STUDY ON EFFECT OF THE ANTIOXIDANTS ON OXIDATIVE STABILITY AND NUTRITIONAL PROPERTY OF RICE BRAN OIL BLENDED WITH OTHER OILS

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ABSTRACT:The objective of this study was to work out different blends of oils amongst rice bran oil (RBO), soybean oil (SBO), and mustard oil (MO) to obtain a unique blend of oil having better shelf life. Further, in present work, the effect of synthetic antioxidant such as butylated-hydroxyanisol (BHA), butyl-hydroxyltoluene (BHT) and t-butyl-hydroxyquinone (TBHQ) were also evaluated by adding upto recommended level (0.02%) on the basis of induction time with the help of Rancimat 743 (Swiss made) as well as better SMP ratio and ω-6/ω-3 ratio.

The results of experiment show that if RBO, SBO and MO are blended in proportion of 3:1:1(blend B-8), it results in maximum oxidative stability up to 11.2h at 110 ^{0}C without adding any antioxidants in comparison to other blends.Oxidative stability of the Blend B-8 by adding of antioxidant TBHQ was improved up to 22.3h at 110 ^{0}C.

From nutritional point of view, the study of SMP ratio and ω-6/ω-3 ratio were also evaluated on the basis of fatty acid composition of blends with the help of GLC. The results show that SMP ratio and ω-6/ω-3 ratio of this blend was approximately 1:2.6:2.2 and 7:1 respectively. This study concludes that the blend of RBO, SBO and MO in proportion of 3:1:1 resulted in proper ratio of ω-6/ω-3, however, there is variation in SMP ratio from the recommended by WHO but still this blend has given good stability.

Key Words: Rice bran oil, Blending, Rancimat, Oxidative stability, Nutrition, Antioxidant

1. INTRODUCTION

The quality of edible oil as a cooking medium is judged by various factors such as oxidation stability and fatty acid composition. Fatty acid composition of a frying oil/fat has a significant effect on the flavor of a fried food [1],more unsaturated the oil; the greater the tendency to form polymeric degradation compounds [2]. The most common method for example, blending polyunsaturated oils with more monounsaturated or saturated oil is an option to adjust fatty acid levels to optimal levels[3].

Blended oils are gaining popularity worldwide due to advantages they offer such as improved thermal stability, oxidative stability, nutritional benefits and an ability to tailor the desired properties. At times, they are cheaper alternatives / substitutes to pure vegetable oils [4]. The nutritional value of frying fats is affected by loss of polyunsaturated fatty acids (PUFA), which supplement the essential fatty acids requirement in human metabolism. Soybean oil, because of its high content of PUFA, is considered to be superior to many vegetable oils and hydrogenated fats from a nutritional stand point, but it is inferior in thermal stability at high temperature [5]. But research shows that the dietary imbalance could be improved by increasing the consumption of ω-3 fatty acids and it would enhance vascular function and it also impairs flow mediated dilation [6]. The ω-3 PUFA found in plant source, alpha-linolenic acid (ALA), may also be protective in heart failure [7]. ALA and linolenic acid (LA) are essential fatty acids because bodies can not make them [8]. RBO, which is rich in tocopherols and

tocotrienols, is expected to improve the oxidative stability of the blends, blending of RBO with other oils have also an economical advantage of lower price [9], Oryzanol is also present in RBO, which has been shown to have hypocholesterlemic activity [10], [11]. Mustard oil is suitable for all type of cooking including frying, but should be used along with other cooking oils to reduce the erucic acid content because dietary erucic acid causes cardiac lipidosis. If erucic acid is present around 10 % of total fatty acid composition, it did not affect heart cholesterol and triglyceride concentration and there is no development of heart lesion [12].

The oxidative stability of oil is enhance by addition of natural or synthetic antioxidant in the recommended level [13]. Oil stability index of Hake liver oil is best when treat with TBHQ followed by PG (propyl gallate)>BHA>BHT>TOC (Tocopherol) [14]. BHT and TBHQ % are the most effective inhibitors of cholesterol oxidation. BHA is less effective and PG is unable to prevent cholesterol oxidation [15].

In the present work, rice bran oil, soybean oil and mustard oil are blended to enhance oxidative stability and fatty acid composition of blended oil. In the blended oil, soybean oil and mustard oil are taken because they are rich source of essential fatty acid ALA and LA. RBO is taken due to its higher stability which stabilizes the oil blends having high percentage of PUFA.

2. MATERIALS AND METHODS

Three different oils were selected according to their nutritional benefits, oxidative stability, availability, functionality, flavor and cost.

