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Vegetable Oil Blends after Frying and Sample Stored in Different Storage Packs

Hamid Nawaz Khan[1*], Jafar Salamat Khan[1], Shakir Ali[2], Kashif Hussain[3], M. S Alam[4], Anwar Habib[5]

[1]Department of Pharmacognosy and Phytochemistry , Research Laboratory, Faculty of Pharmacy, Jamia Hamdard (Hamdard University) Hamdard Nagar, New-Delhi-110062, India
[2]Department of Biochemistry, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062; India
[3]Department of Pharmacy Doon College of Education, Sunderpur, Saharanpur (U.P)
[4]Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062; India
[5]Majeedia Hospital, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062; India

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*Corresponding Author

Tel : +91-9311186432
Fax : +91-1126059663

Email: hamidrumi@gmail.com

Abstract

The work done in the reporting quarter concerns the micronutrient analysis of the blend in fresh oil samples and in samples stored under different storage conditions in various types of containers and as well as before and after frying. Conjugated dienes, p-anisidine value, specific gravity, copper and iron content of the oil samples under different conditions were also estimated, and the oxidative stability of the oils was determined. In particular the retention pattern of micronutrients, conjugated dienes and p-anisidine values in the blend under various storage conditions in different packs was studied.

Key Words: Vegetable oil blend, Micronutrients, Storage, Conjugated dienes, p-anisidine, Specific gravity, Copper, Iron, Oxidative stability

Introduction

Oils such as palm olein oil and rice bran oil are considered as good sources of micronutrients (MN) that include β-carotene, tocopherols and tocotrienols that are known for their beneficial actions in human. Tocotrienols, for example, have been reported for lowering the serum cholesterol level in hypercholesterolemic subjects (1, 2), and have been recommended for metabolic disorders such as coronary heart disease (CHD), diabetes, obesity and hypertension. The American Heart Association recommends (AHA Medical/Scientific Statement, 1990) that total fat in diet should not exceed 30% of energy calories, out of which the energy percent from saturated fatty acids (SFA) should not exceed 8-10%, PUFA (poly unsaturated fatty acid) 10 energy per cent, and monounsaturated fatty acids (MUFA) by difference (that is 10 - 12%). The recommended dietary allowances (RDA) for a balanced diet as per the ICMR Nutrient Requirements and RDA guidelines for Indians (ICMR Expert Group Report: ICMR, 1990) (3) suggests that fat in diet should not exceed 20 energy % with 3 energy % derived from PUFA. The MN present in oils are also recognized for a number of other beneficial effects on body, and have been suggested effective in conditions as wide as cancer and kidney stones, to name a few. However, in spite of a high MN content, the use of unconventional but MN rich oils is limited mainly because of the regional preferences of specific individual oils (4).Blending may reduce the pressure of regional preferences of specific individual oils, and can give a better quality product, which include tailor-made physico-chemical properties as well as nutritional value at affordable prices. In the following section, the effect of two MN rich blends of edible vegetable oils on serum lipid composition. The study is important, as limited data is available on the effects of oil blends on the physiological systems, although extensive research has been conducted on many conventional and unconventional vegetable oils. The oils rich in PUFA, for example, cause a decrease in 'bad' cholesterol and triglycerides. Rice bran oil and palm olein oil are particularly rich in many MN that have been reported for health benefits (5). The impact of palm olein oil on cardiovascular disease and cancer has been investigated (6). Tocotrienols from the palm olein oil inhibit protein oxidation and lipid peroxidation in rat liver microsomes (7). The ω-3 fatty acids (linolenic acid) in oils can increase the level of circulating good cholesterol. The estimation of micronutrients, conjugated dienes, p-anisidine, specific gravity, copper and iron in the MP blend under various conditions, and the study on the micronutrient level (8).

