*Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]*

# ALTERATION IN α-TOCOPHEROL, SOME MINERALS, AND FATTY ACID CONTENTS OF WHEAT THROUGH SPROUTING

**I. Ozturk,[1] O. Sagdic,[2*] M. Hayta,[1] and H. Yetim[1]**

UDC 547.915

*In this study, two wheat cultivars (cvs Demir 2000 and Konya 2002) were sprouted at 17°C and 85% relative humidity for 9 days. Then the wheat sprouts were dried and ground. The wheats and their sprouts were analyzed for α-tocopherol, some minerals, and fatty acid contents during the sprouting. The α-tocopherol contents of cvs Demir 2000 and Konya 2002 were 26.99 and 23.77 mg/kg, respectively, and these values reached 54.62 and 47.19 mg/kg for their sprouts, respectively. Mineral analyses showed that the mineral contents of the sprouts also increased with sprouting. Fatty acids, e.g., 4:0, 6:0, 8:0, and 10:0, could not be detected in the sprouts while some of them, e.g.,* cis-*18:1 and* cis,cis-*18:2, were decreasing during sprouting, but an increase in 18:3 n3 (ω-3) contents was noted. The results showed that sprouting of wheat grains leads to an increase in some significant functional components.*

**Keywords**: wheat sprout, functional components, α-tocopherol, fatty acid composition.

Cereal products provide mainly carbohydrates, proteins, and dietary fibers required for human nutrition [1, 2].

Cereal sprouts have been known to be more nutritious in terms of vitamins, minerals, and phenolic compounds when compared with their native counterparts [3, 4]. Many researchers reported that sprouting in plants or grains results in an increase in some functional components such as vitamins, minerals, and phenolics [4, 5]. However, wheat sprouts are recently being consumed as a salad, juice, and tablet for their vitamin, mineral, and phenolic contents [4–6].

The chemical composition of the sprout is affected by sprouting conditions such as temperature, humidity, and light. For this reason, determination of the optimum sprouting conditions is considered crucial [4]. The majority of reported experimental data have focused solely on the effects of sprouting on the mineral composition of wheat sprouts [4–6], but few studies have appeared on the effect of sprouting on fatty acid composition of wheat sprouts. However, the beneficial effect of fatty acid composition on human health is well known, and thus, alternative additives or processes which could have a positive effect on human health should be continuously investigated. The aim of the present study was to determine the changes in functional components of the two wheat cultivars Demir 2000 and Konya 2002 during sprouting.

Figure 1 shows the α-tocopherol contents of the wheat samples during sprouting. The amount of α-tocopherol was originally 26.99 mg/kg in Demir 2000 cv and 23.77 mg/kg in Konya 2002 cv and reached values of 54.62 and 47.19, respectively, at the end of the sprouting period ($9^{th}$ day). The difference in α-tocopherol contents between the sprouts of two wheat cvs was statistically significant ($p < 0.05$), and the α-tocopherol contents increased approximately 50% as a result of the sprouting processes for both cvs.

The values obtained in the current study are higher than that of the work of Yang et al. [4]. For example, the α-tocopherol content has been reported as 3.77 and 1.95 mg/kg (D.W.) in wheat flour and wheat sprout flour, respectively, after sprouting at 28°C for 96 h. Finney [7] found that the α-tocopherol content increased 25% as a result of sprouting for four days. The variability in the reported results could be caused by the differences in dry matter, genesis of the wheat cvs, and sprouting conditions.

1) Erciyes University, Engineering Faculty, Department of Food Engineering, 38039 Kayseri, Turkey; 2) Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, 34210 Istanbul, Turkey, fax: +90 352 4375784, e-mail: osagdic@erciyes.edu.tr. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 770–772, November–December 2011. Original article submitted June 26, 2010.

 0009-3130/12/4706-0876 

TABLE 1. Composition of Fatty Acid in Wheat Flour and Sprout Flour (total FA, %)

