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## Comparison of Physicochemical Attributes of Einkorn Wheat (*Triticum monococcum*) and Durum Wheat (*Triticum durum*) and Evaluation of Morphological Properties Using Scanning Electron Microscopy and Image Analysis

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### ABSTRACT

Einkorn (*Triticum monococcum* L. subsp. *monococcum*) is a diploid hulled wheat strictly related to durum and bread wheat types. Many farms in northern Turkey, especially the province of Kastamonu, continue to cultivate the oldest type of wheat still in existence, *Triticum monococcum*, also known as Siyez. Although there is great potential for breeding, it's production and consumption has been locally limited and cultivation quantity has been very low. However nowadays, today's consumer demands to traditional or regional food products and grains have been increased einkorn cultivation in Kastamonu, and cultivation area has been reached to approximately from 5 thousand decares to 35 thousand decares. Einkorn is cultivated two times a year in Kastamonu. The variety which is named as "Çatal Siyez" (Kaplica) composed 60-70% of the production of einkorn, is sowed in April and used as animal feed after harvest. Another type which is "Tek Siyez" variety sowed in October is processed to produce einkorn flour and bulgur.

In this study, some physicochemical attributes of Einkorn (*Triticum monococcum*) and Durum Wheat (*Triticum durum*) were compared and their morphological properties were evaluated by using scanning electron microscopy and image analysis. The scanning electron microscopy micrographs shown that Einkorn had opaque but durum wheat had a vitreous appearance. In addition, the starch granules could be easily separated visually in Einkorn texture. In durum wheat, the protein bonds were more compact and the diameter of starch granules located in protein matrix lower than ones of Einkorn. Moreover, it was determined that visually the caryopsis layers of Einkorn were thinner than ones of the durum wheat. The kernel weight (g 1000 pieces<sup>-1</sup>) of Einkorn was found lower (27.94 g) than durum wheat (54.6 g). Also, water absorption was 51.8% of Einkorn and 65.3% of durum wheat. The ash, protein, fat content and antioxidant activity of Einkorn sample were determined higher than durum wheat. Mineral content of the samples was analyzed, and Einkorn contained higher amount of trace and major elements especially in terms of Zn, Fe and Al.

The results show that many foods will able to fortified by einkorn or einkorn products such as semolina, flour, germ or bulgur as a solution of micronutrient malnutrition problem, and to meet today's consumers demand which are clean-label and additive-free food. Moreover, einkorn is a promising candidate for the development of new or special foods such as bakery products, baby food or products with high content of dietary fiber, protein, minerals, carotenoids and tocopherols.

Keywords: Siyez; Einkorn; *Triticum monococcum*; Durum wheat; Image analysis

## 1. Introduction

The archeological findings show that the wheat first occurred in parts of Turkey, Egypt, Ethiopia, Lebanon, Syria and Israel (Lev-Yadun et al 2000). Domesticated Einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*) in Turkey dates back to 9000 B.C (Piperno et al 2004). Einkorn which is a diploid hulled wheat, is the first wheat species cultivated in Karacadağ, Şanlıurfa (Heun et al 1997; Hidalgo & Brandolini 2014) and strictly related to durum and bread wheats. It was most probably spread to Europe during the Agricultural Revolution. Today, traditional einkorn crops are found in mountain areas of the Mediterranean region (Turkey, southern Italy and France, Balkan countries, Morocco and Spain). The changing climate and socio-economic changes have seriously endangered the survival of many traditional foods and native varieties. Most underutilized species and ancestral cereals like einkorn are in danger of disappearing due to agronomic, genetic, economic, and cultural factors. The ancient food grains and cereals have been sustained in specific geographic areas by only small-scale producers. Many farms in the forested northern Turkish province of Kastamonu, continue to cultivate the oldest type of wheat still in existence, *Triticum monococcum*, known as Siyez in Turkey. In recent years the trend towards sustainable agriculture and increased attention to the nutritional attributes of food, has led to the rediscovery of several forgotten cereals and legumes, including einkorn.