Materials & Methods

Experimental diets

Three oils Mustard oil [MO] and Palm olein oil [PO] were tested in this experiment. The oils were either used as the sole source of fat or in a blend. Blends were made with PO which was mixed with MO in a 30:70 ratio respectively. Oil blends used in the study were prepared and supplied by Hamdard University, New Delhi, India. Oils or oil blends were incorporated into the experimental diets which were either high in cholesterol (HCD) or cholesterol frees (CFD). The diets were as follows:

Table 1: The composition of fat free diet

Ingredients	Fat free diet (g/100 g diet)
Casein	15.0
Starch	66.3
MO	10.0
PO	10.0
MP (30:70)	10.0
Salt mixture	04.0
Vitamin mixture	01.0
Cellulose	02.0
Cholesterol	01.0
Cholic acid	00.5
Choline chloride	00.2

Casein (fat free) from Glaxo (India) Ltd. MO- Mustard Oil, MP-Mustard Palm olein oil blends.

Salt mixture contained: 4.6% NaCl; 9.3% $Na_2HPO_4.H_2O$; 25.6% K_2HPO_4; 14.5% $CaH_4(PO_4)_2.H_2O$; 3.3% Fe $(C_6H_5O_7).5H_2O$; 34.9% Ca $(C_3H_5O_3)_2.5\ H_2O$; 7% $MgSO_4$; 0.05% $ZnSO_4.7H_2O$; 0.9% KI; 0.02% Cr $(C_2H_3O_2)_3$. Vitamin mixture contained: 150 mg riboflavin; 100 mg thiamine; 1000 mg nicotinic acid; 100 mg pyridoxine; 1 mg cyanocobalamine; 500 mg pantothenic acid; 50 mg folic acid; 3750 mg ascorbic acid; 100 mg vitamin K; 100 mg vitamin E; 2,50,000 IU vitamin A; 20,000 IU vitamin D, and starch to make up to 100. The full composition of the experimental diets is detailed in Table 1.

Animal experiment

Animals

Seventy two male Wister/NIN albino rats weighing between 120 and 150 g were used for this experiment. The animals were randomly assigned to each of the experimental groups described above. Animals were housed individually in screen bottomed cages under a controlled environment with 12 h light and 12 h dark cycles. Animals were handled using animal Welfare Guidelines outlined by Animal welfare institute, New York.

Feeding

Animals were fed with experimental diets and boiled and purified water on an ad libitum basis. Diets were fed for four weeks. Food intake and body weight were recorded weekly, Tissue collection. During the last three days of the experiment, feces were collected quantitatively for 24 h periods for each animal and stored at -20 °C until analysis. At the end of the four week feeding period, animals were fasted overnight. Blood (4–6 ml) was drawn by cardiac puncture. Following the blood draw, animals were sacrificed using chloroform anesthesia. At that point livers were removed and stored at -20 °C until analysis.

Blood

Blood samples were allowed to stand at room temperature for 1 hour and centrifuged at 2000 r.p.m. for 30 min to separate serum. Samples were labeled and stored at -20 °C until analysis.

Chemical analysis

Oils

Iodine values (IV), saponification value (SV) and acid value (AV) were determined using AOAC Methods [9, 10]. Tocopherols and tocotrienols were estimated by HPLC [11]. For the analysis of oryzanol, retinol has been identified as an important micronutrient in animals that is derived from food. It can be determined with precision by HPLC.

Blood

Serum was analyzed for TC and HDLC [12] and TG [13] using enzymatic kits (Glaxo, India). LDLC and VLDLC were estimated by calculation.

Estimation of conjugated dienes and p-anisidine values

Conjugated dienes

Conjugated diene values were determined by the spectrophotometric method described by Wettasinghe and Shahidi. This method determines the diene conjugation of unsaturated linkage present, expressing the value as a percentage of conjugated dienoic acid. Briefly, 0.02 – 0.04 gm of oil was weighed into a 25 ml volumetric flask and dissolved in iso-octane. The volume was made up to the mark with the same solvent, and the solution was thoroughly mixed before determining the absorbance at 234 nm.

para-anisidine value

This value is defined as 100 times the optical density measure in a one cm cell containing a solution of I g of oil in 100 ml of a mixture of the solvent and reagent. To determine p-anisidine value, the colorimetric method of Jirusova was used.

