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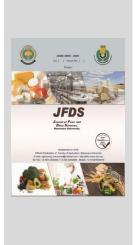
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### Production of Functional Ternary Vegetable Oil Blends Suitable for Dietary and Therapeutic Purposes



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#### ABSTRACT



Cold-pressed vegetable oils are increasingly in demand owing to their superior nutritional quality and health benefits. This investigation aimed to develop functional ternary vegetable oil blends that enhance nutritional, physiochemical, and therapeutic benefits, leveraging the extensive use of virgin vegetable oils. The cold-pressed vegetable oils: flax seed oil (FLO), chia seed oil (CHO), canola seed oil (CAO), sunflower seed oil (SFO), and coconut oil (CNO), as well as their ternary oil blends: Flaxcanochia (FLO+CAO+CHO, 20:40:40), Flaxcanosun (FLO+CAO+SFO, 20:40:40), and Flaxcocosun (FLO+CNO+SFO, 45:10:45), were evaluated for their nutritional and physicochemical properties. The findings confirm that the chosen oilseeds serve as primary sources of vegetable oils with distinctive nutritional and physicochemical properties. The cold-pressed vegetable oils and their ternary oil blends have superior bioactive phytochemicals and antioxidant properties, which may contribute to their health benefits.  $\alpha$ -linolenic acid (omega-3) is the predominant fatty acid in FLO (56.70%) and CHO (59.11%). Linoleic acid (omega-6) is the predominant fatty acid in SFO (54.57%). Oleic acid (omega-9) is the principal fatty acid in CAO (70.23%). Lauric acid is the main fatty acid in CNO (41.21%). The blending process enhanced ternary vegetable oil blends' fatty acid profiles. Flaxcanochia exhibited the highest  $\alpha$ -linolenic acid content (37.85%). Flaxcocosun had the highest linoleic acid content (27.05%). Flaxcanosun had the highest oleic acid content (38.42%). Lauric acid was only present in Flaxcocosun (8.25%). In conclusion, blending different vegetable oils can enhance their nutritional, physiochemical, and therapeutic properties, potentially benefiting the oil industry and preventing chronic and non-chronic diseases.

*Keywords*: Essential fatty acids, endogenous active compounds, vegetable oil blends, nutritional properties, physicochemical properties.

### INTRODUCTION

Fatty acids, whether synthesized or obtained through diet, are essential for our body. They aid in the regulation of membrane architecture and functionality, transcription factor activity, intracellular signaling pathways, and gene expression. However, some, like omega-3 ( $\omega$ -3) and omega-6 ( $\omega$ -6), cannot be synthesized. As a result, the body must consume these essential fatty acids through a diet. The most important dietary fatty acid sources are vegetable oils, oilseeds, meat products, fish oils, and grains (Shahidi and Ambigaipalan, 2018; Li et al., 2019; Monroig et al., 2022; Lichtenstein, 2023).

Edible vegetable oils are indispensable nutritive sources for humans, particularly for essential fatty acids, fatsoluble vitamins, and natural antioxidants. Comprehending the endogenous active compounds present in vegetable oils has become crucial. These bioactive constituents play a vital role in human life because they have many health benefits. Furthermore, they play a crucial role in protecting oils from deterioration that may occur during handling, use, and storage (Sumara et al., 2023; Tuei, 2023; Zhang et al., 2023; Hadidi et al., 2024).

The physicochemical characteristics of vegetable oils are specific indicators for their quality. These properties are related to one or more constituents in vegetable oils' composition. The principal physicochemical properties employed to rate oil quality are their smoke point, acid value, oxidative stability index, peroxide value, saponification value, unsaponifiable substances, para-anisidine value, and iodine value (Paul et al., 1997; Zahir et al., 2017; Bekdeser et al., 2024).

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Food processors and consumers favor cold-pressed vegetable oils due to their low production costs and elevated levels of bioactive constituents. Preserving antioxidant components may confer satisfactory oxidative stability and enhanced health benefits to these vegetable oils. Consequently, there is an increasing demand for cold-pressed vegetable oils due to their superior nutritional quality and health advantages (Chew, 2020; Kaseke et al., 2021; Agah et al., 2024).

Edible vegetable oils are essential ingredients for various food formulations. They lack certain essential characteristics in their natural form since each vegetable oil has its own nutritional and physicochemical characteristics. In other words, no individual oil possesses excellent nutritional and functional characteristics, as well as optimal oxidative stability. Blending vegetable oils is a straightforward, practical, and cost-effective approach to enhance their physicochemical and nutritional characteristics beneficially. Recently, it has emerged as a prevalent practice in the food sector (Sharma and Lokesh, 2013; Choudhary et al., 2015; Hashempour-Baltork et al., 2016; Dhyani et al., 2018; Ndomou et al., 2023; Sharma et al., 2023).

Flax seed oil is a superior functional oil rich in PUSFA (polyunsaturated fatty acids), predominantly  $\alpha$ -linolenic acid

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(40-60%), which is thought to possess numerous advantageous physiological and functional attributes. Flax seed serves as a unique supply of  $\omega$ -3 fatty acids, providing  $\alpha$ linolenic acid for vegetarian diets (Riediger et al., 2008; Ganguly et al., 2021; Al-Madhagy et al., 2023).

Chia seed oil primarily consists of PUSFA (ω-3, αlinolenic acid, 53–66% and  $\omega$ -6, linoleic acid, 12–22%). Therefore, people can refer to this oil as a gourmet oil due to its high-quality and valued flavor, color, and healthful properties. The elevated  $\omega$ -3 content, which exceeds any known plant sources, designates chia seeds as a source of omega-3 functional foods. Besides being used as food, it was also used for medicinal purposes (Fernandes et al., 2021; Agurla et al., 2024).

Canola seed oil contains a lot of tocopherols, phytosterols, and other bioactive substances. It also has a moderate amount of PUSFA and a lot of MUSFA (C18:1 oleic acid, 60%). It possesses a balanced ratio of  $\omega$ -6 and  $\omega$ -3 fatty acids. Canola seed oil is currently considered a significant functional food owing to its function in preventing and managing various disorders and diseases. In addition to cooking oil, people also use canola seed oil to prepare salad dressings, salad oils, shortenings, margarines, and mayonnaises (Jenkins et al., 2014; Loganes et al., 2016; Goyal et al., 2021; Zhang et al., 2023).

Sunflower seed oil has a healthy lipid profile. It contains a high level of linoleic acid (48-74%), an essential fatty acid (C18:2, ω-6). Unlike canola seed oil, sunflower seed oil possesses minimally saturated fatty acid (SFA) contents and negligible a-linolenic acid levels. Several food applications, such as salad dressing, cooking oil, margarine, and shortening, use sunflower seed oil. People prize it for its bland flavor, superior frying quality, and several health advantages (Smith et al., 2016; Khurana and Singh, 2021).

Coconut oil is a rich source of SFA; lauric acid is the major SFA (52.60%). Medium-chain triacylglycerols, readily metabolized, absorbed, and eliminated from the blood, are more abundant in lauric oils like coconut oil than in longchain fatty acids. Consumers have accepted coconut oil as a functional food oil since its introduction to the market, and the demand for this oil is still growing. Coconut oil has recently achieved significant prominence in the health food industry. It has emerged as a potential "miracle" food due to its positive health effects (Kappally et al., 2015; Lima and Block, 2019; Parmar et al., 2021).

The main objective of this investigation is to develop functional ternary vegetable oil blends that enhance nutritional, physiochemical, and therapeutic benefits, leveraging the extensive use of cold-pressed vegetable oils. The cold-pressed vegetable oils (flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil), as well as their ternary oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun), were assessed for their nutritional and physicochemical properties.

#### MATERIALS AND METHODS

#### Materials

The current research utilized freshly harvested four different oilseeds. Brown flax seeds (Linum usitatissimum) were obtained from a local market in Cairo, Egypt. Dr. Ahmed G. Darwesh, USA, kindly provided organic chia seeds (Salvia hispanica). Professor Mohamed Hamam from Shandaweel Agricultural Research Station, Sohag, Egypt, kindly provided canola seeds (Brassica napus) variety Mostawrad-B12. Professor Iman M. Taha of the Field Crops Department, Faculty of Agriculture, Minia University, Minia, Egypt, kindly provided sunflower seeds (Helianthus annuus) variety Giza 120. Coconut oil was obtained from a local market in Minia, Egypt. This research utilized analyticalgrade chemicals obtained from Sigma Aldrich. Methods:

### Preparation of oilseeds for oil extraction:

The used oilseeds (flax seeds, chia seeds, canola seeds, and sunflower seeds) were freshly harvested, manually purified, and cleaned to remove the dust, undesirable seeds, and foreign materials. To extract their oils, the cleaned seeds were immediately crushed and cold-pressed using a mechanical screw press apparatus at room temperature (~ 25°C). The cold-pressed oil samples were collected and filtered. Then transferred into opaque glass containers and preserved below 4°C for analysis and utilization (Agah et al., 2024).

#### Preparation of the ternary vegetable oil blends:

Five different oil types were used to prepare the ternary vegetable oil blends: flax seed oil (FLO), chia seed oil (CHO), canola seed oil (CAO), sunflower seed oil (SFO), and coconut oil (CNO). As shown in Table A, three different ternary vegetable oil blends were prepared using flax seed oil as the base oil and two other oils in different proportions. These proportions were selected to produce new ternary oil blends suitable for both nutritional and therapeutic purposes in Egypt.

Table A. Formulation	of the	ternary	veget	able oi	il blends.
			-		

Ternary	The	The cold-pressed vegetable oils					
oil blends	FLO <sup>1</sup>	CHO <sup>2</sup>	CAO <sup>3</sup>	SFO <sup>4</sup>	CNO <sup>5</sup>		
Flaxcanochia <sup>6</sup>	20	40	40	-			
Flaxcanosun <sup>7</sup>	20	-	40	40	-		
Flaxcocosun <sup>8</sup>	45	-	-	45	10		
<sup>1</sup> Flax seed oil. <sup>2</sup> Ch	ia seed oil. <sup>3</sup> C	anola seed	oil. <sup>4</sup> Sur	nflower s	eed oil. <sup>5</sup>		
					-		

Coconut oil. <sup>6</sup> Flax.canola.chia (ternary oil blend). 8 Flax.coconut.sunflower Flax.canola.sunflower (ternary oil blend). (ternary oil blend).

Blending of the different oil samples was done in suitable flasks with magnetic stirrers for about 60 minutes at a temperature of about 40°C. The oils were mixed well to achieve homogenous blends. The obtained ternary oil blends were placed in opaque glass containers and stored at less than 4°C for analysis and use.

#### Quantitative phytochemicals analysis of oils: **Total phenolics:**

Total phenolics concentration was estimated utilizing the Folin-Ciocalteu reagent (Musa et al., 2011). For extraction, approximately 20 g of each sample was blended with 200 mL of diluted methanol for 5 minutes, followed by centrifugation at 4000 rpm for 15 minutes. The supernatants were obtained and filtered using appropriate filter papers. In each test tube, 1 mL of each extract was combined with 4 mL of distilled water, followed by the incorporation of 5 mL of 0.20 N Folin-Ciocalteu reagent. After 5 minutes, 10 mL of 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> was incorporated and mixed well using a vortex. After 2 hours, the absorption was recorded at 765 nm. An appropriate gallic acid standard curve was prepared to estimate the total phenolics concentration of each sample. The data was presented as mg gallic acid equivalents/100g sample (mg GAE/100g).

