Nutritional, Microbiological and Functional Properties of Unrefined Omega-3 Oils and Natural Essential Oils as Preservatives

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Abstract. This study evaluates the oxidative stability, microbiological parameters, color characteristics, and fatty acid profiles of unrefined omega-3-rich oils (flaxseed, hemp seed, grape seed, chia, algae, fish, and cod liver oils). Volatile compounds from essential oils of coriander, bay leaf, and dill—used as natural antioxidants—were analysed, along with their antimicrobial activity. All oils demonstrated acceptable oxidative stability and typical color values. Coriander oil showed the highest chlorophyll A (32.98 ppm) and carotenoids (61.14 ppm). Infrared spectroscopy indicated the highest omega-3 content in flaxseed, hemp, and chia oils. Fish, cod liver, and algae oils showed unique ester profiles due to their vitamin and phospholipid content. Microbial analysis revealed values below 10 cfu/g for the presence of pathogenic bacteria, moulds, yeasts, and total viable count (TVC), with essential oils—particularly coriander—exhibiting strong antimicrobial effects. Chia oil was richest in omega-3s, followed by flaxseed, algae, fish, and cod liver oil. Grape seed oil had high α- and γ-tocotrienol content, enhancing its antioxidant potential. Phytosterols and triterpenoids were present in all plant oils, with squalene in chia oil and cycloartenol in flaxseed oil. Chia, algae, and cod liver oils show promise for functional dairy applications due to their stability and favourable sensory properties.

1 Introduction

In recent decades, there has been an increasing interest in functional oils rich in omega-3 polyunsaturated fatty acids (PUFAs), due to their significant role in human health and potential use as ingredients in food and technological applications. Among these, cold-pressed vegetable oils and marine or algal oils occupy a leading position, as they contain substantial amounts of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) — compounds associated with cardioprotective, anti-inflammatory, and neuroprotective effects [1]. Cold pressing is a process in which oils are extracted at low temperatures without the use of organic solvents, allowing the preservation of bioactive compounds such as tocopherols, sterols, and carotenoids [2]. This enhances the nutritional value of the final product and differentiates it from refined oils, where these components are often lost or degraded during refining [3]. The fatty acid composition of cold-pressed oils plays a crucial role in determining them nutritional and technological characteristics. High levels of unsaturated fatty acids, particularly omega-3, enhance the biological activity of oils but also make them more prone to oxidative degradation [4]. Therefore, evaluating their chemical stability, microbiological safety, and functional properties is essential for their effective application in the food industry. While a substantial body of data exists on plant-derived cold-pressed oils, relatively few studies have focused on the combined evaluation of vegetable, fish, and algal omega-3 oils with respect to their fatty acid and sterol composition, antimicrobial activity, and functional stability [1, 4].

The aim of the present study is to comprehensively assess the chemical (including fatty acid and sterol profile), microbiological, and functional (antimicrobial and stability) characteristics of a set of cold-pressed oils — plant-based (flaxseed, chia, grape seed, hemp) and marine (fish oil, cod liver oil, algal oil). In this way, the study provides an integrated perspective on the potential of these oils as functional ingredients in modern food systems.

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2 Materials and methods

2.1 Materials

Linseed oil and chia oil from Balcho Agro product Ltd. Grape seeds oil and hemp oil from Sevoa Ltd. Fish oil 18/12, Cod liver oil and Algae oil from BASF SE. Essential oil from coriander, fennel and bay leaf from Eterika Ltd.

2.2 Methods

2.2.1 Determination of acid value, peroxide value and Thiobarbituric acid value

Determination of acid value was made according to ISO 660:2020 [5]. The samples were dissolved in a solvent mixture, and the free fatty acids were quantified by titration with an ethanolic potassium hydroxide solution. The acid value was calculated using the following formula:

$$AV=V.F.5,6104/m, mgKOH/g\ oil \quad (1)$$

Where:

V = the volume of 0.1 N alcoholic potassium hydroxide in mL, consumed in the titration of the oil

F = the factor of 0.1 N alcoholic solution of potassium hydroxide

5.6104 = the volume of potassium hydroxide, in grams, contained in 1mL of 0.1 N alcoholic potassium hydroxide solution

m = the weight in grams of the sample

Peroxide Value (PV) was determined according to Popa et al. [6] with modifications. PV was determined as 0.200±0.0002g of oil was dissolved with 5mL chloroform and 2.5mL acetic acid, after that the sample was stirred until the oil was completely dissolved. Subsequently, 1mL of a 50% potassium iodide solution was added, and the mixture was allowed to stand in the dark for 5 minutes. Afterwards, 15–20mL of distilled water and a few drops of a 1% starch solution were added, resulting in the appearance of a blue-violet coloration. The sample was titrated with 0.002 N sodium thiosulfate solution slowly, under continuous homogenization by shaking, until the coloration in the aqueous (upper) layer disappeared. The volume of sodium thiosulfate solution consumed was recorded in mL. A blank sample was prepared under the same conditions, containing the reagents without the oil.

The peroxide value in meqO_2/kg was calculated using the formula:

$$PV=(V-V_0).0,002.1000/m, meqO_2/kg \quad (2)$$

Where:

V = the volume in mL of 0.002 n sodium thiosulfate used to titrate the oil sample

V_0 = the volume in mL of 0.002 n sodium thiosulfate used to titrate the blank sample

m = the weight in grams of the sample

Thiobarbituric acid value (TBA) was determined according to Wang et al. [7] with modifications. TBA was determined as 0.5g oil was weighted. An oil sample 0.5g was mixed with 50mL of 0.9% NaCl and stirred for 3 min. Then, 50mL of 10% trichloroacetic acid was added and the mixture was filtered through folded filter paper. From the filtrate, 4mL was combined with 1mL of thiobarbituric acid in a test tube. The tubes were incubated in a water bath at 70°C for 30min. Absorbance was measured at 532nm against a blank prepared with water instead of filtrate.

TBA content was calculated from a standard curve of malondialdehyde(MDA) and expressed as MDA mg/per kg sample. The MDA concentration was calculated as:

$$MDA=100.C/g.4, mg/kg \quad (3)$$

Where:

C = concentration MDA

g = grams of the sample

2.2.2 Colorimetric analysis (X,Y,Z, Lovibond's colors)

The transmission spectra of the samples were analysed using an Evolution Pro UV-Vis Spectrophotometer (Thermo Fisher Scientific) in the wavelength range from 250 nm to 750 nm employing a 1mL quartz cuvette at room temperature (20°C). The samples were poured directly into the cuvette and their spectral characteristics were recorded without dilution. The color parameters were calculated using the VISIONlite ColorCalc software. The color parameters of the oils used were determined in the CIELab colorimetric system after pre-tempering of the samples. The brightness (L) and color characteristics (a and b) in the CIELab colorimetric system were measured. along with the color coordinates X, Y, Z and chromaticity coordinates x, y in the XYZ colorimetric system under the following visual reference conditions: standard illuminant D65 (corresponding to a color temperature of 6504 K) and standard CIE 1964 observer (10° viewing angle). For the purposes of this study the CIELab system is more informative. as it is designed for detecting small color differences. The dominant wavelength and color purity were also determined as well as parameters such as lightness (L), color saturation (C) and hue angle (h).

2.2.3 Infrared spectroscopy

We used a Jasco FT/IR-4X FTIR spectrophotometer to get Fourier-transform infrared (FTIR) spectra. The spectral range went from 4000 to 400 cm^{-1}. To reduce background noise from the equipment. Each sample was scanned 32 times at a resolution of 2 cm^{-1}. Each of the seven cold-pressed oil samples was scanned three times to ensure measurement repeatability and spectral consistency and the averaged FT-IR spectrum is presented.

2.2.4 Water content and relative density of oils

Determination of water content was made according to ISO 662:2016 [8]. Water content was determined as 10 g oil was weighted. The sample was dried at 103 °C ± 2 °C until the mass loss between successive weighings were not exceed 2 mg.

