Volume 80(4). Published February, 01, 2021
www.jnsciences.org
E-ISSN 2286-5314

Identification of Fatty Acid Composition by GC-MS Analysis of Pearled Barley Flour

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Abstract - The aim of this paper was to investigate the fatty acid composition of pearled barley flour by using the GC-MS analysis and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test. Seven (7) Tunisian barley cultivars were initially pearled at 20% (w/w) of grain weight corresponding to the outer layers; the residues (80%, w/w) which correspond to the internal fractions were milled at little particles size of 0.1mm of diameter, and were assessed for their fatty acid content. Results suggest that twenty-six (26) fatty acids were identified and confirmed by GC-MS analysis were found for all cultivars. All extracted fractions have an important feature of high level of linoleic acid (46.16 % for 'Lemsi' variety), oleic acid (25.45 % for Rihane variety), palmitic acid and α-linolenic acid (omega 3) methyl esters. These results contribute to enhancing the value of pearled barley flour as a good source of unsaturated and essential fatty acids which serve as functional food ingredients and dietary supplements.

Keywords: *Hordeum vulgare*, pearling, fatty acids, GC-MS

1. Introduction

Increased awareness of the health benefits of soluble dietary fiber and other whole grain constituents, gives barley (*Hordeum vulgare* L.) foods a good opportunity of regaining an important place in the human diet. Among the different cereals, barley is studied in particular as a good source of dietary fibers (DF), especially because of its high natural β-glucans content (Blandino et al. 2015; Hajji et al. 2016). In addition, barley is an important source of other bioactive substances including phenolic compounds, natural antioxidants and vitamin E with antiradical and antiproliferative potentials (Gangopadhyay et al. 2015; Shen et al. 2016; Idehen et al. 2017; Marecek et al. 2017).

Besides their DF and phenolic compounds contents, barley is equally a natural source of lipid bioactives. Generally, lipid fractions in cereals are mainly concentrated in the grains germ; and they determined its greatest extent energy value and had a significant impact on its nutritional attributes through the fatty acid content (Gangopadhyay et al. 2015). Fatty acids are family of lipid molecules, including omega-3 and omega-6 that prevent chronic diseases and have positive antioxidant effects (Cozzolino & Degner 2016). Moreover, fatty acids are organic and bioactive compounds which can be efficiently used as functional ingredients in bakery products and natural foods. Therefore, pearling process can be employed as an effective way to remove external bran fractions, containing coarse fibers which are potentially subjected to safety risks of mycotoxins, pesticides and heavy metal contaminations, from underlying fractions with different health properties, and with detrimental effects on the technological quality owing to their functional composition (Sovrani et al. 2012; Blandino et al. 2013, 2015a, b). Thus, covered barley is typically pearled to discard the husk and external layers, and to obtain pot and pearled barley which represent 80 – 85% of the total grain weight (Bordiga et al. 2016). In addition, pearled barley flour can easily be incorporated into cereal based products (bread, cakes, cookies, noodles and extruded snack foods), and also could be used for the development of accepted dietary and functional food products (Baik and Ullrich 2008; Newman and Newman 2008).

Currently, there is no attempt to improve the functional properties of barley lipids or the quality of lipid requirements for barley food use, owing to the relative insignificance of barley as human food in modern times. However, the composition of lipids and specifically fatty acids in barley flour is still unknown. Thus, the main objective of this work was to identify the composition of individual fatty acids via GC-

MS analysis in order to develop natural and functional cereal ingredients for eventual uses in novel food processing and production of new consumer-friendly products. This paper assesses for the first time the fatty acid composition of pearled barley flour.

2. Materials and methods

Plant Materials and Storage. Plant materials used in this study were 7 hulled and six-rowed barley cultivars. Four registered official varieties (Manel, Rihane, Kounouz and Lemsi) were obtained from the Experimental Research Station of the National Institute for Agronomic Research of Tunisia (INRAT), Field Crop Laboratory located at Beja, 100 Km North-West of Tunisia. All cultivars were grown from 4 December 2014 to 25 June 2015. After harvesting, about 1 Kg of barley grains from the 7 cultivars were cleaned and placed in a plastic box with lid (25 × 15 × 10 cm). Humidity was maintained by placing small beakers of silica gel (50 mL) into the containers, as confirmed by humidity meters. The grain boxes were stored at – 20 °C in the dark for evaluation.

Chemicals. The reagents used for fatty acid analysis were: hexane for HPLC, potassium hydroxide (KOH), methanol, dichloromethane and standard solution of 37 components of fatty acid methyl esters (FAMEs). They were purchased from Sigma–Aldrich, Inc (Sigma Chemical, Co, St Louis, MO, USA). All the other chemicals were used for analytical grade and purchased from Sigma Aldrich (Milan, Italy).

Sample Preparation. Barley grains were pearled according to the protocol as previously described in Sovrani et al. (2012) and Blandino et al. (2015). Pearling process consisted on consecutive passages of barley seeds in an abrasive-type grains testing mill (TM-05C model, Satake, Tokyo, Japan) at a constant speed of 55 Hz. Barley grains were initially pearled to remove 20% (w/w) of the original grain weight, and this resulted in a first fraction (0 – 80% w/w) corresponding to the external layers (husk and bran). The residual 80% of the grains (20 – 100% w/w) were collected as pearled kernels used for evaluation.

Grinding. The residual 80% (w/w) of the pearled kernels were milled using a laboratory mill (3100; Perten-Instruments, Finland) with an opening of 1 mm. Then, milled samples were ground to pass through a 0.1 mm screen at little particles size of 0.1 mm of diameter. After grinding, flours were stored at –20°C until evaluation of their fatty acid composition by GC-MS/MS system and total antioxidant activity.

Fatty Acid Identification by GC-MS

Extraction of lipid bioactives. Lipid fractions were extracted by using the classical and standard method of Soxhlet as described in Gangopadhyay et al. (2015) with some minor changes. Exactly 50g of pearled barley flour of each cultivar were reacted with 125 ml of hexane for HPLC solvent. The reaction was carried out for 2h at 25°C/ pression. Then, lipid solutions were recovered and purified by removing hexane traces by vacuum evaporation with rotavapor (buchi type) (at 110°C, and the material (extracts) was collected and lyophilized at – 20°C for evaluation.

