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### **RESEARCH ARTICLE**

# A novel approach for improvement of Oxidative stability of flaxseed oil by blending with palm oil.

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## Manuscript Info

Key words:

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#### Abstract

..... Manuscript History: Present work is carried out to enhance Flaxseed oil oxidation stability without disturbing its natural properties through blending with Much Received: 15 September 2015 Oxidative stable Palm oil rich in bioactive and antioxidant compounds. Final Accepted: 22 October 2015 Blending unsaturated oil with palm olein is alternative to Partial Published Online: November 20115 hydrogenation Science it also has effect of reducing the percentage content of unsaturated lenoleic and linolenic acids. Method: FNB (Flaxseed oil Blend) was formulated by blending FO flax seed oil, Palm oil, blending, (Flaxseed oil with Palm oil in stablalized ratio. shelf-life (oxidation stability) oxidation stability of pure oils (Flaxseed and plam) as well as oil blends (FNB 1, FNB 2) were evaluated. Oxidation stability was followed up by measuring of physical and \*Corresponding Author chemical properties. ..... Results: It is indicated that OSI of pure oils were 72 (PO) and 6 (FO), and

28.68 (FNB 1) 23.54 (FNB 2) at 100 ŰC which is improved by 22.68 % (FNB 1) 17.54% (FNB 2). Induction period after 1 month storage 42(PO), 3.35(FO) 18.17 (FNB 1) (16.32) FNB 2 and PO blends improved for the Blend FNB 1+PO Compared to pure FO. The blend FNB inhibited formation of PV (31.71%), Flash point (49.46%), FFA (54.17%) and p-AnV (75.29%), FP more significantly (p<0.01) than FSO with inhibition percentage of 9.75%, 29.24%, 35.42% and 20.06% for PV, FFA, MP,FP and *p*-AnV, respectively. Results demonstrated that blending FO with Palm oilen greatly enhanced its oxidation stability. This makes blending a simple promising alternative technique to replace synthetic antioxidants. And also help in balancing the Ratio of omega 3 and omega 6 as required.

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# **INTRODUCTION**

Many studies have suggested that high intake of omega-3 fatty acids could exert a strong positive influence on human health Kolanowski et. al. 1999); however, dietary intake of these fatty acids are usually low Kolanowski et. al, 1999). Flaxseed is a major omega-3 fatty acid source containing a-linolenic acid, along with other non-lipid constituents such as certain sources of phytoestrogens, lignans, and soluble fibers Bloedon, 2004; (3)Kitts et. al, 1999). Another source of omega-3 fatty acid is fish oil, which is known to be the main dietary source of very long chain omega-3 polyunsaturated fatty acids (Gunstone, 1996; Hyunh et. al, 2007). In general, an increase in omega-3 fatty intake in the diet has been associated with a decreased risk of cardiovascular disease, as well as additional benefits in brain development. (Mensink, R.P., M.B. Katan, 1990)

The relatively short shelf-life of most commercially available vegetable oils limits their usefulness in various applications (Merrill et al., 2008). This oxidation is an important problem for food, pharmaceutical and cosmetic industry, specifically when the lipid substrates are composed of unsaturated or polyunsaturated fatty acids (PUFA) that are sensitive to oxidation (Ramadan and Wahdan, 2012). Factors affecting the oxidative stability of vegetable oil include the fatty acid (FA) composition of the oil, antioxidants, oxygen, and light and storage temperature. Flax seed oil with the high levels of PUFA, isomer readily oxidized if stored or handled improperly (Lukaszewicz et al., 2004; 10. Lutterodt, H et al : 2010).Partial hydrogenation, addition of antioxidants and metal cheaters (Warner et al., 1985; Frankel et al., 1985; Snyder et al., 1986), mutation breeding to reduce the linolenic acid content (Anonymous, 1990; Haumann, 1990; Scowcroft, 1990), and interesterification of oils having high content of polyunsaturated fatty acids with oils having high content of saturated fatty acids (Neff et al., 1992, 1993), have been used to improve polyunsaturated (PUFA) vegetable oils resistance to oxidation. Although hydrogenation of PUFA oils improves the stability of the edible oils, one negative effect correlated with hydrogenation is the production of Trans fats which has been associated with risks of heart disease in humans (Lists, 2004; Ascherio, 2002; Mensink and Katan, 1990; Zock and Katan, 1992). Interesterification of PUFA oils with other stable vegetable oils increases processing costs which is not economically acceptable (Chu and Kung, 1998). One way to improve the stability of these oils is by blending with oils of high oleic acid contents (Anwar et al., 2007; Premavalla, Madhura, & Arya, 1998). Compared with PUFA, oleic acid is more resistant toward 2015 5040 oxidation, both at ambient storage and at high temperatures (Warner & Knowlton, 1997). Also, blending with oils rich in natural antioxidants (polyphenols, lignin, vitamins...etc.) has emerged as an economical way of modifying the physicochemical characteristics of vegetable oils and fats besides enhancement in oxidative stability (Gulla and Waghray, 2012). When a specific triacylgyceride is desired, enzymatic interesterification is usually preferred. Although interesterification seems to be desirable in edible oils and fats processing, one negative effect is the high cost associated with this procedure. With the negative effects associated with hydrogenation and costs associated with interesterification, oil blending can be considered another alternative to gain desirable characteristics of certain fats and oils. Blending of oil has been reported successful in various edible oils sources, such as groundnut oil, soybean oil, palm kernel oil, coconut oil, and karanja oil (Semwal, 2001and Rossell, 1985).