The water content value was calculated using the formula:

$$w=((m_1-m_2)/(m_1-m_0)).100 \quad (4)$$

Where:

m_0 = is the mass, in grams, of the dish and the thermometer, or of the glass vessel

m_1 = is the mass, in grams, of the dish, thermometer and test portion, or of the vessel and test portion before heating;

m_2 = is the mass, in grams, of the dish, thermometer and residue, or of the vessel and residue after heating.

Density was determined according to Bilgin et al. [9] with modifications. The relative density of the oil was determined using a pycnometric method. The oil sample was first filtered through sodium sulphate to remove moisture and particulate impurities. A clean, dry, and pre-weighed pycnometer (accuracy ±0.001g) was filled slowly with the oil at approximately 20 °C to prevent the formation of air bubbles. For capillary pycnometers, the oil level was adjusted to the top of the capillary, while for conventional pycnometers it was set to the calibration mark. The pycnometer was then equilibrated in a thermostatic water bath at 20 °C until the oil level stabilized (≈30 min). Excess oil was removed, the exterior of the pycnometer was wiped, and the filled pycnometer was weighed. The mass of distilled water, boiled and cooled to 20 °C, was determined under the same conditions. The relative density of the oil was calculated as the ratio of the oil mass to the mass of an equal volume of water at 20 °C.

The relative density was calculated using the formula:

$$D=(m_2-m_1)/(m_1-m) \quad (5)$$

Where:

m = mass of the empty pycnometer in g

m_1 = mass of the pycnometer with water in g at 20°C

m_2 = mass of the pycnometer with the oil in g

2.2.5 Fatty acid content and content of tocopherols, phytosterols and triterpenoids

Fatty acid content was determined according to Ivanova et al. [10]. For fatty acid analysis, oils were transmethylated using an alkaline method. Approximately 10–20 mg of oil was dissolved in 2.5mL heptane, followed by the addition of 2.5mL of 2N KOH in methanol and vigorous mixing for 1 min. After phase separation, the upper layer was injected into a Thermo Trace GC equipped with a Supelco SP2560 column (100 m × 0.25 mm ID) at a split ratio of 1:100. The oven temperature was programmed from 100 °C (1 min) to 240 °C at 5 °C/min, with a 5 min hold. Injector and FID detector temperatures were maintained at 250 °C. Fatty acid methyl esters (FAMEs) were identified by comparison with a Supelco 37-component standard, and quantification was based on area normalization of peak areas.

Tocopherols were determined following the DGF standard method F-II 4a (00) ((Deutsche_Gesellschaft_für_Fettwissenschaft, e.V., Standardmethode F-II 4a (00)). Briefly, 1 g of oil was weighed (±0.1 mg), diluted to 25mL with hexane, and 20 µL of the solution was injected into an HPLC system equipped with a Supelco NH_2 column (25 cm × 4.6 mm) and a fluorescence detector (excitation 295 nm, emission 340 nm). The mobile phase consisted of 70% hexane and 30% ethyl acetate. Quantification was carried out by external calibration according to the DGF method.

A 100 mg of oil was saponified according to Ulberth and Reich [11]. Phytosterols were separated by GC using a Thermo FOCUS GC with FID detection and an SLB-5 column (30 m × 0.25 mm ID) with a split ratio of 1:25. The oven program was 100 °C for 1 min, ramped at 30 °C/min to 260 °C, then 3 °C/min to 300 °C with a 3 min hold. α-Cholestane was used as an internal standard, and phytosterols were quantified using a calibration curve of cholesterol. Identification of individual phytosterols was previously performed by GC-MS.

2.2.6 Microbiological analysis

2.2.6.1 Microbiological analyses of microorganisms

The microbiological analyses of oils are determined according to the standards:

TVC (Total Viable Count) was determined according ISO 4833-1:2013 [12].

Moulds and yeasts were determined according ISO 21527-1:2011 [13].

Escherichia coli was determined according microbiology of food and animal

feeding stuffs — Horizontal method for the

enumeration of beta-glucuronidase-positive

Escherichia coli — Part 2: Colony-count technique at

44 degrees C using 5-bromo-4-chloro-3-indolyl beta- D-glucuronide [14].

Staphylococcus aureus was determined according ISO 6888-1:2022 [15].

Salmonella was determined according ISO 6579-1:2017/A1:2020 [16].

Listeria monocytogenes was determined according ISO 11290-1:2017 [17].

2.2.6.2 Determination of antimicrobial activities of oils

Antimicrobial activities were determined according to Teneva et al. [18]. The antimicrobial activity was assessed using the disc-diffusion method on LBG agar plates inoculated with standardized suspensions of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Aspergillus niger*, *Mucor* spp., *Penicillium chrysogenum*, *Fusarium verticillioides*, and *Saccharomyces cerevisiae*. Paper discs (6 mm) impregnated with 6 µL of essential oil dilutions (1×, 10×, 100× in saline with 1% Tween 80) were placed on the surface. After incubation at 37 °C for 24–48 h, inhibition zones were measured in millimetres. The minimum inhibitory concentration (MIC) was defined as the lowest dilution showing no visible microbial growth.

2.2.6.3 Determination of minimal inhibitory concentration of oils to starter cultures

Minimal inhibitory concentration of oils was determined according to Teneva et al. [18]. The minimal inhibitory concentration was assessed using the disc-diffusion method on LBG agar plates inoculated with standardized suspensions of starter culture for yoghurt, white cheese, yellow cheese, butter and probiotic yoghurt. Paper discs (6 mm) impregnated with 6 µL of essential oil dilutions (1×, 10×, 100× in saline with 1% Tween 80) were placed on the surface. After incubation at 37 °C for 24–48 h, inhibition zones were measured in millimetres. The minimum inhibitory concentration (MIC) was defined as the lowest dilution showing no visible microbial growth.

2.2.7. Statistical analysis

Data from triplicate experiments were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (± SD). One way ANOVA was applied in order to determine differences between samples and maximum estimation error at significance levels $p<0.05$.

3 Results

In Table 1 are presented the values of the oils reflecting their stability and quality. The lowest peroxide values were observed in flaxseed oil, algal oil, and cod liver oil, indicating high freshness and absence of primary oxidation products. Plant oils from chia and grape seed showed moderate peroxide levels, comparable with literature data; Mikolajczak et al. [20] reported PV values ranging from 3 to 6 meq O_2/kg for cold-pressed chia and grapeseed oils stored at room temperature. Hemp seed oil exhibited a markedly high peroxide value, indicating severe autooxidative degradation and low oxidative stability, as also observed by Shi et al. [21] under accelerated storage conditions. Fish oil had a peroxide value of within the normal range reported for cold-pressed marine oils [19]. These results demonstrate that plant oils with high polyunsaturated fatty acid content, particularly hemp seed oil, are more susceptible to oxidation, whereas animal-derived oils and certain plant oils maintain better oxidative stability.

The thiobarbituric acid (TBA), reflects the formation of secondary lipid oxidation products and is a key indicator of advanced oxidative degradation. The absence of detectable MDA in flaxseed, fish, and algal oils indicates excellent oxidative stability and freshness. Cod liver oil showed slight oxidative progression, consistent with its high polyunsaturated fatty acid content. Chia seed oil and grape seed oil displayed moderate oxidation levels, typical for omega-3-rich cold-pressed plant oils. The extremely high value of thiobarbituric acid in hemp seed oil suggests extensive secondary oxidation, likely due to its high α-linolenic acid content and limited oxidative stability. Comparable results were reported by Shi et al. [21] in their study, where thiobarbituric acid values exceeded 150 mg MDA/kg after 30 days of accelerated storage at 40 °C. Mikolajczak et al. [20] in observed TBA values between 10 and 25 mg MDA/kg for chia and flaxseed oils. Similarly, Choo et al. [19] in reported values below 10 mg MDA/kg for freshly pressed flaxseed oil. Overall, the data confirm that oils rich in polyunsaturated fatty acids are more prone to secondary oxidation, particularly under suboptimal storage conditions. The color of cold-pressed oils reflects their pigment composition, processing degree, and oxidative state.

