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Research Article

Nutritional Quality Perceptions through Fatty Acid Profiling, Health Lipid Indices and Antioxidant Potentialities

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Abstract

Knowing about the fatty acids composition, an important part of the grain lipid fraction, can be important information for both chemotaxonomic and nutritional parameters. Since to date, knowledge of the nutritional and bioactive lipophilic compounds profile of some cereals, and alternative gluten-free grains is limited, the objective of this study was to explore the lipid fraction composition, the antioxidant potentialities and the nutritional quality of the oil derived from wheat and barley as staple foods, millet as gluten-free minor cereals and quinoa as gluten-free pseudocereals. Eleven different FAs were identified in examined samples with a predominant presence of linoleic acid. Considering the content of UFA, the health-related lipid indices, lowest IA and IT and a balanced ω-6/ω-3 ratio as parameters for selection, quinoa and millet would be the species of choice owing to their nutritional adequacy and the great quality of the derived seed oils. Compared to the other cereals, the oils extracted from the grains of quinoa showed the strongest antioxidant capacity, followed by wheat, millet. PCA plot clearly discriminated the four types of grains and clustered from millet and quinoa at the left side of the plot; these grains were specifically characterized by high total oil contents, a high antioxidant activity, high UFA/SFA ratios and high contents of C20:1, C16:1 and C20:0. All this has contributed to the increase of interest and popularization of its consumption among people who seek alternative foods with high nutritional value, especially in developed countries, thus stimulating their agricultural production.

Keywords: Cereals; Pseudo-cereals; Health lipid indices; Nutritional value

Introduction

In African populations, specifically in the Maghreb area (The Northern Region of Africa as Morocco, Algeria, Tunisia, Libya and Egypt) very high incidences of Celiac disease have recently been reported [1,2]. These high frequencies are not surprising since wheat and barley remain the major staple foods in the Maghreb countries [2]. On the other hand, many gluten free products available on the market have low nutritional quality because they are often made from refined starches, which could increase the risk of nutritional deficiencies [3]. Thus, formulating healthy, nutrient rich, gluten free products poses a challenge since the functionality of gluten is hard to duplicate. Thus, searches for alternative gluten free grains with high nutritional value are required.

The cereals play an important role in the human diet, being wheat, corn, rice, barley, oats, rye and sorghum the most important worldwide. Cereals accumulate lipid fractions mostly in their grains as reserve matter known as plant oils [4]. Cereal oils, also, contain essential nutrients for human life, such as clinically important saturated and unsaturated fatty acids [5]. Besides, apart from their application in the food industry, plant seed oils from grains can be used as substitutes for oil and gasoline fuels [6]. Although cereal grains are not recognized as one of the primary unsaturated fatty acids producers, they can be used as additional source of different ω-6 and/or ω-3 FAs. In fact, the new recommendations are trying to insist on balanced intake of ω-6 and ω-3 fatty acids in order to prevent further spread of obesity, especially among the children and adolescents [7]. Although cereals are the most important crops grown, pseudocereals have been recognized as a notable seed, potentially used for human nutrition, whose cultivation developed in various regions of the world [8]. Recently, the pseudocereals have attracted attention because of the proteins with high nutritive value, and their storage proteins are not toxic for celiac patients. Grains, such as quinoa, present themselves as an excellent source of energy and protein with high nutritional value, besides fibers, lipids, vitamins and minerals.

Representing the third main food constituent, lipids are renowned for their large importance to human health [9]. In fact, it has long been considered that lipids are the main source of energy for metabolic processes and closer insight has revealed the versatility of their influence and effect on the health and state of the organism [10]. The lack of information about chemical substances, especially fatty acids, found in cereals and pseudocereals grains and their flour derived, allied to the possibility of obtaining new food sources for unsaturated fatty acids, has stimulated interest in analyzing the lipid composition of fruits from these species.