In this study, high plant based omega-3 fatty acids source is blended into palm oil, a common vegetable oil used in Asia and Africa, to derive two specific omega-3 enriched palm oil blends that contained different omega-3 fatty acids and which could be used as common cooking oils. The purpose of this study was to utilize a quantitative approach for establishing the correct omega- 3: omega-6 ratio that would enable reproducible formulations with oxidation stability.

# Material and Methods

Statistical analysis:

All chemical analyses were performed in three replicates and the results were statistically analyzed. Statistical analysis was performed using the ANOVA, t test and STATA –GLM software's.

#### Materials

Refined, bleached, and deodorized palm oil was purchased by Goyal trader Lucknow (India). Flaxseeds were crushed by laboratory grinder, pressed using laboratory hydraulic press (Carver) under 10,000 Lb/inch for 1 h at room temperature. The resultant oil was filtered through a fine cloth, kept in dark brown bottles and stored in deep freezer at 18 C until analysis and formulation. by Polar Foods, Inc. (Fischer Branch, MB). All reagents were analytical grade. Heptadecanoic acid methyl ester (C17:0) and sodium thiosulphate were obtained from Sigma Aldrich (St. Louis, MO). A Supelco 37 Component FAME mix of C4-C24 (Bellefonte, USA) was used as a fatty acid standard. Upon receiving palm oil, flaxseed oil, both oils were stored at Sub zero temperature of -19 °C. 11 oil samples were covered with aluminum foil and stored in boxes to minimized light exposure (from freezer bulb) during frozen storage.

## Experimental Design

Standard fatty acid calibration curves were constructed to quantify the linoleic acid, linolenic acid, EPA, and DHA content of oil samples for blending calculations. Palm oil was blended with flaxseed oil in two ratios of 2;4 and 3:4.based on weight calculations derived from preliminary GC data on all experimental oils. GC analysis of the new

oil blends was performed to ensure that omega-3: omega-6 ratio was within a 3:4 ratio. All data are presented as standard values.

#### Fatty Acid Analysis - •

The fatty acid compositions of different starter edible oil materials were determined using gas chromatography as reported by (30)(Yuan and Kitts (2002). Fatty acid methyl esters in each sample were measured using a Shimadzu Model GC-17 A flame ionization gas chromatograph (Mandel Scientific Co. Ltd. Guelph, Canada), containing a fused-silica capillary column (J&W Scientific DB-23) of 30 meter length, 0.25 mm internal diameter, and 0.25 um film thickness. The initial column temperature was set at 120 °C with an increase temperature applied at a rate of 3 °C/min until 220 °C was reached. The injector and detector had a working temperature of 250 °C. A fatty acid standard obtained from Supelco (Supelco 37 Component FAME mix C4-C24) was used to identify different fatty acids in each sample. Quantitation of fatty acids was made using an internal standard (heptadecanoic acid methyl ester, C17:0) in comparison with standard curves derived for each of the major fatty acids under investigation. Samples were prepared by adding the internal standard (0.2575 g of heptadecanoic acid methyl ester in 250 ml of hexane) to the oil sample (0.1 g). Then, 5 ml of 0.5 N potassium hydroxide/ methanol was added to dissolve lipids and the test tubes were left overnight at room temperature. The next day, 2.5 ml (one full Pasteur pipette) of petroleum ether was added to each sample and a phase separation wasaccomplished in 20 minutes. After phase separation, the upper layer (contains ether and nonsaponifiable matter) was discarded. The next step was adding 2 or 3 boiling chips and 5 ml of Boron Trifluoride. Samples were heated in a beaker containing 100 ml boiling water. After 15 to 20 minutes, cold water was poured into the beaker to terminate the reaction. Samples were allowed to cool to room temperature for 60 minutes.

