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RESEARCH ARTICLE

CONJUGATED LINOLEIC ACID AND FATTY ACID ISOMERS IN SELECTED COLD PRESSED OILS: ANALYSIS BY GC/FID TECHNIQUE

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ABSTRACT

The conjugated linoleic acid (CLA) isomers in cold pressed oils [*pomegranate seed oil (PGSO), linseed oil (LSO), black cumin seed oil (BCSO), nettle seed oil (NSO),grape seed oil (GSO), sesame seed oil (SSO),safflower oil (SFO), pumpkin seed oil (PSO), wheat germ oil (WGO), fig seed oil (FSO), coriander oil (CO), walnut oil (WO) and coconut oil (CNO)*] extracted with lab–scale screw press machine were further subjected to gas chromatography/flame ionization detection (GC/FID) analysis. The composition of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFAs) of the samples was also determined. The five different positional and geometric isomers of CLA [*cis–9, cis–11 CLA, cis–9, trans–11 CLA, trans–9, cis–11 CLA, trans–9, trans–11 CLA and trans–10, cis–12 CLA*] were also well separated by a highly polar column (100m×0.2µm×0.25mm i.d; HP–88 cyanopropyl) and an applied GC temperature program. It was concluded that the samples were all rich in total CLA (∑CLA) and they were found between 0.14% for PSO and 2.11% for SSO. The most abundant CLA isomer was in general to be *cis*–9, *trans*–11 CLA form, which represented the content of isomer between 3.15% and 72.08% of ∑CLA. Besides, the ∑SFA values were detected between 2.43% and 93.14%, ∑MUFA values were between 4.60% and 71.11% and ∑PUFA values were between 1.79% and 87.59%. Therefore, this study might offer valuable information for the introduction of new food sources, as well as incorporation into medicinal purposes and food formulations which have the potential to be commercially valuable.

Keywords: CLA, Cold press oil, Fatty acid, Gas chromatography

1. INTRODUCTION

Conjugated linoleic acid (CLA) is defined as an expression used for a combination of positional and geometric isomers of linoleic acid (*cis*–C18:2, ω–6), including conjugated dual bonds. The *cis*–C18:2 is a polyunsaturated fatty acids (PUFAs) and comprise two dual bonds divided by a $-CH_2-$ group in the $\Delta^{9,12}$positions [1–4]. In the literature, 56 different isomeric structures of CLA including diverse geometric configurations (*cis–/cis–, trans–/cis–, trans–/trans– and cis–/trans–*) with 14 different positions present in the chain of C18:2 ($\Delta^{2,4}$, $\Delta^{3,5}$, $\Delta^{4,6}$, $\Delta^{5,7}$, $\Delta^{6,8}$, $\Delta^{7,9}$, $\Delta^{8,10}$, $\Delta^{9,11}$, $\Delta^{10,12}$, $\Delta^{11,13}$, $\Delta^{12,14}$, $\Delta^{13,15}$, $\Delta^{14,16}$, and $\Delta^{15,17}$) were reported [5,6]. These isomers are defined as essential since the body couldn't construct these fatty acids, and they are also described as bioactive compounds have significant roles for health like strengthening the immune system, improving bone and cartilage disease, protecting against heart disease, preventing high cholesterol, obesity, cancer, also it could have many benefits many benefits not be determined yet [7–9].

CLA isomers have an important potential for improving the quality of human health, are produced for the commercial purposes to increase the functions of foods and are used for the enrichment of various foodstuffs. The average intake of CLA by people is 15–400 mg.day^{-1}; however, the beneficial effects of these biologically active isomers could be observed clinically over the dosage of 700–6800 mg.day^{-1} [10–12]. For this purpose, functional foods rich in linoleic acid and alternative edible oils with enriched CLA content and high nutritive properties obtained from different seeds are recently offered to consumers. In this sense, increased awareness in cold pressed oils has been detected owing to the

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demand for natural and healthy edible oils including bioactive compounds. The cold pressed oils are produced by conventional screw pressing technology without any chemical usage or heat treatment.

This technology protects antioxidants, special aromatics, phytochemicals and all other fat–soluble bioactive substances in edible oils. Contrary to the refined edible oils containing relatively large amounts of PUFAs, the cold pressed oils in similar nature often have long shelf–life and higher oxidative stability, due to their antioxidant capacity. In our markets, there are different types of cold pressed oils with different amounts and compositions of PUFAs [13–15]. However; up till now, the literatures focused on cold pressed oils have often reported the contents of their bioactive compounds including antioxidants, polyphenols, phytosterols, and so on, and there is no study on the determination of characteristics of CLA isomers with their fatty acid profile. Thus; in this study, the cold pressed oils greatly pleased by consumers owing to their organoleptic and nutritive characteristics was chosen [*pomegranate seed oil (PGSO), linseed oil (LSO), black cumin seed oil (BCSO), nettle seed oil (NSO), grape seed oil (GSO), sesame seed oil (SSO), safflower oil (SFO), pumpkin seed oil (PSO), wheat germ oil (WGO), fig seed oil (FSO), coriander oil (CO), walnut oil (WO) and coconut oil (CNO)*] and the characteristics of their CLA isomers [*cis–9, cis–11 CLA, cis–9, trans–11 CLA, trans–9, cis–11 CLA, trans–9, trans–11 CLA and trans–10, cis–12 CLA*] were concluded by GC/FID method. The composition of SFAs, MUFAs and PUFAs of the cold pressed oil samples was also reported under study. This research might offer valuable information for the introduction of new sources of functional edible oil ingredients, as well as incorporation into medicinal purposes and food formulations which could have potential to be commercially developed.