The Lovibond color analysis revealed marked differences among the samples, consistent with variations in chlorophyll A and carotenoid contents. The highest yellow intensity was observed in hemp seed and algal oil, corresponding to their high carotenoid concentrations. Both hemp oil and algal oil exhibited a greenish tint, attributed to the presence of chlorophyll A. Flaxseed oil displayed low chlorophyll A and high carotenoid content, consistent with its characteristic golden color and the findings of Choo et al. [19]. Chia seed oil exhibited lower yellow intensity and moderate pigment content, possibly due to the natural pigmentation of the seeds. Grape seed oil presented yellow, with relatively high pigment levels, in line with the results of Mikołajczak et al. [20]. Animal-derived oils (fish and cod liver) showed the lowest color

intensity (yellow 1.9 and 0.7) and negligible pigment content, as expected due to the absence of plant pigments. These results agree with Shi et al. [21], who also reported high pigment levels in hemp oil, enhancing its antioxidant capacity but decreasing its photooxidative stability.

Table 1. Key indicators of the stability and quality of vegetable and animal oils

oils / analysis	Linseed oil	Chia oil	Grape seeds oil	Hemp oil	Fish oil 18/12	Cod liver oil	Algae oil
Acidity value	0.74±0.04[a]	2.16±0.30[b]	5.06±0.25[c]	12.86±0.09[d]	0.41±0.05[e]	0.53±0.08[e]	0.87±0.06[f]
Peroxide value	0[a]	4.72±0.17[b]	5.80±0.15[c]	65.76±0.70[d]	5.64±0.20[c]	0[a]	0[a]
Thiobarbituric acid value	0[a]	7.64±0.14[b]	44.81±4.45[c]	173.95±7.36[d]	0[a]	8.83±0.08[e]	4.81±0.07[f]
Lovibond's color							
red	1.23±0.15[a]	1.43±0.07[a]	0.72±0.09[b]	1.20±0.05[a]	0.34±0.08[c]	0.00[d];	3.29±0.24[e]
yellow	33.66±1.52[a]	8.87±0.14[b]	18.17±0.58[c]	77±6.24[d]	1.90±0.05[e]	0.77±0.07[f]	72.33±2.51[d]
neutral	0[a]	0.57±0.08[b]	0.12±0.03[c]	0[a]	0.17±0.08[c]	0[a]	1.91±0.08[d]
chlorophyl A	0.027±0.015ppm[a]	0.188±0.01ppm[b]	8.55±0.08ppm[c]	32.57±1.23 ppm[d]	0.04±0.0001ppm[e]	0.007±0.005ppm[f]	0.013±0.002ppm[g]
Carotenoids	50.82±0.17ppm[a]	22.40±0.58ppm[b]	37.30±0.71 ppm[c]	61.54±0.65 ppm[d]	7.55±0.05ppm[e]	2.90±0.08 ppm[f]	89.63±0.48ppm[g]

a–g letters point out differences ($P < 0.05$) between samples

Table 2 summarized the colorimetric properties of the analysed cold-pressed oils, demonstrating distinct differences in brightness and chromaticity related to their pigment composition and botanical or animal origin. The highest lightness (L* values) were recorded for cod liver oil and fish oil indicating their pale-yellow appearance. Both exhibited low or negative a* values and moderate b* values, consistent with the visual characteristics of refined marine oils. Flaxseed oil and chia oil showed high b* values, corresponding to intense yellow tones, and moderate a* values, reflecting a slight reddish hue typical of natural, unrefined oils. Grape seed oil demonstrated moderate lightness and strong yellow contribution, in agreement with Mikołajczak et al. [20]. Hemp seed oil exhibited the lowest lightness and a highly saturated color, attributed to its high chlorophyll A content, also reflected in the Lovibond results. The deep greenish hue aligns with findings by Shi et al. [21]. Algal oil presented a distinct chromatic profile, and remarkably high a* and b* values, resulting in a vivid orange-yellow appearance due to its exceptionally high carotenoid content. These findings confirm the relationship between the oils' pigment composition and their colorimetric parameters: oils rich in chlorophylls and carotenoids (hemp, algal) exhibit lower lightness and higher chromatic saturation, whereas marine oils are characterized by high lightness and low color intensity. Figure 1 presented a classification of oils based on their color parameters. The clustering was mainly done based on the parameters in the CIELAB colorimetric system based on the parameters L*, a*, b*. Three clusters were obtained, enclosed by ellipses in Figure 1. The first cluster included oils obtained from fish (sample 6 - cod liver oil and sample 7 - fish oil 18/12), characterized by very high lightness (L* > 95) and low b* values, indicative of a pale yellow to almost colorless sample. The second cluster included plant-derived oils with moderate yellow tones (sample 2-grapeseed oil and sample 4-chia oil), showing relatively high lightness L* (83–92) and b* values (61–73), corresponding to the presence of carotenoids or oxidation products typical of chia and grapeseed oils. The third cluster grouped the more intensely pigmented oils (sample 1-linseed oil, sample 3-hemp oil and sample 5-algae oil), which showed elevated b* values (>100 for linseed and algae oils) and unusually low lightness L* values (22 for hemp oil), reflecting a strong yellow-orange coloration due to being rich in carotenoids and the pronounced darkening was probably due to chlorophylls or oxidative changes.

Table 2. Colorimetric analysis of vegetable and animal oils

oils analysis	Linseed oil	Chia oil	Grape seeds oil	Hemp oil	Cod liver oil	Algae oil	Fish oil 18/12
X	83.35±1.23^{a}	93.13±1.65^{b}	71.64±1.07^{c}	4.29±0.06^{d}	101.06±1.54^{e}	94.15±0.98^{b}	99.60±1.72^{e}
Y	70.69±0.56^{a}	80.65±1.56^{b}	63.49±0.98^{c}	3.52±0.12^{d}	91.31±1.89^{e}	74.43±1.23^{f}	89.6±1.56^{e}
Z	1.93±0.08^{a}	8.53±0.09^{b}	4.21±0.10^{c}	0^{d}	26.86±1.34^{e}	0.520±0.05^{f}	19.87±1.08^{g}
x	0.534±2*10^{-3a}	0.511±7*10^{-3b}	0.514±5*10^{-3c}	0.549±1*10^{-3d}	0.461±5*10^{-3e}	0.557±2*10^{-3a}	0.476±1*10^{-3f}
y	0.453±1*10^{-3a}	0.442±5*10^{-3b}	0.456±6*10^{-3c}	0.452±1*10^{-3a}	0.417±2*10^{-3d}	0.440±3*10^{-3e}	0.429±3*10^{-3e}
L	87.34±3.07^{a}	91.98±4.55^{a}	83.70±5.08^{a}	22.01±1.23^{b}	96.54±3.69^{a}	89.12±3.67^{a}	95.85±3.80^{a}
a	8.85±0.01^{a}	5.96±0.06^{b}	2.18±0.04^{c}	5.15±0.07^{d}	-0.670^{e}	19.98±0.45^{f}	-0.03^{e}
b	102.21±5.78^{a}	61.50±3.25^{b}	73.39±6.09^{c}	37.85±1.09^{d}	11.29±1.15^{e}	132.15±7.12^{f}	27.56±3.54^{g}
C	102.59±7.89^{a}	61.79±4.79^{b}	73.42±5.34^{c}	38.20±2.34^{d}	11.29±1.13^{e}	133.66±9.05^{f}	27.56±3.54^{g}
h	85.05±4.23^{a}	84.47±4.09^{a}	88.30±4.09^{a}	82.25±3.76^{a}	93.40±6.12^{a}	81.40±5.14^{a}	90.07±6.34^{a}