| Fatty acid | Demir 2000* | | Konya 2002* | |
|---|---|---|---|---|
| | Wheat | Sprout | Wheat | Sprout |
| 4:0 | $0.13^{b} \pm 0.00$ | Nd. | $0.36^{a} \pm 0.09$ | Nd. |
| 6:0 | Nd. | Nd. | $0.23^{a} \pm 0.06$ | Nd. |
| 8:0 | Nd. | Nd. | $0.14^{a} \pm 0.03$ | Nd. |
| 10:0 | $0.11^{b} \pm 0.00$ | Nd. | $0.32^{a} \pm 0.08$ | Nd. |
| 12:0 | $0.14^{b} \pm 0.00$ | Nd. | $0.38^{a} \pm 0.10$ | Nd. |
| 14:0 | $0.59^{b} \pm 0.00$ | Nd. | $1.39^{a} \pm 0.35$ | $0.98^{b} \pm 0.00$ |
| 15:0 | Nd. | Nd. | $0.21^{a} \pm 0.04$ | Nd. |
| 16:0 | $18.22^{a} \pm 0.46$ | $18.35^{a} \pm 2.10$ | $18.76^{a} \pm 0.51$ | $18.77^{a} \pm 3.40$ |
| 16:1 | $0.17^{c} \pm 0.00$ | Nd. | $0.23^{b} \pm 0.01$ | $0.79^{a} \pm 0.00$ |
| 17:0 | Nd. | Nd. | Nd. | $0.47^{a} \pm 0.00$ |
| 18:0 | $1.3^{a} \pm 0.35$ | $1.25^{a} \pm 0.15$ | $2.44^{a} \pm 0.35$ | $4.13^{a} \pm 3.71$ |
| *cis*-18:1 | $15.90^{a} \pm 0.23$ | $8.56^{a} \pm 0.00$ | $18.56^{a} \pm 0.19$ | $14.35^{a} \pm 5.04$ |
| *trans*-C18:2 | Nd. | $0.75^{a} \pm 0.01$ | Nd. | $0.71^{b} \pm 0.01$ |
| *cis,cis*-C18:2 | $59.09^{a} \pm 1.67$ | $53.90^{a} \pm 3.40$ | $52.22^{a} \pm 1.66$ | $47.44^{a} \pm 14.55$ |
| 18:3 n3 | $4.04^{c} \pm 0.16$ | $16.08^{a} \pm 1.50$ | $3.93^{c} \pm 0.12$ | $12.26^{b} \pm 1.36$ |
| 20:1 | $0.82^{a} \pm 0.04$ | Nd. | $0.72^{b} \pm 0.01$ | $0.65^{b} \pm 0.00$ |
| 21:0 | Nd. | $0.83^{a} \pm 0.01$ | Nd. | $0.84^{a} \pm 0.10$ |

*Mean ± standard deviation. [a–c]Different lowercase letters indicate the statistical difference in the same row ($p < 0.05$). Nd.: not detectable.

TABLE 2. Mineral Contents of Wheat Flour and Sprout Flour, mg/kg*

| Mineral | Demir 2000 | | Konya 2002 | |
|---|---|---|---|---|
| | Wheat | Sprout | Wheat | Sprout |
| Mg | $1792.2^{b} \pm 101$ | $3751.3^{a} \pm 429$ | $1992.2^{b} \pm 188$ | $3270.9^{a} \pm 186$ |
| Ca | $313.4^{c} \pm 18$ | $756.2^{a} \pm 49$ | $277.8^{c} \pm 26$ | $602.3^{b} \pm 60$ |
| Fe | $48^{b} \pm 3$ | $76.4^{a} \pm 8$ | $40.7^{b} \pm 4$ | $84.9^{a} \pm 6$ |
| Na | $155.6^{b} \pm 9$ | $744.1^{a} \pm 16$ | $74.6^{b} \pm 7$ | $899.9^{a} \pm 158$ |
| K | $8169.4^{c} \pm 458$ | $13937.8^{a} \pm 1163$ | $10541.7^{b} \pm 992$ | $12739.4^{ba} \pm 1069$ |
| P | $191.7^{b} \pm 11$ | $443.5^{a} \pm 21$ | $228.9^{b} \pm 21$ | $431.1^{a} \pm 53$ |

*Mean ± standard deviation. [a–c]The different lowercase letters indicate the statistical difference in the same row ($p < 0.05$).

The changes in fatty acid (FA) composition are presented in Table 1. In general, sprouting had a significant effect ($p < 0.05$) on FA composition of the wheats (Table 1). While some saturated fatty acids such as 4:0, 10:0, and 12:0 were determined in the wheat flours, they were absent in their sprouts. Again, while the amount of *cis*-18:1 and *cis,cis*-18:2 fatty acids decreases in wheat sprouts, the linolenic acid (18:3 n3) content increases significantly ($p < 0.05$).

