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Stabilization of Peanut Oil by Pomegranate Peel Extract

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Abstract:

Synthetic antioxidants are widely used as stabilizers and antioxidants in all fatty foods but they are suspected to pose health hazards and illness. Processing of domestic agro wastes can help obtain natural plant antioxidants that could effectively inhibit the extent of lipid oxidation. This current study aims to reveal the superior antioxidant capacity of agro wastes namely pomegranate peels when used in oxidation substrate namely once cooked commercial peanut oil. Pomegranate peel powder was obtained by shade drying and extraction was done by orbital shaking with distilled water. Oil blends containing 200, 400, 600, 800 and 1000 ppm of the hydro-extract were made with cooked oil. Oxidation parameters such as percentage free fatty acids, peroxide value, saponification value and iodine value were assessed for a period of 24 days at 2 day interval. Synthetic antioxidant BHT was (200 ppm) found to exhibit better protection against lipid oxidation than pomegranate peel extract of 200, 400 and 600 ppm. Peel extract of 800 and 1000 ppm showed better results than BHT. By statistical analysis using ANOVA and Student t- test, it was found that optimal performance was exhibited by pomegranate peel hydro-extract at 800 ppm.

Key words: Stabilization, Pomegranate Peel, Hydro Extract, Peanut Oil and Antioxidant

Introduction:

India being an agricultural country is blessed with medicinally and economically important flora. There are a number of agro wastes, fruit and vegetable processing by-products, peels, bran, seed coats, oilseed residues and cereal residues for bioprospecting and screening of natural antioxidants. In recent years, interest has led to produce a great class of foods referred to as nutraceuticals because these type of foods offer either therapeutic or preventive medicinal benefits in addition to nutrition. One among such agro wastes that can be exploited for nutraceutical value is the peels of pomegranate fruit. So this research aims to study the effect of pomegranate peel extract on the stabilization of oxidative rancidity in cooked peanut oil.

Literature review:

The processing of pomegranate fruits produces 46% peels, 14% seeds and 40% juice [31] with by products representing more than 50% of total weight. It has been reported that the pomegranate by-products contains a substantial amount of polyphenols such as sugar-bound flavonoids, quercetin and kaempferol[7], flavone diglycoside, ellagic acid, and tannins[15,19,20]. Lipid oxidation resulting from the reaction between unsaturated fatty acids and molecular oxygen is a severe problem for the oil and fat industry. It not only deteriorates the quality of fatty foods and brings about chemical spoilage; it also produces free radicals and reactive oxygen species which are reportedly associated with carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases[28]. Synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) have been widely used for the preservation and protection of oil based products against oxidation, however, their use is discouraged due to the perceived carcinogenic potential[27]. Hence there is a need to identify new natural antioxidants for prevention of lipid oxidation in the food industry, with the following advantages: (i) Readily accepted by the consumers. (ii) Considered safe. (iii) No safety tests are required by legislation. Natural antioxidant (not as a synthetic chemical antioxidant) is identical to the food which people have been taking for over a hundred years[24]. Peanut seeds (kernels) contain 40 - 50 % fat, 20 - 50 % protein and 10 - 20 % carbohydrate. Major groundnut cultivators are India (26 %), China (19 %) and Nigeria (11 %), while major producers are China (40.1 %), India (16.4 %), and Nigeria (8.2 %). High fat foods, edible oils and fats are prone to spoilage by rancidation, by exposure to heat, light and other external factors. During high thermal

treatments, triglycerides in oils undergo hydrolysis, oxidation, isomerisation, and polymerization, caused by the absorption of oxygen and water[17]. It not only deteriorates the quality of fats, oils and fatty foods and brings about chemical spoilage; it also produces free radicals and reactive oxygen species which contribute to more than a hundred disorders associated with carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases[26,28], atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS[8,18]. Lipid oxidation is the most important factor affecting the shelf life of fatty foods, influencing the chemical, sensory and nutritional properties, thus playing an important role in determining their use and shelf-life[4,14]. The peroxides produced decompose into various smaller volatile compounds like aldehydes, ketones, alcohols, carboxylic acids, etc. that influence flavor, even at very low concentrations, in which both the oil and the food prepared from it become unpalatable[25]. The fruits, leaves and pomaces of the olive plant cultivars were extracted with ethanol and their ability to reduce the oxidation of sunflower oil was tested at 100 °C. At 100, 200 and 400 ppm, the extracts were incorporated and tested for their ability to stabilize sunflower oil. At 400 ppm, the extract exhibited remarkable anti-oxidant activity which was superior to that of BHT in retarding oxidative rancidity in sunflower oil[11]. The antioxidant efficacy methanolic extracts of rosemary (Rosmarinus officinalis L.), sage (Salvia fruticosa L.), and sumac (Rhus coriaria L.) and combinations at 4 % concentrations were applied to peanut oil stored at 80°C fat accelerated storage. Rosemary extract (except for 3 and 4 h) exhibited the most antioxidant effect compared with other individual extracts. Of the blends, the most effective one was sage plus sumac combination[23].

