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Enhancement of oxidative stability and antioxidant potential of flaxseed oil with cinnamon extract

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Abstract

Oxidation in edible oils and fats is one of the main problems faced by the fat and oil industry. Using natural antioxidants is considered the preferred choice to minimize the application of synthetic antioxidants in food products. The present study was conducted to extract cinnamon extract and evaluate its antioxidant potential. The cinnamon extract was incorporated in flaxseed oil samples at different concentrations of 0.5, 0.1, 0.15, 0.2, and 0.25% (v/v) and compared with the control (with no addition of natural/synthetic antioxidant) and another sample with 0.1% (v/v) of synthetic antioxidant (butylated hydroxytoluene [BHT]). The antioxidant activity of the flaxseed oil added with cinnamon extract was carried out by DPPH and FRAP assay. The extraction method, time and temperature treatments, and solvent concentrations significantly affected cinnamon extracts' proximate composition, DPPH, and FRAP activity. Cinnamon extract showed higher flavonoid and total phenolic contents, which led to higher antioxidant activity. Phenolic contents were observed at 313.61 ± 19.83 mg GAE/100 g acetone extract. The DPPH assay showed a significant observation of 84.58 ± 3.80%, while the FRAP assay was 143.82 ± 11.21 µmol/g. During 28 days of storage, there was a significant decrease in free fatty acids, peroxide, iodine, and thiobarbituric acid values for the treatments with higher concentrations of cinnamon extract as compared to the control. The T_1 and $T_{2,}$ exhibited PV of 4.69 and 4.53 milli-equivalents (meq/kg), respectively. The maximum value of peroxide was detected in T_0 (4.78 meq/kg) and the lowest in T_{BHT} (3.50 meq/kg), followed by T_3 (3.97 meq/kg), T_4 (3.94 meq/kg) and T_5 (3.89 meq/kg). As compared to T_0 and T_{BHT}, cinnamon extract was significant in reducing the peroxide value. T_0 showed the highest iodine value (198.51 I_2/100 g), while T_{BHT} and T_5 showed the lowest iodine values of 173.76 and 175.29 g of I_2 / 100 g, respectively. Moreover, T_1, T_2, $T_{3,}$ and T_4 showed iodine values of 194.34,

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195.10, 179.78, and 177.42 g of I_2/100 g, respectively. The results revealed that the TBA value of oil increases with the increase of the storage period. T_0 showed the highest TBA value (6.95 mg MDA/kg) and T_5 had the lowest TBA value (5.92 mg MDA/kg). The TBA values of T_1, T_2, and T_3 were 6.87, 6.63, and 6.68 mg MDA/kg, respectively. Overall, the cinnamon extract improved the oxidative stability of flaxseed oil as an alternative to synthetic antioxidants with no harmful effects on human health.

Keywords: flaxseed oil; oxidation; cinnamon extract; antioxidant potential; free fatty acids; bioactivity

Introduction

Flaxseed is an annual herbaceous plant that is cultivated for its oil and owing to its nutritional as well as therapeutic benefits. It belongs to the family Linaceae and is a member of the genus *Linum.* The Latin name of Flaxseed is *Linum usitatissimum* L., which means very useful and is considered a functional food, originated in Western Asia or the Mediterranean (Suri *et al*., 2020). The world produces 3.06 million tons of flaxseed per year and since ancient times, people have explored flaxseed and its products. Nearly every portion of this plant is used for a variety of purposes including its seed which contains oil consumed for culinary purposes after processing (Kaur *et al*., 2018; Zhang *et al*., 2023). Moreover, in different areas of the world flaxseed has been used for centuries to make linen fiber, but today it is mainly grown for its oil. Flaxseed oil helps reduce cancer cell growth, sink health, reduce inflammation, and treat constipation. The flaxseed oil is exceptional in its polyunsaturated quality (Rahim *et al*., 2023).

The extraction of flaxseed oil is usually nowadays carried out in modern ways which include ultrasound and solvent-based extractions. The most efficient ways include ultrasound- and microwave-assisted extraction methods. As ultrasonic power is increased, flaxseed oil output rises roughly linearly. The yield of flaxseed oil increased from 66.7 to 84.9% (18.2% increase) when the power was raised from 20 to 50 W (1.5× increase). More bubbles formed and bursted when the ultrasonic wave with a greater amplitude passed through a liquid medium. The violent shock wave and high-speed jet that was created could have improved the solvent's penetration into the cell tissues and accelerated the intracellular product release into the solvent by breaking down the cell walls because the temperature and pressure inside the bubbles were extremely high and the bubbles collapsed quickly. Additionally, the strong shock wave and fast-moving jet may have improved molecular mixing and increased the mass transfer rate. The rigid cell walls, which are less permeable, are what caused the significant increase in ultrasonic power to produce a moderate rise in yield (Demrican *et al*., 2023; Tran *et al*., 2023).

Flaxseeds are also good sources of phenolic compounds and flavonoids. The major phenolic acids include chlorogenic acid (7.5 mg/g), gallic acid (2.8 mg/g), and ferulic acid (10.9 mg/g). Flaxseed contains major flavonoids such as flavone C and O-glycosides (Li *et al.*, 2024). The polyphenols in flaxseed-like lignans provide a quantitative advantage to cancer prevention agents, as they can scavenge hydroxyl free radicals during fat and protein oxidation and protect against diseases (Al-Madhagy *et al.*, 2023). Flaxseed is gaining consumers' attention as it exhibits medicinal properties of lipid-lowering agents. Flaxseeds combined with an abundance of omega (ω)-3 fatty acid and phytoestrogen make them a healthy choice to add to the diet (De Lange-Jacobs *et al.*, 2020; Kamyab *et al*., 2021).