Einkorn is highly competitive thanks to having one spikelet and tight hull nature and it can be grown on poor soil with limited fertility, in arid conditions and cold climates with lower input and technology. The studies show that Siyez wheat has high fat, protein and carotenoid content and it contains more lutein compared to bread wheat. As it is traditionally consumed in the form of whole wheat, it has low glycemic index. It is also known for being rich compared to the other wheat varieties in terms of protein, phenolics, tocopheroles and carotenoids. Ancestor wheats are also efficient for treating diseases like cholesterol, colitis and

allergy and their starch is resistant to high enzymes (Strehlow et al 1991; Abdel-Aal et al 2002; Hidalgo & Brandolini 2011). Seed size of Einkorn has an important role for evaluation of the quality and technologic nature of wheat. Because, wheats have bigger and heavier kernels, high endosperm, lower quantities of outer pericarp and aleurone layer. Siyez kernels contain 3.0-3.2% germ while bread wheat has 2.9-3.0%; siyez contains 22.8-23.0% bran while bread wheat has 15.0-17.0%, Siyez's endosperm ratio is 73.0-75.0% while it is 80.0-82.0% for bread wheat. High bran fraction of Siyez wheat stems from its quite small kernel structure, so when Siyez wheat is processed for flour, it will be not appropriate to compare it with bread or durum wheat. Its technologic yield is lower (Hidalgo & Brandolini 2014).

The aim of this study was to draw attention to the issue of dissemination of the ancestral food grains such as einkorn, to increase of demand for its' sustainability. In this research, some physicochemical and morphological properties of einkorn and durum wheat have been compared. In this context, alternative uses of einkorn have been examined and new products have been proposed except usage as bulgur. For this aim, various physicochemical properties such as protein, gluten, fat, moisture, water absorption, antioxidant activity and microtextural properties of einkorn samples cultivated from Kastamonu and durum wheat from Konya were examined.

## 2. Material and Methods

The samples which were named "Tek siyez", were obtained from seeds which were sown in October 2016 and harvested in July 2017 at Devrekani and İhsangazi districts where are the most einkorn cultivation areas of Turkey. Two Einkorn (*Triticum monococcum*) samples from İhsangazi, and two samples from Devrekani district were supplied by the main producers in this study. Every sample was obtained randomly from four locations by the farmers. All samples (from 2 district x 2 sample x 4 location) were mixed homogeneously. The durum wheat (*Triticum durum*) sample (Selçuklu-97)

was supplied from Bahri Dagdas International Agricultural Research Institute, Konya, Turkey. The chemicals used for analysis were purchased from Merck (Germany) and DPPH from Sigma (Germany).

**Physicochemical properties:** The composition of the wheat samples were determined according to AACC (2000) approved methods (Moisture No: 44-15A, Ash No: 08-03, Protein No: 46-10, Wet gluten No: 38-12, Fat No: 30-25) and AOAC (1995) method (Total dietary fiber No: 985.29). The color profile of the samples were determined using a colorimeter (Konica-Minolta, CR400, Japan) as  $L^*$ ,  $a^*$  and  $b^*$ . The measurement was performed on three different points. Farinograph properties (water absorption, degree of softening) were carried out according to AACC (2000) No:54-21 method.

**Antioxidant activity:** The extraction was carried out according to Banu et al (2010). Antioxidant activity was determined by the method of Karamac et al (2002) as inhibition % using the  $\alpha, \alpha$  diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging assay.

**Mineral content:** The mineral content was measured by using the microwave (Milestone MLS 1200, Italy) nitric acid digestion procedure and it was followed by induction-coupled plasma optical emission spectrometry (Spectro Blue ICP-OES) according to slightly modified method of Kilci & Gocmen (2014).

**Scanning Electron Microscopy (SEM):** The samples were adhered on sample holder with double sided tape, the samples coated with about 135 Angstrom Au/Pd (device coating speed  $3 \text{ \AA} \text{ s}^{-1}$ ) with SC 7620 mini Sputter Coater then they were examined by SEM (Aponte et al 2014).

**Image Analysis (IA):** Image Pro Plus 6.0 (Media Cybernetics Inc., USA) software was used for the evaluation of the SEM views. The diameter of starch granules were measured through the software with appropriate calibration.

**Statistical Analysis:** One sample *t*-test (SPSS 17.0.1) were used for the comparison ( $P < 0.05$ ) of

the results. All experiments on the wheat samples were carried out in triplicate on duplicate samples.

