biology and life sciences forum

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Proceedings Paper

Characterisation of the fat profile of different varieties of hemp seeds (*Cannabis sativa* L.) for food use

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† Presented at the 4th International Electronic Conference on Foods. Focus on Sustainable Food Systems: Current Trends and Advances; Available online: https://foods2023.sciforum.net

Abstract: Five varieties of industrial hemp (*Cannabis sativa* L.) (Futura 75, Futura 83, Felina 32, Earlina 08 FC and Henola) were planted to produce grain and then study its potential as an alternative source of nutritional compounds due to its fat, carbohydrate and protein content. The lipid fraction may account the 35% of the total composition of hemp seeds and the aim of this study was to determine the fat content mainly focused in the characterization of the fatty acid profile by gas chromatography with a flame ionization detector of the different varieties of hemp seeds regarding a possible use of the whole grains or the oil extracted from them. The fat content of the seeds ranged between 23 and 31% of total composition, being Earlina 08 FC significantly ($p < 0.05$) the richest in fat content. 87% of total fatty acids were unsaturated, being polyunsaturated acids the main group and linoleic acid the most abundant one, which is a precursor of biological active molecules involved in many physiological processes. Thus, hemp seeds may be considered a valuable source of healthy fatty acids to include in plant-based food formulation, being aware that further studies are being conducted to characterize other nutritional components.

Keywords: fat; hemp seeds; fat acids profile

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Industrial hemp has been cultivated since ancient times with different purposes, but currently, food production is a prospective direction for the hemp seeds mainly due to their high nutritional value. Hemp seeds are a quite balanced food matrix regarding their composition in protein, carbohydrates, fibre and other nutrients such as minerals, vitamins and antioxidants [1]. However, they are considered oilseeds since fat is the most representative component, ranging between 25 and 35% of dry matter depending on the variety studied and the environmental conditions [1,2]. Although there is some scientific information concerning this topic [1,3,4] and about hemp supplementation of food [5], the diversity of industrial hemp seed cultivars requires research to understand which variety exerts a better yield in terms of seed production and nutritional quality of some particular food-derived ingredients, such as fat content, to enhance the production of such components from the seed.

Thus, the aim of this study was to characterise the lipid fraction of different varieties of hemp seeds regarding a potential food use of the whole grain or the oil extracted from them.

2. Materials and Methods

2.1. Raw Materials

Five hemp varieties (Futura 75, Futura 83, Felina 32, Earlina 08 FC and Henola) included in the EU common catalogue of plant species were chosen to carry out this study due to its good field adaptation and productivity. The seeds used as starting material were supplied by Trichome Pharma (Madrid, Spain) for research purposes only. Before planting commercial seeds were disinfected with NaClO 0.58% for 5 min and rinsed with distilled water. Once the crop was fully developed, the seed was collected by variety, and within a representative sampling of 10 m^2 the harvested seeds were manually and mechanically cleaned to eliminate other plant material. Finally, hemp seeds were lyophilized prior to chemical analysis and stored at 4 °C.

2.2. Determination of lipid content and fatty acids profile

Total fat content was determined by acid hydrolysis following the procedure similar to AOAC 922.06 [6].

The fatty acid (FA) profile of the freeze-dried seeds was determined in a Bruker Scion 436 gas chromatograph with flame ionization detector (Bruker, Billerica, MA, USA) equipped with a CP-8400 autosampler and an SP-2560 capillary column (100 m x 0.25 mm x 0.2 μm) (Supelco, Saint Louis, MO, USA) according to Rufino-Moya et al. (2022) [7]. Briefly, 1 μL was injected with 1:125 split relation at 280 °C, oven temperature was set at 125 °C for 15 min and reached 190 °C until 90 min at 5 °C/min. C19:0 was used as an internal standard in the extraction of FA methyl esters (FAME) and identifications were based on FAME retention times that were compared with those of the standard mixtures GLC-532, GLC-401, GLC-643 and GLC-642 (Nu-Chek Prep, Elysian, MN, USA). Quantification was performed as described in ISO 12966-4:2015 [8].

2.3. Statistical Analysis

Analysis of variance (ANOVA), with a confidence level of 95% ($p < 0.05$), using Statgraphics Centurion version 18.1.13 (STSC, Rockville, Md, USA) was applied to evaluate the differences among samples for each analysed compound. LSD posthoc test was used to determine significant differences ($p < 0.05$).