2. EXPERIMENTAL DETAILS

2.1. Chemicals and Instrumentation

High purity chemicals (n–hexane, potassium hydroxide, anhydrous sodium sulphate and methanol) were procured from Sigma–Aldrich Inc. (Zwijndrecht, The Netherlands) and VWR Chemicals BDH Inc. (West Chester, Pennsylvania, US). The reference material of fatty acid methyl esters (FAMEs)(C4–C24, wt.%, mixture) was supplied from Sigma–Aldrich Inc. (Zwijndrecht, The Netherlands). A mix of certified methyl ester isomers of CLA [*cis–9, cis–11 CLA (C18:2,* $\Delta^{cis-9,\ cis-11}$*), cis–9, trans–11 CLA (C18:2,* $\Delta^{cis-9,\ trans-11}$*), trans–9, cis–11 CLA (C18:2,* $\Delta^{trans-9,\ cis-11}$*), trans–9, trans–11 CLA (C18:2,* $\Delta^{trans-9,\ trans-11}$*) and trans–10, cis–12 CLA (C18:2,* $\Delta^{trans-10,\ cis-12}$*)*] reference material was procured from NuChek Prep. Inc. (Elysian, MN, US) and they were prepared in GC grade hexane. An Agilent 7890A GC instrument with a 5975Cmodel FID (Santa Clara, CA, US) and B.03.02–2008 Chemstation software were utilized for the analysis.

2.2. Cold Pressed Oil Samples

The cold pressed oils were extracted with labscale machine (*single head,1.5 kw power,15 kg seed/h capacity, 2hp, screw–press*) from pomegranate seed (*Punica granatum L.*), linseed (*Linum usitatissimum L.*), wheat germ (*Triticum aestivum L.*), nettle seed (*Urtica pilulifera L.*), sesame seed (*Sesamum indicum L.*), grape seed (*Vitis vinifera L.*), fig seed (*Ficus carica L.*), coriander (*Coriandrum sativum L.*), black cumin seed (*Nigella sativa L.*), safflower (*Carthamus tinctorius L.*), pumpkin seed (*Cucurbita pepo L.*), walnut (*Juglans regia L.*) and coconut (*Cocos nucifera L.*) samples. The parameters of screw press machine in our laboratory were set as screw rotation of 30 rpm speed and a temperature of 40°C.The obtained samples were stocked up at –18 °C for GC analysis.

2.3. Operating Conditions of GC–FID Analysis

Prior to the GC–FID analysis, the FAME of cold pressed oil samples [*PGSO, LSO, WGO, NSO, SSO, GSO, FSO, CO, BCSO, PSO, WO, SFO and CNO*] were chemically derivatized using base–catalyzed methanolysis according to our previous studies with some modifications [16–20]. Briefly; n–hexane (10 mL) was mixed with 0.1 g of cold pressed oil; afterwards, 0.1 mL of the base–catalyzation reagent (2N KOH solution in methanol) was poured into the sample and they were mixed for about 2 min. This solution was then centrifugated for 5000 rpm and 10 min, and the upper supernatant was poured into vials for the GC analysis. A highly polar column (100m×0.2μm×0.25mm i.d; Agilent HP–88 cyanopropyl, Santa Clara, CA, US) was used. High purity helium and hydrogen were used as make–up and carrier gases, respectively. The temperature program of GC oven was applied as: begin at 45°C for 4 min, the temperature was then increased to 175°C at a speed of 13 °C/min, detained in this temperature for 27 min, the temperature was then raised to 215°C at a speed of 4°C/min, held at this temperature for 35 min. The FAME of cold pressed oils was injected as 1.0 μL (split; 100:1). The temperatures of detector and injection compartments were set as 250 °C. The findings were reported as percentage of fatty acids by evaluating the values of retention time (t_R) with approved reference materials.

3. RESULTS AND DISCUSSION

The profile of fatty acids in studied oil samples is illustrated in Table 1. The total contents of SFAs, MUFAs and PUFAs are reported as percentage (%, g fatty acid/100 g cold pressed oil). The studied cold pressed oils are mainly characterized by the highest content of PUFAs (1.79%–87.59%), with a predominance of C16:0, C18:0, *cis*–C18:1, *cis*–C18:2 and *cis*–C18:3 fatty acids. As seen in Table 1, the cold pressed SFO, WO, WGO, BCSO, NSO, PSO, SSO and GSO samples have similar types of oils in terms of C18:2$\Delta^{9,12}$ content (40.57%–73.26%). These oils contain the lowest level of SFAs (9.93%–19.90%) and MUFAs (15.60%–39.08%).The fatty acid composition findings of these samples agreed well with the data in earlier studies [13,14,21–24]. The cold pressed PGSO, FSO and LSO contain the high levels of essential C18:3$\Delta^{9,12,15}$ fatty acid (41.29%–76.75%), whereas the CO comprise the low content of C18:1Δ^{9} fatty acid (69.71%). Similarly, these findings matched well with the results of previous literatures [15,25,26]. The cold pressed CNO is characterized by the most different composition of fatty acids, with a predominance of SFAs (93.14%), and the obtained result agreed with the literature [27]. Clearly, some discriminations of the fatty acid composition of studied oils from literature is mainly based on the differences in their seed types, geographical region, growing season, cultivar and production parameters. It is also seen that very low values were obtained in all samples in terms of trans fatty acid (0.10–1.02%) content. Therefore; the ∑SFA values were found between 2.43% and 93.14%, ∑MUFA values were between 4.60% and 71.11% and ∑PUFA values were between 1.79% and 87.59% (Table 1) for the cold pressed oils under study.

Table 1. The CLA and fatty acid data for the cold pressed oils (%, g fatty acid/100 g sample)

