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Chemical Characterisation of New Oils Extracted from Cañihua and Tarwi Seeds with Different Organic Solvents

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Abstract: Vegetable oils contain fatty acids, phenolic compounds, natural antioxidants, and fat-soluble vitamins, which are beneficial against different diseases. Oil extraction methods can, however, affect their composition. This study aims to characterize the chemical composition of oils from two Andean seeds, cañihua (*Chenopodium pallidicaule*) and tarwi (*Lupinus mutabilis*), extracted with different organic solvents, petroleum ether, hexane, and ethanol. This study compares these oils with commercial sunflower, rapeseed, and olive oils. Results showed that oils extracted with hexane had the highest yield, while those extracted with ethanol had higher antioxidant activity and total phenolic compound content. Additionally, using ethanol makes the process more sustainable than non-green solvents. The composition of tarwi and cañahua oils extracted with ethanol includes fatty acids, tocopherols, antioxidants, and phenolic compounds associated with health benefits.

Keywords: Vegetable oils; Andean seeds; green solvents; fatty acid profile; tocopherols

1. Introduction

Vegetable oils extracted from seeds are interesting due to their fatty acid composition, antioxidant capacity, phenolic compound content, and lipid-soluble vitamins like tocopherols. [1,2]. Vegetable oils are rich in saturated, mono- and poly-unsaturated fatty acids, with distinct chemical and functional properties [3]. Each of them has particular effects on health [4]. They are crucial in human nutrition, and some may prevent the promotion of many chronic diseases, such as cardiovascular diseases, cancer, and inflammatory diseases [5–7].

Vegetable oils have health implications, but their extraction methods affect their composition. The most common solvent extraction technology today uses organic solvents like petroleum ether and hexane, which are harmful to the environment, causing air pollution and toxicity [8]. Strict global regulations on petroleum-derived solvents have been introduced, creating a need for more environmentally friendly, bio-based, and renewable solvents for extracting and formulating natural food products [9]. Ethanol is a promising green solvent that also improves the quality of extracted products [10]. There is a need for vegetable oils from new sources to have suitable functional properties and good nutritional value, which are in high demand [11].

Tarwi or andean lupin (*Lupinus mutabilis*) and cañihua or cañahua (*Chenopodium pallidicaule*) seeds are an attractive source of oils. These plants grow mainly in the Andean region of Bolivia, Peru, and Ecuador. Due to the growing conditions in high-altitude areas (3640 m.a.s.l), the seeds are a good source of high-quality proteins, dietary fibre, and polyunsaturated fatty acids [5].

Tarwi is a legume rich in proteins and oils, making it a good crop for applications in food, feed, and cosmetics [12]**.** Potential health benefits have been highlighted in connection to the consumption of lupine oil-containing products, including e.g. cholesterol and triglyceride-lowering effects [13]. Cañihua is an amaranthaceous relative of quinoa and grows under very harsh environmental

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conditions and is even more resistant to frost than quinoa. Cañihua presents high carbohydrate content, and the amount of oil is considerably higher than that found in common cereal grains, with values between 6–7% versus 2–4%, respectively [14].

In this work, we investigate the composition of oils from tarwi (*Lupinus mutabilis*) and cañihua (*Chenopodium pallidicaule*) seeds extracted with different solvents. The three extraction solvents compared in this study were petroleum ether, hexane, and ethanol. Oils were chemically characterized, obtaining the fatty acid profile, antioxidant capacity, total phenolic compounds, and tocopherol content. In addition, all these components were compared to commercial oils available in supermarkets, e.g., sunflower, rapeseed, and olive oil.

2. Materials and Methods

2.1. Seeds Sampling

Seeds were collected from the Andean region of Bolivia in the Department of La Paz. Tarwi (*Lupinus mutabilis*) from the Municipality of Carabuco in La Paz (Puerto Mayor de Carabuco, Camacho Province with the following coordinates 15°44′00″S 69°01′00″O, and Cañihua (*Chenopodium padillicaule*) from a Municipality of Chojñacota in the south of La Paz (Gualberto Villaroel Province with coordinates 17°40′00″S 67°53′00″O).

2.2. Chemicals

Petroleum ether, hexane, ethanol, acetic acid, sulphuric acid, heptane, cyclohexane, potassium and sodium chloride, potassium hydroxide, ethyl acetate, ascorbic acid, standards α, γ, δ- tocopherols and methanol (HPLC – grade), formic acid and acetone were purchased from Sigma Aldrich, the kit MAK-369 USA used for FRAP assay and the chemicals for developing total phenolic compounds like sodium carbonate, gallic acid, Folin-Ciocalteau, were all also purchased from Sigma Aldrich – Merk. The standards used for the Gas Chromatography 37 Component FAME mix and diethyl ether were purchased from Supelco.

The commercial oils were acquired from Swedish supermarkets, and the oils and brands in parenthesis were the following: sunflower oil (Alwaid), rapeseed oil (Zeta Fernando di Lucia), and olive oil (Burcu).

2.3. Oils Extraction

The extraction of the oils was developed using the Soxhlet method, using three different solvents (petroleum ether, hexane, and ethanol). The extraction was carried out from 10 grams of sample seed previously grounded; the sample was added to a filter paper and placed into the Soxhlet apparatus, followed by the addition of 200 mL of extraction solvent at 80 °C. After 5 hours of extraction, the oil was cooled down, and the remaining extraction solvent was subsequently distilled using a rotary evaporator (Buchi B-300) for 5 min at 50 °C in a heating bath [15]. The content of the oil was calculated by the difference between the weight before and after distillation.

2.4. Antioxidant Capacity

Total antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) method, using the Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Colorimetric) from Sigma Aldrich, catalogue number MAK-369 USA. The absorbance was measured at 562 nm, and the results were expressed in the samples as equivalent mmol of ferrous iron (Fe^{+2}).

2.5. Total Phenolic Compounds

Total phenolic compounds (TPH) were determined by the Folin-Ciocalteau reagent. The reagent was diluted with water (1:10 v/v). A gallic acid stock solution was prepared in a solution of 80% methanol-water in (1:1 v/v). From each standard solution and sample, 50 µL was mixed with 1 mL of Folin-Ciocalteu's reagent and 0.5 mL of sodium carbonate solution 7.5% (w/v). The samples were mixed and incubated at 45°C for 30 min. The absorbance was read at 765 nm in a Multiskan Go microplate reader (Thermo Scientific). The results were expressed as gallic acid equivalents (GAE) [16].