GC-MS Analysis. All the samples were analyzed by GC–MS – 2010 Gas Chromatograph – Mass Spectrometry UFLC * R system (Shimadzu – Japan) comprised a Thermo Accela Gas chromatograph coupled to a TSQ Quantum access MAX mass detector monitored by Xcalibur software (Software version: Thermo Xcalibur version 2.2). The extracted lipids (approx 0.1 g) were spiked with 2 ml hexane under stirring, then methylated with 0.2 ml methanolic solution of 2N KOH at 25 °C and kept for 30 seconds for phase separation. Chromatographic separation was performed with a SUPELCOWAX TM10 FUSED SILICA CAPILLARY COLUMN (30mm x 0.25mm x 0.25µm film thickness) at 50 °C, all from Thermo Fisher Scientific Inc. (Supelco, USA). An aliquot (1 µL) was injected into the column and eluted at 250 °C with a flow rate of 1.20 mL/min, and a total flow of 28.2 mL/min. MS/MS detector settings: negative electro-spray ionization mode, spray voltage: 2500 V, vaporizer temperature: 250 °C, sheath gas pressure: 68.3 kPa / min, argon gas pressure: 25 psi, probable temperature: 200 °C. Standard solution was analyzed by GC-MS to reduce the risk of incorrectly identified FAMEs. Total ion chromatograms (TICs) of 37 component FAMEs reference standard are used for individual identification of fatty acids. CYANOPROPYL-POLYSILOXANE COLUMN can provide different retention time of FAMEs.

Statistical Analyses. Extraction and identification of fatty acids by GC-MS analysis were performed as one replicate. TAA analyses were carried out in triplicate; data are reported as the mean of the three replicates. The entire variations coefficients were less than 10. The data were reported as means ± standard error. Statistical analysis was carried out using SAS (V.9.1). Proc ANOVA (Analysis of Variance) with the option of $LSD_{0.05}$ to compare means was used for each trait. Statistical significance was set at $P < 0.05$.

3. Results and discussion

Fatty acids in pearled barley flour were successfully identified and quantified by GC–MS method based on analysis of their molecular structure. The areas of individual fatty acids detected in our extracts are summarized in Figures 1, 2, 3 and 4. Referring to some previous studies (Cozzolino et al. 2013, 2014; Gangopadhyay et al. 2015), twenty-six (26) isolated fatty acids in total were confirmed in the samples investigated. As illustrated in Table 1, all cultivars presented more than eighteen (18) fatty acids. All the extracted fractions showed high contents of linoleic acid, oleic acid and palmitic acid, methyl esters. The α-linolenic acid (omega 3) was also identified in good areas in all samples analyzed, as illustrated by chromatograms in figures 1, 2, 3 and 4 (area, retention time) and Table 1. The most abundant fatty acids shown in our pearled barley flour extracts were linoleic acid, oleic acid and palmitic acid, methyl esters respectively. These results are very interesting and partially consistent with some previous researches (Gangopadhyay et al. 2015) which found that linoleic acid and palmitic acid were the major fatty acids present in oat and barley. Linoleic acid, fatty acid (18:2), was found to be predominant in all fractions followed by oleic acid (18:1) and palmitic acid (16:2). Each fatty acid for each barley cultivar is characterized by a special retention time and area, as shown by the chromatograms in figures 1, 2, 3 and 4. Each fatty acid was present in the pearled barley flour extracts in a large amount. Total area of linoleic acid, methyl ester ranged from 42.51 % for 'Ardhaoui Tataouine' cultivar to 46.16 % for 'Lemsi' variety at the retention times of 16.388 min and 16.213 min respectively. Concerning the oleic acid, concentrations were very important and varied between 21.86 % for Manel variety and 25.45 % for Rihane variety at a retention time of 15.388 min and 15.428 min respectively, which indicated that oleic acid is the second important fatty acids in pearled barley flour lipid extracts. Palmitic acid also shows great percentages in all cultivars; its areas varied from 18.54 % for 'Ardhaoui Tataouine' cultivar to 20.18 % for Manel variety at the retention times of 12.163 min (chromatogram figure 1) and 12.128 min (chromatogram figure 4). These results are in part in agreement with the work of Gangopadhyay et al. (2015) and Cozzolino et al. (2014) who reported that linoleic (18:2) and linolenic (18:3) fatty acids were found to be predominant in most barley and oat fractions followed by palmitic acid (16: 2). Alpha-linolenic acid (omega 3) was also identified with an interesting area, greater than 5.40% at a retention time of an average of 17.277 min for all cultivars, corresponding to the 4th peak identified for all extracts. Other fatty acids such as stearic acid methyl ester and 11-eicosenoic acid (or gondoic acid) were equally shown in all extracted fractions obtained from all cultivars (Table 1), but with lower areas (> 1.53%).

Table I: Main Fatty Acids Identified by GC-MS Analysis (Area %) in Pearled Barley Flour

