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Influence of Chemical Refining Processes on the Total Phenolics and Antioxidant Activity of Sunflower Oil

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Abstract

The raw sunflower oil (SFO) has an undesirable flavour and odour. Therefore, to make it suitable for human consumption, the oil has to undergo a number of refining processes such as degumming, neutralization, bleaching and deodorization. During these refining processes, some of the phytochemicals present in SFO is lost. The aim of this study is to evaluate the loss in total phenolic, flavonoid and non-flavonoid contents and the antioxidant properties of the oil at the different chemical refining stages. The crude SFO oil has the highest total phenolic, flavonoid and non-flavonoid contents. The amount of phenolic compounds decreases as the oil undergoes different chemical refining processes. Results of this study indicated that the highest percentage loss of the phenolic compounds occurred during the deodorizing step. The statistical loss of the deodorized oil was 41.7, 63.9 and 27.6 % for total phenolic, flavonoid and non-flavonoid contents respectively. The free scavenging activity of sunflower oil was determined using DPPH and ABTS assays. The percentage scavenging activity ranged from 55.64 to 35.87 % for the DPPH assay while for ABTS assay the activity ranged from 59.46 to 31.43 % in a 50 mg/ml of SFO sample. This showed a decrease in antioxidant activity from crude to DNW to bleached and deodorized oil. The crude oil having the highest phenolic contents showed the highest antioxidant activity in both DPPH and ABTS assays.

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Introduction

Free radicals are involved in various physiological reactions, causing serious cellular damage resulting in the complete inactivation of biomolecules (proteins, lipids and DNA) and cells, leading to a number of degenerative diseases [1]. Antioxidants scavenge the free radicals thus helping in reducing the free-radical induced damage to the cells.

Oils contribute a major fraction of the human diet and are lipid materials derived from plants. Sunflower (*Helianthus annuus L.*) is one of the most important oilseed crop grown in the world and it contains unsaturated fats, protein, fiber and other important phytochemicals including phenolic compounds [2]. SFO is widely used in nutrition and is a good source of polyphenols that can scavenge the free radicals produced in our body [3, 4]. Crude SFO contains many unwanted compounds such as free fatty acids, odouriferous materials and others which are removed during chemical processing to yield an edible product [5]. Chemical refining includes degumming, neutralizing, washing, bleaching, winterizing and deodorizing stages [6, 7]. However, during these chemical processes many of the bioactive and nutritionally significant compounds are lost. Therefore, the present study was undertaken to assess the antioxidant potential together with the phenolic contents of crude and processed sunflower oil. The anti-oxidative properties of the oils were also correlated in terms of their phenolic, flavonoid and non-flavonoid contents.

Material And Methods

Materials

Oil Samples

Sun flower oil (SFO) samples were collected in the month of November, 2012 from processing lines of Mauritius Oil Refineries (Port Louis, Mauritius). The samples were collected in plastic bottles (200 ml) at four different sampling stations during the manufacturing processes: Crude oil, Degummed/Neutralized/Washed oil (DNW oil), Bleached oil and Deodorized oil. The sample bottles were capped without any headspace and stored away from sunlight in refrigerator at 4 $^{\circ}$C, immediately after sampling.

Chemicals

Ascorbic acid, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) and trolox were purchased from Sigma Aldrich Chemical Co. (USA). Folin-Ciocalteu reagent was bought from BDH Company (UK). All the chemicals and reagents were analytical grade.

Preparation of Extracts

The oil (40.5 g) was extracted with 96 % ethanol in order to extract the phenolic components and the combined extracts were evaporated in a rotary evaporator (Buchi R-3000 Rotavapor) at 40 °C. The

residue was dissolved in 5 ml ethanol and stored at 4 °C for further analysis. The analysis was done in triplicates.

Determination of Total Phenolic Content (TPC)

The total phenolic content of the sunflower oil samples was determined using the Folin-Ciocalteu method [8]. In 0.2 ml of the ethanolic oil sample, 0.5 ml of 0.5 N Folin-Ciocalteu reagent, followed by 2.5 ml of 20 % Na_2CO_3 and 0.8 ml of deionized water were added. The solution was incubated for 40 min at room temperature and the absorbance was read at 725 nm on a Biochrom Libra S22 UV/Vis spectrometer against a blank sample. Gallic acid was used as standard solution and the results were expressed as µg/ml equivalent of gallic acid per 40.5 g of oil extract.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content in the oil samples was determined using the reported procedure [9]. 0.5 ml of ethanolic solution of oil sample was mixed with 2 ml of distilled water and 0.15 ml of $NaNO_2$ solution (15 %). 0.15 ml of $AlCl_3$ solution (10 %) was added to the mixture and allowed to stand. 2 ml of NaOH solution (4 %) was then added and the total volume was brought to 5 ml. The absorbance was measured at 510 nm on a Biochrom Libra S22 UV/Vis spectrometer against a blank. Quercetin was used as the standard solution and the results were expressed as µg/ml equivalents quercetin per 40.5 g of oil extract