Thus, in this work, samples of cereals and pseudocereals commercialized in Tunisia have been characterized for their nutritional value, with a particular focus on their lipid fraction, such as fatty acids, and their antioxidant potentialities in order to increase

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the awareness of their nutritional profile. Moreover, data coming from this study may be included in food nutrient databases.

Material and Methods

Grain material

Grains from organic quinoa (*Chenopodium quinoa*), pearl millet (*Pennisetum glaucum*), common wheat (*Triticum aestivum*) and barley (*Hordeum vulgare L*) commercially available were bought in specialized shops.

Analytical methods

Sample preparation and determination of total lipid content: The collected samples were cleaned from damaged and weed grains, milled to a fine powder, and were packed in vacuum bags and stored at dark and cold place until further analysis. Extraction of total lipids from the whole milled grains was performed using a mixture of n-hexane and isopropanol (60:40, v/v) [11]. The obtained extracts were merged and the solvent was evaporated in an inert atmosphere, at 50°C. The dry residue was measured and the total lipid content was determined as g/100 g of dry matter. Next, samples were kept in a desiccator for further analysis.

Fatty acid analysis: Fatty acid composition of the citrus pulp oils was assessed as recommended by the American Oil Chemists' Society using a Gas Chromatography (GC) system [12]. The separation of the fatty acid methyl esters was done using a Hewlett-Packard 6890 II gas chromatography system equipped with a flame ionization detector and a capillary column (Teknokroma TR-CN100, 60 m × 0.25 mm, film thickness: 0.20 µm) with a stationary phase made of polyethylene glycol. The temperature conditions were as follow: the temperature of oven is 150°C for 1 min, increase from 150°C to 200°C (15°C/min), and then from 200°C to 225°C (2°C/min) and was maintained for 2 min. A flow rate of nitrogen was 1.6 mL/min, injection temperature was 250°C and detector temperature was 275°C. A standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to determine sample peaks. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times [13].

Oil quality

Indexes of lipid nutritional quality: The nutritional quality of the lipid fraction of samples of citrus fruit pulps was determined by examining the fatty acid profile and taking into consideration three Indexes: Atherogenicity (AI), Thrombogenicity (IT) and the ratio between Hypocholesterolemic and Hypercholesterolemic fatty acids (HH). The following calculations were employed:

$$(IA=[(C12:0+4 \times C14:0+C16:0)]/ (\Sigma PUFA+C18:1+\Sigma MUFA)$$

$$(IT) = [(C14:0+C16:0+C18:0)]/ (0.5*MUFA+0.5*n-6+3*n-3+n-3/n-6)$$

$$(HH)=[(C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3)]/ (C14:0+C16:0)$$

The Cox value was calculated based on the percentage of the unsaturated C18 fatty acids following the formula

$$Cox=[1 \times (18:1\%)+10.3 \times (18:2\%)+21.6 \times (18:3\%)]/100$$

DPPH Radical Scavenging assay: The antioxidant properties were assessed by the 2,2-Diphenyl-1-PicrylHydrazyl (DPPH) radical scavenging assay [14]. An aliquot of 2 mL of the oils, at different concentrations (0.1-2000 µg/mL), in methanol was added to 500 µL of a methanolic solution from DPPH at 0.2 mM. The solution was incubated for 30 min, at room temperature, in the dark and the absorbances at 517 nm were recorded. BHA (Butylated hydroxyanisole) was used as positive control. All samples were analyzed in triplicate. The percentage of inhibition was determined using the following equation: DPPH scavenging activity (%)=[(Ac-At)/Ac] × 100; Ac: Absorbance of control; At: absorbance of the test made.

Statistical analysis

All analyses were performed in triplicate and the data were reported as mean ± Standard Deviation (SD). The means were compared using the One-Way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests. All analyses were performed using the "SPSS v.21" software. The differences were considered significant at $p < 0.05$. For the multivariate statistical analyses, the Principal Component Analysis (PCA) was applied to examine the inter-relationships between the investigated species and to explore the characters significantly contributing to the variation. To check for possible correlations between the different data sets, XLSTAT-Pro 7.5.3 software was used. The correlation coefficient (r) was based on Pearson's correlation and p value was used to show correlations and their significance. A probability value of $p < 0.05$ was adopted as the criteria for significant differences.