Fatty Acids	Cultivars						
	Manel	**Rihane**	**Konouz**	**Lemsi**	**Ardhaoui Kerkna**	**Ardhaoui Djerba**	**Ardhaoui Tataouine**
Caproïc acid (hexanoïc)	0.00	0.01	0.00	0.01	0.00	0.00	-
Caprylic acid (octanoïc)	0.01	0.02	0.01	0.01	0.01	0.01	0.01
Capric acid (decanoïc)	-	0.01	0.01	-	-	0.00	0.00
Lauric acid (dodecanoïc)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tridecylic acid (tridecanoïc)	-	0.00	-	-	-	-	-
Myristic acid (tetradecanoïc)	0.33	0.34	0.39	0.33	0.28	0.33	0.34
Pentadecanoïc acid	0.14	0.17	0.14	0.12	0.11	0.13	0.13
Cis-10-pentadecenoïc acid	-	-	-	-	-	-	0.10
Acid palmitic (hexadecanoïc)	20.18	18.75	19.27	18.76	19.95	19.13	18.54
Palmitoleic acid (cis-*9*-hexadecenoïc)	0.30	0.33	0.30	0.16	0.32	0.33	0.37
Heptadecanoïc acid (margaric)	0.16	0.15	0.20	0.13	0.11	0.15	0.15
Cis-10-heptadecenoïc acid	0.07	0.09	-	0.08	0.06	0.09	0.09
Stearic acid methyl ester	3.14	2.69	3.87	1.72	1.66	2.19	2.05
Oleic acid methyl ester	21.86	25.45	22.01	23.96	23.89	23.63	23.96
Linoleic acid methyl ester	44.03	42.95	42.67	46.16	43.17	43.09	42.51
Linolenic acid methyl ester	-	-	-	0.20	-	-	-
α-linolenic acid (omega 3)	6.35	5.40	6.74	6.05	6.93	6.68	7.10
Arachidic acid (icosanoïc)	0.67	0.57	0.94	0.28	0.45	0.62	0.65
Gondoïc acid (11-eicosenoïc)	1.53	1.91	1.69	1.70	2.10	2.24	2.22
11,13-eicosadienoïc acid	-	-	0.21	-	-	-	-
11,14-eicosadienoïc acid	0.19	0.20	-	0.14	-	0.24	0.26
Heneicosanoic acid methyl ester	-	-	0.05	-	-	-	0.04
Docosanoïc acid	0.42	0.28	0.71	-	0.58	0.59	0.73
13-docosanoïc acid -Z-methyl ester	0.13	0.16	0.12	0.16	0.23	0.23	0.22
Lignoceric acid (Tetracosanoïc)	0.26	0.24	0.31	-	0.14	0.27	0.25
15-nervonic acid (cis-15-tétracosénoïque)	0.22	0.25	0.26	-	-	-	0.26

- No defined peak

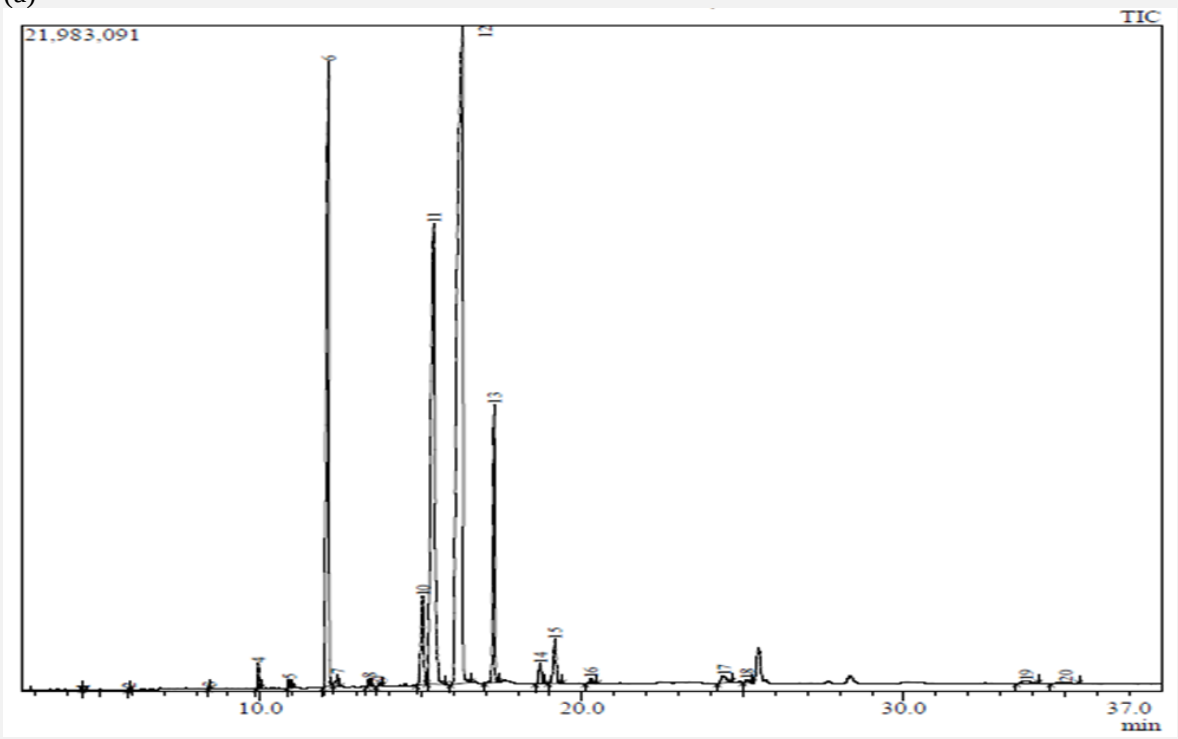
As illustrated in Fig. 1, twenty (20) fatty acids were well detected and identified by GC-MS/MS analysis for the lipid extract obtained from 'Manel' variety. The best yield was recorded for the linoleic fatty acid with an area of 43.03% at a retention time of 16,293 min (peak No. 12), followed by oleic acid with an area of 21.86% at a retention time of 15.388 min (peak 11), followed by the palmitic acid with an area of 20.18% and a retention time of 12.128 min (peak 6), corresponding to the three major fatty acids for this official variety.

Another significant peak (No. 13) was also recorded at a retention time of 17,273 min corresponding to the α-linolenic fatty acid (ALFA), which is an omega-3 polyunsaturated fatty acid corresponding to the all-cis-Δ9 acid, 12,15-octadecatrienoic acid (18: 3). Its crude formula is $C_{18}H_{30}O_2$ and its molar mass is 278.43 g/mol. It is a carboxylic acid with a chain of 18 carbon atoms and three cis double bonds; the first of the double bonds is positioned on the third carbon atom counted from the end of the chain, denoted ω. It is the main omega-3 fatty acid. A-linolenic acid is an essential fatty acid because it is part of the essential foods that are not synthesized by mammals. It is also the main fatty acid that composes the thylakoid membranes of green leaves of plants. So, green plants and animals eating them are sources of this fatty acid. Some seeds and systematically derived oils are rich in ALFA, especially seeds of flax, camelina, rapeseed, hemp, soybeans and nuts. However, these oils and seeds equally contain omega-6 that competes with omega-3 at the cellular level, while their physiological effects are compromised. Studies have suggested that increased use of ALFA reduces the risk of cardiovascular and chronic diseases (Cozzolino & Degner 2016).