These oils were refined, bleached, and deodorized Rice Bran Oil (RBO), KacchiGhani Mustard Oil (MO) and refined, bleached, and deodorized Soybean Oil (SBO) were procured from a local supermarket in Kanpur (India). The other chemicals and reagents used for analysis were of analytical reagent grade.

BHA (SD Fine-Chem) analytical grade, BHT (Qualikems) analytical grade and TBHQ (LobaChemie) analytical grade were obtained from Nath chemical corporation, Kanpur (India).

I. Physico-chemical characteristics

The physico-chemical characteristics of RBO, SBO, MO and their blends were determined according to the procedure given under IS: 548 (part-1)-1964 Indian Standard METHODS OF SAMLING AND TEST FOR OILS AND FATS. Following test were carried out

Acid Value (AV), Saponification Value (SV), Iodine Value (IV), Peroxide Value (PV), unsap matter

II. Preparation of Blends

A 100 g mixture of RBO 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. The temperature during mixing was 65 ^{0}C. Eight different blends having various proportions of RBO and other vegetable oils were prepared in this manner.

The minimum level of incorporation of oil is 20% in an oil blend as per the rules of Prevention of Food Adulteration Act (PFA) in India. The eight blends selected for RBO were 50% RBO + 50% SBO (blend B-1), 50% RBO + 50% MO (blend B-2), 60% RBO + 40% SBO (blend B-3), 60% RBO + 40% MO (blend B-4), 50% RBO + 20% SBO + 30% MO (blend B-5), 50% RBO + 25% SBO + 25% MO (blend B-6), 50% RBO + 30% SBO + 20% MO (blend B-7) and 60% RBO + 20% SBO + 20% MO (blend B-8). These blends were analyzed for fatty acid composition and oxidative stability.

III. Fatty Acid Composition by Gas Chromatography

Fatty acid methyl esters (FAME) of the oil samples were prepared by transesterification, according to AOCS Method No: Ce 1-62, 1998 [16] FAMEs were analyzed on a NUCON 5765 series gas chromatograph (NUCON Industries, New Delhi), equipped with a hydrogen flame ionization detector (FID) and a fused silica capillary column (100 m 9 0.25 mm i.d.), coated with 0.20 µm SP2560 (Supelco Inc., Bellefonte, PA) as the stationary phase. The oven temperature was programmed from 140 to 240 ^{0}C at 2 ^{0}C/min with an initial hold at 140 ^{0}C for 5 min. The injector and FID were at 240 ^{0}C. A reference standard FAME mix (Supelco Inc.) was analyzed under the same operating conditions to determine the peak identity. The FAMEs were expressed as relative area percentage.

IV. Oxidative Stability Measurement

The oxidative stability or induction period is an expression of the stability of oils and fats toward oxidation [17]. In other words, the induction period is the length of time before the resistance of oils and fats toward oxidation is overcome. Oxidative stability was measured using a 743 Rancimat (Herisau, Switzerland) at 110 ^{0}C following the AOCS Official Method Cd 12b-92 [18].

A stream of filtered, cleaned and dried air at flow rate 20 L h^{-1} is bubbled into oil samples 3g contained in reaction vessels. These vessels are placed in an electric heating block which is set at different temperatures (50-220 ^{0}C). Effluent air containing volatile organic acids from the oil sample are collected in a measuring vessel with 60 ml of distilled water. The conductivity of the water is continuously recorded and the OSI (Oil Stability Index) is automatically determined by the apparatus [19].

V. Antioxidant / Oil Preparations

The blended oil having highest oxidative stability is selected for use in the study of the effect of the antioxidants. The supplier recommended levels of antioxidants uses are, 200 ppm for TBHQ, 200 ppm for BHA and 200 ppm for BHT. All the antioxidants added and dissolved with stirring under nitrogen for 24h. Antioxidants are added to the selected blended oil separately and in all possible combinations.

3. RESULTS AND DISCUSSION

I. Analysis of raw materials

Physico-chemical characteristics of used raw materials are observed as follows:

As given in Table-1, the acid value of RBO, SBO and MO are 0.22, 0.15 and 0.17 respectively. The saponification value of RBO, SBO, and MO are 190, 189 and 177 respectively. The Iodine value of RBO, SBO and MO are 106, 130 and 103 respectively. The peroxide value of RBO, SBO and MO are 0.2, 0.4 and 0.3, respectively. The unsaponifiable matter is higher for RBO which is 3.0 % and for SBO and MO it is 0.38% and 0.40% respectively.