The stability of fats and oils is the most important factor that must be kept in mind while handling. The lipid peroxidation is the major cause of the deterioration of flaxseed oil. It not only affects the quality but also causes unfavorable variations in fatty acid profiles (Li *et al*., 2024). It produces free radicals that cause off flavor and color, and affect the nutritional quality of the flaxseed oil. These changes lower the chance of acceptance by the consumer, so the industries suffer from great loss. The oil stability can be increased by using synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), tertiary butyl hydroxy-quinone (TBHQ), and some other food additives (Man *et al*., 2021). Synthetic antioxidants, including BHT, BHA, and TBHQ, are commonly used to prevent oil products from being oxidative degrading, but safety issues have been raised that have led to a change of interest toward their natural counterpart (Cicero and Colletti, 2016). However, it was revealed that the usage of synthetic antioxidants can cause detrimental health hazards that may include enlargement of the liver, hormonal imbalance, and cancer. Therefore, food industries are more focused on using natural antioxidants.

Cinnamon is mainly used in the essence and aroma industries due to its fragrance, which can be incorporated into several varieties of foodstuffs, pharmaceutical products, and perfumes. Cinnamaldehyde and transcinnamaldehyde (Cin), which exist in essential oils, are the most significant constituents of cinnamon and are, therefore, a factor in the fragrance and the diversified biological

activity of cinnamon (Tran *et al*., 2020). A mixture of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and various essential oils are part of cinnamon. With age, cinnamon becomes black, thus improving the resinous compounds in cinnamon extract (Jiang *et al*., 2021). Cinnamon can boost colon health, thereby reducing the risk of cancer of the colon, and is also a blood clotting agent. Cinnamon also stimulates uterine blood circulation and promotes regeneration of the tissue (Isik and Ugraskan, 2021).

Plants plays a very important role as a seasoning, besides the essential oils and other constituents, particularly antimicrobials (Batool *et al*., 2023; Jimayu G, 2022; Okonkwo and Achilik, 2022; Radwan *et al.*, 2022; Raza *et al.,* 2022), antioxidants (Akpinar *et al.*, 2023; Asala *et al.*, 2022; Ghany *et al.*, 2023; Hazafa *et al.*, 2022), antifungals, and antidiabetics, also have significant activities. Several cinnamon extracts, including ether, aqueous, and methanol extracts, have demonstrated significant antioxidant activity (Abd El-Hack *et al*., 2020). The aqueous and alcoholic cinnamon extract (1:1, v/v) ratios theoretically prevent *in vitro* fatty acid oxidation. The free radical-scavenging behaviors and the antioxidant properties of different flavonoids isolated in cinnamon were evaluated for their antioxidant potential. These flavonoids showed the highest potential concerning antioxidant activity as well as characterized by the unique cinnamon flavor of the food products (Bekhit *et al*., 2018; Dzuvor *et al*., 2018). The highest antioxidant activity has been found in a comparative survey of 26 spices, which showed that cinnamon can be applied as a food antioxidant (Hariri and Ghiasvand, 2016).

This study aimed to evaluate the antioxidant activity of cinnamon extract as a natural antioxidant in commercially available flaxseed oil. This study also evaluated the oxidative stability of flaxseed oil after the addition of cinnamon extract during storage conditions.

Materials and Methods

Procurement of materials and chemicals

The study was conducted at the Food Science and Technology Laboratory, University Institute of Diet and Nutritional Science, The University of Lahore, Pakistan. Flaxseeds and cinnamon were purchased from the local market of Lahore. The HPLC grade solvents (acetonitrile, methanol) and all other chemical reagents (acetic acid, sodium hydroxide) were used for experimental work purchased from native suppliers of Sigma Aldrich, MA, USA. The glassware was procured from LAB-101 & 102 Food Science and Technology Laboratory at the University Institute of Diet and Nutritional Science, The University of Lahore, Pakistan. All the raw materials were stored at a specific temperature and the required sample was used for analysis. All treatments were analyzed in triplicate (replicates were analyzed in triplicate for analytical measurements). The results were expressed as mean values ± standard deviations (mean ± STDV).

Preparation of defatted flaxseed extract

The flaxseeds (500 g for each treatment) were mechanically subjected to a traditional oil expeller (electric oil expeller and extraction machine, Tianjin Mikim Technique Co., Ltd, Beijing, China) for separation of flaxseed cake after oil extraction. Flaxseed cake was dried and ground for conversion into powder. Microwave-assisted extraction (MAE) was carried out by using a microwave-assisted extraction system (Milestone ETHOS ONE Advanced Microwave system, Shelton, CT, USA) with an automatic fiber optic temperature control system following the method described by (Rani and Badwaik, 2021; Safdar *et al*., 2020). The sample (500 mg) was placed in a 100 mL quartz tube topped by a vapor condenser that was suspended in 20 mL of 70% (v/v) methanol supplemented with 0.1 or 1 M sodium hydroxide. The power was set to 150 W and the extraction time was 15 min, respectively. The initial MAE conditions for evaluating extraction of the main flaxseed phenolic would be 0.1 M sodium hydroxide at a power of 100 W for 1 min. The extract was then neutralized with acetic acid, centrifuged at 4000 rpm for 10 min and at room temperature, and the supernatant was filtered through Whatman 1 filter paper with coarse or medium porosity.