Similarly, lipid extracts obtained from the official varieties "Rihane and Kounouz" and also from 'Ardhaoui Tataouine' cultivar showed twenty-two (22) fatty acids that are well demonstrated by GC-MS/MS analysis, as illustrated in chromatograms of Figures 1, 2 and 4. Twenty (20) fatty acids were also detected and identified by GC-MS/MS analysis for the lipid extract obtained from 'Ardhaoui Djerba' cultivar, as well shown in Fig.3. Eighteen (18) fatty acids were detected and identified by GC-MS/MS analysis in ‘Lemsi’ forage variety and ‘Ardhaoui Kerkna’ cultivar.

Consequently, 26 fatty acids are identified in the extracted fractions obtained from the 7 cultivars studied. Fourteen (14) fatty acids were detected in all cultivars and twelve (12) other fatty acids were present only in some extracts according to the cultivar genotype (Table 1). Linoleic acid was the major fatty acid in all cultivars (42.51% – 46.16%). It is a polyunsaturated fatty acid corresponding to the unique essential fatty acid of the omega 6 family, involved in the manufacture of the cell membrane. Due to this fatty acid, the body can produce all other lipids of the omega 6 family. Linoleic acid is present in almost all vegetable oils, especially linseed oil (80%). Other cereal oils (corn, sunflower and soya) and plants (evening primrose, borage and grape seed) also contain this omega 6 fatty acid (Gangopadhyay et al., 2015; Cozzolino and Degner 2016). Inside our skin, linoleic acid enters in the composition of ceramides, which in turn, are part of the lipid cement (true protective barrier of the epidermis). A lack of omega 6 causes an intense dryness of the skin, a less dazzling complexion, brittle and dull hair, etc (Zarrouk et al. 2015; Cozzolino et al. 2014).

(a)



(b)

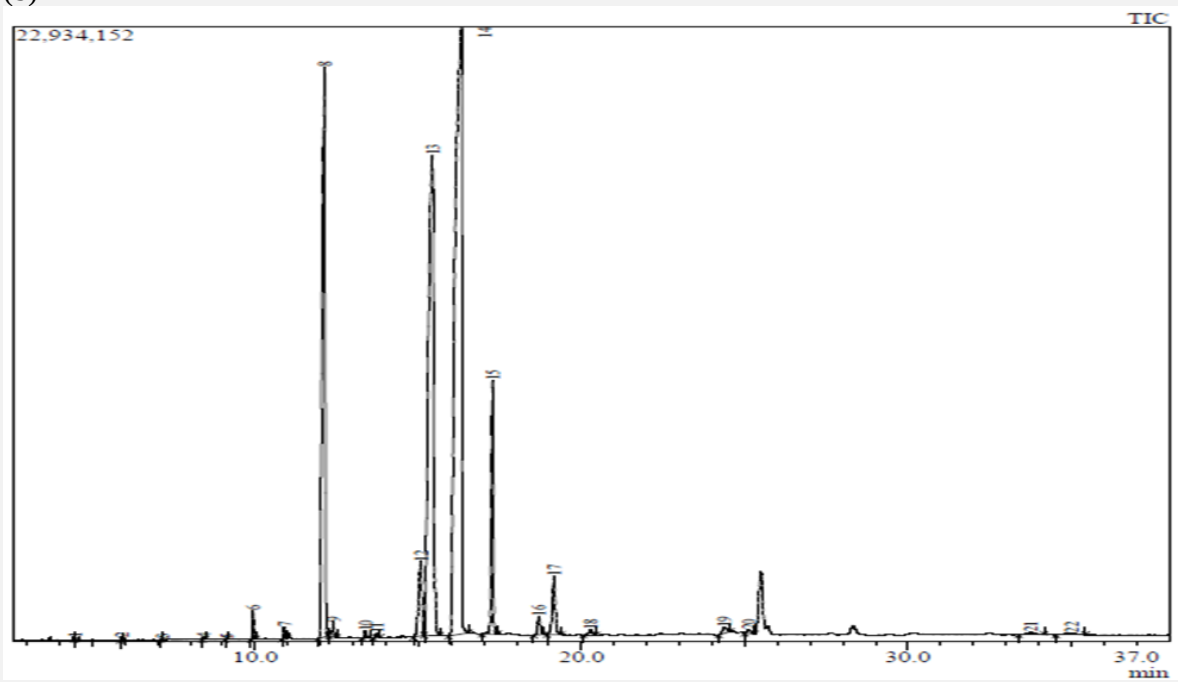
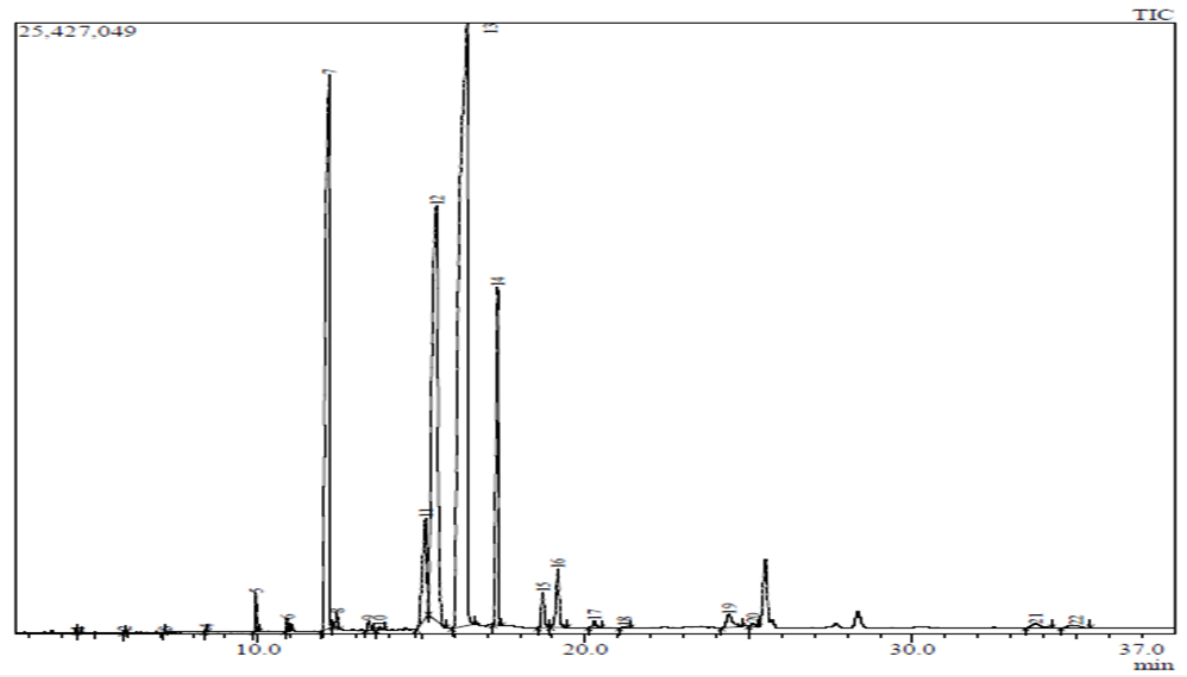