		CLA and fatty acid data for the cold pressed oils (%, g fatty acid/100 g sample)			
t_R (min)		***pomegranate seed oil (PGSO)***	***linseed oil (LSO)***	***wheat germ oil (WGO)***	***nettle seed oil (NSO)***
13.423	butanoic acid (C4:0)	0.1660 ±0.0067	0.0290 ±0.0054	0.0080 ±0.0084	0.0250 ±0.0043
16.861	hexanoic acid (C6:0)	0.2260 ±0.0098	0.0270 ±0.0085	0.0060 ±0.0075	0.0320 ±0.0062
17.72	octanoic acid (C8:0)	0.0660 ±0.0034	0.0280 ±0.0052	0.0080 ±0.0046	0.0280 ±0.0036
20.275	decanoic acid (C10:0)	0.1260 ±0.0057	0.0260 ±0.0048	0.0160 ±0.0062	0.0440 ±0.0037
21.622	dodecanoic acid (C12:0)	0.1660 ±0.0074	0.0260 ±0.0055	0.0080 ±0.0073	0.0300 ±0.0081
23.575	myristic acid (C14:0)	0.1360 ±0.0055	0.0660 ±0.0071	0.0860 ±0.0084	0.0750 ±0.0046
24.459	myristoleic acid, n9(C14:1)	0.0660 ±0.0046	0.0250 ±0.0078	0.0160 ±0.0056	0.0340 ±0.0048
26.188	pentadecanoic acid (C15:0)	0.1160 ±0.0089	0.0280 ±0.0084	0.0060 ±0.0076	0.0240 ±0.0037
27.578	trans–ginkgolic acid (trans–C15:1)	0.0660 ±0.0056	0.0260 ±0.0053	0.0060 ±0.0071	0.0240 ±0.0052
27.81	ginkgolic acid (C15:1)	0.0860 ±0.0044	0.0380 ±0.0061	0.0360 ±0.0055	0.0270 ±0.0042
30.606	palmitic acid (C16:0)	0.7960 ±0.0320	6.3280 ±0.1430	1.2860 ±0.0150	7.4340 ±0.1360
33.043	trans–palmitoleic acid (trans–C16:1)	0.0860 ±0.0074	0.0440 ±0.0041	0.0360 ±0.0082	0.0320 ±0.0064
33.632	palmitoleic acid (C16:1)	0.0960 ±0.0063	0.0960 ±0.0055	0.1560 ±0.0071	0.0820 ±0.0028
35.489	heptadecanoic acid (C17:0)	0.2660 ±0.0110	0.0710 ±0.0130	0.0260 ±0.0170	0.0850 ±0.0150
36.521	heptadecanoleic acid (C17:1)	0.0860 ±0.0051	0.0490 ±0.0042	0.0360 ±0.0057	0.0500 ±0.0066
38.708	stearic acid (C18:0)	2.7060 ±0.1030	5.6280 ±0.1200	0.7160 ±0.1500	3.8020 ±0.1700
40.803	trans–oleic acid (trans–C18:1)	0.4660 ±0.0140	0.0260 ±0.0045	0.0160 ±0.0063	0.0430 ±0.0051
41.805	oleic acid (C18:1, ω9)	5.9760 ±0.2160	20.2980 ±0.9100	7.6560 ±0.1500	19.0460 ±0.1600
42.115	C18:1 izomer	0.5960 ±0.0130	0.6600 ±0.0120	0.5460 ±0.0160	0.7080 ±0.0170
45.302	trans–linoleic acid (trans–C18:2)	0.0560 ±0.0035	0.0960 ±0.0045	0.0460 ±0.0067	0.0650 ±0.0079
46.013	linoleic acid (C18:2, ω6)	4.5560 ±0.1200	13.7380 ±0.2500	8.9960 ±0.1500	67.0830 ±1.2000
46.216	linolenic acid (C18:3, ω6)	76.7560 ±1.300	0.1570 ±0.0093	70.6060 ±1.6000	0.0360 ±0.0027
46.465	arachidic acid (C20:0)	0.2660 ±0.0075	0.4590 ±0.0099	0.1360 ±0.0056	0.3630 ±0.0074
48.052	linolenic acid (C18:3, ω3)	2.4760 ±0.1100	51.7680 ±1.2000	6.8060 ±0.1200	0.5350 ±0.0085
48.728	eicosenoic acid (C20:1)	0.1060 ±0.0054	0.0340 ±0.0025	1.5360 ±0.0850	0.0300 ±0.0074
49.092	**CLA isomer, 9 cis, 11 trans**	**0.0910 ±0.0025**	**0.1330 ±0.0045**	**0.1540 ±0.0032**	**0.3170 ±0.0064**
49.755	**CLA isomer, 10 trans, 12 cis**	**0.0110 ±0.0010**	**0.0250 ±0.0010**	**0.1030 ±0.0087**	**0.0240 ±0.0098**
49.952	**CLA isomer, 9 cis, 11 cis**	**0.0050 ±0.0001**	**0.0270 ±0.0075**	**0.1440 ±0.0079**	**0.0260 ±0.0010**
51.472	**CLA isomer, 9 trans, 11 cis**	**0.0360 ±0.0010**	**0.0640 ±0.0050**	**0.0960 ±0.0020**	**0.0620 ±0.0030**
52.703	**CLA isomer, 9 trans, 11 trans**	**0.0070 ±0.0001**	**0.0280 ±0.0020**	**0.2650 ±0.0090**	**0.0240 ±0.0030**
54.039	eicosadienoic acid (C20:2)	0.2060 ±0.0055	0.0390 ±0.0025	0.1160 ±0.0070	0.0400 ±0.0090
54.681	erucic acid (C22:1)	0.2960 ±0.0078	0.0310 ±0.0045	0.1160 ±0.0065	0.0520 ±0.0041
55.173	lignoceric acid (C20:4)	0.4860 ±0.0095	0.1960 ±0.0053	0.2660 ±0.0092	0.0950 ±0.0014
55.501	tricosylic acid (C23:0)	0.1460 ±0.0053	0.0310 ±0.0045	0.0060 ±0.0005	0.0260 ±0.0030
56.558	cis–13,16–docosadienoic acid (C22:2)	1.4660 ±0.0150	0.0330 ±0.0010	0.0260 ±0.0006	0.0260 ±0.0024
57.877	eicosapentaenoic acid (C20:5)	0.0560 ±0.0025	0.0380 ±0.0024	0.0160 ±0.0010	0.0380 ±0.0030
59.435	lignoceric acid (C24:0)	0.7460 ±0.0180	0.1270 ±0.0087	0.1260 ±0.0099	0.0770 ±0.0025
61.798	nervonic acid (C24:1)	0.1060 ±0.0076	0.0270 ±0.0041	0.0060 ±0.0010	0.0270 ±0.0032
	∑SFAs	**5.9280**	**12.8740**	**2.4340**	**12.0450**
	∑MUFAs	**7.4140**	**21.2580**	**10.1040**	**20.0560**
	∑PUFAs	**86.1470**	**66.2460**	**87.5940**	**68.3060**
	∑trans FAs	**0.6740**	**0.1920**	**0.1040**	**0.1640**

FAs; fatty acids, SFAs; saturated fatty acids, MUFAs; monounsaturated fatty acids, PUFAs; polyunsaturated fatty acids, CLA; conjugated linoleic acid

