Phenolic Compounds, Antioxidant Activity and Fatty Acid Compositions of Commercial Cold-Pressed Hemp Seed (*Cannabis Sativa* L) Oils From Turkey

Zeliha Ustun-Argon

Abstract— Hemp (*Cannabis sativa L*) is one of the oldest plants which has been used throughout history by people for food, textile, and medicine due to its important components. Hemp seed oil has become more popular with a polyunsaturated fatty acids (PUFA) and linoleic/linolenic acid ratio which is ideal for a healhty human diet and cold pressed oil is preffered for supplements and pharmaceuticals. In this study different samples of cold pressed hemp seed oil from Turkey have been evaluated for fatty acid compositions, DPPH radical scavenging activity. Phenolic components determined with LC-QTOF-MS. PUFAs were found between 71.75-74.79% and linoleic/linoleic acid ratios were between 2.96-3.27. DPPH scavenging activity 38.24±2.44-51.23±0.582 %. Phenolic compounds have identified with Metlin_Metabolomic and For_Tox databases and 51-89 different components were defined.

Index Terms— *Cannabis sativa*, Cold press, DPPH radical scavenging, Fatty acid composition, Hemp seed oil, LC-QTOF-MS, Phenolics

1 INTRODUCTION

In the last years, people tend to improve and maintain their health by changing their life styles, their eating habits and by using nutraceuticals. Therefore the researches have focused on nutritional and beneficial properties of non-conventional foods for alternative usages to promote well-being and to prevent, manage or treat diseases. Hemp (*Cannabis sativa* L) belongs to Cannabinaceae family and the known origin is Central Asia. This annual herbaceous plant is probably one of the oldest plants to have been used by people. Due to the plants' fibre structure and nutrients in the seeds, cannabis is revered as an important plant for different purposes from food to textiles and medicine production in history. Hemp seeds are mainly used for feeding purposes but currently hemp seed based oil, protein powder, flour, and meal products tend to be more popular in the human diet due to nutritional and antioxidant properties [1], [2]. Hemp seeds consist of protein (20-25%), carbohydrates (20-30%), insoluble fiber (10-15%), oil (25-35%) and minerals particularly phosphorous, potassium, magnesium, sulfur and calcium, iron, zinc and also a "Vitamin A" precursor carotene [3]. Variety, soil type, climate, and agronomic practices used during seed production processes are the factors which can affect the compositional variations of hemp seed [4]. Hemp seed oil structure is mainly based on polyunsaturated fatty acids with more than 70%. Hemp seed oil is considered important among other oils especially with the optimal linoleic and linolenic acids ratio (3:1) for the human health. Also with the high amount of γ-linolenic acid (GLA) and stearidonic acid (SDA) content hemp seed oil is found to be significant amongst the other industrial crops. These fatty acids and other components in the oil are found to be effective against cancer, inflammations, platelet aggregation, ischemic heart diseases, psoriasis, atopic dermatitis, and mastalgia and also support plasma fatty acid profiles, fat burning and stimulation of general metabolism [3], [5], [6], [7]. Besides human diet, hemp seed oil is preferred as a lighting fuel, wood preservative, printer's ink, soaps, detergents and body-care products' raw material [3].

The virgin hemp seed oil is extracted from the seeds by cold press method. The yield of this method is generally lower than the methods including hot press or solvent extractions but preserve the important phytochemicals and the natural antioxidants and prevent degradative changes, such being possible with this method [2], [3], [8], [9], [10], [11]. High quality cold pressed hemp seed oil is defined with a nutty taste and light to dark green colour due to chlorophyll components which are extracted with the oil [2], [3].

Free radicals are considered important in health problems such as chronic pathologies and cardiovascular diseases with the major effect on lipid peroxidation. The products with antioxidant effects can be preferred to reduce the radicals. Edible oils are also known to be with their antioxidant effects therefore these oils should be taken into consideration as a part of the daily diet [12], [13]. Phenolic compounds in the plants are accepted as the main responsible component of the antioxidant activities by preventing the lipid oxidation related reactions and also have influence on the stability, nutritional and sensory properties and [2], [14], [15].