Figure 1. Chromatograms of fatty acids identified by GC-MS analysis in pearled barley flour for north varieties (North-West of Tunisia): a. Manel variety; b. Rihane variety

(c)



(d)

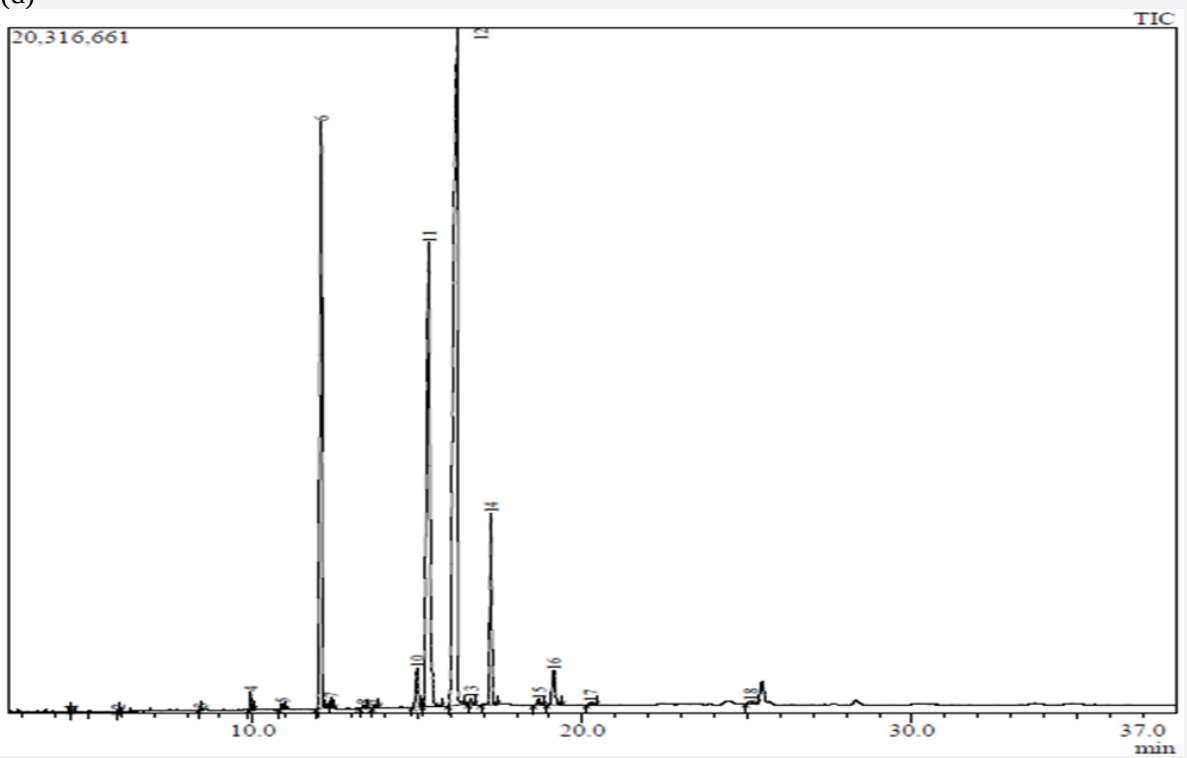
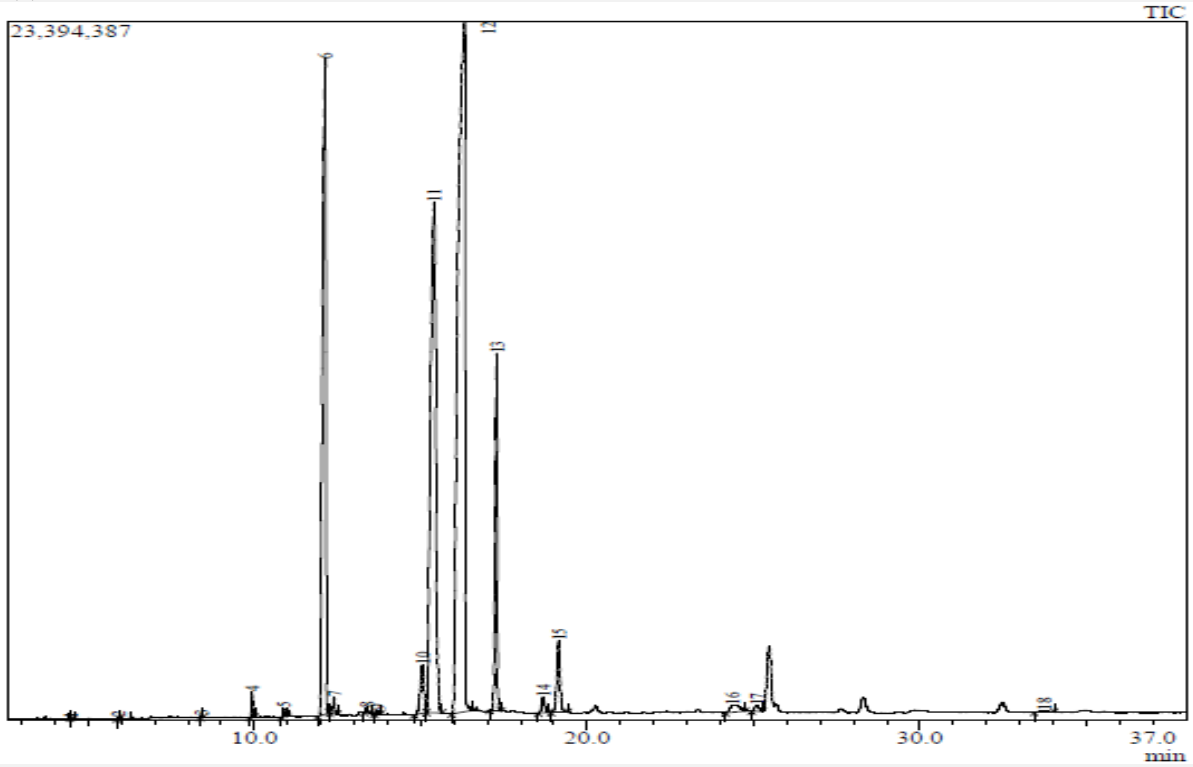


