International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 06 Issue: 02 | Feb 2019 www.irjet.net p-ISSN: 2395-0072

Antioxidant Activity and Oxidative Stability of Some Binary and Ternary Blended Vegetable Oil

SATARUPA GHOSH[1], D.K. BHATTACHARYYA[2], MINAKSHI GHOSH[3]

[1]Resaerch scholar, School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah - 711103, West Bengal, India
[2]Adjunct Professor, School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah - 711103, West Bengal, India
[3]Assistant Professor, School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah - 711103, West Bengal, India

---

Abstract – *The study includes the binary and ternary blending of some vegetable oils in different ratios (1:1,1:2,2:1).Here flaxseed oil, tomato seed oil, olive oil, ricebran oil, soyabean oil were used in blending methods. Analytical values such as iodine value, saponification value and total phenolic content and flavonoid content were determined. Oxidative stability test was done by acid value (A.V.), peroxide value (P.V.), anisidine test (A.T.), T.B.A.value. Antioxidant activity of the blended (binary and ternary methods) oils based on the determination of DPPH Free radical scavenging activity, FRAP assay and ABTS assay were evaluated.*

Keywords – **Blended oils, Oil Extraction, Phytochemical, Antioxidant activity, Oxidative stability test**.

1. INTRODUCTION – Fats and oils play an important role in our diet. National Institute of Nutrition (NIN) ,India has recommended that about 15 -30% fat should be consumed by an adult per day .In India cooking oils play a vital role in every household and right choice of edible oil is needed to maintain a healthy life. But none of these single edible oil can fulfill the recommendation of dietary fatty acids. NIN, India in their recent recommendations state that a correct combination or blend of two or more than two oils should be used to achieve the right proportion of fatty acids .According to the Food Safety and Standards Authority of India binary blending of oil is allowed in India .Cooking oil is subjected to high temperatures and in these conditions the oxidative degradation of oil is accelerated. Shelf life stability and their applicability in food also depends on their oxidative stability .The oxidative stability of PUFA is the highest, followed by MUFA and saturated fatty acids .So it is important to protect the fatty acids from degradation by blendind methods to increase their beneficial effects in our body. Antioxidant helps to prevent oxidation reactions in our body mainly by scavenging free radicals. Different oils contain different antioxidant. So no one antioxidant is sufficient to interact at all levels. Hence blending is essential for achieving different antioxidant to perform at different levels. Ternary blending is established in foreign countries for maintaining healthy life.

2. EXPERIMENTAL –

MATERIALS-

Flaxseed, tomato seed, ricebran oil (RBO), olive oil (OO), soyabean oil (SO) were purchased from local market of Kolkata, India. All the analytical grade chemicals and solvents were used supplied by MERCK, INDIA.

METHODS –

2.1 OIL EXTRACTION –

Flaxseed oil (FO) and tomato seed oil (TO) were extracted from flaxseed and tomato seed respectively by Sohxlet apparatus, using n-hexane as a solvent. Rotary evaporator was used to separate the oil from the solvent.

2.2 PREPARATION OF BLENDS –

Mixture of vegetable oils were placed in beakers for each blend (binary and ternary methods) and were mixed by mechanical stirrer at 180 rpm for 15 minutes. Binary blends of vegetable oils viz. FO+TO,FO + RBO,TO +RBO,FO+SO,SO+O,FO+OO,TO+S0,TO+OO and ternary blends of vegetable oils viz.FO+TO+RBO,FO+SO+RBO,FO+RBO+OO

,FO+TO+SO,FO+TO+OO were prepared in three ratios (1:1,1:2,2:1).These blends of oils were analyzed for oxidative stability and antioxidant activity. The basis of selection of these blends was as follows presence of adequate amounts of PUFA, MUFA, antioxidants and lower cost of blend when compared to individual oil.

2.3 Analytical Characteristics –

Saponification value, iodine value of flax seed oil were estimated according to the AOAC official method.

2.4. Phytochemical Content-

2.4.1. Total Phenolic Content

The total phenolic content was measured using the Folin Ciocalteu reagent Mc Donald S et al. An aliquot of the extract (100 µl) was mixed with 250 µl of Folin Ciocalteu's reagent and incubated in room temperature for 5 minutes.1.5 ml of 20% sodium bi carbonate was added to the mixture and absorbance was measured at 765 nm.