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2.6. High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC was used to visualize the presence of various lipid classes present in the lipid extracts. For this process, 150 µL of a mixture containing chloroform: methanol (2:1 v/v) was added to 50 µL of oil sample, then it was evaporated to dryness under a stream of nitrogen at room temperature. The dried samples were then redissolved in 100 µL of chloroform. Using an automated TLC applicator (Camag; ATS4), 3 µL of this solution was applied to a 20 × 20 cm silica gel 60 plate. The composition of the mobile phase was 66% n-heptane, 33% diethyl ether, and 1% acetic acid (v/v) for the neutral lipids, and 85 % chloroform, 15 % methanol,10% acetic acid, and 3,5 % of water (v/v) for the polar lipids. To detect the separated lipid classes, primulin in acetone: water (8:2 v/v) staining was utilized [17,18].

2.7. Fatty Acid Profile Analysis

The fatty acid composition was determined by conversion to methyl esters (FAME) through acidic methylation. For acidic methylation, 2ml of 2% sulfuric acid in methanol was added to 50 µL of samples, with 50 µL of internal standard C:17 in chloroform (5mg/ml), and then the samples were incubated at 80 °C for 45 min. Lastly, 2 mL of water and 2 mL of heptane were added. The mixture was centrifugated for 5 min at 2500 rpm, and the upper layer was collected and evaporated with N2 until dryness. The sample was reconstituted with 150 µL cyclohexane and kept at -20 °C until analysis. At analysis, 1 µL of the sample was injected into a Trace 1300 gas chromatography system (Thermo Fischer Scientific) equipped with a flame ionization detector with an Al1310 autoinjector. The FAMEs were separated using a Thermo Scientific silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium was used as carrier gas with a flow rate of 0.8 mL/min. The column temperature program started from 140 °C for 5 min and increased to 240 °C. The temperature was programmed to rise at 4 °C/min up to 200 °C, followed by 5 °C /min to 240 °C and then kept until the end of the program, with a total runtime of 40 min. [17]. Data was analysed using the Chromeleon 7.2.10 Chromatography Data System (CDS) software (Thermo Scientific), and the peaks were identified by comparison to the retention times of reference standards (Supelco, Bellefonte, PA, USA). The results were expressed as the relative percentage of each individual fatty acid (FA) present in each sample given by the corresponding retention time.

2.8. Determination of Tocopherols

The analysis was developed using high-performance liquid chromatography (HPLC). As standard solutions, α, γ, and δ – tocopherols were used as standards in concentrations between 1-500 µg/mL dissolved in acetonitrile. As explained below, oil samples were saponified, filtered, and injected into the HLPC.

2.8.1. Saponification of Oils Extracts

The oil samples were saponified according to [17,18]. Briefly, 200 mg of oil samples were weighed, and 0.1 g of ascorbic acid was added, followed by 7.5 mL of ethanol. A solution of KOH 50% was prepared, and 2 mL was mixed with the samples. The samples were incubated for 30 min at 70 °C. After incubation, the samples were cooled down, and 2.5 mL of NaCl (20 g/L) was added. For the extraction, 7.5 mL of hexane-ethyl acetate (85:15, v/v) was added 3 times, and the top organic layer was carefully collected and evaporated with N_2 until dryness. The fatty residue was reconstituted with 1 mL of methanol.

2.8.2. HPLC Analysis Method

The HPLC method was carried out following [órnaś,etal.[20] and Aksoz, *et al.* [21], with some modifications. The equipment used was a Vanquish system (Thermo Scientific). The separation was performed on a Phenomenex silica (C18) column (4µm, 15mm x 4,6 mm) using a mobile phase containing methanol: acidic - water (0.1 % formic acid) (93:7 v/v) with a flow rate of 0.3 mL/min using isocratic elution, with the column oven temperature at 40°C and the UV detector at 295 nm. Data was analysed using the Chromeleon 7.2.10 Chromatography Data System (CDS) software (Thermo Scientific), and the peaks were identified by comparison to the retention times of reference standards.

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2.9. Statistical Analysis

The analysis of the studied samples was performed in triplicate. The results were presented as the mean ± standard deviation (SD). The statistical analysis was carried out with GraphPad Prism software, version 10.2.2. (341) for Mac, GraphPad Software, Boston Massachusetts USA, www.graphpad.com.One-way analysis of variance (ANOVA) was used to compare the differences between the studied extracted oil and were considered significant at $P < 0.05$.

The principal component analysis (PCA) was performed using RStudio Version 2023.12.0+369. RStudio Team (2023). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA*.URL http://www.rstudio.com/.

3. Results and Discussion

Oils from Andean seeds, tarwi, and cañihua were successfully extracted using petroleum ether, hexane, and ethanol. The yields, total phenolic compounds, and antioxidant activity varied among the solvents. Characterization involved analysing the fatty acid profiles and tocopherol compositions of the oil fractions. In addition, the composition of the extracted oils was compared with commercial sunflower, rapeseed, and olive oils.

The conventional solvent method, Soxhlet, is known for its high oil recovery rates, typically from 90% to 98%. However, this method demands significant energy consumption and financial investment. Moreover, using hexane, the most common solvent in this method poses risks due to its toxicity and flammability. In this work, the extraction of tarwi and cañihua with ethanol was studied as a safer and environmentally friendly alternative to hexane [22]. Ethanol has also been used for extracting oil from *Tamarindus indica*, performing a more effective yield than chloroform, methanol, a mixture of chloroform and methanol (2:1 v/v), and isopropanol [23].

3.1. Oils Extraction

The highest oil yield of the oil extraction was obtained with hexane for both seeds, followed by petroleum ether and ethanol as solvents. These findings suggested that the polarity of the solvents affects the extraction of oils. An explanation for these results is attributed to the difference between solvent polarities and the balance achieved in the solvent phase. The alcohols are generally more polar than hexane, and ethanol gave the lowest oil yield due to its inefficient solvation [24,25].

The yield obtained with hexane for tarwi oil extraction (18.31 %) is higher than the other solvents (Table 1), being an expected value according to other studies where they indicate that the percentage of oil in *Lupinus mutabilis* can be between 16 - 20% [26], the value is also within the range reported in similar studies (Table 2).

According to Repo-Carrasco-Valencia, *et al.* [27] cañihua grain can contain 6-11 % of oil content, depending on the ecotype. In this study, the highest yield (6.73%) was obtained with hexane as a solvent. In contrast, the yields were lower for the petroleum ether and ethanol, following the same behaviour as the tarwi samples (Table 1). The yield obtained in this work is lower than the reported in other studies for samples collected from Peru [28]; however, it is within the range of values reported from samples from Amsterdam [29] (Table 2). It should be noted that other factors beyond the type of solvent, such as the number of samples analysed, geographical conditions, varieties, and seasonality of the harvest, can influence the oil content [30].

