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To study the Antioxidative potential of Flaxseed oil at varied temperatures

Promita Gundev[1], Komal Chauhan[1*] and Divya Sachdev[2]

[1] Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana-131028, India

[2] Department of Basic and Applied Science, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana-131028, India

Abstract

The effect of heating flaxseed oil at varied temperatures on the TPC (total phenol content), TFC (total flavonoid content) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was evaluated. Oil was heated at varied temperatures of 25ºC, 60ºC, 100ºC, 140ºC, 180ºC for duration of 30 minutes each. Results indicated that TPC increased upto 100°C followed by a decreasing trend at 140°C and 180°C. On the other hand the TFC content decreased dramatically ($p \leq 0.05$) upon heating. The DPPH scavenging activity showed more or less similar results upto 100 ºC thereafter decreased at 140 ºC. The result suggests that antioxidative and radical scavenging activity deteriorated on heating the oil at higher temperature (at 140 ºC and above).

Keywords: *Phenols, DPPH, Flaxseed oil, Thermal stability, Flavonoids*

1. Introduction

Phenolic compounds are bioactive compounds present naturally in environment comprising of aromatic ring with one or more hydroxyl substituents. They may be present in free acid or ester form, glysosides or conjugated forms (Kasote, 2013; Marina et al., 2009). They exhibit properties like bitterness and astringency in terms of taste, browning reaction and antioxidative potential (Elhamirad and Zamanipoor, 2012; Singleton et al., 1999). The phenolics and flavonoids have been reported to have antimutagenic, anti carcinogenic, anti-inflammatory and antiproliferative properties and helps in treating oxidative stress-related diseases viz. cardiovascular diseases, diabetes mellitus, neurodegenerative diseases and aging (Xuan et al.,2018; Marina et al., 2009). The edible oils including cold pressed oils contains a number of phenolic compounds, thereby lendering antioxidative capacity and oxidative stability to them (Xuan et al.,2018) by preventing oxidative damages due to radicals (Siger et al., 2007). The extraction process affects the quality of oil. Studies have shown that cold-pressed oils are better in terms of thermal and oxidative stability (Prescha et al., 2014).

Flaxseed oil rich in polyunsaturated fatty acids (PUFA) accounting for 73%, containing essential fatty acids: α-linolenic acid and linoleic acid. Moreover it is a good source of phenolic antioxidants and dietary fiber (Hasiewicz-Derkacz et al., 2015; Kasote, 2013).

Flavonoid and phenolic compounds viz. phenolic acids, phenylpropanoids, lignans, secoisolariciresinol, ferulic acid, coumaric acid methyl ester and tannins have been reported in flaxseed oil (Kasote, 2013). These compounds have shown beneficial effects on human health owing to their antioxidant and radical scavenging activities (Chaaban et al., 2016; Rajha et al., 2014).

Flavonoids and phenols are heat sensitive and results in degradation at higher temperature (Chaaban et al., 2016; Sharma et al., 2015). However, the stability of each bioactive compound varies (Elhamirad and Zamanipoor, 2012). Thus, thermal deterioration of bioactives can result in loss of their antioxidant activity. The temperature and duration of heating thus can have varied effects on the antioxidant properties. Elhamirad and Zamanipoor, 2012 had demonstrated that maximum thermal decomposition and loss of antioxidants occurs at the elevated temperatures of cooking and frying.

The study was planned to evaluate the effect of varied temperatures on antioxidant and radical scavenging activity of flaxseed oil.

2. Materials and Methods

2.1 Chemicals

Analytical grade chemicals and standards were used in the study were purchased from Sigma Aldrich. The flaxseed oil sample was provided by SNN Natural Products, Delhi.

2.2 Effect of varied temperature on oil Sample

The flaxseed oil sample (50 ml) was taken in Erlenmeyer flask and gradually heated in the presence of air and moisture on a hot plate with continuous stirring from 25ºC to 180ºC. The oil was heated at varied temperatures of 25 ºC, 60 ºC, 100 ºC, 140 ºC, 180 ºC for 30 minutes each. Thereafter, the 10 ml of each sample was collected after attaining the requisite temperature viz. 25ºC, 60ºC, 100ºC, 140ºC and 180ºC and stored at –18ºC till further analysis.

2.3 Extract Preparation

The extract for TPC and TFC analysis was prepared according to the method used by Kasote et al., 2013 with some modifications. Briefly, 1g of flaxseed oil was dissolved in 10 ml hexane followed by extracting phenols using 60% methanol. The process was repeated thrice and the methanol layers were separated, pooled and concentrated in a vacuum rotary evaporator. The final residue was added with methanol to make up the final volume to 1 ml.