In the flours of both wheat sprouts, 18:2 was the dominant saturated FA with values of 53.90 and 47.44% in Demir and Konya cvs, respectively. While the amount of 16:1 was 0.79% in Konya cv, it was not detected in Demir cv. The major saturated FA in both wheat flour and their sprouts was 16:0, comprising approximately 18% of total FA. The level of 18:1 was low in both cvs, but the amount of *cis*-9-18:1 was higher, reaching values of 15.90 and 18.56% in Demir and Konya sprouts, respectively. While *trans*-18:2 FA was not detected in both Demir and Konya cvs, its level was 0.75 and 0.71% in their sprouts, respectively. The values of *cis*-18:2 in Demir and Konya cvs were 59.09% and 52.22 %, respectively, but its level was 53.90% and 47.44% in their sprouts, respectively. Again, while the 20:1 was not detected in wheat flour, it was present in their sprouts. In recent years, the FA $\alpha$-18:3 has been suggested for the prevention of various human disorders like breast cancer, cardiovascular diseases, and severe cardiac arrhythmias [8].

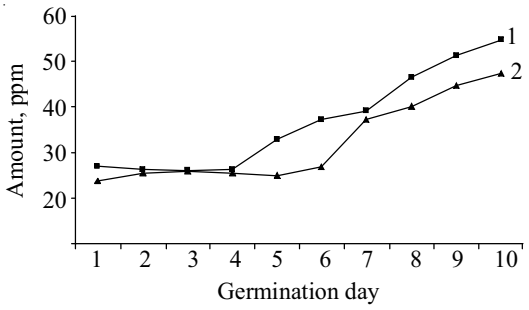


Fig. 1. The $\alpha$-tocopherol contents of the wheat samples during sprouting: DWSF (Demir wheat sprout flour) (1), KWSF (Konya wheat sprout flour) (2).

The present study showed that the amount of 18:3 n3 increased approximately fourfold in both sprouts of wheat cvs Demir and Konya as compared with their native grains. On the contrary, 20:1 FA was not detected in Demir sprout flour, but it was present in Konya wheat cv and its sprout at 0.79 and 0.65%, respectively. The increase in unsaturated FA in wheat sprout could be considered as a significant result in terms of its possible use in human nutrition.

Cereals are a source of some minerals such as K, P, Mg, and Fe that are considered essential in the human diet. However, the amounts of these minerals are significantly decreased as a result of milling [6, 9, 10]. Table 2 shows the mineral content of wheat samples and their sprouts.

Sprouts had significantly higher Mg, Ca, Na, K, and P contents than their wheat kernel counterparts. For instance, the Mg content increased with sprouting in both Demir and Konya cvs by approximately 50% and 40%, respectively. The amount of Fe increased approximately twofold in both wheat cvs after sprouting. Again, K is an essential macronutrient for the plants, and it is the major mineral in both cvs; sprouting resulted in an increase in K content as well. The P content of Demir and Konya grains was 191.7 and 228.9 mg/kg, respectively, but their values reached 443.5 and 431.1 mg/kg in the sprouts, respectively.

Plaza et al. [5] reported an increase in the content of Mg, Fe, and K in wheat kernels (780, 26.2, and 810) and wheat sprout (840, 30.4, and 1430) mg/kg D.W, respectively. However, they reported that Ca and Na contents decreased with sprouting [5]. In another study, Mg, Ca, Fe, Na, and K content of the wheat sprouts increased at the end of 9 days sprouting with values of 1867, 2560, 8.5, 352.2, and 4257 mg/kg, respectively [6]. The differences in reported results of various studies may have originated from the variations in ecological, agronomical, varietal, and sprouting conditions and/or application of the methods.

This research suggest that sprouting of wheat grains leads to increases in some significant functional components such as $\alpha$-tocopherol, some minerals, and fatty acids. The sprouted wheats seemed to have considerable potential for use in functional foods as an ingredient with high biological values. Consequently, sprouting increases the nutritional value of the wheats irrespective of the cvs, particularly in terms of $\alpha$-tocopherol, 18:3 n3 FA, and the minerals (Mg, Ca, Fe, Na, K, and P) studied. Therefore, wheat sprouts can be included in food product formulations as a functional ingredient.

## EXPERIMENTAL

The wheat cvs Demir 2000 and Konya 2002 were obtained from the Research and Development Center of the Ministry of Agriculture, Konya-Turkey.