Table 1. Extracted oil yields % (w/w) with different solvents. Values are expressed as mean values ± standard deviations, n=3. *Values are significantly different ($p < 0.05$).

	Petroleum Ether	Hexane	Ethanol
Tarwi Oil (%)	16.57 ± 0.33	18.31 ± 0.44	13.49 ± 1.69
Cañihua Oil (%)	6.02 ± 0.42*	6.73 ± 0.29*	5.84 ± 0.44*

Table 2. Comparison table of oil Soxhet-extraction yield % (w/w) of tarwi and cañihua in different works (BO=Bolivia, PE= Peru, EC= Ecuador, AM= Amsterdam).

Sample	Content of oil (%)	Solvent	Reference
Tarwi – BO	18.31 ± 0.44	Hexane	Present work

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Tarwi - PE	19.38 ± 0.32	Petroleum ether	[31]
Tarwi - EC	18.3 ± 2.1	Hexane	[32]
Cañihua - BO	6.73 ± 0.29	Hexane	Present work
Cañihua -AM	6.15 ± 0.76	Petroleum ether	[29]
Cañihua - PE	8.50 ± 0.36	Hexane	[28]

3.2. Antioxidant Capacity (FRAP) and Total Phenolic Content (TPH)

The antioxidant capacity in oils is an important characteristic since this property can improve the nutritional and functional value of the oils. Antioxidants are typically used to enhance shelf life, preserve the quality of edible oils and fats, and protect against damage caused by free radicals. Further, they have been shown to play important roles in the development of many chronic diseases, including cardiovascular diseases, ageing, heart disease, anaemia, and cancer [33].

The extraction technique and solvent can play an important role in the antioxidant capacity of oils. The solvents' polarity could determine the solvent's efficiency [34] and lead to a smaller or larger amount of phenolic compounds obtained [35].

The oils extracted with ethanol show a significant difference ($p < 0.05$) compared to the use of other solvents in terms of antioxidant capacity and total phenolic compounds (Table 3). The values obtained from the oil extracted from tarwi are higher than the antioxidant capacity and total phenolic compounds reported in previous studies [5]. In the case of cañihua oils, they are similar to the levels reported by Limachi, *et al.* [36]. In addition, the oils extracted from tarwi and cañihua generally show a higher antioxidant capacity and phenolic compound content than the commercial oils that were also studied (Figure 1 and Figure 2). However, these results were expected since the commercial oils pass through the refined packing and storage process, and some of the antioxidants and phenolic compounds could be lost [37,38].

Table 3. The effect of the solvent regarding the antioxidant capacity and total phenolic compounds in oils extracted from tarwi and cañihua seeds. Values are expressed as mean values ± standard deviations, n=3, the data is expressed in the unit of mM Ferrus Equivalents ($mMFe^{2+}$ equivalent) and mmol Equivalents of galic acid (mmol GAE/L(. *Values in the same row differ significantly ($p < 0.05$). **Values in the same row are significantly different ($p < 0.001$).

	Antioxidant Capacity (FRAP) (mM Ferrus Equivalents)			Total Phenolic Compounds (TPH) (mmol Equivalents of Galic acid)		
Samples	Petroleum Ether	Hexane	Ethanol	Petroleum Ether	Hexane	Ethanol
Tarwi Oil	0.66 ± 0.03	1.13 ± 0.07	1.63 ± 0.20*	0.82 ± 0.08	2.82 ± 0.01	6.12 ± 0.01**
Cañihua Oil	0.80 ± 0.14	0.78 ± 0.04	1.37 ± 0.45*	0.69 ± 0.02	0.39 ± 0.01	2.99 ± 0.03**

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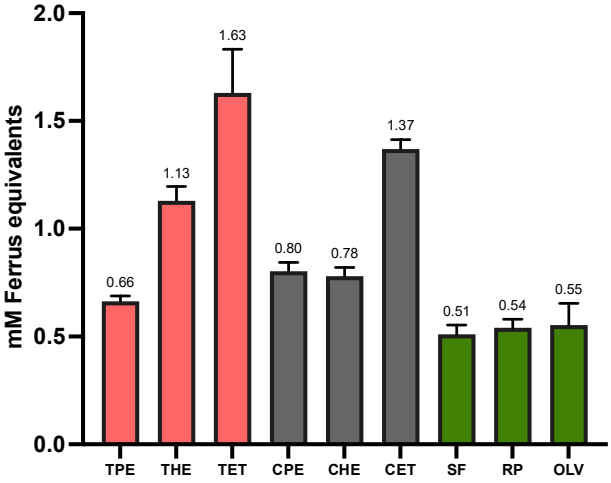

Figure 1. Total antioxidant capacity. The effect of the solvent type on the antioxidant capacity of oils was determined by the FRAP method. (TPE= tarwi oil extracted with petroleum ether, THE= tarwi oil extracted with hexane, TET= tarwi oil extracted with ethanol, CPE= cañihua oil extracted with petroleum ether, CHE= cañihua oil extracted with hexene, CET= cañihua oil extracted with ethanol, SF= sunflower Oil, RP= rapeseed oil, OLV= olive oil). Error bars are expressed as mean values ± standard deviations, n=3.

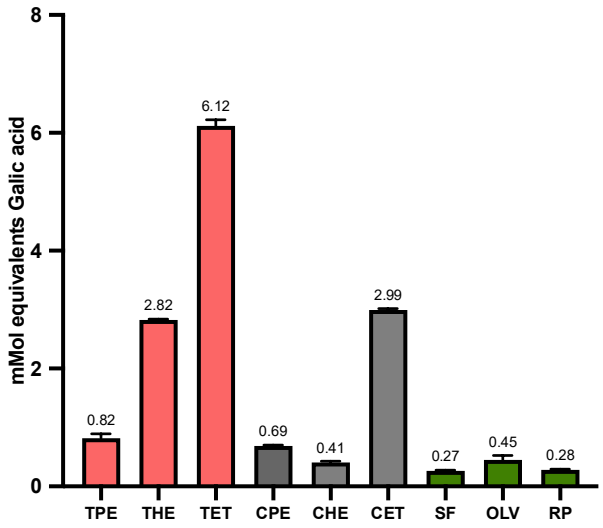

Figure 2. Total phenolic content. The effect of the solvent type on the content of total phenolic compounds. (TPE= tarwi oil extracted with petroleum ether, THE= tarwi oil extracted with hexane, TET= tarwi oil extracted with ethanol, CPE= cañihua oil extracted with petroleum ether, CHE= cañihua oil extracted with hexene, CET= cañihua oil extracted with ethanol, SF= sunflower Oil, RP= rapeseed oil, OLV= olive oil). Error bars are expressed as mean values ± standard deviations, n=3.

