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Physicochemical Characteristics, Inhibition of Lipid Peroxidation, and Oxidative Stability Index by Antioxidant Alternatives of Promising Binary Oil Blend (Canola and Olive oils).

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ABSTRACT

In the present article, three samples (canola seed oil; CSO), (unfiltered olive oil; UFOO) and Canoliven (Canola seed oil: unfiltered olive oil, 80:20 w/w, respectively), were examined for phytochemical screening revealing the presence in all samples: steroids, terpenoids, saponins, tannins, anthocyanins alkaloids, phenols, and flavonoids. The results indicated that CSO variety Giza 56 contained 12 different chemical groups. Four physical characteristics were assayed in three samples. The acidity value ranged from 0.21 for CSO to 0.35 for UFOO and the acidity value for the canoliven is 0.25. Furthermore, the highest level of unsaponifiable matter % was found in CSO, followed by Canoliven and then UFOO. Peroxide value (meq O2/kg oil) ranged from 1.5 for CSO to 8.5 for UFOO, while Canoliven recorded 4.01. The highest level (6.5) of p-anisidine value (p-AV) was reported in the UFOO followed by CSO and Canoliven. Moreover, TOTOX values in Canoliven (11.2) and more than two folds of this value were recorded in UFOO (23.5). Additionally, the concentrations of saturated fatty acids (SFA%) in individual pure oils ranged from 16.29% for UFOO to 17.16% for CSO, while Canoliven recorded 17.01%. Total monounsaturated fatty acids (∑MUFA%) varied from 69.76 to 72.09% for UFOO, and CSO, respectively. The blending process improved the level of MUFA%, whereas its concentration in Canoliven reached 71.56%. The concentrations of polyunsaturated fatty acids (∑PUFA%) are 13.42, 10.57, and 11.43% in UFOO, CSO and Canoliven, respectively. Finally, some specialized analyses were conducted on the samples, including MDA, TPCs, OSI, and FTIR.

KEYWORDS: Antioxidant, Canola seed oil, Edible oils, Fatty acid composition, Unfiltered olive oil.

1. INTRODUCTION

Olive oil may be separated into major and minor portions based on its chemical makeup. Triacylglycerols, one of the principal components, account for more than 98% of the weight of the oil. More than 300 distinct chemical compounds make up minor components found in minimal levels (approximately 2% of oil weight) (**Venturini *et al.*, 2023**). Non-glyceride esters, and triterpene, sterols, hydrocarbons, pigments, lipophilic and hydrophilic phenols, and volatile substances are all included in this portion.

Olive oils are naturally protected by their high content of endogenous antioxidants (polyphenols and tocopherols); whereas some other oils (like sunflower, soybean, and peanut oils) must be enriched with exogenous antioxidants during their production due to the refining process they go through (Viana da Silva *et al.*, 2021) to make them more oxidation-resistant. Edible oils contain phytochemicals and phenolic compounds that have anti-inflammatory, antioxidant, antibacterial, antiviral, antitumor, cardioprotective, antidiabetic, and anti-obesity activities, among other health advantages (Sumara *et al.*, 2023, Xiao *et al.*, 2022, Ali *et al.*, 2022, Goyal *et al.*, 2021, Sey *et al.*, 2020, Chew 2020, Ghazani and Marangoni 2013, and Johnson *et al.*, 2007).

These variables may impact their chemical makeup, physical qualities, functional traits, and sensory characteristics, which may limit both their quality and quantity. To ensure their safety, stability, and compatibility for many culinary applications, it is crucial to optimize the processes used in the manufacturing of canola grains and the products that are created from them (Sabbahi *et al.*, 2023).

Pomegranate peel extract (PPE) contains a variety of bioactive phenolic and flavonoid compounds, including HO-cinnamic acids, ellagi-tannins, gallo-tannins, HO-benzoic acids, and complex polysaccharides (El-Hadary and Taha 2020; Rashid *et al.*, 2022). The antioxidant, antibacterial, and therapeutic characteristics of PPE have been the subject of several studies (Kumar *et al.*, 2022; Rashid *et al.*, 2022; Trigo *et al.*, 2020).

Carotenoids are isoprenoid derivatives soluble in fats and have significant benefits for human health and these substances have antioxidant activities and functional properties. Carotenoids are natural pigments, these compounds are lipophilic isoprenoids associated with a wide range of health benefits and antioxidant properties (Monarca *et al.*, 2023, Venturini *et al.*, 2023, Yu *et al.*, 2022, Basuny *et al.*, 2022 and Kultys & Kurek 2020).

The main objectives of the present work were to do: (1) Analysis of phytoconstituents of three oils. (2) Estimation of the physicochemical properties of oils proposed to be evaluated (3) Determination of levels of some bioactive secondary metabolites in the extracted oils (4) Preparing mixture from oil samples to obtain a specific, compatible and stable composition in the final product and study their physicochemical properties and antioxidant activities of the new mixture by FTC. (5) Estimation of OSI of oil samples by Rancimat method.

2. MATERIALS AND METHODS

In this article two individual oil samples and binary oil was used as follows in Table (1).

Table 1. Samples used in the present work.

1)	Unfiltered olive oil	UFOO	Purchased from Prof. Dr. Sabry Mahfouz, Olive specialist at the National Research Center (NRC)
2)	Canola seed oil *Brassica napus*, L. variety Giza 56	CSO	Canola seeds were provided from Prof. Dr. Mohamed Hamam, Prof. of oil crops in Shandaweel Res. Station, Sohag, Egypt.
3)	CSO + UFOO 80:20%	Canoliven	Newly composed binary blend oils with ratios 80:20 w/w.

2.1.Oils blending.

In general, we resort to mixing oils to take advantage of the properties of the different types involved in the composition of the new mixture with the desired quality. Unfiltered olive oil and canola seed oil were mixed at a ratio of 80:20 w/w. Mixing was done at 40 °C in a conical flask using a magnetic stirrer for approximately 1 h. The oils were mixed well to form uniform blends.

2.2.Qualitative phytochemical analysis

The phytochemical screening of the different oilseed extracts was described by Harborne (1973) as follows: steroids, phenol and alkaloids detection were by Gibbs, (1974). Terpenoids detection was by Ayoola *et al.*, (2008), tannins by Treare and Evans, (1985), saponins by Kumar *et al.,* (2009), anthocyanins by Paris and Moyse (1969), glycosides by Khandewal (2008), emodins by Rizk (1982) and flavonoids by Khandewal, (2008).

