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EXTRACTION AND ESTIMATION OF THE ANTIOXIDANT POTENTIAL OF FRUIT PEELS ON QUALITY OF COOKING MUSTARD OIL

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Abstract
The study was aimed to explore the antioxidant potential and total phenolic content ofapple and banana peel powder extract in cooking oil.

Objective:
(1) To extract and estimate the antioxidant potential of fruit peel.
(2) To study the effect of fruit peel on quality of cooking mustard oil.

Methods: The fruit powders were incorporated at 4,6,8 and 10% level in mustard oil and its effects on various physico-chemical parameters of oil were evaluated against control.

Result: Higher amount of total phenolic content was found in apple peel powder (13.33±1.24mg GAE/g) and DPPHscavenging activity (95.13±2.03%), whereas the FRAP antioxidant capacity value and Vitamin C were higher in banana peel powder. Incorporation of fruit powder significantly ($p<0.05$) increased the oil parameters like density, iodine value, peroxide value and free fatty acid level, whereas no significant effect was observed for refractive index and specific gravity. When compared to the control oil, batch addition of fruit peel powders had a significant ($p<0.05$) reduction on both the peroxide value and free fatty acid level. Hence fruit peel powders can be potentially used as natural preservatives and their functionality can be explored in cooking oil quality.

Keywords: total phenolic content, antioxidant activity, iodine value, peroxide value.

INTRODUCTION

Antioxidants are the chemical compounds found in almost all living organisms which possess the ability to inhibit the destructive process of oxidation. These inhibitory substances oxidize themselves to significantly terminate or at least delay the oxidative processes [1]. Normal metabolism of aerobic cells also often leads to the formation of free radicals [2]. Oxidative stress is caused by an imbalance which produces ROS that affects the biological system's damage. In human body, there are two sources of free radicals: endogenous sources, e.g. nutrient metabolism and ageing process; exogenous

sources, e.g. air pollution [3]. The types of oxygen centered free radicals are: hydroxyl radicals, peroxyl radicals, alkoxyl radicals, phenoxyl radicals, and superoxide radicals. Besides these there are also certain non-radical species which includes: hydrogen peroxide, hypochlorous acid, ozone, singlet oxygen, peroxynitrate, nitrous acid, dinitrogen trioxide, and lipid peroxide [4]. The oxidative stress caused by these radicals results in damages to the biomolecules like lipids, proteins and DNA including LDL oxidation and cancer cell initiation[5]. Epidemiological studies suggest that high intake of dietary polyphenols result in decreased risk of diseases including cardiovascular disease, specific forms of cancer [6] and neurodegenerative diseases [7]. Flavonoids have potential health benefits in reducing various pathogenic processes relevant to chronic disease progression [8]. The phenolics and ascorbic acid present in vegetables also possess the antioxidant properties [9]. The fruits and vegetables are also loaded with various phytoconstituents like vitamins, carotenoids and minerals (selenium and zinc) which can be used as an aid to scavenge free radicals and treat disorders caused by oxidative stress [10].

These antioxidants constitute three types of defense: first line of defense limits on over production of reactive oxygen species by inactivating endogenous cations such as Fe^{2+} or Cu^{+}. The second line of defense is mainly constituted of three enzymes that react synergistically, i.e., superoxide dismutase (found abundantly in mitochondria), catalase (found in peroxisome), and glutathione peroxidase (found in cytoplasm). The third line of defense is constituted of molecules able to scavenge reactive oxygen species such as tocopherols, tocotrienols, carotenoids, ascorbic acid, and other phytochemicals [11].Fruit peel contain several lipophilic and hydrophilic antioxidant compounds which may act together more effectively than singly because they function synergistically and are capable of quenching free radicals in both aqueous and lipid phases [12]. Free radical damage to the body which leads to inflammation and a whole host of problems such as heart disease, disability, Alzheimer's, etc. The regular consumption of foods that is fruits & vegetables from plant origin can provide different antioxidants which can minimize the risks of such diseases. The pulp or endosperm of the fruits often consumed and the peels used as waste or feed, but peels contains maximum antioxidant potential as comparison to pulp [13]. Today food science researchers have studies how to extracts the antioxidant from the fruit peels & is used for various preservative purpose.