3. Results and Discussion

The fat content of industrial hemp seeds makes interesting the study of its fatty acid profile [1]. Table 1 shows the fat content of the five varieties studied and their fatty acid profile. The fat content of hemp seeds ranged from 23 to 31% of total composition. There are significant ($p < 0.05$) differences between them, being Earlina 08 FC the variety with the significant ($p < 0.05$) highest fat content and Futura 75 the lowest one. Looking at the fatty acid profile shown in table 1, linoleic acid (LA) (C18:2 n6) is the major fatty acid in hemp seeds, in agreement with other works [1,2,3,4,9,10]. Like other studies for hemp seed this fatty acid is approximately 55% of the total fatty acids [1,2,3,4,10]. When comparing the linoleic acid content of the five varieties studied, no significant ($p > 0.05$) differences were observed, however, Henola showed a significantly ($p < 0.05$) lower content than Felina 32 or Earlina 08 FC. LA is of great importance in the correct maintenance of human health since it is a precursor of biological active molecules involve in different physiological processes [1]. The following major fatty acids were linolenic acid (ALA) (C18:3 n3) and oleic acid (OA) (C18:1 9c) ranging from 13-15 % and 12-15 % of the total fatty acids, respectively. Similar ranges were generally obtained for hemp seeds by other authors [9]. Earlina 08 FC and Henola showed significantly ($p < 0.05$) higher values of linolenic acid than the other varieties studied. The lowest linolenic acid content was found in Futura 83. However, Futura 75 showed a significantly ($p < 0.05$) higher oleic acid content than Felina 32, Earlina 08 FC and Henola. On the other hand, the lipid fraction was low in saturated fatty acids, which consisted mainly of palmitic (C16:0) and stearic

acid (C18:0) in amounts of approximately 50 mg/g, with palmitic acid as the predominant saturated fatty acid. These values are in the range observed by other works [9]. Futura 83 had the lowest values for these two saturated fatty acids compared to the other varieties.

Table 1. Mean values (and standard deviations) of lipid (g/100g) and fatty acids (mg/g) content of hemp seeds.

Sample	Futura 75	Futura 83	Felina 32	Earlina 08 FC	Henola
Lipid	23.09 (0.15)^{d}	24.44 (0.12)^{c}	25.8 (0.2)^{b}	30.9 (0.3)^{a}	26.1 (0.5)^{b}
C12:0	0.0187 (0.0002)^{b}	0.0173 (0.0012)^{bc}	0.0207 (0.0008)^{a}	0.01657 (0.00013)^{c}	0.0140 (0.0004)^{d}
C14:0	0.2446 (0.0014)^{b}	0.2335 (0.0005)^{bc}	0.242 (0.014)^{b}	0.266 (0.002)^{a}	0.2207 (0.0007)^{c}
C15:0	0.0990 (0.0012)^{a}	0.0956 (0.0007)^{a}	0.097 (0.007)^{a}	0.102 (0.012)^{a}	0.0778 (0.0015)^{b}
C16:0	35.22 (0.03)^{b}	35.1 (0.4)^{b}	37.5 (1.3)^{a}	35.8 (0.5)^{ab}	37.40 (0.17)^{a}
C16:1-9c	0.526 (0.009)^{ab}	0.494 (0.012)^{b}	0.54 (0.03)^{a}	0.516 (0.002)^{ab}	0.517 (0.003)^{ab}
C17:0	0.332 (0.012)^{a}	0.307 (0.0012a	0.34 (0.05)^{a}	0.37 (0.03)^{a}	0.30 (0.06)^{a}
C18:0	13.47 (0.12)^{a}	12.58 (0.08)^{b}	12.8 (0.3)^{ab}	12.76 (0.09)^{b}	12.8 (0.5)^{ab}
C18:1 9c	64.2 (0.5)^{a}	61.5 (1.3)^{ab}	59.4 (3.0)^{bc}	52.5 (0.8)^{d}	56.120 (0.014)^{cd}
C18:1 11c	5.36 (0.03)^{a}	5.09 (0.08)^{ab}	5.3 (0.3)^{a}	5.17 (0.05)^{ab}	4.88 (0.02)^{b}
C18:2 n6	243.2 (0.3)^{ab}	239 (6)^{ab}	248 (10)^{a}	249 (2)^{a}	231.6 (0.2)^{b}
C18:3 n6	8.69 (0.12)^{c}	8.711 (0.008)^{c}	10.2 (0.2)^{b}	13.4 (0.3)^{a}	7.0 (0.2)^{d}
C20:0	2.60 (0.02)^{b}	2.44 (0.04)^{c}	2.65 (0.05)^{ab}	2.77 (0.04)^{a}	2.58 (0.12)^{bc}
C18:3 n3	58.7 (0.4)^{c}	55.8 (1.2)^{d}	63.1 (1.2)^{b}	67.1 (0.8)^{a}	66.3 (1.2)^{a}
C20:1	1.368 (0.012)^{ab}	1.35 (0.04)^{b}	1.43 (0.05)^{a}	1.428 (0.008)^{a}	1.418 (0.002)^{ab}
C18:4 n3	2.03 (0.06)^{c}	1.95 (0.03)^{c}	2.47 (0.03)^{b}	3.45 (0.07)^{a}	1.97 (0.14)^{c}
C21:0	0.028 (0.005)^{ab}	0.0223 (0.0008)^{b}	0.025 (0.004)^{b}	0.036 (0.004)^{a}	0.0277 (0.0008)^{ab}
C22:0	0.70 (0.03)^{a}	0.636 (0.013)^{a}	0.66 (0.03)^{a}	0.702 (0.003)^{a}	0.61 (0.08)^{a}
C22:1	0.064 (0.005)^{a}	0.053 (0.003)^{c}	0.063 (0.002)^{ab}	0.0582 (0.0004)^{abc}	0.0572 (0.0014)^{bc}
C23:0	0.0403 (0.0005)^{a}	0.033 (0.004)^{ab}	0.033 (0.013)^{ab}	0.0226 (0.0012)^{b}	0.0323 (0.0002)^{ab}
C24:0	0.094 (0.002)^{bc}	0.078 (0.013)^{c}	0.103 (0.002)^{ab}	0.117 (0.008)^{a}	0.078 (0.008)^{c}