a–g letters point out differences (P < 0.05) between samples

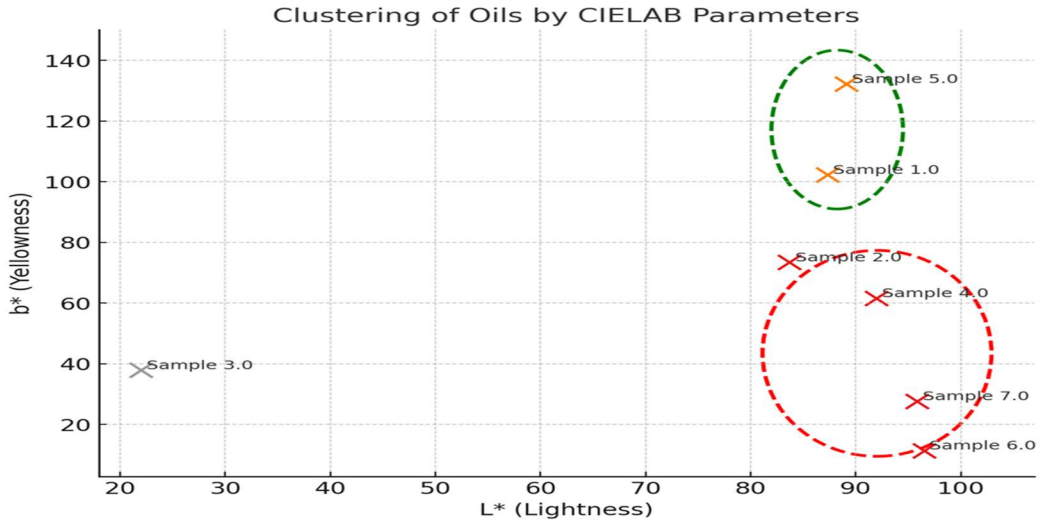


Fig. 1. Clustering according to color parameters in the CILab system

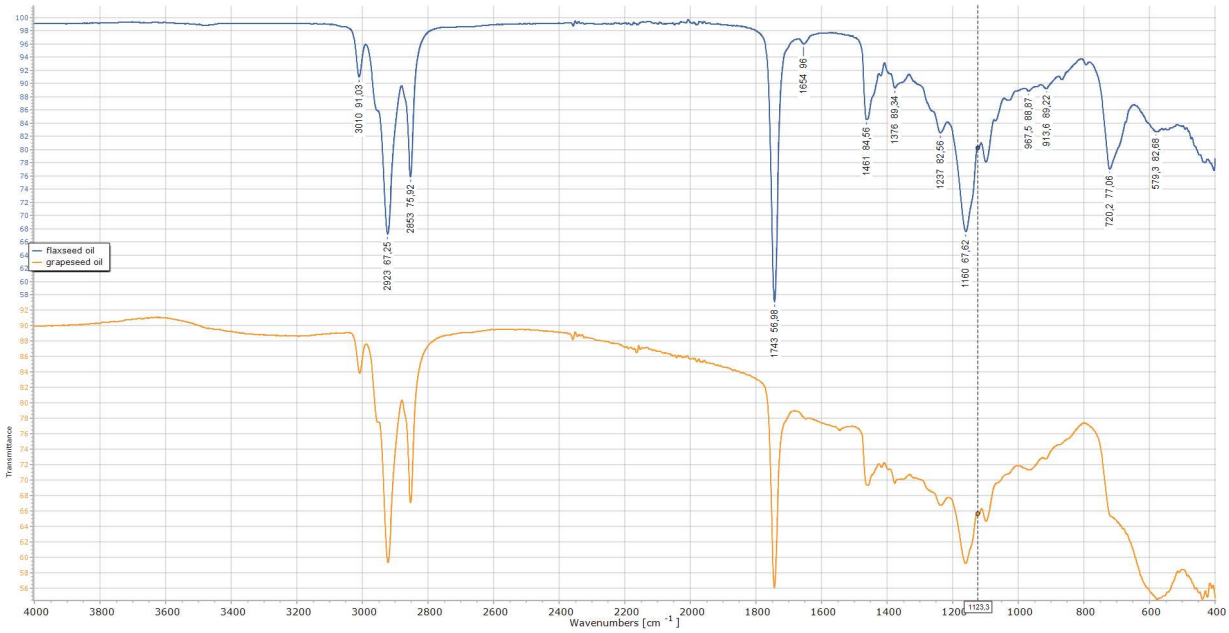


Fig. 2. IR of flaxseed oil and grapeseed oil

Figure 2 presents the FTIR spectra of flaxseed oil and grapeseed oil. Flaxseed oil exhibited a pronounced absorption peak at approximately 3010 cm^{-1}, corresponding to =C–H stretching vibrations in cis-double bonds, indicating a high content of α-linolenic acid (C18:3, ω-3). This observation is consistent with previous reports for flaxseed oil rich in polyunsaturated fatty acids [22, 23]. In contrast, grape seed oil displayed a less intense band in the same region (~3010 cm^{-1}), suggesting a lower proportion of ω-3 polyunsaturated fatty acids and a predominance of linoleic acid (C18:2, ω-6), typical of this oil type [20]. Further differences were observed around 1160 and 1123 cm^{-1}, corresponding to C–O stretching vibrations in the ester groups of triglycerides. The more intense band near 1160 cm^{-1} in flaxseed oil indicates a higher presence of unsaturated ester linkages, while the dominant 1123 cm^{-1} band in grape seed oil suggests a slightly different ester composition and a higher degree of saturation. These findings confirm the distinct fatty acid profiles of flaxseed and grape seed oils, which determine both their nutritional value and oxidative stability.

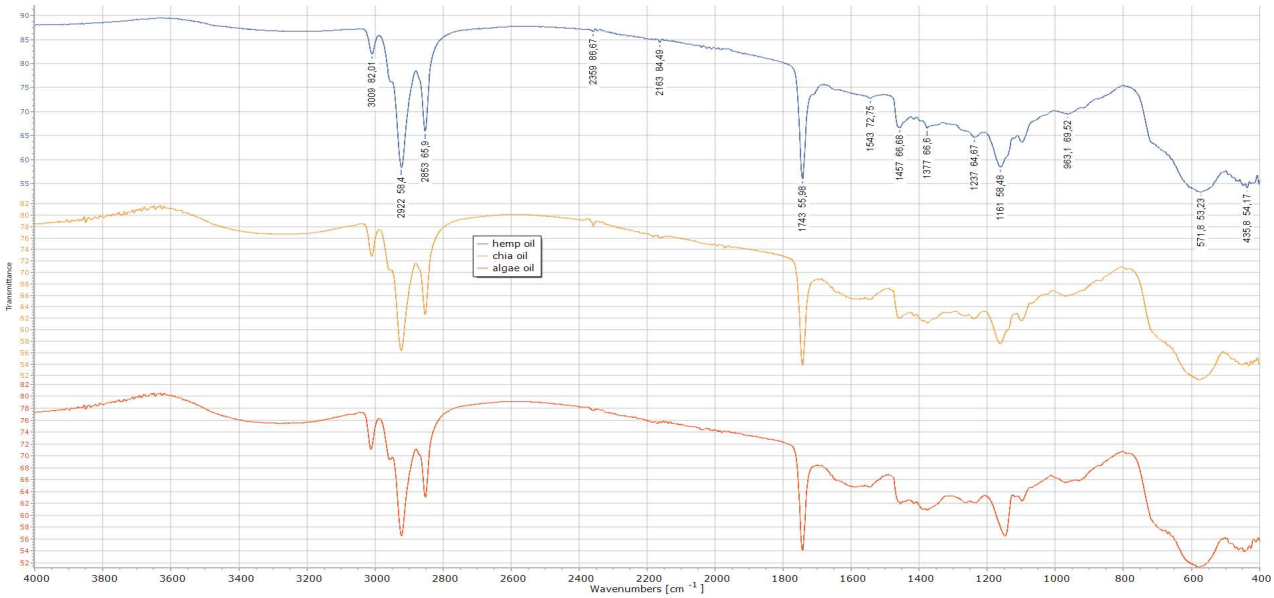


Fig. 3. IR hemp oil, chia oil, algae oil

Figure 3 presents the IR spectra of hemp oil, chia oil, and algal oil. Hemp seed oil exhibited a prominent band at 3009 cm^{-1}, corresponding to =C–H stretching vibrations in cis-double bonds, confirming its high content of α-linolenic (C18:3, ω-3) and linoleic acids (C18:2, ω-6). The strong band at 1743 cm^{-1} is attributed to C=O stretching in ester carbonyl groups, typical of triglycerides rich in polyunsaturated fatty acids [20, 24]. Chia seed oil displayed a similar spectral pattern with well-defined bands at 3009 and 1743 cm^{-1}, but subtle differences were observed between 1237–1161 cm^{-1}, corresponding to C–O stretching vibrations of ester linkages. These variations likely reflect differences in the glyceride structure and a higher ω-3/ω-6 ratio, consistent with previous findings by Reyes-Caudillo et al. [25]. Algal oil, in contrast, presented a flatter spectrum in the 3000–2800 cm^{-1} region and a less intense band at 1743 cm^{-1}, suggesting a distinct lipid profile. This pattern may be attributed to the presence of phospholipids, glycolipids, and pigment-associated molecules, which are typical components of microalgal lipids [26].