Results and Discussion

Fatty acid profiling

The total oil content of the sample analyzed in the present study ranged from 2.5 to 8% with quinoa grains yielding the greatest percentage of oil (Table1). According to literature, there are complex factor combinations that may affect grain lipid content-genetics, amount of intercepted solar radiation, species/variety, climatic and growing conditions, etc [15]. According to the GC analysis of the FA content, eleven different fatty acids were observed in the grain samples. In general, the three main FAs determined in grain samples were linoleic (C18:2n-6), oleic (C18:1n-9) and palmitic (C16:0) acid but with different distributions (Table 1). The number of carbon atoms ranged from C16 to C22. The highest number FA compounds were detected in the quinoa grains (11 FAs); while the lowest number of compounds (9 FAs) were observed in barley and millet grains. The highest total lipid amount was observed in quinoa grains (99.59%), followed by wheat (99.09%), barley (98.08%) and millet (97.87%). All of the FAs were unsaturated (>70%) (Table 1).

The highest Saturated Fatty Acids (SFA) proportion was detected in barley grains (23.97%) while millet grains exhibited the lowest proportion (12.77%) (Figure 1). Among SFAs, palmitic acid (C16:0) was predominant in all samples. Monounsaturated Fatty Acids (MUFA) ranged from 15.25% in barley to 33.86% in quinoa grains. Among MUFA, oleic acid (C18:1) was predominant in the four seed samples (Table 1 and Figure 1). Erucic acid (C22:1) was exclusively detected in quinoa grains, while margaric acid (C17:0) was specific to quinoa and wheat grains. The highest content in term of Polyunsaturated Fatty Acids (PUFA) was detected in millet grains (61.53%), while the lowest content was detected in wheat and quinoa grains, averaging 51%. Linoleic acid (C18:2, n-6) was the main PUFA detected in these grains (Table 1). At species levels, palmitic acid (C16:0), was identified as the major FAs for all the studied grains. Several studies have also demonstrated the abundance of palmitic and oleic acids in cereal (maize, Rye) [16] and pseudo-cereal (buckwheat, lingrains) grains species [15-17].

Table 1: Oil content, fatty acid composition and nutritional quality parameters of the grains oils from the four species analyzed.