In this study, mostly preferred cold pressed hemp seed oils in the Turkish market have been evaluated. The samples have been analysed for their fatty acids compositions (FAME), DPPH radical scavenging activities and phenolic profiles

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extensively. Due to the improvements in the performances of the measuring instruments, conventional and unconventional oils went beyond the known ranges with their differences in their natural compositions [16]. In this respect the analysis has been completed with highly performing instruments, such as LC-Q-TOF-MS, GC-MS to be able to deepen analysis of the properties of hemp seed oil and see the varietion between diffeent brandnames. This study also aimed to identify the trends in the hemp seed oils' compounds by applying principal component (PCA) analysis.

2 Material and Method

2.1 Material

Cold pressed hemp seed oil samples have been chosen amongst the mostly preferred brandnames. Samples have been bought from the natural supplements shops and pharmacies.

2.2 Chemicals

All the reagents were obtained from J.T. Baker, Riedel-de Haën and Sigma–Aldrich and they are either chromatographic or analytical grade. Millipore ultrapure water (Type I) was used for all analysis.

2.3 Fatty acid methyl esters (FAME) analysis

COI/T.20/Doc. No 33 for olive oils method was used for fatty acid methyl esters (FAME) determination [17]. A 37 component mixture of FAME (Supelco) has been chosen as the standard for retention time to identify the fatty acids. Area ratio which is under the relevant peak was used for the quantitative analysis. An Agilent 6890 GC-FID system was used for FAME analysis. The column was a Supelco 2560 capillary column (100 m x 0.25 mm ID x 0.2 µm). Split ratio was 1:100. injection and detector temperatures were 250°C and 260°C, respectively. The temperature program was as follows; the oven temperature is held at 140°C for 1 min and then, increased to 240°C at a rate of 4°C/min and hold for 5 min.

2.4 DPPH Free Radical-Scavenging Assay

The free radical scavenging activity was determined by the DPPH assay spectrophotometrically [18]. Briefly, 1.0 mL of the extract was mixed with 1.0 mL of DPPH (0.8 mmol/L) and was shaken. 30 min later, the absorbance was measured at 517 nm against a reagent blank for 5% solution. Standards were gallic acid and BHT. Triplicate reactions were carried out. The following equation was used to calculate the inhibition percentage for scevenging DPPH radical:

DPPH radical scavenging effect (%) =(A control-A sample)/(A control) x 100

A Control : The initial concentration of the DPPH

A Sample : The remaining concentration of DPPH's absorbance in the presence of the extract and positive controls.

2.5 Phenolics

Chromatographic separation was carried out using an HPLC Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, USA) equipped with a the double pump, degasser, automatic sample dispenser. Poroshell 120 EC-C18 (3.0X50 mm, particle size 2.7 µm) (Agilent) column was preferred to separate the compounds. The mobile phases were used with the gradient washing (elution) steps given in the table. Mobile phase A was the mixture of water and 0.1% formic acid and mobile phase B was the acetonitrile. The column was maintained at 35 ° C, the injected sample volume was 3 µL and the flow rate was determined as 0.4 mL/min.

TABLE 1
Ellution Steps

Time (min)	Mobile phase
0	% 5 B
8	% 15 B
10	% 20 B
13	% 25 B
18	% 30 B
20	% 45 B
24	% 60 B
27	% 80 B
30	% 90 B
32	% 5 B
3	Conditioning cycle

MS analysis was carried out using an Agilent 6550 iFunnel high resolution Accurate Mass QTOF-MS equipped with Agilent Dual Jet Stream operating in positive ion electrospray

ionization (Dual AJS ESI) interface and desiccant gas flow 14.0 L / min; nebilizer gas pressure 35 psi; drying gas temperature 290 ° C; sheath gas temperature 400 ° C; the sheath nitrogen gas flow at 12 L/min. Mass spectra were recorded in the negative ionization mode in a mass range of 50-1700 m/z.

Integration and data detailing were performed using the station MassHunter Workstation "software. Agilent METLIN Metabolomix database, library and full mass personal composite database and library (METLIN_AM_PCDL) have been used to identify analytes. Positive and negative modes are conducted in the same conditions.

