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Utilization of Flaxseed Oil as Releasing For Some Natural Pigments and Antioxidants

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ABSTRACT

Flaxseed oil is a nutritionally important oil because of its high percentage of polyunsaturated essential fatty acids, such as linolenic acid (the linolenic content in this work is about 60%). On the other hand, this feature causes a problem, making it susceptible to oxidation and rancidity more quickly. In this study, flaxseed oil obtained by the cold pressing method was enriched with dryer wastes of carrot, tomato, and olive leaves, where the oil was used directly as a releasing and diffusion medium for some bioactive compounds in these materials. Chlorophyll a and b, lycopene, β-carotene, phenols, and flavonoids were determined in the waste and oil after the enrichment process. Some of the physiochemical properties and fatty acid profiles of the oil were estimated. The antioxidant activity and oxidative stability of the oil on the Rancimat apparatus were also estimated. The results showed that enrichment oil with olive leaves led to an increase in chlorophyll a and b, β-carotene, phenols, and flavonoids by 402.54%, 240.52%, 117.67%, 31.34%, and 23.68%, respectively. While oil enriched with tomato waste showed an increase in lycopene by 200.00%, A decrease in the specific absorption values at 232 and 270 nm was observed for samples of oil enriched with olive leaves (1.56, 0.12) followed by oil enriched with tomato waste (2.65, 0.26) as compared to crude oil (3.63, 1.31), respectively. The oil enriched with olive leaves recorded the highest value of antioxidant activity and the highest stability on the Rancimat apparatus (55.16% and 5.67 h), followed by the oil enriched with tomato waste (48.90% and 4.63 h) as compared to the crude oil (41.10% and 2.76 h). Finally, the salad dressing samples prepared with this oil were acceptable to all. All treatments were recorded; mean scores of tastes and overall acceptability ranged from 7.00 to 7.60 between like moderately and like very much on the hedonic scale, and there were no significant differences between them and the control sample.

1. Introduction

The seeds of the flax plant, which is an annual herb belonging to the Lineaceae family, are mostly consumed in one of these ways: either as raw seeds, after grinding, or as extracted oil. This plant is often believed to have been cultivated for the first time in Egypt (Kakkar et al., 2021), and it is now grown in many countries such as India, China, the United States, Ethiopia, and Canada. It contains about 40–50% oil, and the oil contains a high percentage of polyunsaturated fatty acids, which have health benefits. Flaxseed is high in omega-3 fatty acids and antioxidants, as well as many other nutrients (Kakkar et al., 2021). Flaxseed oil contains monounsaturated, polyunsaturated, and saturated fatty acids. The percentage of unsaturated fatty acids reached 87.8–89.8% of the total fatty acids, compared to a small amount of saturated fatty acids (Yaqoob et al., 2016). α-linolenic acid (ALA), which is an unsaturated fatty acid, is involved in the synthesis of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are among the acids required for the growth and protection of various organs of the human body, such as the brain and skin (Abdul Mueed et al., 2022).

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Prostaglandins and prostacyclins, which are hormone-like substances that are produced by the human body from these polyunsaturated fatty acids, are essential for maintaining vision, helping young children develop their brains and nervous systems, lowering blood pressure and cholesterol, and preventing cancers of the colon, breast, and prostate. It stimulates memory, delays aging, prevents gastrointestinal infections and heart diseases, and controls neurological disorders and diabetes (Ankit et al., 2015). Flaxseed oil has gained great popularity among consumers all over the world due to its numerous nutritional and health benefits. However, its oxidizability is the main limiting factor for its uses in nutrition and other food applications (Abdul Mueed et al., 2022). Due to its high content of polyunsaturated fatty acids (about 75%), flaxseed oil is very sensitive to oxidation in the presence of oxygen, metal ions, and high temperatures, which leads to the formation of peroxide compounds and off-flavor. All these factors limit its use as a cooking or frying oil. Therefore, there is an urgent need to address these defects and produce an oil that is resistant to oxidation and can be added to food products without negatively affecting the taste and smell (Ankit et al., 2015). (Abdul Mueed et al., 2022) showed that some natural antioxidants can slow down the oxidation process of flaxseed oil. Food processing wastes are considered a cheap source of bioactive compounds that can be utilized as a natural source of antioxidants instead of synthetic antioxidants, which have harmful effects on human health. If we look at the global level, we find that nearly a third of the total food production is wasted during operations of transportation, distribution, processing, and storage. The generated waste from vegetables and fruits represents approximately 40–50% of the total generated solid waste (Prabhjot et al., 2022). For example, carrots are a good source of bioactive compounds, especially β-carotene, which acts as a precursor to vitamin A. Carrot wastes contain a reasonable percentage of β-carotene, around 20 mg/100g, considering that the daily recommended amount of vitamin A is 900 mg/day (based on a male adult), equivalent to 10.8 mg of β-carotene (Anne et al., 2016). The processing of carrots, such as carrot juice production, results in a large amount of waste, estimated at about 25% of carrots. It is possible to benefit from these high biological value by-products, such as natural pigments and antioxidants, through the development of extraction and processing methods (Prabhjot et al., 2022). There is also tomato processing waste, which is estimated to account for about 10-15% of the total volume of tomatoes. This waste is rich in antioxidants and is an important source of carotenoids, lycopene, and phenols, which can be used as natural compounds in food additives (Gieraldin et al., 2022). It was found that the lycopene pigment present in tomatoes can reduce the incidence of rancidity and increase the antioxidant capacity of flaxseed oil by 42%, which can extend the shelf life of the treated oil by 31% (Abdul Mueed et al., 2022). There is another waste that contains a large number of antioxidants, which is olive leaves, which accumulate in large quantities during the annual pruning of olive trees. It can also be obtained in large quantities during the olive oil extraction after removing them from olive fruits, which are estimated at about 10% of the weight of the fruits (Leila et al., 2015). These leaves contained as many biophenols as the other parts of olive trees. Olive leaf extracts have been associated with health benefits. Oleuropein is the main phenolic compound in olive leaves (about 60–90 mg/g of dry matter), which has excellent antimicrobial and antioxidant properties (Maha et al., 2021). The traditional method of bioactive component extraction is solvent extraction. The solvent must have the capacity to penetrate the interior of the material to extract the bioactive compounds. The polarity of the solvent also influences the efficiency of extraction (Gieraldin et al., 2022). (Prabhjot et al., 2022) reported that modern methods of extraction known as green technology can be used, which are characterized by high efficiency and minimal impact on biologically active ingredients due to low processing temperature and extraction time. These techniques include microwave extraction, ultrasound-assisted extraction, pulsed electric fields, and high hydrostatic pressure. Supercritical fluid technology can