Mustard Oil is extracted at a low pressure at low temperature (40-60°C). It contains 0.30-0.35 % essential oil (Allyl isothiocynate) which acts as preservative. Mustard Oil is one of the best cooking oil particular for heart patient because it has an omega 3 and 6 fatty acid (Linoleic and alpha Linolenic Acid respectively) in good proportion i.e. rarely found in any other oil [14]. Cooking oil contains unsaturated fatty acids which are easily oxidized, when it exposed to the ambient environment. So there is a need to preserve oil to increase its shelf life and economic benefits. As oxidative rancidity is one if the major cause of oil spoilage, incorporation of addition of factor, which can stop or reduce the rate of oxidation are very useful. Antioxidant compound have such ability to reduce the rate of oxidation and might be useful additives for preservation of oil. Spoilage of oil due to rancidity causes undesirable odors and flavors in processed fried products. Rancidity can also deteriorate the nutritional value of the food [15]. Hence, this work was conducted to extract, estimate the antioxidant activity of fruit peel and it's use for preservation of oil.

2. Material & Methods

Fresh whole fruits of *Mussa acuminata* (Banana), *Malus domestica* (Apple), were taken from the local market. The fruits were chosen for uniformity in shape, size and color. They were washed with tap water and air dried. The edible part of the fruits was separated from the non edible part of the fruit. Further the peel were used for preparation of methanolic extract, which was used for antioxidant analysis and preservation study.

2.1 Preparation of Methanolic Extract

The fruit peels were cut into small pieces and freeze dried in the lyophilizer and powdered. 50g of powder was mixed with 250 ml of methanol using a water bath shaker for 3 hr. Centrifuge the mixture at 7000 rpm and supernatant was collected and then evaporated to dry mass with rotary flash evaporators.Then the partially concentrated extract was freeze dried at-20°C for future analysis.
For the preservation study freshly extracted mustard oil was collected by screw press extractor from Agrawal oil mill, Sambalpur. Before experimental investigation the oil was stored in refrigerator at 4°C. Later on parameter like Density, Specific gravity, Refractive index, free fatty acid, iodine value, and peroxide value were determined to confer the physicochemical properties of above edible oil at fresh stage. The effect of antioxidant activity of the extract was determined by adding several dosage of extract .five sample were taken in different dose of extract was added to each sample in such a way that the extract content in the oil sample in a beaker 50 ml was 4 %, 6 %, 8 %, 10 %, respectively. The peel extract in the oil sample equivalent to 0.14g, 0.22g, 0.29g, 0.37g extracted solid respectively. Further the samples were incubated inside the incubator at 50 °C, with well ventilation, so as to facilitate interaction of oil with air /oxygen for an accelerated oxidation.

2.2 Total phenolic content

Total phenolic content (TPC) by Folin-Ciocalteu reagent as defined by Singleton et al., 1965[16] was adopted. TPC was measured as a milligram of GAE per 100ml.

2.3 Determination of total antioxidant capacity (FRAP method)

Antioxidant property was assessed using the method of ferric reducing antioxidant power Song *et al*., 2004[17]. Test readings were expressed as mg of trolox equivalent antioxidant capacity (TEAC) of juice (mg TE/ 100ml).

2.4 Radical Scavenging Assay(DPPH method)

The free radical scavenging activity of different extracts of banana and apple peel was measured in terms of hydrogen donating or radical scavenging ability of the stable (DPPH) free radical Huang*et al*., 2005 [18] solvent method. The inhibition of DPPH radicals were calculated as:
Scavenging activity (%) = (Control OD – sample OD / control OD) x 100

2.5 Determination of vitamin C

Visual titration method using 2,6-dichlorophenolindophenol dye Ranganna, 2010[19] was used to determine the vitamin C content.

2.6:Determination of physical characteristics of oil

Refractive index: Refractive index of oil sample was determined using. Abbe's refractometer calibrated at 25 °C finding the refractive index with very good accuracy Oderinde *et al*., 2009[20].
Density: Density was determined picnometrically temperatures ranging from 20.0 to 50.0 °C as per Zubr and Matthäus, 2002[21]. The density of the oil was measured by using the formula: $\rho = m/v$
Where, ρ- density of the oil, m- mass of the oil, v- volume of the oil.

Specific gravity: Specific gravity was determined by using specific gravity hydrometer method. The specific gravity was determined a empty cylinder was taken and the oil was poured into the half full of the cylinder and the specific gravity hydrometer was dipped into the cylinder and noted the value [19].

2.7: Determination of chemical properties of the Mustard oil

Peroxide value: Peroxide value was determined by potentiometric titration by Mohammed and Hamza, 2008[22] method.

Iodine value: The iodine value was measured the amount of unsaturation in cooking oil as shown by Coultate (2004)[23].

Free fatty acid content: The free fatty acids, ketones, other aldehydes and peroxides will be produced during the oxidation process of oils and fats as shown by Mohammed and Hamza, 2008[22].