Determination of Total Non-Flavonoid Content (TNFC)

The total non-flavonoid content in the oil samples was determined according to reported method [10]. 1 ml of hydrochloric acid (20 % v/v) and 0.5 ml formaldehyde (37 %) were added to 1 ml of ethanolic oil sample. The mixture was vortexed and left for 24 h and filtered. To 0.5 ml of the filtrate, 3.5 ml of distilled water and 0.25 ml Folin-Ciocaulteu reagent were added followed by 1 ml of Na_2CO_3 (20 %). The resulting mixture was vortexed and heated at 40 °C for 1 h and centrifuged. The absorbance of the solution was read at 685 nm on a Biochrom Libra S22 UV/Vis spectrometer against a blank. Gallic acid was used as the standard and the results were expressed µg/ml of gallic acid equivalents per 40.5 g of oil extract.

Antioxidant Activity

The antioxidant activity of the oil extracts was evaluated according to two assays.

DPPH Radical Scavenging Assay

The free radical activity of the oil samples of concentration ranging from 50000 to 24 mg/ml was assessed using DPPH (2,2'–diphenyl-1-picrylhydrazyl) assay according to the method described in literature [11]. Absorbance was measured at wavelength 492 nm after 30 min using a labsystem multiskan Ms Eliza Reader. Ascorbic acid was used as positive control and

methanol as blank. The percentage radical scavenging capacity was determined using the following formula:

$$\%\ \text{DPPH Scav}enging\ = \left[\frac{\text{Ao} - \text{As}}{\text{Ao}}\right] \times 100$$

Where $^{\text{Ao}}$ is the absorbance of blank and $^{\text{As}}$ is the absorbance of tested samples.

ABTS Assay

The free radical scavenging activity of the different oil samples were assessed using ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay with slight modifications [12]. ABTS diammonium sulphate (0.054 g) and MnO_2 (0.2 g) were dissolved in 100 ml of phosphate buffer and the solution was mixed using an orbital shaker for 15 min. The resulting bluish green solution was filtered. The freshly prepared ABTS radical cation was diluted with phosphate buffer solution to obtain an absorbance of 0.70 ± 0.05 at 734 nm. The SFO extract (0.5 g) was dissolved in 10 ml of chloroform. The solutions of concentration ranging 50000 to 3125 mg/ml were prepared through serial dilution. The ABTS activity was determined by mixing 0.25 ml of the diluted oil sample with 0.5 ml of the diluted ABTS. The decrease in absorbance was then measured at 734 nm using a Biochrom Libra S22 UV/Vis spectrometer. Trolox was used as the positive control. The percentage ABTS activity was determined using the following formula:

$$\%\ \text{ABTS Activity}\ = \left[\frac{\text{Ao} - \text{As}}{\text{Ao}}\right] \times 100$$

Where Ao is Absorbance of blank, As is Absorbance of tested sample

Result and Discussion

Phenolic compounds including flavonoids and non-flavanoids are important components present in SFO extract. The phenolic compounds constitute the main class of natural anti-oxidant present in SFO and they contribute mainly to its anti-oxidative potential. Raw SFO cannot be consumed directly due to its undesirable flavour and odour. It undergoes refining processes in order to make it suitable for consumption. During the chemical refining process the crude sun flower oil undergoes, (Degumming/Neutralization/Washing (DNW); Bleaching and Deodorization treatments [13-16]. During these processes, unwanted components as well as some nutritive compounds including phenols are lost. The solubility of the phenolic compounds is affected by the polarity of the solvent used [17]. The extraction efficiency of phenolic compounds was evaluated using different solvents including acetone (40 %), ethanol (96 %) and methanol (99.5 %). Ethanol was found to have the highest extraction efficiency and was used for further extraction in this study. The mass of the residue obtained after extraction were 1.11 ± 0.05, 0.57 ± 0.04, 0.53 ± 0.05 and 0.43 ± 0.06 g per 40.5 g of crude oil, DNW oil, Bleaching and Deodorized oil respectively. A