2.6 Data Analysis

The multivariate data matrix includes results from the chosen samples represented by fatty acid methyl esters, phenolics and DPPH radical scavenging activity. Results are given means±SD for triplicate analysis. Seed oils were characterized and classified by chemometric methods, PCA (Ward's algorithmic method). Minitab 15 Statistical Software is used for the multivariate analyses. Data and auto scaled variables were standardized prior to the chemometric analysis. Scores and loading plots are used for visualization of PCA results. Score plots provided a clear relevance between principal groupings and observations. Also loadings plot, indicate the significance of each variable for the results. The plots are used to explain the correlation between variables and cluster observations in the score plots.

TABLE 2
ESI-MS VARIABLES

Desiccant gas flow	14.0 L/min
Desiccant gas temperature	35 psi
Nebuliser gas pressure	290 °C
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min

3 RESULTS AND DISCUSSION

3.1 Fatty Acid Composition

FAME analysis showed that main fatty acids in hemp seeds oil are linoleic acid, linolenic acid and oleic acid with the amounts 54.80-55.78%. 16.77-18.85% and 12.44-16.33% respectively (Table 3). Sample B found with the highest linoleic acid rate (55.78%) while sample C with the lowest rate (54.80%). Sample-B also found with the highest linolenic acid (18.85%) and with the lowest oleic acid rate (12.44%). The ratio between linoleic and linolenic acids found 3.13, 2.96, 3.11, 3.27 respectively for the Sample A, B, C, D. This ratios are around 3:1 for linoleic and linolenic acids as recommended optimal for the human health. Sample B found with the highest essential fatty acids (EFAs) (74.64%), polyunsaturated fatty acids (PUFAs) (74.79%) and saturated fatty acids (12.30%) rates while sample D with the highest monounsaturated fatty acids (MUFAs) (12.91%). Results show similarity with other studies changing levels of fatty acids and ratios between the saturated and unsaturated fatty acid and between linoleic and linolenic acids [19],[20], [21], [22], [23], [24], [25], [3]. Variations in fatty acid compositions can depend on variety, differences agronomic and process practices in planting, harvesting, storing and oil extraction steps, soil type and climate [4]. Based on the fatty acid composition it can implicate that the high level of linolenic acid may help to improve coronory health and prevention of cancer and chronic diseases. Also the ratio between polyunsaturated/saturated fatty acids is accepted important for decreasing serum cholesterol level and atherosclerosis [22].

3.2 DPPH Radical Scavenging Activity

TABLE 3
FATTY ACID COMPOSITIONS

	SAMPLES			
FATTY ACIDS	A	B	C	D
Myristic Acid	0.05±0.001	0.03±0.000	0.04±0.001	0.57±0.001
Palmitic Acid	6.38±0.001	6.81±0.001	6.34±0.001	6.49±0.004
Palmitoleic Acid	0.10±0.001	0.16±0.001	0.09±0.001	0.06±0.002
Heptadecanoic Acid	0.06±0.001	0.07±0.001	0.06±0.001	0.08±0.002
Stearic Acid	2.99±0,001	2.88±0.004	2.89±0.001	2.95±0.037
Oleic Acid	15.41±0.017	12.44±0.001	15.50±0.000	16.33±0.203
Linoleic Acid	54.92±0.004	55.78±0.001	54.80±0.001	54.92±0.456
Linolenic Acid	17.41±0.002	18.85±0.001	17.65±0.002	16.77±0.003
Eicosenoic Acid	0.46±0.001	0.44±0.001	0.45±0.001	0.43±0.004
Heneicosanic Acid	0.19±0.002	0.29±0.001	0.18±0.001	0.16±0.003
Eicodadienoic Acid	0.08±0.001	0.08±0.001	0.05±0.001	0.05±0.001
Behenic Acid	0.35±0.001	0.28±0.001	0.32±0.001	0.33±0.006
Tricosanoic Acid	0.06±0.001	0.05±0.001	0.04±0.001	0.04±0.001
Lignoceric Acid	0.14±0.001	0.11±0.001	0.13±0.001	0.13±0.002
EFAs	72.33	74.63	72.42	71.69
Linoleic/Linolenic Acid Ratio	3.15	2.96	3.11	3.27
PUFAs	72.41	74.79	72.53	71.75
MUFAs	15.88	12.91	16.01	16.75
Saturated	11.72	12.30	11.46	11.50
Polyunsaturated/ Saturated Ratio	6.18	6.08	6.33	6.24