	Common Wheat	Barley	Millet	Quinoa	f. value	p. value	-log10(p)	FDR
Fatty-acids composition (%)								
Palmitic acid (C16:0)	10.37	18.254	6.775	7.45	3.80E+31	5.99E-125	124.22	1.48E-124
Margaric acid (C17:0)	1.346	0	0	1.296	3.41E+31	9.19E-125	124.04	2.13E-124
Stearic acid (C18:0)	3.196	2.516	2.605	1.826	2.87E+31	1.83E-124	123.74	3.97E-124
Arachidic acid (C20:0)	1.806	1.476	1.805	1.756	2.77E+31	2.12E-124	123.67	4.36E-124
Behenic acid (C22:0)	1.436	1.726	1.585	1.946	2.51E+31	3.16E-124	123.5	6.15E-124
Palmitoleic acid (C16:1)	1.496	1.306	1.435	1.506	2.36E+31	4.03E-124	123.39	7.45E-124
Oleic acid (C18:1)	26.5	12.5	20.54	26.89	2.27E+31	4.72E-124	123.33	8.31E-124
Gondoic acid (C20:1)	1.736	1.446	1.595	2.806	1.95E+31	8.68E-124	123.06	1.46E-123
Erucic acid (C22:1)	0	0	0	2.666	1.90E+31	9.51E-124	123.02	1.53E-123
Linoleic acid (C18:2 n-6)	48.356	53.258	59.24	42.23	1.61E+31	1.85E-123	122.73	2.85E-123
alpha-linolenic acid (C18:3 n-3)	2.856	5.606	2.295	9.226	1.31E+31	4.25E-123	122.37	6.28E-123
Nutritional quality parameters								
SFA	18.154	23.972	12.77	14.274	3.94E+32	5.17E-129	128.29	1.91E-127
PUFA	51.212	58.864	61.535	51.456	2.93E+32	1.68E-128	127.77	3.11E-127
MUFA	29.732	15.252	23.57	33.868	2.16E+32	5.70E-128	127.24	7.03E-127
UFA	80.944	74.116	85.105	85.324	1.83E+32	1.10E-127	126.96	1.02E-126
PUFA/SFA	2.821	2.456	4.819	3.605	1.63E+32	1.77E-127	126.75	1.31E-126
SFA/UFA	0.224	0.323	0.15	0.167	1.27E+32	4.84E-127	126.31	2.99E-126
UFA/SFA	4.459	3.092	6.664	5.978	6.62E+31	6.48E-126	125.19	3.43E-125
ω6	48.356	53.258	59.24	42.23	5.90E+31	1.02E-125	124.99	4.43E-125
ω3	2.856	5.606	2.295	9.226	5.83E+31	1.08E-125	124.97	4.43E-125
ω-6/ω-3	16.931	9.5	25.813	4.577	4.98E+31	2.02E-125	124.7	6.78E-125
IA	0.113	0.217	0.082	0.082	4.63E+31	2.71E-125	124.57	8.05E-125
IT	0.322	0.432	0.232	0.172	4.52E+31	2.97E-125	124.53	8.05E-125
HH	6.376	3.734	9.445	8.569	4.50E+31	3.04E-125	124.52	8.05E-125
COX	5.863	6.821	6.803	6.611	1.28E+31	4.57E-123	122.34	6.51E-123

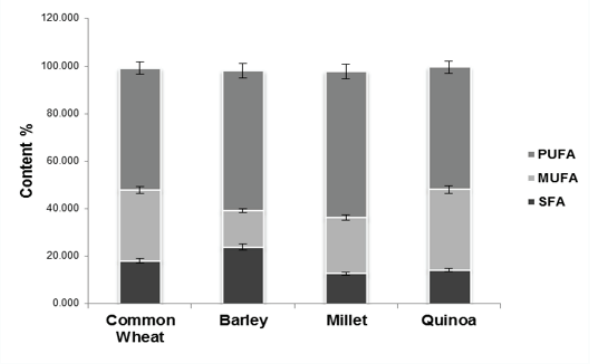

Figure 1: Percentages of the main representative classes of the lipid fraction isolated from the grains oils of the four selected species.

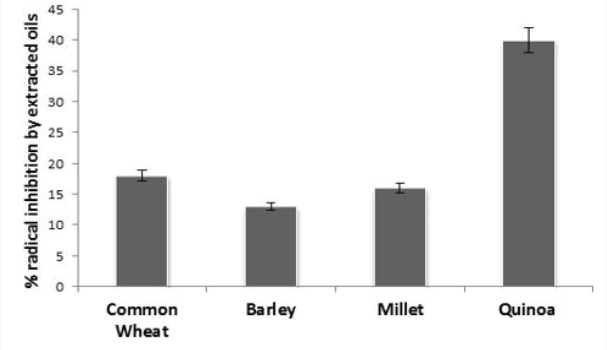

Figure 2: Antioxidant activities of the different citrus fruit essential oils by the DPPH.