Table 1: TPC/TFC/TNFC of SFO during the chemical refining

Oil	TPC GAE/µg/ml	% Loss	TFC Quercetin /µg/ml	% Loss	TNFC GAE/µg/ml	% Loss
Crude oil	95.14 ± 1.20		37.42 ± 2.18		66.47 ± 0.53	
DNW oil	82.99 ± 1.18	12.7	29.39 ± 1.93	21.4	51.52 ± 1.04	22.5
Bleached oil	70.69 ± 1.08	14.8	22.88 ± 0.35	22.1	42.09 ± 1.03	18.3
Deodorized oil	41.18 ± 0.82	41.7	8.24 ± 0.34	63.9	30.44 ± 0.44	27.6

decrease in the mass of the residues was observed for the different refining processes. The obtained residues were then dissolved in 5 ml of ethanol and were used for determining the phenolic, flavonoid and non-flavonoid contents.

Total phenolic (TPC), Flavonoid (TFC) and Non-Flavonoid Content (NFC)

Total phenolic, flavonoid and non-flavonoid contents of the ethanolic oil extracts and the percentage losses between the different oil samples were determined and reported in Table 1. Crude oil possesses higher TPC, TFC and NFC as compared to the refined oil. Significant losses of the phenolic compounds including both the FC and NFC compounds were observed during the different chemical processes. The TPC decreases from 95.14 ± 1.20 to 41.18 ± 0.82 µg/ml, TFC from 37.42 ± 2.18 to 8.24 ± 0.34 µg/ml and the TNFC from 66.47 ± 0.53 to 30.44 ± 0.44 µg/ml. The highest percentage loss in PC and FC was observed during the deodorization step. In this process, the unwanted volatile components such as free fatty acids and the oxidation products of oil which are liable for producing unacceptable odour, colour, taste and flavour in the oil are removed by direct injection of heated steam at a temperature of 180–270 ° C. Due to the vigorous conditions not only the undesirable components are stripped off but some important minor components including the phenolic compounds are also distilled off.

Anti-oxidant Activity

The antioxidant activity is the ability to inhibit the process of oxidation. The DPPH and ABTS radicals are the two most commonly used and stable chromatogens to measure the radical scavenging activity of biologically active plant extracts. The different functional groups present in the bioactive compounds influence the capacity of the sample to react and quench different radicals. ABTS generally gives higher anti-oxidative activity compare to DPPH since ABTS radical reactions involves electron transfer and takes place at a much faster rate than DPPH radical [18].

DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a spectrophotometric method to evaluate the free radical scavenging potential (antioxidant activity) of a sample.

The scavenging ability of DPPH radical is its ability to accept a hydrogen atom or an electron from an antioxidant having RH group such as phenolic and flavonoid compounds. The methanolic-DPPH solution is reduced to the molecular (DPPH-H) by antioxidants [19]. This reduction causes a colour change from purple to yellow. The degree of decolourisation indicates the radical scavenging potential of the antioxidant [20].

The antioxidant activity of the SFO oil samples was determined using the DPPH assay and the absorbances were read at 492 nm. The percentage radical scavenging activity of the ethanolic extracts of the different oil was determined with varying concentrations and is summarized in Table 2 and Figure 1. The free radical scavenging activities were significantly different for different oil samples. It was observed that crude oil has highest antioxidant activity of 55.64 % while the deodorized oil had lowest antioxidant activity of 35.87 % at a concentration of 50000 µg/ml of ethanolic solution.

ABTS Assay

ABTS radical monocation is generated by the oxidation of ABTS with potassium persulphate. Similar to DPPH, the decolorization of ABTS radical reflects the capacity of an antioxidant species to donate electron or hydrogen atoms to inactivate this radical cation. The ABTS results were in good agreement with DPPH assay of the oil samples and decreases as the oil undergoes chemical processes. From the analysis of Table 3 and Figure 2, it can be concluded that the crude oil exhibited higher ABTS radical-scavenging activity (59.46 %) at a concentration of 50000 µg/ml of ethanolic solution.

Both assays showed comparable activities for the different oil extracts. However, DPPH radical being more stable and allows easy handling and manipulation.

Relationship of Total Phenol and Antioxidant

Phenolic compounds are known to display anti-oxidant activity since they can either donates hydrogen or electrons and also form stable radical intermediates. The most important natural phenolics are flavonoids which are responsible for the free radical scavenging activities [21]. There is a significant linear relationship between phenolic compounds and antioxidant activity [22, 23]. Figure 3 highlights the strong positive correlation between PC, FC, NFC and antioxidant activity measured through the DPPH assay. Crude oil having more phenolic compounds showed higher antioxidant activity and vice-versa. A direct relationship was also found for ABTS assay and different phenolic compounds with R^2 values ranging from 0.8877-0.9318.