Materials and Methods:

Fresh pomegranate fruits (var. Kabul) were procured from the local fruit market, checked for damages, which were removed, and washed followed by drying. The peels were manually removed and shade dried for 1 week. The completely dried peels were broken into bits and ground into a fine powder using a blender. The powder was sieved to remove large particles and stored in an air tight container, in refrigerator below 5°C until further use. The fine peel powder was exactly weighed in 10 g each into 3 conical flasks and 100 ml of distilled water was poured into each and mixed well until no lumps were seen. The flasks were plugged tightly using cotton and shaken in orbital shaker for

5 h, each for 2 days. Organic solvents were avoided in order to preserve the purity of the resulting extract. The supernatant was collected and filtered by Whatmann No 1 filter and stored. The residue was re-extracted with 50 ml of distilled water and filtered. Both the supernatants were mixed and filtered again. The final filtrate was sun dried for 2 days, with exposure to light during the day for 6 h each. The crude, dried pomegranate peel extract was stored in sterile Eppendorf tubes in refrigerator below 5°C until further use. Commercial peanut oil was accurately weighed for 100 g and stored in a transparent glass bottle of 200 ml, closed tightly and kept aside. The remaining oil was poured into a large frying pan and heated until it is ready for cooking the pappads. A thermometer was dipped into the oil to check the rise in temperature. Pappads were dropped into the heated oil and fried constantly for 45 min, without replenishment of oil. This heated oil was cooled and weighed at 100 g each into 6 transparent glass bottles of 200 ml and closed tightly. The first bottle was labelled as PNO Negative, which has no additive; the remaining bottles were labelled as PNO Positive, PNO 200, PNO 400, PNO 600, PNO 800 and PNO 1000. The bottle labelled PNO Positive is added with 200 ppm of BHT (w/w). The crude, dried peel extract was separately added to the preheated oil bottles (at 50°C) PNO 200, PNO 400, PNO 600, PNO 800 and PNO 1000 at 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm (w/w), respectively. All the 7 oil blend bottles were stored in ambient room conditions for 24 days. The oil samples named PNO Negative and PNO Positive represent the controls with which the peel extract incorporated oil samples have been compared. All samples were analysed after 2 day interval, i.e, on every 3rd day. All chemical analysis of lipid oxidation parameters were performed according to standard methods, in triplicates, following hygienic conditions. The parameters investigated were percentage Free Fatty Acids (%FFA), Acid Value (AV), Peroxide Value (PV), Saponification Value (SV), and Iodine Value (IV) by AOAC standards (AOAC, 1975).

Result and Discussion:

Effect of Thermal Processing on Commercial Peanut Oil

Peanut oil that has not undergone or has just begun to undergo oxidation has percentage free fatty acids between 0.2 - 2.9 %, and acid value between 0.3 - 4 mg KOH/g , peroxide value [2] below 10 meq /kg saponification value between 188 - 195 mg KOH/g, and iodine value[29] between 82 - 106 g I_2/100g . As oxidation increases, the percentage free fatty acids, acid value, peroxide value and saponification value increase,

and iodine value decreases within the above range. The uncooked oil has lower value of lipid oxidation parameters than the cooked oil. Frying pappads began at 180°C, and gradually reached 190°C which lasted for 45 min. Table 1 shows the various oxidation parameters of cooked and uncooked oil measured on the zeroth day of study.

