

EVALUATION OF TOCOPHEROLS AND TOCOTRIENOLS IN ALBANIAN CULTIVARS

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Abstract

Wheat is a major component in the human diet with an impact on nutritional health due to its significant intake. The aim of this study was to evaluate the quality of Albanian wheat by analysing chemical and nutrition parameters and understanding the health impact of the components in wheat flour.

Five winter wheat cultivars grown during the year 2017 - 2018 on experimental fields of Agriculture Technology Transfer Center (ATTC) in Lushnja were analysed. Protein content (%N x 5.7) was determined by the Kjeldahl method and lipid content was evaluated by Soxhlet extraction method utilizing n-hexane as solvent. The starch content was analyzed following the Megazyme Starch determination procedure (Megazyme International, Ireland, Ltd). The determination of the total content of tocopherols (TP) and tocotrienols (TT) in the five wheat cultivars was carried out using the High Performance Liquid Chromatography (HPLC) analysis. A descriptive statistical analysis was performed for result elaboration. The analysis of variance (ANOVA) and Principal Component Analysis (PCA) was performed using StatSoft Statistica 10.0 software and the significant differences were calculated according to post - hoc Tukey's (HSD) test at $p < 0.05$.

All cultivars showed higher level of protein content ranging from 11.39% to 12.38% and the starch content

ranging from 58% to 62%. Statistical results indicated that the protein content was significantly affected by the wheat cultivars. α -Tocopherol and β -tocotrienol were the most abundant compounds in all samples, ranging, from 9.8 mg/kg DW to 15.6 mg/kg DW and from 8.1 mg/kg to 12.7 mg/kg DW, respectively.

The daily requirement of vitamin E calculated in whole flour ranges from 14.7% to 26%. By selecting the suitable wheat cultivars with high vitamin E content, we can contribute to increasing the content of vitamin E in Albanian wheat flour and supporting the problems of vitamin E deficiency in the human diet.

Key words: *Wheat cultivars, Chemical parameters, Tocopherols, Tocotrienols, Vitamin E.*

1. Introduction

Wheat is a good source of nutrients, minerals and trace elements. The high content of starch, about 60 - 70% of the whole grain and 65 - 75% of white flour, means that wheat is something more than a source of calories. Seeing as how wheat products are the basis of food in Albania, food and safety issue (especially wheat commodity), have recently taken an important focus. In previous study, the content of protein, reported

as an indicator of quality, showed an average value of 14.2 % that is considered a high value [1]. Besides, Albanian wheat samples reported high content of some minerals like Ca, Cu, Zn, Fe and Mn. The most important amino acids in Albanian wheat are glutamic acid, cysteine, lysine and asparagine [2]. Another important component in human diet is the vitamin E. The content of vitamin E and its activity is determined by the content of tocopherols [3]. Tocopherols are molecules with a polar chromanol ring linked to an isoprenoid-derived hydrocarbon chain; tocopherols and tocotrienols differing only in the saturation state of the isoprenoid side chain [4]. In particular, there are eight vitamin E isomers four tocopherols and four tocotrienols: α -tocopherol (α TP), β -tocopherol (β TP), γ -tocopherol (γ TP), and δ -tocopherol (δ TP) and α -tocotrienols (α TT), β -tocotrienol (β TT), γ -tocotrienol (γ TT), and δ -tocotrienol (δ TT). Vitamin E antioxidant activity of the individual isomers differs and decline in the order: α TP > β TP > γ TP > δ TP, [5]. However, recent study considers only α TP as a source of vitamin E activity. Tocotrienols have lower vitamin E activity but higher antioxidant activity [6]. In common einkorn wheat's (*Triticum aestivum* and *Triticum monococcum*) tocopherols are located in the germ fraction and the most abundant tocopherols are α -tocopherol and β -tocopherol [7]. The tocotrienols, α -tocotrienol and β -tocotrienol, are found in the bran, germ and endosperm of wheat [7]. The US Department of Agriculture has reported that the mean vitamin E content for whole wheat flour is 0.71 mg/100g, [8]. This amount clearly indicates that the content of tocopherols in wheat plays an important role in the diet of the humans, especially when using whole wheat flour. Different studies established the antioxidant activity of tocopherols and tocotrienols and their role in health and diseases [9, 10, and 11]. A clear example of this is the relationship between tocopherol nutritional status and the prevention of cardiovascular diseases [12, 13]. A greater knowledge about the quality of Albanian soft wheat, in respect to the content of tocs, vitamin E activity and other nutritional parameters could positively impact a healthy intake of these compounds from wheat.

