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Effect of Refining Process on the Phenol Compound and Antioxidant Activity of Refined and Virgin Oils

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ABSTRACT

Minor components, especially antioxidative constituents, play an imperative role in the nutritional and health impact of edible oils. Traditionally made edible vegetable oils that are unrefined are good sources of natural antioxidants, vitamin E, and polyunsaturated fatty acids which are very important for human health. Polyphenolic compounds naturally present in oils have antioxidant activity and protect the organism against cardiovascular and degenerative diseases. Polyphenols, as thermally unstable compounds, are usually removed from edible oils during various refining processes. The antioxidant potential of polyphenols depends of the type of polyphenol compounds and their ability to give a hydrogen atom from hydroxyl groups and stabilize the phenoxy radical formed by delocalization of free electron within aromatic structure. The oils included in the study were refined and virgin oils of sesame, groundnut, coconut, sunflower and palm oils. The aim of the study was to determine the total phenolic content and antioxidant activity of the selected oils. The results showed that the total polyphenol content for virgin oils ranged from 19.8 to 50.13 mg GAE/100g and for refined oils were in the range of 8.1 to 45.16 mg GAE/100g respectively. The results showed that the antioxidant activity for virgin oils ranged from 28.00 to 63.81 AAEAA/100g and for refined oils were in the range of 14.00 to 32.56 AAEAA/100g respectively. The total polyphenol and DPPH radical scavenging activity of refined oils significantly changed ($P<0.05$) after refining process. However, chemical refining hardly affected the total polyphenol content and antioxidant activity. The results showed that the refining process had the greatest impacts on bioactive minor components of refined oils.

Key words: *Antioxidant activity, total phenol, refined, cold processing, vegetable oil.*

INTRODUCTION

Traditionally made edible vegetable oils that are unrefined are good sources of natural antioxidants, vitamin E, polyunsaturated fatty acids and minerals which are very important for human health[12].

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The refining processes remove undesirable materials (phospholipids, monoacylglycerols, diacylglycerols, free acids, colour and pigments, oxidized materials, flavor components, trace metals and sulfur compounds) but may also remove valuable minor components which are antioxidants and vitamins such as carotene and tocopherols[3].

Antioxidants are used widely in oil to delay the onset of oxidation or slower the rate at which it proceeds. Their role is not to enhance or improve the quality of foods, but they do maintain food quality and extend shelf life. Primary antioxidants react with lipid and peroxy radicals and convert them to more stable, non-radical products. Primary antioxidants donate hydrogen atoms to the lipid radicals and produce lipid derivatives and antioxidant radicals (A•) that are more stable and less readily available to further promote auto-oxidation.

The antioxidant radical produced by hydrogen donation has a very low reactivity with lipids. This low reactivity reduces the rate of propagation, since reaction of the antioxidant radical with oxygen or lipids is very slow. The antioxidant radical is stabilized by delocalization of the unpaired electron around a phenol ring to form stable resonance hybrids. Antioxidant radicals are capable of participating in termination reactions with peroxy, oxy and other antioxidant radicals.

The cold-pressing procedure involves neither heat nor chemical treatments and it is becoming an interesting substitute for conventional practices because of consumers' desire for natural and safe food products. The consumption of new and improved products such as cold-pressed oils may improve human health and may prevent certain diseases. Free radicals may cause reversible or irreversible damages to biological molecules such as DNA, proteins and/or lipids[4]. Minor components, especially antioxidative constituents, play an imperative role in the nutritional and health impact of edible oils[3].

Processing of crude oils to refined oils is generally performed at high temperature, and it can produce oxidized compounds such as cyclic and noncyclic carbon to carbon linked dimers and trimers, hydroxy dimers, and dimers and trimers joined through carbon to oxygen linkage. The refined oil contains 1.2 per cent thermally oxidized compounds[20]. The oxidized compounds formed by hydroperoxide decomposition can act as an emulsifier, which contain both hydrophilic and hydrophobic groups, lower surface tension in the oil, and increase the introduction of oxygen into the oil to accelerate oil oxidation[10].