Comparison of oxidation parameters of both cooked and uncooked peanut oil observed on the zeroth day is shown in Table 1. The uncooked oil has relatively lower values of 0.319 % free fatty acids when compared with that of cooked oil having 1.053 % free fatty acids. The acid values are related to the percentage of free fatty acids. Acid value of cooked oil is only 0.636 mg KOH/g while it is 2.094 mg KOH/g in cooked oil. The peroxide value of cooked oil of 4.006 meq/kg is relatively high that that of uncooked oil of 1.673 meq/kg. Saponification is observed to have increased from 188.87 mg KOH/g in uncooked oil to 190.973 mg KOH/g in the cooked oil. The trend of decrease in iodine value is a vital indication of oxidation. From the value of 99.194 g I_2/100g of uncooked oil, it has reduced to 90.734 g I_2/100g in cooked oil.

OXIDATION PARAMETER*	UNCOOKED PEANUT OIL	COOKED PEANUT OIL
Percentage of Free fatty acids (as oleic acid)	0.319	1.053
Acid value (mg KOH/g)	0.636	2.094
Peroxide value (milli eq./kg)	1.673	4.006
Saponification value (mg KOH/g)	188.87	190.973
Iodine value (g I_2/100g)	99.194	90.734

Table 1- Oxidation parameters of uncooked & cooked commercial oil on zeroth day

* - mean of triplicates

Effect of Storage on percentage of Free Fatty Acids

Formation of free fatty acids (FFA) might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture[12]. Natural antioxidants incorporated in oils and fats help delay rapid oxidation and help preserve the triglycerides from breakdown[34]. Soybean oil containing 1600 and 2400 ppm of potato peel extract, showed lower values of FFA

(0.120 % and 0.109 %) than the control sample (0.320 %) and those containing 200 ppm of BHA and BHA, showing competitive effectiveness of the peel extract with BHA and BHT. A study on the efficiency of garlic extract on stability of soybean oil revealed that the control with no antioxidant exhibited the highest FFA (0.7 %), while oil with 200 ppm BHT and 1000 ppm each exhibited the least (0.13 % and 0.09 %), revealing less interaction among moisture and garlic extract, concluding that garlic at 1000 ppm is more effective over longer storage period than BHT and BHA[16]. A study on the FFA of crude palm oil control samples (without additive) had rapidly increased initially from 10 % to over 40 % in the 7th month. 0.1 % plantain peel extract was better than 0.1 % banana peel extract and brought down FFA better, while 200 ppm of CA was comparatively less efficient in the 7th month, proving higher antioxidant potential of plantain peels in comparison to banana peels and citric acid in reducing oxidation in palm oil[6]. Table 2 gives results of percentage of free fatty acids forming in each sample during the course of study. FFA content went on increasing with the increase in storage period for all the samples, but no regular pattern of increase could be observed. Gradual increase in the free fatty acid is due to oxidative rancidity developing in samples. The PNO negative sample that has no antioxidant additive in it is seen to have undergone the maximum oxidation, evident from the drastic rise in percentage of free fatty acids, from day 3 as 1.165 % to 2.745 % on day 24. The PNO positive sample that has been added with BHT also shows increase in percentage free fatty acids but not to the extent of PNO negative sample. The PNO 200 sample shows similar trends as seen in PNO negative sample. PNO 400, PNO 600, and PNO 800 also exhibit increasing oxidation, while being able to preserve the oil better than PNO negative but not as effectively as done by PNO positive. PNO 1000 is superior in effectiveness when compared to PNO positive, 2.237 % and 2.162 % free fatty acids respectively, in being able to reduce the extent of oxidation, due to the highest level of pomegranate peel antioxidant

Effect of Storage on Acid Value

The acid value (AV) of crude palm oil stored with additive (control) rose from about 20 mg KOH/g in the 1st month to over 90 mg KOH/g in the 7th month. This is very high compared to that of the oil stored with 0.1 % banana peel extract and 0.1 % plantain peel extract in the 7th month, while 0.02 % citric acid was weakly efficient, showing efficiency of plantain and banana peels against citric acid in antioxidation[5]. Orange peel