Proximate analysis of partially defatted seed meal

The samples of partially defatted seed meal (about 250 g for each treatment) were tested chemically according to the techniques for the proximate analysis. The proximate analysis of flax seed oil was carried out with slight modifications in the standardized method of AOAC (2012) (Association of Official Agricultural Chemists, AOAC) and (Thiex, 2009). Moisture content was measured by using air forced drying oven (Drying oven 30L, Model: DO-1-30/02, Dongguan Huanyi Instruments Technology Co., Ltd., Dongguan, China,) at the Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, Pakistan. The crude fiber and protein were determined through the slight modification in the methods of AOAC (2012) and (Lan *et al*., 2020). The crude fat and ash content was also estimated through the described by AOAC (2012) and (Thiex, 2009). The nitrogen-free extract (NFE) was calculated according to the expression (Equation 1) and expressed in percentage as described by AOAC (2012).

$$\text{NFE (\%)} = 100 - (\text{Moisture} + \text{crude fat} + \text{crude protein} + \text{crude fiber} + \text{total ash}) \quad (1)$$

Extraction of flaxseed oil

The electronic measuring balance (Model Kern 440-35N, Kern & Sohn, Germany) was used to weigh the raw flaxseeds (500 ± 0.1 g). Then raw seeds were washed and cleaned to free from dirt and any other external particles. A locally made mini oil presser was used to extract oil by pressing the cleaned flaxseed samples (Zeng *et al*., 2022).

Cinnamon extract preparation

The cinnamon sticks were procured from the local market of Faisalabad, Paksitan. They were cleaned for any external material and stored at moderate room temperature. The extraction was carried out by slight modification in the method described by (Wang *et al*., 2022). Cinnamon sticks were dried in the oven at a temperature of 55°C until the humidity was stable at 8.5 % of overall weight. Afterward, the sample was compressed and moved through the mesh in a powder shape using electric grinders (Brabender: Quadrumate Senior lab mill, Anton Paar, Austria). Then, the powdered sample was subjected to solvent extraction using ethanol. Ten gram powdered cinnamon sample was extracted by using 50 mL ethanol, for 1 h under agitation at room temperature (25°C), and then the mixture was filtered through Whatman 1 filter paper with coarse or medium porosity by using vacuum filtration assembly and residues were extracted again in 50 mL ethanol and filtered thoroughly. The filtrate was combined, packed, and stored in a freezer at a temperature of –18°C until used for further analysis.

Total phenolic contents (TPC)

The phenolic contents (TPC) of cinnamon stick extract were calculated by following proper methods AOAC (2012) and (Akl *et al*., 2020). TPC was expressed as mg gallic acid equivalent (GAE)/100 g extract.

Treatments and experimental design

Cinnamon extract oil was added to flaxseed oil at different levels such as 0.05, 0.10, 0.15, 0.20, and 0.25% (v/v) as T_1, T_2, T_3 T_4, and T_5, respectively, to compare the antioxidant potential of the cinnamon extract with control and synthetic antioxidant added flaxseed oil samples. The control sample (T_0) includes flaxseed oil without any natural or synthetic antioxidants. While one sample of flaxseed oil was added with 0.1% (v/v) synthetic antioxidant (BHT) (T_{BHT}).

Antioxidant activity (AA)

The antioxidant activity (AA) of the flaxseed oil added with cinnamon extract was carried out by free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl; commonly known as DPPH assay) and ferric reducing antioxidant power assay (FRAP assay) as described by (Erickson *et al*., 2023; Pradhananga and Manandhar, 2018). AA was expressed as a percentage of inhibition relative to the control (%) in the case of DPPH while it was expressed as micromolar Fe^{2+} equivalents (μmol/g) relative to an antioxidant standard.

Oxidative stability potential (OSP)

The oxidative stability potential (OSP)/state of oxidation or oxidative rancidity/degree of oxidation of the fat and oils depends upon their stability during storage conditions. The free fatty acids (FFAs), peroxide value (PV), iodine value (IV), and thiobarbituric acid reactive substances (TBARS) assay, simply known as thiobarbituric acid value (TBA), assays were carried out to raise awareness about the oxidative stability of flaxseed oil after addition of cinnamon extract at 0 to 28 days of storage period.

Free fatty acids (FFA)

The free fatty acid (FFA) determination was carried out by a modification in the method (Shahid *et al*., 2018; Zeng *et al*., 2022). The sample (4–5 mL) was dissolved in the solvent (mixture of ethanol and diethyl ether, 1:1, v/v) and heated, if necessary, to increase solubility. After complete dissolution, the sample was titrated with 0.1 mol KOH. The endpoint reading of the burette was noted and the reading was then converted to milli-equivalent (meq)/kg of oil samples for expression of FFA.

Peroxide value (PV)

The peroxide value (PV) of the flaxseed oil and the free fatty acid content of oil samples were measured by the procedures described by AOAC (2012) and (Lu *et al*., 2020). In short, the experiment was carried out by weighing 2 g of flaxseed oil and titration against 0.002 N sodium thiosulfate. The burette reading of the titration endpoint was noted and PV was expressed as meq/kg of oil sample.