Thus, the aim of this study was to evaluate the quality of Albanian wheat, analysing some chemical and nutrition parameters in different soft wheat samples. These results could help the farmers to influence the quantity and composition of tocopherols in wheat flour by selection of appropriate genetic material and growing environment.

2. Material and Methods

2.1 Plant Materials

The wholegrain wheat flour samples were obtained by milling 5 cultivars of soft wheat (Dajti, Progresi, LVS,

L4 and L7) on an experimental automatic mill (Fritch, Pulverisette 14). Five winter wheat cultivar samples were planted during the years 2017 - 2018 on the experimental fields of the State of Seed and Seedlings in Lushnja Region. The soil reaction was slightly alkaline (pH = 7.2). The other soil parameters are described in Table 1.

Table 1. General characterization of soil in experimental field

pH	Humus (%)	Ca ²⁺ (%)	N (%)
7.2	1.85	0.015	0.140

Field trials were carried out by methods of randomized blocks in four replications; the size of each plot was 12 square meters.

2.2 Chemicals and reagents

All the solvents and reagents were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and they were of chromatographic or analytical grade. The standard compounds were supplied by Sigma-Aldrich (Saint Louis, MO, USA).

2.3 Chemical analyses

The moisture content was determined by oven drying a sample at 60 °C for 12 hours [14], whereas ash content was measured according to the dry ashing procedure [15]. Protein content (%N x 5.7) was determined by the Kjeldahl method [16], and lipid content was evaluated by Soxhlet extraction method utilizing n-hexane as solvent [17]. The starch content was analysed following the Megazyme Starch determination procedure (Megazyme International, Ireland, Ltd). All solution, reagents and buffers were prepared as described in the instructions given by Megazyme. All the samples were analysed in triplicate.

2.4 Extraction of lipids

Milled grain samples were freeze dried for three days before lipid extraction. After grounding, lipid fraction was extracted from grain with hexane in a Soxhlet apparatus [18]. The remaining solvent was removed by a stream of nitrogen and the oil was taken up with hexane and stored at -18 °C until use. Each extraction was carried out three times for each seed sample.

2.5 Tocopherol analyses

For the identification and quantification of tocopherols, the normal phase-HPLC method reported by Ben Lajnef was used [18], with slight modification. Approximately

50 mg of lipids were dissolved in 0.5 mL of hexane and filtered through a 0.2 µm nylon filter, and then 2.5 µL were injected in a HPLC 1200 series equipped with a fluorimeter detector (Agilent Technologies, Palo Alto, CA, USA). The excitation wavelength was 290 nm and the emission was 325 nm. The separation of tocopherols was performed by a HILIC Poroshell 120 column (100 mm × 3 mm and 2.7 µm particle size; Agilent Technologies, USA), in isocratic conditions, using an n-hexane/ethyl acetate/acetic acid (97.3: 1.8: 0.9 v/v/v) mobile phase. The flow rate was 0.8 mL/min. To identify the tocopherols, the retention time of tocopherols in seed samples was compared with their retention times in a barley extract obtained by hot saponification; tocopherols were identified by co-elution with the respective standards. The calibration curve used for quantification was constructed with α-tocopherol standard solutions.

2.6 Statistical analyses

Descriptive statistical analyses were performed for all obtained results. All measurements were performed over three repetitions. The analysis of variance (ANOVA) and principal component analysis (PCA) of the obtained results were performed using StatSoft Statistica 10.0 software. Significant differences were calculated according to post-hoc Tukey's (HSD) test at $p < 0.05$ significant levels, 95% confidence limit. Further, principal component analysis (PCA) was applied successfully to classify and discriminate between the different juice samples. PCA was applied within the results' descriptors in order to characterize and differentiate between all samples. Vitamin E activity was calculated based on α-tocopherol content according to the scientific opinion of EFSA [13], while the recommended daily intake (RDI) of vitamin E is set by European Parliament and the Council [19].