extract (1200 and 1600 ppm) inhibited peroxidation of soybean oil 2 times more than BHT and BHA (200 ppm). On the 0^{th} day of storage, the acid value of control (no additive) was 0.064 mg KOH/g, which rose to 0.353 mg KOH/g on the 7^{th} day. The acid values of BHT and BHA samples were 0.257 and 0.267 mg KOH/g respectively. As the level of extract increased, the acid values on the 7^{th} day for 400 ppm, 800 ppm, 1200 ppm and 1600 ppm samples reduced considerably, proviing that inhibition of oxidation increased by increasing peel concentration in treated soybean oil[1] Gradual increase in AV is based on the increase in percentage of free fatty acids [12]. The PNO negative sample has the maximum oxidation and hence the high acid value from 2.319 mg KOH/g on day 3 to 5.461 mg KOH/g on day 24. PNO 200 has similar oxidation pattern to that of PNO negative. PNO positive has relatively less oxidation and is effective than PNO 400, PNO 600 and PNO 800. PNO positive has acid value 4.451 mg KOH/g on day 24, while PNO 1000 shows a relatively lower value of 4.302 mg KOH/g on the same day due to high level of pomegranate peel antioxidant, as shown in Table 3.

SAMPLE	DAYS							
	3	6	9	12	15	18	21	24
	Percentage of FFA (as oleic acid equivalents) *							
PNO NEGATIVE	1.165	1.259	1.429	1.748	1.899	2.162	2.406	2.745
PNO POSITIVE	1.147	1.147	1.165	1.373	1.579	1.729	2.106	2.237
PNO 200	1.165	1.259	1.391	1.673	1.842	2.181	2.350	2.688
PNO 400	1.128	1.222	1.335	1.542	1.729	2.049	2.218	2.501
PNO 600	1.109	1.203	1.297	1.429	1.617	1.899	2.124	2.321
PNO 800	1.109	1.147	1.203	1.373	1.542	1.767	2.049	2.275
PNO 1000	1.090	1.109	1.128	1.316	1.429	1.655	1.937	2.162

Table 2- Effect of Storage on percentage of Free Fatty Acids of Cooked Peanut Oil

*- mean of triplicates (±0.032 standard deviation)

SAMPLE	DAYS							
	3	6	9	12	15	18	21	24
	mg KOH/g *							
PNO NEGATIVE	2.319	2.506	2.842	3.478	3.777	4.302	4.787	5.461
PNO POSITIVE	2.281	2.281	2.319	2.730	3.142	3.441	4.188	4.451
PNO 200	2.319	2.506	2.768	3.329	3.665	4.339	4.675	5.348
PNO 400	2.244	2.431	2.660	3.066	3.441	4.076	4.413	4.974
PNO 600	2.207	2.393	2.581	2.842	3.217	3.777	4.226	4.749
PNO 800	2.207	2.281	2.393	2.730	3.066	3.515	4.076	4.525
PNO 1000	2.169	2.207	2.244	2.618	2.842	3.852	3.852	4.302

Table 3- Effect of Storage on Acid Value of Cooked Peanut Oil

*- mean of triplicates (±0.064 standard deviation)

Effect of Storage on Peroxide Value

Peroxide Value (PV) of any lipid is an index of primary products of oxidation as well as oxidative rancidity or stability of lipids, which indicates oil deterioration in the formation of hydro peroxide. Low peroxide value indicates slow oxidation[9]. The lower the value, more the oil is oxidatively stable[3]. It indicates the amount of peroxides formed in fats and oils during oxidation[23]. Fresh oils have PV less than 10 meq/kg while PV between 20 and 40 meq/kg results in rancid taste[2]. Soybean oil containing 1600 and 2400 ppm of potato peel extract, showed lower PV (10.0 and 9.0 meq/kg) than control (59 meq/kg), in the 7th month. With 200 ppm of BHA and BHT, it showed PV of 8.0 and 6.0 meq/kg respectively[34], showing competitive ant oxidation. Different sources of agro wastes such as peels of pomegranate, apple, banana and citrus, and wheat husk, wheat bran, corncob, rice hull and rice bran have been used at 600 ppm for stabilization of corn oil at 60°C and analysed for 30 days. The rate of decline in PV was highest in samples stabilized with pomegranate peel extract (70.0 %), followed by apple peel (64.6 %), citrus peels (61.6 %), banana peels (58.4 %), corncob (55.8 %), BHT (55.3 %), wheat bran (53.6 %), rice bran (51.3 %), wheat husk (48.8 %), and rice hull (42.5 %). On the 30th day the PV