#### **Total flavonoids:**

The total flavonoids were estimated employing the colorimetric procedure outlined by Abu Bakar et al. (2009). 2.0 mL of each sample extract was combined with 9.0 mL of distilled water in a test tube, followed by the incorporation of 0.60 mL of 5% (w/v) NaNO<sub>2</sub>. After 6 minutes, 1.20 mL of 10% AlCl<sub>3</sub>.6H<sub>2</sub>O was introduced and permitted to react for 5 minutes. Subsequently, 4.0 mL of 4% NaOH solution (1 *M*) was incorporated and well mixed using a vortex mixer. The absorbances were promptly taken at 510 nm. An appropriate quercetin standard curve was prepared to estimate the flavonoids content. The data was reported as mg quercetin equivalents/100g sample (mg QE/100g).

#### Total carotenoids and total chlorophyll:

Total carotenoids and total chlorophyll concentrations were spectrophotometrically quantified applying the methodology described by Rodilla et al. (2023). For pigments extraction, each sample was diluted in cyclohexane to achieve a final volume of 3 mL, followed by spectrophotometric analysis. Total carotenoids were assessed at an absorption of 472 nm, indicative of lutein, the predominant constituent of the fraction. Total chlorophyll was assessed at an absorption of 670 nm, corresponding to pheophytin a, the notable component. Quantification of carotenoids and chlorophyll was estimated using the subsequent equation:

#### $\mathbf{C} = \left[ \left( \mathbf{E} \times \mathbf{V}_{\mathbf{f}} \right) / \left( \mathbf{E}_{1\%} \times \mathbf{W} \right) \right] \times 10000$

Where: C denotes the ultimate concentration of pigments (mg of chlorophyll or carotene/1000 g of sample), E represents the recorded absorbance at each specified wavelength ( $\lambda$ ), V<sub>f</sub> indicates the ultimate volume of each pigment extract (mL), E<sub>1%</sub> refers to the specific absorption of a 1% solution assessed in 1 cm spectrometer cells, with values of 2000 for lutein and 613 for pheophytin a, whereas W represents the weight of each sample (g).

#### Antioxidant activity:

The antioxidant efficacy (DPPH radical scavenging activity) was assessed using the methodology of Musa et al. (2011). Approximately 15.6 mL of DPPH reagent (12 mg DPPH / 500 mL methanol) was combined with 0.40 mL of each sample extract (100 mg sample/mL extracting solvent) or blank (methanol 50%). The mixtures were allowed to stand for 30 minutes at ambient temperature in darkness for the scavenging reaction. The absorbances were spectrophotometrically measured at 516 nm. DPPH scavenging activity (%) was computed applying the subsequent equation:

 $\begin{array}{l} \label{eq:DPPH} \text{ scavenging activity (%)} \\ = \left[ \left( A_{blank} - A_{sample} \right) / \left. A_{blank} \right] \times 100 \\ \text{Where: A denotes the absorbances at 516 nm.} \end{array}$ 

#### Fatty acids composition:

Fatty acids composition was analyzed via gaschromatography, following the AOAC (2016) and ISO 12966-2 (2017) standard methods. The methylation procedure transformed fatty acids into methyl esters following oil extraction. Abdel-Hameed et al. (2023) detailed the procedure.

#### Physicochemical properties of vegetable oils:

#### **Refractive index:**

Oils' refractive index was assessed at a specified temperature (40°C), following the guidelines of the AOAC (2016) and ISO 6320 (2017) standard methods.

#### Relative density:

The relative density of oil samples was measured using the pyknometric method (ISO 279, 1998). Briefly,

equal volumes of distilled water and oil (at 20°C) were weighed successively in a pre-weighed glass pyknometer of minimum nominal capacity of 5 mL. To reach temperature equilibrium, the pyknometer was submerged in a thermostatically controlled water bath at the desired temperature for about 30 min. The following equation gives the relative density:

#### Relative density = $(M_2 - M_0) / (M_1 - M_0)$

Where:  $M_0$  denotes the empty pyknometer's mass (g);  $M_1$  represents the water-filled pyknometer's mass (g);  $M_2$  denotes the oil-filled pyknometer's mass (g). This quantity is dimensionless, and the result is expressed to three decimal places.

#### Kinematic viscosity:

Through a suitably calibrated viscometer, the kinematic viscosity of oil samples was measured at a specified temperature adhering to the guidelines of the ASTM D445 (2017) standard method.

#### Color values:

By achieving optimal correspondence with a typical color slide, Puangsri et al. (2005) and Abdel-Hameed et al. (2023) used the Lovibond tintometer to assess the color values of oil samples.

#### Acid value:

The acid value of oil samples was assessed following the guidelines of the AOAC (2016) and ISO 660 (2020) standard methods. Approximately 10 g of each oil sample was solubilized in 50 mL of a 1:1 volumetric combination of diethyl ether and ethanol in a 250 mL conical flask, with moderate warming, if necessary. After the addition of an indicator (about 3 drops of phenolphthalein), the mixture was titrated with constant swirling using potassium hydroxide (KOH) standard solution. A single drop of alkali, which induces a subtle yet distinct color change that lasts for at least 15 seconds, denotes the titration endpoint. For each oil sample, a separate blank analysis was also performed. The following equation calculates the acid value:

Acid value (mg KOH/g oil) =  $[(V_1 - V_0) \times C \times 56.1] / M$ Where:  $V_1$  or  $V_0$  denotes the KOH standard solution volume (mL);  $V_1$ is used for the oil sample and  $V_0$  is used for the blank; *C* represents the precise concentration for the KOH standard solution (moles/L); the value 56.1 denotes the molecular weight of KOH; and M represents the mass (g) of the oil sample under examination. Iodine value:

Iodine value of oil samples was estimated following the guidelines of the AOAC (2016) and ISO 3961 (2018) standard methods. About 0.10 g of each oil sample was combined with 20 mL of carbon tetrachloride (CCl<sub>4</sub>) in a 500 mL conical flask. Subsequently, 25 mL of Hanus solution was introduced. This was made by mixing 18.20 g of iodine with 1000 mL of glacial acetic acid, followed by the inclusion of 3 mL of bromine water to enhance the halogen concentration. After sealing, the flask was agitated for one minute. The mixture was permitted to rest for one hour at ambient temperature (~ 25°C) in the absence of light, with continuous shaking every 5 minutes. Upon completion of the reaction period, 20 mL of potassium iodide (10%) and 150 mL of distilled water were incorporated and thoroughly mixed. The liberated iodine was titrated against standard sodium thiosulfate solution (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.1*M*) until the iodine's yellow color almost disappeared. Then, a few drops of the starch solution were introduced, and the titration proceeded until the blue color almost vanished following vigorous agitation. For each oil sample, a separate blank test was also performed. The

iodine value was computed and represented as g  $I_2/100$  g of oil using the following equation:

Indine value =  $[(V_0 - V_1) \times C \times 12.69] / M$ 

Where:  $V_1$  or  $V_0$  denotes the  $Na_2S_2O_3$  standard solution volume (mL);  $V_1$  is used for the oil sample and  $V_0$  is used for the blank; *C* is the precise concentration (moles/L) of the  $Na_2S_2O_3$  standard solution; 12.69 serves as the conversion factor from milliequivalents sodium thiosulfate (meq.  $Na_2S_2O_3$ ) to grams of iodine (with iodine's molecular weight being 126.9 g/mol); and M represents the mass (g) of the oil sample under examination.

#### Saponification value:

The saponification value of oil samples was estimated adhering to the guidelines of the AOAC (2016) and ISO 3657 (2023) standard methods. Approximately 2 g of each oil sample was combined with 25 mL of the ethanolic potassium hydroxide solution (EtOH-KOH, 0.5 mol/L) in a 250 mL conical flask, along with some boiling aids. The flask was attached to a reflux condenser and gradually heated with occasional shaking from time to time for about 1 hour. 0.5-1 mL of the color indicator solution was incorporated into the heated solution. The unreacted KOH was then back titrated with a standard volumetric solution of hydrochloric acid (HCl, 0.5 mol/L) until the color of the indicator changes at the equivalence point. For each oil sample, a separate blank test was also performed. The saponification value was computed and expressed as mg KOH/g oil using the subsequent equation:

Saponification value (mg KOH/g oil) =  $[(V_0 - V_1) \times C \times$ Where:  $V_1$  or  $V_0$  denotes the hydrochloric acid standard solution volume (mL);  $V_1$  is used for the oil sample and  $V_0$  is used for the blank; *C* represents the precise concentration (moles/L) for the hydrochloric acid standard solution used; the value 56.1 denotes the molecular weight of KOH; and M represents the mass (g) of the oil sample under examination. Unsaponifiable matter:

Unsaponifiable matter in oil samples was assessed in accordance with the AOAC (2016) and ISO 18609 (2000) standard methods. In a 250 mL conical flask, approximately 5 g of each oil sample was combined with 50 mL of the ethanolic potassium hydroxide solution (EtOH-KOH, 1 mol/L) and some anti-bumping granules. The flask was attached to the reflux condenser and gradually boiled for about one hour. After the heating ended, 50 mL of water was introduced through the upper section of the condenser and agitated. Upon cooling, the solution was transferred to a 250 mL separating funnel, followed by multiple rinses of the flask and anti-bumping granules with 50 mL of hexane. The rinsings were transferred to the separating funnel, then closed and violently agitated for 1 minute. The pressure was released periodically by flipping the separating funnel and carefully opening the stopcock. Subsequently, permit the mixture to remain undisturbed until complete phase separation occurs. As completely as possible, the lower layer was thoroughly transferred into a second separating funnel. The addition of modest amounts of ethanol, concentrated potassium hydroxide, or sodium chloride solution will disrupt the emulsion created. The aqueous ethanolic soap solution was extracted two additional times, using 50 mL of hexane in the same manner each time. The three hexane extracts were consolidated in one separating funnel. All combined extracts were rinsed three times with 25 mL of the ethanol solution (10%), associated with vigorously shaking and drawing off the aqueous ethanolic solution after each wash. This persisted until the washings ceased to produce a pink color upon the inclusion of a drop of phenolphthalein indicator. The hexane solution was quantitatively transferred from the top of the separating funnel into a pre-weighed 250 mL flask, and then the solvent was evaporated using a boiling water bath. The residue, with the flask positioned nearly horizontally, was dried in an oven at 103°C for 15 minutes. After cooling, the residue was weighed to the nearest 0.10 mg. A blank test was conducted with the identical procedure and quantity of all reagents, excluding the test sample. The unsaponifiable matter content was computed and represented as a mass percentage of the sample applying the subsequent equation:

Unsaponifiable matter (%) =  $[(M_1 - M_2) \times 100] / M_0$ Where:  $M_0$  represents the mass (g) of the test oil sample;  $M_1$  denotes the mass (g) of the remains obtained with the test sample;  $M_2$  is the mass (g) of the residue acquired from the blank.