2.4.2. Total Flavonoid Content

The total flavonoids were measured using the Aluminium chloride colorimetric method of Chang C et al. The sample extract (250µl) was added to 4.5 ml distilled water followed by 5% $NaNO_2$ (.03 ml).After incubation of 5 minutes $AlCl_3$ (.03 ml ,10 %) was added at 25^0 C. The reaction mixture was treated with 2 ml of 1 M NaOH. The reaction mixture was then diluted to 10 ml distilled water and absorbance was measured at 510 nm.

2.5. Oxidative Stability Test –

2.5.1. Acid Value (A.V.) - This analysis has been conducted according to AOCS Ca 5a-40 official method.1 gm of oil was mixed with hot ethyl alcohol and 2-3 drops phenopthalein indicator was added to this mixture. The mixture was titrated with standard aqueous solution of alkali, shaking the solution vigorously during titration. Titration was continued till the solution turns pink.

2.5.2 Peroxide Value (P.V.) - This analysis has been conducted according to AOCS Cd 8-53 (1997).

2.5.3 Anisidine Value –This analysis was done according to AOCS Cd 18-90.1 ml of .25% of p-anisidine in glacial acetic acid made upto 100 ml with iso-octane, 1 gm of oil was dissolved in the mixture and allowed to react for 10 minutes at room temperature and absorbance was measured at 350 nm.

2.5.4. TBA Value – This analysis has done according to Hekmat and Mc Hamon (1997).TBA reagent is prepared by dissolving 200 mg TBA in 100 ml 1-butanol and leave it for one night and filter or centrifuge the suspension to remove the undisolved residue and makeup the filtrate to 100 ml with 1-butanol.50-200 mg sample was taken in a volumetric flask(25 ml),dissolved in small amount of 1- butanol and makeup to volume with the same solvent.5 ml sample solution was mixed with 5 ml reagent solution and placed the solution into a thermostat bath at 95 degree Celsius temperature. After 120 minutes remove the solution from thermostat and cooled at running water and absorbance was measured at 530 nm.

2.6. Antioxidant activity –

2.6.1. DPPH Free radical scavenging activity assay –

The oil was assessed using 1, 1-diphenyl 2-picryl hydrazyl (DPPH) radical scavenging assay according to Gorinstein et al. (2004) method..01Mm solution of DPPH in methanol was prepared. An aliquot of .2 ml of sample was added to 2.8 ml of this solution and kept in the dark place for 30 minutes. The absorbance was measured at 517 nm. The ability to scavenge the DPPH radical was calculated with the following equation.

Inhibition percentage (I %)=$(A_0 - A_1)/A_0$

(A_0 =Absorbance of the control, A_1 = Absorbance of the sample.)

2.6.2. Ferric reducing antioxidant power (FRAP) –

The assay was based upon the methodology of Benzie and Strain. The FRAP reagent consist of 10 mM TPTZ in 40 mM HCL, 250 mM sodium acetate buffer (pH -3.6) and 20mM FeCl3.The reagent was freshly prepared by mixing TPTZ solution,FeCl3 solution and acetate buffer in a ratio of 1:1:10.An extract solution (100 µl) was mixed with 900µl of FRAP reagent .The mixture was incubated at 37 degree Celsius for 4 minutes and the absorbance was measured at 593 nm.

2.6.3. ABTS Free radical scavenging activity –

ABTS assay of flax seed oil was measured using the method described by Fatma Bouaziz. A solution of ABTS (7µM) was prepared in distil water and mixed with a solution of potassium per sulphate (2.45 µM).The mixture was kept in the dark place for 16 hours at room temperature. The resulting intense colour matches the ABTS radical cations. The obtained solution was subsequently diluted with distil water and absorbance was measured at 734 nm.1 ml of ABTS diluted solution was mixed with 10 µl of sample at different concentration and the reaction mixture was kept for 6 minutes before measuring the absorbance. ABTS scavenging activity was calculated by the following equation.

Inhibition Percentage (I %) = (1-A/AO) × 100.

(A=Absorbance of the sample. A0 = Absorbance of the ABTS solution.)

3. Results and Discursion

Analytical Characterisics

To determine the analytical charateristics of the blended oil iodine value and saponification value were performed. Table 1andTable 2 show the results of analytical values of blended oils.