Iodine value (IV)

The iodine value (IV) of the flaxseed oil samples was investigated through the method described by AOAC (2012) and (Thiex, 2009). The sample (4–5 mL) was dissolved in CCl_4 and 25 mL of Wijs solution (16.2 g iodine monochloride added in glacial acetic acid and volume up to 1 L in the volumetric flask) was added and kept the sample in the dark for 1 h. After that, deionized water

was added and the excess of iodine was titrated with sodium thiosulphate. The burette reading of the titration endpoint was noted and IV was expressed as grams of iodine (I_2) absorbed per 100 g (g I_2/100 g) of oil sample.

Thiobarbituric acid value (TBA)

The thiobarbituric acid value (TBA) of flaxseed oil samples was determined by slight modifications in the method of (Tobaruela *et al.*, 2018). In a nutshell, 2.5 mL of a mixed solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 M HCl was combined with 0.05 g of material and heated to boiling for ten minutes. After cooling, the mixture was centrifuged for 10 min at 4000 g and room temperature. The absorbance of the supernatant was then measured at a wavelength (λ) 532 nm by using a spectrophotometer (Thermofisher Scientific Spectrophotometer, Evolution Series 201/220, USA). The TBA number was obtained by converting the concentration of malondialdehyde (MDA) as follows (Equation 2):

$$\text{TBA (mg MDA/kg oil)} = \frac{\text{Absorbance (A)} \times 0.003 \times 72.07 \times 1000 \times 100}{1.56 \times 10 \times 0.5} \quad (2)$$

Statistical analysis

Statistical analysis was performed to determine the significance level of the data obtained from each parameter of treatment using a Completely Randomized Design (CRD). The significant difference comparisons were performed by Analysis of variance (ANOVA) and Tukey's test was used to statistically evaluate the data (SAS 9.1 Statistical Software). The $P < 0.05$ was considered to be statistically significant (Montgomery, 2017).

Results and Discussions

Proximate analysis

The proximate analysis of partially defatted flaxseed meal is shown in Table 1. The proximate analysis showed that the moisture, crude fat, and protein and the variation in the composition were affected due to the varieties of flax seed, seasonal or the region of cultivation (Khan *et al.*, 2023). The fat from the defatted meal was found to enhance its functional qualities. The partially defatted seed meal had high protein content and if used in food preparation then protein denaturation improves the emulsifying properties of food products. The foaming capacity and emulsion stability increased by defatting the flaxseed (Zou *et al.*, 2017). The flaxseed meal showed a higher capacity to absorb water and oil. This ability to bind protein, fat, or starches improved good elasticity and plasticity for film formation and good viscosity for food products. Thus, it improved the final product of edible and nonedible bioplastics with smooth texture and rheology. The oilseed meals are not wasted leftovers but rather can be used for food application, fortification, and packaging material (Rani and Badwaik, 2021).

Table 1. Proximate analysis of partially defatted flaxseed meal on dry basis.

Parameter	Quantity (%)
Moisture content	6.52 ± 0.83
Crude protein	20.22 ± 0.12
Crude fat	35.77 ± 0.11
Crude fiber	7.29 ± 0.09
Total ash	3.48 ± 0.83
NFE	23.97 ± 0.19

All values are triplicate means (Mean ± SD) of each treatment.

Total phenolic contents (TPC)

The phenolic compounds contain a broad type of molecules that have a polyphenol structure or molecules in which phenol rings exist such as phenolic alcohols and phenolic acids thus their extraction and identification are still needed (Amna et al., 2023) . The results of the total phenolic contents of the cinnamon extract are depicted in Table 2. Phenolic contents were observed in the range of 313.61 ± 19.83 mg GAE/100 g acetone extract. The cinnamon proved effective in limiting the lipid oxidation of palm oil and successful substitute against synthetic antioxidants (Akl *et al.*, 2020). Natural antioxidants are considered more beneficial for human health and the best alternative to synthetic antioxidants. They have strong antioxidant potential and thus 0can retard lipid oxidation in food systems (Shahid *et al.*, 2018).

The phenolic compounds of cinnamon extract contain a broad type of molecules that have a polyphenol structure

Table 2. Antioxidant properties (mean ± STDV) of cinnamon extract.

Test	Quantity
TPC	313.61 ± 19.83 mg GAE/100 g
DDPH	84.58 ± 3.80%
FRAP	143.82 ± 11.21 µmol/g

All values are triplicate means (Mean ± SD) of each treatment. Where; TPC = Total phenolic contents; DPPH = 2,2-Diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant power; GAE = Gallic Acid Equivalent.