#### Smoke point:

The Cleveland open cup method was used to assess the smoke point of oil samples, as recommended by the AOCS (2017). Briefly, each oil sample was filled into the cup, heated to 40–50°C, and the temperature was adjusted to  $5^{\circ}$ C/min. The smoke point (°C) was identified by producing a steady plume of bluish smoke.

#### Peroxide value:

The peroxide value of oil samples was estimated following the guidelines of the AOAC (2016) and ISO 3960 (2017) standard methods. Approximately 5 g of each oil 56ample was solubilized in a 50 mL mixture of glacial acetic acid and isooctane (3:2 v/v) within a 250 mL glass-stoppered Erlenmeyer flask. Subsequently, 0.5 mL of saturated potassium iodide (KI) solution was introduced. The mixture was agitated for 1 minute and subsequently stored in darkness at ambient temperature. Immediately, 100 mL of distilled water was introduced, and then the flask was sealed and stirred. The mixture was titrated with a standard solution of 0.1 N sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.1 mol/L) after 0.5 mL of starch solution was added. For each oil sample, a separate blank analysis was also performed. The peroxide value was computed and represented in milliequivalents (meq) of active oxygen per kilogram of oil applying the subsequent equation:

#### Peroxide value (meq. $O_2/kg$ oil) = [( $V_1 - V_0$ ) × C × 100] / M

Where:  $V_1$  or  $V_0$  denotes the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>standard solution volume (mL);  $V_1$  is used for the oil sample and  $V_0$  is used for the blank; *C* represents the precise concentration for the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution (moles/L); and M represents the mass (g) of the oil sample under examination.

#### The oxidative stability index:

The oxidative stability index (OSI) was assessed using the Rancimat device at 100°C. The OSI is defined as the duration necessary for a sample of oil to exhibit significant rancidity, according to Malacrida et al. (2011).

#### **Para-Anisidine value:**

Para-anisidine value of oil samples was estimated in accordance with the AOAC (2016) and ISO 6885 (2016) standard methods. In a 25 mL volumetric flask, about 2 g of each oil sample was solubilized in 5–10 mL of isooctane and adjusted to the appropriate level with the same solvent. The anisidine reagent was prepared on the day of use, 0.125 g of the *p*-anisidine crystals (4-Methoxyaniline) was solubilized in the glacial acetic acid within a 50 mL volumetric flask and diluted to the mark with the same solvent, while avoiding exposure to strong light. Three tests were simultaneously conducted: the unreacted test solution, the reacted test

solution and the blank test. For the unreacted test solution, 5 mL was transferred to a test tube using a pipette, followed by the incorporation of 1 mL of glacial acetic acid. For the reacted test solution, 5 mL of the test solution was transferred to a test tube, followed by the incorporation of 1 mL of anisidine reagent. For the blank test, 5 mL of isooctane was transferred to a test tube, followed by the incorporation of 1 mL of anisidine reagent. All these test tubes were closed by their glass stoppers and shaken well, and subsequently placed in darkness at approximately 23°C for 10 minutes. Following a cumulative reaction duration of approximately 10 minutes subsequent to the introduction of glacial acetic acid or anisidine reagent, the solutions were transferred to clean-dried spectrometer cells. The spectrometer's zero absorption was adjusted to 350 nm in a 10 mm cell utilizing isooctane. Three absorbances were measured against isooctane: A1 from the reacted solution, A<sub>0</sub> from the unreacted test solution, and A<sub>2</sub> from the blank. The anisidine value, which has no dimensions, was computed applying the subsequent equation: *p*-Anisidine value =  $[(100 \text{ QV}) / \text{M}] \times$ 

#### $[1.20 (A_1 - A_2 - A_0)]$

Where: V represents the volume in which the test sample is solubilized (25 mL); M denotes the mass (g) of the test component; Q indicates the concentration of the tested solution sample upon which the anisidine value is based (0.01 g/mL); 1.20 serves as a correction factor.

#### Total oxidation value:

Abreu et al. (2010) calculated the total oxidation value (Totox value), which ensures the oxidative deterioration of lipids, applying the subsequent equation:

# The oxidisability value (Cox value):

Fatemi and Hammond (1980) and Abdel-Hameed et al. (2023) estimated the cox value applying the subsequent equation, which was predicated on the quantity of C18-USFA:

#### Cox value = [1 (% oleic) + 10.30 (% linoleic) + 21.60 (% α-linolenic)] / 100

#### Statistical analysis:

Data statistical analysis was performed in accordance with Snedecor and Cochran (1980), employing the Statistical Package for Social Scientists (SPSS) software. All the data are presented in the form of arithmetic averages with standard deviation values (SD).

#### **RESULTS AND DISCUSSION**

#### Quantitative phytochemicals analysis of cold-pressed vegetable oils:

There is an increasing interest in cold-pressed or virgin vegetable oils because they retain higher amounts of bioactive components. Comprehending the endogenous active compounds present in these oils has become crucial. These bioactive constituents play a vital role in human life because they have many health benefits. Moreover, they play a crucial role in protecting oils from deterioration that may occur during handling, use, and storage.

To assess the bioactive phytochemicals (total phenolics, total flavonoids, total carotenoids, total chlorophyll) and antioxidant properties (DPPH scavenging activity) of cold-pressed vegetable oils such as flax seed oil, chia seed oil, canola seed oil, and sunflower seed oil, a quantitative phytochemical analysis was performed. The results presented in Table 1 revealed that the total phenolic values for these vegetable oils ranged from 35.90 to 71.85 mg GAE/100g. The total flavonoids values were 6.20 - 17.80 mg QE/100g. The total carotenoids values were 0.57 - 0.99 mg/100g. The total chlorophyll values were 0.19 - 1.77 mg/100g. There were wide variations of the phytochemicals' composition among the examined vegetable oils. For example, chia seed oil recorded the highest total phenolics content (71.85 mg GAE/100g), followed by sunflower seed oil (43.90 mg GAE/100g) and flax seed oil (38.90 mg GAE/100g). Canola seed oil had the lowest total phenolics Totox value = [(2 × Peroxide value) + (p-Anisidine value) tontent, at 35.90 mg GAE/100g. Chia seed oil recorded the highest total flavonoids content (17.80 mg QE/100g), followed by flax seed oil (8.50 mg QE/100g) and canola seed oil (7.60 mg QE/100g). Sunflower seed oil had the lowest total flavonoids content, measuring 6.20 mg QE/100g. Chia seed oil recorded the highest total carotenoids content (0.99 mg/100g), followed by sunflower seed oil (0.71 mg/100g) and flax seed oil (0.70 mg/100g). Canola seed oil had the lowest total carotenoids content, measuring 0.57 mg/100g. Concerning the total chlorophyll, chia seed oil recorded the highest values (1.77 mg/100g), followed by flax seed oil (0.56 mg/100g) and canola seed oil (0.47 mg/100g). Sunflower seed oil recorded the lowest total chlorophyll content (0.19 mg/100g).

Table 1. Bioactive	phytochemicals and	l antioxidant properti	ies of the cold-presse	d vegetable oils.

A ¥	The cold-pressed vegetable oils				
Phytochemicals*	Flax seed oil (FLO)	Chia seed oil (CHO)	Canola seed oil (CAO)	Sunflower seed oil (SFO)	
Total phenolics (mg GAE/100g)**	$38.90 \pm 0.15$	$71.85 \pm 0.72$	$35.90 \pm 0.14$	$43.90 \pm 0.20$	
Total flavonoids (mg QE/100g)***	$8.50 \pm 0.50$	$17.80\pm0.21$	$7.60 \pm 0.10$	$6.20\pm0.10$	
Total carotenoids (mg/100g)	$0.70 \pm 0.04$	$0.99 \pm 0.08$	$0.57 \pm 0.04$	$0.71 \pm 0.05$	
Total chlorophyll (mg/100g)	$0.56 \pm 0.05$	$1.77 \pm 0.07$	$0.47 \pm 0.05$	$0.19 \pm 0.01$	
DPPH scavenging activity (%)	$29.30 \pm 2.00$	$34.18 \pm 0.83$	$26.50 \pm 1.90$	$33.10 \pm 2.10$	
* Means of three determinations ± SD.	** (mg gallic acid equivale	nts/100g sample).	*** (mg quercetin equival	ents/100g sample).	

The antioxidant properties (DPPH scavenging activity) of vegetable oils showed a similar trend to the contents of total phenolics and total flavonoids. Their values ranged between 29.30 and 34.18%. Chia seed oil recorded the highest value of the DPPH scavenging activity (34.18%), followed by sunflower seed oil (33.10%) and flax seed oil (29.30%). Canola seed oil recorded the lowest DPPH scavenging activity (26.50%). These findings indicated that cold-pressed vegetable oils are excellent sources of natural antioxidants because they contain phenolic compounds. The

phytochemicals present in these vegetable oils may be largely responsible for their health benefits. Total phenolic compounds are bioactive plant secondary metabolites. They present considerable amounts in oilseeds and their oils and are valuable for human health. It is widely recognized that the functional groups of phenolic compounds widely differ in their antioxidant properties, which could explain these results. Literature previously reported related observations (Bayram et al., 2012; Rodilla et al., 2023).

# Quantitative phytochemicals analysis of the ternary vegetable oil blends:

The quantitative phytochemicals analysis was performed to assess the bioactive phytochemicals and antioxidant properties (DPPH scavenging activity) of the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun). The results are presented in Table 2. From which it could be observed that the total phenolic values for these ternary vegetable oil blends ranged from 46.20 to 54.80 mg GAE/100g. The total flavonoids values were 9.20-12.50 mg QE/100g. The total carotenoids values were 0.46 - 0.96mg/100g. The total chlorophyll values were 0.32 - 0.70mg/100g. There were close variations in the phytochemicals' composition among the examined oil blends. For example, Flaxcocosun recorded the highest total phenolics content (54.80 mg GAE/100g), followed by Flaxcanochia (53.70 mg GAE/100g). Flaxcanosun recorded the lowest total phenolics content (46.20 mg GAE/100g). A similar trend was also noted for the total flavonoids content. Flaxcocosun recorded the highest total flavonoids content (12.50 mg QE/100g), followed by Flaxcanochia (11.30 mg QE/100g). Flaxcanosun had the lowest total flavonoids content, measuring 9.20 mg QE/100g. In terms of total carotenoids and total chlorophyll, Flaxcocosun also recorded the highest values (0.96 and 0.70 mg/100g), followed by Flaxcanochia (0.89 and 0.60 mg/100g). Flaxcanosun, on the other hand, recorded the lowest values of 0.46 and 0.32 mg/100g, respectively.

The amounts of total phenolics and total flavonoids correlated with the antioxidant activity of the ternary vegetable oil blends. The DPPH scavenging activity values ranged between 34.80 and 41.30%. Flaxcocosun had the highest DPPH scavenging activity (41.30%), followed by Flaxcanochia (40.40%). Flaxcanosun recorded the lowest DPPH scavenging activity (34.43%). All the ternary oil blends had higher content of total phenolics and total flavonoids, resulting in better antioxidant properties than their individual oils. These results indicated that the ternary vegetable oil blends are superior sources of natural antioxidants, owing to the existence of phenolic substances. The phytochemicals present in these oil blends may be largely responsible for their health benefits.

Table 2. Bioactive phytochemicals and antioxidant	properties of the ternary yegetable oil blends.