2.3.Determinations of physicochemical properties of oils

The density, refractive index, kinematic viscosity and color were determined using the procedures described by Erwa *et al*. (2019). Acid value (AV) was determined and calculated according to the method described by Erwa *et al*. (2019). Saponification value (SV) was performed according to ISO I. 660: 2009(E)). Unsaponifiable matter (UM %) was determined according to ISO 3657:2013(E) with slight modifications described by Ishag, *et al.*, (2019). Ester value (EV) was calculated according to Akpan *et al.,* (2006), using the following equation Ester Value = SV–AV. Assay of peroxide value (PV) was assayed and calculated according to ISO 3960: (2007). Iodine value (IV) was determined according to ISO 3960, (2007). *p*-Anisidine values (*p*-AV) were determined was determined by standard method according to AOCS (1998). Total oxidation (TOTOX) was calculated using the equation TOTOX= (2PV+*p*-AV) (Samaram *et al.*, 2013). The smoke point (°C) was determined according to the method by Tarmizi and Ismail (2008). To determine the total phenolic content, the method described by (Ahmed *et al.*, 2021, Abdelaty & Desoukey 2021, Darwish *et al.*, 2020) were used with by slight modifications. Total flavonoids (TFs) were extracted and assayed according to the method of Zhuang *et al.*, (1992) and Nagaty *et al.*, 2023.

2.4.Pigment Content

The pigment content of extracted oil samples was measured following the protocol described by Mazaheri *et al*. (2019).

2.5.Preparation of antioxidants alternatives

Fine powders from turmeric (Cur-E), hybrid yellow corn husks (FPH-YCH) and black pepper (BP) were mixed and extracted by ethanol (70%). Pomegranate fruits were provided by a Pomegranate Farm in Manfalot, Assiut. The sound fruits were cut and the pomegranate seeds were carefully separated. The fleshly mesocarp layers were cut and left to dry. After the samples were air-dried at room temperature (10 days), the dried samples were ground in a mixer grinder (Moulinex, France) and mixed with 70% ethanol at a ratio of 1:10 (fine powder/solvent) and then soaked for extraction. The samples were shaken by Earlene shaker at 250 rpm overnight at room temperature. Then, the resulting solutions were filtered and concentrated by a rotary evaporator (35-40 °C). The solvent was then eliminated by evaporating the solvent residue in a vacuum oven at a temperature of 40 °C. The resulting extracts were kept at −10 °C for the next analyses (Bazargani-Gilani, 2021; Hassan *et al.*, 2021; Pan *et al.,* 2008, and Albu *et al.,* 2004).

2.6.Lipid peroxidation inhibition assay (LIPIA) by FTC

Determination of Lipid peroxidation inhibition was carried out by using the linoleic acid model system (Osawa and Namiki, 1981).

2.7.Procedure of lipid peroxidation inhibition

Linoleic acid model system was applied to determine the total antioxidant activities according to Osawa and Namiki (1985).

2.8.Thiobarbituric acid reactive substances (TBARS) assay

This method is performed to measure malondialdehyde (MDA) found in oil samples and MDA generated from the lipid peroxidation using TBA reagent were determined according

to the method described by Samy *et al.,* (2022), Hamed & Wahid, 2021 and Mendes *et al.,* (2009).

2.9.Instrumental analyses

2.9.1. Determination of oxidative stability (OSI)

OSI of UFOO, CSO and Canoliven were evaluated by Metrohm Professional Rancimat model 892 (Herisau, Switzerland) according to (Anwar *et al.,* 2003, Kaseke *et al.,* 2020).

2.9.2. GC-Mass identification of fatty acids profile:

The gas chromatography-mass spectrometry (GC-MS) method was used. Fatty acids profile of samples (UFOO, CSO and Canoliven) according to Qian, (2003). Fatty acids were recognized and enumerated with the help of FAME 37 Kit, Sigma-Aldrich, Chemical Company.

2.9.3. Calculations of the nutritional quality UFOO, CSO and Canoliven:

Calculations of some nutritional quality indicators using mathematical equations such as atherogenicity index; thrombogenicity index are calculated according to the equation proposed by Ulbricht and Southgate (1991). Hypocholesterolimic / hypercholesterolemic (h/H), calculated according to equation of Fernández *et al.,* (2007). The HPI (health promoting index) was proposed by Chen *et al*., (2004) as an indicator of the health value of dietary oil and is largely focused on the effect of some FA on cardiovascular diseases.

2.9.4. COX value

The value of calculated oxidisability was calculated according to equation proposed by Fatemi and Hammond, (1980).

2.10. FTIR Spectral analysis:

FTIR spectroscopy is an excellent tool for analysis as the intensities of the bands in the spectrum are proportional to concentration according to (Jaggi, and Vij, 2006 and Fan *et al.,* 2012).

2.11. Statistical analysis

All experiments mentioned above were repeated three times. Data analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSION

3.1. Qualitative screening of bioactive secondary metabolites in CSO, UFOO and Canoliven:

A phytochemical screening was carried out by specific coloring and precipitation reactions. The type of extract non-aqueous methanol was used as extracting solvent, and the phytochemical screening revealed the presence of various bioactive constituents. In the present article, three samples of oilseeds were examined for phytochemical screening revealed the presence of following secondary metabolites in all samples: steroids, terpenoids, saponins, tannins, anthocyanins alkaloids, phenols, flavonoids and fatty acids. The results given in **Table (2)** indicated that CSO contain 12 different groups and all samples i.e., these oils are edible oils which mainly constituting triglycerides. The purposes of identification of these constituents are to evaluate thermal stability and oil quality.

3.2.Physicochemical characteristic of CSO, UFOO and Canoliven:

The present results of 12 physicochemical indices are presented in Table (3). The physical characteristics such as density (g/cm^3) 20 °C/water at 20 °C) values for CSO is (0.937) higher than canoliven (0.933).

Values of refractive index (40 °C) were changed in very close extent and the highest level of RI was reported for CSO 1.4670. Smoke point (°C) in three samples is higher than 195 °C i.e., these oils valid for cooking or frying especially CSO (240 °C). Unfiltered olive oil is said to have a greater flavor by some. But both filtered and unfiltered oil offer health advantages. The shelf life of filtered and unfiltered olive oil is the main distinction between the two. Unfiltered olive oil will continue to ferment in the bottle as long as the olive particles are there.

Table 2. Qualitative screening of bioactive secondary metabolites in CSO, UFOO and Canoliven.

Group	CSO	UFOO	Canoliven	Group	CSO	UFOO	Canoliven
Steroids	present	present	present	**Emadins**	present	absent	absent
Terpenoids	present	present	present	**Alkaloids**	present	present	present
Tannins	present	present	present	**Glycosides**	present	present	present
Saponins	present	present	present	**Phenols**	present	present	present
Anthocyanin	present	present	present	**Flavonoids**	present	present	present
Coumarins	present	absent	absent	**Glycerol**	present	present	present

CSO = Canola seed oil, UFOO = Unfiltered olive oil.

Table 3. Physicochemical properties of cold pressed canola seed oil, unfiltered olive oil and Canoliven.