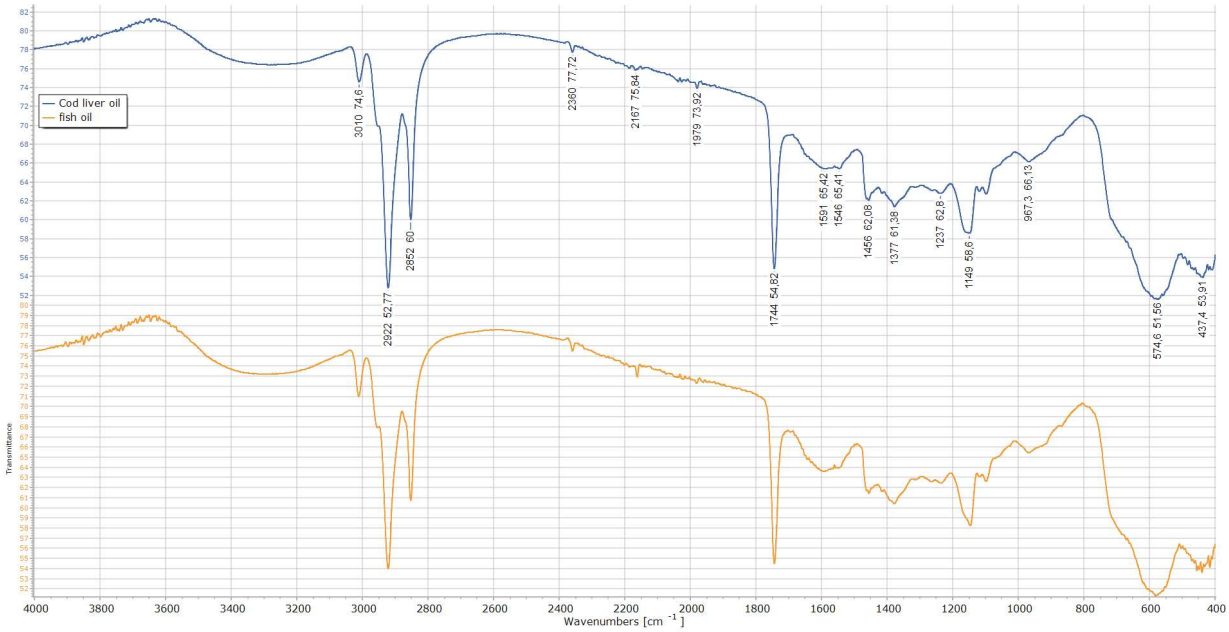


Fig. 4. IR cod liver oil, fish oil.

Figure 4 presents the IR spectra of cod liver oil and fish oil. Both samples showed lower intensity of the =C–H stretching band at 3010 cm^{-1}, indicating a lower proportion of polyunsaturated double bonds compared to vegetable oils. This is consistent with the presence of long-chain ω-3 fatty acids, primarily eicosapentaenoic acid and docosahexaenoic acid, as reported by Knothe [27] and Guillen & Cabo [22]. Cod liver oil exhibited characteristic absorption bands at ~1456 cm^{-1} and 1377 cm^{-1}, associated with CH_3 and CH_2 bending vibrations, as well as possible contributions from vitamin A/D compounds or phospholipids, typical of liver-derived oils [28]. Additional weak bands observed below 1000 cm^{-1} in both samples may correspond to secondary bioactive compounds such as vitamins, phospholipids, and sterols, commonly present in marine lipid extracts [29].

Table 3. Water content and density of vegetable oils.

oils analysis	Linseed oil	Chia oil	Grape seeds oil	Hemp oil
Water content, %	0.20±0.015[a]	0.20±0.015[a]	0.20±0.015[a]	0.16±0.006[b]
Density g/cm^3	0.9123±0.007[a]	0.9230±0.008[a]	0.8850±0.01[b]	0.8952±0.02[b]

a–g letters point out differences (P < 0.05) between samples

Table. 4. Water content and density of fish oils and algae oil.

oils analysis	Fish oil 18/12	Cod liver oil	Algae oil
Water content, %	0.16±0.006[b]	0.18±0.006[a]	0.14±0.006[c]
Density g/cm^3	0.8870±0.006[a]	0.8850±0.01[a]	0.9230±0.006[b]

a–g letters point out differences (P < 0.05) between samples

The moisture content of all oil samples in Table 3 and Table 4 was low, which is typical for cold-pressed oils. The lowest moisture was observed in algal oil, whereas the highest values were recorded in flaxseed, chia, and grape seed oils. Low moisture content is crucial for oil stability, as it minimizes hydrolysis and microbial growth, consistent with observations reported by Choo et al. [19] for cold-pressed plant oils. Oil density is the highest values in chia and algal oils and the lowest in grape seed, fish, and cod liver oils. These differences reflect variations in fatty acid composition and the proportion of unsaturated fatty acids, which influence the density and viscosity of oils [20, 21]. Overall, the measured moisture content and density confirm the high quality of the cold-pressed oils and their suitability for long-term storage under proper conditions.

Table 5. Fatty acid profile of vegetable oils.

oils fatty acids mg/100g	Linseed oil	Grape seeds oil	Hemp oil	Chia oil
Myristic acid (C14:0)	2.30	2.08	n.d.	n.d.
Pentadecanoic acid (C15:0)	0.40	n.d.	n.d.	n.d.
Anteiso-pentadecanoic acid (C15:0ai)	0.10	n.d.	n.d.	n.d.
Palmitic acid (C16:0)	14.20	10.10	7.50	7.30
Iso-hexadecanoic acid (C16:0i)	0.80	n.d.	n.d.	n.d.
Heptadecanoic acid (C17:0)	0.40	n.d.	n.d.	n.d.
Stearic acid (C18:0)	8.31	5.61	3.10	3.30
Palmitoleic acid (C16:1, n-7)	0.80	0.70	0.90	0.80
Oleic acid (C18:1, n-9)	21.40	15.80	17.00	5.60
Linoleic acid (C18:2, n-6)	11.40	66.20	54.00	18.20
α-Linolenic acid (C18:3, n-3)	39.40	0.50	12.90	63.90
γ-Linolenic acid (C18:3, n-6)	0.10	n.d.	2.20	0.20
Arachidic acid (C20:0)	n.d.	n.d.	1.00	0.30
Gondoic acid (C20:1, n-9)	n.d.	0.20	0.40	0.10
Eicosadienoic acid (C20:2)	n.d.	n.d.	0.50	n.d.
Behenic acid (C22:0)	0.10	n.d.	0.40	0.10

The general analytical error, which is <1% (as % RSD) for fatty acids of > 1% and < 3% for fatty acid contents between 0.1 and 1 %.

