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Effect of Storage on Physico-chemical Characteristics and Fatty Acid Composition of Selected Oil Blends

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ABSTRACT An attempt was made to prepare oil blends with SFA:MUFA:PUFA ratios very close to the recommended ratio by admixing oils with different fatty acid composition, in the ratios of 80:20 and 20:80 because of its important health benefits and the possibility of developing nutritionally superior oils with recommended fatty acid ratios and their effect during storage in rice bran and mustard based blends with sesame oil as control, was studied. These blends were stored for 12 months and their physico-chemical changes and fatty acid composition were studied every month till the end of the storage period. Significant changes ($p<0.005$) were observed. Slight variations of increase in saturated fats and decrease in unsaturated fats were seen over time. Fatty acid composition and changes during storage of control indicated that control was efficient in C-18:3. During storage there is a gradual increase in C-16:0 from 10.03 to 11.89, C-18:0 from 5.26 to 5.86, and a gradual decrease in C-18:1 from 37.94 to 33.05, C-18:2 from 46.74 to 44. The principal fatty acid seen in sesame-rice bran blends were C-18:1 at a level of 45.65 and 38.35 followed by C-18:2, 29.66 and 39.16 and C-16:0 at a level of 16.54 and 13.35 respectively while C-18:0 was present in low quantities and C-18:3 was found to be negligible. The major fatty acids seen in sesame-cottonseed blends were C-18:2 at 43.82 and 37.95 followed by C-18:1 at 38.55 and 40.77 and C-16:0 at 11.8 and 17.58 respectively for the blends of 80:20 and 20:80. Lesser quantities of C-18:0 of 4.45 and 2.44 and C-18:3 of 0.68 and 0.77 were also observed.

INTRODUCTION

For many years, modification of original fats by means of direct blending with other fats, fractionation, hydrogenation and inter-esterification has been attempted to improve the fat functionalities and thus optimize their application in food products. Of the modification techniques, direct blending of fats is the method of choice as it has been considered to be a cheap and non-destructive technique. The technique has been used to modify the suitability of several underutlised fats and oils (Nor Aini and Sabariah 2005 and Nor Hayati et al. 2002). There are basically three parameters to adjudge any oil as the healthiest cooking oil that is, ratio of saturated / mono unsaturated / polyunsaturated fatty acid, ratio of essential fatty acids (Omega 6/ Omega 3) and presence of natural antioxidants (White 2000).

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The oils can be blended even to derive the protective advantage due to the presence of specific ingredients that offer protection against oxidation to improve frying recyclability (Toliwal et al. 2005).

Several studies have been carried out on elucidating the physical and chemical properties of oil blends involving antioxidant properties of sesame oil which provides high levels of antioxidants and lignins. Because of this, sesame oil is stable, and when mixed with other oils, actually increases the stability of the blend (Nirmala et al. 1996 and Shankar et al. 2003). Rice bran is considered to be an important edible oil due to the presence of high percentage of unsaturated fatty acids and certain nutritionally and medicinally important minor constituents such as tocopherols, tocotrienols, sterols, oryzanol etc. Taking advantage of the valuable fact that micronutrient levels are so adequate in rice bran oil, it was considered that value of any other edible oil could be remarkably increased by addition of even small amounts of rice bran oil (Adhikari 2002). It is this characteristic of rice bran oil coupled with sesame oil's naturally present antioxidants that rice bran oil had been blended with sesame oil. The amount of monounsaturated content as isomers and absence of conjugated double bonds in mustard are another important criteria for establishing their stability upon deep frying. Taking advantage of this property, mustard was chosen to be blended with sesame oil.

Oxidative stability of oil can be improved by modification of fatty acid composition (Tatum and Chow 2000). Fatty acid composition and functional properties of oils can be modified by hydrogenation, inter-esterification, genetic modification, and blending of different oils. Blending of oils modifies fatty acid composition without any chemical or biological process (Liu and White 1992).

The properties of the related blends have not yet been investigated and reported in detail, and it is essential to understand the physical properties in the function of the chemical composition of the blends in order to better predict their functionality in more complex food systems (Nor Hayati et al. 2009). Therefore, this study was carried out to determine the physico-chemical characteristics of rice bran oil , mustard oil blends with sesame oil in terms of fatty acid composition, specific gravity (30°C), refractive index, lovibond colour (Y+5R), peroxide value (meq/kg), free fatty acids (percent oleic acid), paraanisidine value (PAV), totox value, (TV), thiobarbituric acid value (TBA), kreis test, free fatty acid value (FFA), iodine value (IV).

MATERIALS AND METHODS

Materials

All oils were available locally and were purchased in bulk from the Oil Millers Association of Hyderabad, India. Sesame oil has been used as control and rice bran oil and mustard oil were used as experimental oils. Sesame oil was blended with rice bran, and mustard in the ratios of 80:20 and 20:80 in the laboratory using a blender cum mixer and stored in PET bottles at room temperature.

Analysis

All individual blends were analysed in triplicates for their physico-chemical parameters initially and therein every month for 12 months. Analysis was done using standardized methods for specific gravity, refractive index, iodine value (Raghuramulu et al. 2003), Lovibond colour (Mathur 1983), Peroxide value (AOCS 1973). Free Fatty Acids (AOCS 1990), Para - anisidine value (IUPAC 1987), Totox value (Akoh and Min 1998), Thio barbituric acid value (BSI 2000), Kreis test (Toteja 1990) and Fatty acid analysis wherein the methyl esters of fatty acids were separated and determined quantitatively by gas chromatography (AOCS 1990). The data was tabulated and subjected to two way analysis of variance (ANOVA), tests of significance, means and standard deviation.

RESULTS AND DISCUSSION

Physical Characteristics

Specific gravity, refractive index and lovibond colour units measured physical characteristics of the oils and their blends. Results of the study are reported in Table 1. A steady increase in specific gravity was observed in the control and the blends. The specific gravity of fresh sesame oil was 0.922 units and the specific gravity of oil blends ranged from 0.854 units in sesame-mustard (80:20) to 1.19 units for a period of one year. It was observed that sesame-mustard blends were significantly different from control throughout the analytical period and sesame- rice bran (80:20) was only different in the first month of analysis though clearly an increasing trend in specific gravity on storage was seen. The rise in the specific gravity observed in the study may be attributed to the formation of polymeric fractions of high molecular weight.

