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Research Article

COMPARISON OF SOME BIOACTIVE COMPONENTS OF EMMER WHEAT [*Triticum dicoccum* (SCHRANK) SCHÜBLER] CULTIVARS FROM TWO DIFFERENT ORIGINS GROWN UNDER THE SAME CONDITIONS

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ABSTRACT

The chemical composition (lipids, fatty acids, proteins, amino acids, starch, dietary fiber, sterols and tocopherols) of two Emmer wheat [*Triticum dicoccum* (Schrank) Schübler=*Triticum dicoccon* Schrank] cultivars grown under the same condition in Greece was analyzed. Starch accounted for the highest percentage of the detected substances (67.1-69.4%), followed by proteins (16.1-17.5%) and dietary fiber (ADF 2.1-2.5% and αNDF 5.7-12.0%). The main fatty acids in the lipid fractions (1.36-1.62%) were oleic (35.4-37.0%), palmitic (28.0-31.5%) and linoleic (23.3-28.9%) acids. γ-Tocotrienol (46.1-53.2%), α-tocopherol (28.6-34.4%) and β-tocopherol (15.9-17.8%) predominated in the tocopherol fraction, and β-sitosterol (61.3-67.0%) and campesterol (31.3-37.3%) in the sterol fraction. Arginine (10.8-13.2 g/100 g protein), proline (8.7-13.0 g/100 g protein) and tyrosine (8.3-9.2 g/100 g protein) dominated in the amino acids.

Keywords: Emmer wheat [*Triticum dicoccum* (Schrank) Schübler], Bioactive components, Dietary fiber, Sterols, Fatty acids, Amino acids

Introduction

Cultivated emmer wheat (*Triticum dicoccum* (Schrank) Schübler) from the Poaceae family is one of the oldest crops in the world and is cultivated by organic farmers in many countries in Central Europe (Arzani and Muhamad, 2017; Čurná and Lacko-Bartošova, 2017; Konvalina *et al.,* 2012; Koutis, 2015; Lacko-Bartošova *et al.,* 2015). Emmer is tetraploid wheat (AABB genome, 2n=4x=28 chromosomes) characterized by specific properties owing to the starch, proteins and fiber contained in it, therefore it plays a role as a functional food ingredient.

Different studies on the chemical composition of emmer have determined the effect of the soil and climatic conditions, crop culture, its varieties and cultivars (Castagna *et al.,* 1996; Fares *et al.,* 2008; Hejtmankova *et al.,* 2010; Serpen *et al.,* 2008; Suchowilska *et al.,* 2012).

According to the study of Čurná and Lacko-Bartošova (2017), emmer wheat has higher levels of protein (13.5-19.05%), starch (55.4-73.3%), dietary fiber (10-12%), lipids (2.4-3.0%) and total tocopherols (19.7-69.85 mg/g). The primary fatty acid is linoleic (60% of the total fatty acids), followed by oleic (19% of the total fatty acids), and palmitic (16% of the total fatty acids).

A study by Konvalina *et al.* (2008) investigated the amino acid composition of six varieties of emmer from different geographical regions in the Czech Republic. The authors established that the emmer grains were characterized by high protein and amino acid content. No significant differences were observed in the lysine, threonine, leucine, tyrosine and phenylalanine content, which are the limiting amino acids among the tested varieties.

Lachman *et al*. (2011) established that the selenium content in emmer grains (58.9-68.4 µg/kg) was related to varieties. Total polyphenols (expressed in gallic acid equivalents) prevailed in emmer varieties (584-692 mg/kg).

Suchowilska *et al.* (2012) analyzed the concentrations of macro- and microelements in the whole emmer grain and observed that the main macroelements were P (5.12 g/kg) and K (4.39 g/kg), and the main microelements were Zn (54 mg/kg), Fe (49 mg/kg), and Mn (24 mg/kg).

Despite the studies carried out, there is different information about the chemical composition of emmer wheat cultivars. Therefore, the specific object of this study was to determine the chemical composition of two Emmer wheat cultivars (*T. dicoccum* (Schrank) Schübler) grown in Greece, as a potential source for the isolation of bioactive compounds due to their prospective utilization in ***various*** industries.