Properties/ Indices	CSO	UFOO	Canoliven
Density (g/cm^3) 20 C/water at 20 °C)	0.937±0.03	0.914±0.01	0.933±0.02
Kinematic viscosity 20 °C, mm^2/sec)	78.8±1.5	84.25±1.6	79.89±1.5
Refractive index (40 °C)	1.4670±0.005	1.4626±0.003	1.4661±0.004
Color index	41.5±0.4	67.1±0.7	47.2±0.5
Smoke point (°C)	240±5	196±3	231±4
Acid value (mg KOH/g oil)	0.21±0.01	0.35±0.03	0.25±0.02
Saponification value (mg KOH/g oil)	191±1.9	190.26±2.0	192.50±1.9
Unsaponifiable matter (%)	1.542±0.01	1.514±0.01	1.519±0.01
Ester value (SA-AV)	190.79	189.91	192.25
Iodine value (g/100g)	96±1.2	85.12±1.1	94±1.2
Peroxide value (meq O_2/kg oil)	1.5±0.01	8.5±0.5	4.01±0.04
***p*-Anisidine Value (*p*-AV)**	5.11±0.2	6.5±0.3	4.91±0.1
TOTOX value	8.11	23.5	11.2

Ester Value = (ES = SV - AV), Total oxidation (TOTOX) value was calculated from the PV and p-AV using the equation TOTOX =(2PV+p-AV) (**Samaram *et al.*, 2013**).

By estimating the physicochemical properties of the individual oils and its mixture, it is found that the acidity value ranges from 0.21 for canola seed oil to 0.35 for unfiltered olive oil, and the acidity number for the mixture of canoliven oil is 0.25. The differences are close in the saponification values of the oils under the experiment and range from 190.26 for unfiltered olive oil to 192.5 for the Canoliven mixture. By estimating the percentage of unsaponifiable matter %, the highest level was found in canola seed oil, followed by the Canoliven and then the UFOO.

Levels of iodine values are under 100 g of I_2 and the lowest level (85.12±1.1) for unfiltered olive oil while the highest one (96±1.2) was recorded for the CSO. After calculation of ester value (EV) it ranged from 189.91 for unfiltered olive oil to 192.25 for canoliven. EV depends on the value of saponification and acid value of the individual oils and the blended one. These results are in agreement with those reported by **Kılıc *et al.*, (2007)** who stated that edible oils have various colors according to concentration of pigments.

The presence of pigments like lutein, chlorophyll, and carotenoids that move from oil seeds is what gives most vegetable oils their colour. These pigments are quickly converted during storage to coloured or colourless products, changing the colour of the oil (Zhang *et al.*, 2023; Alonso-Salces *et al.*, 2021).

3.3. Levels of peroxide values, p-anisidine values and TOTOX values of individual oils and their blend Canoliven.

Results of three important chemical indices are presented in Table 3 and Fig. 1. Peroxide value (meq O_2/kg oil) ranged from 1.5 for CSO to 8.5 for unfiltered olive oil while the binary blend recorded 4.01. The highest level (6.5) of *p*-anisidine value (*p*-AV) was reported in the unfiltered olive oil followed by CSO and Canoliven. TOTOX values in the binary blend (11.2) and more two folds of this value were

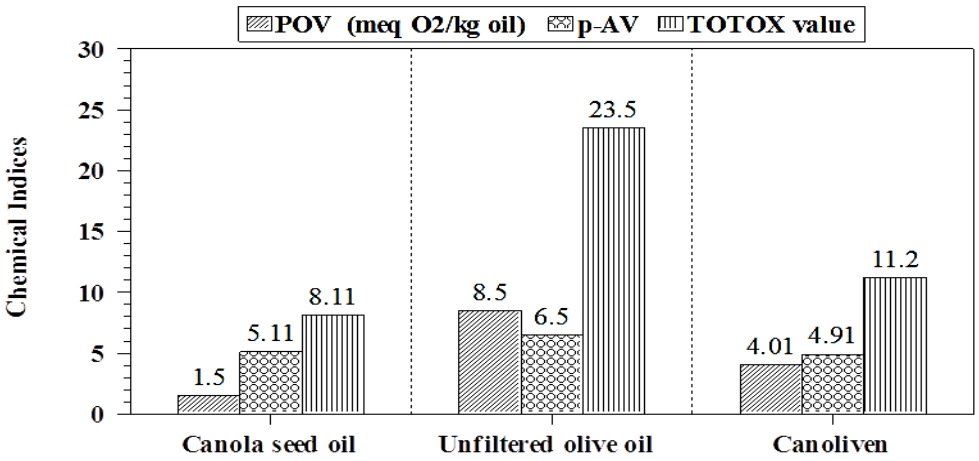

Fig 1. Levels of peroxide values, *p*-anisidine values and TOTOX values of individual oils and their blend Canoliven.

recorded in unfiltered olive oil (23.5). The present results also showed that blending process improved of *p*-anisidine value and TOTOX values.

Aldehydes, carbonyls, trienes, and ketones are some examples of secondary oxidation products measured by the *p*-methoxyaniline value. When combined with tests like peroxide levels, it can actually provide detailed information about the condition of animal or vegetable oils and fats (Dijkstra, 2016; Steele, 2004).

The PV is important indicator for evaluating hydroperoxides and peroxides which dominate the early stages of lipid oxidation. According to **Kasek *et al.*, (2020)** and Cong *et al.*, (2020), the better the oxidative stability of the seed oil, the slower the rate of hydroperoxide formation and PV increase. Among the oils utilized in this article, olive oil has one of the highest PVs. According to **Codex (2015)**, virgin olive oils that are sold can contain up to 15 meq O_2/kg of peroxide. However, because flaxseed oil contains a lot of polyunsaturated fatty acids, it can also oxidize quickly and have a high PV.

3.4. Fatty acids profile of CSO, UFOO and their blend Canoliven

Results of fatty acids analyses are given in Table (4) and Fig. (2). The concentrations of saturated fatty acids (SFA%) in individual pure oils ranged from 16.29% for unfiltered olive oil to 17.16% for canola seed oil (CSO), while blended oil Canoliven recorded 17.01%.

The present results also showed ∑SFAs in CSO is higher than both unfiltered olive oil and Canoliven (Fig. 3). Total monounsaturated fatty acids (∑MUFA %) varied from 69.76% to 72.09% for unfiltered olive oil and CSO respectively. Blending process improve the level of MUFA % whereas its concentration in Canoliven reached to be 71.56 %. Results indicated that oleic acid concentration ($C_{18:1}$) was the most dominant fatty acid in MUFA group. The concentrations of polyunsaturated fatty acids (∑PUFA %) are 13.42, 10.57 and 11.43 in unfiltered olive oil, CSO and Canoliven respectively. PUFA level in CSO is lower than those reported in both unfiltered olive oil and Canoliven. These results are in agreement with reported by Dorni *et al.*, (2018).

3.5. PUFA/SFA ratios

PUFA/SFA ratios for pure oils ranged from 0.615 for CSO to 0.823 for UFOO while blended oil Canoliven recorded average value reached to be 0.671, i.e., the blending process led to improving the fatty acids profiles. Results given in Table (4) also, indicated to existence of odd chain saturated fatty acid OCS-FAs ($C_{17:0}$ Margaric acid) and odd monounsaturated fatty acid ($C_{17:1}$ Heptadecenoic acid). The existence of odd chain fatty in the oils such as $C_{17:0}$ and $C_{15:0}$ have important roles in human health in biological and nutritional observations (Jenkins *et al.*, 2015).