3.3. High-Performance Thin Layer Chromatography

The determination of lipids classes was carried out using HPTLC, and lipids were separated based on polarity using different mobile phases. The extraction solvent had no significant effect on the analysis of neutral lipids (Figure 3), and a similar composition of Diacylglycerol (DAG), Free fatty acids (FFA,) and Triacylglycerol (TAG) was extracted in all the samples of tarwi and cañihua, respectively.

Oils from tarwi and cañihua extracted with ethanol showed higher amounts of polar lipids than those extracted with hexane and petroleum ether (Figure 4). Phospholipids such as phosphatidylcholine (PC) and phosphatidylinositol (PI) HPTLC bands are more intense for the oils

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extracted with ethanol and less visible for those extracted with the other solvents. Recent studies reported that polar lipids might benefit human health, e.g. reducing the risk of cardiovascular diseases and managing the blood lipid level, if they are present in our diet [39]. Other studies suggest phospholipids are potential emulsifiers for developing additives and other food products [40]. Phospholipids reduce the surface tension in the oil-water interface and improve the stability of the emulsions [41].

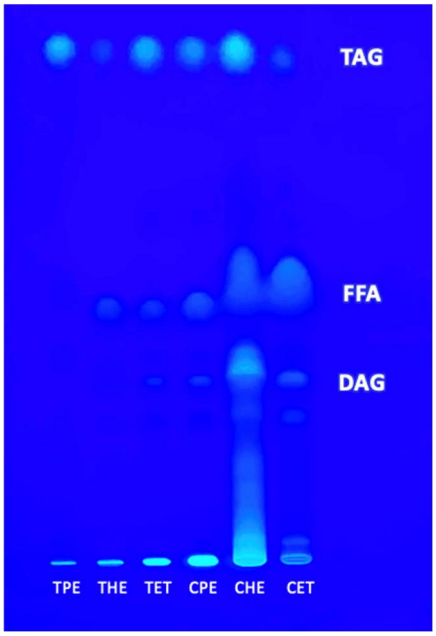

Figure 3. Neutral Lipids separated by HPTLC. TPE= tarwi oil extracted with petroleum ether, THE= tarwi oil extracted with hexane, TET= tarwi oil extracted with ethanol, CPE= cañihua oil extracted with petroleum ether, CHE= cañihua oil extracted with hexene, CET= cañihua oil extracted with ethanol, DAG= diacylglycerols, FFA= free fatty acids, TAG= triacylglycerols).

The most abundant phospholipids in the tarwi oil were phosphatidylinositol (PI) and phosphatidylcholine (PC) (Figure 4). The oils extracted with ethanol showed the most intense HPTLC bands. These results are consistent with other studies on lupine phospholipids [42,43]. In the cañihua oil samples, several bands corresponding to unknown phospholipids were observed, which are interesting molecules to investigate in future work.

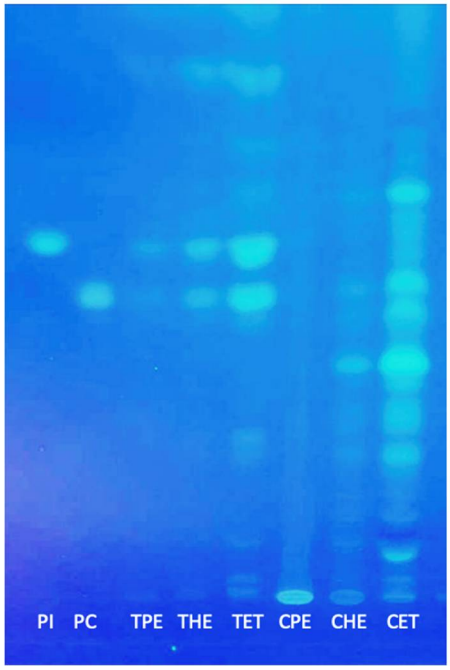

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Figure 4. HPTLC separates polar lipids. TPE= tarwi oil extracted with petroleum ether, THE= tarwi oil extracted with hexane, TET= tarwi oil extracted with ethanol, CPE= cañihua oil extracted with petroleum ether, CHE= cañihua oil extracted with hexene, CET= cañihua oil extracted with ethanol, PI= phosphatidylinositol Standard, PC= phosphatidylcholine Standard.

3.4. Fatty Acid Profile

Saturated fatty acids (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) of tarwi and cañihua oils were analysed by gas chromatography. The MUFA and PUFA are of special interest as they are essential in nutrition [44]. Traditionally, most studies have been interested in the health impact of fatty acids related to cardiovascular diseases, but recently, the influence on other diseases has been highlighted, such as type 2 diabetes, inflammatory diseases, and cancer [45].

3.4.1. Fatty Acid Composition in Tarwi Oils

The composition of fatty acids in oils extracted from tarwi with the three solvents is similar to each other, with the main representative fatty acids being palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2-n6), and oleic acid (C18:1-n9) (Table 5).

The values obtained of the main fatty acids are higher in comparison with values reported by Al-Amrousi, *et al.* [46] for lupin seed oils from different varieties were extracted with petroleum ether and showed values between 42.65 – 50.87 % of oleic acid, 5.61 – 8.89 % of palmitic acid and 0.61 – 3.52 % of stearic acid. Also, other values are lower than the values obtained in the present study with 42.33 and 54.33 % for oleic acid [31]. This study was carried out through the extraction by Soxhlet with hexane. On the other hand, Rodríguez, *et al.* [47] reported a linoleic acid content of 25.7 %, which is higher than those obtained in the extraction with ethanol.

Table 4 shows all the fatty acids extracted with the different solvents. Hexane and ethanol extracted a total of 10 fatty acids, among them erucic acid (C22:1-n9), tricosanoic acid (C23:0), and cis-11-eicosenoic acid (C20:1-n9), while the oils extracted with petroleum ether only present 8 in their total composition.

The total saturated fatty acids (SFA) were higher in the oil extracted with hexane (15.4 %) compared with other solvents (Table 4). In this group, we can find fatty acids like palmitic acid (C16:0), one of the most abundant saturated fatty acids in nature. It is present in animal and human tissues, plants, algae, fungi, yeasts, and bacteria [48]. The average dietary intake of this fatty acid is around 20-30 g/day. It can be found in different vegetable and animal fats sources, with 20-30% in animal lipids and 8-45% in vegetable oils, making palm oil the primary source [49].