Materials and Methods

Plant Material

The emmer (*Triticum dicoccum* (Schrank) Schübler) cultivars Nari Nigrocyat (Greek origin) and Farro (Italian origin) were collected from the village of Kukos (North Greece) in October 2017.

The plant species were identified as *Triticum dicoccum* (Schrank) Schübler=*Triticum dicoccon* Schrank by the Botany Department of Paisii Hilendarski University of Plovdiv, Bulgaria.

The grain moisture was determined according to the method of Russian Pharmacopoeia (1990). The biologically active substances were analyzed in the samples, and the values were represented on the basis of absolute dry weight.

Dietary Fiber

Acid Detergent Fiber (ADF) was determined after acid hydrolysis at 100 ºC with reflux condenser of the milled grain with 1.00 N H_2SO_4 in the presence of cetyltrimethylamonium bromide for 1 hour (Undersander *et al.,* 1993).

Neutral Detergent Fiber (αNDF) was determined after boiling milled grain in phosphate borate buffer with pH=6.95-7.05 in the presence of disodium EDTA and sodium lauryl sulfate, and after 10 min treatment with thermostable α-amylase (Termamyl®) was applied for 1 hour (Undersander *et al.,* 1993).

Starch

Starch content was subjected to polarimetric evaluation after partial hydrolysis in the presence of 1.124% H_2SO_4 and elimination of proteins with 5% water solution of phosphotungstic acid (BIS 13488, 1974).

Protein Content

Determination of the total protein content was carried out according to the Kjeldahl method described by AOAC (2016). A UDK 152 System (Velp Scientiffica, Italy) was used for the analysis.

Amino Acids

For the hydrolysis of the protein to free amino acids was used the method of Nair *et al.* (1976). Subsequently, the chemical score was counted, based on the FAO (1985) pattern (threonine=3.4; valine=3.5; leucine=6.6; isoleucine=2.8; tyrosine + phenylalanine=6.3; lysine=5.8) (FAO/WHO, 1991).

Lipid Fraction Isolation

The grains were processed according to standard methods (ISO 659, 2014).

Fatty acids. The total fatty acid composition of the lipid fraction was determined using gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in absolute CH_3OH at 50°C (ISO 12966-2, 2011). The analysis was conducted as it was described in the method of ISO 12966-1 (2014). Determination of fatty acid methyl esters (FAMEs) was performed on HP 5890 gas chromatograph equipped with a 75 m x 0.18 mm x 25 µm (film thickness) capillary Supelco column and a flame ionization detector. The column temperature was programmed from 140°C (hold 5 min), at 4°C/min to 240°C (hold 3 min); the injector and detector temperatures were set at 250°C. Identification was performed by comparison of the retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions.

Sterols. The unsaponifiable matter were determined by weight after saponification of the lipids and extraction with hexane (ISO 18609, 2000). The identification of sterols was carried out by standard method (ISO 12228-2, 2014).

Tocopherols. Tocopherols were determined directly in the lipids by HPLC using a Merck-Hitachi unit equipped with a 250 mm x 4 mm Nucleosil Si 50-5 column and a Merck-Hitachi F 1000 fluorescent detector. The operating conditions were as followed by the standart method (ISO 9936, 2016). The mobile phase was hexane: dioxane, 96:4 (v/v) and the flow rate was 1 mL/min. The oil was diluted with hexane (2% solution) and 20 µL were injected. Tocopherols were identified by comparing the retention times to those of authentic individual tocopherols standards.

Statistical Analysis

The measurements were performed in triplicate for the reliability and comparability of the data. The obtained values are presented as mean value ± standard deviation (SD). The Microsoft Exel 2003 software was used to summarize the data.

Results and Discussion

The chemical characteristics of Emmer wheat cultivars have been shown in Table 1. The obtained results showed that there were differences in the chemical composition of the cultivars, which could be explained by their plant origin. Both cultivars were characterized by higher starch (67.1-69.4%) and protein (16.1-17.5%) content, and lower lipid (1.4-1.6%) content. The values are barely distinguishable from Lacko – Bartošová and Čurná (2017), who determined chemical composition in four varieties emmer wheat grown under conditions of organic farming system during 2011 and 2012. Although our results differ slightly from those of Giacintucci *et al*. (2014), it could nevertheless be argued that the variability could be explained by the method performed in the analyses, as well as the growing conditions with respect to geographical, soil, and climatic conditions and genetic background of the cultivars (Čurná and Lacko-Bartošova, 2017).