2.8 Statistical Analysis

For the statistical analysis, three replicates of each sample were used and the data were expressed as mean ± S.D. To identify significant differences among the mean values (at $p < 0.05$) analysis of variance (ANOVA) and Tukey's tests were performed.

3. RESULT & DISCUSSION

3.1: Total phenolic content of fruit peel

Methanolic extract of fruit peel sample was analysed for total phenolic content as per Folin-Ciocalteu method. The total phenolic content was found to be 11.33 ± 1.69 mg GAE/gand 13.33± 2.03 mg GAE/g, for banana and apple peel extract respectively. The data obtained in this study is similar to the result obtained by Someya et al., 2002 [24]. Extraction of total phenolic was also done by Aurore *et al* in (2009)[25] and TPC of banana was found to be 12mg GAE/g extract.

DPPH scavenging activity of banana and apple peel was found to be 86.44 ± 0.13 and 95.13 ± 2.03, respectively. Alothman *et al.*[26]conducted similar experiment and found DPPH scavenging activity of banana is 68 ± 0.5. Study made by Musa *et al*, 2010 [27] also supports the result of this study. The statistical t-value and p value was found to be 6.02 and 0.013, shows statistically significant different. The ferric reducing antioxidant power was found to be 113.3 ± 1.82 µg QE and 53.3 ± 1.38 µg QE/ g of extract for banana and apple peel respectively. In this study the banana peel extract was found to be higher values of FRAP as compared to apple peel extract.

Initially the physical characteristics' of raw mustard oil 1.474± 0.002, 0.878±0.0.012, 878.6± 1.26 of refractive index, specific gravity, and density respectively where as the chemical characteristics of raw mustard oil was 94.51± 1.60 gI_2 /100g oil, 19.33± 2.19 milimoles /g, 4.90± 0.32% oleic acid of iodine value, peroxide value, free fatty acid respectively.

In table-1 shows that the physico- chemical properties of the mustard oil stored at 50 °C in well ventilated incubator. After 1 month the physical properties of the oil was 1.460± 0.002, 0.812± 0.012,812±1.37 for refractive index, specific gravity, density respectively. The chemical properties like iodine value was 78.01± 2.08 gI_2 /100g oil,peroxide value was 33.08± 1.34 milimoles/g and free fatty acid was 21.09± 0.72%. The result suggested that mustard oil stored at 50 °C well ventilated incubator, will increases the rate of oxidation. The oxidation will increase the free fatty acid, peroxide value and decreases the iodine value.

Dosing with peel extract stored at 50°C in well ventilated incubator after 1 month the physical properties of 8% apple extract oil was 1.470± 0.003, 0.868± 0.010, 868± 1.22 for refractive index specific gravity,density, respectively where as chemical propertieswere 93.49± 0.58 gI_2 /100g, 20.18± 1.98 milimoles/g, 5.13± 0.69% of iodine value, peroxide value and free fatty acid value respectively.Thus the result suggested that the rate of oxidation increased in control oil i.e. without addition of peel extract increases the free fatty acid peroxide value when adding different doses of peel extract the rate oxidation decreases as compared to the control mustard oil.

Table- 1: Effect of Apple peel extract on physicochemical properties in mustard oil