The refractive index of a substance varies with the wavelength of light used in its measurements as seen in Table 1. No significant differences were observed during storage for refractive index of oil blends up to 12 months and it ranged from 1.465 to 1.63 units in the oil blends which were however seen to be significantly different from control. Unlike the present study, Murthi et al. (1987) analysed the refractive index only at the initial stage, whereas (Premavalli et al. (1998), Madhura and Arya (1998) , Agarwal et al. (2000), Semwal and Arya (2001), Padmavathy et al. (2001), reported the changes in refractive index on storage which did not change significantly during the entire period of storage at lower temperatures, but increases were higher at high temperatures. The data on refractive index showed a consistency without any change, which reflected the stability of the oil blend.

Table 1: Changes in the specific gravity, refractive index, and lovibond colour value of the chosen oil blends during storage

Parameter	*Sample*	*Ratio*	*Storage period-months*				
			0	*3*	*6*	*9*	*12*
Specific Gravity	Control	100	0.922±0.001	1.018±0.035	1.05 ±0.035	1.135±0.029	1.162±0.036
	Sesame-Rice bran	80:20	0.901 0.001*	1.002±0.025	1.04 ±0.035	1.155±.0251	1.19 ±0.0368
		20:80	0.922±0.0018	1.025±0.030	1.05 ±0.030	1.149±0.035	1.18 ±0.0368
	Sesame-Mustard	80:20	0.8540.0025*	0.9080.0003*	1.090.0007*	1.1280.0005*	1.18 ±0.0007*
		20:80	0.896±0.0021*	1.02 ±0.0005*	1.19 ±0.0063*	1.12 ±0.0007*	1.19 ±0.0006*
Refractive Index	Control	100	1.4650.0002	1.465±0.0001	1.465±0.00009	1.465±0.0001	1.465±0.0001
	Sesame-Rice bran	80:20	1.460.0001*	1.46 ±0.00005*	1.46±0.00004*	1.46 ±0.00005*	1.46±0.00005*
		20:80	1.46 ±0.0002*	1.46±0.0001*	1.46±0.0002*	1.46 ±0.0001*	1.46±0.00009*
	Sesame-Mustard	80:20	1.35 ±0.106*	1.45±0.0056*	1.46±0.0007	1.47 ±0.0005*	1.63±0.0009*
		20:80	1.44 ±0.0675*	1.47 ±0.0007*	1.48±0.0014*	1.50 ±0.0007*	1.52±0.0228*
Lovibond Colour Value	Control	100	15.26 ±0.305	102.3 ±0.3000	110.8 ±0.3	115.18 ±0.236	116.35±0.312
	Sesame-Rice bran	80:20	12.56 0.152*	104.70.246*	108.780.24*	110.3 ±0.3	112.7 ±0.2*
		20:80	12.53 ±0.351*	80.70 ±0.200*	87.76±0.305*	90.2 ±0.2	92.3 ±.0.300*
	Sesame-Mustard	80:20	15.530.305*	103.430.152*	110.430.907*	114.950.589*	116.15±0.653*
		20:80	12.63 ±0.378*	80.46 ±0.585*	87.36±0.945*	90.00±0.529*	92.10 0.634*

Mean±S.D
*Significant at 5% level.

Pure fats and fatty acids are colourless and devoid of spectral properties in the visible range. However, all natural fats and oils contain pigments, which have more or less characteristic absorption patterns. In the past, oils have almost invariably been graded for colour by visual comparision with such standards as the Lovibond red and yellow glasses (Gupta 2005). The changes in the lovibond colour value of selected oil blends during storage are presented in Table 1. The change in the colour in control was seen to be from 15.26 to 116.35 units from the initial to the final 12 months of storage. All the oil blends showed an increase in colour as the storage period increased, ranging from 12.53 and 116.1 units and were seen to be significantly different from control. The intensity of the colour was seen to be lighter in rice bran-sesame (20:80), probably because of its refined state and were seen to have lower lovibond colour values of 92.3 units. The colour of sesame was deep yellow and that of rice bran, a lighter yellow. Darkening of the colour may be attributed to several factors such as storage conditions, condition of sterilization, and oxidative effects during storage. Similar observations were made by Sundararaj et al. (2005) who studied the effect of storage on lovibond colour units on rice bran oil at two different temperatures for nine months.

Chemical Characteristics

Chemical changes in oils were measured by peroxide value, free fatty acid value, para-anisidine value, totox value, thiobarbituric acid value, kreis test, and iodine value.

Peroxide Value (PV)

Storage changes in vegetable oil blends were measured by the peroxide formation, the final products of oxidation. The changes in the peroxide values of selected oil blends during storage are seen in Table 2. PV of oil blends and control stored for 12 months registered a progressive increase with the storage period. Steady increase in the blends according to the extent of oxidation caused by the formation of hydro peroxides during fat oxidation was observed. It was observed that the increase in PV of control was from 1.83 meq/kg to 14.79 meq/kg during the 12 months of storage.

Table 2: Monthly changes in the peroxide value (meq/kg) of oil blends during storage

Month	*Control*	*Sesame: rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	1.83±0.0088	3.65±0.0045*	3.68±0.0020*	7.48±0.055*	5.86±0.050*
1	6.20±0.0896	5.99±0.1625	5.91±0.1800*	8.01±0.025*	6.65±0.540*
2	8.14±0.1563	8.08±0.1625	7.42±0.0896*	10.37±0.05*	7.21±0.04*
3	9.50±0.1625	9.07±0.0896*	8.13±0.1001*	12.88±0.05*	9.87±0.05*
4	10.37±0.0896	10.03±0.1625*	9.75±0.1800*	14.32±0.05*	11.87±0.05*
5	10.82±0.1625	10.64±0.0750	9.96±0.0896*	15.29±0.06*	12.68±0.04*
6	11.49±0.1620	11.04±0.1819*	10.94±0.100*	16.26±0.03*	14.72±0.05*
7	12.14±0.0350	11.80±0.0971*	11.7 ±0.1338*	17.03±0.02*	14.11±0.04*
8	12.92±0.0890	11.94±0.1625	11.99±0.180	19.31±0.04*	15.22±0.05*
9	13.64±0.1800	13.08±0.0896*	12.78±0.162*	19.88±0.05*	16.72±0.05*
10	13.93±0.0890	13.59±0.1625*	13.13±0.180*	20.41±0.05*	17.81±0.04*
11	14.34±0.0960	14.21±0.2433	13.67±0.112*	22.19±0.05*	18.22±0.06*
12	14.79±0.1560	14.74±0.1625	13.91±0.089*	25.70±0.09*	19.91±0.04*

Mean±S.D
*Significant at 5% level.