The radical-scavenging activity of antioxidants may be influenced by the testing conditions and radical systems. DPPH is preferred due to its stable properties for determination of

antioxidant's radical scavenging capasities and to estimate the thermodynamical and kinetical characteristics of antioxidant-radical reaction [26]. In our study sample A found with the highest percentage while sample D with the lowest rate (Table 4). The results are generally are higher than the Casoni et al's [27] study which is found the radical scavenger activity for hemp seed oil 41.90±1.11%. Another study conducted by Siger et al. [2] found the DPPH scavenging activity of hemp seed oil 76.2 ± 4.5%. This result showed that hemp seed oil has the highest level of antioxidant capasity among the other plant oil extracts including pumpkin seed oil (65.3±3.1%), rapeseed oil (51.2±4.1%), soybean (17.4±3.2%), sunflower(23.8±2.1%), grapeseed (13.4±2.0%), flax (19.3±2.1%), rice bran (23.7±2.6%) . Our study results were also higher than the other cold pressed oils like nutmeg (31.69 ± 1.27%), white mustard (7.39 ± 0.29%), anise (12.52 ± 0.66%), coriander (4.96 ± 0.22%) and caraway (8.81 ± 0.41%) [25]. Another study which analysed the cold pressed oils found the antioxidant activities as black caraway> cranberry> hemp> carrot. The implication for these seed oils is that they may considered novel natural antioxidants for the protection of proteins, DNA and membrane lipid against oxidative damage caused by free radicals for a wide range different applications from foods to supplements and pharmaceutials [26]. Two other studies also found some components in the hull, seed and sprouts of the hemps seeds as valuable natural antioxidants for different pharmaceutical applications [28], [1].

3.3 Phenolics

Phenolic compounds are known as natural antioxidants and antimicrobial compounds to be present in all vegetable oils, which is very critical for the stability of the polyunsaturated fatty acids oxidation of these oils and sensorial and nutritional characteristics. In this content, not only the total amount of phenolic components is affecting the nutritional characteristic of oils but also the composition of phenolic compounds are effective [2], [11]. Phenolic components have been determined with LC-QTOF-MS and have identifed with Metlin_Metabolomic and For_Tox databases. According to the results sample D contains 89 different components while sample A is with 61, sample B 54, sample C 51 components including THC / delta9-Tetrahydrocannabinol, Stearic acid, Physcion, Norethindrone acetate, Methylsalicylate, Linolenic acid, Diosmetin, Citric acid, CBN / Cannabinol, Azelaic acid, Apigenin, Vanillin, TPPA / Triphenyl phosphate, THCA-A / delta9-Tetrahydrocannabinol-2-carboxylic acid, THC / delta9-Tetrahydrocannabinol, Syringaldehyde, Stearamide, Pimozide, Hexylnicotinate, Papaverine, Palmidrol, Noscapine, Norharman, Δ2-cis-Hexadecenoic Acid, α-Linolenic Acid, trans-EKODE-(E)-Ib, Sucrose, Stearidonic Acid, Stearic acid, Sinapyl aldehyde, Petroselinic acid, Norethindrone Acetate, Methyl N-(a-methylbutyryl)glycine, Genkwanin, Galactonic acid, Elaidic Acid, α-Linolenoyl Ethanolamide, Triphenyl phosphate, Trigonelline, Sucrose, Stearidonic Acid, Stearamide, Phthalic acid. These results, in our knowledge, are the widest range of identification for hemp seed oils samples. Other studies are determined limited phenolic acids for this oil, such as p-hydroxybenzoic, vanilic, p-coumaric, sinapic acids [2] or generally analysed the total phenolic content (TPC). TPC level for hemp seed oil is found 0.44±0.01 mg GE/g [26], 2.45±0.05 mg CAE/100 g [2] and 3.21±0.27 mg GA/g oil [25]. The hemp seed hull also identified with two different phenolic compounds by using high performance liquid chromatography methods, which were identified as N-trans-caffeoyltyramine and cannabisin B by high-resolution nuclear magnetic resonance spectra, mass spectra, and ultraviolet data [28]. Because phenolic compounds may contribute to overall antioxidant activities, the implication is that the compositions of the components in hemp seed oil make it very useful for different food, pharmaceuticals, nutraceuticals applications.