3. Results and Discussion

3.1 Chemical analyses

Summary of descriptive statistics of chemical parameters of the whole flour obtained from selected cultivars are presented in Table 2.

The humidity content in the samples ranged from 12.5% (Dajti) to 12.99% (L7), highlighting how those cultivars can be stored for a longer time. Since whole flour wheat was analysed in this study, the cultivars presented a high level of ash content, ranging from 1.65% (Progresi) to 1.82% (L7). The most important components of wheat grain are proteins, which affect the end-use quality [20]. The total protein content in the analysed samples ranged from 11.39% (L4) to 12.38% (Progresi) and the starch content ranged from 58.00% (L7) to 62% (Progresi). Generally, the whole wheat flour contains about 70% starch and the protein content range from 8 - 15% on weight basis [21]. The lipid content in five cultivars of wheat ranged from 1.86% (LVS) to 1.94% (Dajti). The highest lipid content was observed in Dajti cultivar, while the lowest lipid content was found in LVS cultivar. The statistical analysis of the lipid content demonstrates that there is no significant difference ($p < 0.05$) between cultivars of wheat. Although lipids are in small amount in wheat flour, they are considered to have significant impact in the performance of the wheat flour in bread making and in the storage stability of wheat flour and cereals [22].

These results are in agreement with other earlier studies on Albanian wheat genotypes [2] and the protein and starch content reported by the samples showed how these cultivars could be considered of high quality.

The tocopherols (TP) and tocotrienols (TT) content in whole grains, as well as TT/TP ratio is shown in Table 3.

Table 2. Descriptive statistics for chemical parameters of the whole flour

Parameters	Humidity (%)	Ash (%)	Protein (%)	Starch (%)	Lipids (%)
Dajti	12.5 ± 0.23 ^a	1.77 ± 0.02 ^{bc}	11.72 ± 0.04 ^b	60.5 ± 0.71 ^a	1.94 ± 0.01 ^a
Progresi	12.95 ± 0.09 ^a	1.65 ± 0.03 ^a	12.38 ± 0.07 ^c	62 ± 0.00 ^a	1.92 ± 0.02 ^a
LVS	12.69 ± 0.09 ^a	1.79 ± 0.01 ^{bc}	12.23 ± 0.01 ^c	58.5 ± 2.12 ^a	1.86 ± 0.02 ^a
L4	12.95 ± 0.09 ^a	1.72 ± 0.03 ^{ab}	11.39 ± 0.04 ^a	62.5 ± 0.71 ^a	1.9 ± 0.02 ^a
L7	12.99 ± 0.03 ^a	1.82 ± 0.02 ^c	11.84 ± 0.01 ^b	58 ± 1.41 ^a	1.91 ± 0.01 ^a

Legend: The results are expressed as mean ± SD values, followed by different letters in the same column are significantly different ($p < 0.05$), according to Tukey's HSD test.

Table 3. Tocopherols (TP) and tocotrienols (TT) content (mg/kg) in the whole grain of selected cultivars