The initial PV was seen to be higher in the blends in comparison to the control between 0 month to 12 months of storage, showing the oxygen uptake by the oil in the blends studied. However, the increase in PV was seen to be the highest in sesame-mustard (80:20) which increased before from 7.4 8 meq/kg to 25.70 meq/kg followed by sesame-mustard (20:80) from 5.86 meq/kg to 19.91 meq/kg. Although peroxides are possibly not directly responsible for the taste and odour of rancid fats, their concentration as represented by the PV is often useful in assessing the extent to which the rancidity has advanced. Fresh oils usually have peroxide values below 10 meq/kg, and a rancid taste often begins to be noticeable when the PV is above 20 meq/kg (PFA 2005). Though there is a progressive increase in PV upto 12 months of storage it did not exceed the limits specified by PFA in most of the blends except sesame-mustard (80:20), which has exceeded by 11 months of storage.

Among the 4 blends sesame-rice bran (20:80) had the lowest mean PV (3.68 -13.91 meq/kg.) with a significant difference from control after a period of 12 months. Contradictorily, Murthi et al. (1987) reported the changes in the PV of edible oils stored at room temperature were not regular, and Schnepf (1991) reported the changes to be slow but consistent throughout the 20 week (5 month) study. The rate of degradation of hydro peroxides was seen to be higher than control in case of the sesame-mustard blends. There was a steady increase in PV of sesame-rice bran oil blends during storage, but this increase was seen to be the least in comparison to control. The nutritional contribution of the three minor components of tocopherol, tocotrienols and g-oryzanol in rice bran oil blends may have conferred this greater oxidative stability.

Para-anisidine Value (PAV)

The formation of secondary oxidation products under storage conditions every month was determined by PAV and is presented in table 3. The blended oils were compared for their PAV with control during storage. The value for fresh sesame oil (control) was 1.04 units and after one month. After 10 months, the PAV increased slightly to 1.28, thereafter 1.46 and 1.63 units. The same trend was seen in the blends. It was seen that sesame-rice bran (20:80) had a very high PAV of 4.67 units, which rose to 5.56 units after 12 months of storage. This high initial value could be attributed to higher storage values of the bran, though there aren't any specifications in regard to PAV in Bureau of Indian Standards or Prevention of Food Adulteration act for either single or blended oils.

The initial PAV of sesame-mustard (80:20) was seen to be 1.86 units and for sesame-mustard (20:80) is 1.32 and the increase after a storage period of 12 months was seen to be 3.13 and 2.23 units respectively. The usefulness of the PAV in predicting the quality of oils remains a matter of debate. Typically the PAV will increase as aldehydes are produced and then decrease when the aldehydes reach a certain level and subsequently are further oxidized or participate in dimerization or condensation reactions (Schnepf 1991).

Table 3: Monthly changes in the para-anisidine value of oil blends during storage

Month	*Control*	*Sesame:Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	1.04±0.0165	1.16±0.005*	4.67±0.016*	1.86±0.011*	1.32±0.06*
1	1.04±0.0188	1.17±0.051	4.67±0.039*	2.03±0.02*	1.47±0.05*
2	1.05±0.0173	1.17±0.248*	4.67±0.018*	2.21±0.04*	1.72±0.04*
3	1.05±0.025	1.18±0.018*	4.67±0.005*	2.34±0.004*	1.77±0.004*
4	1.07±0.018	1.18±0.024	4.67±0.039*	2.42±0.006*	1.82±0.055*
5	1.07± 0.024	1.19±0.008*	4.72±0.018*	2.58±0.005*	1.85±0.005*
6	1.08±0.024	1.19±0.032*	4.74±0.005*	2.56±0.11*	1.92±0.004*
7	1.09±0.056	1.20±0.082*	4.74±0.202*	2.64±0.05*	1.94±0.34*
8	1.09±0.018	1.20±0.024	4.74±0.039	2.75±0.012*	1.96±0.005*
9	1.15±0.039	1.20±0.018*	4.74±0.024*	2.79±0.004*	2.06±0.005*
10	1.28±0.018	1.25±0.024*	4.92±0.039*	2.94±0.005*	2.18±0.004*
11	1.46±0.012	1.32±0.049*	5.28±0.021*	3.04±0.005*	2.18±0.005*
12	1.63±0.017	1.44±0.024*	5.56±0.018*	3.13±0.005*	2.23±0.06*

Mean±S.D
*Significant at 5% level.

Totox Value (TV)

Similarly changes in PV and PAV, TV showed significant concomitant rise during the entire storage period. Total oxidation value, the so called Totox value, calculated from twice the peroxide value plus the p-anisidine value, is another useful indicator of measuring the onset of progressive deterioration in oil and provides information regarding progression of the formation of primary and secondary oxidation products (Akoh and Min 1998). In Table 4 the changes in the TV of the oil blends during storage is presented and compared with control. The TV of control was seen to increase from 4.7 to 31.21 units. These increases that could be seen were a parameter of the increase of peroxide value and p-anisidine value, hence the increases were significant to that of peroxide values of the oil blends. The lowest increase was seen in sesame-rice bran (80:20) from 8.46 to 30.92 units and the highest increase was seen in sesame-mustard(80:20) from 16.83 units to 54.54 units. The other two blends of rice bran and mustard showed intermediate values.