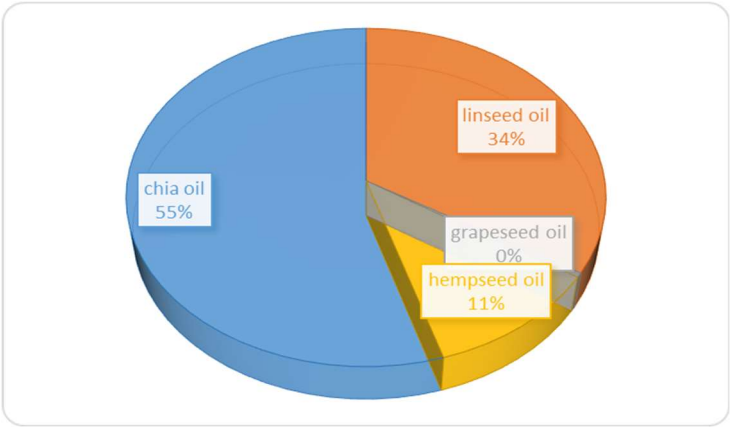


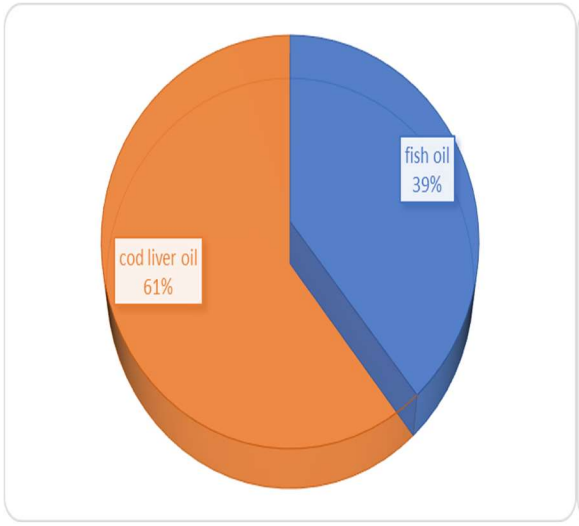
Fig. 5. α-Linolenic acid content in vegetable oils

Table 5 presents the fatty acid profile of the four cold-pressed plant oils. The fatty acid composition of the four cold-pressed plant oils revealed distinct, botanically consistent patterns that determine their nutritional applications and technological constraints. Fig. 5 presented the α-linolenic acid (ALA) content in vegetable oils. Chia oil is strongly ALA–rich, followed by linoleic and oleic acids, confirming chia as one of the most potent plant sources of ALA and a preferred ingredient where plant-based omega-3 enrichment is desired [30, 31]. Flaxseed oil also is dominated by α-linolenic acid (ALA), with moderate amounts of oleic and linoleic acid. This profile positions flaxseed oil among the richest plant sources of omega-3 (ALA), suitable for functional food enrichment. The relatively high palmitic and stearic contents affect physical properties relevant to processing [32, 33]. Grape seed oil in the dataset is clearly omega-6 dominant, while ALA is negligible. Such a high linoleic proportion is typical for grape seed oils and implies pronounced oxidative susceptibility, requiring antioxidant strategies and careful storage in practical use. Oleic and palmitic acids are secondary constituents [34] various compositional studies. Hemp oil contains mainly linoleic acid and a moderate ALA fraction, with oleic acid around. This composition offers a favourable ω-6:ω-3 balance valued nutritionally, and presence of small amounts of γ-linolenic acid and C20 species indicates additional nutraceutical potential [35, 36].

Table 6. Fatty acid profile of animal oils and algae oil.

oils fatty acids mg/100g	Fish oil 18/12	Cod liver oil	Algae oil
Myristic acid (C14:0)	4.70	8.10	9.40
Myristoleic acid (C14:1, n-5)	0.10	n.d.	n.d
Pentadecanoic acid (C15:0)	0.30	n.d.	0.50
Iso-pentadecanoic acid (C15i)	0.20	n.d.	n.d
Palmitic acid (C16:0)	10.10	19.30	19.90
Palmitoleic acid (C16:1, n-7)	7.40	10.10	7.40
Hexadecadienoic acid (C16:2)	0.20	1.30	n.d.
Hexadecatrienoic acid (C16:3)	2.50	1.60	n.d.
Iso-hexadecanoic acid (C16i)	0.10	n.d.	n.d.
Heptadecanoic acid (C17:0)	0.30	n.d.	n.d.
Iso-heptadecanoic acid (C17i)	0.30	n.d.	n.d.
Stearic acid (C18:0)	1.90	4.00	0.80
Vaccenic acid (C18:1, n-7)	4.31	3.50	7.20
Oleic acid (C18:1, n-9)	13.20	8.20	0.50
Linoleic acid (C18:2, n-6)	1.90	3.70	0.10
α-Linolenic acid (C18:3, n-3)	0.10	n.d.	n.d.
γ-Linolenic acid (C18:3, n-6)	0.10	n.d.	n.d.
Stearidonic acid (C18:4, n-3)	1.90	3.50	n.d.
Arachidic acid (C20:0)	0.20	n.d.	n.d.
Gondoic acid (C20:1, n-9)	9.40	1.50	n.d.
Eicosenoic acid (C20:1)	5.50	n.d.	n.d.
Eicosadienoic acid (C20:2)	0.60	n.d.	n.d.
Eicosatrienoic acid (C20:3)	0.10	n.d.	n.d.
Arachidonic acid (C20:4, n-6)	0.30	1.50	0.20
Eicosapentaenoic acid (C20:5, n-3)	9.10	19.80	1.60
Heneicosapentaenoic acid (C21:5, n-3)	0.40	0.60	n.d.
Erucic acid (C22:1, n-9)	0.80	n.d.	n.d.
Nervonic acid (C24:1, n-9)	9.70	n.d.	n.d.
Docosapentaenoic acid (C22:5)	1.10	1.70	n.d.
Adrenic acid (C22:4, n-6)	0.10	n.d.	7.80
Docosahexaenoic acid (C22:6, n-3)	9.30	11.60	43.90

The general analytical error, which is <1% (as % RSD) for fatty acids of > 1% and < 3% for fatty acid contents between 0.1 and 1 %.



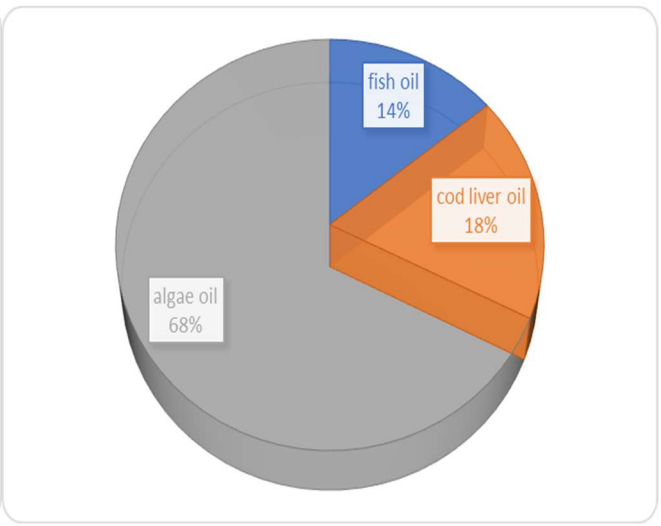


Fig. 6. Docosapentaenoic acid content in animal oils. **Fig.7.** Docosahexaenoic acid content in animal oils and algae oil

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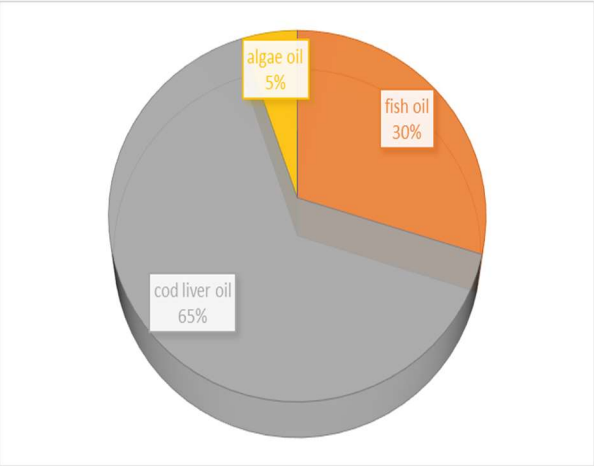

Fig. 8. Eicosapentaenoic acid content in animal oils and algae oil.