also be used to extract these compounds from fruit and vegetable by-products. Also, studies have shown that edible vegetable oils can be used very efficiently as an extraction solvent for biologically active compounds such as carotenoids. Among the recommended techniques for extraction with vegetable oils is the magnetic stirring technique, which is used to extract lycopene, for example, from some plant residues, as this technique is considered less harmful and does not generate free radicals that lead to the oxidation of lycopene or other extracted compounds (Gieraldin et al., 2022). The main objectives of this study were to utilize flaxseed oil as a releasing and diffusion medium for some natural pigments and bioactive compounds (chlorophyll, carotene, lycopene, phenols, and flavonoids) from the residues of carrot, tomato, and olive leaves. Also study the effect of these compounds as natural antioxidants on some physiochemical properties, prolonging the preservation period and protecting the oil from rancidity, thus expanding its nutritional uses.

2. Materials and Methods

Raw materials

1-The waste of yellow carrots was obtained from the local market after pressing carrots to obtain juice at the carrot juice shops.

2- Tomato wastes were obtained from one of the major tomato processing plants located on 6 October City, Egypt.

3- The fresh olive leaves were collected (in late January) from the planted trees on the farm of the the Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

These materials were dried at 50°C for 24 hours, ours, then ground, sieved at 60 mesh, and stored in vials protected lightlightunder refrigeration at 5°C.

4-Flaxseed oil: Egyptian flaxseed (*Linumusitatissimum*, Sakha-11, Season 2022) was obtained from the Fiber Crops Institute, Agricultural Research Center. After cleaning, the seeds were pressed by cold hydraulic pressing methods, according to (Haumann 1997). The obtained oil was filtrated and kept at -20°C until use.

Preparation of enriched flaxseed oil

The extraction method with agitation (magnetic stirring techniques) was used with some modifications, as follows: 100 g of dried raw materials (waste) and 500 mL of flaxseed oil were weighed. The waste and oil were placed in a 1000-mL conical flask with a tight-fitting lid. Inside the conical, a magnetic stirrer was placed. The conical was placed on a Corning brand stirring rack, followed by agitation for 17 hours at room temperature (Gieraldin et al., 2022).

The enriched flaxseed oil was filtered through two layers of cloth and kept at refrigerator temperature and in the dark until tests were done. At the same time, another sample of flaxseed oil was mixed with butylated hydroxyanisole (BHA, 200 mg/kg oil) as a control sample.

Chlorophyll and carotenoids determination:

The spectra of the samples were obtained with a spectrophotometer equipped with a quartz cell with a path length of 1cm **(**Gieraldin et al., 2022).

1– In the used plant material waste materials, 0.5g of olive leaves, tomato and carrot powder were extracted with acetone-hexane (4:6) three times. After filtration, the extracts were combined and reached a known volume of 25 ml.

2- In the crude and enriched oil samples, 0.5 ml of the oil samples were mixed with 10 ml of the acetone and hexane (4:6) in a test tube.