Table 1.Analytical Characteristics of binary blended oils

Binary blended oils	Iodine value	Saponification value
FO+TO		
1:1	134±.33	172±.27
1:2	130±.67	165±.29
2:1	136±.42	176±.38
FO+RBO		
1:1	120±.33	180±.62
1:2	112±.56	182±.45
2:1	128±.87	183±.59
TO+RBO		
1:1	105±.71	184±.66
1:2	102±.56	188±.87
2:1	109±.47	189±.93
FO+SO		
1:1	140±.65	188±.76
1:2	128±.44	192±.67
2:1	156±.48	194±.65
SO+OO		
1:1	84±.67	180±.54

1:2	89±.54	182±.65
2:1	88±.61	183±.61
FO+OO		
1:1	135±.29	178±.51
1:2	129±.54	180±.45
2:1	140±.69	181±.57
TO+SO		
1:1	110±.76	184±.67
1:2	107±.56	186±.69
2:1	114±.66	185±.56
TO+OO		
1:1	106±.76	175±.67
1:2	102±.56	178±.54
2:1	111±.55	176±.59

Table 2 Analytical Characteristics of ternary blended oils

Ternary blended oils	Iodine value	Saponification value
FO+TO+RBO		
1:1:1	132±.77	176±.62
1:2:1	134±.56	178±.67
2:1:1	135±.45	181±.43
FO+SO+RBO		
1:1:1	140±.51	180±.61
1:2:1	136±.56	184±.54
2:1:1	134±.76	187±.41
FO+RBO+OO		
1:1:1	139±.31	188±.61
1:2:1	143±.54	184±.54
2:1:1	145±.91	187±.47
FO+TO+SO		
1:1:1	142±.67	174±.56
1:2:1	137±.54	175±.75
2:1:1	145±.49	172±.67
FO+TO+OO		
1:1:1	145±.45	182±.66
1:2:1	134±.39	187±.32
2:1:1	147±.33	189±.67

Phytochemical content of blended oils –

The phytochemical analysis revealed the presence of phenolics and flavonoids content shown in table 3 and table 4.

Table3 Phytochemicals Content of binary blended oils

Binary blended oils	Phenolic content	Flavonoid content
FO+TO		
1:1	1556±.29	412±.75
1:2	1534±.37	387±.45
2:1	1587±.33	401±.59
FO+RBO		
1:1	1496±.66	423±.37
1:2	1487±.76	412±.49
2:1	1566±.45	433±.54
TO+RBO		
1:1	1385±.69	339±.56
1:2	1352±.53	335±.61
2:1	1388±.43	354±.43
FO+SO		
1:1	1397±.29	357±.39
1:2	1386±.56	368±.54
2:1	1422±.54	324±.47
SO+OO		
1:1	1243±.51	301±.67
1:2	1256±.45	322±.61
2:1	1267±.39	329±.56
FO+OO		
1:1	1545±.71	412±.53
1:2	1434±.51	418±.61
2:1	1497±.69	407±.73
TO+SO		
1:1	1345±.58	334±.39
1:2	1324±.41	356±.47
2:1	1335±.69	343±.56
TO+OO		
1:1	1354±.29	356±.58
1:2	1335±.62	324±.61
2:1	1345±.38	333±.87

Table 4.Phytochemical content of ternary blended oils

Ternary blended oils	Phenolic content	Flavonoid content
FO+TO+RBO		
1:1:1	1345±.71	335±.53

1:2:!	1339±.56	324±.49
2:1:1	1325±.41	323±.71
FO+SO+RBO		
1:1:1	1256±.66	235±.45
1:2:1	1245±.61	221±.38
2:1:1	1241±.54	220±.29
FO+RBO+OO		
1:1:1	1267±.57	223±.73
1:2:1	1279±.49	226±.69
2:1:1	1256±.77	234±.47
FO+TO+SO		
1:1:1	997±.41	223±.54
1:2:1	1007±.37	239±.49
2:1:1	1012±.49	247±.73
FO+TO+OO		
1:1:1	990±.73	243±.41
1:2:1	980±.69	247±.49
2:1:1	1012±.51	283±.53

Oxidative stability test

Among oxidative stability test acid value, peroxide value, anisidine value and TBA test were performed.

A.V. measures the content of free fatty acids formed upon the hydrolytic degradation of lipid molecules, thus contributing the reduction of shelf life of oil (Warner K,2008,).According to Codex Alimentarius Commission standard acid value upto 5 mg KOH/gm of oil is safe for consumption. Here the acid value of blended oil(table 5 and table 6) is bellow 5 mh KOH/gm of oil so they are safe for human consumption.