Thiobarbituric Acid Value (TBA)

Thiobarbituric acid value of control and the blends are displayed in Table 5, Significant increase in TBA values could be noticed between the initial and final periods of storage in the blends indicating the development of off -flavour, but there were not large enough to cause perceptible changes upto 12 months of storage at room temperature. The value ranged from 0.007 units to 0.619 units in control for a period of one year. Sesame-rice bran (80:20) recorded a value of 0.1 units initially and 0.594 units after 12 months of storage which recorded the lowest

Table 4: Monthly changes in the totox value of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	4.7 ±0.034	8.46±0.0066*	12.03±0.018*	16.83±0.08*	13.05±0.16*
1	13.64±0.1903	13.15±0.3469	16.49±0.396*	18.05±0.070*	14.78±1.04*
2	17.33±.2.162	17.33±0.3287	19.51±0.346*	22.95±0.140*	16.15±0.120*
3	20.05±.3469	19.32±0.190*	20.93±0.204	28.10±0.115*	21.56±0.105*
4	21.81±.1903	21.24±0.346*	24.17±0.396*	31.06±0.117*	25.51±0.155*
5	22.71±0.346	22.47±0.151	24.64±0.19*	33.16±0.125*	27.31±0.094*
6	24.06±0.346	23.27±0.392*	26.62±0.204*	34.91±0.232*	31.68±0.359*
7	25.37±0.098	24.8±0.162*	28.14±0.468*	36.71±0.100*	30.04±0.107*
8	26.93±0.190	25.08±0.346	28.72±0.396	41.37±0.070*	32.30±0.115*
9	28.43±0.396	27.36±0.190*	30.3±0.346*	42.55±0.104*	35.42±0.105*
10	29.14±0.018	28.43±0.024*	31.18±0.039*	43.77±0.115*	37.68±0.085*
11	30.14±0.198	29.74±0.533	32.62±0.24*	47.42±0.105*	38.63±0.127*
12	31.21±0.32	30.92±0.346*	33.38±0.19*	54.54±0.204*	42.0±0.145*

Mean±S.D
*Significant at 5% level.

Table 5: Monthly changes in the thiobarbituric acid value of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	0.007±0.0015	0.01 ±0.0015*	0.20 ±0.0041*	0.852±0.001*	0.962±0.001*
1	0.097±0.0015	0.091±0.002	0.189±0.003*	0.933±0.002*	1.04 ±0.003*
2	0.121±0.0020	0.012±0.002*	0.28 ±0.0015*	1.05 ±0.003*	1.29 ±0.001*
3	0.211±0.0024	0.195±0.001*	0.32 ±0.0008*	1.103±0.002*	1.39 ±0.002*
4	0.295±0.0015	0.252±0.0024	0.396±0.0035	1.27 ±0.002*	1.25 ±0.001*
5	0.32 ±0.00247	0.29 ±0.0007*	0.41 ±0.0015*	1.31 ±0.001*	1.57 ±0.001*
6	0.399±0.002	0.3 ±0.00306*	0.49 ±0.0008*	1.484±0.009*	1.67 ±0.081*
7	0.427±0.013	0.372±0.028*	0.545±0.042*	1.502±0.001*	1.745±0.003*
8	0.49 ±0.0015	0.41 ±0.0024	0.59 ±0.0035	1.626±0.003*	1.825±0.004*
9	0.516±0.003	0.482±0.001*	0.615±0.002*	1.752±0.001*	1.944±0.002*
10	0.57 ±0.0015	0.51 ±0.0024*	0.67 ±0.0035*	1.892±0.001*	2.012±0.003*
11	0.592±0.001	0.568±0.004*	0.692±0.001*	1.954±0.003*	2.105±0.002*
12	0.619±0.0020	0.594±0.024*	0.72 ±0.0015*	2.054±0.003*	2.282±0.001*

Mean±S.D
*Significant at 5% level.

values and sesame-mustard blends TBA value ranged from 0.852 units to 2.282 units for a period of 12 months. The results of the present study are in agreement with that of Semwal et al. (1996), and Semwal and Arya (2001) who reported thiobarbituric acid values on storage for a few oil blends and confirmed the increase as storage period increased.

Kreis Test

The changes in the kreis test value of selected oil blends during storage can be seen in Table 6. Control and the blends had a kreis test value of 0.13 initially and as the period of storage increased the kreis test value increased. The kreis test, which is qualitative or roughly quantitative, depends on the presence of a specific class of compounds, namely acetals of epihydrin aldehyde or its homologs (Toteja et al. 1990).

Sesame- rice bran (80:20) recorded lower values than control and hence was significantly better than control through-out the period of storage except during the seventh and eighth month of storage, whereas in the sesame-rice bran (20:80) it was seen that except during 8th month of storage all were significantly better than control. According to kreis test value, the blends and control were seen to be within the normal limits and only incipient rancidity could be seen, though however by the end of storage period all the blends were significantly superior to control. Relevant studies regarding kreis test values in blends have not been reported.

Free Fatty Acid Value

Free fatty acids occur in fats as a result of enzymatic hydrolysis by lipases, metal ions acting as free radicals or at an elevation of tempera-

Table 6: Monthly changes in the Kreis test value of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	0.13 ±0.00208	0.11 ±0.0026	0.10 ±0.0011	1.63 ±0.003*	1.944±0.003*
1	0.14 ±0.0025	0.12 ±0.0041*	0.127±0.005*	1.843±0.001*	2.053±0.001*
2	0.188±0.0035	0.162±0.004*	0.150±0.002*	2.045±0.003*	2.292±0.001*
3	0.258±0.0041	0.199±0.002*	0.187±0.001*	2.102±0.002*	2.322±0.002*
4	0.294±0.0025	0.228±0.004*	0.213±0.005*	2.385±0.003*	2.633±0.002*
5	0.31 ±0.0041	0.29 ±0.001*	0.27 ±0.002*	2.464±0.002*	2.743±0.001*
6	0.396±0.004	0.31 ±0.0051*	0.299±0.001*	2.475±0.048*	2.785±0.101*
7	0.439±0.019	0.446±0.039	0.371±0.023*	2.512±0.002*	3.182±0.001*
8	0.49 ±0.0025	0.46 ±0.0041	0.40 ±0.0057	2.634±0.003*	3.283±0.001*
9	0.52 ±0.0057	0.51 ±0.0025*	0.46 ±0.0041*	2.513±0.001*	3.384±0.003*
10	0.583±0.002	0.564±0.004*	0.492±0.005*	2.643±0.002*	3.414±0.002*
11	0.618±0.002	0.579±0.007*	0.511±0.003*	2.703±0.001*	3.553±0.002*
12	0.624±0.0035	0.59±0.0041*	0.543±0.002*	2.853±0.002*	2.614±0.002*

Mean±S.D
*Significant at 5% level.