Phytochemicals <sup>*</sup>		The ternary oil blends	
<b>J</b>	Flaxcanochia <sup>1</sup>	Flaxcanosun <sup>2</sup>	Flaxcocosun <sup>3</sup>
Total phenolics (mg GAE/100g)**	$53.70 \pm 0.45$	$46.20 \pm 0.16$	$54.80 \pm 0.56$
Total flavonoids (mg QE/100g)***	$11.30 \pm 0.50$	$9.20 \pm 0.20$	$12.50 \pm 0.60$
Total carotenoids (mg/100g)	$0.89 \pm 0.08$	$0.46 \pm 0.04$	$0.96 \pm 0.08$
Total chlorophyll (mg/100g)	$0.60 \pm 0.05$	$0.32 \pm 0.05$	$0.70 \pm 0.06$
DPPH scavenging activity (%)	$40.40 \pm 2.70$	$34.80 \pm 2.50$	$41.30 \pm 2.80$
* Moong of three determinations   SD ** (mg	callie agid aquivalents/100g comple)	*** (ma granatin agrinalanta/	100g commle) 1 Flow cood oil

\* Means of three determinations  $\pm$  SD. \*\* (mg gallic acid equivalents/100g sample).\*\*\* (mg quercetin equivalents/100g sample). <sup>1</sup> Flax seed oil + Canola seed oil + Chia seed oil + Chia seed oil + Colored (20:40:40). <sup>2</sup> Flax seed oil + Canola seed oil + Sunflower seed oil (20:40:40). <sup>3</sup> Flax seed oil + Coconut oil + Sunflower seed oil (45:10:45).

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In general, total phenolics are more dominant than total flavonoids in the studied vegetable oil samples. The current findings show that the blending process clearly increased the amounts of total phenolics and total flavonoids. These naturally occurring bioactive substances, which are strong antioxidants, enhance the product's resistance to oxidation and extend its shelf life. In detail, researchers have investigated the known functions of total phenolics in oilseeds. These functions could include high-potential antioxidants, anti-rancidity, antimicrobial, anti-insect, and defensive compounds.

#### Fatty acids composition of the cold-pressed vegetable oils:

Fatty acid methyl esters of cold-pressed vegetable oils (flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil) were examined employing gas chromatography. Table 3 and Figs. 1–2 detail the findings. The analysis reveals that  $\alpha$ -linolenic acid (C18:3, 56.70%), oleic acid (C18:1, 18.50%), linoleic acid (C18:2, 14.01%), palmitic acid (C16:0, 5.80%), and stearic acid (C18:0, 4.42%) are the predominant fatty acids in flax seed oil. The minor fatty acids are arachidic acid (C20:0, 0.16%), gadoleic acid (C20:1, 0.16%), margaric acid (C17:0, 0.09%), palmitoleic acid (C16:1, 0.08%), myristic acid (C14:0, 0.04%), and Cis-10-Heptadecanoic acid (C17:1, 0.04%).

The data in Table 3 indicate that saturated fatty acids (SFA) comprise approximately 10.51%, while unsaturated fatty acids (USFA) constitute around 89.49% of the total fatty acids. The monounsaturated fatty acids (MUSFA) content is 18.78%, and the polyunsaturated fatty acids (PUSFA) content is 70.71%. The ratio of PUSFA to SFA, reflecting the unsaturation degree of flax seed oil and its susceptibility to oxidative processes, is around 6.73. The omega-6 to omega-3

ratio is 0.25. Flax seed oil is a significant source of  $\alpha$ -linolenic acid (omega-3), which is advantageous for human health.

Table	3.	Fatty	acids	composition	of	the	cold-pressed
		veget	able oi	ls.			

		The cold-pressed vegetable oils					
Ту	pe of fatty acids <sup>*</sup>	FLO <sup>1</sup>	CHO <sup>2</sup>	CAO <sup>3</sup>	SFO <sup>4</sup>	CNO 5	
~	C8:0 Caprylic acid	-	-	-	-	0.27	
acids	C10:0 Capric acid	-	-	-	-	3.91	
	C12:0 Lauric acid	—	-	-	—	41.21	
E.	C14:0 Myristic acid	0.04	0.06	0.01	0.10	23.90	
1 fa	C16:0 Palmitic acid	5.80	6.43	14.17	11.30	16.50	
Saturated fatty	C17:0 Margaric acid	0.09	0.06	0.10	0.07	-	
nra	C18:0 Stearic acid	4.42	4.01	2.45	1.83	3.14	
Sat	C20:0 Arachidic acid	0.16	0.42	0.43	0.40	-	
•1	C22:0 Behenic acid	-	0.08	—	—	_	
	C16:1 Palmitoleic acid	0.08	0.09	1.49	0.11	_	
acids	C17:1 Cis-10- Heptadecanoic acid	0.04	0.05	0.06	0.01	-	
fatty	C18:1 Oleic acid (omega-	18.50	7.44	70.23	29.30	9.47	
rated	ŹC18:2 Linoleic acid ≂(omega-6)**	14.01	20.54	8.65	54.57	1.60	
Unsaturated fatty	C18:3 Linolenic acid (omega-3)**	56.70	59.11	0.92	0.90	-	
	C20:1 Gadoleic acid	0.16	0.23	0.31	0.25	-	
SF.	A (%)	10.51	11.06	17.16	13.70	88.93	
US	FA (%)	89.49	87.46	81.66	85.14	11.07	
	onounsaturated fatty acids USFA) (%)	18.78	7.81	72.09	29.67	9.47	
	yunsaturated fatty acids JSFA) (%)	70.71	79.65	9.57	55.47	1.60	
	SFA/SFA	6.73	7.20	0.56	4.05	0.02	
On	nega-6 / Omega-3	0.25	0.35	9.40	60.63		
* 1	·	1 . 6 4 . 4	-1 f-44-		E 4*	1 6 44	

\* Expressed as a percentage (%) of total fatty acids.\*\* Essential fatty acids.<sup>1</sup> Flax seed oil.<sup>2</sup> Chia seed oil.<sup>3</sup> Canola seed oil.<sup>4</sup> Sunflower seed oil. <sup>5</sup> Coconut oil.

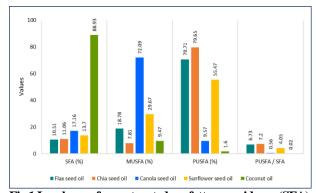


Fig.1.Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFA), and the PUSFA/SFA ratio for the cold-pressed vegetable oils.

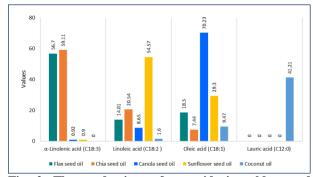


Fig. 2. The predominant fatty acids in cold-pressed vegetable oils (flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil).

It is well known that omega-3 ( $\omega$ -3) and omega-6 ( $\omega$ -6) fatty acids cannot be synthesized in the human body. As a result, the body must consume these essential fatty acids through a diet. For example,  $\alpha$ -linolenic acid serves as a forerunner for long-chain derivatives like eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are the two most important  $\alpha$ -linolenic acid derivatives for the human body. The optimal ratio for  $\omega$ -3 and  $\omega$ -6 fatty acids is approximately 5  $\omega$ -6 to 1  $\omega$ -3, according to nutritionists (Shahidi and Ambigaipalan, 2018; Li et al., 2019; Monroig et al., 2022; Lichtenstein, 2023).

Flax seed oil is a superior functional oil rich in USFA, predominantly  $\alpha$ -linolenic acid (40 – 60%), which is thought to possess numerous advantageous physiological and functional attributes. The remaining fatty acids present in flax seed oil are oleic acid (13 – 19%), linoleic acid (12 – 17%), palmitic acid (5 – 8%), and stearic acid (2 – 4.5%). The  $\omega$ -6 to  $\omega$ -3 ratio in flax seed oil is about 0.30. Flax seed serves as a unique supply of  $\omega$ -3 fatty acids, providing  $\alpha$ -linolenic acid for vegetarian diets. The distinctive chemical composition of flax seed oil, the principal component of flax seeds, is primarily responsible for its numerous therapeutic benefits (Riediger et al., 2008; Ganguly et al., 2021; Al-Madhagy et al., 2023).

According to the data in Table 3, chia seed oil has an almost identical fatty acids profile to flax seed oil. The principal fatty acids in chia seed oil are  $\alpha$ -linolenic acid (C18:3, 59.11%), linoleic acid (C18:2, 20.54%), oleic acid (C18:1, 7.44%), palmitic acid (C16:0, 6.43%), and stearic acid (C18:0, 4.01%). The minor fatty acids are arachidic acid (C20:0, 0.42%), gadoleic acid (C20:1, 0.23%), palmitoleic

acid (C16:1, 0.09%), behenic acid (C22:0, 0.08%), myristic acid (C14:0, 0.06%), margaric acid (C17:0, 0.06%), and Cis-10-Heptadecanoic acid (C17:1, 0.05%). The SFA account for about 11.06%, and the USFA account for about 87.46% of the total fatty acids. The MUSFA content is 7.81%, and the PUSFA content is 79.65%. The PUSFA/SFA ratio is approximately 7.20, reflecting the high degree of unsaturation in chia seed oil and its susceptibility to oxidation. The ratio of  $\omega$ -6 to  $\omega$ -3 is 0.35. Chia seed oil is a rich source of  $\alpha$ -linolenic acid ( $\omega$ -3), advantageous for human health.

Chia seed oil primarily consists of PUSFA, particularly  $\alpha$ -linolenic acid (53 – 66%) and linoleic acid (12 -22%). The intake of these essential fatty acids can help prevent various chronic diseases. Both  $\alpha$ -linolenic ( $\omega$ -3) and linoleic ( $\omega$ -6) acids are vital for cardiovascular health, and they reduce the risk of hypertension, diabetes, obesity, arthritis, and autoimmune disorders, among other conditions. Alongside these essential fatty acids, palmitic acid, oleic acid, and stearic acid are found in minor quantities. Therefore, people can refer to this oil as a gourmet oil due to its high-quality and valued flavor, color, and healthpromoting attributes. Furthermore, chia seed oil can undergo micro- and nanoencapsulation to facilitate the integration and conservation of fatty acids in food products (Ixtaina et al., 2011; Ixtaina et al., 2015; Timilsena et al., 2017; Fernandes et al., 2021; Agurla et al., 2024).

Unlike flax seed oil and chia seed oil, different fatty acids profile was observed for canola seed oil. It is primarily composed of oleic acid (C18:1, 70.23%), palmitic acid (C16:0, 14.17%), and linoleic acid (C18:2, 8.65%). The minor fatty acids are stearic acid (C18:0, 2.45%), palmitoleic acid (C16:1, 1.49%),  $\alpha$ -linolenic acid (C18:3, 0.92%), arachidic acid (C20:0, 0.43%), gadoleic acid (C20:1, 0.31%), margaric acid (C17:0, 0.10%), Cis-10-Heptadecanoic acid (C17:1, 0.06%), and myristic acid (C14:0, 0.01%). The SFA account for about 17.16%, while the USFA account for about 81.66% of the total fatty acids. The MUSFA content is 72.09%, and the PUSFA content is 9.57%. The PUSFA/SFA ratio, reflecting the unsaturation degree of canola seed oil and its propensity for oxidative processes, is approximately 0.56. The ratio of  $\omega$ -6/ $\omega$ -3 is 9.40.