of control increased to 22 meq/kg from an initial value of 1 meq/kg. Sample containing rice hull extract had PV of 12.5 meq/kg, while that with wheat husk had 11 meq/kg, and with rice bran had PV of about 10 meq/kg. Samples stabilized with extracts of wheat bran, BHT, banana peel, citrus and apple peel had PVs between 10 meq/kg and 6 meq/kg. Sample stabilized with pomegranate peel extract had the lowest PV among all samples (5 meq/kg), showing varying levels of antioxidation[30]. A fish-rapeseed oil blend studied at 55°C for 3 days, showed that the PV of control (no additive) increased from 1.02 meq/kg to 25 meq/kg, while PV of sample with 200 ppm of BHT, increased from 0.85 meq/kg to 21 meq/kg. Samples containing 800 ppm, 1600 ppm and 2400 ppm of potato peel extract had PV of 1 meq/kg to 23 meq/kg, 0.6 meq/kg to 22 meq/kg and 0.5 meq/kg to 4 meq/kg respectively, proving that increasing extract level shows higher antioxidation than BHT [13]. Table 4.4 represents the results of formation of peroxides during the 24 day storage period. As expected PNO negative has the maximum peroxide value of 28.673 meq/kg on day 24 from 8.006 meq/kg observed on day 3, showing drastic increase in oxidation. Each sample undergoes oxidation, but the most effective reduction is seen in PNO 1000 from day 3 at 4.673 meq/kg to 19.340 meq/kg on day 24, followed by PNO 800 which seems to be superior in effectiveness than PNO positive. It is observed that PNO 600 and PNO positive show similar effect on 24th day followed by PNO 400. PNO 200 shows similar oxidation as seen in PNO negative, with almost no antioxidant protection. PNO 1000 proves to show a better due to high level of pomegranate peel antioxidant.

Effect of Storage on Saponification Value

High saponification value indicates the presence of greater number of ester bonds, suggesting that the fat molecules are intact [10] and formation of lower molecular weight oxidation products like aldehydes and carboxylic acids. The saponification value[29] of peanut oil is 188 - 195 mg KOH/g. Based on the degree of oxidation and antioxidant used to protect the oil, the saponification value of oil changes within this given range[33]. The results of change in saponification values of samples are shown in Table 5 represent the change in saponification value. Rise in saponification is an indicator of the formation of soap and free fatty acids, and hence rise in saponification value indicates increasing oxidation. Sample with no antioxidant shows highest saponification value while sample with the most effective antioxidant shows a relatively

lower extent of oxidation and hence lower saponification value. PNO negative has maximum saponification value of 193.311 mg KOH/g. PNO 200 has a slightly lesser value of 193.077 mg KOH/g. PNO 400 and PNO 600 show similar value of 192.610 mg KOH/g while PNO positive and PNO 800 also show similar value of 192.377 mg KOH/g, showing better effect against oxidation. Maximum oxidative protection is seen in PNO 1000 with value of 191.674 mg KOH/g, due to high level of pomegranate peel antioxidant, and hence proving to have a lower value than that of PNO positive.

SAMPLE	DAYS							
	3	6	9	12	15	18	21	24
	meq peroxides/kg *							
PNO NEGATIVE	8.006	10.340	12.673	15.006	19.006	22.673	25.340	28.673
PNO POSITIVE	5.340	7.673	8.673	10.673	12.673	15.006	16.673	23.673
PNO 200	8.006	10.006	12.006	14.673	17.673	21.673	23.340	28.673
PNO 400	7.673	7.673	11.340	14.006	16.673	19.673	21.673	26.673
PNO 600	7.006	7.340	10.006	12.673	14.673	16.673	19.673	23.673
PNO 800	6.006	7.006	8.673	11.006	13.673	14.673	17.673	21.673
PNO 1000	4.673	6.006	7.340	10.006	11.673	13.673	15.673	19.340

Table 4.4- Effect of Storage of Peroxide Value of Cooked Peanut Oil

*- mean of triplicates (±0.577 standard deviation)