The total monounsaturated fatty acids (MUFA) are similar in oils extracted with petroleum ether and ethanol (62.7 and 62.3 %), respectively. Erucic acid (C22:1-n9), only extracted with ethanol, is a monounsaturated fatty acid (MUFA) with a chain length of 22 carbon atoms and one double bond in the omega-9 position. This fatty acid has beneficial properties such as being anti-inflammatory, has shown neuroprotective activity and can be a carrier of drugs [50]. Oleic acid is one of the most abundant MUFA in the extracted oils (C18:1-n9). This fatty acid is an omega-9 fatty acid. It is considered health-beneficial as it has been connected to decreased cholesterol levels and reduced some inflammation in the body [51,52].

The polyunsaturated fatty acids (PUFA) (29.3 %) were obtained with hexane. Linoleic acid (C18:2-n6) is an essential fatty acid nutrient containing 2 double bonds at the 9th and 12th carbons. It is known as an omega-6 fatty acid and is the most highly consumed PUFA in the human diet. Evidence shows that this fatty acid improves insulin sensitivity and blood pressure and reduces total and LDL cholesterol [53,54].

Table 4. Fatty acid composition expressed as % of total fatty acids detected of tarwi oil extracted with different solvents. Values are expressed as mean values ± standard deviations, n=3. (SFA=saturated fatty acids, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids) ND = not detected. *Values in the same row differ significantly ($p < 0.05$).

	Composition in % of total fatty acids		
Fatty acid	Petroleum Ether	Hexene	Ethanol

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C14:0 (myristic acid)	0.13 ± 0.04	0.12 ± 0.01	0.13 ± 0.03
C16:0 (palmitic acid)	8.43 ± 0.11	8.69 ± 0.68	8.57 ± 0.52
C16:1 (palmitoleic acid)	0.20 ± 0.03	0.21 ± 0.02	0.21 ± 0.01
C18:0 (stearic acid)	5.17 ± 0.11	5.59 ± 0.37	4.75 ± 0.11
C18:1-n9 (oleic acid)	62.49 ± 0.80	55.02 ± 1.31*	62.03 ± 1.60
C18:2-n6 (linoleic acid)	21.57 ± 0.35	28.15 ± 1.64*	21.79 ± 1.33
C18:3-n3 (α-linolenic acid)	1.04 ± 0.07	1.11 ± 0.08	1.42± 0.18
C21:0 (henicosanoic acid)	0.96 ± 0.03	0.79 ± 0.17	0.89 ± 0.05
C20:1n9 (cis-11-eicosenoic acid)	ND	0.10 ± 0.80	0.11 ± 0.01
C23:0 (tricosanoic acid)	ND	0.22 ± 0.70	ND
C22:1n9 (erucic acid)	ND	ND	0.11 ± 0.01
Total number of fatty acids extracted	8	10	10
SFAs	14.7	15.4	14.3
MUFAs	62.7*	55.3	62.3*
PUFAs	22.6	29.3*	23.3

3.4.2. Fatty Acid Composition in Olis from Cañihua

The composition obtained of fatty acids of oils extracted from cañihua is similar in the three solvents, with the fatty acids as palmitic acid (C16:0), a-linoleic acid (C18:3-n3), linoleic acid (C18:2-n6), and oleic acid (C18:1-n9) being the most abundant (Table 6). The oil from cañihua extracted with ethanol shows a slightly higher concentration of oleic acid, linoleic acid, and palmitic acid than the oil samples extracted with other solvents. The values obtained for oleic acid (C18:1-n9) are higher than the values reported by Carpio-Jiménez, *et al.* [55] where the findings are around 24.4% and are lower than the values reported for linoleic acid (C18:3-n3) and palmitic acid (C16:0) with 41.1 % and 17.5 % respectively. Oils of cañihua extracted with hexane by Soxhlet shows values of 12.9 %, 27.8 %, and 45.8 % of palmitic acid (C16:0), oleic acid (C18:1-n9), and linoleic acid (C18:2-n6) respectively [28]. These values are close to our findings, with differences in lower palmitic acid (C16:0) and higher oleic acid (C18:1n-9) than reported.

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Table 5 shows all the fatty acids present in the different samples of cañihua oils. The Oils extracted with petroleum ether and hexane present 16 and 15 other fatty acids in their composition, respectively, among them cis-4,7,10,16,19-docosahexaenoic acid (DHA) and lignoceric (C24:0) fatty acids that are only present in the extraction with petroleum ether; and cis-8.11.14-eicosatrienoic acid (C20:3-n6) and elaidic acid (C18:1n9t) that are presented in the samples extracted with hexane.

The total saturated fatty acids (SFA) are similar in oils extracted with petroleum ether and ethanol (13.5 and 13.7 %), respectively. In this group, one of the most important SFA is palmitic acid (C16:0) for its important characteristics previously mentioned. Another SFA that is found in higher abundance in oils extracted from cañihua is stearic acid (C18:0). Stearic acid is a long-chain fatty acid in animal fat and plant oils [56]. Some scientific evidence suggests that including stearic acid in the diet may positively influence the prevention of cardiovascular diseases [57]. Moreover, consuming fats rich in stearic acid appears to have favourable effects on blood lipids and coagulation [58].

The total monounsaturated fatty acids (MUFA) are similar to the three solvents, with an average of (41,02 %). The oils extracted from cañihua show a significant amount of oleic acid (C18:1-n9) compared to other studies, as mentioned before. Still, other MUFAs like cis-11-eicosenoic acid (C20:1-n9) were also found. It is a monounsaturated fatty acid that, although found in small quantities, being MUFA, may have potential health benefits, particularly for heart health, as it decreases LDL cholesterol levels while maintaining or even increasing HDL (good) cholesterol levels [59].

Table 5. Fatty acid composition expressed as % of total fatty acids detected of cañihua oil extracted with different solvents. Values are expressed as mean values ± standard deviations, n=3. (SFA=saturated fatty acids, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids) ND = not detected. *Values in the same row differ significantly ($p < 0.05$).