TABLE 4
DPPH RADICAL SCAVENGING ACTIVITY

Sample	Antioxidant Activity (%)
A	51.23±0.582
B	49.01±0.698
C	42.52±1.98
D	38.24±2.44

3.4 Principal Component (PCA) Analysis

It can be seen with PCS score plot graphics (Fig. 1) PC 1 and PC 2 have %58.32 and % 22.79 effects respectively. Hemps seed oils with from four different samples are seperated from each other and Sample A and C are clustered in the same area. Effective parameters for separation can be seen in a loading plot.

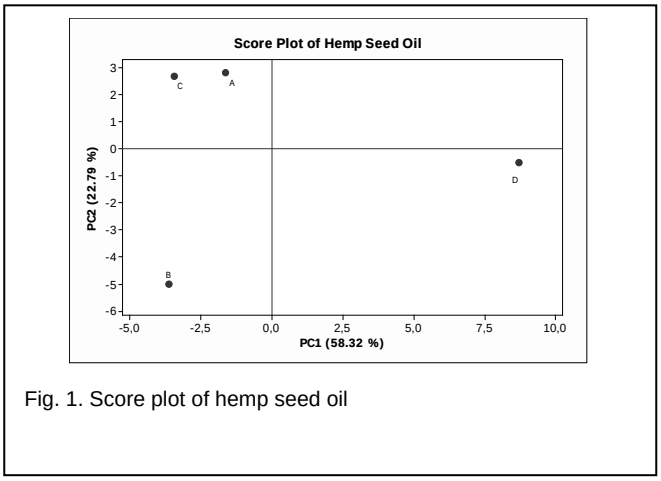

Fig. 1. Score plot of hemp seed oil

Sample A and Sample C are separated with 2-Pyrimidine Acetic Acid, THC/delta-9-Tetrahydrocannabin, pimozide, glycerophosphocholine, isopimpinellin, cannabidiol, hexylnicatinate, phthalic acid Mono-2-ethylhexyl ester components. Sample B is identified and sperated with the effect of apigenin, physcion, genkwanin, carnosol, linoleoyl ethanolamide, piperine, methyl linolenate, elaidic acid and galactonic acid (Figure

2).

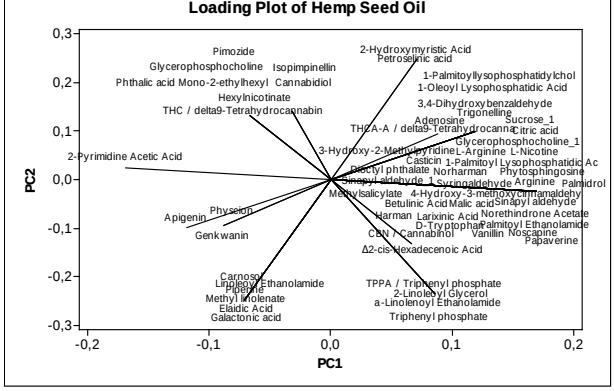

Fig. 2 Loading plot of hemp seed oil

4 Conclusion

In the present study, cold pressed hemp seed oils from the Turkish market are evaluated according to their fatty acid compositions, DPPH radical scavenging activity and phenolic components. The fatty acid content showed that Turkish hemp seed oils are rich with PUFA and MUFA and the linoleic/linolenic acid ratio is in the optimal level for a healthy daily diet. With its antioxidant properties, hemp seed oils have important health properties also impoved product safety with a high level antioxidants. Phenolic compounds also showed great variety in the products which are important for health studies and to develop novel food ingredients or supplements. The obtained data from this study could be important to deepen the knowledge about hemp seed oil and its alternative usages to improve the human diet quality, nutraceutical and pharmaceuticals.

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5 References

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