Table 1. Continue

t_R (min)		CLA and fatty acid data for the cold pressed oils (%, g fatty acid/100 g sample)			
		sesame seed oil (SSO)	***grape seed oil (GSO)***	***fig seed oil (FSO)***	***coriander oil (CO)***
13.423	butanoic acid (C4:0)	0.0310 ±0.0020	0.1070 ±0.0087	0.0390 ±0.0012	0.1010 ±0.0057
16.861	hexanoic acid (C6:0)	0.0250 ±0.0012	0.0320 ±0.0022	0.0280 ±0.0014	0.7440 ±0.0120
17.72	octanoic acid (C8:0)	0.0380 ±0.0022	0.0580 ±0.0013	0.0520 ±0.0015	0.0260 ±0.0034
20.275	decanoic acid (C10:0)	0.0370 ±0.0015	0.1630 ±0.0085	0.0290 ±0.0021	8.6600 ±0.2400
21.622	dodecanoic acid (C12:0)	0.0330 ±0.0010	0.0330 ±0.0023	0.0310 ±0.0014	0.6750 ±0.0105
23.575	myristic acid (C14:0)	0.0370 ±0.0013	0.0940 ±0.0057	0.0550 ±0.0061	0.0450 ±0.0042
24.459	myristoleic acid, n9(C14:1)	0.0320 ±0.0023	0.1160 ±0.0058	0.0260 ±0.0010	0.0470 ±0.0035
26.188	pentadecanoic acid (C15:0)	0.0300 ±0.0028	0.0940 ±0.0079	0.0340 ±0.0026	0.0610 ±0.0041
27.578	trans–ginkgolic acid (trans–C15:1)	0.0320 ±0.0024	0.0360 ±0.0021	0.0390 ±0.0036	0.0480 ±0.0027
27.81	ginkgolic acid (C15:1)	0.0270 ±0.0025	0.0340 ±0.0023	0.0380 ±0.0027	0.0460 ±0.0035
30.606	palmitic acid (C16:0)	9.5230 ±0.1800	8.8750 ±0.2000	7.3500 ±0.1700	3.1920 ±0.0750
33.043	trans–palmitoleic acid (trans–C16:1)	0.0450 ±0.0032	0.0470 ±0.0037	0.0360 ±0.0031	0.2310 ±0.0096
33.632	palmitoleic acid (C16:1)	0.1440 ±0.0088	0.1880 ±0.0069	0.0860 ±0.0029	0.1660 ±0.0069
35.489	heptadecanoic acid (C17:0)	0.0700 ±0.0085	0.0810 ±0.0065	0.0780 ±0.0081	0.0460 ±0.0027
36.521	heptadecanoleic acid (C17:1)	0.0490 ±0.0026	0.0520 ±0.0033	0.0500 ±0.0028	0.0660 ±0.0046
38.708	stearic acid (C18:0)	5.4210 ±0.1800	4.6780 ±0.1030	2.9870 ±0.0950	0.7290 ±0.0100
40.803	trans–oleic acid (trans–C18:1)	0.1230 ±0.0085	0.1080 ±0.0092	0.0390 ±0.0028	0.1250 ±0.0093
41.805	oleic acid (C18:1, ω9)	37.7390 ±0.9500	17.7440 ±0.7500	16.5030 ±0.8500	69.7160 ±1.3000
42.115	C18:1 izomer	0.8580 ±0.0200	0.7640 ±0.0270	1.0150 ±0.0100	0.7630 ±0.0100
45.302	trans–linoleic acid (trans–C18:2)	0.0790 ±0.0090	0.4860 ±0.0100	0.1010 ±0.0095	0.2470 ±0.0097
46.013	linoleic acid (C18:2, ω6)	42.6020 ±1.2000	64.5750 ±1.1000	29.3460 ±0.9500	13.6980 ±0.1850
46.216	linolenic acid (C18:3, ω6)	0.0350 ±0.0060	0.1580 ±0.0098	0.0630 ±0.0095	0.0300 ±0.0037
46.465	arachidic acid (C20:0)	0.5940 ±0.0150	0.1940 ±0.0095	0.4280 ±0.0096	0.0920 ±0.0066
48.052	linolenic acid (C18:3, ω3)	0.3710 ±0.0088	0.4320 ±0.0092	41.2970 ±1.1000	0.1960 ±0.0082
48.728	eicosenoic acid (C20:1)	0.0310 ±0.0050	0.0400 ±0.0025	0.0450 ±0.0030	0.2500 ±0.0096
49.092	**CLA isomer, 9 cis, 11 trans**	**0.1850 ±0.0025**	**0.1800 ±0.0039**	**0.2740 ±0.0024**	**0.0700 ±0.0010**
49.755	**CLA isomer, 10 trans, 12 cis**	**0.2950 ±0.0021**	**0.1290 ±0.0010**	**0.0280 ±0.0010**	**0.0290 ±0.0010**
49.952	**CLA isomer, 9 cis, 11 cis**	**0.2810 ±0.0027**	**0.1700 ±0.0023**	**0.0310 ±0.0010**	**0.0280 ±0.0010**
51.472	**CLA isomer, 9 trans, 11 cis**	**0.6340 ±0.0054**	**0.1220 ±0.0032**	**0.0300 ±0.0010**	**0.0440 ±0.0015**
52.703	**CLA isomer, 9 trans, 11 trans**	**0.7210 ±0.0028**	**0.2910 ±0.0022**	**0.0270 ±0.0010**	**0.0350 ±0.0021**
54.039	eicosadienoic acid (C20:2)	0.0420 ±0.0031	0.0540 ±0.0026	0.0460 ±0.0037	0.0850 ±0.0041
54.681	erucic acid (C22:1)	0.0360 ±0.0018	0.0700 ±0.0058	0.0500 ±0.0036	0.0310 ±0.0021
55.173	lignoceric acid (C20:4)	0.1410 ±0.0088	0.0500 ±0.0032	0.1000 ±0.0110	0.0500 ±0.0098
55.501	tricosylic acid (C23:0)	0.0260 ±0.0023	0.0380 ±0.0020	0.0290 ±0.0010	0.0370 ±0.0010
56.558	cis–13,16–docosadienoic acid (C22:2)	0.0330 ±0.0021	0.0360 ±0.0032	0.0290 ±0.0015	0.0260 ±0.0017
57.877	eicosapentaenoic acid (C20:5)	0.0390 ±0.0018	0.0440 ±0.0026	0.0410 ±0.0028	0.0420 ±0.0039
59.435	lignoceric acid (C24:0)	0.1040 ±0.0099	0.1500 ±0.0087	0.0570 ±0.0048	0.0570 ±0.0035
61.798	nervonic acid (C24:1)	0.0260 ±0.0024	0.0450 ±0.0022	0.0340 ±0.0020	0.0340 ±0.0015
	∑SFAs	**15.9690**	**14.5970**	**11.1970**	**14.4650**
	∑MUFAs	**38.9420**	**19.0530**	**17.8470**	**71.1190**
	∑PUFAs	**45.3790**	**66.2410**	**71.3120**	**14.3330**
	∑trans FAs	**0.2790**	**0.6770**	**0.2150**	**0.6510**