	Composition in % of total fatty acids		
Fatty acids	Petroleum Ether	Hexane	Ethanol
C14:0 (myristic acid)	0.15 ± 0.01	0.22 ± 0.00	0.16 ± 0.01
C16:0 (palmitic acid)	9.49 ± 0.08	9.30 ± 1.30	9.60 ± 0.35
C17:1 (cis-10-heptadecanoic acid)	0.25 ± 0.01	0.30 ± 0.03	0.29 ± 0.01
C18:0 (stearic acid)	1.43 ± 0.09	1.75 ± 0.09	1.54 ± 0.04
C18:1-n9T (elaidic acid)	ND	0.91 ± 0.00	ND
C18:1-n9 (oleic acid)	37.85 ± 0.15	38.12 ± 1.54	40.07 ± 1.04
C18:2-n6 (linoleic acid)	43.2 ± 0.31	41.94 ± 0.88	43.39 ± 1.09
C18:3-n6 (γ-linoleinic acid)	0.20 ± 0.02	0.27 ± 0.00	0.16 ± 0.01
C18:3-n3 (α-linolenic acid)	2.40 ± 0.05	2.31 ± 0.40	2.60 ± 0.08
C20:1-n9 (cis-11-eicosenoic acid)	1.14 ± 0.01	1.16 ± 0.16	0.11 ± 0.01
C20:3-n6 (cis-8.11.14-eicosatrienoic acid)	ND	0.21 ± 0.00	ND
C20:3-n3 (cis-11.14.17-eicosatrienoic acid)	0.34 ± 0.01	0.37 ± 0,04	ND
C21:0 (henicosanoic acid)	1.00 ± 0.01	1.19 ± 0.11	1.05 ± 0.04
C22:0 (behenic acid)	0.53 ± 0.01	0.52 ± 0.66	0.55 ± 0.01
C22:1-n9 (erucic acid)	0.47 ± 0.02	0.68 ± 0.19	0.47 ± 0.01
C22:6-n3 cis-4,7,10,16,19-docosahexaenoic acid	0.63 ± 0.07	ND	ND
C23:0 (tricosanoic acid)	0.54 ± 0.01	0.73 ± 0.03	0.57 ± 0.07
C24:0 (lignoceric acid)	0.28 ± 0.01	ND	ND

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Total number of fatty acids extracted	16	15	13
SFAs	13.5	13.7	12.8
MUFAs	39.71	41.17	41.02
PUFAs	46.76	45.10	46.15

The total PUFAs in oils extracted from cañihua are around 46 %. Among this group of fatty acids, besides the well-known linoleic acid (C18:2-n6), were founding a-linolenic acid (C18:3-n3), cis-11.14.17-eicosatrienoic acid (C20:3-n3) and cis-4,7,10,16,19-docosahexaenoic acid (C22:1-n3) were found. The a-linolenic acid (C18:3-n3) is an essential omega-3 fatty acid connected to a wide range of health benefits, among them decreasing the risk of heart disease, helping to maintain normal heart rhythm and pumping, and promoting brain development and function [60]. Cis-4,7,10,16,19-docosahexaenoic acid (C22:1-n3), also known as DHA (docosahexaenoic acid), is abundantly found in fish oils and as well as in certain algae [61]. DHA plays a vital role in brain development and function, eye health, and overall cognitive function throughout life. It is also associated with cardiovascular health, reducing inflammation, and supporting the optimal functioning of various organs and systems in the body [62,63].

3.4.3. Comparison of the Fatty Acid Composition of Tarwi and Cañihua Oils with Commercial Oils.

In Figure 5 we can see the fatty acid composition of the oils extracted from tarwi and cañihua with ethanol as a solvent. Ethanol previously presented the best fatty acid profile in comparison with the other solvents and is compared with the fatty acid profile of commercial sunflower, rapeseed, and olive oils (Figure 6). The results are in line with what was expected: the percentage of the main fatty acids as oleic acid (C18:1-n9) and linoleic acid (C18:2-n6) is higher in commercial oils than the values obtained in tarwi and cañihua oils. However, tarwi and cañihua oils also show a considerable amount of these fatty acids, and that allows these oils to compete on the market and be a good source of health-beneficial fatty acids. Also, Figure 5 shows another interesting finding regarding the number of fatty acids obtained in the extraction of tarwi and cañihua oils, which are 10 and 13, respectively. Among the fatty acids that can be observed are omega 3 and 9 acids, as mentioned in more detail above, which have a beneficial potential for health if we include them in the diet [64].

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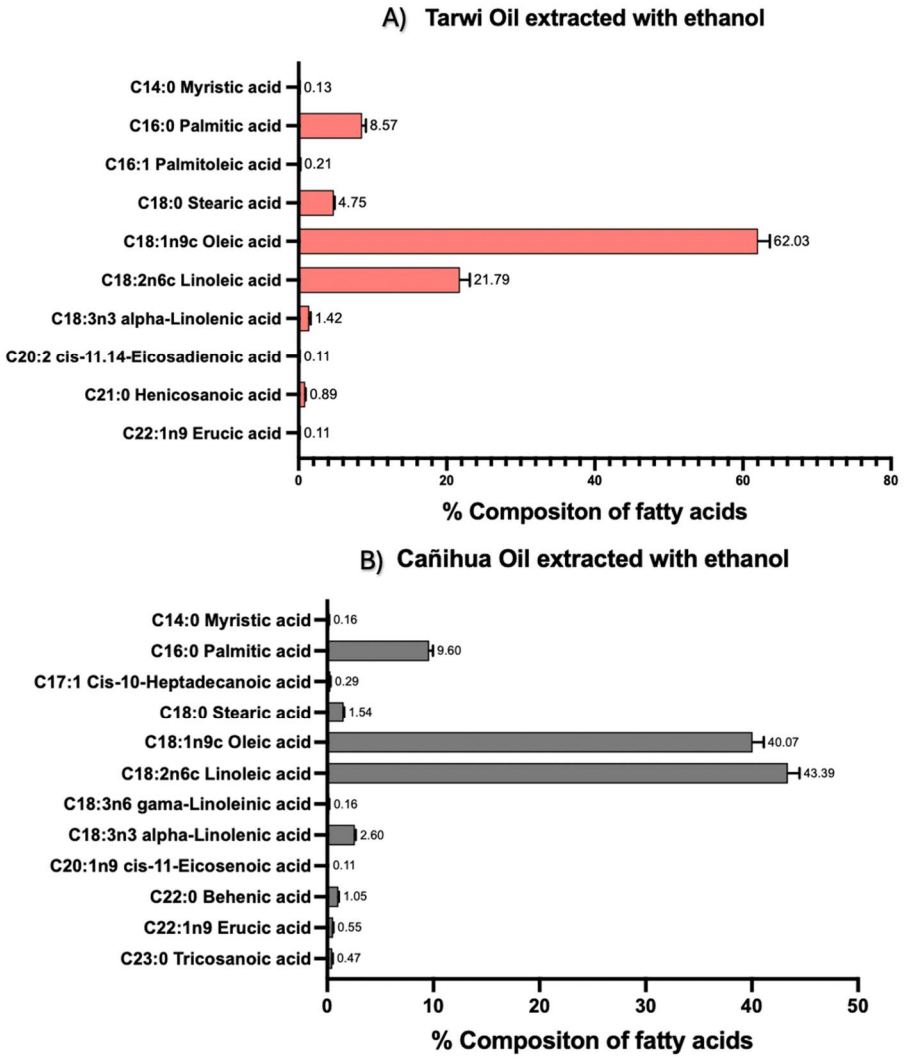

Figure 5. The fatty acid composition expressed as % of total Fatty acid detected(A) Tarwi oil extracted with ethanol. (B) Cañihua oil extracted with ethanol. Error bars are expressed as mean values ± standard deviations, n=3.