Conclusions

There is a direct relationship between the phenolic compounds and the oil extracts obtained from the different stages of processing. It is clear from the results that the loss of phenolic compounds cause a decrease in radical scavenging activity. The present study indicated

Table 2: Average % DPPH scavenging activity of oil extracts

Conc	Ascorbic Acid	Crude oil	DNW oil	Bleached oil	Deodorized
50000	78.09 ± 0.59	55.64 ± 1.01	50.29 ± 0.25	43.92 ± 0.29	35.87 ± 1.01
25000	81.22 ± 0.89	53.00 ± 0.25	48.48 ± 0.66	40.46 ± 0.66	31.76 ± 2.25
12500	81.54 ± 0.74	51.13 ± 0.63	45.93 ± 0.95	31.48 ± 1.15	20.76 ± 1.56
6250	82.27 ± 0.59	47.70 ± 0.43	40.22 ± 0.80	20.87 ± 2.67	6.49 ± 2.25
3125	82.16 ± 1.33	41.48 ± 1.52	35.19 ± 0.29	11.75 ± 0.90	2.03 ± 1.50
1562	81.22 ± 0.00	35.35 ± 1.13	11.98 ± 3.99	7.05 ± 0.66	0.00 ± 0.00
781	82.86 ± 1.33	29.78 ± 0.66	4.92 ± 0.95	1.69 ± 1.30	0.00 ± 0.00
390	82.68 ±0.59	25.19 ± 0.25	1.61 ± 1.01	0.00 ± 0.00	0.00 ± 0.00
195	82.68 ± 0.00	13.53 ± 3.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
97	82.68 ±0.59	1.00 ± 1.28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
48	82.05 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
24	77.68 ± 2.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

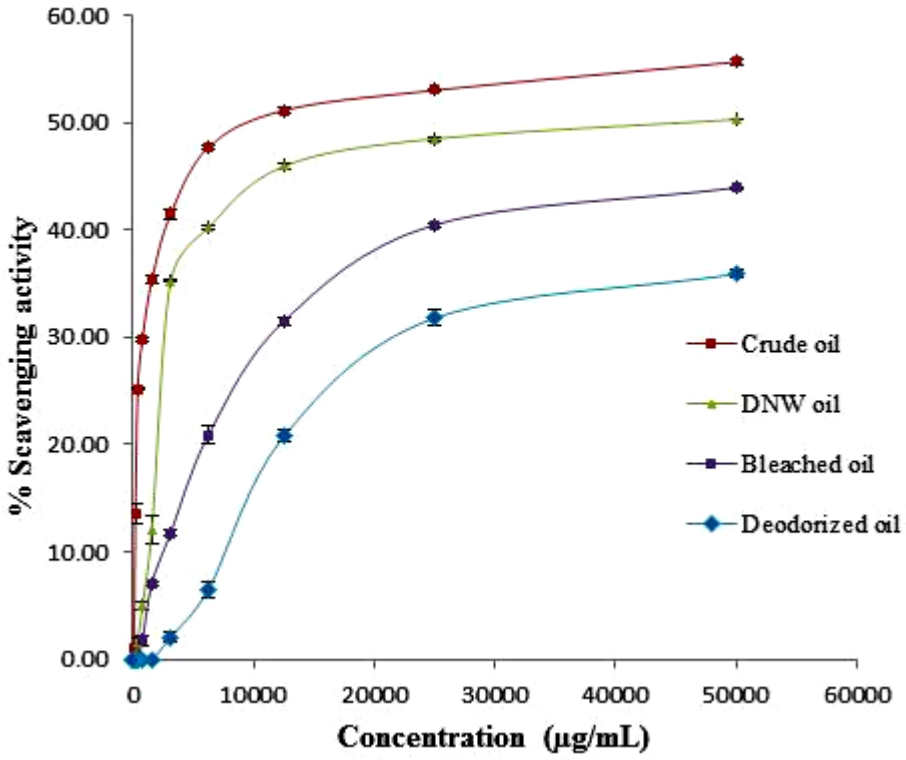

Figure 1: Percentage of radical scavenging activity of sunflower oil using the DPPH Assay

Table 3: Average % scavenging activity of oil extracts using ABTS assay

Conc (mg/ml)	Crude oil	DNW oil	Bleached oil	Deodorized oil
50000	59.46 ± 0.51	53.72 ± 0.43	38.17 ± 0.36	31.43 ± 0.51
25000	56.61 ± 0.57	50.92 ± 0.57	32.48 ± 0.43	26.84 ± 0.43
12500	53.67 ± 0.30	44.99 ± 0.59	24.66 ± 0.43	16.22 ± 0.43
6250	48.41 ± 0.57	42.25 ± 0.57	20.86 ± 0.67	10.91 ± 0.43
3125	45.00 ± 0.64	38.41 ± 0.57	15.6 ± 0.36	3.84 ± 0.38