Table 4. Fatty acids profile of CSO, UFOO and Canoliven.

Fatty acid (%)	UFOO (20%)	CSO (80%)	Canoliven
$C_{14:0}$ Myristic acid	0.01±0.001	0.01±0.00	0.01±0.001
$C_{16:0}$ Palmitic acid	13.78±0.14	14.17±0.14	14.1±0.14
$C_{16:1}$Palmitoleic acid	1.36±0.014	1.49±0.015	1.47±0.015
$C_{17:0}$Margaric (OCS-FAs)	0.05±0.001	0.10±0.001	0.10±0.001
$C_{17:1}$Heptadecenoic (OCS-FAs)	0.10±0.01	0.06±0.02	0.06±0.02
$C_{18:0}$ Stearic acid	2.41±0.024	2.45±0.024	2.44±0.024
$C_{18:1}$ Oleic acid Ω-9	68.05±0.69	70.23±0.71	69.77±0.70
$C_{18:2}$ Linoleic acid Ω-6	12.68±0.13	8.65±0.87	10.45±0.11
$C_{18:3}$ Linolenic acid Ω-3	0.74±0.008	0.92±0.009	0.98±0.010
$C_{20:0}$ Arachidic acid	0.04±0.001	0.43±0.004	0.36±0.003
$C_{20:1}$ Gadoleic acid	0.25±0.003	0.31±	0.26±
∑Saturated fatty acid (∑SFA)	16.29±0.16	17.16±0.17	17.01±0.17
∑Monounsaturated (∑MUFA)	69.76±0.70	72.09±0.72	71.56±0.71
∑Polyunsaturated (∑PUFA)	13.42±0.14	10.57±0.11	11.43±0.12
∑PUFA/∑SFA ratio	0.823±0.008	0.615±0.006	0.671±0.007
Ω -6/ Ω-3 ratio	17.13±0.17	9.40±0.10	10.66±0.11
Calculated Oxidisability (COX) value	19.875	22.411	25.149
Atherogenicity index (AI)	0.1657	0.1736	0.1700
Thrombogenicity index (TI)	0.2057	0.2083	0.2059
Hypocholesterolimic/ Hypercholesterolemic	5.907	5.627	5.754
Health promoting index (HPI)	6.0144	6.1816	5.8067
Total FA	99.47	98.82	100

Calculated Oxidisability (COX) value (Fatemi and Hammond, 1980), Atherogenicity index (AI) and Thrombogenicity index (TI) according to Ulbricht and Southgate (1991), Hypocholesterolimic/Hypercholesterolemic (*h*/H), based on Fernández *et al.*, (2007), Health promoting index (HPI) described by Chen *et al.*, (2004).

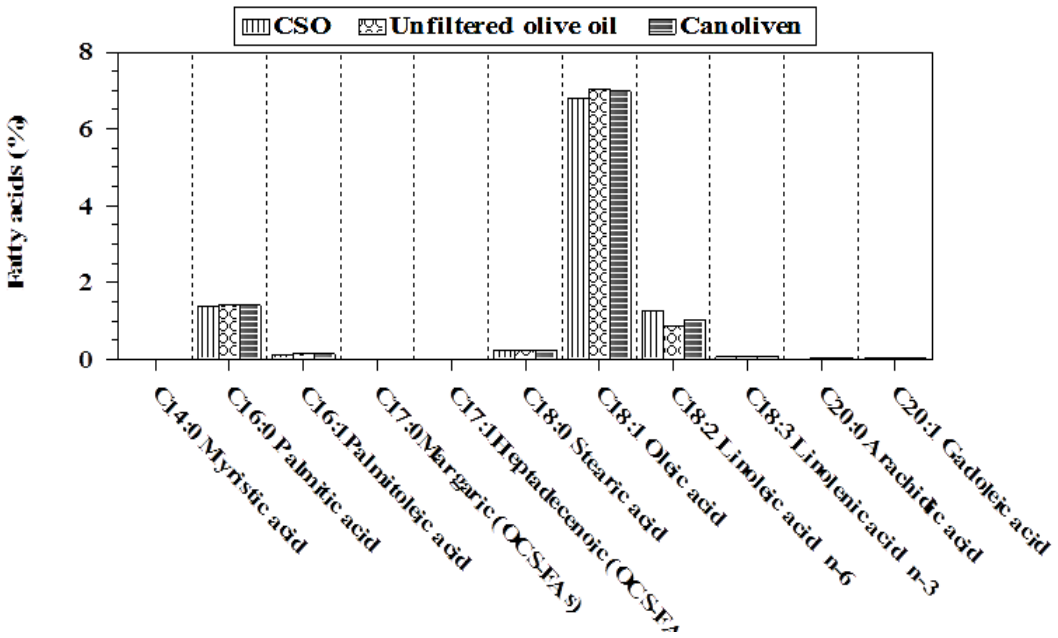

Fig. 2. Fatty acid profile of canola seed oil, unfiltered olive oil and their blend Canoliven

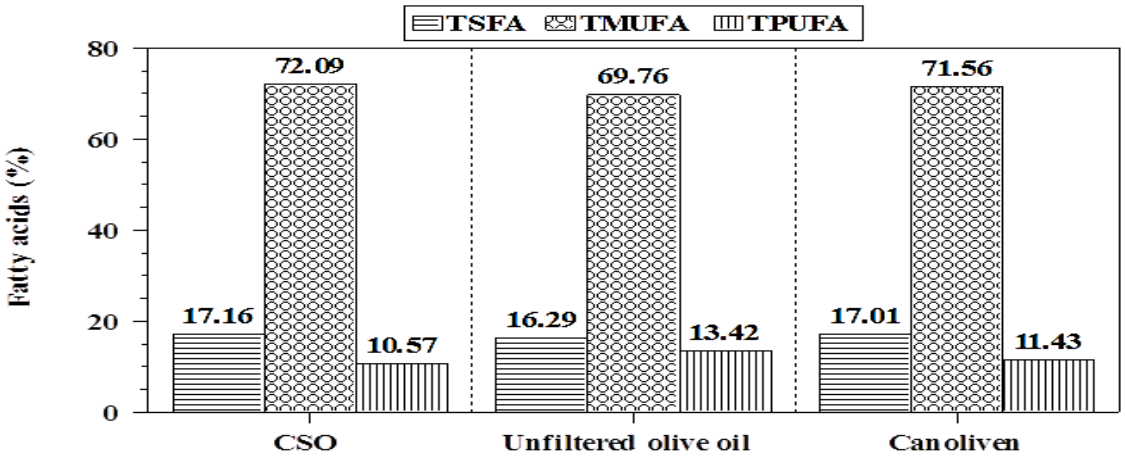

Fig. 3. Concentrations of TSFAs, TMUFA and TPUFA in CSO, UFOO and their blend Canoliven.