Canola seed oil is an abundant source of oleic acid, which is advantageous for human health. It can improve the human body's nutritional assimilation. In addition, oleic acid serves as a marker of elevated stability in frying oils. Canola seed oil possesses a balanced ratio of  $\omega$ -6 and  $\omega$ -3 fatty acids. Nowadays, it is considered a significant functional food due to its role in preventing the risks of various diseases (i.e., hypercholesterolemia, cardiovascular diseases, type II diabetes, etc.). Researchers have employed genetic engineering to boost the oleic acid content from the standard 60% to 85%, thereby improving the oxidative stability of canola seed oil and extending its shelf life. Additionally, varieties abundant in α-linolenic acid were produced using plant breeding procedures to provide health advantages (Jenkins et al., 2014; Loganes et al., 2016; Goyal et al., 2021; Zhang et al., 2023).

Concerning sunflower seed oil, the main fatty acids are linoleic acid (C18:2, 54.57%), oleic acid (C18:1, 29.30%), and palmitic acid (C16:0, 11.30%). The minor fatty acids are stearic acid (C18:0, 1.83%),  $\alpha$ -linolenic acid (C18:3, 0.90%), arachidic acid (C20:0, 0.40%), gadoleic acid (C20:1, 0.25%), palmitoleic

acid (C16:1, 0.11%), myristic acid (C14:0, 0.10%) margaric acid (C17:0, 0.07%), and Cis-10-Heptadecanoic acid (C17:1, 0.01%). The SFA account for about 13.70%, and the USFA account for about 85.14% of the total fatty acids. The MUSFA content is 29.67% and the PUSFA content is 55.47%. The PUSFA/SFA ratio, reflecting the unsaturation level of sunflower seed oil and its propensity for oxidative reactions, is approximately 4.05. The ratio of  $\omega$ -6/ $\omega$ -3 is 60.63.

It is essential to acknowledge that conventional sunflower seed oil is notably abundant in linoleic acid (C18:2,  $\omega$ -6, 48–74%). In contrast to canola seed oil, it possesses minimal levels of SFA and negligible amounts of  $\alpha$ -linolenic acid. Mutation and/or breeding techniques led to the development of different varieties of sunflower seeds. Just one or two fatty acids differentiated these new varieties from each other, catering to distinct functional and nutritional needs for various food applications. Based on the principal fatty acids present, they can be divided into three types: linoleic, mid-oleic, and high oleic. Mid- and high-oleic sunflower seed oils have superior oxidative stability compared to conventional sunflower seed oil. People prize sunflower seed oil for its bland flavor, exceptional frying quality, and abundance of health advantages (Khurana and Singh, 2021).

In the case of coconut oil, the predominant fatty acids are lauric acid (C12:0, 41.21%), myristic acid (C14:0, 23.90%), palmitic acid (C16:0, 16.50%), and oleic acid (C18:1, 9.47%). The minor fatty acids are capric acid (C10:0, 3.91%), stearic acid (C18:0, 3.14%), linoleic acid (C18:2, 1.60%), and caprylic acid (C8:0, 0.27%). The SFA account for about 88.93%, and the USFA account for about 11.07% of the total fatty acids. The MUSFA content is 9.47% and the PUSFA content is 1.60%. The PUSFA/SFA ratio, reflecting the unsaturation level of coconut oil and its propensity for oxidative processes, is approximately 0.02. The ratio of  $\omega$ -6/ $\omega$ -3 is not estimated for coconut oil due to the absence of  $\alpha$ linolenic acid ( $\omega$ -3).

Coconut oil is a rich source of SFA; lauric acid is the predominant (52.60%) saturated fatty acid. Medium-chain triacylglycerols, readily metabolized, absorbed, and eliminated from the blood, are more abundant in lauric oils like coconut oil than in long-chain fatty acids. Consumers have accepted coconut oil as a functional food oil since its introduction to the market, and the demand for this oil is still growing. Coconut oil has recently achieved prominence in the health food sector. It has emerged as a potential "miracle" food due to its positive health effects (Kappally et al., 2015; Lima and Block, 2019; Parmar et al., 2021).

Numerous studies have demonstrated the potent bactericidal activities of lauric acid (C12:0), a medium-chain fatty acid (MCFA) prevalent in natural products like coconut oil. Because it is the precursor to monolaurin, lauric acid exhibits a wide range of antimicrobial activities against enveloped viruses and diverse bacteria. It may be beneficial to safeguard against microbial infections and regulate the balance and distribution of bacteria in the human gut microbiota (Matsue et al., 2019; Parmar et al., 2021).

# Fatty acids composition of the ternary vegetable oil blends:

In comparison with the individual vegetable oils, the fatty acids composition of the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun) are shown in Table 4 and Figs. 3 - 8. The main fatty acids in Flaxcanochia are  $\alpha$ -linolenic acid (C18:3, 37.85%), oleic acid (C18:1, 25.95%), linoleic acid (C18:2, 22.89%), palmitic acid (C16:0,

7.04%), and stearic acid (C18:0, 3.95%). The lesser fatty acids include palmitoleic acid (C16:1, 0.43%), arachidic acid (C20:0, 0.33%), gadoleic acid (C20:1, 0.25%), margaric acid (C17:0, 0.07%), behenic acid (C22:0, 0.05%), Cis-10-Heptadecanoic acid (C17:1, 0.03%), and myristic acid (C14:0, 0.02%). The SFA account for about 11.46%, and the USFA account for about 87.40% of the total fatty acids. The MUSFA content is 26.66% and the PUSFA content is 60.74%. The PUSFA/SFA ratio, reflecting the unsaturation level of Flaxcanochia oil blend and its propensity for oxidative processes, is approximately 5.30. The ratio of  $\omega$ -6 to  $\omega$ -3 is 0.60.

Regarding the fatty acids profile of Flaxcanosun, the predominant fatty acids are oleic acid (C18:1, 38.42%), linoleic acid (C18:2, 25.74%),  $\alpha$ -linolenic acid (C18:3, 20.00%), and palmitic acid (C16:0, 11.0%). The minor fatty acids are stearic acid (C18:0, 2.79%), palmitoleic acid (C16:1, 1.09%), arachidic acid (C20:0, 0.30%), gadoleic acid (C20:1, 0.20%), myristic acid (C14:0, 0.11%), margaric acid (C17:0, 0.08%), and Cis-10-Heptadecanoic acid (C17:1, 0.05%). The SFA account for about 14.28%, and the USFA account for about 85.50% of the total fatty acids. The MUSFA content is 39.76% and the PUSFA content is 45.74%. The PUSFA/SFA ratio is 3.20. The  $\omega$ -6 to  $\omega$ -3 ratio is 1.29.

In the case of Flaxcocosun, the predominant fatty acids are linoleic acid (C18:2, 27.05%), oleic acid (C18:1, 26.10%),  $\alpha$ -linolenic acid (C18:3, 23.04%), palmitic acid (C16:0, 9.24%), and lauric acid (C12:0, 8.25%). The minor fatty acids are stearic acid (C18:0, 2.82%), myristic acid (C14:0, 2.46%), capric acid (C10:0, 0.40%), arachidic acid (C20:0, 0.23%), gadoleic acid (C20:1, 0.17%), palmitoleic acid (C16:1, 0.10%), margaric acid (C17:0, 0.08%), caprylic acid (C8:0, 0.03%), and Cis-10-Heptadecanoic acid (C17:1, 0.03%).

 Table 4. Fatty acids composition of the ternary vegetable oil blends.

on bienus.					
Туре	of	The t	ernary oil bl	ends	
fatty	acids*	Flaxcanochia <sup>1</sup>	Flaxcanosun <sup>2</sup>	Flaxcocosun <sup>3</sup>	
	C8:0 Caprylic acid	-	_	0.03	
	C10:0 Capric acid	_	_	0.40	
C III	C12:0 Lauric acid	_	_	8.25	
l fa FA	C14:0 Myristic acid	0.02	0.11	2.46	
S	C16:0Palmitic acid	7.04	11.0	9.24	
Saturated fatt acids(SFA)	C17:0 Margaric acid	0.07	0.08	0.08	
Sat	C18:0 Stearic acid	3.95	2.79	2.82	
•1	C20:0 Arachidic acid	0.33	0.30	0.23	
	C22:0 Behenic acid	0.05	_	_	
	6:1 Palmitoleic acid	0.43	1.09	0.10	
acids	Cis-10-Heptadecanoic acid	0.03	0.05	0.03	
l fatty FA)	C18:1 Oleic acid (omega-9)	25.95	38.42	26.10	
Unsaturated fatty acids (USFA)	C18:2 Linoleic acid (omega-6)**	22.89	25.74	27.05	
Unsat	C18:3 Linolenic acid (omega-3)**	37.85	20.00	23.04	
<u> </u>	C20:1 Gadoleic acid	0.25	0.20	0.17	
SFA (	%)	11.46	14.28	23.51	
USFA	A (%)	87.40	85.50	76.49	
	unsaturated fatty acids SFA) (%)	26.66	39.76	26.40	
(PÚSI	nsaturated fatty acids FA) (%)	60.74	45.74	50.09	
PUSF	A/SFA	5.30	3.20	2.13	
Omeg	ga-6 / Omega-3	0.60	1.29	1.18	

\* Expressed as a percentage (%) of total fatty acids. \*\* Essential fatty acids. <sup>1</sup>Flax seed oil+Canola seed oil+Chia seed oil (20:40:40). <sup>2</sup>Flax seed oil+Canola seed oil+Sunflower seed oil (20:40:40). <sup>3</sup>Flax seed oil+Coconut oil+Sunflower seed oil (45:10:45).

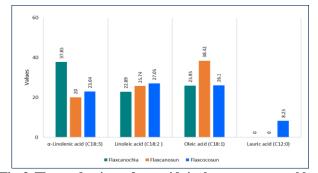


Fig. 3. The predominant fatty acids in the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun).

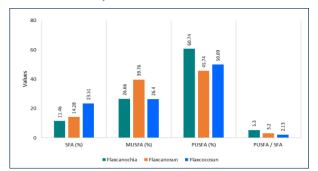


Fig.4.Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFA), and the PUSFA/SFA ratio for the ternary vegetable oil blends.

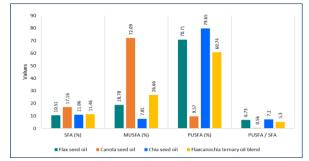


Fig. 5. Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFA), and the PUSFA/SFA ratio for flax seed oil, chia seed oil, canola seed oil, and their Flaxcanochia ternary oil blend.

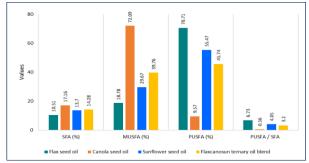


Fig. 6. Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFA), and the PUSFA/SFA ratio for flax seed oil, canola seed oil, sunflower seed oil, and their Flaxcanosun ternary oil blend.

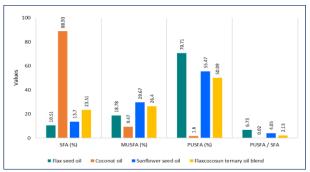


Fig. 7. Levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUSFA), polyunsaturated fatty acids (PUSFA), and the PUSFA/SFA ratio for flax seed oil, coconut oil, sunflower seed oil, and their Flaxcocosun ternary oil blend.