Estimation of metals in the oil sample

Iron

For determining iron, the oil sample (10-20g) was first ashed in an accurately weighed silica crucible. The dish was ignited, and the material was ashed below 525˚C for 4-6 hrs in a muffle furnace. The dish was then cooled and weighed. The total ash content was expressed in percentage.

Copper

Copper is isolated and determined calorimetrically as copper diethyl dithocarbonate at pH 8.5 in the presence of EDTA. Copper reacts with sodium diethyl dithiocarbonate in alkaline solution producing a yellow to brown colour depending on the amount of metal present.

Determination of the density/specific gravity of oil samples

In can be determined using a pyknometer fitted with a thermometer or a density bottle balance. The pyknometer or empty density bottle is accurately weighed (W1) and then filled with the sample and then weighed.

Determination of the antioxidant efficacy using differential scanning calorimetry (DSC)

Differential scanning calorimetric (DSC) method is used for studying various heat-related phenomena in materials by monitoring associated changes in enthalpy. Oxidation is an exothermic process and the heat of reaction evolved makes it possible to employ DSC for the evaluation of oxidative stability of oils. The analysis was carried out in a Mettler Toledo DSC 821 instrument (Schwerzenbach, Switzerland), which was calibrated with indium before the analysis with an empty pan as reference. The sample (10 mg) was loaded into the aluminum sample pan. The oil without additives was first studied under dynamic heating regime from 90°C to 200°C and the temperature of onset of oxidative changes was noticed from the DSC curve as the point of inflection. The samples were then analyzed isothermally at 150°C at a temperature 10°C below the said onset temperature for 45', under a stream of oxygen at 40 ml/minute. The flow of nitrogen was 200 ml/min. The extracts were tried at 100, 200, 500 and 1000 ppm levels. The time at which the onset of oxidation occurred was noted and this induction period was taken as an indicator of the oxidative stability of the oil sample. The observations are shown in the results section.

Statistical analysis

Data were analyzed by ANOVA to ascertain if the dietary treatments were a source of variance related to various lipid parameters measured. Significance was accepted at the $p<0.05$ levels.

Results and discussion

Micronutrient levels in the blend

Methodology for the analysis of tocopherol, tocotrienols, oryzanols and phytosterols in oil samples (14). All these estimations were based on the HPLC technique. Results are depicted in Tables 1 – 5. These results clearly suggest that the vegetable oil blend has improved micronutrient content in comparison to the mustard oil, and that the micronutrients retained to a larger extent even after frying the blend.

Micronutrients present in vegetable oils such as tocols, oryzanols, and phytosterols, have several functions. Oryzanol in rice bran oil, for example, helps reduce blood cholesterol, improve capillary actions of blood vessels, and has anti-aging effect similar to tocopherols. Oryzanol I spastically a mixture containing ferulic acid esters of triterpene alcohols and plant sterols. Tocols are the natural antioxidants in vegetable oils and help to protect the oil against auto-oxidation. Gamma- and delta- tocopherols and their corresponding trienols are particularly very effective in preventing auto-oxidation. Alpha-tocopherol is a good antioxidant against photoxidation. The oils rich in tocols such as palm oil have good frying stability because of the presence of gamma- and delta- tocopherols. Tocotrienols contain three unsaturated double bonds as compared to the corresponding tocopherols. This makes tocotrienols more effective as antioxidant against auto-oxidation in the frying process. In our study we measured the levels of tocols, oryzanols and phytosterols in fresh oil samples, oil samples taken after frying and the samples stored for six months in various containers. The micronutrient levels were estimated by chromatographic method, and the results are depicted in Tables 1- 5. These results clearly suggest that the MP blend had improved micronutrient content in comparison to the mustard oil, and that the micronutrients retained to a larger extent even after frying the blend. Studies on the effect on the micronutrient levels of oils stored in different containers for six months also provide evidence that the retention of micronutrients in the blend was better than the mustard oil alone. Amber colored pet bottle were able to preserve more micronutrients.