Unfortunately, no natural vegetable oil has this ideal ratio some have too much monounsaturated fatty acids; others are rich in monounsaturated fatty acids. When vegetable oil is hydrogenated, the product is Vanaspati Ghee, consisting of 100% SFA, since all unsaturated bonds (MUFA, PUFA) are fully saturated during the process. A proper mix of cooking oils such as rice bran and safflower/sunflower oil; coconut oil and sesame oil; canola oil and flax seed oil are good choices. Blended oils are more heat stable, provide better nutrition, and have anti-inflammatory properties (Lele, 2022).

Vegetable oils with a high C18:0 ratios have higher oxidative stability than ordinary vegetable oils (White, 2000). The protective effect may be mediated by polyunsaturated ω-3 fatty acids (ω-3 PUFA). These data suggest that the protective effects of the Mediterranean diet on cognitive function may be partially mediated by higher plasma ω-3 PUFA (Rabail *et al.,* 2021, Barberfer-Gateau *et al.,* 2011, and Nehdi *et al.,* 2019).

3.6. Assessment of the nutritional quality UFOO, CSO and Canoliven:

Data obtained by GC-Mas fatty acid analysis could be used in some derived calculations using mathematical equations such as atherogenicity index which ranged from 0.1657 to 0.1736; thrombogenicity index ranged from 0.2057 to 0.2083 and hypocholesterolimic/hypercholesterolemic which from 5.754 to 5.907 (Table 4). These indexes are lower than those recorded in unhealthy oils. The present results indicate that the use of UFOO, CSO and Canoliven in human consumption is safe and health according to nutritional quality indicators. The HPI was proposed by Chen *et al*. (2004) as an indicator of the health value of dietary oil and is largely focused on the effect of some FA on cardiovascular diseases. Oils with a high HPI value are assumed to be more beneficial to human health. In the present work, the highest HPI value (6.1816) was detected in the canola seed oil, followed by UFOO (6.0144), while the blend oil Canoliven recorded the lowest value (5.8067). These finding are in agreement with those reported by Khalili-Tilami and Kouˇrimská, (2022).

3.7. Levels of malondialdehyde (MDA), in fresh and stored oils:

2-Thiobarbituric acid method (2-TBA) was used to measure the concentrations of malondialdehyde in fresh and 60-stored oil samples and the data are presented in **Table (5)**. Concentration of unsaturated aldehyde (malondialdehyde) in fresh oil samples ranged from 0.21 µg/g for CSO to 0.42 for UFOO sample. Moreover, MDA concentrations increased after storage for 60 days at different rates, as they increased by about 11 to 17 folds and the highest increase was 17 folds in the Canoliven, and the least increase was about 12 folds in UFOO oil.

Table 5. Concentrations (µg/g) of MDA in fresh and 60-D stored oils of CSO, UFOO and Canoliven.

Oil samples	Malondialdehyde (µg/g)		MDA formation rate	PUFA (%)
	Fresh oil	60-D stored oils		
CSO	0.21±0.05	3.26±0.12	3rd	10.57
UFOO	0.42±0.05	5.02±0.15	1st	13.42
Canoliven	0.26±0.05	4.56±0.14	2nd	11.43

CSO= Canola seed oil, UFOO= Unfiltered olive oil, Canoliven= binary oil blend composed from Canola + unfiltered olive oil MDA, malondialdehyde (µg/g), MDA formation rate = MDA-FR, Polyunsaturated PUFA

MDA is a widely used oxidative stress biomarker in organisms and an evaluation index of the degree of lipid oxidation in edible oils (Ma *et al.*, 2019 and Ma and Liu, 2017).

3.8. Endogenous bioactive compounds in CSO, UFOO and Canoliven

The concentrations of bioactive compounds existed in two pure individual oils and their mixture Canoliven are given in Table (6). Levels of total phenolic compounds (µg GAE/100 g) in UFOO reached to be 6.3-folds those found in CSO and 3 folds than in Canoliven. Total flavonoids (760±8 µg RE)/00g) in CSO are higher than those found in UFOO and Canoliven and the same trend is observed also in total carotenoids.

Table 6. Some endogenous bioactive compounds act as antioxidants in CSO, UFOO and Canoliven.

Antioxidants	CSO	UFOO	Canoliven
TPCs µg GAE/100 g	38900±389	35020±349	10116±100
TFs (µg RE)/100g	760±8	331±3.0	675±7.0
TCs µg/100 g oil	594±6.0	127±1.3	516±5.0
TChls µg/100 g oil	371±4.0	296±3.0	356±4.0
∑EAS	40625±406	35774+358	11663+116
Order	1st	2nd	3rd

∑EAS=Summation of Endogenous antioxidant sources (µg), CSO= Canola seed oil, UFOO = Unfiltered olive Canoliven= Binary oil blend composed from Canola + unfiltered olive oil (80:20 respectively)

Total chlorophylls (TChls µg/100 g oil) ranged from 296±3.0 for UFOO to 371±4.0 for Canoliven and UFOO contain averaged value. These bioactive compounds play very important roles as antioxidants, in preservation of vegetable oils and inhibit the lipid peroxidation by dependency reduce the rancidity rates. These results are in agreement with the results of Elsorady *et al.*, (2017) who compared filtered and unfiltered olive oil in terms of fatty acid composition, TPCs, TFs, and total carotenoids (Peres *et al.*, 2016). It could be concluded that the higher the phenolic content is the stronger the antioxidative activity of the extract. The antioxidant sources could be (a) endogenous antioxidants, which naturally existed in oil (b) exogenous sources synthetic antioxidants which add to the oils during refining process (Viana da Silva *et al.*, 2021).

3.9. Oxidative stability index (OSI h)

The oxidative stability index (h) at 100 °C and 110 °C was determined with rancimat method and the results are present in Fig. (2). Stability was expressed as the oxidation induction time (h). UFOO recorded the highest oxidation induction time reach to be 29 at 100°C followed by Canoliven blend and CSO. At 110 °C CSO ranked the first position followed by Canoliven and UFOO. The oils with the highest total phenols content have the highest oxidative stability, confirming the positive direct proportion between these parameters (Beltrán *et al.*, 2016).

The differences in OSI values for oils based on a group of factors, including the fatty acids composition of the raw materials, primary moisture, seed moisture, mechanical damage, the presence of pollutants, the maturity of seed, and oil treatment conditions (Ratusz *et al.*, 2016).

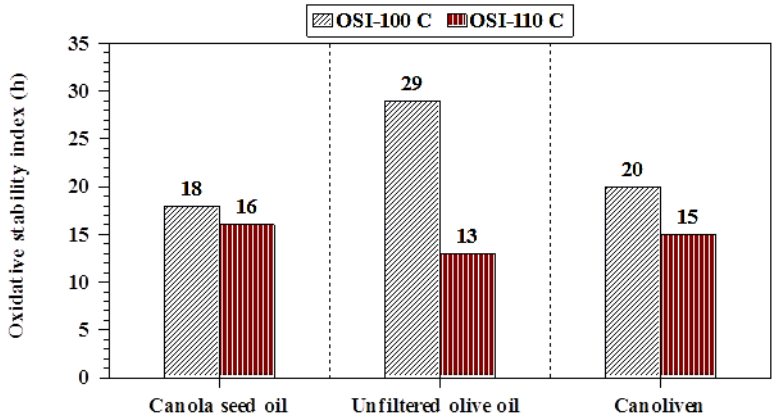

Fig. 4. Oxidative stability index (h) at 100 °C and 110 °C of CSO, UFOO and their blend Canoliven.