FAs; fatty acids, SFAs; saturated fatty acids, MUFAs; monounsaturated fatty acids, PUFAs; polyunsaturated fatty acids, CLA; conjugated linoleic acid

Table 1. Continue

		CLA and fatty acid data for the cold pressed oils (%, g fatty acid/100 g sample)				
t_R (min)		*black cumin seed oil (BCSO)*	*pumpkin seed oil (PSO)*	*walnut oil (WO)*	*safflower oil (SFO)*	*coconut oil (CNO)*
13.423	butanoic acid (C4:0)	0.0360 ±0.0024	0.0250 ±0.0010	0.1300 ±0.0095	0.0290 ±0.0031	0.0390 ±0.0022
16.861	hexanoic acid (C6:0)	0.0320 ±0.0017	0.1260 ±0.0084	0.5690 ±0.0120	0.0250 ±0.0030	0.7040 ±0.0087
17.72	octanoic acid (C8:0)	0.0390 ±0.0023	0.0280 ±0.0010	0.0980 ±0.0057	0.0900 ±0.0098	8.6270 ±0.1500
20.275	decanoic acid (C10:0)	0.0280 ±0.0020	0.0820 ±0.0050	1.1400 ±0.0105	0.0300 ±0.0069	6.8370 ±0.1400
21.622	dodecanoic acid (C12:0)	0.0480 ±0.0033	0.0320 ±0.0024	1.5590 ±0.0105	0.0250 ±0.0020	49.5710 ±1.2000
23.575	myristic acid (C14:0)	0.1850 ±0.0088	0.1340 ±0.0093	0.0480 ±0.0020	0.1400 ±0.0089	17.0580 ±1.1000
24.459	myristoleic acid, n9(C14:1)	0.0290 ±0.0025	0.0310 ±0.0030	0.0250 ±0.0010	0.0250 ±0.0015	0.0340 ±0.0020
26.188	pentadecanoic acid (C15:0)	0.0310 ±0.0030	0.0530 ±0.0042	0.0280 ±0.0030	0.0250 ±0.0025	0.0270 ±0.0031
27.578	trans–ginkgolic acid (trans–C15:1)	0.0480 ±0.0020	0.0300 ±0.0030	0.0360 ±0.0025	0.0260 ±0.0021	0.0420 ±0.0030
27.81	ginkgolic acid (C15:1)	0.0360 ±0.0027	0.0440 ±0.0035	0.0380 ±0.0020	0.0340 ±0.0015	0.0280 ±0.0030
30.606	palmitic acid (C16:0)	12.0440 ±0.2500	11.2600±0.9700	6.4430 ±0.0850	6.6300 ±0.0750	7.2610 ±0.0680
33.043	trans–palmitoleic acid (trans–C16:1)	0.0360 ±0.0020	0.0350 ±0.0018	0.0730 ±0.0021	0.0430 ±0.0018	0.0410 ±0.0030
33.632	palmitoleic acid (C16:1)	0.2290 ±0.0120	0.1260 ±0.0100	0.0900 ±0.0099	0.1080 ±0.0089	0.0300 ±0.0026
35.489	heptadecanoic acid (C17:0)	0.0900 ±0.0085	0.1040 ±0.0075	0.0680 ±0.0056	0.0540 ±0.0028	0.0250 ±0.0023
36.521	heptadecanoleic acid (C17:1)	0.0770 ±0.0036	0.0640 ±0.0040	0.0500 ±0.0035	0.0500 ±0.0039	0.0250 ±0.0030
38.708	stearic acid (C18:0)	3.1380 ±0.0950	7.1630 ±0.0860	2.3890 ±0.0150	2.3140 ±0.0105	2.8340 ±0.0100
40.803	trans–oleic acid (trans–C18:1)	0.1130 ±0.0098	0.1210 ±0.0085	0.0520 ±0.0020	0.1530 ±0.0095	0.1230 ±0.0086
41.805	oleic acid (C18:1, ω9)	22.4240 ±1.2000	38.1060±1.0500	16.4770 ±1.3000	14.5680 ±1.0200	4.3520 ±0.0250
42.115	C18:1 izomer	1.0140 ±0.0095	0.6340 ±0.0058	0.7410 ±0.0084	0.7210 ±0.0069	0.0550 ±0.0026
45.302	trans–linoleic acid (trans–C18:2)	0.1150 ±0.0050	0.0690 ±0.0045	0.1820 ±0.0039	0.1120 ±0.0054	0.8230 ±0.0063
46.013	linoleic acid (C18:2, ω6)	56.8640 ±1.4000	40.5760±1.2000	58.3950 ±1.3000	73.2610 ±2.0050	0.1460 ±0.0095
46.216	linolenic acid (C18:3, ω6)	0.0340 ±0.0025	0.0270 ±0.0015	0.0310 ±0.0010	0.0440 ±0.0021	1.3380 ±0.0084
46.465	arachidic acid (C20:0)	0.2200 ±0.0095	0.5060 ±0.0105	0.0510 ±0.0050	0.3870 ±0.0100	0.0910 ±0.0057
48.052	linolenic acid (C18:3, ω3)	0.2530 ±0.0084	0.2190 ±0.0063	10.6220 ±0.5200	0.1250 ±0.0085	0.0270 ±0.0015
48.728	eicosenoic acid (C20:1)	0.0330 ±0.0036	0.0250 ±0.0024	0.1440 ±0.0058	0.0290 ±0.0013	0.0240 ±0.0023
49.092	**CLA isomer, 9 cis, 11 trans**	**0.3280 ±0.0105**	**0.0320 ±0.0022**	**0.0250 ±0.0030**	**0.1850 ±0.0095**	**0.0470 ±0.0037**
49.755	**CLA isomer, 10 trans, 12 cis**	**0.0290 ±0.0021**	**0.1340 ±0.0099**	**0.1960 ±0.0085**	**0.0680 ±0.0052**	**0.0240 ±0.0010**
49.952	**CLA isomer, 9 cis, 11 cis**	**0.0380 ±0.0015**	**0.0270 ±0.0010**	**0.1050 ±0.0069**	**0.1790 ±0.0099**	**0.0250 ±0.0030**
51.472	**CLA isomer, 9 trans, 11 cis**	**0.0300 ±0.0020**	**0.0250 ±0.0015**	**0.0660 ±0.0020**	**0.2540 ±0.0105**	**0.0270 ±0.0010**
52.703	**CLA isomer, 9 trans, 11 trans**	**0.0300 ±0.0015**	**0.0270 ±0.0021**	**0.4010 ±0.0120**	**0.2100 ±0.0103**	**0.0260 ±0.0015**
54.039	eicosadienoic acid (C20:2)	0.0440 ±0.0052	0.0390 ±0.0031	0.0480 ±0.0024	0.0400 ±0.0015	0.0290 ±0.0010
54.681	erucic acid (C22:1)	2.6110 ±0.0105	0.0260 ±0.0021	0.0450 ±0.0027	0.0420 ±0.0034	0.0330 ±0.0015
55.173	lignoceric acid (C20:4)	0.0580 ±0.0057	0.1500 ±0.0095	0.0410 ±0.0034	0.2660 ±0.0105	0.0280 ±0.0023
55.501	tricosylic acid (C23:0)	0.0390 ±0.0015	0.2870 ±0.0100	0.0270 ±0.0010	0.0280 ±0.0015	0.0270 ±0.0025
56.558	cis–13,16–docosadienoic acid (C22:2)	0.0330±0.0010	0.0300 ±0.0010	0.0340 ±0.0025	0.0310 ±0.0025	0.0400 ±0.0015
57.877	eicosapentaenoic acid (C20:5)	0.0490 ±0.0023	0.0390 ±0.0025	0.0390 ±0.0027	0.0260 ±0.0010	0.0380 ±0.0015
59.435	lignoceric acid (C24:0)	0.0560 ±0.0032	0.1080 ±0.0097	0.0360 ±0.0024	0.1620 ±0.0035	0.0410 ±0.0025
61.798	nervonic acid (C24:1)	0.0280 ±0.0020	0.0260 ±0.0015	0.0290 ±0.0015	0.0300 ±0.0020	0.0250 ±0.0010
	∑SFAs	**15.9860**	**19.9080**	**12.5860**	**9.9390**	**93.1420**
	∑MUFAs	**26.4810**	**39.0820**	**17.6390**	**15.6070**	**4.6060**
	∑PUFAs	**57.7900**	**41.3250**	**70.0030**	**74.6890**	**1.7950**
	∑trans FAs	**0.3120**	**0.2550**	**0.3430**	**0.3340**	**1.0290**