Deionized water (2 drops) and 5 ml of hexane was added into each tube, FAMEs were dissolved in the top hexane layer and the hexane layer was transferred to a tube containing a drying agent ( $Na^2SO^4$ :  $NaHCO^3$ , 4:1) before being dispensed into a GC vial for analysis.

#### **Fatty Acid Calibration Curves**

Gas chromatography was performed to derive fatty acid area measurement (linoleic acids,  $\alpha$ -linolenic acids, EPA, and DHA) using an internal standard (heptadecanoic acid). Calibration curves were obtained by comparing the area ratio of FAMEs (linoleic acid,  $\alpha$ -linolenic acid, EPA, and DHA) with the internal standard on X - axis and weight ratio of FAMEs (linoleic acid,  $\alpha$ -linolenic acid, EPA, and DHA) with the internal standard on the Y-axis. A linear regression was used to derive the equation for mass to mass ratio and area to area ratio for the internal standard relative to the specific fatty acid.

#### **Blending Procedure**

A quantitative measure of the amount of omega-3 fatty acids derived from flaxseed oil respectively, when added to palm oil was determined by absolute calculation based on quantitative measurement of fatty acid composition of palm oil, flaxseed oil. Upon blending, a palm oil sample was removed from frozen storage and placed at room temperature overnight. Flaxseed were added to the palm oil sample, and mixed together in stainless steel vessel, gently warmed at 40–50 C with continuous steering until complete solublization and homogeneity were achieved (5–10 min)using a regular spoon stirrer for 5 minutes. Blending was performed in a cold room set at 4  $^{\circ}$ C to minimize the heat friction from the stirrer.

#### **Peroxide Value**

The AOAC official method (AOAC 965.33, 2003), with minor modification, was used to determine sample oil peroxide values. Samples weighing  $5.00 \pm 0.05$  g were transferred to a 250 ml Erlenmeyer flask, to which 30 ml of an acetic acid-chloroform mixture was added. Saturated potassium iodide (1ml) was added to the flask and the sample was mixed by swirling the flask for 1 minute. Samples were stored in the dark for 5 minutes, after which, 30 ml of deionized water was added and followed by the addition of 1% starch solution (0.5 ml). The mixture was then titrated with 0.01 N sodium thiosulphate until the blue color disappeared. The peroxide value (P.V.) was calculated using the following equation:

P.V. (meq OVkg fat) = [(Volume (ml) of sodium thiosulphate)\*(N of sodium thiosulphate used)]\* 1000

## **Determination of p-anisidine Value**

The IUPAC (1987) method was used to quantify aldehydes (mainly 2-alkenes) Present in oil using the p-anisidine values. Oil samples were weighed to approximately 2 g (recorded to 3 decimal places) and placed in a 25 ml volumetric flask, then filled to Volume with iso-octane. Absorbance readings at 350 nm were taken in a 1.0 cm cell using a Shimadzu UV-160 spectrophotometer (Canada), employing iso-octane as the blank.

The next step was to measure 5 ml of oil solution into a glass test tube and add 1.0 ml of

a 0.25% p-ariisidine solution (p-anisidine in acetic acid). Samples were shaken to mix and then stored in darkness for 10 minutes. Absorbance was taken at 350 nm against a sample blank (5ml of iso-octane plus 1 ml p-anisidine reagent). The p-anisidine value (p-A.V) was calculated according to the following equation:

## P-A.V. = [15(1.2As-Ab)]/m

where, As = the absorbance of the fat solution with the addition of p-anisidine reagent; Ab = the absorbance of the fat solution; and m = mass (g) of oil used.

## **1.9 Total Oxidation Number (Totox)**

Totox is a measure of the total oxidation (Che Man and Hussin, 1998; Rossell, 1994), and is calculated according to the following equation: totox = (2\*P.V.) + p-A.V.

