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Biochemical characterization of blended mustard oils and their physico-chemical attributes for nutritional enrichment

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Abstract

Blending technology has the potential to put the puissance of two or more edible oil in order to offer balanced fatty acid ratio and natural antioxidants thereby increasing the stability of oil. The objective of the present study was to develop healthier and stable blends using fresh crude mustard oil (MO),crude palm oil (PO) and crude rice bran oil (RBO).Therefore, three mustard oil blends were prepared as blend A, blend B and blend C in the ratio i.e., 40:40:20, 50:30:20 and 60:20:20, respectively. These blends as well as individual oils (mustard oil, palm oil, and rice bran oil) were analyzed for their physico-chemical and biochemical characteristics. Peroxide value and free fatty acid parameters of oils and blends were studied at regular interval i.e., 0, 7,14,21,28 days. All the parameters were evaluated at room temperature. Consequently, mustard oil and blend C (60:20:20) contained highest amount of monounsaturated and polyunsaturated fatty acid i.e., 60.81% and 26.98% and 61.325% and 21.847%, respectively. Saturated fatty acids were found to be highest in palm oil (47.682 %) and blend A (26.354%).Oil stability index indicated that palm oil (4.17) and blend A (1.88) to be highly stable. Minimum peroxide value and free fatty acid increase was observed in mustard oil (3.25 to 6.83 mEq/kg and 1.20 to 5.17%) and blend A(8.04 to 13.06 mEq/kg and 4.07 to 6.77%). Highest DPPH radical scavenging activity and total antioxidant capacity was displayed by mustard oil (9.36 µg/ml and 58.66 mg/g) and blend A (8.44 µg/ml and 60.86 mg/g).The observed results suggested that blend A displayed better nutritional output.

Key words: *Antioxidant activity, blending, fatty acid composition, mustard oil, physico-chemical characteristics*

Introduction

In India, since time immemorial, the oils used in the kitchens are to a great extent subjected to where you originated from. Diverse societies eat distinctively and the kind of oil fits delightfully into the food landscape of that region. Most of the vegetable oils have constrained technological application in their original form due to their particular chemical and physical features. Blending of edible oils with distinctive properties is a simple strategy to create products with desired textural and oxidative properties. Blending technology is a widely accepted concept and showed a healthy and steady growth during the last five years from 2009-2014 in Indian oil markets (Ken Research, 2015). Blending different edible oils can alter the fatty acid composition and give more elevated amounts of natural antioxidants and bioactive lipids in the blends and in this manner, can enhance dietary value and stability of oils (Aladedunye and Przybylski, 2013).

Mustard oil is traditional oil that has been used for centuries in Indian homes and suitable for all sort of cooking. In spite of the fact that mustard oil gives many advantages it is restricted and sold only for external use in countries like United States, Canada and European Union. The erucic acid content in mustard oil is higher than 50% which against the globally acknowledged standards (Downey, 1983). Therefore, mustard oil quality can be adjusted with other edible oil to get a coveted product. Palm oil is an imperative and versatile raw material for edible and non-edible industries. Palm oil rich in saturated fatty acid make it suitable for frying purposes. The beta carotene found in palm oil is a precursor of vitamin A and function as a scavenger to free radicals (Basu *et al*., 2001). Palm oil has a tendency to crystallize amid winter (low temperature). It has been observed that blending palm oil with edible oils with a higher magnitude of unsaturation, brought about blends that are more steady (do not crystallize) at low temperatures. The blends remain clear for a longer timeframe. Rice bran oil is comparatively new oil, demonstrating extraordinary guarantee for wide use in India, due to the presence of oryzanol, which are known to shield from cardiovascular diseases. Tocopherols and tocotrienols present in crude and physically refined oils, for example palm oil and rice

bran oil, have hypocholestrolemic and antioxidative characteristics and are advantageous in averting cardiovascular diseases (Shahidi and Wanasundara, 1992). Blending unconventional oil like rice bran oil with other edible oils is permissible so as to accomplish distinctive aims to lessen the cost and meet industrial requirements (Choudhary *et al*., 2015). In the present study, fresh crude mustard oil (MO) was blended with crude palm oil (PO) and crude rice bran oil (RBO) in order to derive blends with improved nutritional and physico-chemical qualities.

Material and Methods

Fresh crude mustard oil (MO) was procured from Kichha. Crude palm oil (PO) was procured from B.L Agro Pvt. Ltd, Bareilly and crude rice bran oil (RBO) was obtained from Rudra Solvents Pvt. Ltd., Rudrapur.