Cold-pressed oils may retain higher levels of natural antioxidants that may be removed during the refining steps of a conventional oil processing procedure, and exhibit acceptable shelf stability and improved safety without added synthetic antioxidants. In addition, cold pressing involves no organic solvents[1]. This study was carried out to determine the antioxidant activity and total phenolic content of refined (sesame, peanut, coconut, sunflower and palm) oil and virgin oils (sesame, peanut, coconut, sunflower and palm).

MATERIALS AND METHODS

The selected oils were the traditionally processed vegetable oils (virgin oil) such as sesame oil, groundnut oil, sunflower oil and palm oils and refined oil such as sesame oil, groundnut oil, coconut oil, sunflower oil and palm oil. The virgin oils were purchased from the Sathasivam cold pressed oil mill, Madurai and the refined oils were purchased from the local market, Madurai. Antioxidant activity and total phenolic content of selected oils were analyzed at the Department of Food Science and Nutrition, Home Science College and Research Institute, Madurai.

Determination of total phenolic content (TPC)

Total phenolic content was determined using Folin-Ciocalteu's colorimetric method and expressed as mg of gallic acid equivalents (GAE) per gram extract. Total polyphenolic contents were determined according to the spectrophotometric method of Sadasivam and Manickam[13]. 0.5 – 1.0 g of sample was weighed and add 10 time volume of 80 per

cent ethanol or methanol and then it was centrifuged at 10,000 rpm for 20 min. The supernatant was collected. The residue was re-extracted with five times the volume of 80 per cent ethanol or methanol, centrifuged and the supernatants were collected and made up to a known volume.

Pipette out different aliquots of (0.2 - 2 ml) of methanolic extract was taken into test tubes and it was made up the volume to 3 ml with distilled water. To this 0.5 ml of Folin-Ciocalteau reagent was added and mixed well. After 3 min, 2 ml of 20 per cent sodium carbonate was added and mixed well again and measured the absorbance at 650 nm against reagent blank using Double beam UV-VIS spectrophotometer 2201, Systronics. A set of standard solutions of gallic acid prepared using distilled water (10 µg - 100 µg per ml) was treated in the same manner as described earlier and read against blank. Total polyphenolic content was expressed as mg of gallic acid equivalent (GAE) per 100 g on DWB.

Assessment of antioxidant activity DPPH radical-scavenging activity

The scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to method described by Madhujith and Shahidi[8]. Each oil extract (2_10 mg/ml) in methanol was mixed with 1 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark, and then the absorbance was read at 517 nm against a blank. A percentage inhibition activity was calculated as [(A0 _ A1)/ A0] x 100, where A0 is the absorbance of the control (contained no sample extract) and A1 is the absorbance of the extract. The EC_{50} (mg/ml) value was calculated based on the amount of coconut oil extracts necessary to decrease the initial DPPH radical concentration by 50 per cent a-tocopherol was used as a positive control. a-tocopherol is commonly used to act against free radicals in foods and biological systems, and often serves as a reference antioxidant.

Antioxidant potential of the oil extract was measured using DPPH and ABTS radical scavenging assays and β-carotene/linoleate model system. α-tocopherol was used as the reference antioxidant. Results of the DPPH and ABTS radical scavenging assays were expressed as IC_{50} value and percentage discoloration, respectively. The dose dependence behavior of the extract towards DPPH and ABTS radicals was also determined by using varying quantities (0.1 – 0.5 g) of the extract[7,8]. The antioxidant activity as measured by β-carotene/linoleate model system was calculated as percentage discoloration[9].

RESULTS AND DISCUSSION

Total Polyphenol Content

Polyphenolic compounds naturally present in oils have antioxidant activity and protect the organism against cardiovascular and degenerative diseases[2]. Polyphenols, as thermally unstable compounds, are usually removed from edible oils during various refining processes. The antioxidant potential of polyphenols depends of the type of polyphenol compounds and their ability to give a hydrogen atom from hydroxyl groups and stabilize the phenoxy radical formed by delocalization of free electron within aromatic structure.