SAMPLE	DAYS							
	3	6	9	12	15	18	21	24
	mg KOH/g *							
PNO NEGATIVE	191.207	191.674	192.143	192.376	192.844	192.844	193.077	193.311
PNO POSITIVE	190.740	190.740	190.973	190.973	191.207	191.674	191.909	192.377
PNO 200	191.207	191.674	192.143	192.377	192.610	192.844	193.077	193.077
PNO 400	190.973	190.973	190.973	191.207	191.909	192.143	192.610	192.610
PNO 600	190.973	190.973	190.973	191.207	191.909	192.143	192.610	192.610
PNO 800	190.973	190.973	190.973	190.973	190.973	191.874	192.377	192.377
PNO 1000	190.740	190.740	190.740	190.973	190.973	191.440	191.674	191.674

Table 5- Effect of Storage on Saponification Value of Cooked Peanut Oil

*- mean of triplicates (±0.404 standard deviation)

Effect of Storage on Iodine Value

Reduction in the iodine number or iodine value is a vital indicator of lipid oxidation, since there is a decrease in unsaturation of the fatty acids of triglycerides during oxidation[21]. After 60 days, storage at 45 °C, iodine values of soy bean oil containing 1600 and 2400 ppm of potato peel extract were 71 and 77 g/100g, respectively, which were higher than the control sample (58g/100g). However, iodine values for soy bean oil treated with 200 ppm of BHA and BHT were 80 and 84 g/100g, respectively, after 60 days storage at 45 °C, illustrating that potato peel extract, at various concentrations exhibited very strong antioxidant activity which was almost equal to BHA and BHT. Therefore, potato peel extract in oils, fats and other food products can safely be used as natural antioxidant to suppress lipid oxidation[34]. Table 6 shows that drastic

decrease in iodine value indicates rapid oil deterioration while a gradual drop indicates delayed oxidation, due to better antioxidant effect of the antioxidant added in samples. Iodine value of peanut oil is between 82 - 106 g I_2/100g (Stauffer, 2005). Drastic drop in iodine value from day 3 at 90.09 g I_2/100g to 87.13 g I_2/100g on day 24 is seen in PNO negative due to absence of any antioxidant, hence maximum oxidation. The next in the high oxidation series is PNO 200 at 90.09 g I_2/100g on day 3 to 87.34 g I_2/100g on day 24. The extent of oxidation is lesser in PNO 400 than in PNO 200. PNO positive and PNO 600 have the same iodine value of 88.18 g I_2/100g, showing that PNO 600 is as effective in antioxidation as PNO positive. PNO 800 has relatively higher iodine value of 88.40 g I_2/100g proving to be superior to PNO positive. PNO 1000 has the least change in iodine value indicating very narrow rise in oxidation due to the presence of highest level of pomegranate peel antioxidant.

SAMPLE	DAYS							
	3	6	9	12	15	18	21	24
	g I_2/100g *							
PNO NEGATIVE	90.09	89.67	89.04	88.83	88.40	87.77	87.77	87.13
PNO POSITIVE	90.52	90.31	89.88	89.46	88.61	88.40	88.18	88.18
PNO 200	90.09	89.88	89.25	89.04	88.61	87.98	87.98	87.34
PNO 400	90.31	90.31	90.09	89.88	89.67	89.04	88.61	87.98
PNO 600	90.31	90.31	90.31	90.31	89.67	89.04	88.40	88.18
PNO 800	90.52	90.52	90.52	90.31	89.67	89.46	89.04	88.40
PNO 1000	90.73	90.73	90.52	90.31	89.88	89.67	89.25	89.04

Table 6- Effect of Storage on Iodine Value of Cooked Peanut Oil

*- mean of triplicates (±0.363 standard deviation)

Optimization of Concentration of Pomegranate Peel Extract for Stabilization of Peanut Oil

Statistical analyses of the data were performed by Analysis of Variance (ANOVA) and Student t- test. A probability value of $P \leq 0.05$ was considered to denote the statistically significant differences, in order to compare the mean values of the investigated parameters. The results of statistical analysis by ANOVA and Student t- test shows that at the 0.05 level, the means are NOT significantly different ($P \geq 0.05$), i.e., there is no significant difference between PNO Positive and PNO 800 (Appendix 12). This denotes that whether 200 ppm of BHT or 800 ppm of the pomegranate peel extract is added to the peanut oil, it will not significantly affect the formation of free fatty acids. Hence both will have similar effect on the percentage of free fatty acids. Hence it can be concluded that BHT can be eliminated and substituted with 800 ppm of pomegranate peel extract. The results of statistical analysis by ANOVA and Student t- test showing that at the 0.05 level, the means are NOT significantly different ($P \geq 0.05$), i.e., there is no significant difference between PNO Positive and PNO 800 (Appendix 13). This denotes that whether 200 ppm of BHT or 800 ppm of the pomegranate peel extract is