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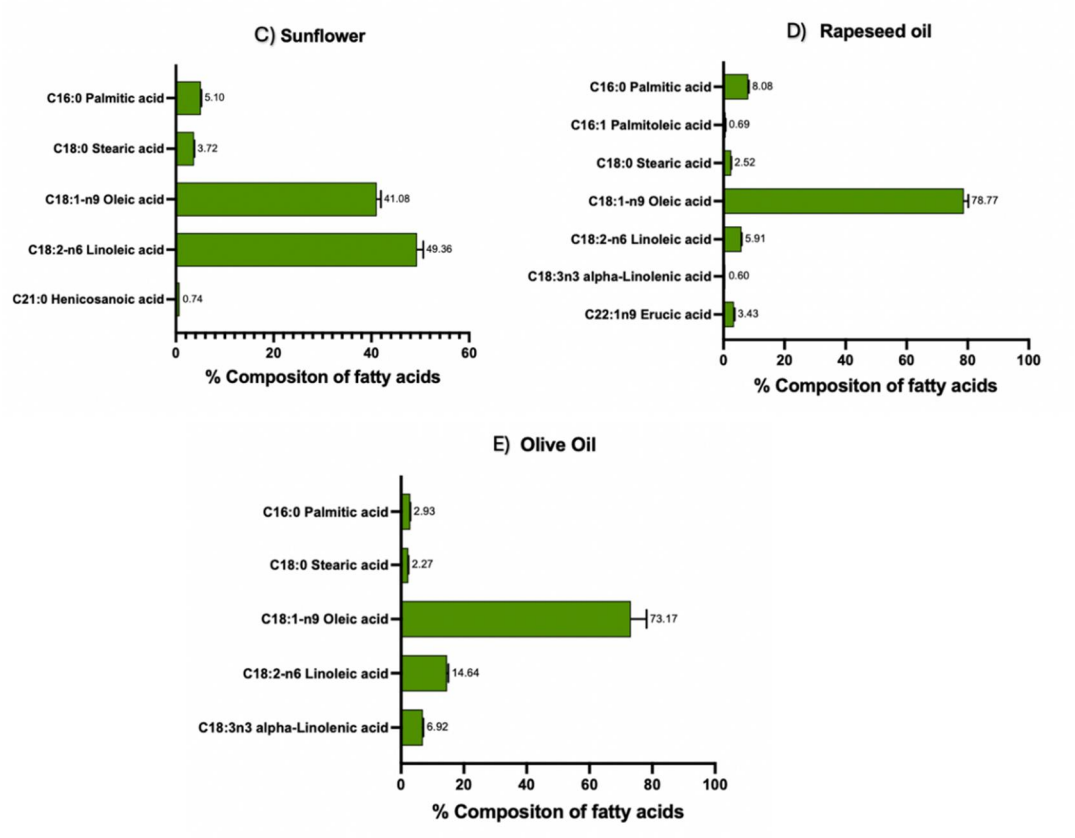

Figure 6. The fatty acid composition expressed as % of total fatty acid detected (C) Sunflower oil. (D) Rapeseed oil. (E) Olive oil. Error bars are expressed as mean values ± standard deviations, n=3.

3.5. Tocopherols

Tocopherols belong to the vitamin E family. Tocopherol isomers (a-, b-, g, and d) are the most potent natural fat-soluble antioxidants [13]. The most common and biologically active form of vitamin E is a-tocopherol. The primary biochemical function of tocopherols is believed to protect polyunsaturated fatty acids against peroxidation due to the chromanol ring and a hydrophobic side chain. This structure allows tocopherols to reduce free radicals [65,66]. In tarwi and cañihua oils, a-, b-, g-, and d-tocopherols were identified, where g-tocopherol is dominant (Table 6). The highest amount of g-tocopherol was present in the oil extracted from tarwi with ethanol as a solvent, with 205.1 mg/kg, while the extraction with petroleum ether yielded 22.2 mg/kg. These values are similar to those reported in other studies in the 192 to 234 mg/kg range by Estivi, *et al.* [67] and 103 mg/kg observed by Boschin and Arnoldi [68]. Regarding the levels of a-tocopherol obtained, the oil samples from tarwi extracted with hexane and ethanol are higher than those obtained in other studies that show values between 0.26 – 2.7 mg/kg [1]. The tocopherol values obtained from the oil from cañihua are higher than the values reported in Repo-Carrasco-Valencia [5] where the values of a-, and g-, tocopherols were 1,6 and 6,9 mg/100 g of dry weight respectively in cañihua grain samples. Other studies report values of g a and d- tocopherols in different seeds like quinoa, with values of 48.4, 22.74, and 1.62 mg/100 g of oil, respectively [69].

Table 6. Effect of the solvent in the concentrations of α, γ, and δ- tocopherols in oils extracted from tarwi and cañihua seeds. (TPE= tarwi oil extracted with petroleum ether, THE= tarwi oil extracted with hexane, TET= tarwi oil extracted with ethanol, CPE= cañihua oil extracted with petroleum ether, CHE= cañihua oil extracted with hexene, CET= cañihua oil extracted with ethanol) dw= dry weight. Values are expressed as mean values ± standard deviations, n=3.