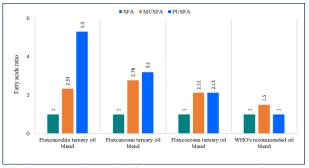


Fig. 8. Fatty acids ratio of the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun).

The SFA account for about 23.51%, and the USFA account for about 76.49% of the total fatty acids. The MUSFA content is 26.40% and the PUSFA content is 50.09%. The PUSFA to SFA ratio is 2.13. The  $\omega$ -6 to  $\omega$ -3 ratio is 1.18.

The Flaxcocosun ternary oil blend naturally produces medium-chain fatty acids (MCFAs) from coconut oil. Caprylic, capric, and lauric acids are examples of MCFAs. These MCFAs inhibit cell signaling, regulate glucose and lipid metabolism, and break down substrates more quickly than long-chain fatty acids (LCFAs). This has led to more clinical benefits, including potential use in treating metabolic and neurological disorders, as reported by Jadhav and Annapure (2023).

Figs. 2 and 3 summarize the predominant fatty acids in cold-pressed vegetable oils (flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil) and the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun, and Flaxcocosun), respectively. As can be seen,  $\alpha$ -linolenic acid  $(\omega$ -3) is the predominant fatty acid in flax seed oil (56.70%) and chia seed oil (59.11%). Linoleic acid ( $\omega$ -6) is the predominant fatty acid in sunflower seed oil (54.57%). Oleic acid ( $\omega$ -9) is the predominant fatty acid in canola seed oil (70.23%). Lauric acid is the predominant fatty acid in coconut oil (41.21%). As a result, the Flaxcanochia ternary oil blend had the highest  $\alpha$ -linolenic acid content (37.85%), followed by Flaxcocosun (23.04%) and Flaxcanosun (20%). Flaxcocosun had the highest linoleic acid content (27.05%), followed by Flaxcanosun (25.74%) and Flaxcanochia (22.89). Flaxcanosun had the highest oleic acid content (38.42%), followed by Flaxcocosun (26.10%) and Flaxcanochia (25.95%). Lauric acid was only present in

Flaxcocosun (8.25%). These findings showed that the blending process improved the fatty acid profiles for the produced ternary vegetable oil blends. It adjusts fatty acids to stabilize vegetable oils for commercial frying, preventing oxidation and hydrolysis at high temperatures. Previous literature has reported related observations (Dhyani et al., 2018).

In the diet, the WHO advises a ratio of 1:1.5:1 for saturated, monounsaturated, and polyunsaturated fatty acids and a ratio of 5–10:1 for linoleic acid (omega-6) to  $\alpha$ -linolenic acid (omega-3). Numerous studies advocate for the specified fatty acid ratios to sustain optimal health; nevertheless, no natural oil exhibits such a balanced composition. Consequently, researchers sought to produce oil blends with enhanced chemical characteristics and balanced fatty acid compositions (WHO, 2008; Grover et al., 2021).

Fig. 8 shows that the Flaxcocosun ternary oil blend has the most balanced mix of fatty acids. It has 23.51, 26.40, and 50.09% SFA, MUSFA, and PUSFA, with a ratio of 1: 2.12: 2.13, respectively. Flaxcanosun's fatty acid composition is the second-most balanced. Their SFA, MUSFA, and PUSFA amounts are 14.28, 39.76, and 45.74%, indicating a ratio of 1: 2.78: 3.20, respectively. Flaxcanochia recorded the highest amounts of SFA, MUSFA, and PUSFA, which are 11.46, 26.66, and 60.74%, with a ratio of 1: 2.33: 5.30, respectively. For humans, the WHO optimal ratio for SFA, MUSFA, and PUSFA is 1:1.5:1, respectively. Among the ternary vegetable oil blends produced, Flaxcocosun is the ternary oil blend that comes closest to the WHO's recommended oil blend, followed by Flaxcanosun and Flaxcanochia.

To lower the risk of autoimmune, cardiovascular, and other chronic diseases, a PUSFA/SFA ratio greater than 0.40

is advised. Furthermore, a higher quantity of dietary SFAs, which are one of the primary risk factors for cardiovascular disease, is indicated by a lower PUSFA/SFA ratio. In all studied vegetable oils and their ternary oil blends, this ratio is beyond the minimum advised limit, except for coconut oil. Literature previously reported related observations (Simopoulos, 2002; Rahman et al., 2023).

The optimal dietary  $\omega$ -6 to  $\omega$ -3 ratio necessary for human evolution is approximately one, whereas Western diets have a 15/1–16.7/1 ratio. Omega-6 fatty acids are more prevalent in modern Western diets, leading to diseases like cancer, cardiovascular disease, inflammatory diseases, and autoimmune disorders. A low  $\omega$ -6/ $\omega$ -3 ratio can suppress these diseases. Research suggests that the ideal proportion may vary depending on the disease, as chronic diseases may have multiple causes and factors. A lower  $\omega$ -6/ $\omega$ -3 ratio is advised to reduce the likelihood of multiple health problems. The ideal ratio is approximately 5  $\omega$ -6 to 1  $\omega$ -3, according to nutritionists (Simopoulos, 2002; Shahidi and Ambigaipalan, 2018; Li et al., 2019; Monroig et al., 2022; Lichtenstein, 2023).

#### Physicochemical properties of the cold-pressed vegetable oils:

The physicochemical properties of vegetable oils are specific indicators for their quality. Therefore, the coldpressed vegetable oils (flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil) were evaluated for their physicochemical properties, including refractive index, kinematic viscosity, relative density, color index, peroxide value, acid value, iodine value, unsaponifiable matter, smoke point, saponification value, oxidative stability index, paraanisidine value, ester value, total oxidation value (Totox value), and oxidisability value (Cox value). Table 5 and Fig. 9 summarize the results.

Table 5. Physicochemical properties of the cold-pressed vegetable oils.

Parameters*	The cold-pressed vegetable oils						
r al ametel s	FLO <sup>1</sup>	CHO <sup>2</sup>	CAO <sup>3</sup>	SFO <sup>4</sup>	CNO <sup>5</sup>		
Refractive index (40°C)	1.4689±0.006	1.4767±0.001	1.4669±0.005	1.4731±0.009	1.4543±0.003		
Relative density (20°C)	$0.920 \pm 0.01$	$0.929 \pm 0.02$	$0.917 \pm 0.02$	$0.921 \pm 0.02$	$0.941 \pm 0.04$		
Kinematic viscosity (20°C, mm <sup>2</sup> /sec)	$67.35 \pm 1.30$	$80.02 \pm 1.50$	$78.19 \pm 1.50$	$42.60 \pm 1.10$	$48.51 \pm 1.30$		
Color index	$34.60 \pm 0.60$	$42.50 \pm 0.60$	$41.50 \pm 0.60$	$31.10 \pm 0.40$	$32.85 \pm 0.40$		
Acid value (mg KOH/g oil)	$0.75 \pm 0.02$	$0.79 \pm 0.01$	$0.71 \pm 0.01$	$0.19 \pm 0.01$	$0.56 \pm 0.03$		
Peroxide value (meq. O <sub>2</sub> /kg oil)	$0.80 \pm 0.01$	$1.09 \pm 0.12$	$1.88 \pm 0.01$	$0.11 \pm 0.01$	$0.76 \pm 0.01$		
Iodine value (g $I_2/100$ g oil)	$173 \pm 1.20$	$202 \pm 5.00$	$97 \pm 1.10$	$119 \pm 1.10$	$21 \pm 0.20$		
Saponification value (mg KOH/g oil)	$187.83 \pm 5.00$	$197.10 \pm 4.00$	$180.56 \pm 4.00$	$197.49 \pm 7.00$	$241 \pm 5.00$		
Unsaponifiable matter (%)	$1.72 \pm 0.02$	$1.29 \pm 0.05$	$1.46\pm0.01$	$1.33 \pm 0.01$	$1.55 \pm 0.02$		
Smoke point (°C)	$107 \pm 2.00$	$214 \pm 4.00$	$238 \pm 5.00$	$227 \pm 4.00$	$204 \pm 2.00$		
Oxidative stability at 100°C (hr)	$3.50 \pm 0.03$	$5.67 \pm 0.04$	$18.50 \pm 0.25$	$7.20 \pm 0.06$	$54.20 \pm 1.20$		
<i>p</i> -Anisidine value	$1.55 \pm 0.10$	$3.56 \pm 0.50$	$4.01 \pm 0.50$	$3.75 \pm 0.30$	$2.75 \pm 0.20$		
Ester value (mg KOH/g oil)**	187.08	196.31	179.85	197.30	240.44		
Total oxidation value (Totox)***	3.15	5.74	7.77	3.97	4.27		
Oxidisability (Cox) value	13.88	4.06	1.79	6.11	0.26		

\* Means of three determinations  $\pm$  SD. \*\* = (Saponification value – Acid value). \*\*\* = [(2 × Peroxide value) + (p-Anisidine value)].

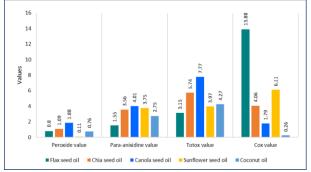


Fig. 9. Peroxide, *p*-Anisidine, Totox, and Cox values for the cold-pressed vegetable oils.

As can be seen, the refractive index (at 40°C) and relative density (at 20°C) values for these vegetable oils were found to be 1.4543 - 1.4767 and 0.917 - 0.941, respectively. Coconut oil recorded the lowest refractive index (1.4543) and the highest relative density (0.941). Chia seed oil recorded the highest refractive index (1.4767). These results revealed that the fatty acids composition of vegetable oils had a notable effect on their refractive index and relative density. The refractive index increases with the elongation of fatty acids chain length and unsaturation degree. Conversely, as fatty acid chain length and unsaturation degree increase, the relative density decreases. People utilize the refractive index to evaluate the purity of oil and to signify the extent of unsaturation and chain length. An elevated refractive index

signifies a greater abundance of unsaturated materials. The refractive index standard limits recommended for edible oils are 1.40 - 1.47 (Timilsena et al., 2017; Rahman et al., 2023).

The kinematic viscosity (at 20°C) for the examined vegetable oils was found to be  $42.60 - 80.02 \text{ mm}^2/\text{sec.}$ Among the vegetable oils examined, there was a wide variation in kinematic viscosity. For example, chia seed oil recorded the highest kinematic viscosity (80.02 mm<sup>2</sup>/sec), followed by canola seed oil (78.19 mm<sup>2</sup>/sec), flax seed oil (67.35 mm<sup>2</sup>/sec), and coconut oil (48.51 mm<sup>2</sup>/sec). Sunflower seed oil recorded the lowest kinematic viscosity at 42.60 mm<sup>2</sup>/sec. Many factors, such as temperature, fatty acid chain length, and the number and nature of double bonds, influence the kinematic viscosity of vegetable oils. The physicochemical properties of oils link to the change in viscosity. Consequently, it can serve as a criterion for assessing the global quality and stability of vegetable oils. Consumers typically place a high value on oils with reduced viscosity values (Fasina and Colley, 2008; Zahir et al., 2017).