The table 1 showed that the total polyphenol content for virgin oils ranged from 19.8 to 50.13 mg GAE/100g and for refined oils were in the range of 8.1 to 45.16 mg GAE/100g respectively. The VGO had the highest total polyphenol content of 50.13 followed by VPO 40.30, VSO 39.21, VSUO 26.13 and VCO 19.8 mg GAE/100g. The RGO had the highest total polyphenol content of 40.16 followed by, RSO 25.82, RPO 18 mg GAE/100g which is due to the very high temperature is usually applying in the refining process in which the polyphenolic compounds were destroyed. According to the results presented on Table 1, the virgin oils showed highest value of total polyphenolics compared with refined oils. The statistical analysis revealed highly significant difference ($P \leq 0.01$) between the refined and virgin oils.

The findings of the present study was in confirmation with the studies of

Velickovska and Mitrev[19] (2013) in total polyphenol of virgin and refined palm oil where it was found to be decreased (40 to 19.42 mg GAE/100g) after refining the oil. This agrees with the studies performed in RBD palm oil by Tuberoso *et al*[16]. They also reported that the decrease in total polyphenol was observed in case of all the refined oil samples. However, the fall was highest in refined palm oil and least in refined groundnut oil.

Similar results were reported by Velicˇkovska *et al*[18]., in refined groundnut oil where it was found to be decreased from 65.31 to 30.13 mg GAE/100g.

Antioxidant Activity

DPPH radical scavenging activity can be used to evaluate the antioxidative capacity of oily materials. The absorbance of DPPH decreases proportionally to the concentration of the radical scavenging compounds.

The fig 1 showed that the antioxidant activity for virgin oils ranged from 28.00 to 63.81 AAEAA/100g and for refined oils were in the range of 14.00 to 32.56 AAEAA/100g respectively. The VGO had the highest antioxidant activity of 63.81 followed by VSO 49.16, VPO 48.73, VSUO 38.13 and VCO 28.00 AAEAA/100g. The RPO had the highest antioxidant activity of 32.56 followed by, RGO 32.29, RSO 22.36, RSUO 17.14 and RCO 14.00 mg/100g which is due to the very high temperature is usually applying in the refining process. The results indicate the vigin oils had the highest level of antioxidant activity compared with refined oils.

The results showed that the RSA of the refined oils significantly decreased the statistical analysis revealed highly significant difference ($P \leq 0.05$) between the refined and virgin oils.

Shahina *et al*[15]., opined that the deterioration of oils due to air, light, heat and deep frying may be reduced with antioxidants. Lee *et al*[5]., observed the sesame oil was highly stable to oxidation compared with other plant oils. The main sesame lignans namely sesamin and sesamolin, which are found in sesame oil, possess no antioxidative activity. During sesame oil manufacturing, however, sesamilin can be converted to other lignans, such as sesamol, sesaminol and sesamol dimer. These components are believed to play an important role in the oxidative stability of sesame oil Lee *et al*[6]., Sesamin also affects the lipid metabolism. It was reported that sesamin inhibits cholesterol absorption from the intestine, reduces 3-hydroxy-3-methyl-glutaryl reductase activity in liver microsomes, and affects the incorporation of linoleic acid into lipid sub-fractions in rats[14].

Nahidi *et al*[11]., investigated the antioxidant effect of palm oil α-carotene on comparing with β-carotene in organic solutions containing egg-yolk phosphatidycholine (EYPC) in the presence of lipid soluble 2, 2'-azobis (2,4 – dimethyl valeronitrile) (AMVN) generated peroxyl radicals by measuring the formation of phosphatidyl choline hydroperoxide (PCOOH) and thiobarbituric acid reacting substances (TBARS). Suggested that α-carotene, a carotenoid abundantly present in human diets, especially red palm oil, may better attenuate peroxyl radical – dependent lipid peroxidation than β-carotene in organic solution.