Parameters	Raw Oil	Control			Apple Peel extract											
		---			4%			6%			8%			10%		
		10th Day	20th day	30th day	10th Day	20th day	30th day	10th Day	20th day	30th day	10th Day	20th day	30th day	10th Day	20th day	30th day
Refractive Index	1.474± 0.002	1.472± 0.005^{a}	1.469± 0.002^{a}	1.460± 0.002^{b}	1.472± 0.001^{a}	1.467 ± 0.001^{a}	1.463 ± 0.003^{b}	1.470± 0.002^{a}	1.469± 0.002^{a}	1.467 ± 0.005^{a}	1.471± 0.003^{a}	1.470± 0.002^{a}	1.470± 0.003^{a}	1.474± 0.002^{a}	1.474± 0.006^{a}	1.474± 0.003^{a}
Specific Gravity	0.878± 0.012	0.860± 0.21^{a}	0.845± 0.010^{e}	0.812± 0.012^{g}	0.870± 0.010^{b}	0.860 ± 0.012^{d}	0.841 ± 0.005^{f}	0.871± 0.025^{b}	0.864± 0.013^{c}	0.854 ± 0.032^{e}	0.871± 0.022^{b}	0.868± 0.010^{c}	0.860± 0.014^{d}	0.876± 0.012^{a}	0.876± 0.014^{a}	0.871± 0.012^{b}
Density	878.6± 1.26	860± 1.55^{a}	845± 1.29^{e}	812± 1.37^{g}	870± 1.22^{b}	860± 1.42^{d}	841± 1.34^{f}	871± 1.20^{b}	864± 1.31^{c}	854± 1.23^{e}	871± 1.35^{b}	868± 1.22^{c}	860± 1.48^{d}	876± 1.53^{a}	876± 1.39^{a}	876± 1.22^{b}
Iodine Value (g I_2 /100g)	94.51± 1.60	93.17± 1.51^{a}	87.25± 1.21^{c}	78.01± 2.08^{e}	93.29± 1.13^{a}	87.21 ± 1.63^{c}	81.69 ± 2.60^{d}	93.11± 1.63^{a}	92.36± 1.32^{b}	87.19 ± 1.66^{c}	93.23± 1.30^{a}	92.96± 1.73^{b}	92.77± 1.58^{b}	94.07± 1.26^{a}	94.11± 2.18^{a}	94.11 ± 1.20^{a}
Peroxide Value (milimoles /g)	19.33± 2.19	21.62± 2.18^{b,c}	27.81± 1.19^{b}	33.08± 1.34^{a}	19.58± 1.63^{d}	23.66 ± 1.46^{c}	28.26 ± 1.38^{b}	19.60± 2.19^{d}	22.73± 1.06^{c}	26.98 ± 1.94^{b}	19.46± 1.22^{d}	20.07± 1.16^{d}	20.18± 1.98^{d}	19.28± 1.25^{d}	19.26± 1.40^{d}	19.39± 2.02^{d}
Free fatty Acid (% oleic acid)	4.90± 0.32	4.97± 0.13^{f}	8.99± 0.20^{d}	21.98± 0.72^{a}	4.94±0. 42^{f}	6.19± 0.34^{e}	17.48 ± 2.1^{b}	4.83±1. 33^{f}	6.11± 0.32^{e}	10.32 ± 1.13^{c}	4.63±0.12 ^{f}	4.98± 1.3^{f}	5.13±0.69 ^{e}	4.69± 1.21^{f}	4.78± 1.1^{f}	4.82± 1.10^{f}

Table- 2: Effect of Banana peel extract on physicochemical properties in mustard oil

Parameters	Raw Oil	Control			Banana Peel Extract											
		---			4%			6%			8%			10%		
		10th Day	20th day	30th day	10th day	20th day	30th day	10th Day	20th day	30th day	10th Day	20th day	30th day	10th Day	20th day	30th day
Refractive Index	1.474± 0.002	1.470± 0.005^{a}	1.469± 0.003^{a}	1.460 ± 0.002^{c}	1.472 ± 0.002^{a}	1.466 ± 0.001^{b}	1.461± 0.003^{c}	1.470 ± 0.002^{a}	1.469 ± 0.003^{a}	1.469 ± 0.006^{a}	1.471 ± 0.002^{a}	1.471± 0.002^{a}	1.473± 0.003^{a}	1.474± 0.005^{a}	1.474± 0.002^{a}	1.474± 0.003^{a}
Specific Gravity	0.878± 0.012	0.860± 0.010^{a}	0.845± 0.012^{b}	0.812 ± 0.010^{c}	0.862 ± 0.010^{a}	0.843± 0.012^{b}	0.829± 0.009^{d}	0.864 ± 0.027^{a}	0.853 ± 0.018^{b}	0.838 ± 0.012^{c}	0.876 ± 0.20^{a}	0.872± 0.010^{a}	0.868± 0.014^{a}	0.878± 0.012^{a}	0.875± 0.014^{a}	0.871± 0.012^{a}
Density	878.6± 1.26	860± 1.22^{d}	845±1.1 9^{d}	812± 1.28^{h}	862± 1.56^{d}	843± 1.42^{f}	829± 1.34^{d}	864±1 .24^{d}	853± 1.41^{e}	838± 1.29^{g}	876± 1.38^{a}	872± 1.22^{c}	868± 1.39^{d}	878±1.5 3^{a}	875± 1.39^{b}	871± 0.122^{c}
Iodine Value (g I_2 /100g)	94.51± 1.60	93.17± 1.51^{a}	87.25± 1.21^{b}	78.01 ± 2.08^{c}	94.41 ± 1.51^{a}	86.27± 0.63^{b}	79.0± 2.6^{c}	94.11 ± 1.67^{a}	90.36 ± 1.32	83.19 ± 1.93	93.19 ± 1.32^{a}	93.62± 1.89^{a}	92.39± 1.63^{a}	94.16± 1.29^{a}	93.93± 2.1^{a}	93.80± 1.23^{a}
Peroxide Value (milimoles/g)	19.33± 2.19	21.62± 2.18^{c}	27.81± 1.19^{b}	33.08 ± 1.34^{a}	19.58 ± 2.32^{c}	23.63± 1.16^{c}	29.26± 1.93^{a}	19.72 ± 2.11^{c}	22.93 ± 1.06^{c}	26.29 ± 1.96^{b}	19.96 ± 1.02^{c}	20.29± 1.11^{c}	22.23± 0.98^{c}	20.09± 1.62^{c}	21.26± 1.63^{c}	21.33± 2.14^{c}
Free fatty Acid (% oleic acid)	4.90± 0.32	4.97± 0.18^{d}	8.99± 1.21^{b}	21.98 ± 1.89^{a}	4.97± 0.42^{d}	6.39± 1.14^{c}	19.23± 2.13^{a}	4.89± 1.33^{d}	6.11± 0.32^{c}	7.32± 2.13^{b}	4.63± 2.1^{d}	5.63± 1.3^{c}	5.93±2. 69^{c}	5.1± 1.66^{c}	4.91± 2.1^{d}	5.32± 1.13^{c}