Table 6 presents the fatty acid profile of the two cold-pressed animal oils and one cold-pressed algae oil. The fatty acid composition of marine and algal omega-3 oils revealed substantial variation reflecting their biological origin and biosynthetic pathways. All three oils are characterized by a predominance of long-chain polyunsaturated fatty acids, particularly those of the omega-3series eicosapentaenoic acid (EPA), docosapentaenoic acid, and docosahexaenoic acid (DHA) which are key contributors to their nutritional and functional properties. Fish oil demonstrated a balanced fatty acid profile with moderate levels of saturated and monounsaturated fatty acids, alongside substantial amounts of polyunsaturated omega-3 fatty acids—EPA and DHA. This composition is typical of cold-water marine species and supports the recognized cardioprotective and anti-inflammatory properties of fish-derived lipids [37, 38]. Cod liver oil exhibited the highest proportion of total omega-3 fatty acids, dominated by EPA and DHA, as well as the highest DPA contribution (61% relative distribution among the studied samples). The elevated palmitic and palmitoleic acid contents stabilize the lipid matrix but also increase susceptibility to oxidation [39, 40]. This profile confirms that liver oils, unlike muscle oils, accumulate higher amounts of long-chain PUFAs and fat-soluble vitamins, making cod liver oil a superior nutraceutical ingredient. Algal oil, in contrast, was dominated by DHA and showed a relatively low EPA content, consistent with the lipid profiles reported for Schizochytrium sp. and Crypthecodinium cohnii [41]. The predominance of palmitic and myristic acid is characteristic of microalgal lipids, which serve as the primary biosynthetic source of DHA in the marine food web [42]. The exceptionally high DHA concentration, highlights algal oil as a sustainable and vegetarian alternative to fish oils for applications in infant nutrition, functional foods, and clinical formulations [43]. Figs. 6–8 presented the content of long-chain omega-3 fatty acids in animal and algal oils. Comparative evaluation of the three oils indicates distinct compositional signatures: cod liver oil provides the highest EPA and DPA levels, algal oil is the richest in DHA, while conventional fish oil offers a more balanced ratio of long-chain omega-3s. These results align with recent findings suggesting that fish and cod liver oils complement each other in EPA provision, whereas algal oil represents the most concentrated natural source of DHA [44]. Consequently, blending or selective application of these oils may optimize the omega-3 profile in functional formulations depending on the desired physiological benefits.

Table 7. Tocopherol contents of vegetable and animal oils.

Oils analysis	Linseed oil	Chia oil	Grape seeds oil	Hemp oil	Fish oil 18/12	Cod liver oil	Algae oil
α-tocopherol	Trace	n.d.	14.91	4.60	n.d.	2.50	8.00
γ-tocopherol	49.00	22.00	36.24	80.16	n.d.	8.40	32.00
δ-tocopherol	n.d.	n.d.	n.d.	n.d.	n.d.	2.30	4.00
α-tocotrienol	n.d.	n.d.	14.00	n.d.	n.d.	n.d.	n.d.
γ-tocotrienol	n.d.	n.d.	14.00	n.d.	n.d.	n.d.	n.d.

The analytical error was < 1 % (as %RSD) for tocopherol, tocotrienol and phytosterol contents above 10 mg/100 g.

The content of vitamin E derivatives in the tested oils is shown in Table 7. Among the plant-based oils, the highest total concentration was observed in hemp seed oil, followed by flaxseed oil and algal oil. Grape seed oil also contained notable levels, along with detectable α- and γ-tocotrienols. In chia oil, only γ-tocopherol was detected. Fish oil showed no detectable tocopherols, while cod liver oil contained low levels, which is consistent with its known high content of vitamin A and D rather than tocopherols. The high γ-tocopherol levels in hemp and flaxseed oils align with literature reports describing these oils as rich sources of natural antioxidants and omega-3 fatty acids, contributing to enhanced oxidative stability [45, 46]. The exclusive presence of tocotrienols in grape seed oil is noteworthy, since tocotrienols possess stronger antioxidant activity than tocopherols, thereby enhancing the oil's functional value [47]. Overall, the differences in vitamin E profiles reflect plant origin and cold-pressing extraction characteristics. Oils rich in γ-tocopherol (flaxseed, hemp) and tocotrienols (grape seed) exhibit increased antioxidant potential, reinforcing their nutritional significance.

Table 8. Phytosterol contents of vegetable and animal oils.

oils analysis	Linseed oil	Chia oil	Grape seeds oil	Hemp oil	Fish oil 18/12	Cod liver oil	Algae oil
Squalene	n.d.	4.00	n.d.	n.d.	n.d.	7.00	n.d.
Campesterol	59.00	71.00	15.00	57.00	n.d.	n.d.	n.d.
Stigmasterol	24.00	24.00	21.00	12.00	n.d.	n.d.	n.d.
β-Sitosterol	112.00	279.00	153.00	234.00	n.d.	n.d.	n.d.
Cycloartenol	161.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The analytical error was < 1 % (as %RSD) for tocopherol, tocotrienol and phytosterol contents above 10 mg/100 g.

Table 8 presented the phytosterol contents of vegetable and animal oils. The sterol profile of the oils showed marked differences between plant and marine sources. β-Sitosterol was the predominant phytosterol in all plant oils, with the highest concentrations in chia oil and hemp oil, followed by flaxseed and grape seed oils. These findings agree with Ryan et al. [48] and Orsavová et al. [49], who reported β-sitosterol as the major sterol in polyunsaturated seed oils. Campesterol levels were highest in chia and hemp oil, consistent with values reported by Hanuš et al. [50] for cold-pressed hemp oil. Stigmasterol was present in all plant oils, ranging from 12 mg/kg in hemp oil to 24 mg/kg in chia oil, aligning with sterol distributions described by Piironen et al. [51]. Squalene was detected only in chia oil and cod liver oil, confirming literature reports that marine oils—particularly liver oils—are natural squalene sources [52]. No cycloartenol was detected in any sample, consistent with the sterol profile of typical cold-pressed seed oils [53]. No sterols were detected in fish oil, supporting previous evidence indicating negligible phytosterol levels in animal lipids [54]. Overall, chia and hemp oils exhibited the richest sterol profiles, supporting their use as functional foods due to the hypocholesterolaemia and antioxidant properties of phytosterols [55, 56].

Table 9. Microbiological analysis

oils analysis	Coriander oil	Fennel oil	Bay leaf oil	Linseed oil	Chia oil	Grape seeds oil	Hemp oil	Fish oil 18/12	Cod liver oil	Algae oil
Salmonella	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g
Listeria monocytogenes	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g	Not detected in 25g
Ecsherichia coli, cfu/g	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Staphylococcus aureus, cfu/g	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Moulds and yeasts, cfu/g	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
TVC, cfu/g	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

Table 9 presented the microbiological analysis of vegetable and animal oils. The examined essential oils (coriander, fennel, and bay leaf) and omega-3 rich oils (flaxseed, chia, grape seed, hemp, fish oil, cod liver oil, and algae oil) demonstrated an absence of microbiological contamination. All analyses for *Salmonella spp.*, *Listeria monocytogenes*, and *Escherichia coli* were negative, with no pathogenic microorganisms detected in the standard tested sample quantities. Yeasts, mould, and total viable counts (TVC) were below 10 CFU/g, confirming the microbiological safety of the investigated samples. The low microbial status is consistent with established scientific evidence indicating that vegetable oils, especially cold-pressed oils, maintain minimal microbial activity due to their low water content and lipid-rich matrix, which creates an unfavourable environment for microbial growth [57, 58]. The essential oils of coriander, fennel, and bay leaf demonstrated complete absence of microorganisms, likely due to their content of terpene-based compounds with documented antimicrobial effects [59].