Table 7: Monthly changes in the free fatty acid value (% oleic acid) of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	0.53±0.0066	0.58± .0050*	0.64± .0107*	1.06±0.035*	0.59 ±0.002*
1	0.65±0.0000	0.66± .0005*	0.69± .0010*	1.17±0.002*	0.73 ±0.003*
2	0.89±0.0000	0.81±0.000*	0.86±0.000*	1.28±0.002*	0.86 ±0.0005*
3	0.99±0.0005	0.91±0.000*	0.98±0.000*	1.45±0.03*	0.912±0.0005*
4	1.02±0.0005	1.04± .0005*	1.10± .0005*	1.50±0.005*	0.966±0.003*
5	1.19±0.0005	1.16±0.000*	1.14± .0005*	1.61±0.002*	1.21 ±0.001*
6	1.35±0.0005	1.23±0.001*	1.26± .0000*	1.73±0.004*	1.45 ±0.001*
7	1.47±0.0352	1.34± .0971*	1.37± .1338*	1.89±0.001*	1.67 ±0.001*
8	1.54±0.0003	1.5 ±0.0004	1.48±0.0007	1.94±0.004*	1.83 ±0.002*
9	1.71± .0005	1.59± .0005*	1.62± .0005*	2.46±0.003*	2.09 ±0.002*
10	1.82±0.0000	1.69± 0058*	1.82± .0005*	2.61±0.003*	2.32 ±0.003*
11	1.93±0.0000	1.82±0.001*	1.91± .0005*	2.89±0.001*	2.58 ±0.001*
12	2 ±0.0003	1.93± .0004*	2.01± 0003*	3.04±0.001*	2.74 ±0.004*

Mean±S.D
*Significant at 5% level.

ture. The changes in free fatty acid values expressed in percent oleic acid during storage for a period of 12 months is depicted in Table 7. The initial free fatty acid value of control was seen to be 0.53, which increased slowly and steadily to 2 after a storage period of 12 months. The free fatty acid values of the rice bran blends with sesame were seen to range from 0.58 to 1.93 in 80:20 blend and 0.64 to 2.01 in the 20:80 blend. The free fatty acid value of the rice bran blends were seen to be on the higher side but significantly lower than control. This may be due to the presence of an active lipase in rice bran, which upon milling is activated and quickly begins to hydrolyse triglycerides into free fatty acids, diglycerides and mono glycerides, and the lipase eventually will decompose all the triglycerides present over a period of several months. Similar changes were reported by Handoo et al. (1992 a, b,) Sarojini and Bhavani (1997), Semwal and Arya (2001). The hydrolytic changes though not predominant, the formation of free fatty acids was found to increase with increase in time of storage. Though initial levels of free fatty acids were found to be different in the blends, rate of formation was found to be almost parallel, recording a drastic increase after 12 months of storage.

Iodine Value

Iodine value is an index of the unsaturation, which is the most important analytical characteristic of an oil. Data on changes in the iodine value of the selected oil blends during storage is presented in Table 8. It was observed that iodine value decreased gradually during storage in the oil blends studied. Maximum iodine value was 111.9 units initially in sesame-mustard (20:80) and

Table 8: Monthly changes in the iodine value of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	106.9±0.264	92.4±0.132*	97 ±0.264*	110.65±0.035*	111.94±0.025*
1	106.8±0.100	92.2±0.050*	96.8±0.100*	109.5 ±0.100*	111.33±0.057*
2	106.3±0.100	92 ±0.050*	96.4±0.100*	109.26±0.208*	110.33±0.251*
3	106.1±0.100	91.9±0.050*	96.3±0.100*	108.74±0.035*	109.33±0.152*
4	105.8±0.086	91.8±0.043*	96.1±0.086*	108.22±0.020*	109.13±0.015*
5	105.4±0.100	91.7±0.050*	95.9±0.100*	107.93±0.010*	108.94±0.030*
6	105.3±0.086	91.6±0.043*	95.7±0.264*	107.74±0.020*	108.25±0.025*
7	105.1±0.264	91.4±0.13*	95.4±0.100*	107.14±0.025*	108.03±0.015*
8	104.8±0.100	91.3±0.050	95.2±0.100*	106.93±0.015*	107.83±0.015*
9	104.5±0.100	91.1±0.050*	95 ±0.100*	106.45±0.030*	107.63±0.020*
10	103.8±0.100	91 ±0.050*	94.8±0.086*	105.93±0.015*	107.04±0.020*
11	103.5±0.086	90.9±0.043*	94.5±0.100*	105.63±0.020*	106.83±0.015*
12	103.2±0.100	90.6±0.050*	94.3±0.0866*	103.66±0.051*	106.63±0.015*

Mean±S.D
*Significant at 5% level

110.6 units in sesame-mustard (80:20) which decreased to 103.6 and 106.6 units by the end of the storage period. Minimum iodine value was 92.4 units in sesame-rice bran (80:20), which further decreased to 90.6 units on storage. Slow decrease in iodine value of oil blends may be due to induction period where fat was oxidized slowly showing initiation stage of auto oxidation reaction. Rapid changes in iodine value of oil blends may be attributed to propagation of auto oxidation process where hydro peroxides are formed from free radicals in fatty acids generated in initiation stage or auto oxidation reaction. During the end of storage period slight change in iodine value was observed which might be due to termination stage of reaction. (Nasirullah et al.1991).

Effect of Storage on Fatty Acids

Nearly 91 people die every hour in the world due to heart attacks, as per WHO statistics. In urban India Coronary Artery Disease prevalence is around 7 percent compared to 3 percent in USA and less than 1 percent in Japan. Occurrence of first myocardial infarction before the age of forty is 5-10 times higher than in most populations worldwide. Quality of fat consumed can be the key factor that can explain this. It is necessary to obtain energy from fat in the right ratio (Raghu 2003).

Edible oils are important components of human foods and their variety and consumption depends on the availability in each country or region. Fatty acid composition of edible oils does not follow a standard pattern because this composition is modified by the amount of saturated, mono unsaturated and polyunsaturated fatty acids (Valenzuela 2002). According to current concepts, the influence of high fat intake on cardiovascular status depends on the fatty acid profile and the P:S ratio, both of which can be modified by fatty acids (Rao 1992). Use of different edible oils and blending them to make up for the deficient factors is essential. The possibility of developing nutritionally more suitable oils with recommended fatty acid ratios and their effect during storage is the point of discussion selected oil blends.