Chlorophyll a and b, lycopene, and β-carotene were estimated in the previous extracts (wastes materials and oil extracts) by measuring the optical density at wavelengths of 663nm, 645nm, 505 nm, and 453nm**.**

From these values, the content of chlorophyll a and b and lycopene could be estimated as mg/100 g sample using the equations of **(**Nagata and Yamashita 1992) as follows**:**

Chlorophyll a = 0.999A663 - 0.0989A645

Chlorophyll b = - 0.328A663 + 1.77A645

Lycopene= -0.0458A663+ 0.204A645 + 0.372A505 - 0.0806A453

While the content of β-carotene was determined according to the equation of (Prabhjot 2022) as

follows: C = Amax *V*D * 10^{4} / $A^{1\%}$ 1cm*W

Total Phenolic Content (TPC)

the total phenolic content was determined by the Folin- Ciocalteu method according to (Naeem et al., 2019). The absorbance was measured at 765 nm after 30 min. the results were expressed as mg of Gallic acid equivalent (GAE) per 100 g of oil sample.

Total Flavonoids content (TFC)

The aluminum chloride method was used for the determination of the total flavonoid content according to (Khatiwora et al., 2010). Absorbance at 415 nm was recorded after 30 minutes of incubation. The concentrations of flavonoids were calculated as mg quercetin equivalent /100g of sample.

Physio-chemical parameters of crude and enriched oil samples

Refractive index, Peroxide value and Iodine number were determined according to (AOAC, 2016). And acid value was determined according to (PN-ISO 660:2020).

Conjugated constituents

Values of specific extinctions at 232 nm（K232） and 270 nm（K270）for conjugated dienes and trienes, were determined according to **(**PN-EN ISO 3656:2011).

Antioxidant activity of oil samples by DPPH

The antioxidant activities of the oil samples were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reported by **Zahran and Najafi (2020)**. The absorbance of the mixture was recorded at 517 nm, and the percentage inhibition was calculated from the following equation:

%DPPH = [(Abs. control - Abs. sample) / Abs. control] ×100

IC_{50} values of the plant extracts were calculated from the inhibition percent against concentration plot. The IC_{50} value indicates the concentration in µg/mL of the extract, which is required to scavenge 50% of DPPH free radicals.

Oxidative stability of oil samples by Rancimat method

Oxidative stability of oils was evaluated according to **(**Salta et al., 2007) using Rancimat 679 apparatus, a sample weighs 5g set at constant temperature 100° C with an air flow of 20 L/h and measures the induction period (by hours).

Fatty acids composition: were converted into methyl ester and determined by GC according to (PN-ISO 12966-2:2017).

Fatty acids composition: were converted into methyl ester and determined by GC according to (PN-ISO 12966-2:2017).

Preparation of salad dressing

Mustard-vinaigrette salad dressing were prepared with an oil: vinegar ratio of 3:1 according to methods of **(**Gendreau and Ruiz, 2019). The other ingredients include: 5% mustard, 4% garlic powder, 1% black paper powder, 5% salt, 10% sugar. All ingredients were gentle stirred and mixed at 60 rpm with a homogenizer at room temperature for 10 minutes. All samples were kept at 5°C for7 days.

Sensory evaluation

Samples were evaluated using a 9-point hedonic scale (9- extremely liked; 1- extremely disliked), when to their general appreciation. A total of ten panelists from food technology research institute were asked to make their evaluations on the basis of appearance, taste, aroma, texture and overall acceptability, (Mihov et al., 2012).

Statistical analysis

The results were expressed as mean ± SD and the statistical analysis performed using one-way analysis of variance. The obtained data were exposed to proper statistical analysis according to the SPSS software (SPSS Inc., Chicago, IL, USA) (Gieraldin et al., 2022).

3. Results and discussions

Fruits and vegetable waste are exclusive due to their capacity for human consumption and the high value of bioactive components such as chlorophyll, carotenoids, polyphenols, flavonoids, and other antioxidants. The data in Table 1. show some of these compounds determined in the dried powder of