Nutritional quality parameters

From the data generated for the fatty acid profiling, some conclusions could be drawn about the nutritional quality and characteristics of the grains flour from these species. Accordingly, four parameters are required to be calculated such as the ratio between the ω-6/ω-3 ratio [18], the PUFA/SFA ratio, and the IA and the IT values [19]. Because a high ω-6/ω-3 ratio is associated with overweight/obesity, where as a balanced ratio decreases obesity and weight gain, it is essential that every effort is made to decrease the ω-6 fatty acids in the diet, while increasing the omega-3 fatty acid intake. The recommended ratio of ω-6 to ω-3 fatty acids is estimated to be from 3 to 5 [20]. Accordingly, if we include recommendation for ω-6/ω-3 ratio as a selection parameter it is obvious that quinoa grains are a potential source of balanced intake of ω-6 and ω-3 FAs.

Based on recommended UFA and SFA daily intakes (WHO/FAO, 2003) the suggested and desirable UFA/SFA ratio should be equal or higher than 1.6 [21]. Since the predominance of UFAs (>70%) in all examined samples was determined the calculated UFA/SFA ratios (Table 1) were by far higher than 1. Based on these results, it can be concluded that all cereals/pseudo-cereals studied are excellent UFAs sources since all UFA/SFA values were higher than 1.6, where millet and quinoa displayed the highest ratios. In addition, the PUFA/SFA ratios were significantly different for these species and ranged from 2.45 to 4.81, where the highest ratio was detected for millet grains and the lowest for barley grains. It has been recognized [5] that a diet rich in PUFAs may be an alternative choice to a low-fat diet, which may lower blood cholesterol levels, modulate immune function, decrease susceptibility of oxidation of Low-Density Lipoprotein (LDL) cholesterol and improve the fluidity of High-Density Lipoprotein

(HDL) cholesterol. The IA and IT indexes take into consideration the different effects that single fatty acid might have on human health and on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation. The results showed that the IA were significantly different among the four grains flour types, where the lowest IA was calculated for millet and quinoa grains (0.082), followed by wheat (0.113), while the highest index was detected in barley seed (0.217). Similarly, considering the IT values, quinoa grains exhibited the lowest index (0.172), followed by millet flour (0.232), while barley displayed the highest value (0.432). These indexes report the relationship between fatty acids in food and their contribution to the prevention of coronary diseases, thus, the lower the IA and the IT index values, the healthier the food which indicates a sample's potential for plaque aggregation [22]. Considering the HH ratios, the highest ratio was determined for millet grains flour (9.44), followed by quinoa flour (8.56), while the lowest ratio was found in barley seed oil (3.73). Consequently, indicating the nutritional adequacy and the great quality of millet grains. Based on the percentages of UFAs present in the oils, the COX value is a beneficial element usually taken to evaluate the oil's tendency to undergo autoxidation [23]. Results showed that COX values ranged from 5.86 to 6.82, where the lowest value was obtained for wheat seed oil (5.82), followed by quinoa (6.61), millet (6.80) and barley (6.82) seed oils (Table 1). These results indicated that barley and millet seed oils had a great tendency towards autoxidation in comparison with the other species.

Antioxidant activity - DPPH assay

The antioxidant activity of the studied plant seed oils was evaluated using the DPPH assay and the results obtained were expressed as percentages of antioxidant capacity. The oils extracted from the grains of quinoa showed the strongest antioxidant capacity, followed by wheat, millet and the lowest activity was displayed by barley seed oils (Figure 2). A significant influence of the species on the antioxidant activities of the seed oils was perceived. Compared to other studies, the obtained values were in the same range of those obtained for grains of different cereals and pseudo-cereals [24].