Fatty acid composition and changes during storage of control is shown in Table 9 and the results indicated that control is deficient in C-18:3. During storage there is a gradual increase in C-16:0 from 10.03 to 11.89, in C-18:0 from 5.26 to 5.86, and a gradual decrease in C-18:1 from 37.94 to 33.05, in C-18:2 from 46.74 to 44.24 units upto nine months of storage and faster changes from 10 to 12 months of storage and the proportion of SFA:MUFA:PUFA is 1:2.4:3 for the same. Saha (2001) in his studies in fatty acid composition of sesame oil derived on an S:M:P ratio of 1.0:1.6:1.7.

The fatty acid composition and changes during storage for sesame-rice bran blends studied is shown in Table 10 and sesame-mustard blends in Table 11. It was observed that there was a gradual increase in C-16:0, and C-18:0 but a decrease in C-18:1, C-18:2 and C-18:3. Murthy et al. (1996) studied the changes in fatty acid composition of oil blends during storage for 12 months and their results correlate to the present study.

Table 9: Changes in the fatty acid composition of control during storage (%)

Month	*16:0 (Palmitic acid)*	*18:0 (Stearic acid)*	*18:1 (oleic acid)*	*18:2 (linoleic acid)*	*18:3 (linolenic acid)*
0	10.03±0.0194	5.26 ±0.00305	37.94 ±0.0114	46.74±0.0187	Negligible
1	10.28±0.2516	5.29 ±0.17054	37.40 ±0.4633	46.78±0.1654	Negligible
2	10.59±0.2516	5.54 ±0.2757	36.95 ±0.9614	46.58±0.2203	Negligible
3	10.63±0.3013	5.57 ±0.3058	36.35 ±0.5473	46.33±0.8458	Negligible
4	10.67±0.3167	5.60 ±0.2019	36.15 ±1.8704	46.19±1.0010	Negligible
5	10.68±0.301	5.63 ±0.3	35.71 ±0.547	46.09±1.07	Negligible
6	10.83±0.301	5.69 ±0.381	35.28 ±0.440	45.89±1.072	Negligible
7	10.86±0.155	5.72 ±0.1705	34.87 ±0.900	45.75±1.091	Negligible
8	10.86±0.251	5.73 ±0.3058	34.21 ±0.717	44.89±1.988	Negligible
9	10.53±0.278	5.76 ±0.21	33.99 ±1.308	44.35±0.379	Negligible
10	11.17±0.251	5.79 ±0.2174	33.74 ±1.377	44.3 ±0.1450	Negligible
11	11.28±0.246	5.835±0.160	33.113±2.75	44.29±3.05	Negligible
12	11.89±0.316	5.867±0.156	33.05 ±2.106	44.24±1.06	Negligible

Mean±S.D
*Significant at 5% level

Table 10: Changes in the fatty acid composition of sesame- rice bran blends during storage (%)

Month	*16:0 (Palmitic acid)*		*18:0 (Stearic acid)*		*18:1(oleic acid)*		*18:2 (linoleic acid)*		*18:3 (linolenic acid)*	
	80:20	*20:80*	*80:20*	*20:80*	*80:20*	*20:80*	*80:20*	*20:80*	*80:20*	*20:80*
0	16.54±0.016*	13.35±0.029*	3.86±0.0452*	2.83±0.0030*	45.65±0.237*	38.35±0.254*	29.66±0.063*	39.16±0.006*	Negligible	0.7±0.0079
1	16.86±0.301*	13.45±0.307*	4.09±0.3058*	2.94±0.1618*	45.39±0.440*	38.21±0.130*	29.47±0.269*	39.10±0.125*	Negligible	0.69±0.0032
2	16.93±0.301*	14.12±0.251*	4.16±0.523*	3.26±0.1705*	44.37±0.139*	38.00±0.444*	29.46±1.023*	38.84±0.425*	Negligible	0.65±0.0371
3	17.17±0.251*	14.64±0.281*	4.25±0.5973*	3.84±0.1537*	44.01±0.150*	38.26±0.062*	29.40±0.684*	39.02±0.098*	Negligible	0.63±0.0513
4	18.15±0.301*	15.09±0.307*	4.36±0.3486*	3.909±0.381*	42.79±0.139*	38.26±0.027*	29.99±1.290*	39.21±0.379*	Negligible	0.62±0.0652
5	18.22±0.448*	15.25±0.251*	4.56±0.486*	4.03±0.17*	41.98±0.106*	38.24±0.15*	29.91±0.083*	39.07±0.165*	Negligible	0.61±.0074
6	18.24±0.305*	15.79±0.281*	4.80±0.118*	4.30±0.1537*	40.71±0.19*	38.08±0.062*	29.27±0.332*	38.01±0.098*	Negligible	0.60±.00045
7	18.59±0.505*	15.95±0.288*	4.88±0.1499*	4.52±0.3125*	39.77±0.078*	37.93±0.008*	28.80±0.472*	37.85±0.361*	Negligible	0.599±0.092
8	18.78±0.180*	16.46±0.307*	4.94±0.3058	4.64±0.2704	39.06±0.139*	37.48±0.461*	28.16±1.023*	37.54±0.409*	Negligible	0.589±0.054
9	18.79±0.251	17.29±0.301	4.96±0.17*	4.88±0.523*	38.57±0.15*	37.39±0.139*	27.57±9.425	37.22±1.023	Negligible	0.57±0.0472
10	19.27±0.301*	17.42±0.307*	5.21±0.3058*	4.69±0.3819*	38.01±0.139*	36.65±0.767*	27.36±0.332*	37.15±0.778*	Negligible	0.55±0.0466
11	19.85±0.971*	18.12±0.238*	5.506±0.314	5.025±0.216*	37.159±0.29*	36.208±0.55*	26.83±0.792*	36.68±0.41*	Negligible	0.5308±0.02
12	20.004±0.67*	18.59±0.251*	5.56±0.305	5.43±0.17	36.75±0.139*	35.83±0.15*	26.64±0.269*	35.99±0.837*	Negligible	0.521±0.006

Mean±S.D
*Significant at 5% level

Table 11: Monthly changes in the fatty acid composition of sesame-mustard blends during storage (%)