FAs; fatty acids, SFAs; saturated fatty acids, MUFAs; monounsaturated fatty acids, PUFAs; polyunsaturated fatty acids, CLA; conjugated linoleic acid

The GC chromatograms of WGO, GSO and SFO samples are illustrated in Figures 1–3. These oil samples are commonly consumed, and the zoomed chromatograms (upper side) depict the separation of CLA isomers; the main chromatograms show the separation of all fatty acids in *cis–/trans–* configurations of the same sample. It can be seen from the representative chromatograms, the fatty acid separations were occurred because of the elution principle of highly polar HP–88 cyanopropyl capillary GC column and the applied temperature program, namely all unsaturated fatty acids which have the chain length of *X* were eluted between the retention times (t_R) of SFAs (*CX:0*) and (*CX+2:0*). Identification of the other positional and geometric isomers was based on the direct comparison with the target reference fatty acids and our earlier studies [16–20]. The order of retention time (t_R) in GC method was also given in Table 1.

The GC data were gained with a highly polar column (100m×0.2μm×0.25mm i.d; HP–88 cyanopropyl) by a similar temperature program applied for FAME analysis. This column was very practical and currently recommended for the identification of SFAs, *cis*–MUFAs and *cis*–PUFAs based on their chain–lengths, as well the number and position of their dual bonds. Of special interest in this GC column was the identification of five different CLA isomers [cis–9, cis–11 CLA, cis–9, trans–11 CLA, trans–9, cis–11 CLA, trans–9, trans–11 CLA and trans–10, cis–12 CLA]. The positional and geometric isomers of CLA were also well separated, and they were eluted as *cis–/trans–*, *trans–/cis–*, *cis–/cis–*, and *trans–/trans–*, then relative to the carbon atom where the double bond is located, expressed as Δ for positional isomers within these groups. In positional CLA isomers, the geometric isomers of *cis–/trans–* were eluted prior to the *trans–/cis–* form. Hence; their t_R was recorded in order: cis–9, trans–11 CLA (t_R=49.963 min), trans–10, cis–12 CLA (t_R=50.285 min), cis–9, cis–11 CLA (t_R=50.487 min), trans–9, cis–11 CLA (t_R=50.793 min) and trans–9, trans–11 CLA (t_R=50.963 min). This elution order obtained under study is in accordance with the literature [1–4,7–12].