The studies performed on the micronutrient content of various oils following frying indicate that frying caused a decrease in the level of micronutrients. The reduction in the level of tocols was much severe with the tocols levels brought down to 35.8 ppm from 655 ppm as observed in fresh mustard oil samples; that is only 5% tocols were retained on frying the mustard oil. However, in MP blend the retention was 22% (Table 2) after an 8 hour frying. After an 8hr frying the retention of phytosterols was 56% in the MP blend in comparison to 40% retention in mustard oil alone.

Table – 1: Tocols measured in fresh oil samples and after frying in various oil blends of six months study

		After frying			
Oil sample	Fresh oil	2 hr	4hr	6hr	8hr
MO	655.14	518.8	209.9	61.7	35.8
PO	1204.0	805.12	590.10	390.12	180.3
MP	829.9	523.0	425.0	325.0	165.20

Values in ppm.

Table – 2: Tocols analyzed in oil samples stored in different containers in various oil blends of six months study

		Pet Bottle		Glass Bottle		Tin
Oil	Fresh oil	Amber	Transparent	Amber	Transparent	
MO	655.14	463.1	409.5	418.1	380.7	413.2
PO	1204.0	524.0	500.0	580.1	450.0	525.3
MP	829.94	535.0	550.0	535.0	565.0	560.0

Values in ppm

Table – 3: Phytosterols measured in fresh oil and after frying performance various oil blends of six months study

Oil sample	Fresh oil	After frying 4hr	8hr
MO	0.7874	0.516	0.3152
PO	0.8583	0.635	0.5023
MP	0.8012	0.602	0.4012

% = ppm/10,000

Table – 4: Phytosterols in fresh oil samples and in samples stored in different containers various oil blends in six months study

Oil	Fresh oil	Pet Bottle Amber	Pet Bottle Transparent	Glass Bottle Amber	Glass Bottle Transparent	Tin
MO	0.7874	0.7058	0.6564	0.7271	0.571	0.684
PO	0.8583	0.7520	0.6921	0.8231	0.813	0.831
MP	0.8012	0.6510	0.6120	0.7012	0.651	0.711

% = ppm/10,000

Table – 5: Carotenoids measured in fresh oil samples and after frying

Oil sample	Fresh oil	After frying 2 hr	4hr	6hr	8hr
PO	50.0	40.0	35.0	20.0	15.0
MP	32.3	28.3	21.3	18.2	15.2

Values in ppm

Table-6: Totox value measured in fresh oil samples and following frying

Oil Sample	0-Hour	After frying 2 hr	4 hr	6 hr	8 hr
MO	7.1±0.05	18.4± 0.06	28.3±0.30	36.8±0.20	49.7±0.19
PO	13 ±0.41	21.3±0.87	24.2±0.71	31.3±0.43	33.2±0.58
MP	9.2±0.15	30.2±0.18	31.8±0.62	35.3±0.25	37.2±0.69

Values shown as Mean ± SD, measurement of unit, Total oxidation (TOTOX), and values were calculated as 2PV + AnV.

Table-7: Totox value measured in oil samples stored in different containers this self-life study duration six-month study

Oil	Fresh oil	Plastic Bottle Amber	Plastic Bottle Transparent	Glass Bottle Amber	Glass Bottle Transparent	Tin
MO	8.4±0.05	77.4±0.6	73.2±0.2	72.7±0.54	70.6±0.6	65.2±0.3
PO	13.4±0.2	41.2±0.2	40.2±0.15	39.8±0.5	38.5±0.3	40.5±0.21
MP	9±0.15	30±0.18	29.8±0.2	30.3±0.25	30.0±0.6	30.1±0.3

Table – 8: Conjugated dienes measured in fresh oil samples before and after frying

Oil sample	Fresh oil	After frying 2 hr	4hr	6hr	8hr
MO	0.57±0.05	---	1.41±0.32	--	3.0±0.76
PO	0.57±0.52	---	1.41±0.62	--	3.0±0.63
MP	0.86±0.79	1.07±0.55	1.40±0.51	1.4±0.65	2.5±0.05