Month	*16:0 (Palmitic acid)*		*18:0 (Stearic acid)*		*18:1(oleic acid)*		*18:2 (linoleic acid)*		*18:3 (linolenic acid)*	
	80:20	20:80	80:20	20:80	80:20	20:80	80:20	20:80	80:20	20:80
0	5.5 ±0.0052*	5.72±0.0051*	5.55±0.0115*	5.42 ±0.007*	20.25±0.059*	20.43±0.010*	24.88±0.067*	24.78±0.110*	0.576±0.002*	0.596±0.002*
1	5.53±0.0015*	5.73±0.0012*	5.57±0.0110*	5.433±0.011*	20.25±0.027*	20.40±0.001*	24.86±0.011*	24.76±0.011*	0.575±0.001*	0.594±0.002*
2	5.56±0.0017*	5.74±0.0021*	5.58±0.0110*	5.45 ±0.011*	20.25±0.022*	20.36±0.022*	24.84±0.011*	24.75±0.009*	0.572±0.001*	0.592±0.001*
3	5.57±0.0021*	5.75±0.0027*	5.60±0.002*	5.47 ±0.011*	20.24±0.010*	20.33±0.007*	24.82±0.011*	24.73±0.012*	0.572±0.0007*	0.592±0.001*
4	5.61±0.0088*	5.77±0.0022*	5.62±0.007*	5.48 ±0.011*	20.16±0.026*	20.30±0.001*	24.81±0.002*	24.71±0.01*	0.573±0.001*	0.593±0.001*
5	5.63±0.0083*	5.80±0.0025*	5.63±0.0115*	5.80 ±0.001*	20.14±0.021*	20.27±0.027*	24.80±0.002*	24.70±0.001*	0.571±0.001*	0.592±0.001*
6	5.65±0.0001*	5.82±0.0052*	5.65±0.0001*	5.82 ±0.0001*	20.13±0.002*	20.26±0.002*	24.79±0.000*	24.68±0.00001*	0.570±0.0001*	0.591±0.0002*
7	5.64±0.0021*	5.83±0.0115*	5.66±0.0167*	5.82 ±0.0162*	20.03±0.017*	20.24±0.016*	24.75±0.021*	24.63±0.0120*	0.566±0.002*	0.590±0.0001*
8	5.66±0.0016*	5.84±0.0012*	5.67±0.0200*	5.85 ±0.0110*	19.83±0.012*	20.15±0.021*	24.73±0.017*	24.61±0.0047*	0.565±0.002*	0.586±0.002*
9	5.67±0.0100*	5.84±0.0158*	5.70±0.002*	5.87 ±0.0162*	19.60±0.001*	20.03±0.011*	24.71±0.012*	24.60±0.002*	0.563±0.002*	0.584±0.002*
10	5.68±0.0105*	5.86±0.0120*	5.70±0.0056*	5.88 ±0.0085*	19.47±0.020*	19.83±0.011*	24.70±0.002*	24.57±0.0156*	0.562±0.001*	0.583±0.001*
11	5.71±0.0020*	5.87±0.0167*	5.71±0.0066*	5.90 ±0.0025*	19.20±0.002*	19.76±0.025*	24.67±0.021*	24.57±0.010*	0.561±0.0007*	0.583±0.001*
12	5.72±0.0110*	5.90±0.0062*	5.73±0.0115*	5.92 ±0.0066*	19.02±0.012*	19.54±0.026*	24.67±0.010*	24.55±0.0105*	0.560±0.0002*	0.580±0.0001*

Mean±S.D
*Significant at 5% level

Gradual increase in saturated fat and a decrease in unsaturated fats were seen over time, which were significantly different from the control in the oil blends studied. This may probably be due to oxidative cleavage of these fatty acids on storage.

Sesame-rice bran blends showed a significant difference of both saturated and unsaturated fatty acids during storage. 16.54 units and 13.35 units of C-16:0 were seen in sesame-rice bran (80:20) and (20:80), which were seen to increase to 20.004 and 18.59 units. For C-18:0 in the same blend it was seen to be 3.86 and 2.83 units initially and finally by the end of storage period it was noted to increase to 5.56 and 5.43 units respectively. The principal fatty acid seen in sesame-rice bran blend was C-18:1 at a level of 45.65 and 38.35 units, followed by C-18:2 of 29.66 and 39.16 units and C-16:0 at a level of 16.54 and 13.35 units respectively, while C-18:0 was present in low amounts of 3.86, and 2.83 units and C-18:3 was found to be negligible. The ratio of fatty acid composition was 1:2.2:1.4 and 1:2.3:2.4 before storage as seen in Table 12 for sesame-rice bran blends of 80:20 and 20:80 respectively. Semwal and Arya (2001) contradicted the changes in the fatty acid composition during storage as not significant.

The objective of this part of the study was to determine the optimal fatty acid composition to achieve a balance between the storage stability, frying property and health aspect. Among the fatty acids, C-18:2 was the highest in the blends. The higher the sesame oil content, the higher the C-18:0 and C-18:1, and lower the 16:0, 18:2 and 18:3. This resulted in higher un-saturation ratio, which is a content ratio of unsaturated fatty acids to saturated fatty acids in sesame oil blended samples. Based on the fatty acid composition of the blends proportion of saturated, mono unsaturated and poly-unsaturated fatty acids is calculated and shown in Table 12. These values indicate that though the ideal proportion of 1:1:1 is not achieved there is an improvement in the proportion of fatty acids when compared to control.

CONCLUSION

The blends could be stored for a period of 12 months without any adverse changes in their peroxide values, which were seen to be under the limits specified by regulations mentioned by PFA. Based on the fatty acid composition of the blends proportion of S:M:P calculated showed that no blend had achieved the ideal ratio of 1:1:1. It was seen that as the storage period increased the proportion of S:M:P came closer to the ideal ratios, which could not be taken as the correct measure for the ideal ratios since storage showed a gradual increase in saturated fats and a decrease in unsaturated fats over time due to oxidative cleavage of fatty acids.