The same letter in superscript within the row indicates homogeneous groups established by ANOVA ($p < 0.05$).

Fatty acids are classified according to their chemical structure. Figure 1 shows the content of FAs grouped into saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FAs in the hemp seeds studied. The five varieties studied showed no significant ($p > 0.05$) differences in their total FA content. Between 71.5 and 74.7 percent of the FAs are PUPA in the hemp seeds studied. Earlina 8 FC showed the highest PUFA content without showing significant ($p > 0.05$) differences with Felina but did show significant ($p < 0.05$) differences with the other varieties studied. In the case of MUFA, their value corresponds to 13.4 -16.4 % of total FAs. Overall, a high content of unsaturated fat in relation to total fat is obtained (87.23 – 88.1%). The content of SFA in relation to total FA was between 11.90 and 12.77 %. Futura 83 showed a significantly ($p < 0.05$) lower SFA content than the other varieties.

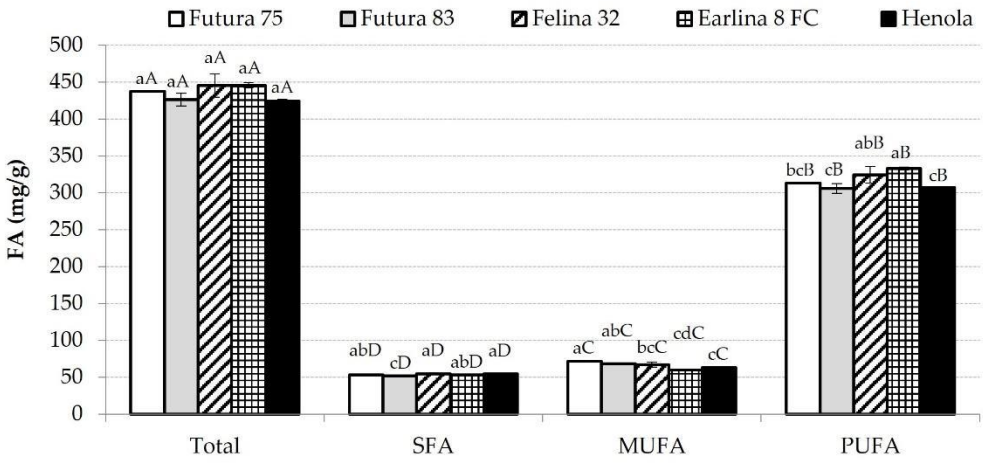

Figure 1. Mean values and standard deviations (error bars) of fatty acids (FA). For each FA group, small letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) by comparing hemp seeds. For each kind of hemp seed, capital letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) by comparing FA groups. Total: total sum of FA; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids.

According to Commission Regulation (EU) No 116/2010 of 9 February 2010 [11] which amends the Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims [12], the hemp seeds studied may be considered as "high polyunsaturated fat" and "high unsaturated fat" due to its high content of PUFAs.

4. Conclusions

Hemp grain is a valuable vegetable source of healthy fatty acids, so food products manufactured including a certain quantity of hemp seed could be considered as "high polyunsaturated fat" according to Commission Regulation (EU) No 116/2010 of 9 February 2010. Looking for possible food uses of the grain derived products rather than of the whole seed, research is being developed in the extraction of hemp seed oil regarding the substitution of other seed or nuts oils in food production. Finally, regarding the complexity of the matrix, further analysis is being conducted to characterize other remarkable food components such as protein and fibre, to better evaluate a potential addition of hemp grain in the formulation of plant-based products.

Author Contributions: Conceptualization, M.I. and J. N-R.; data curation, D. G-M., M.I. and J. N-R.; writing—original draft preparation, D. G-M. and M.I.; writing—review and editing, P.G.-S., J.M.-M., M.I. and J. N-R. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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