In table-2 the physico- chemical properties of the mustard oil stored at 50 °C in well ventilated incubator with banana peel extract. After 1 month the physical properties of the oil was 1.460± 0.002, 0.812± 0.012 and 812±1.37 for refractive index, specific gravity, density respectively. The chemical properties like iodine value78.01± 2.08 gI_2 /100g,peroxide value 33.08± 1.34 milimoles/g,free fatty acid 21.09± 0.72%. Dosing with peel extract stored at 50°C in well ventilated incubator after 1 month the physico- chemical properties of 8% Banana extract oil 1.473± 0.003, 0.868± 0.014, 868± 1.39 for refractive index specific gravity ,density, respectively and 92.39± 0.63 gI_2 /100g,, 22.23± 0.98 milimoles/g, 5.93± 2.69% of iodine value, peroxide value and free fatty acid value.Thus the result suggested that the rate of oxidation increased in control oil i.e. without addition of peel extract increases the free fatty acid peroxide value when adding different doses of peel extract the rate oxidation decreases as compared to the control mustard oil. The results were supported by the finding of other researchers [27, 28].

After 30th day of storage period the control oil had iodine value 78.01± 2.08gI_2 /100g, peroxide value 33.08± 1.34 milimoles/gand free fatty acid 21.08± 0.72%. Similarly the 8% Apple and Banana extract was incorporated with the same raw mustard oil sample and stored same as the control, after 30th day the result was noted. The chemical properties of oil was found to be 93.49± 0.58gI_2 /100g,20.18±1.98 milimoles/g,5.13±0.69 for iodine value, peroxide value, free fatty acid in 8% apple peel extract and 92.39±0.63gI_2 /100g,22.23±0.98 milimoles/g, 5.93±2.69 for the Iodine value peroxide value free fatty acid in 8% banana peel extract respectively. The values were found to be statistically significant.

It was observed in control oil the % of iodine value reduced 17.4% as compared to the raw oil, the % of peroxide value and free fatty acid value increased 71.13%, 34.69% respectively. Similarly, the %

of iodine value was reduced in 8% banana and apple peel extract was 2.2% and 1.07% respectively. The peroxide value and free fatty acid value were increased 15% and 4.93% in 8% banana peel extract added oil. The peroxide value and free fatty acid value were increased 21.02%, 4.69% in 8% apple peel extract added oil. The peel extracts act as natural preservatives and can be used to improve in cooking oil quality [29].

CONCLUSION

In this research, apple and banana fruit peel extract has been used to reduce the FFA levels in fried mustard oil in order to improve its quality characteristics. Experimental investigations were carried out to determine the optimum values of 8% peel extract. The changes in the quality parameters include free fatty acid, peroxide value, iodine value were investigated during the use of mustard oil for 30 days. It was found that the free fatty acid was 4.90±0.32% in raw cooking oil when it stored at 50°C, the accelerated temperature results an increase in free fatty acid up to 21.98±0.72%. The result suggested that the oxidation of oil increases the free fatty acid content. Dose of peel extract on free fatty acid was reduced by 8% apple extract oil to 5.13±0.69% and with 8% banana extract oil reduced to 5.93±2.69%. The changes in the quality parameters were acceptable. So it was recommended that mustard oil quality can be rectified by the peel extracts and can be used for deep fat frying for healthy consumption.

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