



South African Journal of Animal Science 2020, 50 (No. 4)

# Arbuscular mycorrhizae inoculation of einkorn wheat affects fatty acid, nutrient and mineral concentrations

T. Ayaşan<sup>1#</sup>, S. Esen<sup>2</sup>, V. Kader Esen<sup>3</sup>, H. Eseceli<sup>4</sup> & E. Cabi<sup>2</sup>

Osmaniye Korkut Ata University, Kadirli Academy of Applied Sciences, Osmaniye, Turkey
 <sup>2</sup> Tekirdağ Namık Kemal University, Department of Biology, Tekirdağ, Turkey
 <sup>3</sup> Bursa Uludağ University, Faculty of Agriculture, Department of Animal Science, Bursa, Turkey
 <sup>4</sup>Kastamonu University, Faculty of Veterinary, Department of Animal Nutrition and Nutritional Diseases, Kastamonu, Turkey

(Submitted 5 May 2020; Accepted 25 June 2020; Published 26 September 2020)

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#### Abstract

The purpose of this study was to determine the effect of arbuscular mycorrhizae (AM) inoculation, which was administered to increase the quality of einkorn wheat in its fatty acid composition, quality and mineral concentration. Wheat grown from AM inoculated seed (AM+) had increased the dry matter (DM), iron (Fe), monounsaturated fatty acids (MUFA), especially oleic acid, and alpha-linolenic acid, and cis-5,-8,-11,-14, and cis-17 eicosapentaenoic acid from polyunsaturated fatty acids (PUFA) (P <0.05), but decreased crude ash (CA), potassium (K), and total saturated fatty acids (UFA), especially myristic acid, palmitic acid, stearic acid and monounsaturated fatty acids, especially cis-palmitoleic acid, cis-10-heptadecenoic acid, cis-11 eicosenoic acid, erucic acid, and linoleic acid (P <0.05) compared with einkorn wheat grown from non-inoculated seed (AM-). Crude protein (CP) content ranged from 10.56% to 10.68%, and ether extract (EE) content varied between 2.03% and 2.41%, neither of which was significantly different between treatments. Significant increases were observed in UFA, and in the ratios of UFA to SFA and PUFA to SFA in AM+compared with AM-.

**Keywords**: mycorrhizal inoculation, nutrient composition, *Triticum monococcum* \*Corresponding author: tayasan@gmail.com

# Introduction

Arbuscular mycorrhizal fungi are naturally ubiquitous and beneficial fungi in agricultural ecosystems. These fungi have symbiotic relationships with many cereal crops (Smith & Smith, 2011), in which they have an active role in providing mineral nutrients to plants (Somenahallya *et al.*, 2018). Akpınar (2011) reported that the most important benefit of mycorrhiza is the expanded surface area of roots of plants that have coarse and weakly structured root systems, thus increasing the uptake of nutrients from the soil. Thus, mycorrhizal inoculated barley had greater CP content, but less ADF and ADL than a control group that was not inoculated (Sabia *et al.*, 2015).

Einkorn wheat (*Triticum monococcum*) was one of the first cultivated wheat varieties. Although it originated in Turkey, it is cultivated in the Balkans. Einkorn wheat is important in human health, and human and animal nutrition and is one of the most popular products of the organic market. Einkorn wheat bread has not been gene edited and has a low risk of inducing allergic reactions. It is rich in vitamins, minerals, and antioxidants and has some positive effects on human health (Hidalgo & Brandolini, 2014). It has higher levels of CP, phosphorus (P), and K compared with other varieties of wheat. It is also used as an animal feed and as the main ingredient in cakes and breads (Nakov *et al.*, 2016). Unlike modern cultivars, einkorn wheat has a pair of each of 14-chromosomes. It can be consumed as bulgur by humans and its grain and straw can be fed to animals. With its tight husk structure, einkorn wheat is resistant to diseases and pests and is highly competitive in dry conditions and in infertile soils (Kastamonu Chamber of Commerce, 2019). These attributes make it a putative source of new and useful genes, and a crop that has good agri-biological properties (Nakov *et al.*, 2016).