P.V. defines the content of lipid hydroxides in oil formed under conditions of auto and photo –oxidation. Here the oil shows the very low P.V.(table 5 and table 6)which does not exceed the recommended limit of P.V. of oil (10 mequ O2/Kg oil.)

Anisidine value indicates the content of secondary products of lipid oxidation resulting from the decomposition of hydroxides. Anisidine value along with P.V. indicates the rancidity of oil(Frankel EN 2007).Both binary and ternary blended oils show low anisidine value(table 4).

TBA Value indicates the degradation of oil. Flax seed oil shows the low TBA value (table 4) which is good for health.

Table 4. Oxidative stability test of binary blended oils

Blended oils	A.V.	P.V.	A.T.	TBA
FO+TO				
1:1	.88±.23	.96±.21	3.23±.81	3.87±.76
1:2	.86±.31	.99±.26	3.53±.66	3.99±.54
2:1	.89±.25	.93±.33	3.17±.37	3.76±.59
FO+RBO				
1:1	.85±.63	1.03±.25	3.57±.42	3.41±.56

1:2	.87±.39	.99±.23	3.87±.49	3.65±.61
2:1	.84±.27	.97±.36	3.99±.47	3.78±.38
TO+RBO				
1:1	.92±.28	.95±.29	2.87±.54	3.54±.69
1:2	.89±.26	.91±.21	2.99±.49	3.98±.61
2:1	.87±.19	.90±.17	3.01±.41	3.67±.48
FO+SO				
1:1	.99±.71	1.07±.26	3.17±.33	3.99±.76
1:2	.93±.67	1.05±.23	3.33±.29	3.89±.61
2:1	.97±.49	.99±.19	3.69±.37	3.83±.52
SO+OO				
1:1	.94±.17	1.02±.21	3.78±.51	3.57±.41
1:2	.88±.11	1.05±.13	3.79±.46	3.89±37
2:1	.82±.13	1.03±.18	3.97±.37	3.97±.23
FO+OO				
1:1	.86±.23	.99±.34	3.99±.29	4.01±.31
1:2	.93±.28	1.02±.33	3.87±.33	3.97±.39
2:1	.97±.33	1.05±.38	3.83±.41	3.99±.43
TO+SO				
1:1	.91±.13	1.11±.22	3.33±.43	3.97±.35
1:2	.85±.16	1.09±.29	3.63±.31	4.07±.41
2:1	.80±.23	.98±.37	3.76±.38	4.01±.39
TO+OO				
1:1	.79±.17	1.04±.23	3.89±.39	3.99±.43
1:2	.87±.19	1.01±.26	3.91±.31	4.03±.39
2:1	.83±.28	1.03±.29	3.97±.41	4.09±.51

Table 5.Oxidative stability test of ternary blended oils

Blended oils	A.V.	P.V.	A.T.	TBA
FO+TO+RBO				
1:1:1	.77±.23	.91±.56	3.31±.66	4.06±.56
1:2:1	.93±.27	.95±.43	3.19±.63	4.11±.61
2:1:1	.87±.34	.97±.32	3.29±.56	3.92±.52
FO+SO+RBO				
1:1:1	.99±.11	1.01±.05	3.77±.23	3.93±.16
1:2:1	.93±.14	.91±.07	3.68±.34	4.11±.11
2:1:1	.88±.12	.97±.09	3.85±.37	3.91±.19
FO+RBO+OO				
1:1:1	.76±.09	.94±.14	3.29±.29	4.02±.16
1:2:1	.89±.06	.82±.13	3.09±.36	3.73±.23

2:1:1	.81±.07	.88±.05	3.35±.31	3.96±.28
FO+TO+SO				
1:1:1	.91±.03	1.01±.09	3.97±.41	3.67±.19
1:2:1	.97±.02	.99±.11	4.02±.57	3.84±.26
2:1:1	.85±.03	1.03±.13	3.99±.54	3.97±.22
FO+TO+OO				
1:1:1	.79±.03	1.05±.02	3.46±.31	4.04±.30
1:2:1	.88±.06	.97±.04	3.96±.36	3.73±.33
2:1:1	.93±.04	1.03±.05	3.72±.29	3.19±.27

Antioxidant activity - The antioxidant activity of blended oil samples were evaluated using DPPH, FRAP, ABTS assay and the results are shown in table 6 and table 7.