Table -1: Physico-chemical characteristics of raw materials

Characteristic*	RBO	SBO	MO
Acid value (mg KOH/g)	0.22	0.15	0.17
Saponification value (mg KOH/g)	190	189	177
Iodine value (wijs)	103	138	110
Peroxide Value ($meqO_2$ /kg)	0.20	0.40	0.30
Unsaponifiable Matter	3.0	0.38	0.40

*average of three determinations

RBO; Rice Bran Oil, SBO; Soybean Oil and MO; Mustard Oil

II. Analysis of blends

As depicted in Table-2, the blend B-1, B-2, B-3, B-4, B-5, B-6, B-7 and B-8 have approximately same acid value, it is approximately 0.20, 0.20, 0.21, 0.21, 0.19, 0.19, 0.19 and 0.20 respectively. The saponification value of blend B-3 is highest in all blends which is approximately 189, and approximately same for blend B-2, B-4, B-5, B-6, B-7 and B-8, it is approximately 184, 185, 186, 187, 187 and 187 respectively and lower for blend B-1 which is approximately 180. The Iodine value of blend B-1 is highest which is 121, and approximately same for blend B-3, B-5, B-6, B-7 and B-8, it is approximately 117, 115, 114, 116 and 112 respectively and lower for blend B-2 and B-4 it is 107 and 106 respectively. The blend B-1, B-2, B-3, B-4, B-5, B-6, B-7 and B-8 approximately same peroxide value, it is 0.32, 0.28, 0.29, 0.27, 0.30, 0.28, 0.30 and 0.26 respectively. The blend B-3, B-4 and B-8 have highest unsaponifiable matter it is 1.98 %, 1.96% and 1.97% respectively and approximately same for blend B-1, B-2, B-5, B-6 and B-7, it is approximately 1.70%, 1.71%, 1.69%, 1.70% and 1.70% respectively.

Table - 2: Physico –chemical characteristics of different blends

Characteristic	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8
Acid value (mg KOH/g)	0.20	0.20	0.21	0.21	0.19	0.19	0.19	0.20
Saponification value (mg KOH/g)	180	184	189	185	186	187	187	187
Iodine value (wijs)	121	107	117	106	115	114	116	112
Peroxide Value ($meqO_2$ /kg)	0.32	0.28	0.29	0.27	0.30	0.28	0.30	0.26
Unsaponifiable Matter	1.70	1.71	1.98	1.96	1.69	1.70	1.70	1.97

Blend B-1; 50% RBO + 50% SBO, Blend B-2; 50% RBO + 50% MO, Blend B-3; 60% RBO + 40%, Blend B-4; SBO, 60% RBO + 40% MO, Blend B-5; 50% RBO + 20% SBO + 30% MO, Blend B-6; 50% RBO + 25% SBO + 25% MO, Blend B-7; 50% RBO + 30% SBO + 20% MO and Blend B-8; 60% RBO + 20% SBO + 20% MO

III. Effect of blending on fatty acid composition

The fatty acid composition and chemical characteristics of the RBO, SBO, MO and also those of the RBO blended with SBO and MO in different ratio to make blend of these oils are shown in Table-3. It is observed that the oils distinguished from each other mainly due to the significant differences in the percentage of palmitic (C16:0), oleic (C18:0), linoleic (C18:2), and linolenic (C18:3) acids. The percentage saturated fatty acid (SFA) for the RBO is significantly higher than that of the SBO and MO. The percentage mono-unsaturated fatty acid (MUFA) is highest in MO (63.90%) and the MUFA percentage of RBO and SBO are 44.28% and 24.19% respectively. The SBO has the highest percentage poly-unsaturated fatty acid (PUFA), followed by the RBO and MO. From above, it is clear that the PUFA/SFA ratio (also known as polyene index) [20] is the greatest for the SBO followed by the RBO and MO.

As depicted in Table-3, the blend of RBO with single oil SBO and MO, blend B-1 and B-3 has higher percentage level of SFA, it is 17.8% and 18.4% respectively, and blend B-2 and B-4 has lower percentage level of SFA, it is approximately 15.0% and 16.1% respectively, this can be explained as a consequence of higher percentage saturated fatty acid present in MO. The percentage of MUFA is approximately similar for blend B-1 and B-3 but higher for blend B-2 and B-4 due to presence of MO which contains more erucic acid. The MUFA percentage of blend B-1, B-2, B-3 and B-4 is 34.2%, 54.1%, 35.2% and 51.1% respectively. The PUFA for blend B-1 and B-3 is 48.0% and 45.4% respectively which is higher in comparatively to blend B-2 and B-4, it is 31.0% and 31.7% respectively, this can attribute due to the fact that blend B-1 and B-3 is the blend of RBO with SBO and SBO has higher percentage of PUFA. The blend of RBO with SBO and MO, the blend B-5, B-6, B-7 and B-8 have approximately similar percentage level of SFA, it is 16.1%, 16.4%, 17.6% and 17.3% respectively, the percentage of MUFA is approximately similar for B-6, B-7 and B-8 but slightly higher for B-5 due to presence of high presence of MO which contains more erucic acid. The MUFA percentage of blend B-5, B-6, B-7 and B-8 is approximately 46.1%, 44.1%, 42.1% and 44.1% respectively. The blend b-7 shows higher