### 3. Results

**Morphological properties:** The scanning electron microscopy (SEM) micrographs shown that Einkorn had opaque but durum wheat had a vitreous appearance. In addition, SEM images were evaluated by Image ProPlus software, the diameters of starch granules were measured after appropriate calibration. Both wheat samples contained starch granules of spherical and lenticular shapes that were distributed throughout the protein matrix. The starch granules which were small as well as large could be easily separated visually in Einkorn texture. But, the protein bonds were more compact in the image of durum wheat and the diameter of starch granules located in the protein matrix lower than ones of Einkorn (Figure 1).

Wheat starch granules generally exhibit a bimodal distribution in the structure and can be classified as A-type: 20-35  $\mu\text{m}$  and B-type: 2-10  $\mu\text{m}$  (Delcour & Hoseneay 2010). In Figure 2, the B type of granules was captured in SEM images of both wheat types. But the A-type starch granules were seen as separate phase and captured clearly in einkorn images, they were not captured in durum wheat images due to vitreous texture. Moreover, the diameter of A-type granules was lower than the classification of literature (Delcour & Hoseneay 2010). But the diameters of granules of einkorn for both types granules were higher than ones of the durum wheat.

The SEM micrograph shows that Einkorn kernels had a higher proportion of endosperm and smaller amounts of the external pericarp and aleurone layers (Figure 1). The seed size of einkorn had a marked difference from durum wheat. The sizes were smaller than durum wheat (Figure 1 and Table 1). Although the bran fraction of einkorn is thinner than durum wheat (Figure 1 and Table 2), the percentage of this layer is higher than other haploid wheat types. This phenomenon is related to its smaller and lightweight seed structure (Borghini

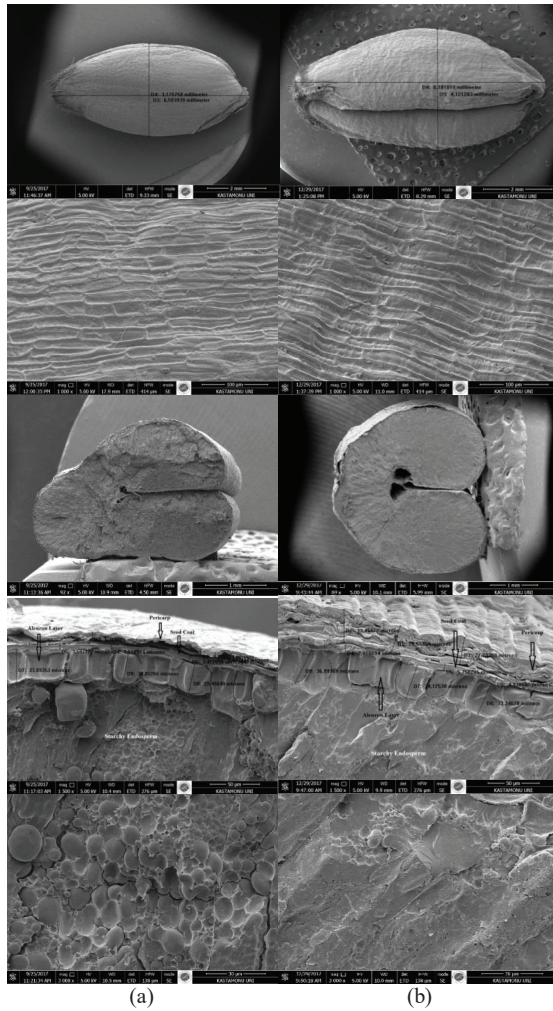


Figure 1- Scanning Electron Microscopy (SEM) micrographs of wheat samples (a, einkorn; b, durum wheat)

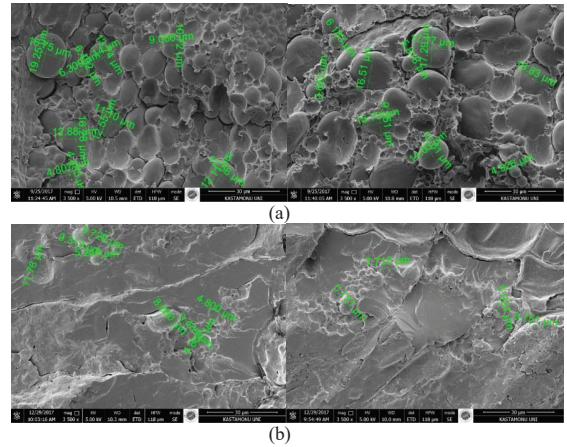


Figure 2- Scanning Electron Microscopy (SEM) micrographs (3500X); Diameter of starch granules of wheat samples (a, einkorn; b, durum wheat)

et al 1996; Løje et al 2003), whose average weight was in the range  $27.94 \pm 2.10$  g. It was  $54.6 \pm 8.45$  g for durum wheat per 1000 kernels.