URL: http://www.sasas.co.za

ISSN 0375-1589 (print), ISSN 2221-4062 (online) Publisher: South African Society for Animal Science Recent concerns have led to increased demand for healthy foods and products, which has increased the requirement for bulgur made from einkorn wheat (Erba *et al.*, 2011; Karabak *et al.*, 2019). Ertop (2019) reported that einkorn wheat contained 12.74% CP, 89.91% DM, 2.48% ash, 2.51% fat, and 10.76% total cellulose. An analysis of the fatty acid composition of einkorn wheat distinguished at least 14 compounds (Hidalgo & Brandolini, 2014; Nakov *et al.*, 2016). Gabrovská *et al.* (2002) found the fatty acid contents in einkorn wheat (g/100 g) included myristic acid, 0.18; myristoleic acid, 0.08; palmitic acid, 13.9; palmitoleic acid, 0.21; stearic acid, 0.87; oleic acid, 25.3; linoleic acid, 54; linolenic acid, 3.60; arachidic acid, 0.15; cis-11-eicosenoic acid, 1.36; behenic acid, 0.14; and erucic acid, 0.23. Hidalgo and Brandolini (2014) asserted that linoleic acid (C18:2n6), oleic acid (C18: 1n9 + C18:1n7), and palmitic acid (C16:0) acid were the most prevalent fatty acids in einkorn wheat, comprising 50.9 - 54%, 24.8 - 26.4%, and 13.9 - 16.7%, respectively, of total fatty acids.

In Turkey, einkorn wheat is grown mostly in Kastamonu (Emeksizoğlu, 2016). In 2016, einkorn wheat was sown on 783.5 ha in the region and produced 1959 tons (Kastamonu Chamber of Commerce, 2019). In this study, the effect of AM inoculation on the fatty acid, nutrient, and mineral contents of einkorn wheat was examined.

# Materials and methods

The field experiment was conducted at a commercial farm in Pinarbasi, Kastamonu (41.55 N, 33.10 E), Turkey. There were two treatments, namely wheat grown from seed with and without AM inoculation (AM+ and AM-, respectively). The einkorn wheat was planted in mid-November 2018 and the grain was harvested in late July 2019. A total of 5 ha was cultivated with einkorn wheat. The seed used on 4.5 ha was AM-, but the seed used on the remaining 0.5 ha was AM+. Three random samples were collected from each treatment while harvesting.

The einkorn wheat seed was inoculated with AM fungi before sowing. The AM spores were collected from the rhizosphere soil of maize plants (Gerdemann & Nicolson, 1963) and were identified as a mixture of *Glomus* spp. according to their morphological characteristics (Schenck & Perez, 1990). The *Glomus* spp. was mixed with silicon dioxide (1: 1 w/w), which served as an aid to coating the einkorn wheat seed (Merck, Darmstadt, Germany). Abnormal grains were removed and then the remainder were coated by gradually adding the inoculum-coating mixture, according to the pan-coating method (Scott *et al.*, 1991) and air-dried at  $24 \pm 2$  °C for 72 hours. Non-inoculated control seeds were coated only with silicon dioxide.

After harvesting, all samples were milled through a 1-mm sieve, transported to the laboratory, and stored in glass jars for further chemical analyses. All chemical analyses were carried out in triplicate. In the laboratory, the DM, CP, CA, EE, and crude fibre (CF) content of the samples were determined using AOAC methods (AOAC, 1990).

The Zn, Cu, Fe, sodium (Na), and K contents of the milled wheat were measured in this study. Approximately 0.5 g of sample was weighed into a vessel that was resistant to temperature and pressure, with 8 mL nitric acid and 2 mL hydrogen peroxide for microwave digestion. The digested sample was allowed to cool before being transferred quantitatively into clean Falcon tubes, completed to 25 mL final volume with deionized water, and analysed with a Shimadzu AA-7000 (Shimadzu Corporation, Kyoto, Japan) flame atomic absorption spectrophotometer (Milestone, 2009).