carrot, tomato, and olive leaf wastes. It seems that olive leaf showed the highest values of chlorophyll a, chlorophyll b, β-carotene, and total phenolic compounds, which recorded 27.50, 12.72, 9.68, and 147.50 mg/100 g, respectively. Also, tomato waste showed the highest values of lycopene and flavonoids, at 2.04 and 44.47 mg/100 g, respectively, while carrot waste had the lowest values of these compounds. The presence of chlorophyll a and b in tomato waste was 3.86 and 4.25 mg/100 g, respectively. It may be due to the degree of maturity of the fruits or the presence of remaining green parts with the fruits during processing. Also, the presence of chlorophyll in carrot juice residues may be due to the green color of the inner axis of the root. While the detection of lycopene in olive leaves in this measurement may be due to color interference resulting from the conversion of some chlorophyll molecules to pheophytin, This happens when the coordinated magnesium in the chlorophyll molecule (which is easily lost during extraction and processing) is lost, yielding a yellow-brown pigment (Ngamwonglumlert et al., 2017). There are significant variances between these findings and those of other studies, which can be mostly attributed to variations in cultivars, nations, climates, and production methods that alter the concentration of these bioactive chemicals. (Markov et al., 2021). (Ribas et al., 2022) found the total chlorophyll content in the dry matter of some varieties of olive leaves ranged from 0.958 to 0.833 mg/100 mL extract, the total polyphenol content from 13.27 to 22.81 mg gallic acid/g sample, and the total flavonoid content from 6.50 to 7.65quercetin/gn/ g sample. He added that all types of olive leaves had a higher percentage of chlorophyll as compared to chlorophyll b. The difference in total chlorophyll content was attributed to several factors, such as the age of the leaves, temperature, exposure to light, and harvest time. (Anne et al., 2016) observed losses in β-carotene of 57% after drying waste of carrots which were 4.43 mg of β-carotene/100g. He explained these losses by the time and the temperature of drying, also attributed this result to the vegetal geographical origin, the harvesting period and the time of storage. (Gieraldin et al., 2022) reported that, during the processing of various tomato products, large amounts of peel and seeds are left behind. It was found that the peels have a greater amount of fiber, carotenoids, and phenols, while the seeds contain mainly amounts of oil and proteins. It was also found that the concentration of lycopene in processed tomato waste is extremely low, about 30 mg/kg. This shows that the processes used in manufacturing, such as high temperatures, juicing processes, and final production steps, lead to the release of large amounts of lycopene in the juices (Asmaa et al., 2017). Table 1. also showed the IC_{50} value which is defined as the concentration of a substrate that causes 50 % loss of the DPPH activity. According to the rule "the lower the IC_{50} value, the higher is the scavenging potential" (Riaz et al., 2012), it's clear that, olive leaves extract had the highest scavenging potential which recorded 0.88 ug/ml followed by tomato and carrot waste extracts, (1.35 and 1.60 ug/ml) respectively. This may be due to the high content of β-carotene, total phenolic and flavonoids compounds.

Table 1. Some bioactive compounds in the used plant waste materials (mg/100g on dry basis)

Samples	Chl a	Chl b	Lycopene	β-carotene	TPC	TFC	IC_{50} µg/mL
Carrot waste powder	0.29	0.36	0.208	2.71	87.45	25.59	1.60
Tomato waste powder	3.86	4.25	2.04	5.31	102.72	44.47	1.35
Olive leaves powder	27.50	12.72	0.59	9.68	147.50	37.26	0.88

Chl: Chlorophyll, TPC: Total phenolic content, TFC: Total flavonoid content, IC_{50}: Half maximal inhibitory concentration.

One of the main objectives of this study is to use flaxseed oil to release natural fat-soluble pigments such as chlorophyll, lycopene, and β-carotene and some bioactive compounds from wastes resulting from their plant sources. Table 2. illustrates the concentrations of these compounds after releasing them into the crude flaxseed oil. Beginning, the crude oil contains 1.18, 1.90, 0.55, 2.15, 67.00and 4.18 mg/100g of chlorophyll a, chlorophyll b, lycopene, β-carotene pphenols,and flavonoids, respectively. The oil enrichment with olive leaves showed an increase in these values by 402.54, 240.52, 54.54, 117.67, 31.34, and 23.68%,% respectively. The oil enriched with tomato waste showed an increase of 155.93, 114.21, 200.00, 79.06, 19.40, and19.85%,% respectively. While the oil enriched with carrot waste came in third, where recorded increases were 16.94, 12.10, 21.81, 20.93, 5.22, and 12.91%, respectively. From the data in Table 2., it could be noticed that the main components in crude flaxseed oil were phenolics and flavonoids, which were 67.00 and 4.18 mg/100 g, respectively. These results are based on the results of (Choo et al., 2007) on seven cold-pressed flaxseed oils traded for sale in New Zealand, who found that total phenolic acids and total flavonoids ranged from 76.80 to 307.30 and 12.70 to 25.60 mg/100g, respectively. But (Miguel et al., 2019) who reported that flaxseed oil has a relatively low content of phenolic compounds (56.39 mg/kg), explained that these molecules are not directly extracted during cold pressing or solvent extraction but remain in the defatted residue. This variation may be due to oil extraction methods, genetics, varieties, and the ripening stage of the seed. This finding also appliesflavonoids,oids which are secondary metabolites with different phenolic structures.

It is also clear that enrichment with olive leaves showed the highest increase in chlorophyll a, chlorophyll b, β-carotene, phenolic, and flavonoids compounds, while enrichment with tomato waste showed the highest increase in lycopene.

