Mysuru for their kind help and support during this research work. The first author in this MS expresses her gratitude and heartfelt thanks to Department of Science & Technology (DST), New Delhi for awarding INSPIRE Fellowship to her.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

Authors' Contributions

AP: Experimentation, data collection and compilation, Preparation of the draft; RPS: Verification of the data and review of the draft.

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