Lipids were extracted from the einkorn wheat with diethyl ether according to AOAC (1990). The diethyl ether extracts were evaporated to dryness and resuspended in 10 mL hexane and 0.5 mL 2 N KOH for at least two hours in a dark place. Then, the clear upper phase was passed through the 0.45  $\mu$ m PTFE filter and transferred to the vials (Tanacı, 2015). A GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID) and a TR-CN100 (Teknokroma, Barcelona, Spain) capillary column (60 m x 0.25 mm x 0.20  $\mu$ m) was used for analyses. Hydrogen was used as the carrier gas and the flow rate was controlled at 40 ml min<sup>-1</sup>. The injector temperature was kept at 250 °C and the injection amount of the sample was 1.0  $\mu$ L. The column temperature procedure was started at 90 °C and held for 4 min, then heated up to 240 °C at 3 °C per min, and held at 240 °C for 12 min. The peaks were identified with Supelco 37 component FAME mix according to area normalization.

One-way analysis of variance was performed using SAS (JMP version 13.2, SAS Institute Inc., Cary, North Carolina, USA) to determine the effects of treatment on the nutritive value, concentrations of selected minerals and fatty acids composition. The level of significance between individual means was determined using student's t-test. Differences were considered significant at  $P \le 0.05$ .

# **Results and Discussion**

The effects of AM inoculation on the nutrient composition and mineral content of einkorn wheat are shown in Table 1.

	Control	Inoculated	SE	<i>P</i> -value
Dry matter, g kg <sup>-1</sup>	915.3 <sup>b</sup>	949.0ª	2.12	0.0004
Crude protein, g kg <sup>-1</sup>	105.6	106.8	0.56	0.2159
Ether extract, g kg <sup>-1</sup>	20.3	24.1	1.67	0.2491
Crude fibre, g kg <sup>-1</sup>	104.3	95.5	3.05	0.1123
Crude ash, g kg <sup>-1</sup>	62.3 <sup>a</sup>	58.1 <sup>b</sup>	0.37	0.0013
Zinc, mg 100 g <sup>-1</sup>	3.71	3.70	0.03	0.7284
Copper, mg 100 g <sup>-1</sup>	0.24	0.26	0.01	0.1979
Iron, mg 100 g <sup>-1</sup>	4.93 <sup>b</sup>	7.79 <sup>a</sup>	0.17	0.0003
Sodium, mg 100 g <sup>-1</sup>	3.14	2.36	0.40	0.2348
Potassium, mg 100 g <sup>-1</sup>	600.2 <sup>a</sup>	558.8 <sup>b</sup>	5.39	0.0056

**Table 1** Comparison between nutrient composition of einkorn wheat grown from seed inoculated with arbuscular mycorrhizae and control

The DM content of AM+ and AM- differed significantly in this study. However, both were greater than the 89.81%, 89.69%, and 88.95% DM found by Ertop (2019), Ertop and Atasoy (2019), and Vrancheva *et al.* (2019), respectively.

The treatments in this study did not affect the CP content of the einkorn wheat significantly (P > 0.05). Sabia *et al.* (2015) reported that AM+ barley contained more CP than AM- barley (9.5% and 8.1%,). They also found that when berseem clover seed was inoculated with mycorrhizae the crop contained more CP than the crop from non-inoculated seed (12.7% and 12.2%). Numerous studies found CP levels in einkorn wheat that were comparable with or higher than those observed in the present study (Sachambula *et al.*, 2015, 15.8%; Arzani & Ashraf, 2017, 15.5 - 22.8%; Patijn *et al.*, 2018, 10.5 - 11.7%; Costanzo *et al.*, 2019, 12%).

The treatments did not affect the EE content of the einkorn wheat (P > 0.05). Similarly, Sabia *et al.* (2015) observed that the EE contents of the mycorrhizal inoculated barley and berseem clover were similar to the non-inoculated controls. In a variety of studies (e.g. Şanal, 2018; Costanzo *et al.*, 2019; Abdel-Aal & Hucl, 2014), the fat content or EE of einkorn wheat was found to vary between 1.8% and 4.4%. This variation might stem from the differences in analytical methods, places of cultivation, and country, and whether it was the main or the second crop. Brandolini and Hidalgo (2011) reported that the fat content of einkorn wheat was higher than that of other wheat varieties (4.2 g/100 g vs. 2.8 g/100 g).