Preparation of blends: Three mustard oil blends were prepared in the ratio(v/v): Blend A(40% MO+40% PO+ 20% RBO), blend B(50% MO+ 30% PO + 20% RBO) and blend C(60% MO + 20% PO + 20% RBO).These blends along with the individual oils(MO,PO,RBO) were analyzed for physico-chemical and biochemical properties.

***Physico-chemical properties*:** Density was measured using R.D bottles. Viscosity was recorded with the help of Ostwald viscometer. Refractive index was determined using Abbe's Refractometer. Peroxide value and free fatty acid value were determined by IS: 548 (part-1) 1964(Reaffirmed 2010) methods.

Fatty acid composition: Samples were analyzed for their fatty acid composition by gas chromatography using fatty acid methyl esters (FAMEs). FAMEs were prepared by the esterification method proposed by Morrison and Smith (1964). A NUCON gas chromatograph (Model GC-5765) equipped with quartz jet FID (flame ionization detector) and stainless steel capillary column was used. The initial column temperature was set at 150^0C and raised up to 240^0C to remove any impurities left in the column due to the previous run (conditioning mode).Oven temperature fall down to 120^0C (run mode). The carrier gas was nitrogen at a flow rate of 30 ml/min. The temperature of oven, injector and detector temperature was 75^0C, 110^0C and 120^0C. A reference standard FAME mixture (Supelco Inc.) was also examined under the same operating conditions. The FAMEs were expressed as relative percentage area.

***Antioxidant activity*:** To analyze the antioxidant activity of oils and blends total antioxidant capacity and radical scavenging activity towards DPPH radicals were determined.

***Total antioxidant activity*:** The total antioxidant activity was determined by phosphomolybdenum assay stated by Prieto *et. al*., 1999. An aliquot of 100µl of oil samples were combined with 900µl of methanolic extract (80%) in different test tubes. 1ml of phosphomolybdenum reagent (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to each sample. The samples were incubated in boiling water bath for 90 mins at 95^0C.The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The antioxidant activity of the sample was determined using a standard curve of ascorbic acid. The antioxidant activity is expressed as mg of ascorbic acid equivalents (AAE).

DPPH radical scavenging activity: The DPPH radical scavenging activity of oils and blends was determined by the procedure set out by Braca *et al*. (2001). Various concentrations of methanolic extract of oil samples were taken in test tubes and to each test tube 5ml of 0.4mM DPPH methanolic solution were added. The samples were allowed to stand in dark for 30 minutes. Disappearance of color was read at 517 nm using UV spectrophotometer. Similar steps were taken for control and standard except the addition of oil sample. Free radical scavenging activity was calculated as follows:

$$\% \text{ inhibition} = [(A_o - A_1)/A_o] \times 100$$

A_o - absorbance at ë = 517 nm for blank

A_1 - absorbance at ë = 517 nm for samples

The standard used was ascorbic acid. The IC_{50} (the concentration of antioxidant which scavenges the free radical DPPH to 50%) value was determined using Microsoft Excel 2007. On linear graph section of the percentage of inhibition according the concentration of the sample and standard, a trend line equation: $y = ax + b$ were determined. IC_{50} DPPH was calculated by changing the equation mentioned and the expression x at which y-value is 50% was accepted as unknown.

Statistical analysis: All the determinations were carried out in triplicate and the results were expressed as mean± standard deviation.Two way analysis of variance (ANOVA), factorial completely randomized design (CRD) and their statistical significance (p±0.05) was ascertained.

Results and Discussion

***Biochemical analysis*:** fatty acid composition and antioxidant activity of parent and blended oils were estimated.

Fatty acid composition: The fatty acid composition of oils and blends presented in Table 1.The gas chromatogram of blend A represented in fig.1. It can be seen that the oils are differentiated from each other chiefly due to the significant contrasts in percentage of palmitic acid, oleic acid, linoleic acid and linolenic acid. The percentage of saturated fatty acid (SFA) was highest in PO (46.12%) than MO and RBO i.e., 9.77 and 22.29%, respectively. Highest monounsaturated fatty acid (MUFA) was observed in MO (60.81%) as compared to PO and RBO i.e., 42.48 and 41.08%, respectively. RBO had the highest polyunsaturated fatty acid (PUFA) followed by mustard oil i.e., 36.6 and 26.4%, respectively. Prominent fatty acids in mustard oil, palm oil and rice bran oil were erucic acid, palmitic acid and oleic acid.