Physicochemical properties: Actually the wheat kernels are a good source of dietary fibers which some of them are been resistant to digestion and absorption in the small intestine, but some of them are been totally or partially fermented in the large intestine. In this study, it was found that einkorn had lower dietary fiber than the durum wheat. In conducted various studies, it was indicated that the dietary fiber content of *Triticum monococcum* varied to 93-128 g kg<sup>-1</sup> (Loje et al 2003; Grausgruber et al 2004; Gebruers et al 2008). But it was higher in free-threshing wheats, as reported by Gebruers et al(2008) (durum wheat, 134 g kg<sup>-1</sup> DM; bread

Table 1- Diameter of starch granules and thickness of layer of wheat samples

Property	Granule/Layer	Einkorn ( $\mu\text{m}$ )/1000	Durum wheat ( $\mu\text{m}$ )/1000	P value*
Diameter of granules	Class A	16.77 $\pm$ 2.31	9.18 $\pm$ 2.01	0.005
	Class B	5.07 $\pm$ 1.41	4.20 $\pm$ 0.71	0.091
	Pericarp	5.77 $\pm$ 0.34	29.74 $\pm$ 3.29	0.000
Thickness of layer	Seed coat	3.54 $\pm$ 0.12	5.91 $\pm$ 1.05	0.010
	Aleuron	30.88 $\pm$ 1.94	34.83 $\pm$ 1.93	0.072

\*, P<0.05 means statistically different

**Table 2- Physicochemical properties of wheat samples**

Property	Einkorn	Durum wheat	P value*	
Kernel weight(g/1000 pieces)	27.94±2.10	54.60±8.45	0.000	
Softening degree (BU)	186.00±1.50	28.00±0.90	0.000	
Water absorbtion (%)	51.80±0.20	65.30±0.00	0.000	
Protein (%)	12.74±0.80	11.85±1.05	0.194	
Wet gluten (%)	6.75±0.20	9.82±0.50	0.000	
Gluten index (%)	42.00±1.10	85.00±0.30	0.000	
Ash (%)	2.34±0.13	1.96±0.079	0.037	
Fat (%)	2.64±0.21	1.85±0.090	0.023	
Moisture (%)	10.31±0.05	11.18±0.07	0.001	
Total dietary fiber (%)	10.76±1.36	13.10±0.02	0.228	
Total carotenoids (µg/g dw)	2.79±0.21	3.58±0.10	0.023	
Antioxidant activity (Inhibition%)	18.60±0.20	9.23±0.55	0.000	
Color	<i>L</i> *	50.16±1.13	60.19±0.93	0.000
	<i>a</i> *	8.14±0.12	3.32±0.09	0.000
	<i>b</i> *	18.85±0.16	22.44±0.79	0.001

\*, P<0.05 means statistically different

wheat, 147-152 g kg<sup>-1</sup> DM) and Andersson et al (2013) (bread wheat, 134 g kg<sup>-1</sup> DM). Einkorn was poor in dietary fiber compared to durum wheat, but rich in proteins, lipids and mostly all elements (including Zn and Fe).

It was indicated that by Hidalgo & Brandolini (2014), the good concentration of several antioxidant compounds (carotenoids, conjugated polyphenols, tocots, and phytosterols) and low lipoxigenase and β-amylase activities (which limit antioxidant degradation during food processing) contribute to the excellent nutritional properties of einkorn.

In the study, it was found that einkorn sample was softer than durum wheat and its water absorption quite lower than durum wheat. These parameters are directly related to baking and milling technology, and they should be taken into account when the einkorn processes to cereal products such as flour, semolina, bulgur or various doughs.