Parameters	αTP	αTT	βTP	βTT	δTP	δTT	TT/TP	Total
Dajti	13.21 ± 0.17 ^c	3.46 ± 0.03 ^b	3.32 ± 0.13 ^a	12.72 ± 0.21 ^a	0.00 ± 0.00 ^a	0.73 ± 0.02 ^a	1.02 ± 0.03 ^a	33.43 ± 0.11 ^a
Progresi	15.6 ± 0.11 ^d	4.04 ± 0.02 ^c	3.47 ± 0.89 ^a	10.79 ± 2.79 ^a	0.00 ± 0.00 ^a	1.13 ± 0.28 ^a	0.84 ± 0.13 ^a	35.04 ± 3.88 ^a
LVS	8.83 ± 0.59 ^a	2.86 ± 0.20 ^a	2.07 ± 0.18 ^a	9.92 ± 0.77 ^a	0.00 ± 0.00 ^a	0.83 ± 0.15 ^a	1.25 ± 0.11 ^a	24.51 ± 0.29 ^a
L4	9.87 ± 0.15 ^{ab}	2.38 ± 0.05 ^a	2.97 ± 0.52 ^a	10.54 ± 2.13 ^a	0.45 ± 0.13 ^b	0.85 ± 0.02 ^a	1.03 ± 0.11 ^a	27.06 ± 3.01 ^a
L7	10.49 ± 0.44 ^b	5.13 ± 0.19 ^d	1.07 ± 1.51 ^a	8.05 ± 11.38 ^a	0.11 ± 0.15 ^{ab}	0.64 ± 0.90 ^a	1.14 ± 0.92 ^a	25.48 ± 13.32 ^a

The results are expressed as mean ± SD values, followed by different letters in the same column are significantly different ($p < 0.05$), according to Tukey's HSD test.

Total tocol concentration of samples ranged between 24.51 mg/kg (LVS) to 35.04 mg/kg (Progresi) for whole flour and Progresi was found to have the highest concentration among the evaluated cultivars. In all samples, α -tocopherol was the predominant tocol (8.83 mg/kg - 15.60 mg/kg), followed by β -tocotrienols (8.05 mg/kg - 12.72 mg/kg). The data provided agree with the results reported by Labuschagne and co-workers, [23]. Unsaturated / saturated tocol ratio (TT/TP) ranged between 0.84 (L4) to 1.25 (LVS).

This ratio is lower compared to the other study conducted in the Balkan area (4.47) [24]. These differences may be explained by the variation of concentration of tocotrienols among cultivars. Wong and Radhakrishnan, [25], reported that cultivar and cultivation location could affect the composition of tocotrienols, although it is still unclear what affect the relationship of the variation of tocotrienols and the ratio between tocotrienols and tocopherols is playing. δ -Tocopherols was not found in cultivars Dajti, Progresi and LVS, and was present in small amounts in cultivars L4 (0.45 mg/kg) and L7 (0.11 mg/kg). This study shows that the quantity and composition of tocols could be governed in wheat flour with the selection of genetic material.

The daily requirement of vitamin E calculated in whole flour ranged from 14.7 % to 26 % (Table 4).

For cultivars Dajti and Progresi, a higher level of vitamin E was analyzed, resulting in the presence of more than 20% of the daily requirement of vitamin E in 200 g of whole wheat flour (22% and 26 %, respectively). These results agree with other studie conducted in whole wheat flour [26]. These results could help to suggest the most suitable wheat cultivars with high vitamin E content (Dajti, Progresi) and so contribute to increase the content of vitamin E in Abanian wheat flour and solve in part the problems of vitamin E deficiency in the human diet. Taking into account the evidence provided here and the level of consumption of whole wheat flour, which amounts 200 g/day per person, the contribution of vitamin E in the human diet is very low. Recent studies showed a reduction of tocopherols content when food is heated [27, 28]. days, most wheat is consumed after cooking, thereby resulting

in reduction of tocopherols content. To secure high intake of vitamin E in the human diet, flour based products should be combined with other foods with high level of tocols.

The PCA allows a considerable reduction in the number of variables and the detection of structure in the relationship between measuring parameters and different samples that give complimentary information. The number of factors retained in the model for proper classification of experimental data, in the original matrix regarding loading and scores, was determined by applying Kaiser and Rice's rule (Figure 1). This criterion retains only principal components with Eigenvalues > 1.