PCA analysis

PCA was carried out to determine the relationships between samples and original variables, and to check the usefulness of these substances to discriminate between related species. According to the PCA analysis (Figure 3), the first two principal components (PC1 and PC2) explained 82.01% of the total variation of observed data and allowed a clear differentiation of all the studied species based on their FA composition and their according parameters. Principal component 1 explained 52.47% of the total variance, where as principal component 2 explained 29.54% of the total variance (Figure 3a). The PCA plot clearly discriminated the four types of grains. Grains from *Pennisetum glaucum* species clustered together at the upper left side of the plot where as *Chenopodium quinoa* grains were clustered at the bottom left side of the plot; these grains were specifically characterized by high total oil contents, a high antioxidant activity, high UFA/SFA ratios and high contents of C20:1, C16:1 and C20:0. In addition to presenting high nutritional quality, these two species are characterized by being gluten-free feature allowing to obtain a greater variety of foods more suitable and nutritious to holders of celiac disease, accordingly, making quinoa and millet appropriate for the production of food products commonly referred as "glutenfree", an important aspect that allows greater variety and supply of foods that are more nutritious and suitable for patients with celiac disease. At the right side of the plot, clustered *Triticum durum* and *Hordeum vulgare* grains and were characterized by their higher contents of C16:0 and their high IA and IT indexes (Figure 3b).

Correlation analysis

Correlation coefficients (r) of fatty acids and the related parameters are shown in Table 2. Considering the individual compounds, the highest positive correlation ($p<0.05$) was observed between palmitoleic acid (C16:1) and oleic acid (C18:1) ($r=0.995$). Gondoic acid (C20:1) and erucic acid (C22:1) were also positively correlated ($r=0.981$). In contrast, a negative correlations ($p<0.05$) was detected for stearic acid (C18:0) and behenic acid (C22:0) ($r=-0.978$) (Table 2). On the other hand, it is also interesting to mention the presence of interesting pairs of correlations between the antioxidant activity

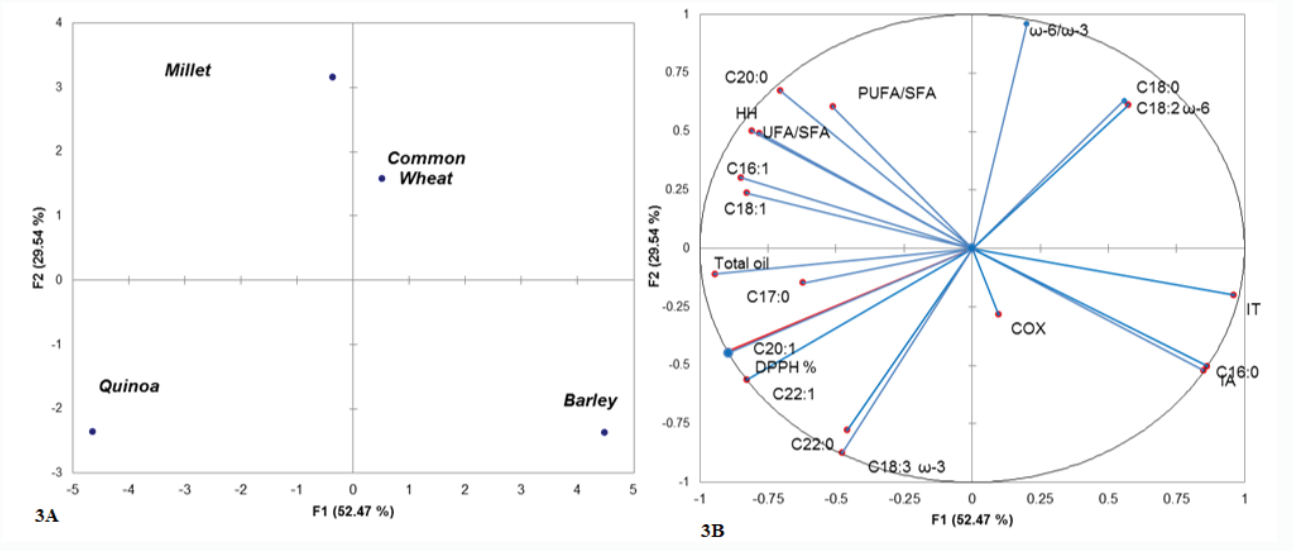

Figure 3: 3A: Principal Component Analysis (PCA) of the grains types based on the lipid fraction profile; 3B: Score plot from the analysis of the compounds from the different grains oils.

Table 2: Correlation coefficients all identified fatty acids and related parameters.