The AM+ did not affect the CF content of the resulting plants ( $\dot{P}$  >0.05). Sabia *et al.* (2015) found similar values (P >0.05) for the CF content of mycorrhizal inoculated barley (25.7%) and the non-inoculated one (25.7% and 26.0%). However, Sabia *et al.* (2015) observed an increase in the CF content of berseem clover (17.6%) grown from inoculated seed compared with clover grown from seed that was not inoculated (16.5%). Luje *et al.* (2013), Abdel-Aal and Hucl (2014) and Ertop (2019) reported the cellulose levels in einkorn wheat were 7.6-9.9%, 8.7%, 10.76%, respectively.

The AM+ treatment decreased the CA content in the grain that was produced (P <0.05). The levels of CA observed in the present study were greater than those observed in previous studies of einkorn wheat (e.g. Abdel-Aal & Hucl, 2014; Şanal, 2018; Ertop, 2019; Vrancheva *et al.*, 2019), in which the average CA content was 2.2%. Luje *et al.* (2013) found the CA content of einkorn wheat was only slightly higher than was observed in the previously mentioned studies at 2.3 - 2.8%. In contrast with the present results, Sabia *et al.* (2015) did not detect significant differences in the CA content of barley owing to the mycorrhizal inoculation of the seed.

Adıgüzel (2019), stated that the mineral content of einkorn wheat grain varied, depending on the climate, soil, and genotype. In examining the treatment effects on the content of Zn, Cu, Fe, Na, and K, only Fe and K differed as a result of seed inoculation (P < 0.05), with the Fe content being increased whereas the K content was decreased. Ozkan *et al.* (2007) stated that the Fe content in einkorn wheat varied depending on the genotype and averaged 4.70 mg 100 g<sup>-1</sup>, which is a lower level than was observed in the present study. Likewise, Şanal (2018) reported that the Fe content of einkorn wheat was 4.21 mg 100 g<sup>-1</sup>. In other wheats, Kariithi *et al.* (2016) observed that the Fe content was in the range of 1.11 to 3.05 mg 100 g<sup>-1</sup>. Čurna and Lacko-Bartošová (2017) reported it was between 3.82 and 5.55 mg 100 g<sup>-1</sup> (4.59 on average), and

Vrancheva *et al.* (2019) found it was 4.80 mg 100 g<sup>-1</sup>. In contrast, Ertop and Atasoy (2019) observed that wheat had a higher Fe content of 7.31 mg 100 g<sup>-1</sup>.

Abdel-Aal *et al.* (1995), Gabrovská *et al.* (2002), Grain Nutrition Comparison (2009), and Şanal (2018) observed that the K content of einkorn wheat was approximately 390 - 400 mg 100  $g^{-1}$ . Hidalgo and Brandolini (2014) observed a range of values for the K content of einkorn wheat from 390 to 466 mg 100  $g^{-1}$ , and Ertop and Atasoy (2019) and Vrancheva *et al.* (2019) found K levels in einkorn wheat of 496.3 and 420.1 mg 100  $g^{-1}$ , respectively.

The contents of Zn, Cu, and Na in the einkorn wheat in this study were not affected by AM+. The average level of Zn observed here was less than the observations of 4.2 mg 100 g<sup>-1</sup> (Gabrovská *et al.*, 2002), 5.32 mg 100 g<sup>-1</sup> (Şanal, 2018), 5.48 mg 100 g<sup>-1</sup> (Ozkan *et al.*, 2007) and 6.79 mg 100 g<sup>-1</sup> (Ertop & Atasoy, 2019). However, Ozkan *et al.* (2007) observed that the Zn content of einkorn wheat depended on its genotype. They found that the Zn content of 54 samples of einkorn wheat grown in the highlands was between 3.6 and 7.6 mg 100 g<sup>-1</sup> (5.1 mg 100 g<sup>-1</sup> on average) and those grown in the lowlands had Zn content of 4.4–8.4 mg 100 g<sup>-1</sup> (5.9 mg 100 g<sup>-1</sup> on average). Kariithi *et al.* (2016) asserted that the Zn content in other varieties of wheat was from 2.61 to 9.15 mg 100 g<sup>-1</sup>. In contrast to the present results, Pellegrino *et al.* (2015) reported that AM+ increased the Zn content of wheat grain by 10%.