In terms of the color quality of vegetable oils, the results indicated that each oil has its own specific color index due to the presence of various pigments (i.e., carotenoids, chlorophyll, etc.). The color values of these vegetable oils ranged from 31.10 for sunflower seed oil to 42.50 for chia seed oil. Flax seed oil and coconut oil recorded nearly the same color index (34.60, 32.85) as sunflower seed oil. Canola seed oil shares a nearly identical color index (41.50) with chia seed oil.

The acid value, an explicit indicator of the concentration of free fatty acids in a specific quantity of oil, was found to be 0.19 - 0.79 mg KOH/g oil. Chia seed oil recorded the highest acid value (0.79 mg KOH/g oil), followed by flax seed oil (0.75 mg KOH/g oil), canola seed oil (0.71 mg KOH/g oil), and coconut oil (0.56 mg KOH/g oil). Sunflower seed oil recorded the lowest acid value (0.19 mg KOH/g oil). The low levels of free fatty acids in oils render them appropriate for use as edible oils. Vegetable oils with low acid values are more stable over a long period of time. Previous literature has reported related observations for various types of vegetable oils (Aremu et al., 2015; Onu and Mbohwa, 2021; Sruthi et al., 2021).

The peroxide value, a measure of the peroxides present in vegetable oils, was found to be 0.11 - 1.88 meq.  $O_2/kg$  oil. Canola seed oil recorded the highest peroxide value (1.88 meq.  $O_2/kg$  oil), followed by chia seed oil (1.09 meq.  $O_2/kg$  oil), flax seed oil (0.80 meq.  $O_2/kg$  oil), and coconut oil (0.76 meq.  $O_2/kg$  oil). Sunflower seed oil recorded the lowest peroxide value (0.11 meq.  $O_2/kg$  oil). The number of peroxides present in edible oils reflects their oxidative levels and, consequently, their susceptibility to rancidity. The high peroxide value (> 10 meq  $O_2/kg$ ) indicates high levels of oxidative rancidity of oils. The relatively low peroxide value (< 10 meq  $O_2/kg$ ) indicates that the oils are resistant to oxidation (Canneddu et al., 2016; Buthelezi et al., 2019).

The iodine value, reflecting the degree of unsaturation in oils and potential changes under intensive conditions, was found to be 21 - 202 g I<sub>2</sub>/100 g oil. Among the vegetable oils examined, there was a wide variation in the iodine value. For example, chia seed oil recorded the highest iodine value (202 g I<sub>2</sub>/100 g oil), followed by flax seed oil (173 g I<sub>2</sub>/100 g oil), sunflower seed oil (119 g I<sub>2</sub>/100 g oil), and canola seed oil (97 g I<sub>2</sub>/100 g oil). Coconut oil recorded the lowest iodine value (21 g  $I_2/100$  g oil). These findings revealed that the greater the iodine value, the higher the degree of unsaturation and sensitivity to oxidation. The iodine value estimates these quality parameters because the unsaturation degree influences the oxidative stability and melting points (Onu and Mbohwa, 2021).

The saponification value, that is the quantity (milligrams) of alkali necessary to saponify a specific amount (one gram) of oil under designated conditions, was found to be 180.56 – 241 mg KOH/g oil. Coconut oil recorded the highest saponification value (241 mg KOH/g oil), followed by sunflower seed oil (197.49 mg KOH/g oil), chia seed oil (197.10 mg KOH/g oil), and flax seed oil (187.83 mg KOH/g oil). Canola seed oil recorded the lowest saponification value (180.56 mg KOH/g oil). These results indicate that short-chain fatty acids possess higher saponification values than long-chain fatty acids due to a greater number of carboxyl groups per mass unit.

A comparative analysis of fatty acid chain lengths in oils benefits greatly from the saponification value. It quantifies the average molecular weight of all fatty acids included in oils as triglycerides. A higher saponification value corresponds to a shorter average length of fatty acids. The vegetable oils (i.e., sunflower, soybean, canola oils) mostly comprise long-chain fatty acids (C16–C18). Therefore, they possess approximately identical saponification values (168– 196 mg KOH/g oil). Certain vegetable oils, like palm kernel oil and coconut oil, are rich in lauric acid (C12:0) and myristic acid (C14:0). This results in significantly elevated saponification values (235–260 mg KOH/g oil). In practice, oils with high saponification values are more suitable for soap making (Sajjadi et al., 2016; Onu and Mbohwa, 2021; Ivanova et al., 2022).

The unsaponifiable matter comprises substances that are insoluble or unable to form soaps with alkali. These substances comprise 1 to 2% of edible oils. The examined vegetable oils contained about 1.29 - 1.72% unsaponifiable matter. Flax seed oil recorded the highest unsaponifiable matter (1.72%), followed by coconut oil (1.55%), canola seed oil (1.46%), and sunflower seed oil (1.33%). Chia seed oil recorded the lowest unsaponifiable matter (1.29%). Researchers reported that the main components of unsaponifiable matter are tocopherols, carotenoids, hydrocarbons, and sterols. The resistance of vegetable oils against oxidation depends on their fatty acids pattern and these unsaponifiable substances (Matecka, 2002; Fontanel, 2013; Aremu et al., 2015).

The smoke point, which is the temperature at which an oil emits a thin, clearly visible bluish smoke, was found to be 107 – 238°C. The generation of smoke signifies the onset of the oil's quality deterioration. Among the examined vegetable oils, there was a wide variation in the smoke point. For example, canola seed oil recorded the highest smoke point (238°C), followed by sunflower seed oil (227°C), chia seed oil (214°C), and coconut oil (204°C). Flax seed oil recorded the lowest smoke point (107°C). This parameter is crucial for assessing the appropriateness of oils for frying purposes. Regulations often stipulate 200°C as the minimum temperature. As can be seen, the examined vegetable oils, except flax seed oil, demonstrated a smoke point exceeding 200°C. Therefore, these vegetable oils are suitable for frying applications and demonstrate superior frying stability.

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Reports indicate that the smoke point of oils can range from a relatively low (162°C) to a very high (271°C). The smoke point of oils is significantly associated with their free fatty acid content; as the acid value increases, the smoke point decreases. Generally, low thermal stability is associated with a low smoke point. In other words, a higher smoke point signifies superior oil quality and reduced quantities of free fatty acids (Yen et al., 1997; Alvarenga et al., 2018; Khor et al., 2019).

Oxidative stability is a crucial criterion for assessing oil quality. The oxidative stability index, which indicates the duration necessary for an oil sample to exhibit significant rancidity during the Rancimat test, was found to be 3.50 -54.20 hours at 100°C. Coconut oil recorded the highest oxidative stability index (54.20 hour at 100°C), followed by canola seed oil (18.50 hour at 100°C), sunflower seed oil (7.20 hour at 100°C), and chia seed oil (5.67 hour at 100°C). Flax seed oil recorded the lowest oxidative stability index (3.50 hour at 100°C). These results clearly demonstrated an inverse relation between the oxidative stability of oils and their unsaturation levels. The PUSFA/SFA ratio reflects the unsaturation degree of oils and their susceptibility to oxidative reactions. For example, vegetable oils with high levels of PUSFA (i.e., flax seed oil, chia seed oil) are less stable against oxidation and vice versa. As a result, the low PUSFA (1.60%) and high SFA (88.93%) levels of coconut oil can explain its high oxidative stability. The fatty acid composition and minor constituents such as tocopherols, phytosterols, and phenolic substances greatly influence the oxidative stability of oils.

Unlike peroxide value and free fatty acid analyses, which assess the quality of oil at a certain moment, the oxidative stability index possesses predictive significance. This can be utilized to compare various oils and predict their respective shelf lives. Therefore, it can assess the efficacy of antioxidants or ascertain the duration of oil use before spoilage (Malacrida et al., 2011; Kumar and Sharma, 2015; Symoniuk et al., 2022).

The p-Anisidine value gives an indication of the secondary oxidation of fats and oils, which may cause a rancid taste and affect physical properties. The p-Anisidine values for the examined vegetable oils were found to be 1.55-4.01. Canola seed oil recorded the highest p-Anisidine value (4.01), followed by sunflower seed oil (3.75), chia seed oil (3.56), and coconut oil (2.75). Flax seed oil recorded the lowest p-Anisidine value of 1.55. The p-Anisidine value quantifies the aldehyde and ketonic degradation products of peroxides, while the peroxide value measures the components produced during the first stages of oxidation. Together with peroxide value measurements, the p-Anisidine value measurements are commonly used to quantify the total extent of oxidation using the Totox value (Gordon, 2001; Zuo et al., 2017; Bekdeser et al., 2024).

Esters, naturally occurring constituents in vegetable oils, contribute to flavor development and pleasant aromas. Ester value, denotes the quantity of alkali necessary to saponify the esters found in fats or oils, was found to be 179.85 – 240.44 mg KOH/g oil. Coconut oil recorded the highest ester value (240.44 mg KOH/g oil), followed by sunflower seed oil (197.30 mg KOH/g oil), chia seed oil (196.31 mg KOH/g oil), and flax seed oil (187.08 mg KOH/g oil). Canola seed oil had the lowest ester value (179.85 mg KOH/g oil).

The total oxidation value (Totox value), defined as the sum of the *p*-anisidine value and twice the peroxide value was found to be 3.15 - 7.77. Canola seed oil recorded the highest

Totox value (7.77), followed by chia seed oil (5.74), coconut oil (4.27), and sunflower seed oil (3.97). Flax seed oil recorded the lowest Totox value (3.15). The calculated Totox values are used to provide a comprehensive picture of the overall oxidation profile. A lower Totox rating indicates superior oil quality. The Totox value, on the other hand, is an empirical parameter as it results from the summation of two parameters with distinct units. For edible oils of satisfactory quality, the limiting Totox value is 10. Fortunately, all tested vegetable oils did not exceed this limit, which indicates their high quality (Gordon, 2001; Bojanowska and Lamorska, 2016; Zuo et al., 2017; Bekdeser et al., 2024).

The oxidisability value (Cox value) for these vegetable oils, computed predicated on the quantity of C18-USFA (oleic, linoleic, and  $\alpha$ -linolenic), was found to be 0.26 – 13.88. Flax seed oil recorded the highest Cox value (13.88), followed by sunflower seed oil (6.11), chia seed oil (4.06), and canola seed oil (1.79). Coconut oil recorded the lowest Cox value (0.26). A low Cox value indicates the strong oxidative stability of vegetable oils, and vice versa.

The results observed are comparable to those mentioned in the literature. For example, Timilsena et al. (2017) reported that the physicochemical properties of chia seed oil were found to be 1.48 for refractive index (at 40°C), 0.93 for specific gravity (at 25°C), 43.23 mPa.s for viscosity (at 25°C), 2.54 mg KOH/g oil for acid value, 197 mg KOH/g oil for saponification value, 204 g I<sub>2</sub>/100 g oil for iodine value, 4.33 meq.  $O_2$ /kg oil for peroxide value, 1.12% for unsaponifiable matter, and 2.4 hours at 90°C for oxidative stability index.