Figure 2. Chromatograms of fatty acids identified by GC-MS analysis from pearled barley flour for north varieties (North-West of Tunisia): c. Konouz variety; d. Lemsi 'forage variety'

(e)



(d)

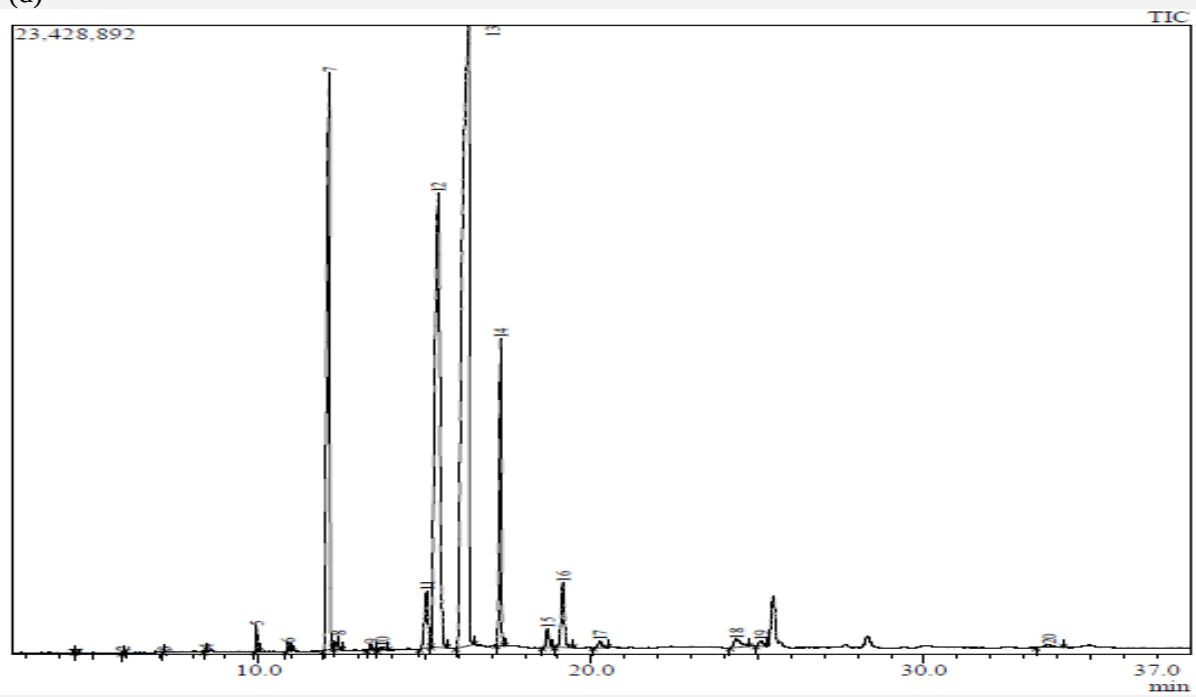


Figure 3. Chromatograms of fatty acids identified by GC-MS analysis from pearled barley flour for south cultivars (Islands of South East of Tunisia): e. Ardhaoui Kerkna; f. Ardhaoui Djerba

(g)