In the current research, oil showed a high OSI. The OSI values of the oil samples were arranged as follows: unfiltered olive oil > Canoliven > CSO. The OSI of oil samples contains a high amount of PUFA and can be increased by blending them with high MUFA oils (Bhatnagar *et al.,* 2009). Therefore, the oxidative stability of mixed oils is depended on the MUFA and PUFA contents (Bhatnagar *et al.,* 2009 and Arranz *et al.,* 2008).The contents of both fatty acids composition, in addition to tocopherol were the most important factors affecting the oxidation and thermal stabilities of the oils (Nehdi *et al.,* 2019).

3.10. FTIR of unfiltered oil (UFOO):

Figure 3 shows the FTIR spectrum of unfiltered olive oil (UFOO). There are 12 beaks, of which 8 beaks are in the area confined from 1746.81 to 722.35 cm^{-1}, and four beaks are in the area between 3423.92 and 2854.25 cm^{-1}. The spectrum also, shows that no peaks existed in the area between 2855 and 1747 cm^{-1}.

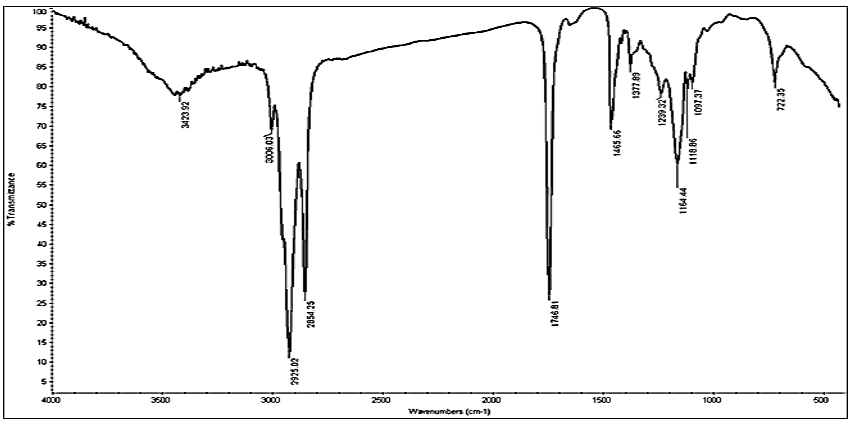

Fig. (5): FTIR spectrum of UFOO.

3.11. FTIR spectrum of CSO:

Figure 4 shows the FTIR spectrum of canola seed oil (CSO) variety Giza 56. There are 17 beaks, of which 13 beaks are in the area confined from 1746.62 to 410 cm^{-1}, and four beaks are in the area between 3299.92 and 2854.33 cm^{-1}. The spectrum also, shows that no peaks existed in the area between 2853 and 1747 cm^{-1}.

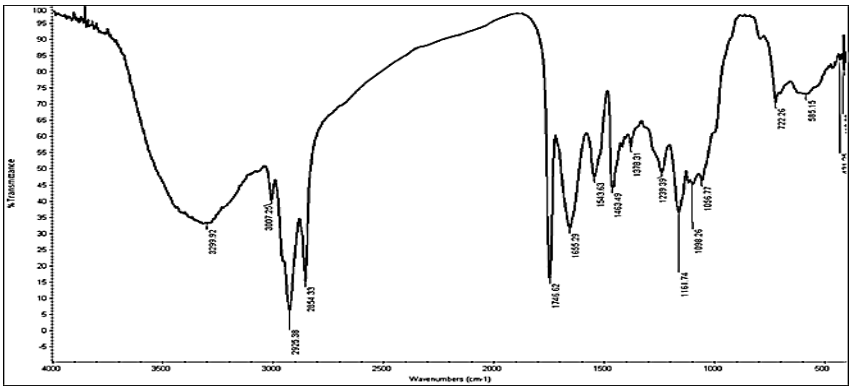

Fig. (6): FTIR spectrum of canola seed oil (CSO) variety Giza 56.

The spectrum of CSO revealed some differences to other vegetable oils including flax seed oil, sunflower seed oil and coconut oil, especially in the regions around 3007.25 and 1655 cm^{-1} and at frequency regions of 1161-1099 cm^{-1}. The present results are in agreement with those found by Che Man and Rohman, (2013).

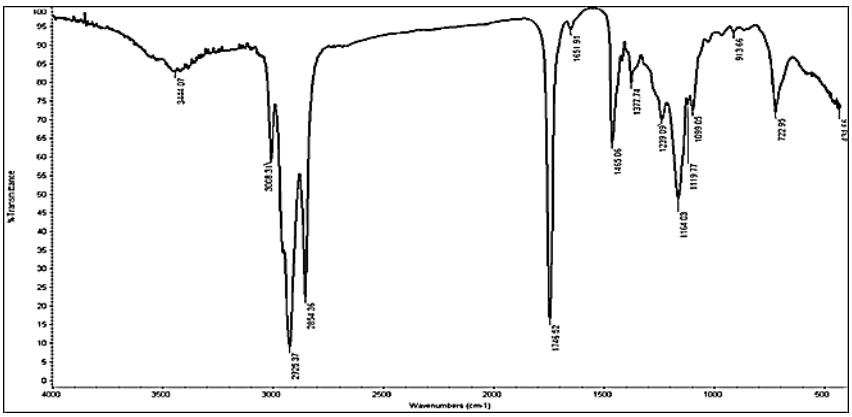

Fig. (7): FTIR spectrum of Canoliven.

3.12. Analysis of FTIR spectra of TPN, LFBs and HFBs of vegetable oil samples

Table (7) show the total peaks number (TPN) in the spectra of oil samples. The individual pure oil CSO variety Giza 56 presented the highest peak number (17) followed by Canoliven (15) and UFOO (12). In the region of lower frequencies (500 to 1746 cm^{-1}) the peaks ranged from 8 for UFOO to 13 for CSO. From the FTIR-spectra could be noticed that the peaks at frequencies from 2800 to 3300 cm^{-1} only four peaks are observed.

Table 7. Analysis of FTIR spectra, total number of peaks (TPN), lower frequencies bands (LFBs) and higher frequencies bands (HFBs) of vegetable oil samples

Samples	LFBs 500 to 1746 cm^{-1}	HFBs 2800 to 3500 cm^{-1}	TPN
UFOO	8	4	12
CSO	13	4	17
Canoliven	11	4	15

TPN = Total peak numbers

Recent developments in FTIR spectroscopy instrumentation extend the application of this technique to the field of food research, facilitating particularly the studies on edible oils and fats. The possible antioxidant effect of oregano is also discussed by Vlachos *et al.*, (2006).