The extracted lipid fraction was observed to be a yellow liquid. The content of their biologically active components is presented in Table 2. The data showed that unsaponifiable matter were found to be 9.8 and 6.8% respectively in the oil from the Farro and Nari Nigrocyat cultivars. The sterol content in the investigated fractions (2.2-2.7%) was higher but still close to that of most plant oils, i.e. corn, sunflower, safflower, in which the respective quantities were 0.4-0.9% (Popov and Ilinov, 1986). The tocopherol quantity was found to be considerably higher than that of other common oils (Nosenko, 2017; CODEX STAN 210-1999). There were observed differences in the total tocopherol content of the oil from the two examined cultivars. Total tocopherols in the oil from Farro cultivar were 2676 mg/kg, while in the oil from Nari Nigrocyat cultivar were almost two times lower (1546 mg/kg).

The fatty acid composition of the lipid fraction is presented in Table 3. The data show that 15 fatty acids were determined in the lipid fraction from the samples, constituting 100% of the total oil content. In the Farro cultivar, the main fatty acids were oleic (37.0%), palmitic (31.5%) and linoleic (23.3%). The saturated:unsaturated fatty acid ratio was 35.9:64.1. The main fatty acids in the lipid fraction from the Nari Nigrocyat cultivar were oleic (35.4%), linoleic (28.9%) and palmitic (28.0%). The saturated:unsaturated fatty acids ratio was 32.1:67.9. The fatty acid composition of the oil from Farro and Nari Nigrocyat cultivars was slightly different. The only differences were observed in the content of palmitic and linoleic acid. The amount of the palmitic acid in the triacylglycerols from Farro cultivar was higher than in the lipids from Nari Nigrocyat, while the quantity of the linoleic acid in the first cultivar was lower. No considerable differences were observed in the content of the other fatty acids.

Table 1. Chemical composition of emmer grains

Composition, %	*Farro Cultivar*	*Nari Nigrocyat Cultivar*
Moisture	9.7 ± 0.12	10.1 ± 0.15
Lipids	1.6 ± 0.01	1.4 ± 0.01
Protein	17.5 ± 0.25	16.1 ± 0.21
Starch	67.1 ± 1.20	69.4 ± 1.30
Dietary fiber (ADF*)	2.1 ± 0.02	2.5 ± 0.02
Dietary fiber (αNDF**)	5.7 ± 0.06	11.9 ± 0.16

* Acid Detergent Fiber; ** Neutral Detergent Fiber

Table 2. Lipid fraction composition

Compounds	*Farro Cultivar*	*Nari Nigrocyat Cultivar*
Sterols (%)	2.2 ± 0.02	2.7 ± 0.02
Tocopherols, mg/kg	2676 ± 40.10	1546 ± 20.11
Unsaponifiable matter (%)	9.8 ± 0.09	6.8 ± 0.06

Table 3. Fatty acid composition of the lipid fraction

Fatty acids, % (w/w)		*Farro Cultivar*	*Nari Nigrocyat Cultivar*
$C_{12:0}$	Lauric	0.2 ± 0.00	0.1 ± 0.00
$C_{14:0}$	Myristic	0.4 ± 0.00	0.3 ± 0.00
$C_{15:0}$	Pentadecanoic	0.3 ± 0.00	0.2 ± 0.00
$C_{16:0}$	Palmitic	31.5 ± 0.28	28.0 ± 0.26
$C_{16:1}$	Palmitoleic	0.2 ± 0.00	0.2 ± 0.00
$C_{17:0}$	Margaric	0.2 ± 0.00	0.2 ± 0.00
$C_{18:0}$	Stearic	2.2 ± 0.02	2.5 ± 0.02
$C_{18:1}$	Oleic	37.0 ± 0.31	35.4 ± 0.30
$C_{18:2}$	Linoleic (cis)	23.3 ± 0.20	28.9 ± 0.27
$C_{18:2}$	Linoleic (trans)	0.8 ± 0.00	0.8 ± 0.00
$C_{18:3}$	α-Linolenic	1.2 ± 0.01	1.1 ± 0.02
$C_{20:0}$	Arachidic	0.4 ± 0.00	0.3 ± 0.00
$C_{20:1}$	Gadoleic	0.2 ± 0.00	0.2 ± 0.00
$C_{20:2}$	Eicosadienoic (cis)	1.4 ± 0.01	1.3 ± 0.01
$C_{22:0}$	Behenic	0.7 ± 0.00	0.5 ± 0.00
Saturated fatty acids		35.9	32.1
Unsaturated fatty acids		64.1	67.9