Values are shown as mean ± SD

Table – 9: Conjugated Dienes measured in oil samples stored in different containers

Oil	Fresh oil	Plastic Bottle Transparent	Plastic Bottle Amber	Glass Bottle Transparent	Glass Bottle Amber	Tin
MO	0.575±0.05	2.45±0.14	2.33±0.85	2.26±0.05	2.15±0.92	2.15±0.45
PO	0.675±0.59	2.45±0.24	2.33±0.05	2.26±0.35	2.15±0.09	2.15±0.25
MP	0.871±0.87	1.25±0.87	1.12±0.86	1.37±0.39	1.07±0.25	1.10±0.59

Values are shown as mean ± SD

Table – 10: The p-anisidine value measured in fresh oils and after frying performance

Oil sample	Fresh oil	After frying 2 hr	4hr	6hr	8hr
MO	2.38±0.25	6.26±0.52	9.17±0.14	11.23±0.35	13.4±0.26
PO	2.41±0.45	5.26±0.65	8.17±0.10	10.23±0.55	12.4±0.36
MP	2.10±0.05	3.81±0.86	4.65±0.05	5.51±0.46	6.6±0.18

Table – 11: The p-anisidine values measured in oils stored in different containers

Oil	Fresh oil	Pet Bottle Amber	Pet Bottle Transparent	Glass Bottle Amber	Glass Bottle Transparent	Tin
MO	2.38±0.15	18.2±0.19	16.73±0.70	16.73±0.20	17.16±0.52	15.3±0.39
PO	2.1±0.35	13.2±0.32	14.73±0.40	15.73±0.50	16.16±0.59	15.3±0.46
MP	1.7±0.25	10.23±0.75	11.33±0.95	12.53±0.85	14.43±0.50	12.9±0.85

The values indicate AV units [AOCS method cd-18-90 (97)]

Table – 12: Specific gravity measured in fresh oil samples

Oil	Specific Gravity
MO	0.9040±0.05
PO	0.9097 ±0.26
MP	0.9075±0.115

The values indicate AV units [AOCS method cd-18-90 (97)]

Table – 13: Copper and iron measured in various oil

Oil	Copper	Iron
MO	0.740±0.39	0.673±0.06
PO	2.0±0.070	4.12±0.86
MP	3.0±0.97	4.1±0.65

Values are shown as ppm

Studies on the oxidative degradation of oils under various conditions

Conjugated dienes (CD) and p-anisidine values (AnS) describe oxidative degradation of oils and resistance to oxidation. Higher the value, faster is the degradation (15). When oils are oxidized, polyunsaturated fatty acids (mainly linoleic acid or linolenic acid) form conjugated dienes. The CD formation is mainly because of the isomerization of unsaturated peroxides. Therefore, increase in the CD values can be attributed to the oxidation during storage or other conditions. Only traces on conjugated dienes are desired in oil, and a maximum level up to 0.5% is permissible. In the present study, we observed an increase in the value of CD on frying. The high value of CD in the experiment is, therefore, justified by the high content of linoleic acid. In the blend the increase was 87%, an increase much lower than the value exhibited by mustard oil (Table 8). Storage conditions also affect the CD values of oils (Table 9). The increase in CD stored in amber colored glass bottle was just 23%. The values were slightly higher in transparent plastic and glass bottles.

Aldehydes are the secondary oxidation products of decomposition of hydro peroxides, and can be used as marker to determine how much per oxidized material has already broken down (16). The anisidine value quantities important aldehydes. It is defined as 100 times the absorbance (at 350 nm) of a solution resulting from reaction of 1 g of fat in 100 ml of solvent. The value describes the oxidative ad thermal degradation of oil. The anisidine values of all oils increase during heating. For some oils such as olive oil, this increase is much lower than for other oils. AnS values increase as the frying time is extended. The p-anisidine value in oil must not exceed6; <4.0 is the desired level. High anisidine values may be an indication that a fat has been oxidized even when TB and other aldehydes tests give low results because volatile aldehydes may incidentally or intentionally be removed during processing. In the present study (Table 10, 11), an increase in anisidine value is evident in all oil samples following 8hr frying. The increase in mustard oil was 63%, but in the blend it was 88%. The anisidine value also increased in various oil samples stored for six months in pet bottles, glass bottles and tin (Table 11). The increase was least in oil samples stored in tin and amber colored glass bottles.