3.13. Effect of some antioxidants on lipid peroxidation inhibition of Canoliven

The lipid peroxidation inhibition activities (LIPIA) of Canoliven were determined by the FTC method. The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation. During handling, storage or cooking edible oils may undergo deterioration processes due to lipid peroxidation. Antioxidant activities of four natural antioxidant extracts, namely (1) Extract of fine powder of pomegranate peels (PPE), (2) Curcumin extract from turmeric (Cur-E), (3) Fine powder of hybrid yellow corn husks (FPH-YCH) (4) Black pepper (BP). In addition to α-Tocopherol, (α-Terol), and two synthetic antioxidants i.e., BHA, BHT, were measured by ferric thiocyanate (FTC) method which measure the amount of peroxide formed during the initial stage of linoleic acid oxidation (Table 8 and Fig. 8).

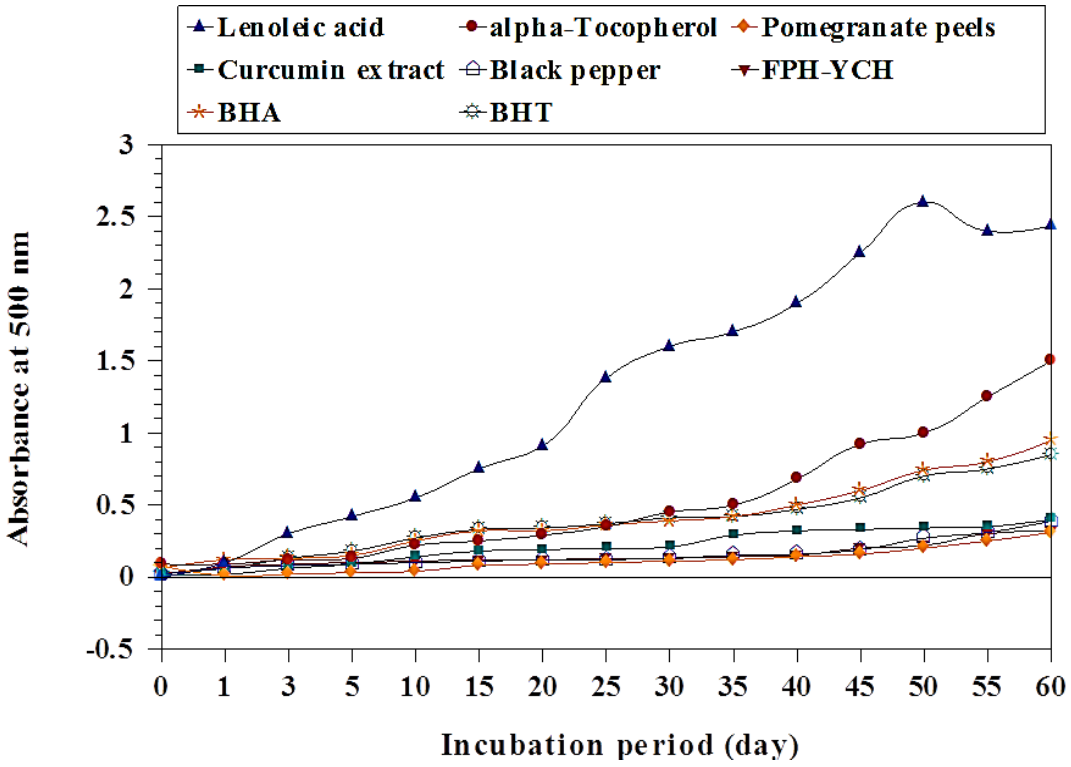

Fig. 8. Effect of some antioxidants on lipid peroxidation inhibition of Canoliven.

The individual activity of PP extracts showed low absorbance values at 500 nm (Fig. 8) which indicated marked inhibition of peroxidation of linoleic acid by all extracts. All of them were more effective than α-tocopherol, a common natural antioxidant. Results generally showed that the PPE the most efficient than the other antioxidants while absorbance values were lower than those reported for synthetic antioxidants. It could be concluded that the higher the secondary metabolites is the stronger the antioxidative activity of the extract. Therefore, lower absorbance indicates a higher level of antioxidant activity. It can be concluded that PPE showed superior efficiency than the all studied antioxidants (Javani-Seraji *et al.*, (2023). A large number of studies related to the use of by-products, such as pomegranate peel, which contain secondary metabolic products, biologically active substances (30%), such as flavonoids, polyphenols, caffeic acid, phenolic acids, anthocyanins, hydrolyzable tannins, and anthocyanins and all of these substances possess antimicrobial and antioxidant activities. It is a highly effective antioxidant and prevents lipid peroxidation (Selahvarzi *et al.*, 2022; Kaderides *et al.*, 2021). Generally, PPE has the potential to improve the functional features of many food products (Javani-Seraji, *et al.*, 2023; Selahvarzi *et al.*, 2022 and Trigo *et al.*, 2020).

Table (8). Effect of some antioxidants on lipid peroxidation inhibition of Canoliven

Incubation period (d)	Linoleic acid (ω-6)	ω-Terol	Natural antioxidant				Synthetic antioxidant	
			PPE	Cur-E	BP	FPH-YCH	BHA	BHT
0	0	0.09	0.08	0.02	0.02	0.02	0.06	0.03
1	0.1	0.09	0.01	0.02	0.06	0.07	0.12	0.06
3	0.3	0.12	0.02	0.06	0.08	0.09	0.13	0.13
5	0.42	0.13	0.03	0.09	0.09	0.1	0.15	0.18
10	0.55	0.22	0.04	0.14	0.1	0.11	0.25	0.27
15	0.75	0.25	0.08	0.18	0.11	0.12	0.32	0.33
20	0.91	0.29	0.09	0.19	0.12	0.12	0.32	0.34
25	1.38	0.35	0.1	0.2	0.12	0.13	0.36	0.37
30	1.6	0.45	0.11	0.21	0.13	0.13	0.39	0.41
35	1.7	0.5	0.12	0.29	0.15	0.14	0.42	0.42
40	1.9	0.68	0.14	0.32	0.16	0.15	0.5	0.47
45	2.25	0.92	0.16	0.33	0.19	0.2	0.6	0.55
50	2.6	1	0.2	0.34	0.27	0.22	0.74	0.7
55	2.4	1.25	0.25	0.35	0.31	0.3	0.8	0.75
60	2.44	1.5	0.31	0.4	0.39	0.32	0.95	0.85
$\sum A_{500}$nm	19.3	7.84	1.74	3.14	2.30	2.22	6.11	5.86
Orders	Prooxidant	7th	1st	4th	3rd	2nd	6th	5th

ω-Terol =α-tocopherol, BHA= Butylated hydroxy anisole, BHT= Butylated hydroxy toluene, (1) Fine powder of pomegranate peels (FP-PP), (2) Curcumin extract from turmeric (Cur-E), (3) Fine powder of hybrid yellow corn husks (FPH-YCH) and (4) Black pepper (BP).