or molecules in which phenol rings exist such as phenolic alcohols and phenolic acids thus their extraction and identification are still needed. The main groups of polyphenols are flavonoids and nonflavonoid compounds. Flavonoids have strong antioxidant, anti-microbial and anti-inflammatory potential (Ramaiyulis *et al.*, 2022). Non-flavonoid compounds include benzoic acid and cinnamic acid. Numerous derivatives of hydroxybenzoic (HB) and hydroxycinnamic acid (HCA) are present in cinnamon extracts (Pongsumpun *et al.*, 2020). Some derivatives of HB are gallic acid, p-hydroxybenzoic acids, protocatechuic and syringic. While caffeine, ferulic acids chlorogenic, and p-coumaric are other derivatives of HCA. Such phenolic compounds of cinnamon extracts prevent oxidation, extend shelf life, and improve the quality of food products (Kiralan *et al.*, 2019; Ozcan and Uslu, 2022). The phenolic compounds are liable for the flavor, odor, color, and acidity of foods as they protect food against invading pathogens and radiation. Phenolic compounds possess therapeutic potential like prevention from cancer, diabetes, osteoporosis, cardiovascular and neurodegenerative diseases. Cinnamon extract has a higher total phenolic content than other parts of fruit (seed/pulp) and showed high antioxidant activity. Its extracts displayed better antimicrobial activity against bacterial strains as compared to fungal strains (Abdul Qadir *et al.*, 2017; Ereifej *et al.*, 2016). The best results were obtained when phenolic components were extracted from the bark of various plants using a total of 80% aqueous ethanol. When aqueous ethanol and acetone were used as extractants, the highest concentration of phenolic compounds was extracted from barley flour. Our results are consistent with research showing that phenolic chemicals could be extracted more successfully from various plant materials using aqueous methanol and aqueous ethanol extraction solvents (Isik and Ugraskan, 2021).

Antioxidant activity

The antioxidant activity of flaxseed oil with cinnamon extract was estimated by the DPPH and FRAP assays (Table 2). The DPPH assay showed a significant observation of 84.58 ± 3.80%, while the FRAP assay was 143.82 ± 11.21 µmol/g. The preservative effects of some cinnamon extract as natural herbs oleoresins stabilize the flaxseed oil in comparison to synthetic antioxidants. The DPPH free radical scavenging activity is a reliant method for the evaluation of the antioxidant capacity of the extract and some elected bioactive compounds (Ashraf *et al.*, 2023). Usually, free radical scavenging power is computed by DPPH and FRAP free radical which is normally a proton radical. Subsequently, losing hydrogen atoms from double bonds of unsaturated fatty acids resulted in the formation of free radicals which might accelerate the process of lipid oxidation (Khan *et al.*, 2024). In such a way, the scavenging of free radicals initiates the mechanism of oxidation as well as preventing lipid oxidation by inhibiting the chain reactions by the phenolic compound of cinnamon extract (Erickson *et al.*, 2023; Pradhananga and Manandhar, 2018).

The results showed that inhibition of free radicals increased due to the higher concentrations of cinnamon extract. A direct correlation between antioxidant activity and the effectiveness of cinnamon in controlling the ranitidine value and peroxide value of the flaxseed oil was observed. The DPPH and FRAP activities were affected by the extraction time, concentrations of the solvent used for extraction, and microwave power level or extraction methods. These antioxidant assays are simple, rapid, and easy methods and can be used as a standard to check the free radical scavenging activity. At the optimum time and temperature conditions, higher inhibition of free radicals was observed by cinnamon extract (Besharati *et al.*, 2020; Spitalniak-Bajerska *et al.*, 2018). Nowadays, it is well acknowledged that one of the phytochemicals that are present in large quantities in cereals, grains, fruits, and several other plant sources is phenolics and antioxidant potentials. Due to their numerous biological activities, including anticancer and antioxidant capacities, as well as other health-promoting qualities, flaxseeds have garnered significant interest from the general public and scientific community (Akl *et al.*, 2020).

Oxidative stability potential (OSP)

Peroxide value (PV)

Peroxide value is the measurement of primary oxidation products such as peroxide and hydroperoxides generated during the initial stage of oil and fat oxidation and is used as a sign to verify the oxidative rancidity of fats and oils. The results of PV of all blended and control samples during storage are given in Table 3. There was a statistically significant effect of storage on the PV value of flaxseed oil added with cinnamon extract. The lower PV was observed in cinnamon extract samples comparable to the controlled one and T_{BHT} but with time PV may tend to increase again due to a decline of the antioxidant potential of antioxidants significantly after 28 days of storage. The T_1 and $T_{2,}$ exhibited PV of 4.69 and 4.53 milli-equivalents (meq)/kg (meq/kg), respectively. The maximum value of peroxide was detected in T_0 (4.78 meq/kg) and the lowest in T_{BHT} (3.50 meq/kg), followed by T_3 (3.97 meq/kg), T_4 (3.94 meq/kg) and T_5 (3.89 meq/kg). As compared to T_0 and T_{BHT}, cinnamon extract was significant in reducing the peroxide value. Therefore, the natural extract proved to be effective against primary oxidation products.

Table 3. Effect of cinnamon extract on peroxide value of flaxseed oil.

Treatment/ Days	PV (meq/kg)	
	0 days	28 days
T_0	4.49 ± 0.48[a]	4.78 ± 0.98[a]
T_{BHT}	3.95 ± 0.98[e]	3.50 ± 0.68[e]
T_1	4.40 ± 0.77[a]	4.69 ± 0.6[b]
T_2	4.34 ± 0.59[b]	4.53 ± 0.22[c]
T_3	4.23 ± 0.85[c]	3.97 ± 1.12[d]
T_4	4.20 ± 0.97[c]	3.94 ± 0.73[d]
T_5	4.14 ± 0.67[d]	3.89 ± 1.16[d]

All values are triplicate means (Mean ± SD) of each treatment. Different superscript letters in the same column show statistically significant differences among the different treatments (P < 0.05). Where: PV = Peroxide value, meq/kg = milli-equivalents (meq)/kg, T0 = Control (flaxseed oil without any antioxidant), TBHT = 200 mg/kg concentration of synthetic antioxidant BHT added in flaxseed oil, T_1 = Cinnamon extract 0.05% (v/v) added in flaxseed oil, T_2 = Cinnamon extract 0.10% (v/v) added in flaxseed oil, T_3 = Cinnamon extract 0.15% (v/v) added in flaxseed oil, T_4 = Cinnamon extract 0.20% (v/v) added in flaxseed oil, T_5 = Cinnamon extract 0.25% (v/v) added in flaxseed oil.