Variables	C16:0	C17:0	C18:0	C20:0	C22:0	C16:1	C18:1	C20:1	C22:1	C18:2 n-6	C18:3 n-3	PUFA/SFA	UFA/SFA	ω-6/ω-3	IA	IT	HH	COX	Total oil	DPPH %
C16:0	**1**																			
C17:0	-0.389	**1**																		
C18:0	0.188	-0.024	**1**																	
C20:0	-0.929	0.516	0.157	**1**																
C22:0	-0.018	0.069	**-0.978**	-0.301	**1**															
C16:1	-0.843	0.817	-0.049	0.900	-0.032	**1**														
C18:1	-0.784	0.872	-0.036	0.858	-0.024	**0.995**	**1**													
C20:1	-0.525	0.682	-0.733	0.352	0.718	0.661	0.676	**1**												
C22:1	-0.413	0.555	-0.843	0.191	0.838	0.509	0.523	**0.981**	**1**											
C18:2 n-6	0.124	-0.866	0.411	-0.117	-0.504	-0.539	-0.607	-0.825	-0.788	**1**										
C18:3 n-3	0.043	0.359	-0.865	-0.273	0.936	0.104	0.144	0.805	0.890	-0.761	**1**									
PUFA/SFA	-0.798	-0.242	-0.233	0.624	0.027	0.350	0.253	0.143	0.115	0.411	-0.232	**1**								
UFA/SFA	-0.950	0.113	-0.334	0.788	0.142	0.641	0.562	0.450	0.388	0.067	-0.024	0.933	**1**							
ω-6/ω-3	-0.347	-0.416	0.612	0.460	-0.754	0.035	-0.038	-0.616	-0.693	0.809	-0.918	0.598	0.393	**1**						
IA	**0.997**	-0.460	0.166	-0.945	-0.006	-0.884	-0.832	-0.551	-0.431	0.185	0.028	-0.747	-0.922	-0.312	**1**					
IT	0.936	-0.419	0.514	-0.765	-0.368	-0.792	-0.743	-0.756	-0.691	0.322	-0.301	-0.729	-0.922	-0.038	0.931	**1**				
HH	**-0.973**	0.191	-0.301	0.834	0.113	0.703	0.629	0.478	0.402	0.012	-0.026	0.905	**0.996**	0.381	**-0.952**	-0.936	**1**			
COX	0.152	-0.753	-0.639	-0.479	0.585	-0.582	-0.634	-0.039	0.128	0.404	0.283	0.364	0.157	-0.064	0.223	-0.035	0.075	**1**		
Total oil	-0.771	0.365	-0.762	0.519	0.651	0.635	0.597	0.869	0.858	-0.439	0.573	0.606	0.803	-0.233	-0.762	-0.945	0.804	0.234	**1**	
DPPH %	-0.521	0.659	-0.754	0.338	0.736	0.645	0.658	**1.000**	**0.986**	-0.812	0.813	0.155	0.457	-0.617	-0.546	-0.759	0.482	-0.008	0.877	**1**

and gondoic acid and erucic acid ($p<0.05$). Palmitic acid (C16:0) was positively correlated with the IA index ($r=0.997$), whereas negatively correlated with the HH index ($r=-0.973$) ($p<0.05$).

Conclusion

To the best of our knowledge, this is the first detailed report on the fatty acids composition and related lipid health indices of different types of three cereals (wheat, barley, millet) and pseudo-cereals (quinoa) grains. Total oil content ranged from 1.5% in barley grains to 7% in quinoa grains and the type of fat, in all examined foods, and was predominantly unsaturated. Besides, the data pointed to species-dependent variations in the fatty acid composition and the nutritional quality of the grains. The PCA showed that fatty acids are very useful components to discriminate between related species and contribute to point out the specificity of each grain types. In conclusion, the present study indicates that grains of cereal and pseudo-cereal grains are good natural sources of fatty acids profiled as favorable from a cardio-protective perspective.

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