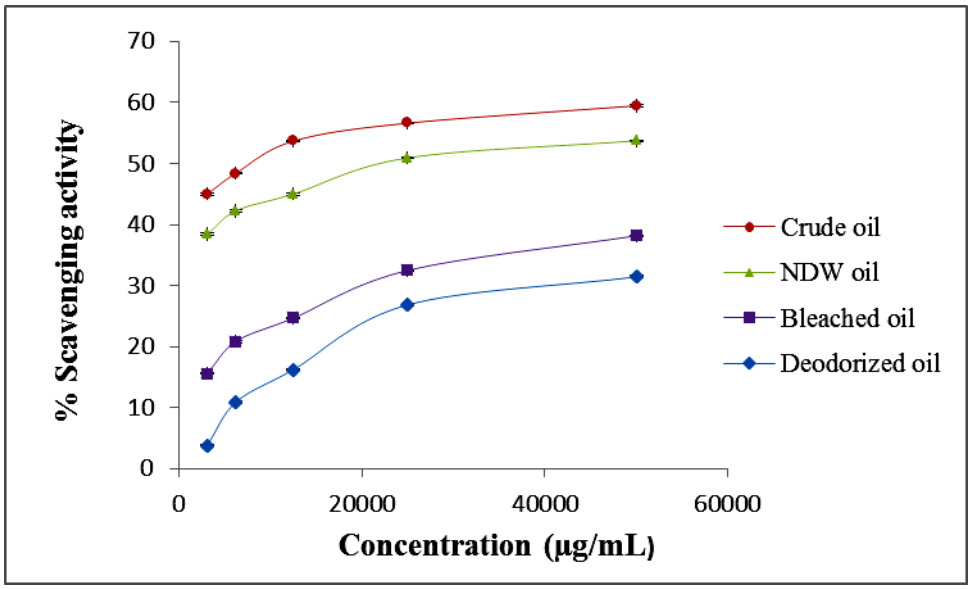

Figure 2: Percentage of radical scavenging activity of oils using the ABTS Assay

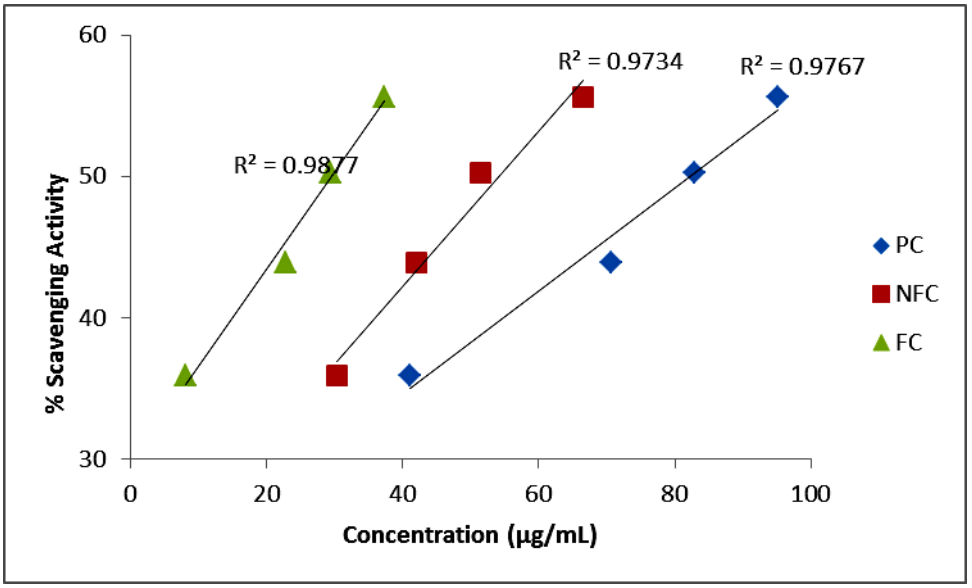

Figure 3: Correlation among phenolic compounds and antioxidant activity of oil samples measured according to DPPH assay

that sunflower oil can contribute to the dietary intake of antioxidants depending on the processing conditions. It is observed in the present work, that the unrefined oil possesses better activity which reduces as the oil is processed. This suggests the importance of optimizing and/or modifying the existing steps in the sunflower oil processing to retain the polyphenolic content in oil.

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