Maximum SFA content was observed in blend A (24.72%), monounsaturated and polyunsaturated fatty acid in Blend C (56.34% and 21.16%). Blend A contained palm oil volume higher than the other blends. Blend C contained higher volume of mustard oil which has maximum percentage of monounsaturated fatty acid. Erucic acid in blend A, B and C was found to be 20.41%, 28.04% and 32.32%, respectively, lower than mustard oil. Many studies suggested the advantageous impact of MUFA on cardiovascular and diabetic risk components (Schwingshackl and Hoffmann, 2012). Oleic acid is associated with reduction of low density lipoprotein (LDL) cholesterol (Lopez, 2010).

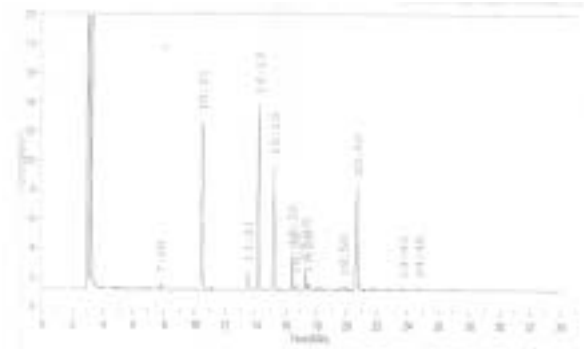

Fig. 1: Gas chromatogram of Blend A (40:40:20).

As per the WHO (2008) recommendations, SFA: MUFA: PUFA and omega 6: omega 3 ratio must be in 1:1.5:1 and 5-10 in the diet. The SFA: MUFA: PUFA ratio of RBO was found to be closer to the recommendation. No single oil showed omega 6: omega 3 ratios closer to the recommended one. The SFA: MUFA: PUFA and omega 6: omega 3 ratio of blend A i.e., 1:2.0:0.8 and 4.5 was close to the recommendation by WHO.

The oil stability index (OSI) is the ratio of oleic acid to linoleic acid. Higher value of OSI indicates longer shelf life. Among the individual oils, palm oil displayed higher OSI. In case of blends, highest OSI was observed in blend A.

Antioxidant activity: DPPH radical scavenging activity and total antioxidant activity of oils and blends is summarized in Table 2.

***DPPH radical scavenging activity*:** IC_{50} value is inversely proportional to the antioxidant activity (Onder *et. al*., 2009).The IC_{50} value of ascorbic acid was 4.75 µg/ml indicating high antioxidant activity. Highest radical scavenging activity was observed in crude mustard oil followed by rice bran oil i.e., 9.36 and 10.12 µg/ml. Blend A showed highest scavenging activity i.e., 8.45 µg/ml than the other blends. High radical scavenging activity of blend A might be due to presence of high oleic acid. It

Table 1: Fatty acid composition of edible oils and their blends

Fatty acid	MO	PO	RBO	BLEND A	BLEND B	BLEND C
Palmitic acid(C16)	2.00	42.00	20.07	21.39	16.35	13.11
Stearic acid(C18)	1.07	4.12	2.22	2.59	2.14	1.87
Oleic acid(C18:1)	10.37	42.48	41.08	30.15	26.63	24.02
Linoleic acid(C18:2)	15.82	10.31	35.01	16.01	15.45	15.98
Linolenic acid(C18:3)	10.58	ND	1.59	3.59	4.31	5.18
Arachidic acid(C20)	6.70	ND	ND	0.74	0.89	0.83
Erucic acid(C22:1)	50.44	ND	ND	20.41	28.04	32.32
SFA	9.77	46.12	22.29	24.72	19.38	15.81
MUFA	60.81	42.48	41.08	50.56	54.67	56.34
PUFA	26.4	10.31	36.6	19.6	19.76	21.16
SFA:MUFA:PUFA	1:6.2:2.7	1:0.9:0.2	1:1.8:1.6	1:2.0:0.8	1:2.8:1.0	1:3.6:1.3
Omega6:omega 3	1.5	ND	22.01	4.5	3.5	3.0
Oil stability index	0.66	4.12	1.17	1.88	1.72	1.50

MO-*Mustard oil*, PO-*Palm oil*, RBO-*Rice bran Oil*, ND-*Not detected*

Table 2: IC_{50} value and total antioxidant capacity of oils and blends (Mean±SD)

Sample	IC_{50} of DPPH radical scavenging activity (µg/ml)	Total antioxidant activity (mg/g ascorbic acid)
Mustard Oil	9.36±0.08	58.66 0.23
Palm Oil	11.01 0.24	51.13 1.10
Rice Bran Oil	10.12 0.40	56.86 0.80
Blend A	8.45 0.05	60.86 0.11
Blend B	9.15 0.57	56.6 0.20
Blend C	9.94 0.39	54.86 0.11
CD*	0.564	1.167

has been reported that oleic acid is more stable towards oxidation during storage and cooking (Bastida and Muniz, 2001).The IC_{50} values of mustard oil and rice bran oil corroborated by earlier finding (Siger *et. al*., 2007).The order of effectiveness of oils in inhibiting free radicals was compared with the standard and as follows: ascorbic acid > mustard oil > rice bran oil > palm oil and of blends was: ascorbic acid > blend A > blend B > blend C. However, all blends showed improved antioxidant activity in comparison to individual oils.