The antimicrobial properties of essential oils against *Salmonella spp.*, *Escherichia coli*, and *Listeria monocytogenes* are well described in the literature [60]. These findings are in agreement with Burt et al. [59], who also reported effective inhibition of pathogenic microorganisms by natural plant extracts in food matrices. Marine oils (fish oil, cod liver oil, and algae oil) likewise demonstrated high microbiological purity, which can be attributed both to their lipid nature and to proper production and storage practices. Similar trends have been reported in previous studies, where isolated fish oils exhibited low microbial activity [53]. The obtained results confirm that all tested oils possess excellent microbiological safety and pose no risk to consumers, making them suitable for nutritional applications as well as potential carriers of natural bioactive compounds.

Table 10. Antimicrobial activities of vegetable and animal oils.

Analysis oils	Escherichia coli	Staphylococcus aureus	Salmonella	Listeria monocytogenes	Aspergillus niger	Mucor sp.	Penicillium chrysgenum	Fusarium verticillioides	Sascharomyces cerevisiae
	Diameter of inhibition zone (IZ), mm								
Coriander oil- pure oil	15	17	9	12	9	n.d.	13	12	10
10-fold dilution	10	n.d.	8	9	n.d.	n.d.	n.d.	n.d.	9
100-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fennel oil-pure oil	12	18	11	12	n.d.	n.d.	10	8	9
10-fold dilution	8	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bay leaf-pure oil	10	13	8	10	9	n.d.	10	10	10
10-fold dilution	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Linseed oil-pure oil	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chia oil-pure oil	n.d.	n.d.	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Grapeseed oil-pure oil	10	n.d.	8	8	n.d.	n.d.	n.d.	8	n.d.
10-fold dilution	8	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hemp oil-pure oil	9	10	8	8	n.d.	n.d.	n.d.	n.d.	n.d.
10-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fish oil-pure oil	n.d.	n.d.	8	n.d.	n.d.	8	n.d.	n.d.	n.d.
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cod liver oil-pure oil	n.d.	n.d.	8	n.d.	n.d.	8	n.d.	8	n.d.
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Algae oil-pure oil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8	n.d.
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 10 presented the antimicrobial activities of vegetable and animal oils. The antimicrobial properties of cold-pressed omega-3 oils (flaxseed, chia, grape seed, hemp, fish oil, cod liver oil, and algae oil) and essential oils (coriander, dill, and bay leaf) were evaluated against pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes*. The results revealed that essential oils exhibited pronounced antimicrobial activity, particularly in their undiluted form. Coriander and dill essential oils showed the largest inhibition zones, while diluted samples (1:10 and 1:100) demonstrated significantly reduced or no inhibitory effect. This concentration-dependent antimicrobial behaviour is consistent with previous findings highlighting that monoterpenes such as linalool, limonene, and eugenol disrupt bacterial membrane integrity [59, 60]. Among the tested pathogens, *S. aureus* and *E. coli* were the most sensitive to coriander and dill essential oils, whereas *Listeria monocytogenes* and *Salmonella* were less susceptible, showing inhibition only at full-strength oil concentrations. In contrast, none of the cold-pressed omega-3 oils demonstrated inhibitory activity, in agreement with literature reports indicating that plant and marine lipids do not exhibit direct bactericidal properties and may instead serve as nutrient sources for microorganisms [49]. These findings emphasize the potential use of essential oils as natural antimicrobial agents for food preservation and safety enhancement. The antifungal potential of cold-pressed omega-3 oils and essential oils was assessed against food-associated moulds (*Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium verticillioides*, *Mucor* sp.) and yeast (*Saccharomyces cerevisiae*).

The results showed that essential oils exhibited weak to moderate antifungal effects, with the most pronounced activity observed for undiluted coriander and bay leaf oils. Dilution (1:10 and 1:100) resulted in the complete loss of antifungal action. Dill essential oil showed minimal or no inhibition, consistent with its lower content of fungicidal terpenes [59]. Among mould species, *A. niger* and *P. chrysogenum* were the most sensitive to coriander oil, while *Mucor* sp. and *F. verticillioides* were resistant to all tested oils except for slight inhibition at undiluted concentrations. *S. cerevisiae* exhibited moderate sensitivity to undiluted coriander and bay leaf oils but no response at lower concentrations. In contrast, none of the cold-pressed omega-3 oils showed any antifungal or antiyeast activity, confirming their microbiological safety and functional neutrality when used in food systems [60]. These findings align with literature data indicating that essential oils act as natural fungicides through disruption of fungal cell membranes and inhibition of ergosterol biosynthesis, while lipid oils lack such bioactive compounds [45, 2]

Table 11. Minimal inhibitory concentration.

Starter culture oils	Yoghurt	White brain cheese	Yellow cheese	Buter	Probiotic
	Diameter of inhibition zone (IZ), mm				
Coriander oil	12	15	8	18	8
10-fold dilution	10	n.d.	9	10	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.
Fennel oil	14	12	10	13	11
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.
Bay leaf	13	12	n.d.	15	9
10-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.
100-fold dilution	n.d.	n.d.	n.d.	n.d.	n.d.

Table 11 presented the minimal inhibitory concentrations. The effect of cold-pressed omega-3 oils (flaxseed, chia, grape seed, hemp, fish oil, cod liver oil, and algae oil) and essential oils (coriander, dill, bay leaf) on the growth of starter cultures used in yogurt, white brined cheese, yellow cheese, butter, and starter for probiotic products was evaluated. The results demonstrated that none of the tested omega-3 oils inhibited the growth of lactic acid bacteria. This finding is consistent with published literature indicating that lipid matrices do not suppress lactic acid bacteria growth and may even serve as substrates for lipolytic microbiota and beneficial metabolic activity [49]. These results confirm that omega-3 oils can be safely incorporated into fermented dairy systems without compromising starter activity. In contrast, essential oils exhibited antimicrobial activity in a concentration-dependent manner. Pure essential oils generated clear inhibition zones, whereas dilution at 1:10 resulted in reduced or absent inhibition, and a 1:100 dilution produced no inhibitory effect against any tested culture. Such behaviour aligns with previous studies reporting that essential oils inhibit lactic acid bacteria at higher doses [59, 60], while lower concentrations can be compatible with fermentation systems [60]. This suggests that essential oils could be considered as natural antimicrobial agents in dairy formulations, though careful dosing is required to avoid interference with starter culture performance**.**

4 Conclusion

The results of the analyses demonstrated that all examined plant-based and animal-derived cold-pressed oils exhibited low acid and peroxide values, indicating good quality and absence of oxidative degradation. These parameters confirm the freshness and oxidative stability of the oils during storage, which is crucial for their use as functional ingredients in food formulations. The colorimetric parameters varied depending on the botanical or animal origin of the oils. Plant-derived oils showed higher b* (yellow) values, while animal-derived oils exhibited lower color intensity, reflecting differences in pigment and lipid composition. Microbiological evaluation revealed the absence of pathogenic microorganisms (*Salmonella spp*., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*), as well as low levels of total viable count, moulds, and yeasts, confirming the microbiological safety of all tested samples. The fatty acid profile showed significant differences between plant and animal oils. Plant oils (flaxseed, chia, hemp, and grape seed oils) were characterized by a predominance of polyunsaturated fatty acids, mainly linoleic acid and α-linolenic acid, which are essential and biologically active components. In contrast, animal-derived oils (fish oil, cod liver oil, and algal oil) contained high levels of long-chain omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. The highest docosahexaenoic acid content was found in algal oil, followed by cod liver oil and fish oil. The eicosapentaenoic acid content was highest in cod liver oil, moderate in fish oil, and lowest in algal oil. These findings indicate that plant-based oils represent valuable sources of essential omega-3 and omega-6 fatty acids of botanical origin, while animal-derived oils, particularly cod liver and algal oils, serve as concentrated sources of long-chain polyunsaturated fatty acids with well-documented physiological benefits. Plant and animal oils could therefore provide an optimal fatty acid balance and expand their potential applications in the development of functional and nutritionally enriched food products.

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