It was concluded that the cold pressed oil samples were all rich in total CLA (∑CLA) contents and they ranged from 0.14% (for PSO) to 2.11% (for SSO). The contents of ∑CLA in cold pressed PGSO (0.14%), LSO (0.27%), WGO (0.76%), NSO (0.45%), SSO (2.11%), GSO (0.89%), FSO (0.3900%), CO (0.20%), BCSO (0.45%), PSO (0.24%), WO (0.79%), SFO (0.89%) and CNO (0.14%) were commonly in similar concentrations. The major CLA isomer in tested cold pressed oils was concluded as *cis*–9, *trans*–11 CLA, which represented the content of isomer between 3.15% and 72.08% of ∑CLA. The contents of trans–10, cis–12 CLA isomer were between 5.29% and 54.69% of ∑CLA, cis–9, cis–11 CLA isomer were between 3.33% and 19.97% of ∑CLA, trans–9, cis–11 CLA isomer were between 6.59% and 29.96% of ∑CLA, trans–9, trans–11 CLA isomer were between 4.66% and 50.56% of ∑CLA. Thus, the present study contributed valuable information for the introduction of new sources of functional cold pressed oils, as well as incorporation into medicinal purposes and food formulations which could have potential to be commercially developed.

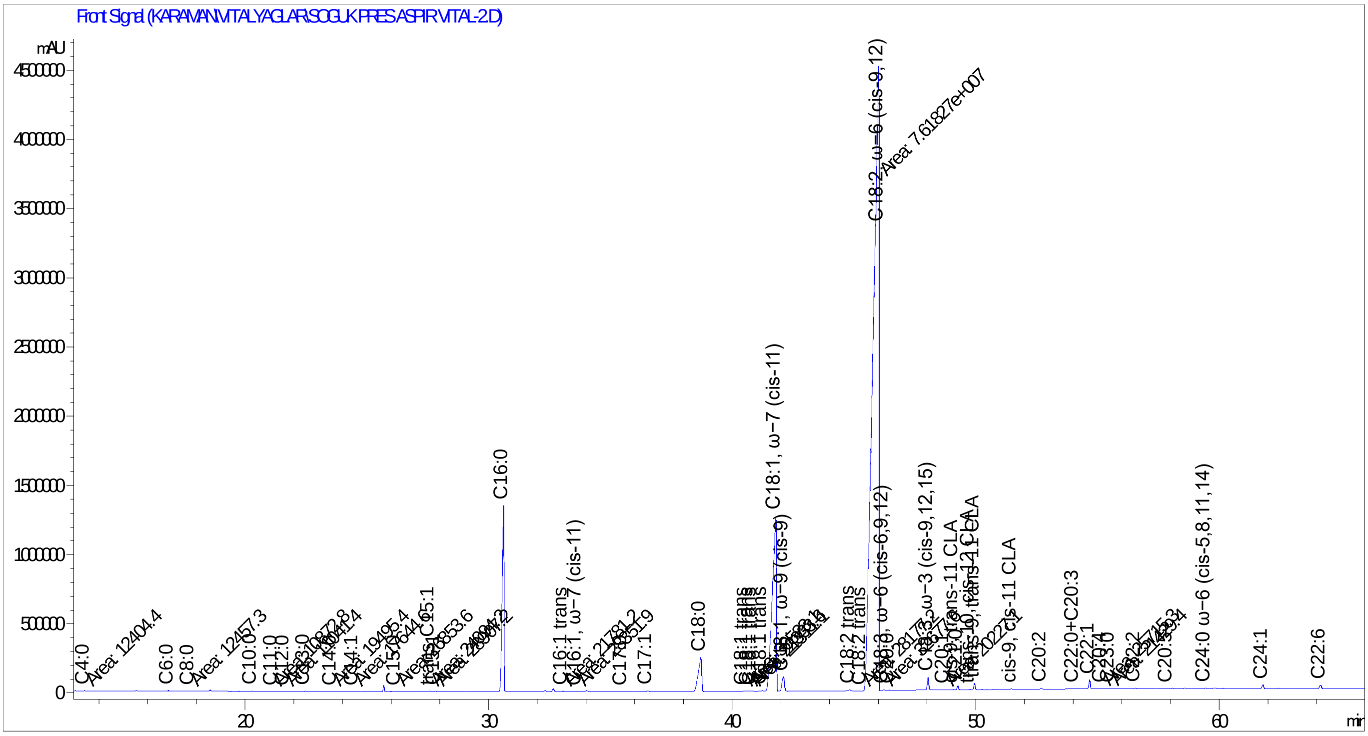

Figure 1. GC/FID chromatogram for the CLA and fatty acid analysis of cold pressed safflower oil (SFO) sample