Mineral content: Statistically significant differences were detected among einkorn and

durum wheat in terms of both major and trace elements (Table 3). Especially the contents of Fe, Zn and Al were great significantly higher (P<0.05) in einkorn. Einkorn is considered a good source of micronutrients (Ozkan et al 2007). The genotype, climate and soil all play significant roles in mineral

**Table 3- Mineral contents of wheat samples**

Minerals	Einkorn	Durum wheat	P value*
Na, mg kg <sup>-1</sup>	59.70±0.01	26.30±0.01	0.000
K, mg kg <sup>-1</sup>	4963.60±0.05	4769.52±0.09	0.000
Ca, mg kg <sup>-1</sup>	549.45±0.03	479.21±0.05	0.000
Fe, mg kg <sup>-1</sup>	73.10±0.00	27.82±0.16	0.000
Zn, mg kg <sup>-1</sup>	67.90±0.00	14.74±0.12	0.000
P, mg kg <sup>-1</sup>	3924.30±0.17	3348.90±0.22	0.000
Mg, mg kg <sup>-1</sup>	1294.70±0.06	1118.60±0.09	0.000
Al, µg kg <sup>-1</sup>	5343.50±0.44	<100	0.000
Co, µg kg <sup>-1</sup>	< 31.70	< 31.70	-
Cu, µg kg <sup>-1</sup>	6120.00±0.30	5890.32±0.12	0.000
Cd, µg kg <sup>-1</sup>	< 6.30	< 6.30	-
Ba, µg kg <sup>-1</sup>	1746.20±0.08	698.50±0.02	0.000

\*, P<0.05 means statistically different

content levels in cereals. In addition, most mineral elements are more abundant in the bran fraction. Therefore the higher trace and major mineral contents of einkorn may be partially attributed to the smaller size of its seeds, which increases the (bran+germ)/endosperm ratio and leads to a non-genotypic or non-climatic concentration effect.

Cereals and especially their refined flours are poor in concentration and bioavailability of some of the trace and major minerals. So they may fail to satisfy human daily requirements, and various regions where have cereal based daily diet in the world suffer from micronutrient deficiencies.

#### 4. Conclusions

In the study, it has been found that einkorn has a high-nutritional value, especially considered for its high protein and antioxidant content. The grain differs from conventional varieties due to its low level of gluten, high protein content. Also, it has high fat and mineral content level. Despite its extraordinary characteristics, the cultivation of Einkorn, has been declining constantly. Current trends toward slow-impact and sustainable agriculture as well as an increase in the utilization of “biological” and “functional” products suggest that this cereal may still play a role in human consumption. The results and several studies concluded that einkorn is a promising candidate for the development of new or special foods such as bakery products, baby food or products with high content of dietary fiber, carotenoids and tocopherols. Durum wheat is rich in lutein due to selection for yellow color. Einkorn wheat has also high contents of the carotenoid. Furthermore, because of its high protein content, Siyez wheat gives larger particulate flour like fine semolina. For this reason, Einkorn can be a good supplement raw-material to durum wheat and it should be tried for several types of pasta. Already traditional Siyez erişte (traditional einkorn pasta), Siyez ezmesi (flake) and Siyez bread have just been produced in Kastamonu for many years. The functional properties of these products should be examined and used for the developing of the new products.

On the other hand, the content of major and trace elements such as Fe and Zn were higher statistically significant in einkorn than durum wheat. The micronutrient malnutrition effects over two billion people in the developing countries. Iron (Fe) deficiency alone affects most of the women globally and >47% of all preschool-aged children, often leading to impaired physical growth, learning capacity and mental development. Zinc (Zn) and iron (Fe) deficiency are thought to affect billions of people, hampering growth and development, and destroying immune systems. In many micronutrient-deficient shown countries, wheat and cereals are the dominant staple food of the diet. For that reason, many foods can be fortified through einkorn or einkorn products such as semolina, flour, germ or bulgur for the regions affected by malnutrition. The natural fortification is the power and a new approach to improve the nutritional quality of foods consumed in the daily diet for today’s consumers who demand clean-label and additive-free food.

To ensure the sustainability of Siyez wheat, the main steps should to make the direct sale of the product possible domestic and abroad, increase harvesting area and production rate, to investigate functional and nutritional qualities and to find alternative usage areas. Moreover, agricultural production incentives should be provided by the government and domestic or abroad advertisement should be made about Siyez wheat and its products.

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