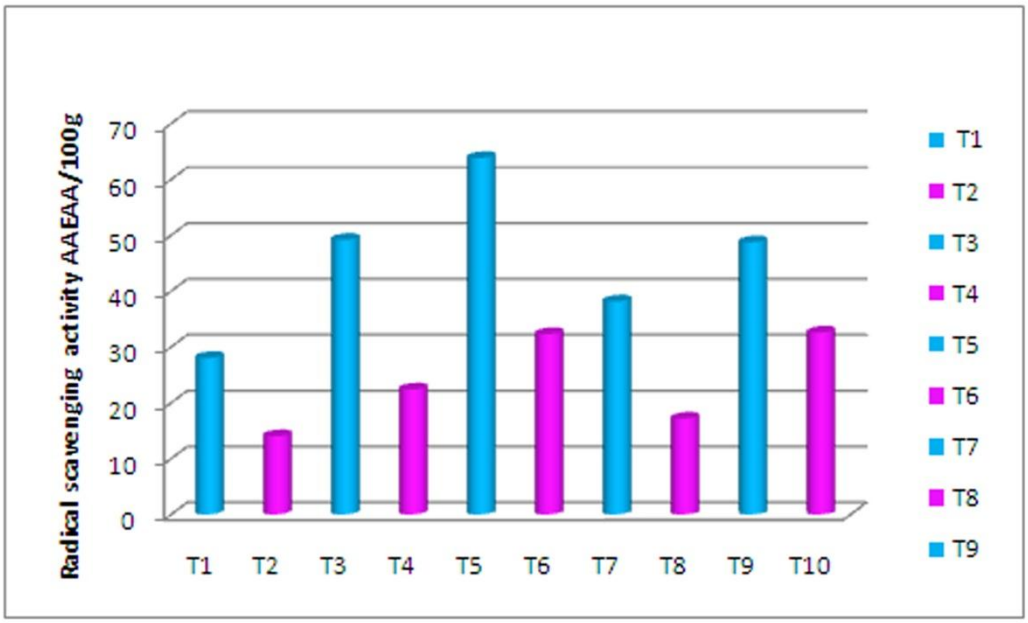
Antioxidants are used to absorb destructive free radical oxygen molecules from the oil to slow down the degradation process. Antioxidants, both synthetic and natural have been used in oil to extend their shelf life. Synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), Tertiary Butylated Hydroxyl Quinone (TBHQ) and Propyl Gallate (PG), are used as antioxidants in food stuffs against rancidity. These synthetic substances have been shown to cause several disorders, i.e. enlargement of liver, reduced food intake, growth inhibition etc[17].

Table 1: Total polyphenol content of the selected oils

Treatments	Total polyphenol mg GAE/100g
T_1	**19.8 ± 0.12**
T_2	8.1 ± 0.19
T_3	**39.21 ± 0.2**
T_4	25.82 ± 0.16
T_5	**50.13 ± 0.14**
T_6	40.16 ± 0.56
T_7	**26.13 ± 0.13**
T_8	14.33 ± 0.28
T_9	**40.30 ± 0.74**
T_{10}	18.76 ± 0.15
SED	0.0726
CD (0.01)	0.2067**
CD (0.05)	0.1515**

T_1 : Virgin coconut oil
T_2 : Refined coconut oil
T_3 : Virgin sesame oil
T_4 : Refined sesame oil
T_5 : Virgin groundnut oil
T_6 : Refined groundnut oil
T_7 : Virgin sunflower oil
T_8 : Refined sunflower oil
T_9 : Virgin Palm oil
T_{10} : Refined palm oil

Fig 1. Total antioxidant activity of selected oils



T_1	:	Virgin coconut oil	T_6	:	Refined groundnut oil
T_2	:	Refined coconut oil	T_7	:	Virgin sunflower oil
T_3	:	Virgin sesame oil	T_8	:	Refined sunflower oil
T_4	:	Refined sesame oil	T_9	:	Virgin Palm oil
T_5	:	Virgin groundnut oil	T_{10}	:	Refined palm oil

CONCLUSION

The total polyphenol and DPPH radical scavenging activity of refined oils significantly changed ($P<0.05$) after refining process. However, chemical refining hardly affected the total polyphenol content and antioxidant activity. The results showed that the refining process had the greatest impacts on bioactive minor components of refined oils. Therefore, it could be suggested that degumming–neutralization process parameters (e.g. temperature, heating time, NaOH solution concentration) should be optimized to meet the basic requirements for oil and minimize the bioactive minor components losses towards high-quality refined oil production for human consumption.

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