Total antioxidant activity: Antioxidant activity of ascorbic acid has been used as a standard reference from which the plants extracts with potent antioxidant capacity are being compared (Aderogba *et. al*., 2005). Highest total antioxidant activity was found in fresh crude mustard oil followed by rice bran oil i.e., 58.66 mg/g and 56.66 mg/g, respectively (Table 2). Blend A showed maximum total antioxidant capacity i.e., 60.86 mg/g. The total antioxidant activity is contributed by combination of various compounds namely phenols with flavonoid content, anthocyanins, glucosinolates and vitamin C (Alessendra, 2017). Mustard oil is rich in tocopherol that acts as a preservative towards rancidity. Crude mustard oil also contains sulphur compounds that are broken down into thermo labile and non-volatile compounds (Devinat *et. al*., 1980). These sulfur compounds contribute to oxidative stability of the oil by scavenging free radicals (Barnard *et. al.,* 1958). Crude palm oil contain carotenoids (â-carotene) and vitamin E (á, â and ã tocotrienols) as minor components. Crude palm oil or mildly refined palm oil is believed to have has seventeen times more carotenoids than carrots. It is believed that both carotenoids and vitamin E confer synergistic protection against auto-oxidation and photo-oxidation of unsaturated triglycerides (Haila *et. al*.,1996). This might be the reason for the highest total antioxidant capacity of blend A (40:40:20).

***Physical characteristics*:** Density, refractive index and viscosity of oils and blends is reported in table 3. Densities of the ternary blends were found to be comparable to those of single oils. Highest density was observed in mustard oil and blend C i.e., 0.9218 and 0.9181 g/ml, respectively. Maximum density indicated the presence of unsaturated fatty acid in highest amount. Refractive index was highest in mustard oil and blend C i.e., 1.4697 and 1.4770, respectively. Refractive index varied with the degree of unsaturation in the fatty acid chains and its length. As the unsaturation level and chain length increased, refractive index also increased. Palm oil (43.30 cP) was found to be most viscous. Maximum viscosity was also observed in blend A (34.78cP). The phenomenon is dependent upon the molecular structure. Reduction in viscosity is highly influenced by the presence of unsaturated fatty acid (Kim *et. al*., 2010).The findings were corroborated with earlier report demonstrating that viscosity of natural oils and fats did not contrast much

Table 3: Physical characteristics of oils and blends (Mean±SD)

Sample	Density (g/ml)	Refractive Index	Viscosity (cP)
Mustard Oil	0.9218±0.05	1.4697±0.01	36.74±0.11
Palm oil	0.9062±0.01	1.4554 ±0.02	43.30 ±0.16
Rice bran oil	0.9156±0.03	1.4681±0.05	38.00±0.10
Blend A	0.9012±0.09	1.4673±0.01	34.78±0.12
Blend B	0.9153±0.02	1.4677±0.03	32.94±0.09
Blend C	0.9181±0.03	1.4770±0.01	30.28±0.07
CD*	0.112	0.115	0.190

cP-centipoise,*=Significant at 5%

(Timms, 1985). As shown in table 3, oils and blends with higher density and refractive index showed lower viscosities.

***Chemical characteristics*:** Peroxide value and free fatty acid of oils and blends were determined at room temperature stored for 28 days.

***Peroxide value*:** The change in peroxide values of oils and blends during the storage period are illustrated in Table 4. Highest peroxide increase was seen in rice bran oil and blend C from 19.01 mEq/kg to 27.06 mEq/kg and 11.15 to 16.19 mEq/kg, respectively. Although, high peroxide value was observed in palm oil and rice bran oil. However, no rancidity was noticed for these oils. High peroxide values of palm oil and rice bran oil were reported earlier (Sheila *et. al*., 2004). The greatest upper limit for peroxide value is 20 mEq/kg (Bureau of Indian Standards, 2000) and fresh oils generally have peroxide value below 10 mEq/kg. Although the initial peroxide value of palm oil and rice bran oil was higher, the peroxide values of blends did not increase to a considerable degree i.e., not above the limit. This stability of blends might be due to the nutritional contribution of rice bran oil minor components (tocopherol, tocotrienols and oryzanol). Blends with higher palm oil, lowered the peroxide value. As shown in the table, blends having high volume of palm oil followed the similar trend (Naghshineh *et. al.,* 2010).