2.4 Total Phenol Content

TPC was evaluated by modifying the Folin–Ciocalteu colorimetric method described by Singleton et al., 1999. To 0.1 ml of methanoilc extracts of samples, distilled water (5 ml) and Folin–Ciocalteu (FC) reagent (0.5 ml) were mixed and waited for 3 minutes. Afterwards, it was added with 1 ml of saturated Na_2CO_3 (35% w/v) and final volume was made up using distilled water to 10ml. The solution was incubated for 60 minutes at room temperature. The absorbance was measured at 725 nm. The gallic acid standard curve was used to calculate results, expressed as mg of gallic acid equivalent per 100 gram of flaxseed oil (mg GAE/100 g of flaxseed oil).

2.5 Total Flavonoid Content

TFC was determined using aluminium chloride spectrophotometric method described by Baba and Malik, 2015 with some minor modifications. 0.5 ml extract was diluted to 1 ml using methanol and added with distilled water (4 ml). Thereafter, 5% $NaNO_2$ (0.3 ml) was added. After 5 minutes, 10% $AlCl_3$ (0.3 ml) and NaOH (2 ml) was added followed by making up the volume to 3 ml using distilled water. The solution was kept for 15 minutes at room temperature before reading the absorbance at 510 nm. The quercetin calibration curve was used to calculate flavonoid content, expressed as mg quercetin equivalent per 100g of flaxseed oil.

2.6 DPPH Radical Scavenging Activity

The antioxidant activity of the samples was determined using DPPH free radical scavenging method described by Malacrida et al, 2012. Firstly, the oil samples were diluted in ethyl acetate (1:10 w/w). 1 ml of the above solution was added with 3 ml DPPH solution (10^{-4} M) and the mixtures were shaken and kept in dark for 15 minutes. Thereafter, the absorbance was taken using UV/VIS spectrophotometer (Shimadzu, UV-2600, Japan) at 517 nm. The DPPH radicals scavenging activity of the oil samples were calculated as follows:

$$\% \text{ DPPH Scavenging} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

3. Results and Discussion

3.1 Total Phenol Content (TPC)

The changes in the TPC content of the flaxseed oil during the heating process at various temperatures are shown in Figure 1. It was observed that TPC increased with the increase in heating temperature till 100 ºC thereafter, it showed a decreasing trend. The TPC content of fresh oil was 32.2 mg GAE/100 g (25ºC) which increased to 37.86 mg GAE/100 g at 100 ºC and then decreased to nearly half (16.7 mg GAE/100 g at 180ºC). The TPC showed an increasing trend initially up to 100ºC probably owing to the release of the polyphenols upon heating. The flax seed oil used for the study was obtained from cold pressed extraction resulting in incomplete release of bioactives as they are in the bound form and upon heating got released thereby increasing the content. The results are in accordance with Lohani and Muthukumarappan, 2015 demonstrating that during heating, the phenolic compounds are liberated due to the cleavage of covalent bound in phenolic compounds. On further heating to 140 ºC and 180ºC,

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the TPC reduced to 24.5 mg GAE/100 g and 16.7 mg GAE/100 g respectively due to the degradation of phenolic compounds at higher temperature. The study performed by Gomez-Alonso et al., 2003 also showed that the degradation of phenols occur rapidly during frying confirming that at high temperatures total phenolic content reduces.

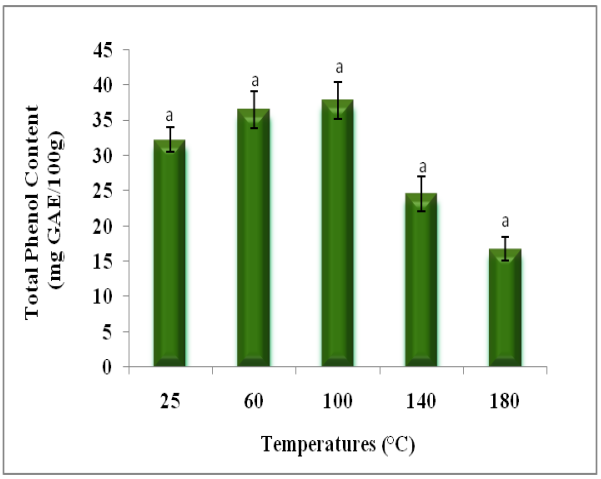

Figure 1: Effect of Varied Temperature on Total Phenol Content of Flaxseed oil. The superscript 'a' denotes the significant effect ($p \leq 0.05$) of temperature on TPC content of oil.