	Tocopherols mg/Kg of dw		
Sample	Delta (δ)	Gamma (γ)	Alpha (α)

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TPE	11.3 ± 0.20	22.2 ± 1.43	11.5 ± 0.05
THE	13.9 ± 0.19	161.6 ± 2.90	15.5 ± 0.11
TET	13.5 ± 0.10	205.1 ± 0.53	16.6 ± 0.43
CPE	13.6 ± 0.13	13.8 ± 2.73	15.6 ± 0.47
CHE	22.5 ± 0.58	26.1 ± 0.95	20.1 ± 0.46
CET	23.7 ± 0.50	28.3 ± 1.99	21.0 ± 0.54

3.6. Principal Component Analysis (PCA).

The principal component analysis (PCA) was applied to identify the variability and the patterns in data obtained in the present work. Figure 8 presents the biplot of the samples of oils from tarwi and cañihua seeds, extracted with different solvents, and all the parameters studied (antioxidant capacity, total phenolic compound, fatty acid profile, and concentration of tocopherols). PC1 and PC2 together explained 72,5% of the total variance. It showed an effect due to the solvent, where PC2 distinguished two groups. The PC1 showed that the observations close to component one (X-axis) had similar profiles to the oil samples from tarwi extracted with hexene and ethanol and related to the variables close to a principal component. It was also possible to confirm that samples extracted with ethanol as a solvent had high antioxidant and phenolic content and a high amount of a and g-tocopherol. In contrast, the sample of oil from cañihua extracted with hexene showed a high amount of PUFA.

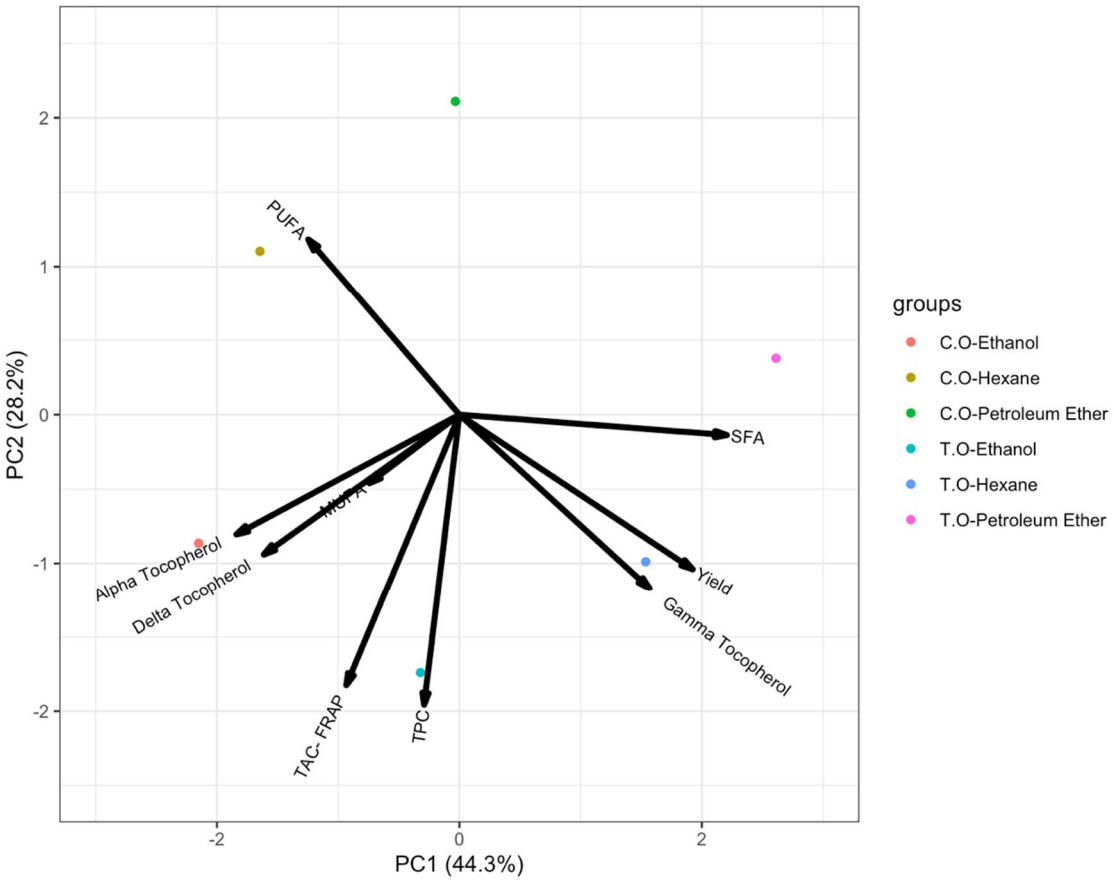

Figure 7. PCA biplot represents the 3 solvents used in oil extraction from tarwi and cañihua seeds and their relations with all the parameters developed in this study. (C.O - Ethanol= Cañihua oil extracted with ethanol, C.O-Hexane = Cañihua oil extracted with hexane, C.O – Petroleum ether = Cañihua oil extracted with petroleum ether, T.O - Ethanol= Tarwi oil extracted with ethanol,T.O – Hexane = Tarwi oil extracted with hexane, T.O – Petroleum ether = Tarwi oil extracted with petroleum ether).

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This is in line with the expected results; the solvents used for the extraction of tarwi and cañihua oils were demonstrated to affect different aspects of their composition in the characterization that was carried out. One of the most evident effects in the determination of antioxidants was that the values obtained by extraction with ethanol for both seeds were significantly higher than by the other solvents. The same was seen for the concentration of tocopherols and expected since the solvent effect on tocopherols follows the same chemical principles. It could also be seen that the type of solvent did not have much influence on the determination of the fatty acid profile since the total values of SFA, MUFA, and PUFA for both seeds did not show any significant difference.

These results show great potential in oils extracted from tarwi and cañihua seeds. Highlighting the extraction with ethanol as a solvent, which is not only a green solvent alternative but can also give added value to the extracted oil, such as significantly increased level of antioxidants, a greater concentration of α-γ-tocopherol, and interesting omega-3 fatty acids in its composition.

4. Conclusions

Vegetable oils extracted from tarwi and cañihua seeds are a promising alternative to diversify the traditional vegetable oil market. The results show the potential of these oils in terms of nutritional and functional levels. Extraction with ethanol, a green alternative, was the best way to obtain the highest levels of antioxidants and phenolic compounds. In the same way, the concentration of α-γ-δ-tocopherols was higher with this solvent.

Tarwi and cañihua oils are natural food components with high nutritional value. In comparison, commercial oils show a more diverse profile of fatty acids. The fatty acids found are connected to a positive effect on human health and are highly recommended in a well-balanced diet. In addition, the oils extracted with ethanol also show an interesting polar lipid profile, which should be studied in detail in future research. These polar lipids might offer unique functional properties that make the oils suitable for specific applications, potentially opening new markets and opportunities for innovation.

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Data Availability Statement: All data are available in the article. The datasets generated during and/or analysed during the current study are available from the corresponding authors.

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