Table 6.Antioxidant activity of binary blended oil

Blended oils	DPPH	FRAP	ABTS
FO+TO			
1:1	76±.78	.52±.05	39±.11
1:2	79±.71	.50±.09	38±.19
2:1	77±.66	.53±.11	40±.23
FO+RBO			
1:1	78±.73	.55±.06	41±.27
1:2	74±.56	.59±.09	43±.41
2:1	78±.69	.60±.13	42±.36
TO+RBO			
1:1	66±.56	.42±.03	39±.33
1:2	62±.61	.40±.07	37±.29
2:1	64±.66	.43±.03	36±.23
FO+SO			
1:1	71±.66	.44±.02	38±.51
1:2	73±.76	.46±.07	37±.59
2:1	74±.81	.49±.09	40±.61
SO+OO			
1:1	69±.56	.38±.03	33±.47
1:2	72±.64	.37±.07	35±.41
2:1	68±.71	.33±.06	36±.33
FO+OO			
1:1	71±.66	.54±.11	40±.59
1:2	70±.71	.52±.03	38±.41
2:1	73±.69	.57±.06	41±.56
TO+SO			
1:1	60±.81	.37±.07	37±.53

1:2	63±.67	.39±.05	35±.51
2:1	61±.61	.41±.04	37±.49
TO+OO			
1:1	62±.71	.39±.06	36±.54
1:2	61±.64	.42±.03	35±.61
2:1	62±.66	.40±.05	36±.56

Table 7.Antioxidant activity of ternary blended oil

Blended oils	DPPH	FRAP	ABTS
FO+TO+RBO			
1:1:1	75±.19	.44±.03	30±.35
1:2:1	74±.27	.43±.05	32±.29
2:1:1	75±.23	.47±.08	32±.36
FO+SO+RBO			
1:1:1	66±.32	.38±.11	29±.23
1:2:1	65±.28	.35±.07	29±.39
2:1:1	63±.33	.34±.09	30±.32
FO+RBO+OO			
1:1:1	70±.46	.40±.07	31±.27
1:2:1	69±.28	.39±.06	32±.31
2:1:1	72±.22	.42±.02	31±.29
FO+TO+SO			
1:1:1	66±.28	.37±.09	28±.21
1:2:1	65±.61	.33±.03	29±.68
2:1:1	67±.56	.36±.07	29±.83
FO+TO+OO			
1:1:1	64±.81	.31±.08	31±.49
1:2:1	66±.37	.35±.06	30±.47
2:1:1	63±.49	.36±.04	30±.59

4. Statistical Analysis -

All the experiments done in triplicates and results were shown as mean ± Standard Deviation (S. D.) of three samples. Differences were tested for significance using the ANOVA procedure.

5. Conclusion - From the present study it can be concluded that in binary blending system flax seed and tomato seed oil combination shows the highest antioxidant activity and their phyto chemical content was also very high while tomato seed oil and olive oil combination shows the lowest antioxidant activity and phyto chemical content compared to any other blended oils in binary blending system. In ternary blending system flax seed oil ,tomato seed oil and rice bran oil combination show the highest antioxidant activity and phyto chemical content than other ternary blended oils. Both binary and ternary blended oils are oxidatively stable.

6. Acknowledgement -

I would like to acknowledge my institute Indian Institute of Engineering Science And Technology (IIEST), Shibpur, Howrah, Westbengal, India and my supervisors Prof. D.K.Bhattacharyya and Dr. M.Ghosh.

7. Reference -

1) AOAC International, Official methods of analysis of AOAC International, 16th ed., GAITHERSBURG, 1999.

2) Benzie, I.F.F., Strain, J.J.The ferric reducing ability of plasma (FRAP) AS A MEASSURE OF "antioxidant power" the FRAP assay.Anal Biochem 239,70-76 (1996)

3) Chang C, Yang M, Wen H, Chern J.Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 2002, 10:178-182.

4) Fatma Bouaziz, Mohamed Koubaa, Francisco J.Barba, Sahin Roohinejad and Semia Ellouz Chaabouni. Antioxidant properties of water soluble gum from flax seed hull, 2016

5) Gorinstein, Oscar J. Medina Vargas, Nicolas Jaramillo O, Simon Trakhtenberg. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. European Food Research and Technology 225(3):321-328 · January 2007

6) Mc Donald S, Prenzler PD, Antolovich M,Robards K.Phenolic content and antioxidant activity of olive extract. Food Chemistry 2001, 73:73-84.