The obtained results about the fatty acid composition are not in agreement with the data found in the literature (Čurná and Lacko-Bartošova, 2017).

The Emmer wheat lipid fraction was found to contain very high amounts of the saturated palmitic acid (28.0-31.5%), which was close to the levels in other oils (O'Brien *et al.,* 2004).

Tocopherols are a class of organic chemical compounds, many of which have vitamin E activity, where the main dietary sources are olive and sunflower oils, soybean and corn oil (Popov and Ilinov, 1986). The tocopherol composition of the lipid fraction has been presented in Table 4. The quantity of tocopherols in the examined oils was found to be considerably higher than that of other common oils (CODEX STAN 210-1999). γ-Tocotrienol (46.1-53.2%) and α-tocopherol (28.6-34.4%) predominated in the tocopherol fraction, followed by β-tocopherol (15.9-17.8%). Our results are

compatible to those reported by Konopka *et al*. (2012). Some of the genotypes were more sensitive to the effects of weather conditions in the growing cultivation year, whereas others were relatively stable. It is reported that temperature and moisture influenced the α-tocopherol content (Konopka *et al*., 2012).

Sterols were present in the so-called non-saponificated part of the lipid fraction. The individual sterol composition of the lipid fraction has been presented in Table 5. *β*-Sitosterol (61.3-67.0%) and campesterol (31.3-37.3%) predominated in the sterol fraction. No significant differences were observed in the sterol composition of the lipids from the examined cultivars. The data demonstrated that regarding its sterol content and composition, emmer oil was similar to the findings for other seed oil (CODEX STAN 210-1999).

The amino acid composition of the protein fraction has been presented in Table 6. Leucine was the first limiting amino acid (the chemical score varied from 0.1 to 0.2) and lysine was the second limiting amino acid (the chemical score was 0.3 and 0.7). It was followed by valine (the chemical score was 1.1 and 1.6), tyrosine and phenylalanine (the chemical score was 1.6 and 1.7), threonine (the chemical score was 1.6 and 2.0), and isoleucine (the chemical score was 1.7 and 2.5). Obtained results were comparable to those reported in literature. Konvalina *et al.* (2008) reported no considerable differences in the content of the limiting amino acid lysine between the tested varieties (the chemical score varied from 0.37 to 0.44); the second limiting amino acid threonine (the chemical score varied from 0.66 to 0.73), leucine (the chemical score varied from 0.80 to 0.84), tyrosine and phenylalanine (the chemical score varied from 0.92 to 0.96).

Table 4. Tocopherol composition of the lipid fraction

Tocopherols % (w/w)	*Farro Cultivar*	*Nari Nigrocyat Cultivar*
α-Tocopherol	28.6 ± 0.25	34.4 ± 0.31
β-Tocopherol	15.9 ± 0.14	17.8 ± 0.16
γ-Tocopherol	2.3 ± 0.03	1.7 ± 0.02
γ-Tocotrienol	53.2 ± 0.30	46.1 ± 0.22

Table 5. Sterol composition of the lipid fraction

Sterols % (w/w)	*Farro Cultivar*	*Nari Nigrocyat Cultivar*
Cholesterol	0.4 ± 0.00	0.3 ± 0.00
Campesterol	37.3 ± 0.35	31.3 ± 0.30
Stigmasterol	0.4 ± 0.00	0.5 ± 0.00
β-Sitosterol	61.3 ± 0.59	67.0 ± 0.62
Δ^5-Avenasterol	0.4 ± 0.00	0.9 ± 0.00
Δ^7-Stigmasterol	0.2 ± 0.00	-*

-* Not identified

The comparative analysis of both varieties shows that they are with close content of biologically active substances. Differences are found in the values of dietary fiber (αNDF), which is higher for Nari Nigrocya cultivar (11.9%) and tocopherols for Farro cultivar (2676 mg/kg). These differences can be explained in part by the variety features, although the plants are grown in the same region.