added to the peanut oil, it will not significantly affect the development of acidity. Hence both will have similar effect on the acid value. It can be inferred that BHT can be eliminated and substituted with 800 ppm of pomegranate peel extract. The results of statistical analysis by ANOVA and Student t- test showing that at the 0.05 level, the means are NOT significantly different ($P \geq 0.05$), i.e., there is no significant difference between PNO Positive and PNO 800 (Appendix 14). This denotes that whether 200 ppm of BHT or 800 ppm of the pomegranate peel extract is added to the peanut oil, it will not significantly affect the formation of peroxides. Hence both will have similar effect on peroxide value. Hence it can be inferred that BHT can be eliminated and substituted with 800 ppm of pomegranate peel extract. The results of statistical analysis by ANOVA and Student t- test showing that at the 0.05 level, the means are significantly different ($P \leq 0.05$), i.e., there is significant difference between PNO Positive and PNO 800. This denotes that whether 200 ppm of BHT or 800 ppm of the pomegranate peel extract is added to the peanut oil, it will significantly affect saponification. 200 ppm of BHT could lead to lower saponification value of the oil while being similar to that by 800 ppm of pomegranate peel extract, with very less difference; hence the inference is that 800 ppm of the peel extract can be used as a BHT substitute. The results of statistical analysis by ANOVA and Student t- test showing that at the 0.05 level, the means are significantly different ($P \leq 0.05$), i.e., there is significant difference between PNO Positive and PNO 400 (Appendix 16). This denotes that whether 200 ppm of BHT or 400 ppm of the pomegranate peel extract is added to the peanut oil, it will significantly affect unsaturation. 200 ppm of BHT could lead to lower iodine value of the oil while compared to 400 ppm of pomegranate peel extract, which will not lower the iodine value to the extent of BHT. Hence 400 ppm is the optimum concentration for maintaining the iodine value. From the results of statistical analysis, the pomegranate peel extract at 800 ppm concentration satisfies the requirements of an antioxidant against BHT, for four out of five dependent variables, i.e., percentage free fatty acids, acid value, peroxide value, and saponification value, while 400 ppm of the extract is found to be more efficient than BHT in case of maintaining the iodine value. In the case of comparing the extract treated oils with that of control (no additive), almost all the extracts exhibit antioxidant activity, in controlling oxidation. On the whole, it can be concluded that pomegranate peel extract at 800 ppm can be a vital candidate and a most suitable substitute for BHT, for use as antioxidant against lipid oxidation in the substrate of peanut oil.

Conclusion:

The results of the current investigation reveal the superior antioxidant capacity of agro wastes namely pomegranate peels when used in oxidation substrate namely once cooked commercial peanut oil. Pomegranates (var, Kabul) were procured, cleaned, washed and peeled followed by shade drying. The dry peels were cut into pieces and ground to a fine powder which was extracted by orbital shaking with distilled water and refrigerated until use. Oil blends containing 200 ppm to 1000 ppm of the hydro-extract were made with cooked oil. Oil with 200 ppm of BHT was used as positive control, while that without any additive was the negative control. Oxidation parameters such as percentage free fatty acids, peroxide value, saponification value and iodine value were used to assess oxidation in peanut oil, for a period of 24 days, analyzed at 2 day interval, using standard protocols. In a few cases, synthetic antioxidant BHT was (200 ppm) found to exhibit better protection against lipid oxidation than pomegranate peel extract (200 ppm, 400 ppm, 600 ppm), while in others the peel extract (600 ppm) was better than BHT. Peel extract (800 ppm and 1000 ppm) showed better and/or equal antioxidation than BHT in a few results. By statistical analysis using ANOVA and Student t- test, it was found that optimal performance was exhibited by pomegranate peel hydro-extract at 800 ppm. This property of waste pomegranate peels can be domestically made use of in order to aid in the reduction of oxidation in cooking oils, especially peanut oil. The processing of the peels is simple and also economic when it comes to waste utilization. The natural antioxidants present in waste peels provide higher antioxidation effect along with additional health benefits in the form of functional foods with abundant nutraceutical value.

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