(Tarchoune et al., 2019), in a study on adding a variety of olive leaves to their olive fruits prior to crushing and during oil extraction, found that the addition of leaves increased total phenolic and total flavonoid concentrations by 44 and 22% in the oils, respectively, and this is somewhat consistent with our results. Carotenoids have high antioxidant activity, which mainly includes β-carotene, α-carotene, lycopene, and other fractions. (Miguel et al., 2019) reported that flaxseed oil showed relatively small amounts of β-cryptoxanthin, lutein, and zeaxanthin by total content of carotenoid 0.61 mg/100g, while the addition of tomato lycopene-rich extract only led to significant changes in the concentration of lycopene. (Tańska et al., 2016) reported that the content of carotenoids in commercial flaxseed oil from Poland varied from 1.21 to 2.95 mg/100g, while another study showed that carotenoid pigment content was much lower, ranging from 0.06 to 0.7 mg/100g. In contrast, the results obtained by other researchers found the content of these compounds in flaxseed oils was much higher, at 2.00–11.5 mg/100g (Daun et al., 2003).

Table 2. Some bioactive compounds in the crude and enriched flaxseed oil (mg/100g sample)

Samples	Chl a	Chl b	Lycopene	β- carotene	TPC	TFC
Crude Oil	1.18	1.90	0.55	2.15	67.00	4.18
Oil enriched with carrot waste	1.38	2.13	0.67	2.60	70.50	4.72
Oil enriched with tomato waste	3.02	4.07	1.65	3.85	80.00	5.01
Oil enriched with olive leaves	5.93	6.47	0.85	4.68	88.00	5.17

Chl: chlorophyll, TPC: total phenolic content, TFC: total flavonoid content

The data in Table 3. showed the recovery rates of some bioactive compound from the used plant waste materials after releasing it by flaxseed oil. It is clear that the highest recovery of chlorophyll (a and b) were found in the oil enriched with carrot waste (68.96 and 63.88%) followed by (47.66 and 51.05 %) and (17.27 and 35.92%) for oil enriched with tomato waste and olive leaves respectively.

Lycopene showed the highest recovery in oil enriched with carrot waste, 57.69 followed by 53.92 and 50.84 % for tomato waste and olive leaves respectively. While the highest recovery of β-carotene was found in oil enriched with tomato waste 32.01% followed by 26.13% and 16.60% for oil enriched with olive leaves and carrot waste respectively. The highest recovery of phenolic and flavonoids compounds were observed in oil enriched with olive leaves, 14.23 and 2.65 % respectively. It seems that by using flaxseed oil as extraction solvent the recovery rate may dependent on, plant sources and industry processing hence influence on release of these components. Also polarity and solubility of these compounds in the oil may be affecting of the recovery rate. (Asmaa et al., 2017), reported that optimizing conditions for lycopene extraction found the recovery with different experimental conditions varied between 8.49 and 93.59%. Lowering in the recovery rate in our study may be either due to lower solubility of the some, such as phenolic and flavonoids or to lower efficiency of the extraction method as insufficient time and stirring power with this extracting technique. Superiority of lycopene compared to the rest of other compounds may be due to the highly lipophilic nature which it is a highly unsaturated, containing 13 double bonds, (straight chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds), in against 11 conjugated for β-carotene. These lipophilic nature of lycopene has a higher antioxidant activity at the cell membrane level by interacting with lipid components, (Gieraldin et al., 2022).

Table 3. Concentration and recovery rates of some bioactive compound from plant waste materials after releasing it in flaxseed oil (mg/100g).

Items	Chl. A	Chl. b	Lycopene	β-carotene	TPC	TFC
Crude oil	1.18	1.90	0.55	2.15	67.00	4.18
Carrot waste	0.29	0.36	0.208	2.71	87.45	25.59
Oil with carrot waste	1.38	2.13	0.67	2.60	70.50	4.72
Increasing value	0.2	0.23	0.12	0.45	3.50	0.54
Increasing percentage	16.94	12.10	21.81	20.93	5.22	12.91
Recovery %	**68.96**	**63.88**	**57.69**	**16.60**	**4.00**	**2.11**
Tomato waste	3.86	4.25	2.04	5.31	102.72	44.47
Oil with tomato waste	3.02	4.07	1.65	3.85	80.00	5.01
Increasing value	1.84	2.17	1.10	1.70	13.00	0.83
Increasing percentage	155.93	114.21	200.00	79.06	19.40	19.85
Recovery %	**47.66**	**51.05**	**53.92**	**32.01**	**12.65**	**1.86**
Olive leaves	27.50	12.72	0.59	9.68	147.50	37.26
Oil with olive leaves	5.93	6.47	0.85	4.68	88.00	5.17
Increasing value	4.75	4.57	0.30	2.53	21.00	0.99
Increasing percentage	402.54	240.52	54.54	117.67	31.34	23.68
Recovery %	**17.27**	**35.92**	**50.84**	**26.13**	**14.23**	**2.65**

Increasing value = concentration in enriched oil – concentration in crude oil
Recovering % = Increasing value / concentration in plant waste materials x 100