The essential oils and eugenol from cinnamon bark showed strong *in vivo* and *in vitro* antioxidant activity by lowering the production of nitrotyrosine and peroxy nitrite-induced lipid oxidation. Furthermore, it was found that cinnamon bark extract was effective in scavenging free radicals and that these substances also chelated superoxides, hydroxyl, and DPPH radical cations (Khan *et al.*, 2023). Our study results showed that the antioxidant activity of cinnamon extract was associated with its phenolic component and other antioxidant contents. Therefore, it follows that cinnamon can be used to provide food products with a natural source of antioxidants to enhance human health and nutrition. The cinnamon extract also inhibits the rancidity of oil by lowering the peroxide value (Shahid *et al.*, 2018). An empirical measure of oxidation that is helpful for samples that are oxidized to relatively low levels (peroxide values of less than 50) and in mild enough conditions to prevent hydroperoxides from decomposing noticeably is the oil's peroxide value. The oil's peroxide value reaches a maximum during autoxidation and then decreases at later phases, depending on the oxidation conditions and the oil's fatty acid content. The p-anisidine test is frequently used to find secondary oxidation products and offers helpful information on nonvolatile carbonyl compounds generated in oils during processing. The p-anisidine value of high-quality oil should be less than two. The antioxidant ability of cinnamon extract is higher than synthetic antioxidants (T_{BHT}) in lowering peroxide value and stabilizing flaxseed oil at storage for 28 days. The variety of flavonoid chemicals found in cinnamon is thought to be responsible for its antioxidant capacity. The measurement of conjugated diene hydroperoxides resulting from polyunsaturated lipids is a sensitive technique to track the early stages of lipid oxidation under circumstances where hydroperoxides undergo little to no decomposition. The hydroperoxides' strong absorption maximum at 234 nm allows for a quantitative determination of the hydroperoxides. The potential effects of cinnamon essential oils, such as linalool, eugenol, and cinnamaldehyde on lipid peroxidation and peroxynitrite-induced nitration showed that cinnamon has more antioxidant activity than other spices (Lu *et al.*, 2020; Rangani and Ranaweera, 2023).

Free fatty acids (FFA)

The FFA content measured the degree of extent at which the glycerides compound in the oil have been deteriorated by the lipase activity. The results regarding the value of FFA from 0 to 28 days of storage are tabulated in Table 4. The FFA were observed less in cinnamon extract treatments and comparable to the controlled one but with time, FFA increased again due to a decline of the antioxidant potential of antioxidants significantly after 28 days of storage. The highest free fatty acid value was detected for the control sample followed by T_1, T_2, T_3, T_4, and T_5, while T_{BHT} showed the lowest value of FFA after 28 days of storage. The natural extract significantly lowered the FFA at a comparable rate and showed an inhibitory effect as compared to synthetic antioxidants. Such natural extract significantly reduces the free fatty acids value of flaxseed oil, prevents rancidity, and prolongs the shelf-life of oil (Zeng *et al.*, 2022).

Table 4. Effect (mean ± STDV) of cinnamon extract on FFA of flaxseed oil.

Treatments	FFAs (meq/kg)	FFAs (meq/kg)
Days	0 days	28 days
T_0	1.37 ± 0.54[a]	1.50 ± 0.98[a]
T_{BHT}	0.83 ± 0.98[d]	1.05 ± 0.68[bc]
T_1	1.27 ± 0.77[b]	1.10 ± 0.60[b]
T_2	1.28 ± 0.59[b]	1.08 ± 0.22[b]
T_3	1.25 ± 0.85[bc]	1.06 ± 1.12[c]
T_4	1.22 ± 0.97[bc]	1.02 ± 0.73[bc]
T_5	1.23 ± 0.97[bc]	1.03 ± 1.16[bc]

All values are triplicate means (Mean ± SD) of each treatment. Different superscript letters show statistically significant differences among the different treatments (P < 0.05). Where: meq/kg = milli-equivalents (meq) / kg, T_0 = Control (flaxseed oil without any antioxidant), T_{BHT} = 200 mg/kg concentration of synthetic antioxidant BHT added in flaxseed oil, T_1 = Cinnamon extract 0.05% (v/v) added in flaxseed oil, T_2 = Cinnamon extract 0.10% (v/v) added in flaxseed oil, T_3 = Cinnamon extract 0.15% (v/v) added in flaxseed oil, T_4 = Cinnamon extract 0.20% (v/v) added in flaxseed oil, T_5 = Cinnamon extract 0.25% (v/v) added in flaxseed oil.

The amount of FFA depends upon several factors such as as the nature of fats and oils, the action (activity) of lipases, the method of extraction, humidity, temperature, and storage conditions. It has been ascertained that oil was susceptible to its color and flavor which ultimately leads to rancidity process. Hence, in edible oil products, the free unsaturated fat generation is seen as a crucial record for the estimation of rancidity. The FFA are formed by the proliferated response and hydrolysis of triglycerides of oil with water, the FFA are an indispensable source of fuel for specific tissues, mainly they can give an ample amount of adenosine triphosphate (ATP). Hence, natural antioxidant extract can prevent the breakdown of triglycerides and hence can prolong the shelf-life of flaxseed oil (Rangani and Ranaweera, 2023; Shahid *et al*., 2018).