Table 4: Changes in peroxide value (mEq/kg) of oils and blends over a storage period of 28 days. (Mean±SD).

SAMPLE	0 day	7 days	14 days	21 days	28 days
Mustard oil	3.25±0.11	4.178±0.19	4.84±0.11	5.12±0.11	6.83±0.11
Palm oil	11.94±0.19	13.09±0.11	13.96±0.19	14.72±0.11	15.65±0.11
Rice bran oil	19.0±0.01	23.32±0.19	24.81±0.11	25.41±0.23	27.06±0.11
Blend A	8.04±0.11	9.31±0.11	10.40±0.11	11.62±0.11	13.06±0.01
Blend B	8.89±0.11	10.63±0.11	12.88±0.11	14.42±0.10	16.62±0.11
Blend C	9.44±0.37	12.21±0.22	14.27±0.06	16.42±0.11	18.85±0.01

***Free fatty acid value*:** The changes in free fatty acid during the storage period are reported in Table 5.Free fatty acid levels are expressed in % as oleic acid. Highest increase in free fatty acid was found in rice bran oil and blend C from 2.75 to 4.74 % and 2.43 to 4.13 %, respectively. It was observed that the formation of free fatty acids increased as the period of storage increased in oils and blends. It was reported that rice bran oil is highly influenced by the presence of lipase enzyme, thereby, elevating the rate of hydrolysis by 10-20% in a day or up to 70% in a month imparting a dark color to the oil. Blend A with low free fatty acid indicated that it contained less polyunsaturated fatty acids which were more vulnerable to oxidation. According to Krishna *et. al*., (2006), higher levels of oryzanol in rice bran oil contribute to the higher free fatty acid values. As per the study conducted by Gulla *et al.,* (2010), the blends with lower palm oil volume showed highest increase in free fatty acid levels. As of the vulnerability of oil to enzymatic hydrolysis, the free fatty acid levels may vary with age and storage history. In spite of the fact that rice bran oil showed high initial free fatty acid, the blends showed low free fatty acid than the single oil. Melton et al (1994) had contradicted that the estimation of free fatty acid as a measurement of deterioration in oils and fats quality is polemical, because he discovered that free fatty acids are volatile and can be lost by means of steam distillation.

Conclusion

Blending mustard oil with palm oil and rice bran oil resulted in oil blends with improved physico-chemical and biochemical characteristics. According to the results,

Table 5: Changes in free fatty acid (%) over a storage period of 28 days (Mean±SD)

Sample	0 days	7 days	14 days	21 days	28days
Mustard oil	1.20±0.15	2.57±0.15	3.05±0.41	4.41±0.16	5.17±0.16
Palm oil	4.62±0.41	5.25±0.15	6.11±0.16	7.87±0.01	8.343±0.01
Rice bran oil	5.48±0.16	6.86±0.01	7.22±0.16	8.44±0.41	9.43±0.16
Blend A	4.07±0.16	4.46±0.01	4.98±0.02	5.85±0.01	6.77±0.21
Blend B	4.55±0.16	5.003±0.01	5.61±0.00	6.29±0.01	7.78±0.16
Blend C	4.83±0.16	5.46±0.01	6.28±0.27	7.19±0.16	8.23±0.16

*=Significant at 5%

blend A (40:40:20) had better oxidative stability than the other blends as the increase in peroxide value, free fatty acid during the storage was least. Viscosity of blend A was lowest. Lower value of viscosity and low melting point are highly desirable to the consumers. Blend A can be categorized as oil with highest antioxidant potential. Blend A contained mustard oil which has adequate amount of omega 3 fatty acids and high palm oil volume in comparison to other blends that exhibited better polymorphic stability. Also, contained rice bran oil in appreciable amount which is rich in antioxidants. Blend A contained lesser amount of polyunsaturated fatty acids which are supposed to be more susceptible to oxidation than monounsaturated fatty acid. As per the WHO recommendation, blend A ratio of SFA:MUFA:PUFA and omega 6:omega 3 was closer to ideal ratio of 1:1.5:1 and 5-10, respectively. Taking into account the merits and demerits of individual oil in culinary purposes, blended oils appear to be just as or even more suitable than single oil for cooking. The summarized conclusion of the above investigation is that mixing mustard oil with palm oil emphatically improved the stability of the blends and it turn out to be nutritionally rich by adding appropriate amount of rice bran oil.

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