3.2 Total Flavonoid Content (TFC)

The flavonoid content of flaxseed oil was 22.82 mg QE/100g at 25°C. The flavonoid content of the oil samples dramatically decreased upon heating. The Figure 2 clearly depicts that the flavonoid content of flaxseed oil degraded at a faster rate with increase in temperature. At 100°C the initial concentration (22.82 mg QE/100g) of flavonoid was reduced by 53.19% (10.68 mg QE/100g) which further reduced by 78.43% (4.92 mg QE/100g) at 180°C. Similar results were shown by Chaaban et al., 2016 where 50% of the flavonoid content was lost at a temperature of 90°C. The results were supported by the study Elhamirad and Zamanipoor, 2012 reporting that the concentration of quercetin and catechin were drastically reduced upon heating.

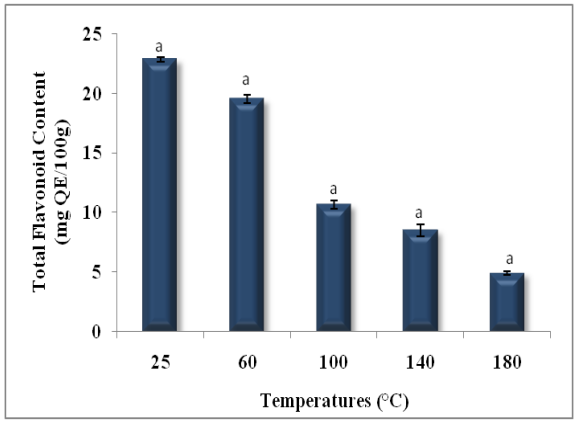

Figure 2: Effect of Varied Temperature on Total Flavonoid Content of Flaxseed oil. The superscript 'a' denotes the significant effect ($p \leq 0.05$) of temperature on TFC content of oil.

3.3 DPPH Radical Scavenging Activity

Figure 3 indicates the capacity of the flaxseed oil to scavenge the DPPH radicals. The radical scavenging capacity slightly increased initially followed by a decreasing trend. The antioxidant activity of the oil kept at 25°C was 74.7%, which slightly increased (75.4%) ($p \leq 0.05$) upon heating at 60°C (1% increase). However, with further increase in temperature to 100°C, the DPPH activity decreased (73.9%) though the decrease was only 1.07%. The enhancement of antioxidant activity as reported earlier (Sharma et al., 2015) may be contributed to the formation of novel antioxidant components or the structure alteration of the existing antioxidants. There was a significant decrease ($p \leq 0.05$) in antioxidant activity by 11.49% at 140°C (66.15 %) and by 29.64% 180°C (52.58%). Similar findings were reported by Kalantzakis et al., 2006 where the DPPH scavenging activity of various vegetable oils decreased during heating at 180°C. The antioxidant activity of oil can be directly related to the amount of phenols present owing to the presence of hydroxyl groups that have the ability to destroy the radicals and form stable phenoxyl radicals (Bajalan et al., 2017), thus rendering free radical scavenging activity. The results confirm with the earlier studies showing an increase in DPPH scavenging activity with the increase in TPC content while heating to 100°C and thereafter the phenolic content and DPPH activity declined significantly ($p \leq 0.05$) indicating positive correlation between DPPH scavenging activity and phenolic compounds present in the flaxseed oil.

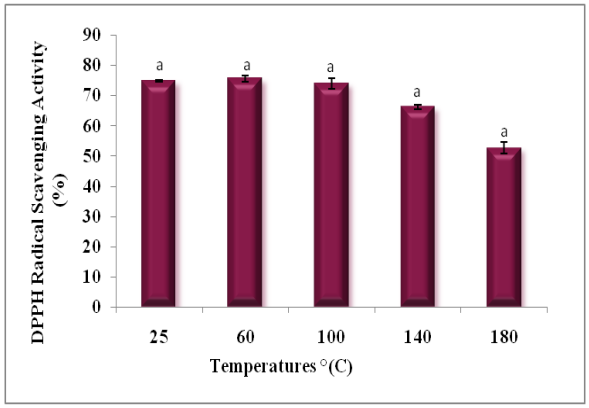

Figure 3: Effect of Varied Temperature on DPPH radical scavenging activity of Flaxseed oil. The superscript 'a' denotes the significant effect ($p \leq 0.05$) of temperature on DPPH content of oil.

4. Conclusions

The stability of flaxseed oil at varied temperature was evaluated. The study has shown that flaxseed oil is thermally stable upto 100°C and can contribute to nutraceutical potential owing to its bioactive compounds.

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