When ALA in flaxseed oil increased, oleic acid decreased in proportion. 51.80 and 22.21%, respectively, of the oils had the lowest ALA and highest oleic concentrations; these percentages differed markedly from the other oils. Water has a role in the hydrolysis of oil during different stages of handling and processing, producing compounds like glycerol and free fatty acids. Therefore, low moisture content in oils is preferable. Different flaxseed varieties, their origins, and the related environmental variations could be the cause of this. The primary mechanism that causes edible oils to deteriorate during manufacture, shipping, and mostly storage is lipid oxidation. Exposure to light can hasten the oxidation of oils, especially poly-unsaturated vegetable oils like flaxseed oil. An essential method for creating hydroperoxides from unsaturated fatty acids and esters in the presence of oxygen, light energy, and a photosensitizer is photooxidation. Oil's chlorophyll pigments have the ability to start photosensitized oxidation. Although the high quantity of unsaturated fatty acids in flaxseed oil is expected to make it sensitive to rapid oxidation and rancidity, this may not have happened because of the strong antioxidant content (Zeng *et al*., 2022).

Iodine value (IV)

The iodine value of all allocated oil samples at 0 to 28 days of storage are shown in Table 5. The results displayed that the iodine value of oil decreases with the increase of the cinnamon extract concentrations and storage period. T_0 showed the highest iodine value (198.51 I_2/100 g), while T_{BHT} and T_5 showed the lowest iodine values of 173.76 and 175.29 g of I_2/100 g, respectively. Moreover, T_1, T_2, T_3, and T_4 showed iodine values of 194.34, 195.10, 179.78, and 177.42 g of I_2/100 g, respectively. There was a remarkable decline in iodine values, which might be due to the change in fatty acid profile throughout storage (Jang *et al*., 2020). In edible oils or fats, the degree of unsaturation is considered higher if more iodine value is observed. Normally, the oil oxidizes when in interaction with oxygen and air. As a result of this oxidation the iodine value decay constantly. At the point when the oil is repeatedly used for deep-fat frying, there is a significant increase in the absorption of saturated fat that influences the iodine value (Verma *et al*., 2021).

Table 5. Effect (mean ± STDV) of cinnamon extract on iodine value of flaxseed oil.

Treatments	IV (g I_2/100 g)	IV (g I_2/100 g)
Days	0 days	28 days
T_0	185.48 ± 9.08^{a}	198.51 ± 9.98^{a}
T_{BHT}	181.02 ± 8.98^{bc}	173.76 ± 9.68^{a}
T_1	183.00 ± 8.77^{b}	194.34 ± 10.06^{ab}
T_2	183.73 ± 9.59^{b}	195.10 ± 11.22^{ab}
T_3	182.69 ± 8.85^{b}	179.78 ± 10.12^{ab}
T_4	181.85 ± 9.97^{c}	177.42 ± 10.73^{ab}
T_5	180.51 ± 8.98^{bc}	175.29 ± 10.16^{b}

All values are triplicate means (Means ± SD) of each treatment. Different superscript letters show statistically significant differences among the different treatments ($P < 0.05$). Where: g I_2/100 g = grams of I_2 absorbed per 100 g of oil sample, T_0 = Control (flaxseed oil without any antioxidant), T_{BHT} = 200 mg/kg concentration of synthetic antioxidant BHT added in flaxseed oil, T_1 = Cinnamon extract 0.05% (v/v) added in flaxseed oil, T_2 = Cinnamon extract 0.10% (v/v) added in flaxseed oil, T_3 = Cinnamon extract 0.15% (v/v) added in flaxseed oil, T_4 = Cinnamon extract 0.20% (v/v) added in flaxseed oil, T_5 = Cinnamon extract 0.25% (v/v) added in flaxseed oil.

Because flaxseed oil is more prone to oxidation, which raises the peroxide value, and because it has a high concentration of alpha-linolenic acid, it has a high iodine number. Different kinds of commercial flaxseed have varying iodine values, ranging from approximately 150 to 200 or more. Iodine values of 185 or higher often indicate outstanding drying qualities, while those below 165 are typically regarded as oils of clearly lower quality. Because so much flaxseed oil with poor drying properties and low iodine numbers has been marketed in huge amounts, the significance of iodine number in the oil from the l manufacturing business has expanded significantly in recent years. A straightforward technique for anticipating the iodine number of oil that may be extracted from a specific lot of flaxseed would be beneficial to the linseed-oil sector, given the significant variance in iodine numbers across oils made from distinct lots of the grain (El-feky *et al*., 2024).

Cinnamon extract considerably prevents the increase of the iodine value of oil samples. The effect of the antioxidant potential of cinnamon extract on the flaxseed oil under accelerated storage conditions for 28 days was higher than that of the control sample. However, the iodine value observed was higher if the concentration of cinnamon extract increased than it was effective more

than synthetic antioxidants in preventing lipid oxidation (El-feky *et al*., 2024; Sharma *et al*., 2020).