## Oil satiability index:

A 5.0g sample of oil or melted fat is weighed into a disposable glass test tube. The test tube is then placed in a heating block at a temperature of 110°C. Clean, dry air is bubbled through the sample, and the effl uent stream of air is bubbled through a collection tube fi lled with ultra pure water. An electrode is placed in the water, and the instrument monitors the conductivity. As the oil oxidizes, volatile organic acids are given off, trapped in the collection tube, and increase the conductivity of the water. The instrument generates a plot of conductivity vs. time, and determines the inflection point in the conductivity curve. This inflection point is defined as the OSI time. A mathematical conversion can be used to convert the OSI time into a corresponding AOM time.

## **Results:**

## Fatty acid composition:

Fatty acid composition of substrate oils and oil blends is presented in Table 1. The main fatty acids in NO and SO were palmitic, oleic, and linoleic acids with 13 %, 21,08% and 57.7% (NO) and 10.47%, 40,18% and 42,59% (SO), respectively. While, The main fatty acids in FO were oleic, linoleic and linolenic acid with 16,95 %, 15.02 % and 58.11 %, respectively. As a result of blending, major changes were noted in the contents of C18:1 and C18:2 of the substrate oils. Blending of FO with 20 % of NO or SO resulted in significant (p < 0.05) increase in oleic acid and linoleic acid content whereas significant (p < 0.05) decrease in linolenic acid content was observed (Table 1). Oleic acid and linoleic acids were increased by more than 10 % for FNO and FSO. On the other hand linolenic acid content exhibited a decrease by more than 8 % upon blending.

Table 1 : Fatty acid profile of pure oils and oils of formulated oil blends.					
	Vegetable oils		oil blends		
	Palm oil	Flaxseed oil	40% palm oil	60% palm oil	
			FPB2	FPB3	
C12:0	0.17	ND	0.1	0.1	
C14:0	1.04	ND	0.61	0.61	
C16:0	41.21	5.71	27.41	26.61	
C16:1	0.18	0.07	0.13	0.13	
C17:0	0.1	ND	0.08	0.08	
C17:1	0.02	ND	0.02	0.02	
C18:0	4.44	4.84	5.21	4.38	
C18:1	36.21	14.99	27.88	27.25	

C18:2	10.03	15.11	12.25	12.32	
C18:3	0.17	59.02	29.74	27.45	

## **Total oxidation value**

Lipid oxidation involves the continuous formation of primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products hydroperoxides. The formation rate of hydroperoxides exceeds their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages (Fereidoon Shahidi and Ying Zhong, 2015). Therefore, PV is a very important indicator of stability at the first stages of oxidation. PV measured for pure oils, and oil blends were shown in Table 2. Results indicated that pure oils showed very lower PV compared with Blended ones which assessed the antioxidative effect of the minor polar components (including phenolic compounds, sterols and tocopherols and carotenoids). Regarding oil blends our results revealed that 60% FNO oil blend was significantly (p > 0.01 %) more stable than 40% FSO blend through the entire storage period, which may be due to the oxidative stability of palm oil. Also because of Flaxseed lignans SDG, SECO, ED and EL are found to be equal or somewhat more potent than BHT, vitamin E. Thus, they could have commercial potential as an alternative to these antioxidants. oil moiety which proved superior antioxidant effects . (fig1, fig2)

Table 2: Physical and chemical characteristics of pure oils and oils of formulated oil blends.					
	Vegetable oils		Oil Blends		
	Palm oil	Flaxseed oil	40% palm oil	60% palm oil	
			FPB2	FPB3	
Refractive index	1.4631	1.4651	1.4669	1.4662	
Melting point (°C)	36	11.50	33	33.5	
Slip point (°C)	34	16.70	31.5	32	
P-Anisidine value	1.85	2.66	2.54	2.23	
Flash point	320	135	210	250	
Free fatty acid (as oleic acid %)	0.04	0.32	0.733	0.532	
Peroxide value	0.49	0.98	0.89	0.79	
Acid value	0.35	0.39	0.47	0.49	
Iodine value	54.39	195.98	130.98	121.3	
Saponification number	230.61	188.98	189.76	191.87	



Fig 1 : peroxide value profile of pure and blended oil



## Fig 2: p-AV profile of pure and Blended oil

## **Oxidative stability:**

Lipid oxidation may be assessed in many ways, among which changes in the initial reactants and formation of new oxidation products are most commonly assessed. Meanwhile, sensory analysis assesses both the subjective and, in some cases, objective measurements of oxidative changes in foods. Each method shows both advantages and disadvantages, thus it is highly recommended to use two or more methods assessing both primary and secondary oxidation products.