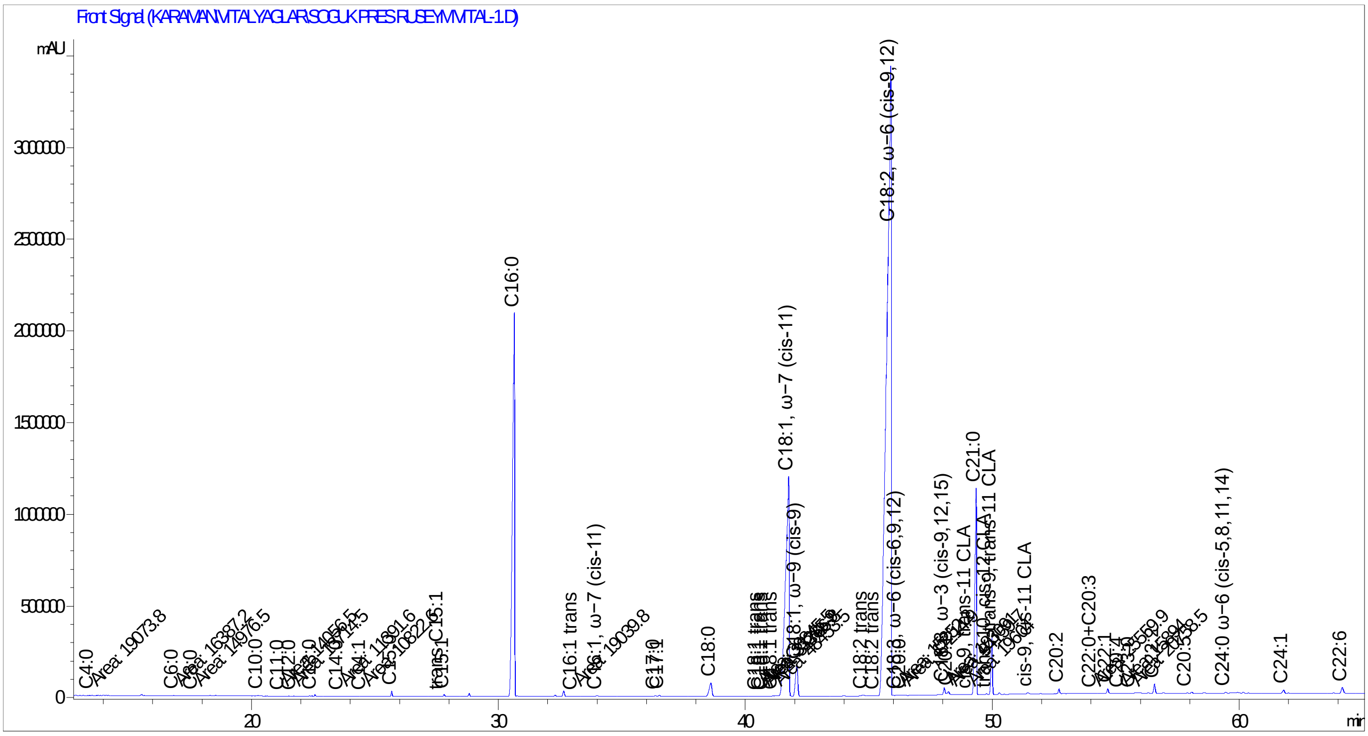

Figure 2. GC/FID chromatogram for the CLA and fatty acid analysis of cold pressed wheat germ oil (WGO) sample

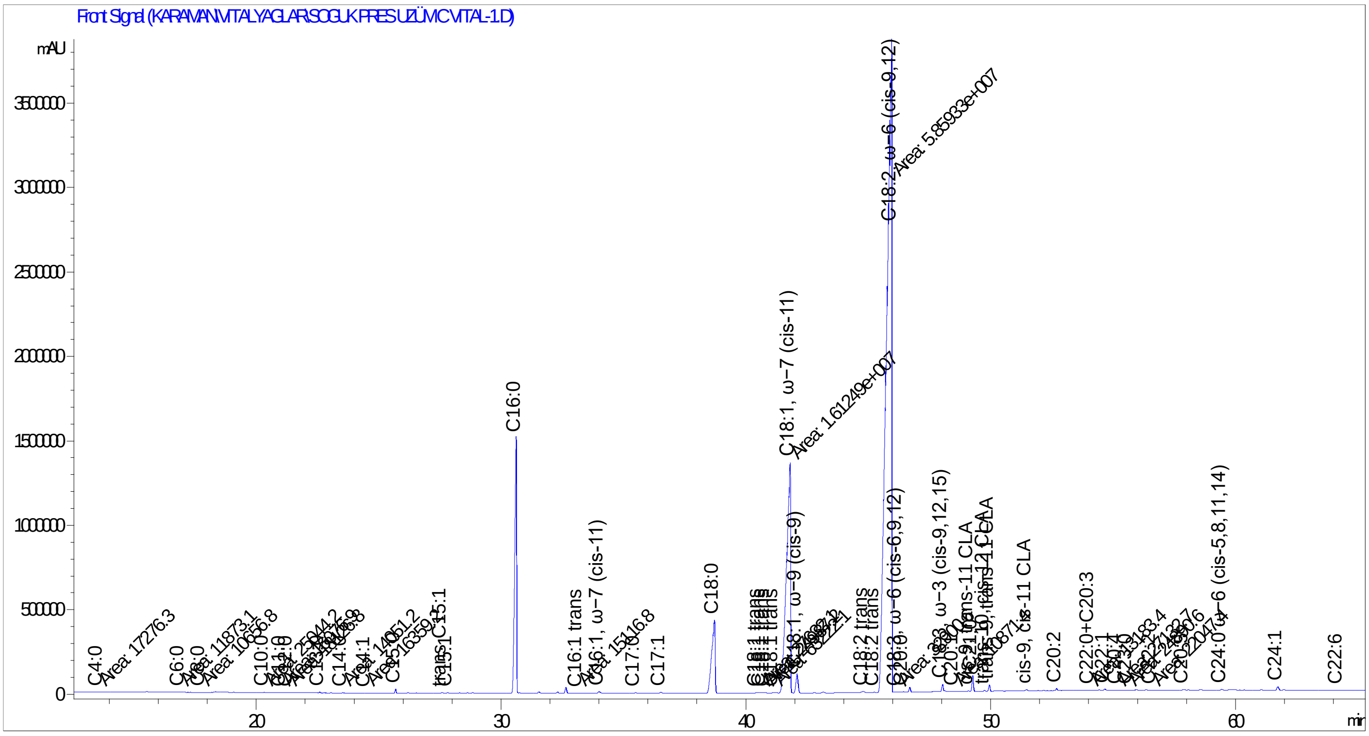

Figure3. GC/FID chromatogram for the CLA and fatty acid analysis of cold pressed grape seed oil (GSO) sample

4. CONCLUSIONS

In conclusion, we aimed to determine the composition and content of CLA isomers, which have been proven to support a health and life quality with many scientific studies, and other valuable fatty acids (SFAs, *cis*–MUFA and *cis*–PUFAs) in some cold pressed oils (*PGSO, LSO, WGO, NSO, SSO, GSO, FSO, CO, BCSO, PSO, WO, SFO and CNO*) extracted with lab–scale screw press machine in our research laboratory. The CLA and fatty acid isomers were profiled by GC/FID method based on the fatty acid composition analysis. The five valuable isomers of CLA [*cis–9, cis–11 CLA, cis–9, trans–11 CLA, trans–9, cis–11 CLA, trans–9, trans–11 CLA and trans–10, cis–12 CLA*] were also well separated according to their geometric and positional structure. The studied samples were all rich in ∑CLA content and they were found between 0.14% and 2.11%. The major isomer was to be *cis*–9, *trans*–11 CLA (ranged from 3.15% to 72.08% of ∑CLA). The ∑SFA values were also detected between 2.43% and 93.14%, ∑MUFA values were between 4.60% and 71.11% and ∑PUFA values were between 1.79% and 87.59%. Therefore, we are of the opinion that the study will be a valuable source for studies that will make it possible to obtain low–cost, high value–added functional food products, and it will also be a guide for the industry of cold press oil.

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CONFLICT OF INTEREST

The researcher declared that she had no conflicts of concern relating to the publication of this research

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