Specific gravity of oil samples

Values shown in Table 12 denote the specific gravity of oil measured in fresh oil samples. The values are well within the acceptable limits.

Trace metal analysis in oil

Trace metals are present in crude vegetable oils at ppm level. Whenever oil containing unsaturated fatty acids is heated in presence of a metal initiator such as iron, nickel or copper, free radicals are formed during frying (17). Oils such as

soybean can deteriorate from auto-oxidation even when iron content is as low as 0.3 ppm. Metal initiators initiate auto-oxidation in all vegetable oils and animals fats. The desired level of iron in oil is <0.2 ppm and the maximum level should not exceed 0.5 ppm. In Table 13, the value of iron in the blend was 0.150 ppm ± 0.15. This value was value was much below the value measured in mustard oil; in mustard oil the value was 0.134 ppm. Copper content of the blend was 0.28 ppm – around 40% below the copper level in mustard oil.

Oxidative stability studies

We have measured the retention of micronutrients following frying and under different storage containers. Frying makes the food palatable and desirable to the consumer by imparting texture, flavor, mouth feel and after taste. Thus, the cooked product's flavor, texture, appearance, mouth feel and after taste are important criteria applied for the selection of oil for household use in general and industrial use in particular. Shelf life of the product, availability of oil, cost and nutritional requirements are other criteria (18). It has therefore been recommended that to meet consumer desire, the frying oil must possess the following attributes (19):

Low in saturated fatty acids
Low in linolenic acid,
High oxidation and flavor stability, and
Trans-fat free (that is, not hydrogenated)

This is difficult as oils such as palm olein oil, for example, has no trans-fat but has saturated fats. Canola and soybean, on the other hand, must be hydrogenated for industrial frying; thus they will have trans-fat. Further, heating the oil produce changes in the food and oil, both as shown below

The changes occurring in the food	The changes occurring in the oil
The food loses moisture breaking	The fresh oil passes through a
The food surface gets darker, sometimes developing a hard crust	Fried food flavor develops as the frying process continues
The food develops fried flavor and aroma	Along with flavor development, the oil undergoes chemical reaction.

Conclusion

The chemical reactions taking place during heating of oil include hydrolysis, auto-oxidation, oxidative polymerization, and thermal polymerization. All these reactions alter the chemical structure of the molecules in oil. The unsaturated fatty acids are mostly affected. Some desirable as well as undesirable chemical compounds are also formed in oil. Oil in the freshly fried foods contains the same compounds that are present in the fryer oil. The desirable compounds help provide good flavor to the freshly fried product. Sometime, the undesirable oil components can affect the fresh product flavor. In many instances, a fried product with good initial flavor may develop oxidized or rancid flavor during storage. This is because the products of oil oxidation are strong catalysts and cause further degradation of the oil (contained in the product) during storage. This phenomenon is quite pronounced when the oil is abused in the frying process. This is even more evident in products fried in oil with poor fresh oil quality, and low oxidative stability. In this study, we have measured the oxidative stability of the oil samples. The oxidative stability of oils depends primarily on its PUFA content such as linoleic acid and linolenic acid. Linoleic acid is less reactive than linolenic acid. As the number of double bonds increase in the fatty acid, the relative rate of oxidation increases at higher than at lower rate. For example, the relative rate of oxidation of Stearic acid is 1, oleic acid is 10, linoleic acid is 100, and linolenic acid is 150. The oxidative stability result of the MP blend can be correlated to the content of these fatty acids in the blend.

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