Thiobarbituric acid value (TBA)

The TBA value expressed the amount of oxidation present in samples of fat and oil. The results of the TBA value of all flaxseed oil treatments from 0 to 28 days of storage are presented in Table 6. The results revealed that the TBA value of oil increases with the increase of the storage period. T_0 showed the highest TBA value (6.95 mg MDA/kg) and T_5 had the lowest TBA value (5.92 mg MDA/kg). The TBA values of T_1, T_2, and T_3 were 6.87, 6.63, and 6.68 mg MDA / kg, respectively. This indicated that T_4 and T_5 were strong enough to prevent oil from secondary oxidation products.

The cinnamon extract showed a higher TBA value which retarded the secondary oxidation products in the flaxseed oil during the storage period. The natural extract stabilized the flaxseed oil in comparison to synthetic antioxidants during accelerated storage. The presence of resinous compounds, such as cinnamaldehyde, cinnamate, cinnamic acid, and other phenolic compounds, provide the antioxidant effect but time storage conditions affected the oxidative stability of oil, and higher TBA was observed after 28 days of storage in each oil sample (Hadad and Goli, 2019; Lu *et al*., 2020). The formation of primary and secondary oxidation products occurs throughout the complex process of lipid oxidation. TBA was taken into consideration in this investigation for secondary oxidation products. The aldehydes, ketones, epoxides, hydroxy compounds, oligomers, and polymers are among the byproducts of secondary oxidation of lipids; TBA is the most widely used marker compound among them. The best compound to detect the precise and accurate secondary oxidation problems in food goods is colored trimethadione, which is formed when the TBA reagent reacts with the TBA reactive compounds (TBARS) in the sample (Hadad and Goli, 2019). These free radical-scavenging behaviors and the antioxidant properties of the cinnamon extract were due to the presence of flavonoids and phenolic compounds. Thus, cinnamon stick extract proved a better source of natural antioxidants than synthetic antioxidants with no harmful effects on human health (Edo *et al.,* 2022; Shahid *et al.*, 2018).

Table 6. Effect (mean ± STDV) of cinnamon extract on TBA value of flaxseed oil.

Treatments	TBA (mg MDA/kg)	TBA (mg MDA/kg)
Days	0 days	28 days
T_0	3.98 ± 0.87^a	6.95 ± 0.98^a
T_{BHT}	3.37 ± 0.98^d	2.31 ± 0.68^e
T_1	3.70 ± 0.85^b	3.57 ± 0.60^b
T_2	3.46 ± 0.77^c	3.53 ± 0.52^b
T_3	3.42 ± 0.97^c	2.88 ± 0.62^c
T_4	3.38 ± 0.97^d	2.73 ± 0.73^d
T_5	3.34 ± 0.59^e	2.33 ± 0.66^e

All values are triplicate means (Means ± SD) of each treatment. Different superscript letters show statistically significant differences among the different treatments ($P < 0.05$). Where: MDA = Content of Malondialdehyde present in oil, T_0 = Control (flaxseed oil without any antioxidant), T_{BHT} = 200 mg/kg concentration of synthetic antioxidant BHT added in flaxseed oil, T_1 = Cinnamon extract 0.05% (v/v) added in flaxseed oil, T_2 = Cinnamon extract 0.10% (v/v) added in flaxseed oil, T_3 = Cinnamon extract 0.15% (v/v) added in flaxseed oil, T_4 = Cinnamon extract 0.20% (v/v) added in flaxseed oil, T_5 = Cinnamon extract 0.25% (v/v) added in flaxseed oil.

Conclusions

The appliance of the cinnamon extract showed a negative effect on the rancidity of flaxseed oil during storage. Cinnamon extract showed higher flavonoids contents, which led to higher antioxidant activity. Cinnamon extract has a higher total phenolic content than other parts of fruit (seed/pulp) and showed high antioxidant activity. A direct correlation between antioxidant activity and the effectiveness of cinnamon in controlling the ranitidine value and peroxide value of the flaxseed oil was observed. The DPPH and FRAP activities were affected by the extraction time, concentrations of the solvent used for extraction, and microwave power level or extraction methods. At the optimum time and temperature conditions, higher inhibition of free radicals was observed by cinnamon extract. The amount of FFA depends upon several factors like as the nature of fats and oils, the action (activity) of lipases, the method of extraction, humidity, temperature, and storage conditions. The cinnamon extract thus enhanced the antioxidant potential of flaxseed oil but it depends on the varieties, season, or region of cultivation and method of extraction and storage conditions of cinnamon extracts. The cinnamon extracts have also been recognized for their antimicrobial activity against bacterial strains as compared to fungal strains. The higher concentration of cinnamon extract showed higher antioxidant activity to prevent lipid oxidation during storage conditions. Thus, cinnamon extract proved to be a significant alternative to synthetic antioxidants with no harmful effects on human health.

Authors' Contribution

Conceptualization, M.R., A.A.K and A.R.; methodology, M.T.N., S.T and U.M.K; software, K.S and Y.B.; validation, T.A and F.S.S.; formal analysis, M.Z.K., S.M. and S.G.; investigation, A.A.K., and A.R; resources, J.M.R. and

T.A. data curation, M.R., and K.S; writing – original draft preparation, M.R., K.S and A.A.K; writing – review and editing, M.Z.K., U.M.K., Y.B., T.A., and J.M.R; supervision, A.A.K. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Ethical approval

Not applicable.

Competing Interests

The authors have no conflict of interest to declare.

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Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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