The physico-chemical characteristics of crude and enriched flaxseed oil samples are shown in Table 4. Acid value (mg KOH/g oil), Iodine value (g Iodine / kg oil), Refractive index (at 20°C) and Peroxide value (meq.O_2/kg oil) in the crude oil were 0.62, 183.24, 1.48 and 2.79 respectively. These results are consistent with many previous studies (Zhang et al., 2011), (Anwar et al., 2013), (Nahed and Enaam 2017) and (Miguel et al., 2019),

Compared to the control sample (oil with BHA) and the rest of the other treatments, we find that there hasn't been much change in these values, where the acid value ranged from 0.66 to 0.69 mg KOH/g oil and peroxide value ranged from 2.68 to 2.88 meq.O_2/kg oil. Peroxide values in all treatments were lower than the limit (15 meq O_2/kg oil) established by the Codex Alimentarius for vegetable oils obtained by cold pressing (Codex Alimentarius Stan 19-1981). Also according to the Codex Standard for edible fats and oils cold pressed oils should have acid value less than 4.0 mg KOH/g oil. The lower acidity value and peroxide value indicate that the oil have a better quality and longer shelf life (Zhang et al., 2011). This may be due to enrichment of the flaxseed oil with more bioactive compounds (chlorophyll, lycopene, β-carotene, phenols and flavonoids).

On the other hand, the higher values of iodine number which ranged from 179.66 to 184.44 (g Iodine/ kg oil) indicated that the oil contained higher amount from polyunsaturated fatty acids (linolenic acid, C18:3), (Zhang et al., 2011) and (Nahed and Enaam, 2017). These amount reached to about 60% in our results as it will be shown in Table 7.

Table 4. Some physio-chemical properties of crude and enriched flaxseed oil samples

Samples	Acid value	Iodine value	Refractive index	Peroxide value
Crude oil	0.62	183.24	1.48	2.79
Oil with BHA	0.68	180.25	1.47	2.68
Oil enriched with carrot waste	0.67	182.66	1.48	2.88
Oil enriched with tomato waste	0.69	184.44	1.48	2.88
Oil enriched with olive leaves	0.66	179.66	1.48	2.86

(Orwa et al., 2014) reported that, the quality of oil samples can be evaluated by measuring absorbance in the region between 200 and 300 nm. These are the wavelengths related to the diene and triene compounds. A low absorption in this area indicates the presence of high-quality oil. When high absorption occurs at 232 nm, this is due to aqueous peroxides (the initial stage of oxidation) and conjugated dienes. While high absorption occurs at 270 nm, is due to carbonyl compounds (the secondary stage of oxidation) and conjugated trienes.

The data in Table 5. show the degree of oxidations of crude and enriched flaxseed oils which are reflected by its specific extinction at 232 and 270 nm. The lower values for dienes and trienes (K233 and K270) were observed in oil samples enriched with olive leaves which were 1.56 and 0.125 respectively. Followed by tomato waste which recorded 2.65 for dienes and 0.26 for trienes. This may be due to the higher scavenging potential of olive leaves (IC_{50} was 0.88 ug/ml as shown in table 1). These results are partly consistent with (Choo et al., 2007) in a study on seven different varieties of flaxseed oil who found that K232 values ranged between 1.7 to 2.5 and about 0.2 to 0.4 for K270.

It was observed that also increasing in absorption value measured at 233 and 270 nm for crude oil, oil enriched with carrot and oil with BHA which were (3.63 and 1.31), (3.39 and 0.99) and (3.11 and 0.43) respectively. These values employed to monitor the formation of conjugated dienes and presence of secondary oxidation products, trienes and it may be related to high concentration of alpha linolenic acid. Carotenoids inhibit the photosensitivity of oil to oxidation through physical or chemical capture of single oxygen. During the physical capture of singlet

oxygen, its structure is not destroyed. However, in the case of chemical capture, a breakdown occurs in its structure, followed by the breaking of the double bonds and the transformation into epoxide or carbonyl derivatives, and this appears in the form of colorless (Tańska et al., 2016).

Table 5. Specific extinctions at 232 nm (K232) and 270 nm (K270) for conjugated dienes and trienes respectively

Samples	K232 (dienes)	K270 (trienes)
Crude oil	3.63	1.31
Oil with BHA	3.11	0.43
Oil enriched with carrot waste	3.39	0.99
Oil enriched with tomato waste	2.65	0.26
Oil enriched with olive leaves	1.56	0.12

Oxidation occurs in fats during the process of rancidity, resulting in free radicals that give off undesirable tastes and odors. One way to measure fat oxidation is by inhibiting the DPPH radical (2,2-Diphenyl-1-picrylhydrazyl).The results for the inhibition of the DPPH radical were shown in (Table 6). It was observed that oil enriched with olive leaves resulted in higher (55.16%) values of inhibition followed by oil enriched with tomato waste, oil enriched with carrot waste, oil with BHA and crude oil which were 48.90, 46.80, 42.83and 41.10% respectively.