Considering the merits and demerits of oil as a cooking medium, blended oils seem to be just as or even more suitable than single oil for culinary purposes. Blending of course could be so designed as to achieving an ideal fatty acid combination. Oil blends having nutritional merits and have more stability during heating could thus be expected to receive acceptance if sufficient time is given for adjustment. Based on the results of the present study, the following recommenda-

Table 12: Proportion of saturated fatty acid composition: Monounsaturated fatty acid composition: Polyunsaturated fatty acid composition of the selected oil blends

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100 S:M:P	*80:20 S:M:P*	*80:20 S:M:P*	*20:80 S:M:P*	*20:80 S:M:P*
0	1:2.4:3	1:2.2:1.4	1:1.8:2.3	1:1.8:2.3	1:2.3:2.4
1	1:2.4:3	1:2.1:1.4	1:1.8:2.3	1:1.8:2.3	1:2.3:2.4
2	1:2.2:3.8	1:2.1:1.4	1:1.8:2.3	1:1.8:2.3	1:2.1:2.2
3	1:2.2:2.8	1:2:1.3	1:1.8:2.3	1:1.8:2.3	1:2:2.1
4	1:2.2:2.8	1:1.9:1.3	1:1.8:2.3	1:1.8:2.2	1:2:2
5	1:2.1:2.8	1:1.8:1.3	1:1.8:2.3	1:1.7:2.2	1:1.9:2
6	1:2.1:2.7	1:1.7:1.2	1:1.8:2.2	1:1.7:2.2	1:1.8:1.9
7	1:2:2.7	1:1.6:1.2	1:1.8:2.2	1:1.7:2.2	1:1.8:1.8
8	1:2.1:.2.7	1:1.6:1.1	1:1.8:2.2	1:1.7:2.2	1:1.7:1.8
9	1:2.1:2.7	1:1.6:1.1	1:1.7:2.2	1:1.7:2.2	1:1.6:1.7
10	1:2:2.6	1:1.5:1.1	1:1.7:2.2	1:1.7:2.1	1:1.6:1.7
11	1:1.9:2.5	1:1.4:1	1:1.7:2.2	1:1.7:2.1	1:1.5:1.6
12	1:1.9:2.5	1:1.4:1	1:1.7:2.2	1:1.7:2.1	1:1.4:1.5

tions can be suggested. The blends chosen should be based on fatty acid composition to obtain a nearer balance of fatty acid proportion of 1:1:1 as per the recommendations of American Heart Association. Suitability of blending of unconventional oils with traditional oils to obtain an ideal fatty acid combination can be done and it also reduces the demand of traditional oils.

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APPENDIX

Appendix (only for review)

Supplementary Table 1: Monthly changes in the specific gravity of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame: Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	0.922±0.0015	0.901±0.001*	0.854±0.0025*	0.896 ±0.0021*	0.92 ±0.00185
1	0.965±0.2517	0.952±0.0351	0.859±0.0286*	0.906 ±0.0028*	0.933±0.293
2	0.994±0.0368	0.956±0.0351	0.906±0.0006*	0.97 ±0.0080*	0.964±0.0251
3	1.018±0.0351	1.002±0.0251	0.908±0.0003*	1.02 ±0.0005*	1.025±0.0304
4	1.038±0.0251	1.034±0.0351	0.93 ±0.0021*	1.07 ±0.0003*	1.025±0.0293
5	1.05 ±0.0351	1.03 ±0.0264	0.98 ±0.0003*	1.09 ±0.0003*	1.03 ±0.0251
6	1.05 ±0.0351	1.04 ±0.0351	1.09 ±0.0007*	1.12 ±0.0063*	1.05 ±0.0304
7	1.115±0.006	1.06 ±0.0693	1.1 ±0.0058*	1.15 ±0.0058*	1.13 ±0.0324
8	1.13 ±0.0251	1.15 ±0.0351	1.11 ±0.0004*	1.17 ±0.0007*	1.13 ±0.0293
9	1.135±0.029	1.155± .0251	1.128±0.0005*	1.19 ±0.0007*	1.149±0.0351
10	1.14 ±0.0251	1.17 ±0.0351	1.14 ±0.0110*	1.191 ±0.0008*	1.16 ±0.0293
11	1.151±0.028	1.181±0.037	1.16 ±0.0009*	0.19 ±0.0006*	1.18 ±0.027
12	1.162±0.0368	1.19 ±0.0368	1.18 ±0.0007*	1.19 ±0.0006*	1.18 ±0.0368

Mean±S.D
*Significant at 5% level.

Supplementary Table 2 : Monthly changes in the refractive index of oil blends during storage

Month	*Control*	*Sesame: Rice bran*		*Sesame:Mustard*	
	100	*80:20*	*20:80*	*80:20*	*20:80*
0	1.465±0.0002	1.46 ±0.0001*	1.46±0.0002*	1.35±0.106*	1.44±0.0675*
1	1.465±0.0001	1.46 ±0.0005*	1.46±0.0001*	1.45±0.0003*	1.47±0.0004*
2	1.465±0.0001	1.4 ±0.00005*	1.46±0.0001*	1.45±0.0005*	1.47±0.0038*
3	1.465±0.0001	1.4 ±0.00005*	1.46±0.0001*	1.45±0.0056*	1.47±0.0007*
4	1.46 ±0.00009	1.4 ±0.00004*	1.4 ±0.00009*	1.46±0.0126	1.47±0.0003*
5	1.465±0.0001	1.4 ±0.00005*	1.46±0.0001*	1.46±0.0005	1.47±0.0116*
6	1.46 ±0.00009	1.4 ±0.00004*	1.46±0.0002*	1.46±0.0007	1.48±0.0014*
7	1.465±0.0002	1.46 ±0.0001*	1.46±0.0001*	1.46±0.0007	1.48±0.0004*
8	1.465±0.0001	1.4 ±0.00005*	1.46±0.0001*	1.47±0.0005*	1.48±0.010*
9	1.465±0.0001	1.4 ±0.00005*	1.46±0.0001*	1.47±0.0005*	1.50±0.0007*
10	1.465±0.0001	1.4 ±0.00005*	1.4 ±0.00009*	1.48±0.0003*	1.50±0.0006*
11	1.465±0.0009	1.4 ±0.00004*	1.4 ±0.0001*	1.57±0.0004*	1.51±0.0033*
12	1.465±0.0001	1.4 ±0.00005*	1.4 ±0.00009*	1.63±0.0009*	1.52±0.0228*

Mean±S.D
*Significant at 5% level.