In this study the Cu content of the einkorn wheat was substantially less than previous observations. Ozkan *et al.* (2007), Abdel-Aal and Hucl (2014), Ertop and Atasoy (2019), and Vrancheva *et al.* (2019) all observed that the Cu content of einkorn wheat was 6 mg kg<sup>-1</sup> or slightly higher.

Previous research showed the Na content of einkorn wheat was variable, with levels in the range of 1.8 mg 100 g<sup>-1</sup> (Gabrovská *et al.*, 2002) to 7.4 mg 100 g<sup>-1</sup> (Vrancheva *et al.*, 2019). The results from the present study are within this range.

The treatment effects on fatty acid contents of AM grown from inoculated and non-inoculated seed are detailed in Table 2. In this study, the major constituent fatty acids were palmitic acid, oleic acid, and linoleic acid, all of which were affected ( $P \le 0.01$ ) by the inoculation of einkorn wheat seed with AM. However, the differences between the treatments were fairly small ( $\le 4\%$ ).

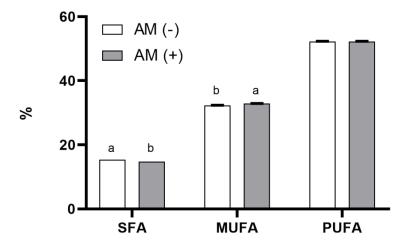
Table 2 Comparison of fatty acid composition of einkorn wheat grown from seed inoculated with arbuscular mycorrhizae and control

Fatty acids	Control	Inoculated	SE	<i>P</i> -value
Lauric acid	0.06	ND	0.001	
Myristic acid	0.14 <sup>a</sup>	0.12 <sup>b</sup>	0.002	0.0020
Pentadecylic acid	0.08	0.09	0.001	0.1145
Palmitic acid	13.38 <sup>a</sup>	12.93 <sup>b</sup>	0.004	< 0.0001
Margaric acid	0.14 <sup>b</sup>	0.15 <sup>a</sup>	0.001	0.0007
Stearic acid	1.14 <sup>a</sup>	1.10 <sup>b</sup>	0.001	< 0.0001
Arachidic acid	0.23 <sup>a</sup>	0.22 <sup>b</sup>	0.001	0.0006
Behenic acid	0.20	0.20	0.001	0.2943
Cis palmitoleic acid	0.25 <sup>a</sup>	0.18 <sup>b</sup>	0.001	<0.0001
Cis-10 heptadecenoic acid	0.13 <sup>a</sup>	0.05 <sup>b</sup>	0.003	< 0.0001
Oleic acid	26.83 <sup>b</sup>	27.93 <sup>a</sup>	0.008	< 0.0001
Cis-11 eicosenoic acid	4.73 <sup>a</sup>	4.40 <sup>b</sup>	0.006	< 0.0001
Erucic acid	0.26 <sup>a</sup>	0.19 <sup>b</sup>	0.004	0.0040
Nervonic acid	0.15	0.14	0.002	0.1098
Linoleic acid	50.49 <sup>a</sup>	50.42 <sup>b</sup>	0.011	0.0115
Gamma linolenic acid	ND	0.06	0.002	
Alpha linolenic acid	1.46 <sup>b</sup>	1.49 <sup>a</sup>	0.006	0.0144
Cis-11,-14 eicosadienoic acid	0.07	0.07	0.002	0.5300
Arachidonic acid	0.09 <sup>a</sup>	0.07 <sup>b</sup>	0.003	0.0089
Cis-5, -8, -11, -14, cis-17 eicosapentaenoic acid	0.18 <sup>b</sup>	0.19 <sup>a</sup>	0.001	0.0020

In the present study, AM inoculation of einkorn wheat seed reduced the palmitic acid level and increased the levels of oleic and linoleic acids in the grain that was produced. In comparison, Vrancheva *et al.* (2019) found the palmitic, oleic and linoleic acid levels in einkorn wheat were 13.15%, 29.70%, and 49.57%, respectively.