percentage of PUFA in comparison to B-5, B-6 and B-8 due to presence of high percentage of RBO.

Table - 3: The fatty acid composition (%) of RBO, SBO, MO and their blends

Parameter	RBO	SBO	MO	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8
Palmitic	18.83	10.53	01.43	14.68	10.13	15.51	11.87	11.95	12.40	12.86	13.69
Stearic	01.73	03.81	00.59	2.80	01.16	02.56	01.27	01.81	01.97	02.43	01.92
Oleic	44.28	24.19	10.76	34.24	27.52	35.24	30.87	30.20	30.87	31.55	33.55
Linoleic	33.08	55.03	14.53	44.06	23.81	41.86	25.66	31.90	33.93	35.95	33.76
Linolenic	01.52	06.44	12.76	03.98	07.14	03.49	06.02	06.52	05.56	05.24	04.75
Arachidic	00.57	-	06.81	00.29	03.69	00.34	03.07	02.33	01.99	02.33	01.71
Erucic	-	-	53.14	-	26.57	-	21.26	15.94	13.28	10.62	10.62
SFA	21.13	14.34	08.83	17.77	14.98	18.41	16.16	16.09	16.36	17.62	17.32
MUFA	44.28	24.19	63.90	34.24	54.09	35.24	51.13	46.14	44.15	42.17	44.17
PUFA	34.60	61.47	27.29	48.04	30.95	45.35	31.68	38.42	39.49	41.19	38.51
PUFA/SFA	01.63	04.28	03.09	02.70	02.07	02.46	01.96	02.38	02.40	02.33	02.22

SFA; Saturated Fatty Acid, MUFA; Monounsaturated Fatty Acid and PUFA; Polyunsaturated Fatty Acid

The erucic acid percentage for blend B-2 and B-4 are very high, it is approximately 26.6 % and 21.3 % respectively, and nil for blend B-1 and B-3 because it is only blend of two oil RBO and SBO. It is clear from the table that the blend B-7 and B-8 has around 10 % erucic acid of total fatty acid which a good achievement of blend B-7 and B-8

On the basis of fatty acid composition the blend of RBO with single oil MO and SBO, the blend B-1 and B-3 have approximately similar SMP ratio, it is approximately 1:1.9:2.7 and 1:1.9:2.5 respectively, the SMP ratio of blend B-2 and B-4 have slightly different SMP ratio, it is approximately 1:3.7:2.1 and 1:3.2:2.0 respectively this can be explained as a consequence of high percentage of MUFA presence in MO (erucic acid) than the SBO and high percentage of PUFA presence in SBO than the MO . The blend B-5, B-6 and B-8 have approximately similar SMP ratio, it is approximately 1:2.9:2.4. 1:2.7:2.4 and 1:2.6:2.2 respectively, the SMP ratio of blend B-7 is slightly different and it is 1:2.4:2.3. The blend B-1 and B-3 have higher ω-6/ω-3 ratio, it is approximately 11.1:1 and 12:1 respectively and blend B-2 and B-4 have lower ω-6/ω-3 ratio, it is approximately 3.3:1 and 4.3:1 respectively, the blends B-1, B-2, B-3 and B-4 are not lie in WHO recommended level which is 5-10:1. The ω-6/ω-3 ratio for blends B-5, B-6, B-7 and B-8 are lie in WHO recommended level. The ω-6/ω-3 ratio of blend B-5, B-6, B-7 and B-8 are 5:1, 6:1, 7:1 and 7:1 respectively.