Our results showed that seven antioxidants could be ordered in the following orders: PPE> FPH-YCH > BP> Cur-E> BHT> BHA> α-Tocopherol as according to the ∑absorbance value at 500 nm, These findings are in consistent with those reported by (Im *et al.,* 2014; Choe and Min, 2006 and Hraš *et al.,* 2000). The effect of turmeric on heat-induced lipid peroxidation of edible oils was studied by Souparnika *et al.,* (2015) who showed that a household practice of adding a pinch of turmeric to oil before heating retards the heat induced lipid peroxidation thereby reducing the ill-effects of oxidants on health. Sunflower oil has increase lipid peroxidation and antioxidant status on heating in comparison to coconut oil (Souparnika *et al.,* 2015). Chattopadhyay *et al.,* 2004 reported that turmeric and curcumin have many biological actions and medical applications. In conclusion, the natural extracts of plants exhibited antioxidant activity in linoleic acid. Therefore, the extraction of phenolic compounds from different plants and the seed coasts and their possible use as natural antioxidants to retard lipid oxidation would present new opportunities for one of the waste products of food industry.

Vegetable oils (VOs) have effects on the uptake of zeaxanthin and lutein by adult retinal pigment epithelial (ARPE) cells19. VOs can be used to treat and protect against age-related macular degeneration, in addition to their positive roles in increasing the bioavailability of lutein and zeaxanthin, and their cell-protective activity against oxysterols. The uptake of lutein and zeaxanthin by ARPE-19 cells was found to depend on the type of oil, with the highest uptake of carotenoids observed with coconut oil (Baek *et al.,* 2023).

Each oil combination had high-quality indicators. As a result, synthetic oil blends with high levels of antioxidants, suitable ω-6/ω-3 ratios, and suggested FA compositions can have an impact on human health. In a ternary diagram, the composition of healthy oil blends with ideal ω-6/ω-3 ratios were stated quantitatively (Nehdi *et al.,* 2019).

4. CONCLUSION

In the present article we introduce a newly oil formulation (Canoliven) composed from CSO and UFOO with 80:20 % w/w respectively. Also, the phytoconstituents, physicochemical properties, fatty acid profiles and endogenous bioactive compounds (antioxidants) were evaluated. The present results also show that blending process improve of *p*-AV and TOTOX values, i.e., the blending process led to improving the fatty acids profiles.

The lipid peroxidation inhibition activities (LIPIA) of Canoliven were determined by the Ferric thiocyanate (FTC) method. Antioxidant activities of four natural antioxidant extracts, namely (1)-Extract of fine powder of pomegranate peels (PPE), (2)-Curcumin extract from turmeric (Cur-E), (3)-Extract of fine powder of hybrid yellow corn husks (FPH-YCH) (4)- Extract of black pepper (EBP). In addition to α-Tocopherol, (α-Terol), and two synthetic antioxidants i.e., BHA and BHT, were emulated by FTC method. The individual activity of PPE extract shows low absorbance values at 500 nm which indicated marked inhibition of peroxidation of linoleic acid by all extracts. All of them were more effective than α-tocopherol. Results generally show that the PPE the most efficient than the other antioxidants while absorbance values were lower than those reported for synthetic antioxidants. It can be concluded that PPE show superior efficiency than the all studied antioxidants. Our results show that seven antioxidant inhibitors could be ordered in the following orders: PPE> FPH-YCH > BP> Cur-E> BHT> BHA> α-Tocopherol as according to the $\sum A_{500}$ nm.

The process of mixing CSO and UFOO in the Canoliven mixture improved the oxidative stability parameters and improved the nutritional quality of the final product. This research demonstrates that the use of UFOO in vegetable oil blends can produce an effective level of bioactive compounds, a balanced ω6: ω3 ratio and suitable stability.

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المخلص العربي

الخصائص الفيزيائية والكيميائية، تثبيط بيروكسيد الدهون، ومؤشر الثبات التأكسدي بواسطة البدائل المضادة للأكسدة لمزيج الزيت المختلط الواعد "زيت الكانولا وزيت الزيتون"

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في هذا البحث، تم فحص ثلاث عينات نباتية كيميائياً لزيت بذور الكانولا، زيت الزيتون غير المفلتر وخليط الزيتين الكانولا والزيتون (الكانوليفن بنسبة ٢:٨ على الترتيب). وكشف عن وجود هذه المجاميع الكيميائية في جميع العينات: الستيرويدات، التربينات، الصابونين، العفصيات، قلويدات الأنثوسيانين، الفينولات والفلافونويدات. أشارت النتائج إلى أن زيت بذور الكانولا صنف جيزة ٥٦ يحتوي على ١٢ مجموعة كيميائية مختلفة للمركبات. وتم تقييم أربع خصائص فيزيائية في الثلاث عينات. تتراوح قيمة الحموضة من ٠,٢١ لزيت بذور الكانولا إلى ٠,٣٥ لزيت الزيتون غير المفلتر، وقيمة الحموضة للكانوليفن كانت ٠,٢٥. تم أيضاً تقييم أعلى مستوى من المادة غير القابلة للتصبن في زيت بذور الكانولا، يليه الكانوليفن ثم زيت الزيتون غير المفلتر. وتراوحت قيمة البيروكسيد من ١,٥ لزيت بذور الكانولا إلى ٨,٥ لزيت الزيتون غير المفلتر بينما سجل المزيج الثنائي ٤,٠١. وقد تم تقييم أعلى مستوى (٦,٥) لقيمة بارا–أنيسيدين في زيت الزيتون غير المفلتر يليه زيت بذور الكانولا والكانوليفن. علاوة على تسجيل قيم الأكسدة الكلية في المزيج الثنائي (١١,٢) وأكثر من ضعفين من هذه القيمة في زيت الزيتون غير المفلتر (٢٣,٥).

وتراوحت تركيزات الأحماض الدهنية المشبعة في الزيوت النقية الفردية من ١٦,٢٩% لزيت الزيتون غير المفلتر إلى ١٧,١٦% لزيت بذور الكانولا، بينما سجل زيت كانوليفن ١٧,٠١%. وقد تراوح إجمالي الأحماض الدهنية الأحادية غير المشبعة من ٦٩,٧٦ إلى ٧٢,٠٩% في زيت الزيتون غير المفلتر وزيت بذور الكانولا على التوالي. أدت عملية الخلط إلى تحسين مستوى إجمالي نسبة الأحماض الدهنية الأحادية غير المشبعة حيث وصل تركيزه في الكانوليفين إلى ٧١,٥٦%. بالإضافة لتركيزات الأحماض الدهنية المتعددة غير المشبعة هي ١٣,٤٢، ١٠,٥٧ و١١,٤٣% في زيت الزيتون غير المفلتر، زيت بذور الكانولا والكانوليفن على الترتيب. وأخيراً تم إجراء بعض التحاليل المتخصصة على العينات منها مالونديالدهيد، المحتوى الفينولي، مؤشر استقرار الأكسدة وطيف التحويل فوريه للأشعة تحت الحمراء.