Conclusions

Consumers' concerns about the food quality, the nutritional value, methods of food production and the conditions under which food are grown have increased. We have presented the parameters such as fatty acids, proteins, amino acids, starch, dietary fiber, sterols and tocopherols which were determined in order to learn the nutritional value of the studied two Emmer wheat cultivars grown in Greece. According to the analyses conducted, the lipid extract contained unsaponifiable substances, sterols, and tocopherols. Fifteen fatty acids were identified, and the main ones were oleic (35.4-37.0%), palmitic (28.0-31.5%) and linoleic acid (23.3-28.9%). *γ*-Tocotrienol (46.1-53.2%), *α*-tocopherol (28.6-34.4%) and *β*-tocopherol (15.9-17.8%) predominated in the tocopherol fraction, and *β*-sitosterol (61.3-67.0%) and campesterol (31.3-37.3%) in the sterol fraction. Arginine (10.8-13.2 g/100 g protein), proline (8.7-13.0 g/100 g protein) and tyrosine (8.3-9.2 g/100 g protein), predominated in amino acids. The results show that cultivars are with close content of biologically active substances. Differences are found in the values of dietary fiber (αNDF), which is higher for Nari Nigrocya cultivar (11.9%) and tocopherols for Farro cultivar (2676 mg/kg). After suitable chemical treatment, Emmer wheat cultivars (*Triticum dicoccum* (Schrank) Schübler) grown in Greece could be used as an alternative source of starch and other biologically active substances such as proteins and fiber. Based on the results, the two Emmer wheat cultivars could be established as a potential source for the isolation of bioactive compounds with possibilities for application in food, cosmetics, pharmaceutical and other products, and their studying is a potential subject for future research.

Table 6. Amino acid composition of the protein fraction

Amino acids	*Farro Cultivar*		*Nari Nigrocyat Cultivar*	
	Content, g/100 g protein	*Chemical score*	*Content, g/100 g protein*	*Chemical score*
Asp	1.9 ± 0.01	-	0.3 ± 0.00	-
Ser	0.8 ± 0.00	-	2.9 ± 0.01	-
Glu	0.8 ± 0.02	-	1.5 ± 0.01	-
Gly	2.3 ± 0.01	-	6.7 ± 0.02	-
His	0.7 ± 0.00	-	0.6 ± 0.00	-
Arg	10.8 ± 0.20	-	13.2 ± 0.10	-
Thr	5.5 ± 0.04	1.6 ± 0.00	6.7 ± 0.01	2.0 ± 0.00
Ala	4.0 ± 0.02	-	7.3 ± 0.02	-
Pro	8.7 ± 0.07	-	13.0 ± 0.13	-
Cys	3.3 ± 0.00	-	3.3 ± 0.01	-
Tyr	8.3 ± 0.07	-	9.2 ± 0.12	-
Val	3.7 ± 0.02	1.1 ± 0.00	5.7 ± 0.00	1.6 ± 0.00
Met	0.8 ± 0.01	-	1.5 ± 0.00	-
Lys	1.8 ± 0.00	0.3 ± 0.01	4.2 ± 0.01	0.7 ± 0.00
Ile	4.8 ± 0.02	1.7 ± 0.00	7.0 ± 0.02	2.5 ± 0.01
Leu	0.8 ± 0.01	0.1± 0.00	1.3 ± 0.00	0.2 ± 0.00
Phe	1.5 ± 0.00	1.6* ± 0.01	1.5 ± 0.01	1.7* ± 0.01

* tyrosine + phenylalanine

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

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