The most common fatty acid in bread-type wheats is linoleic acid but, unlike einkorn wheat, these varieties contain more palmitic acid than oleic acid. Ruibal-Mendietaa *et al.* (2004) asserted that the main fatty acid contents of winter and spring wheat varieties were linoleic, palmitic, and oleic acids. The  $\alpha$ -linolenic and stearic fatty acids were relatively minor contributors to these wheats. Demir *et al.* (2015) found five major contributors to the fatty acid profile in einkorn wheat, namely linoleic acid (49.43%), oleic acid (34.34%), palmitic acid (10.27%), linolenic acid (3.59%), and eicosenoic acid (2.38%). Likewise, Gabrovská *et al.* (2002) described the fatty acid profile of einkorn wheat as myristic acid, 0.18; myristoleic acid, 0.08; palmitic acid, 13.9; palmitoleic acid, 0.21; stearic acid, 0.87; oleic acid, 25.3; linoleic acid, 54.0; linolenic acid, 3.60; arachidic acid, 0.15; cis 11 eicosenoic acid, 1.36; behenic acid, 0.14; and erucic acid, 0.23 g/100 g<sup>-1</sup>. Hidalgo and Brandolini (2014) reported that linoleic, oleic and palmitic acids were found in einkorn wheat at higher levels than 11 other fatty acids.

By forming groups of SFAs, MUFAs and PUFA, AM+ influenced SFAs significantly (AM+ =  $14.81 \pm 0.01$  vs. AM- =  $15.37 \pm 0.01$ ) and MUFAs (AM+ =  $32.89 \pm 0.01$  vs. AM- =  $32.34 \pm 0.01$ ), but not the PUFA content (AM+ =  $52.30 \pm 0.02$  vs. AM- =  $52.29 \pm 0.02$ ) (Figure 1). These changes led to an increased quantities of UFAs (AM+ =  $85.19 \pm 0.01$  vs. AM- =  $84.63 \pm 0.01$ ) and the ratios of UFA and PUFA to SFA.



**Figure 1** Comparison of total saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids of einkorn wheat grown from seed that inoculated with arbuscular mycorrhizae and control

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid a,b Values within fatty acid groups with different superscripts differ significantly at  $P \le 0.001$ 

Although the relative ratios in einkorn wheat and bread-type wheat varieties are the same, einkorn wheat contains 50% more fat than bread-type wheat (Hidalgo & Brandolini, 2014). Compared with bread-type wheat, einkorn wheat has more MUFAs, fewer PUFAs, and fewer SFAs (Hidalgo *et al.*, 2009). In feeds and foods, the coexistence of high MUFA and PUFA concentrations and low SFA content contribute to the prevention of cardiovascular diseases. Monounsaturated fatty acids and PUFAs affect fat and cholesterol synthesis through reducing atherosclerosis and thrombosis. On the other hand, high MUFA and low PUFA contents ensure greater resistance to oxidation and longer shelf life (Hidalgo *et al.*, 2009).

Decreasing the consumption of SFAs and increasing that of MUFAs is important in reducing cardiovascular diseases in people (Çakmakçı & Tahmas Kayaoğlu, 2012). Armanino *et al.* (2002) and Kan (2015) reported that the total UFA levels of wheat were 75.31 - 77.34%. Dinu *et al.* (2018) stated that because high levels of MUFA and PUFA and a low level of SFA coexist in einkorn wheat, the total cholesterol and low density lipoprotein cholesterol levels were low.

Seferoğlu *et al.* (2006) reported that the fatty acid composition of seeds was affected to a great extent by ecological conditions. Dolde *et al.* (1999) suggested that the fatty acid composition of wheat was related to the differences in temperature at which the crop matured. However, it was not anticipated that ecological conditions would contribute to the present results in that the AM+ and AM- crops were grown in close proximity.

## **Conclusions**

Inoculation of einkorn wheat seed with AM played an effective role in increasing the levels of DM, Fe and some UFAs. Thus, this treatment could improve the healthfulness qualities of bulgur made from einkorn wheat.

### **Acknowledgements**

The authors thank Mr. N. Bodur for cooperation during to field trial.

#### **Authors' Contributions**

TA, SE and EC contributed to the project idea, design and execution of the study. SE, VKE, HE and EC were in charge of laboratory analyses. TA and SE were responsible for supervision and writing the manuscript.

#### **Conflict of Interest Declaration**

The authors declare that they have no competing interests.

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