(Tarchoune et al., 2019) found that olive leaves addition to two different types of olive oil by 3%, increased antioxidant capacity by 15% and 87%. He added, this wide change was probably due to the increases in chlorophyll, carotenoid, total phenolic and flavonoid concentrations and its existence has been proven a good correlation between the total phenolic amount and the radical scavenging power.

(Gieraldin et al., 2022) reported that DPPH radical inhibition of 50% was found in lycopene-enriched sunflower oil from tomato waste. He added that the reduced inhibition of this oils may be also due to other antioxidant molecules such as phenols, flavonoids and carotenoids present in the native oils. In our study enriched oil with carrot, tomato waste and olive leaves lead to more amount of these compounds.

Table 6. show also the oxidative stability of flaxseed oil before and after enriched with carrot, tomato and olive leaves wastes compared to BHA treatment measuring by Rancimat method. The induction time of flaxseed oil increased in all treatments compared to the crude oil (2.76 h). The initial test performed at 100 °C showed that the enriched oil with olive leaves allowed an increase in oxidative stability(5.67 h) close to that obtained with BHA (5.16 h), also the other enrichment oils confirmed a higher induction time than in the crude flaxseed oil. Where oil enriched with tomato waste come in followed place (4.63 h) and oil enriched with carrot waste (4.18 h). These results may consistent with those of (Tańska et al., 2016) in the study on selected cold pressed flaxseed oils samples. They found the Rancimat test showed induction time ranged from 2.00 h to 4.35 h. (Szterk et al., 2010) found that induction time for flaxseed oil heated at a lower temperature (100℃) and 2-fold slower air flow(10 L/h) was 5.85 h , while using the temperature of 110℃ the induction time was (1.57 h).

(Miguel et al., 2019) reported that in flaxseed oils, the high temperatures of the Rancimat method may affect induction times due to the occurrence of polymerization, with the release of volatile compounds and the possible formation of a dry layer that may prevent air from reaching the sample, and this process may lead to a reduction in shelf life. Also they found that, the presence of 8.00 mg lycopene/100g flaxseed oil led to the same induction time obtained using 200 mg/kg of the antioxidant artificial BHT, and the addition of tomato lycopene-rich extract increased the shelf life of flaxseed oil by 31%.

In our study, the induction time increase by 105.43, 67.75 and 51.44% in oil enriched with olive leaves, tomato waste and carrot waste respectively as compared to 86.95% for oil with BHA. It can be said that the presence of, lycopene, β-carotene, phenolic, and flavonoids compounds in flaxseed oil at the concentrations shown in Table (2) gave good results and might outperform the antioxidant artificial BHA if these quantities were increased by enhancement the recovery rate.

Table 6. Antioxidant activity by (DPPH) and Oxidative stability by Rancimat test of crude and enriched flaxseed oil samples

Samples	% DPPH	IT(h)
Crude oil	41.10	2.76
Oil with BHA	42.83	5.16
Oil enriched with carrot waste	46.80	4.18
Oil enriched with tomato waste	48.90	4.63
Oil enriched with olive leaves	55.16	5.67

DPPH :Diphenyl-1-picrylhydrazyl, IT: induction time

The fatty acid content of crude and other enrichment flaxseed oil samples are shown in Table (7). Taking a general look at the table we find that there are no noticeable differences in the composition of fatty acids and their ratios to each other in all samples. The total saturated fatty acids represented about 10%, while unsaturated fatty acids represented about 89% where the percentage of linolenic acid (C18:3) constituted only about 60% of the total fatty acids. These results agree with (Nahed and Enaam 2017) who found that linolenic acid is the main unsaturated fatty acid in flaxseed oil with a percentage of up to (54.9%). Differences in oil components are due to genotype, environment and growing conditions. There was also a strong effect of temperature and humidity levels on the content of fatty acids: in cold climates, the proportion of unsaturated acids is greater. The fatty acids composition depends also on the extraction process (Miguel et al., 2019).

(Tańska et al., 2016) evaluated the properties of flaxseed oils from two different types: fiber-flax and oil-flax seeds, they found that more linolenic acid (58.03%) in the fatty acids of the oil-type flax against (47.37%) for fiber-type flax. Our results are agree also with (Miguel et al., 2019) who found the fatty acid content of linseed oil and of enriched linseed oil with lycopene don't showed significant differences in the acid profile, except for linoleic acid, which had little variation.