IV. Effect of blending on oxidative stability

Oxidative stability of RBO, SBO, MO and their blends are analyzed by Rancimat, as depicted in Table-4 the Induction time (IT) of raw material at 110 ^{0}C of RBO, SBO, MO, are 13.7h, 4.2h, 6.4h respectively due to presence of oryzanol in RBO which enhance the oxidative stability and Table-5 shows the IT of blended oil at 110 ^{0}C of different blends B-1, B-2, B-3, B-4, B-5, B-6, B-7 and B-8 which are 10.3h, 10.8h, 9.0h, 10.0h, 10.5h, 10.7h, 9.5h and 11.2h respectively. Above result concluded that RBO has highest IT than SBO, MO and their blends after RBO, blend B-8 has highest oxidative stability which is 11.2h due to presence

of higher percentage of RBO than the other blends. The induction time is depicted in Table-4. RBO, SBO and MO shows 13.7, 4.2, 6.4h induction time measured by Rancimat at 110 ^{0}C. RBO had maximum induction time(oxidation stability).

Table - 4: Oxidative stability of raw material

Oil Sample	Induction Time(h) ± SD[a]
RBO	13.7 ± 0.10
SBO	04.2 ± 0.29
MO	06.4 ± 0.10

a : Standard deviation (n=3)

The induction time of different blends is given in Table-5. It is clear from results that B-8 gives good shelf life as well as having approx recommended level parameters and compositions. The oxidative stability of blend B-8 is lower than the RBO due to presence of higher percentage of natural antioxidant gamma-Oryzanol in RBO.

Table – 5: Oxidative stability of blended oil

Blended Oil	Induction Time (h) ± SD[a]
Blend -1	10.3 ± 0.15
Blend -2	10.8 ± 0.10
Blend - 3	09.0 ± 0.10
Blend - 4	10.0 ± 0.10
Blend – 5	10.5 ± 0.06
Blend – 6	10.7 ± 0.06
Blend – 7	09.5 ± 0.15
Blend - 8	11.2 ± 0.06

a : Standard deviation (n=3)

V. Effect of synthetic antioxidants on oxidative stability of blended oil

On the basis of oxidative stability of blended oils measured by Rancimat at 110 ^{0}C, blend B-8 having highest oxidative stability is selected for further evaluation of the effect of antioxidants on oxidative stability of blend B-8. Table-6 shows the effect of antioxidant addition on oxidative stability of blend B-8 measured by Rancimat at 110 ^{0}C the oil antioxidant combination having maximum IT (22.3h), B-8 containing 200 ppm TBHQ. B-8 contains 200 ppm BHA and B-4 containing 200 ppm BHT the IT is 14.3h and 12.9h respectively. Effect of antioxidants combinations (100 ppm TBHQ + 100 ppm BHA, 100 ppm TBHQ + 100 ppm BHT and 100 ppm BHA + 100 ppm BHT) with blend B-8 are 18.9h, 17.9h and 13.4h respectively. Above result concluded that TBHQ alone and in combination with other antioxidants, is most effective in increasing the stability of the blend B-8 than the other antioxidants.

Table -6: Oxidative stability of Blend - 8 with different antioxidant

Blend- 8 with antioxidant	Induction Time (h) ± SD[a]
200 ppm TBHQ	22.3 ± 0.11
200 ppm BHA	14.3 ± 0.27
200 ppm BHT	12.9 ± 0.1
100 ppm TBHQ + 100 ppm BHA	18.9 ± 0.1
100 ppm TBHQ + 100 ppm BHT	17.9 ± 0.32
100 ppm BHA + 100 ppm BHT	13.4 ± 0.15

a : Standard deviation (n=3)

4. CONCLUSION

The following conclusion can be drawn on the basis of result and discussion:

The SMP ratio of the blend B-8 is 1:2.6:2.2, which is almost nearest to the WHO recommendation

(below 33%: above 33%: about 33% respectively).

The blend B-8 has sufficient ω-3 and ω-6 fatty acid. As WHO suggested "Ratio of omega-6 and omega-3 in the diet should be 5-10:1" and we observed that it is 7:1 in case of blend B-8.

The shelf life of blend B-8 found 11.2h, which has better than the other blends. Shelf life of blend B-8 is lower than RBO (13.7h) but it is consider better oil than the RBO, reason is that, blend B-8 has good fatty acid composition which is directly associated with human health and it has all beneficial properties of rice bran, soybean and mustard oil. Blend B-8 has better shelf life while it contain sufficient amount of ω-3 fatty acid. It is a good achievement of any healthiest cooking oil that it maintains both oxidative stability and fatty acid composition for increasing its nutritional value.

Considering the effect of individual antioxidants on blends, addition of TBHQ resulted in dramatic increase in the stability of blend B-8. TBHQ alone provided as much protection against oxidation as any other combination of antioxidants which is 22.3h. The addition of TBHQ to either BHA or BHT resulted in greater stability than either single antioxidant.

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