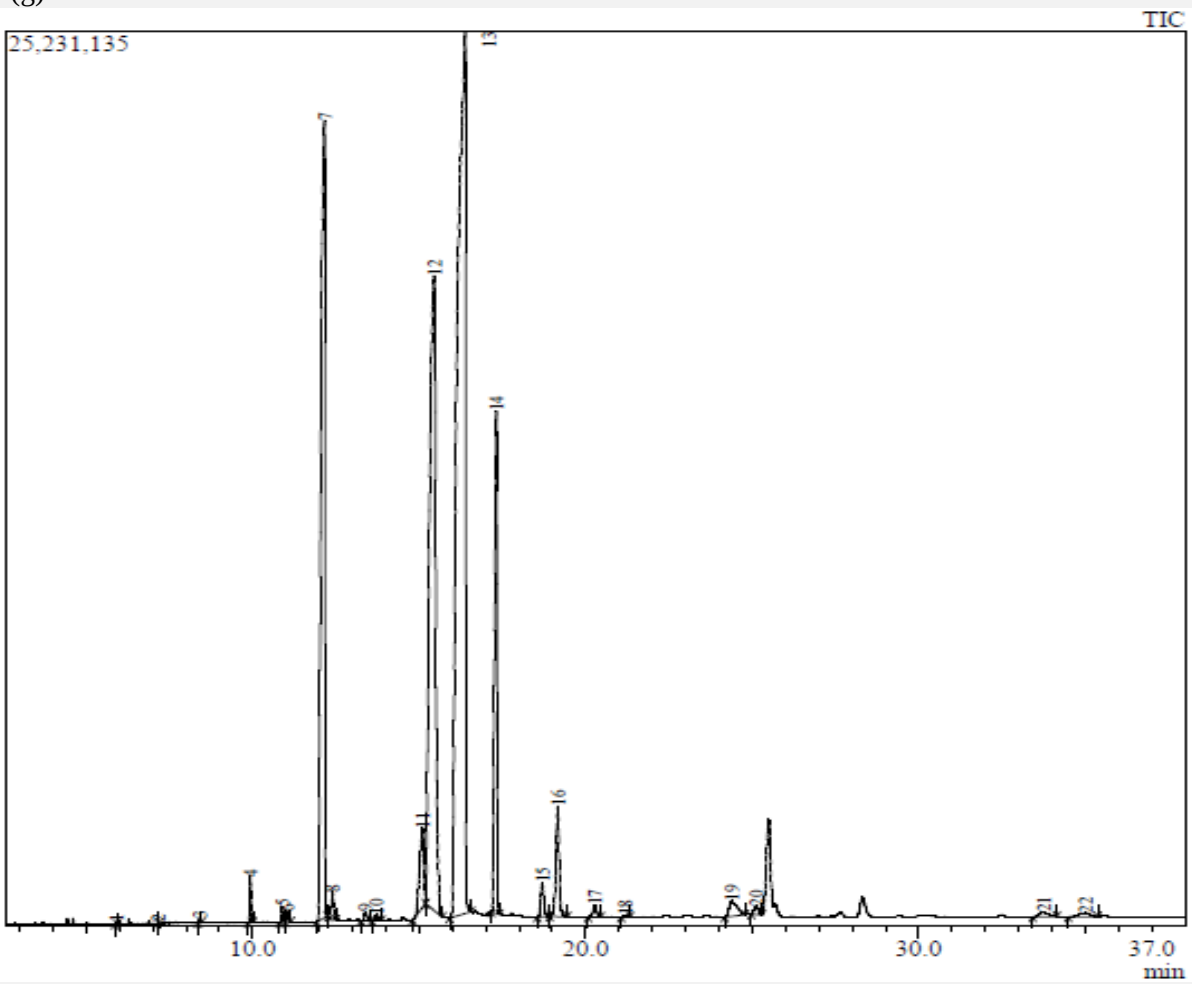


Figure 4. Chromatogram of fatty acids identified by GC-MS analysis from pearled barley flour for South West cultivar (Desert area): g. Ardhaoui Tataouine

Oleic acid was equally identified for all cultivars with great areas (21.86% – 25.45%). It is the most abundant long chain mono-unsaturated fatty acids in our body, also known as omega-9. It is a monounsaturated fatty acid that is found in many foods. Olive oil contains from 55% to 80%. Oleic acid proves to be an excellent skin cell regenerator, highly beneficial to delay the onset of wrinkles. It is very present in the human body, and protects the cardiovascular system and reduces cholesterol. In cosmetics, oleic acid is a very commonly used ingredient, known for its nourishing properties. It participates in the strengthening of hydrolipidic film which helps retention of elasticity and suppleness for the skin. It has equally remedial and healing properties. The saturated form of this acid is stearic acid (Cozzolino and Degner 2016).

Palmitic acid, also known as hexadecanoic acid or cetyl acid, is one of the most common saturated fatty acids in animals and plants, and was identified in all extracts with important areas (18.54% – 20.18%). It is a monounsaturated fatty acid which is a common constituent of glycerides in adipose tissues, which are part of the composition of lipids. Palmitic acid is a saturated fatty acid, of animal origin or present in certain vegetable oils such as vegetable oil of palm or coconut. It is an important component of the skin barrier and the acidic layer of the epidermis; it has emollient, emulsifying and cleansing powers. It also comes in the composition of certain perfumes (Cozzolino et al. 2014, 2015).

The ALFA was demonstrated in all extracts obtained with an area varying from 5.40% for 'Rihane' variety to 7.10% for 'Ardhaoui Tataouine' cultivar. Alpha-linoleic acid is the unique acid of omega 3 families known as "essential" since it can't be synthesized by the human body. In cosmetics industry, alpha-linoleic acid is known for its moisturizing power that gives the skin suppleness. It is anti-inflammatory and soothes redness and skin irritations. In addition, stearic acid of methyl acid was identified in all extracts with a variable area among 1.66% for 'Ardhaoui Kerkna' cultivar and 3.87% for 'Kounouz' variety. Naturally present in butter and vegetable oils, stearic acid is a saturated fatty acid very popular in cosmetic compositions. It makes it possible to enrich the emulsions to give them a more creamed consistency, to stabilize the formulations or to harden certain cosmetic balms and butters. Its

emollient properties make it possible to hydrate the epidermis or the hair while protecting them due to its film-forming power (Cozzolino and Degner 2016).

As the same thing, 11-eiconenoic acid (or gondoic acid) was present in all extracted fractions and identified with minor but interesting areas ranging from 1.53% for 'Manel' variety to 2.24% for 'Ardhaoui Djerba' cultivar. 11-eiconenoic acid, also known as gadoleic acid or erucastic acid is an omega-9 unsaturated fatty acid. It is found in little quantities in triglycerides of various vegetable edible oils, but in a significantly higher proportion in camelina oil (Camelina sativa), where it accounts for 15 to 20% of fatty acids. In addition, arachidic acid (icosanoic acid) was detected with small variable areas of 0.28% for the extract obtained from the 'Lemsi' forage variety to 0.94% for 'Konouz' variety.