The oxidation stability of the stripped oils, pure oils (crude oils) and oil blends were evaluated by following up the formation of primary oxidation products (PV, CD and CT) as well as formation of secondary oxidation products (p-AnV). Induction period for 60% were 23.4 h while 40% FOB 28.4

From the result it has been concluded that 60% blend of palm provide the good stability to the flaxseed oil in comparison to 40% blend (see Table 3).Depend upon the blending concentration should provides the desirable value of Omega 3 required for the further process.

Table 3: The oxidative stability of pure oils and oils of formulated oil blends.					
Oxidative stability at 100 $\hat{A}^\circ C$	Vegetable oils		oil blends		
	Palm oil	Flaxseed oil	40% Total fat	60% Total fat	
			FPB2	FPB3	
Induction period in h	76	6	23.54	28.54	
Induction period in months (Validity period)	42	3.35	16.14	18.17	



Fig 3 : The Oxidative Stability of pure and blended oil .

# **Discussion:**

The big problem with processed vegetable oils is at the cell level. Since these fats don't occur in nature, our bodies don't know how to deal with them. The body tries to use them, thinking they are normal fats, and they wind up in cell membranes and other places where they behave strangely. Oil these man-made fats weaken the cell membrane. particularly their protective structure and function. The big threat to males is coronary heart disease. In males, processed vegetable oils appear to activate the body's immune responses when they enter the artery walls, because these fats do not resemble anything that the body recognizes, so the body attacks it. This directly leads to an inflammatory response in the arteries which leads to the formation of dangerous plaque build-up. In the case of women it's a bit different. These processed vegetable oils manage to somehow bypass the immune response at the artery wall. However they move further along into the body, and are deposited into the fatty tissues such as in the breasts, where they directly contribute to cancer. here in this study naturally expelled flaxseed oil is used for blending. This may leads to make human body tissue to recognize in its natural form, modifications mainly as discussed esterification may leads to change the natural properties of the fatty acid present in flaxseed oil. Oil blending can be considered another alternative to gain desirable characteristics of certain fats and oils. Blending of oil has been reported successful in various edible oils sources, such as groundnut oil, soybean oil, palm kernel oil, coconut oil, and karanja oil (Fatouh Said 2012; Semwal, 2001; De et al., 1999; and Rossell, 1985) In this study, Plant based omega-3 fatty acids source (flaxseed oil) were blended into palm oil, a common vegetable oil used in Asia and Africa, to derive two specific omega-3 enriched palm oil blends that contained different omega-3 fatty acids and which could be used as common cooking oils. The purpose of this study was to utilize a quantitative approach for naturally establishing the 2:4 or 3; 4 omega- 3: omega-6 ratio that would enable reproducible formulations.

# **Conclusion:**

Currently research efforts are focused on identifying the factors/pathways that mediate the protective effects of omega-3 fatty acids and elucidating the molecular mechanism of their action. But firstly we want to work on how to utilize maximum Plant based sources of EPA and DHA and produce maximum amount of Omega3 fatty acids using different methods of Blending and extractions. One of the most serious problems facing edible oil and food industry is Oxidation. Flax seed oil (FO) is very important oil both from a nutritional or medicinal point of view due to its high content of omega-3 and omega-6 fatty acid. Unfortunately, its high content of PUFA makes it very susceptible

to oxidation and hence limits its use. Such a problem could be solved through blending with other oils rich in natural antioxidants like N. sativa oil. Seeking new stable oils and proper ratio of omega 3 and omega 6 blending should be a research point depending on the target oil as well as the application it will be used in. Accumulating evidences suggest that flaxseed is a rich source of natural antioxidants. Thus far, antioxidant potential of flaxseed and their phenolic constituents must have been studied in both in vitro and in vivo models.

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#### **Conflict of Interest:**

Authors declare that there are no conflicts of interest.

#### **Ethical approval:**

This article does not contain any studies with human participants or animals performed by any of the authors.

#### Informed consent:

Not applicable.

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