The physicochemical properties of canola oil were found to be 1.465 - 1.467 for refractive index (at 40°C), 0.914 – 0.920 for relative density, 78.20 mm<sup>2</sup>/sec for kinematic viscosity (at 20°C), 182 – 193 mg KOH/g oil for saponification value, 91 – 126 g I<sub>2</sub>/100 g oil for iodine value, 0.5 - 1.2% for unsaponifiable matter, 1 - 3 for anisidine value, and  $220 - 230^{\circ}$ C for smoke point (Shahidi, 2005).

# Physicochemical properties of the ternary vegetable oil blends:

In comparison with the individual vegetable oils, the physicochemical properties of the ternary vegetable oil blends (Flaxcanochia, Flaxcanosun and Flaxcocosun) are shown in Table 6 and Figs. 10 - 13. From which, it could be seen that the refractive index values (at  $40^{\circ}$ C) for these ternary vegetable oil blends were found to be 1.4656 - 1.4699. Flaxcocosun recorded the lowest refractive index (1.4656). Flaxcanochia and Flaxcanosun had nearly the same refractive index (1.4699 and 1.4690). Flaxcanosun recorded a lower relative density (0.876, at 20°C) than Flaxcanochia and Flaxcocosun, which recorded nearly the same value of relative density (0.918 and 0.920).

The kinematic viscosity (at 20°C) for these ternary vegetable oil blends was found to be 52.82–75.90 mm<sup>2</sup>/sec. There was a wide variation of the kinematic viscosity among the examined ternary vegetable oil blends. For example, Flaxcanochia recorded the highest kinematic viscosity (75.90 mm<sup>2</sup>/sec), followed by Flaxcanosun (59.50 mm<sup>2</sup>/sec). Flaxcocosun recorded the lowest kinematic viscosity (52.82 mm<sup>2</sup>/sec).

The color values of these ternary vegetable oil blends were found to be 32.85 - 37.30. Flaxcanochia recorded the highest color index (37.30), followed by Flaxcanosun (35.20). Flaxcocosun recorded the lowest color index (32.85).

Parameters*	The ternary oil blends				
rarameters	Flaxcanochia <sup>1</sup>	Flaxcanosun <sup>2</sup>	Flaxcocosun <sup>3</sup>		
Refractive index (40°C)	$1.4699 \pm 0.001$	$1.4690 \pm 0.001$	$1.4656 \pm 0.006$		
Relative density (20°C)	$0.918 \pm 0.02$	$0.876 \pm 0.02$	$0.920 \pm 0.02$		
Kinematic viscosity (20°C, mm <sup>2</sup> /sec)	$75.90 \pm 1.00$	$59.50 \pm 1.00$	$52.82 \pm 1.40$		
Color index	$37.30 \pm 0.30$	$35.20 \pm 0.20$	$32.85 \pm 0.10$		
Acid value (mg KOH/g oil)	$0.62 \pm 0.05$	$0.36 \pm 0.05$	$0.53 \pm 0.04$		
Peroxide value (meq. O <sub>2</sub> /kg oil)	$1.01 \pm 0.05$	$0.31 \pm 0.05$	$0.56 \pm 0.03$		
Iodine value (g $I_2/100$ g oil)	$161 \pm 2.00$	$110 \pm 1.10$	$157 \pm 2.50$		
Saponification value (mg KOH/g oil)	$188 \pm 1.50$	$155 \pm 5.00$	$208.8 \pm 3.00$		
Unsaponifiable matter (%)	$1.58 \pm 0.01$	$1.35 \pm 0.01$	$1.53 \pm 0.02$		
Smoke point (°C)	$191 \pm 3.00$	$184 \pm 3.00$	$179.50 \pm 2.00$		
Oxidative stability index at 100°C (hr)	$10.37 \pm 0.11$	$10.98 \pm 0.12$	$12.20 \pm 0.13$		
<i>p</i> -Anisidine value	$3.25 \pm 0.50$	$3.45 \pm 0.30$	$2.65 \pm 0.20$		
Ester value (mg KOH/g oil)**	187.38	155.64	208.27		
Total oxidation value (Totox)***	5.27	4.07	3.77		
Oxidisability (Cox) value	10.79	7.36	8.02		

\* Means of three determinations  $\pm$  SD. \*\* = (Saponification value – Acid value). \*\*\* = [(2 × Peroxide value) + (p-Anisidine value)]. <sup>1</sup>Flax seed oil + Canola seed oil + Chia seed oil + Chia seed oil + Chia seed oil + Canola seed oil + Sunflower seed oil (20:40:40). <sup>3</sup>Flax seed oil + Coconut oil + Sunflower seed oil (45:10:45).

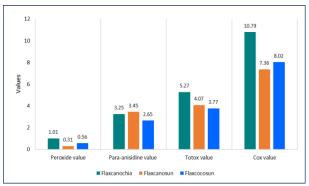


Fig. 10. Peroxide, *p*-Anisidine, Totox, and Cox values for the ternary vegetable oil blends.

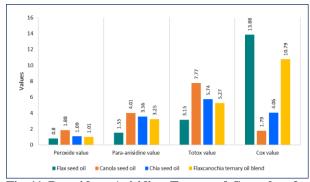


Fig. 11. Peroxide, p-Anisidine, Totox, and Cox values for flax seed oil, canola seed oil, chia seed oil, and their Flaxcanochia ternary oil blend.

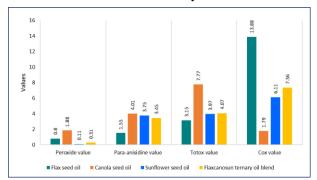


Fig. 12. Peroxide, p-Anisidine, Totox, and Cox values for flax seed oil, canola seed oil, sunflower seed oil, and their Flaxcanosun ternary oil blend.

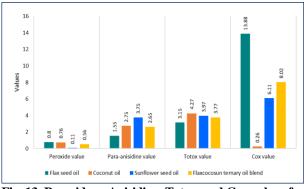


Fig. 13. Peroxide, *p*-Anisidine, Totox, and Cox values for flax seed oil, coconut oil, sunflower seed oil, and their Flaxcocosun ternary oil blend.

The acid values for these ternary vegetable oil blends were found to be 0.36 - 0.62 mg KOH/g oil. Flaxcanochia recorded the highest acid value (0.62 mg KOH/g oil), followed by Flaxcocosun (0.53 mg KOH/g oil). Flaxcanosun recorded the lowest acid value (0.36 mg KOH/g oil).

The peroxide values for these ternary vegetable oil blends were found to be 0.31 - 1.01 meq. O<sub>2</sub>/kg oil. Flaxcanochia recorded the highest peroxide value (1.01 meq. O<sub>2</sub>/kg oil), followed by Flaxcocosun (0.56 meq. O<sub>2</sub>/kg oil). Flaxcanosun recorded the lowest peroxide value (0.31 meq. O<sub>2</sub>/kg oil).

The iodine values for these ternary vegetable oil blends were found to be  $110-161 \text{ g I}_2/100 \text{ g}$  oil. Flaxcanochia recorded the highest iodine value (161 g I<sub>2</sub>/100 g oil), followed by Flaxcocosun (157 g I<sub>2</sub>/100 g oil). Flaxcanosun recorded the lowest iodine value (110 g I<sub>2</sub>/100 g oil).

The saponification values for these ternary vegetable oil blends were found to be 155 - 208.8 mg KOH/g oil. Flaxcocosun recorded the highest saponification value (241 mg KOH/g oil), followed by Flaxcanochia (188 mg KOH/g oil). Flaxcanosun recorded the lowest saponification value (155 mg KOH/g oil).

The unsaponifiable matter values for these ternary vegetable oil blends were found to be 1.35 - 1.58%. Flaxcanochia recorded the highest unsaponifiable matter (1.58%), followed by Flaxcocosun (1.53%). Flaxcanosun recorded the lowest unsaponifiable matter (1.35%).

The smoke point for these ternary vegetable oil blends was found to be 179.50 – 191°C. Flaxcanochia recorded the highest smoke point (191°C), followed by Flaxcanosun (184°C). Flaxcocosun recorded the lowest smoke point (179.50°C).

The oxidative stability index for these ternary vegetable oil blends was found to be 10.37 - 12.20 hours at 100°C. Flaxcocosun recorded the highest oxidative stability index (12.20 hour at 100°C), followed by Flaxcanosun (10.98 hour at 100°C). Flaxcanochia recorded the lowest oxidative stability index (10.37 hour at 100°C).

The *p*-Anisidine values for these ternary vegetable oil blends were found to be 2.65 - 3.45. Flaxcanosun recorded the highest *p*-Anisidine value (3.45), followed by Flaxcanochia (3.25). Flaxcocosun recorded the lowest *p*-Anisidine value (2.65).

The ester values for these ternary vegetable oil blends were found to be 155.64 – 208.27 mg KOH/g oil. Flaxcocosun recorded the highest ester value (208.27 mg KOH/g oil), followed by Flaxcanochia (187.38 mg KOH/g oil). Flaxcanosun recorded the lowest ester value (155.64 mg KOH/g oil).

These ternary vegetable oil blends had total oxidation values (Totox values) ranging from 3.77 to 5.27. Flaxcanochia recorded the highest Totox value (5.27), followed by Flaxcanosun (4.07). Flaxcocosun recorded the lowest Totox value (3.77).

The oxidisability values (Cox value) for these ternary vegetable oil blends were found to be 7.36 - 10.79. Flaxcanochia recorded the highest Cox value (10.79), followed by Flaxcocosun (8.02). Flaxcanosun recorded the lowest Cox value (7.36).

As shown in Fig. 10, there were variations in the *p*anisidine, peroxide, and Totox values of the ternary vegetable oil blends. The changes were stronger in Flaxcanochia than in Flaxcanosun. This is because Flaxcanochia contains FLO and CHO, which are rich in PUSFA. Factors like temperature, moisture, light, oxygen, and metals are recognized as factors influencing the rate of oxidation.

The above results confirm that the chosen oilseeds serve as the primary sources of vegetable oils with distinctive physicochemical properties. Blending processes improved some important physicochemical properties of the resulting ternary vegetable oil blends. For example, coconut oil improved the Flaxcocosun ternary oil blend's stability. Given their relatively high smoke points, all ternary oil blends are safe to use as cooking oil.

#### CONCLUSION

This investigation aimed to develop functional ternary vegetable oil blends that enhance nutritional, physiochemical, and therapeutic benefits, leveraging the extensive use of coldpressed vegetable oils. The results highlight the superior bioactive phytochemicals and antioxidant properties of coldpressed vegetable oils and their ternary oil blends as natural antioxidant sources. The phytochemicals present in these vegetable oils and their blends may be largely responsible for their health benefits. They are also excellent sources of necessary fatty acids for the human body, conferring various health benefits. Combining various vegetable oils, like flax seed oil, chia seed oil, canola seed oil, sunflower seed oil, and coconut oil, in a ternary form can significantly balance their fatty acid composition, improving their nutritional and physiochemical properties. This could be a cost-effective way to improve and protect health, potentially leading to therapeutic benefits. Future recommendations should consider other oil varieties and variable vegetable oil characteristics.

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### إنتاج خلطات ثلاثية وظيفية من الزيوت النباتية مناسبة للأغراض الغذائية والعلاجية

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#### الملخص

*الكلمات الدالة*: الأحماض الدهنية الأساسية – المركبات النشطة الذاتية – خلطات الزيوت النباتية – الخصائص الغذائية – الخصائص الفيزيو كيميائية.