Table 7. GC Analyses of the crude and enriched flaxseed oil

Fatty acids		Samples				
		Crude oil	Oil with BHA	Oil with carrot waste	Oil with tomato waste	Oil with olive leaves
C14:0	Myristic	0.046	0.048	0.047	0.045	0.044
C16:0	Palmitic	5.54	5.48	5.52	5.49	5.47
C17:0	Margaric	0.058	0.058	0.056	0.059	0.057
C18:0	Stearic	4.38	4.35	4.29	4.47	4.32
C20:0	Arachidic	0.132	0.202	0.135	0.185	0.240
C22:0	Behenic	0.117	0.122	0.115	0.018	0.118
Total	**SFA**	**10.27**	**10.26**	**10.16**	**10.26**	**10.24**
C18:1	Oleic	14.52	14.57	14.62	14.90	14.62
C18:2	Linoleic	14.85	14.93	14.95	15.03	14.86
C18:3	Linolenic	60.06	59.94	59.98	59.49	59.94
Total	**USFA**	**89.43**	**89.44**	**89.55**	**89.42**	**89.42**

Table (8) show the results of sensory evaluation of salad dressing prepared by enriched flaxseed oil samples. According to the scores given by the organoleptic panelists, there was no significant ($P<0.05$) difference among all treatments in all parameters except the appearance in the sample of oil enriched with olive leaves which showed a significant reduction (6.80), and this may be due to its high content of chlorophyll which makes the oil appear cloudy. According to the hedonic scale method which ranked from 9- extremely liked to 1- extremely disliked, all treatments were recorded mean score of taste and overall acceptability ranged from 7.00 to 7.60 (between like moderately and like very much).

Table 8. Sensory evaluation of salad dressing prepared with enriched flaxseed oil compared with olive oil.

Samples	Appearance	Texture	Aroma	Taste	Overall acceptability
COL	7.70	7.50	7.70	7.60	7.60
CFL	7.60	7.80	7.30	7.30	7.50
FLC	7.40	7.30	7.40	7.30	7.20
FLT	7.20	7.30	7.00	7.00	7.10
FLO	6.80	7.50	7.20	7.50	7.10

COL: Dressing with crude olive oil, CFL: Dressing with crude flaxseed oil, FLC: Dressing with flaxseed oil enriched with carrot waste, FLT: Dressing with flaxseed oil enriched with tomato waste, FLO: Dressing with flaxseed oil enriched with olive leaves.

The data in Table 9. show the oxidative stability of salad dressing prepared by enriched flaxseed oil were evaluated by measuring their peroxide value and acid value after storage for seven days at 5C°. it could be observed that slight increasing in peroxide value in salad dressing with flaxseed oil compared to that with olive oil (control). While the enriched flaxseed oil samples did not differ nearly from the control. This also applies to the acidity value, this is due to the presence of some spices that were added to all samples, which can act as powerful antioxidants, in addition to the antioxidants that came from the waste of carrots, tomatoes, and olive leaves. (Mihov et al., 2012) reported that mayonnaise samples with olive oil containing natural spices and herbs or their extracts were more resistant to oxidation. (Gendreau and Ruiz, 2019) in their study on mustard-vinaigrette salad dressing (with corn oil) found that, the peroxide content showed primary values in a range of 0.83 to 1.56 meq O_2/kg that increased to 1.73-2.56 meq O_2/kg during four weeks and to 5.26-8.79 meq O_2/kg in eight weeks. On a commercial scale the peroxide values of dressing should not exceed 20 meq O_2/kg, this tracking value is important for determining the shelf life of the dressings. They added, similar peroxide values of 1.85 to 5.67 meq O_2/kg for the fresh prepared salad dressings, with higher oxidation (3.70- 30.91 meq O_2/kg) after five days of storage.

Salad dressing is one of the applied examples of uses, as there are many products in which flax oil can be included after improving its physical and chemical properties by enriching it with the bioactive compounds, where it is possible to study storage periods at different temperatures and track the microbial status of these products.

Table 9. Peroxide value and acid value in salad dressing prepared with enriched flaxseed oil compared with olive oil.

Samples	Peroxide value	Acid value
Dressing with crude olive oil	2.96	0.66
Dressing with crude flaxseed oil	3.16	0.68
Dressing with flaxseed oil enriched with carrot waste	3.01	0.69
Dressing with flaxseed oil enriched with tomato waste	2.98	0.69
Dressing with flaxseed oil enriched with olive leaves	2.97	0.68

4. Conclusion

Flaxseed oil is very sensitive to oxidation, especially when the appropriate conditions are available such as heat, oxygen, or presence of metal ions. This leads to formation of unwanted compounds that affect the taste and smell. This ultimately limits its uses as cooking or frying oil and reduces the shelf life and nutritional quality. The bioactive compounds from by-products such as, chlorophyll, lycopene, β-carotene, phenolic and flavonoids can be used as sources of natural antioxidant instead of artificial to enhances the flaxseed oil properties. This can be done by releasing it in the oil directly and this might be less cost and more effective than extracting with organic solvents and re-adding to the oil. In this regard, oil enriched with olive leaves had the best results, followed by oil enriched with tomato waste, then oil enriched with carrot waste, where the induction time by Rancimat test increased by 105.43, 67.75 and 51.44% respectively. Using oil as an extraction medium for natural antioxidants and bioactive compounds is an effective and economical method, but we may need more studies to develop this method to obtain the highest recovery rate while maintaining the nature of these compounds, with ensuring that the oil is not exposed to oxidation during extraction and without leading any free radicals.

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