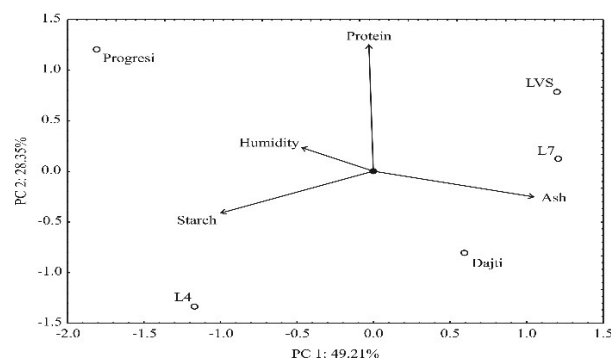


Figure 1. PCA biplot showing the different samples

There was a neat separation of the samples in Figure 1, according to the applied assays. Quality results showed that the first two principal components, accounting for 77.56% of the total variability could be considered sufficient for data representation. PC1 correlated positively with ash content (the variable contributed 46.9% for PC1 calculation) and negatively to starch content (43.3%); the second component PC2, correlated positively with protein content (84.7%) and negatively with starch (9%).

The full autoscaled data matrix consisting of eight variables was submitted to PCA. For visualizing the data trends and the discriminating efficiencies of the used descriptors, a scatter plot of samples using the first two principal components (PCs) issued from PCA of the data matrix was obtained (Figure 2).

Table 4. Vitamin E activity of whole wheat flour in different cultivars, calculated as tocopherol equivalent and percentage of recommended intake from the average flour consumption of 200 g/ person/day

Cultivars	Vitamin E activity (mg/kg)	Recommended Daily Intake (mg/day)	Percentage of recommended Vit. E from 200 g wheat flour (%)
Dajti	13.21	12	22.0
Progresi	15.60	12	26.0
LVS	8.83	12	14.7
L4	9.87	12	16.5
L7	10.49	12	17.5

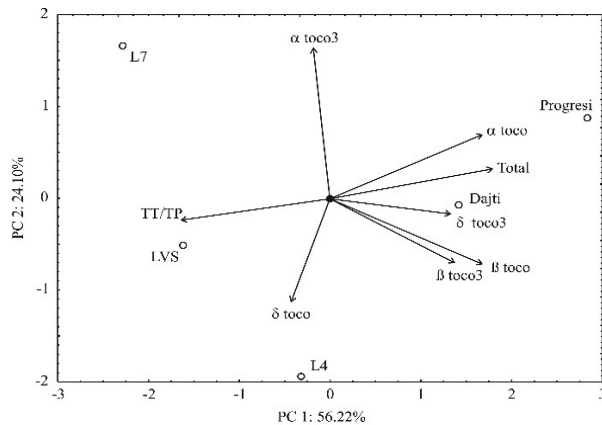


Figure 2. PCA biplot showing the different samples

As could be seen, there was a clear separation of the samples, according to the applied assays. Quality results showed that the first two principal components, accounting for 80.31% of the total variability could be considered sufficient for data representation. PC1 correlated positively with α -TP (the variable contributed 18.2% for PC1 calculation), Total (20.7%), δ -TT (11.5%), β -TP (18.3%) and β -TT (12.1%), while TT/TP was negatively correlated to PC1 (17.7%). The second component PC2 was correlated positively with α -TP (8.9%), α -TT (48.2%), while β -TP (18.3%), β -TT (12.1%) and δ -TP (22.2%) were negatively correlated to the PC2 coordinate.

4. Conclusions

- This study was focused on the quality of five Albanian wheat cultivars, focusing on some chemical parameters and tocopherols content. All cultivars of wheat were characterized by high level of protein and optimum level of humidity and starch.

- The concentration of total tocopherols was found to vary from 24.51 mg/kg to 35.04 mg/kg for whole flour. In all samples, the predominant tocol was α -tocopherols (8.83 mg/kg - 15.6 mg/kg) followed by β -tocotrienols (8.05 mg/kg - 12.72 mg/kg).

- This study shows that the quantity and composition of tocols can be governed in wheat flour with the selection of genetic material. By doing so, 14.7% to 26% of the human daily requirement of vitamin E could be received by an average consumption of 200 g of wheat flour per day.

- Taking into account the results provided, selecting the suitable wheat cultivars with high vitamin E content (Dajti, Progresi), we can contribute to increase the content of vitamin E in Albanian wheat flour and contribute to solving the problems of vitamin E deficiency in the human diet.

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