The icosanoic acid or arachidic acid is a saturated C_2 fatty acid of the semi-developed formula $CH_3(CH_2)_{18}COOH$. It is found in peanut oil and other vegetable oils, and in fish oils. Myristic acid was homogeneously identified in all extracts with small areas among 0.28% for 'Ardhaoui Kerkna' cultivar and 0.39% for 'Kounouz' variety (Table 1). It is a saturated fatty acid naturally present in dairy products, and is also used in the composition of coconut oil and palm oil. It is used in cosmetics for its cleansing, smoothing and protective powers. Palmitoleic acid (cis-9-hexadecenoic acid) was identified for all extracts with smaller areas (0.16% – 0.37%). It is a monounsaturated fatty acid which is a common constituent of the glycerides of human adipose tissues, having a very high penetrating power in the skin, especially present in macadamia nut oil. Close to human sebum, it hydrates and fortifies the epidermis without greasing. Palmitoleic acid reduced insulin resistance and decreased blood glucose levels, also reduced the production of fats and its accumulation, normalized abnormal lipid profiles with increasing high-density lipoprotein (HDL) cholesterol, positive action against obesity and a powerful suppression of inflammation that leads to the metabolic syndrome. The 13-fatty acid docosanoic acid was equally identified in all extracts at lower areas (0.12% – 0.23%). It is involved in the treatment of Alzheimer's disease (Zarrouk et al. 2012). Heptadecanoic acid or margaric acid which is a saturated fatty acid of the semi-developed formula $CH_3(CH_2)_{18}COOH$, has equally been identified in all extracts with smaller areas among 0.11% for 'Ardhaoui Kerkna' cultivar and 0.20% for 'Kounouz' variety. It is a mixture of palmitic acid and stearic acid. Pentadecanoic acid was equally detected for all extracts with minor areas (0.11% - 0.17%). It is a saturated fatty acid of the formula $CH_3(CH_2)_{18}COOH$. It is rare in nature, representing 1.2% of the cow's milk fat. This fat is the first food source of pentadecanoic acid. It is also used as a marker of fat consumption. Pentadecanoic acid was also present in hydrogenated sheep meat fat. This acid may increase the risk of transmission of the human immunodeficiency virus (HIV) from mother to child through breastfeeding (Zarrouk et al. 2012, 2015). Caprylic acid (or octanoic acid) was identified in all extracts with the same area of 0.01%, excepting the extract of barley 'Rihane' which showed an area of 0.02%. Caprylic acid is known for its antifungal properties. It is a saturated fatty acid naturally present in dairy products, and also in breast milk; Caprylic acid is additionally included in the composition of coconut oil and palm oil. In cosmetics, it is used for its emollient, moisturizing virtues, as well as for its recognized antifungal properties (Cozzolino and Degner 2016). N-dodecanoic acid, more commonly known as lauric acid, was identified in our extracts with the same area of 0.02% for all cultivars. It is a saturated fatty acid with 12 carbon atoms, of the semi-developed formula: CH_3-$(CH_2)_{10}$-COOH. It is a saturated fatty acid predominantly present in coconut oil, used in cosmetics for its cleansing properties, emulsifying and surface-active (reduced surface tension and promotes uniform distribution of the product during its use). The lauric acid additionally has an antimicrobial action and makes it possible to harden the balsams, soaps and body butters. Some fatty acids have been identified only for certain extracts but not for others. For example, cis-10-heptadecenoic acid was detected for official varieties' Manel and Lemsi 'and for 'Ardhaoui Kerkna cultivar' with a 0.06% area, and to the 'Rihane' variety and the two local populations 'Ardhaoui Djerba and Tataouine' with an area of 0.09%. On the other hand, it was absent in 'Kounouz' variety. Similarly, cis-10-pentadécenoïque acid was detected only for 'Ardhaoui Tataouine' cultivar. Also, the 11-13-acid eicosadienoic fatty acid was identified solely for 'Kounouz variety' with an area of 0.21%. In the same way, linolenic acid was detected only for the forage variety 'Lemsi' with an area of 0.20%. Therefore, we find that Tunisian barley is very rich in fatty acids including essential fatty acids. This research work is being studied for the first time, and suggested that pearled barley flour is considered to be a good source of several fatty acids, including unsaturated and essential fatty acids which could be used for new food processing and therapeutics.

Unsaponifiable lipid fractions of Plant-based diet were equally a possible source of phytosterols. These are natural components incorporated into plant cell membranes. High levels of phytosterols been detected in pearls (outer grains) of barley even they are known to be moderate in cereals. (Lampi et al. 2004). More recently, a special milling process (Fitzpatrick Comminuting mill) has been used to produce

germ-rich fractions rich in barley phytosterols (Moreau and Hicks 2013). The potential use of beading / grinding as a method for generating fractions with high levels of phytosterols has been proposed in some studies. Another study (Piironen et al. 2002), has compared the level of sterols in different cereals, including oats and barley. Furthermore, phytosterols should be widely studied owing to their ability to control serum cholesterol levels and thus protect against cardiovascular disease. While lowering low density lipoprotein (LDL) cholesterol levels, levels of "good" HDL cholesterol and triacyglycerol remain unchanged (Lagarda et al. 2006; Madhujith & Shahidi 2007). Beneficial health effects of plant sterols have led to the development of functional foods enriched of sterols. Thus, possibility of using sterols as adjuvant therapy for anti-cholesterol pharmaceuticals has additionally been proposed (AbuMweis et al. 2014; Idehen et al. 2017).

4. Conclusion

Our findings showed that pearled barley flour could be interesting concentrated source of unsaturated and essential fatty acids, which can be successfully incorporated into food formulations, and therefore might be used as natural ingredients in foods and therapeutics. Thus, it should be necessary to explore the possibility of increasing consumption of pearled barley fractions and derived end products as a good source of fatty acids and natural antioxidants for human foods. However, incorporation of these materials would enhance their nutritional, physiological and technological properties.

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