**Sprouting**. Before sprouting, the wheat kernels were submerged in water containing 1.5% sodium hypochlorite (NaOCl) (seed–water ratio of 1:5, w/v) at room temperature for 30 min to disinfect contaminating microorganisms on the surface. The grains were rinsed with fresh tap water at approximately 15°C for 1 h. Then the grains were steeped in tap water at 17°C for 24 h. For sprouting, wheat samples were placed in an aluminum foil pan with drain holes at the bottom to keep the moisture constant without overwatering, then sprinkled with tap water at 12 h intervals and turned (aerated) by gloved hands once every 24 h in order to prevent rootlets from matting. Sprouting was carried out in dark incubators at 85 ± 5% relative humidity (RH) and 17°C for nine days according to the procedure described by Yang et al. [4]. Sprouted wheat samples were harvested and then freeze-dried (Labconco, FreeZone 2.5, USA) for $\alpha$-tocopherol analysis. For fatty acid and mineral analysis,

the sprouts were dried at 55°C in a conventional oven (Nuve, ID 501, Turkey). All the samples were powdered by a Waring blender (Waring blender, USA) and screened with a 35 mesh sieve (Ultralab, Turkey) before analyses.

**$\alpha$-Tocopherol Content**. The $\alpha$-tocopherol content of dried wheat sprouts was determined by HPLC according to the procedure described by Yang et al. [4]. The extraction was preceded by alkaline saponification. Freeze-dried wheat sprout powder (1 g) was mixed with ethanol (10 mL) containing 0.1% pyrogallol and sodium hydroxide solution (2 mL, 40%), and the mixture was incubated for 30 min at 70°C. After cooling, 10 mL of hexane–ethyl acetate (9:1, v/v) and 10 mL of 1% sodium chloride were added and the whole remixed. The upper layer of the mix was separated and evaporated under vacuum at 60°C. The dried sample was then redissolved in 5 mL of methanol, vortexed, and centrifuged at 1200 rpm for 10 min, and the clean upper layer was collected. Then $\alpha$-tocopherol content of the extract was determined by a HPLC (Agilent 1100, USA). In the HPLC analysis of tocopherols we used a C18 reverse-phase column. The flow rate was set at 1.5 mL/min. The mobile phase consisted of methanol–acetonitrile (50:50 v/v). Detection was performed with a fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm.

**Fatty Acid Composition**. The fatty acid composition of the samples was determined according to [11]. For the analyses, the samples were extracted with ether and stored in Eppendorf tubes, and then the air was removed and replaced with nitrogen. The samples were stored at –60°C until analysis. The oil (100 mg) was saponified with 100 μL 2 N KOH, and 3 mL hexane was added to the mixture. The mixture was vigorously shaken with a vortex mixer for 1 min and then centrifuged at 5000 rpm for 5 min. The fatty acid composition was analyzed by a GC (Agilent 6890, USA) equipped with an FID and a 100 m × 0.25 mm ID HP-88 column. Injector temperature was 250°C. The oven temperature was kept at 103°C for 1 min, then programmed from 103°C to 170°C at 6.5°C/min, from 170°C to 215°C for 12 min at 2.75°C/min, and finally 230°C for 5 min. The carrier gas was helium with a flow rate of 2 mL/min; split rate was 1/50. The fatty acid was identified by comparison of retention times to known standards. The results were expressed as g fatty acid/100 g total fatty acids (%).

**Mineral Content**. The Mg, Ca, and Fe contents of the samples were determined by flame atomic absorption spectrometry [12], Na and K content by flame photometry [13], and P content by spectrophotometry [14].

**Statistical Analysis**. The statistical analyses were performed using SAS 8.0 statistical packaged software on Windows-based SAS [15]. Two-way ANOVA was used for the assessment of significance, and Duncan multiple comparison tests were utilized for the determination of differences among the means.

## ACKNOWLEDGMENT

The financial support from the Scientific Research Project Unit of Erciyes University is greatly acknowledged.

## REFERENCES

1. A. Lebiedzinska and P. Szefer, *Food Chem.,* **95**, 116 (2006).
2. P. R. Shewry, *J. Cereal Sci.*, **46**, 239 (2007).
3. T. V. Price, *Can. Inst. Food Sci. Technol. J.,* **21**, 57 (1988).
4. F. Yang, T. K. Basu, and B. Ooraikul, *Int. J. Food Sci. Nutr.,* **52**, 319 (2001).
5. L. Plaza, B. Ancos, and M. P. Cano, *Eur. Food Res. Technol.,* **216**, 138 (2003).
6. S. D. Kulkarni, R. Acharya, A. G. C. Nair, N. S. Rajurkar, and A. V. R. Reddy, *Food Chem.,* **95**, 699 (2006).
7. P. L. Finney, *Recent Adv. Phytochem.*, **17**, 229 (1985).
8. P. K. Rudra, S. S. D. Nair, J. W. Leitch, and M. L. Garg, *Handbook of Nutraceuticals and Functional Foods*, CRC Press, United States of America, 2001.
9. H. W. Lopez, V. Krespine, C. Guy, A. Messager, C. Demigne, and C. Remesy, *J. Agr. Food Chem.*, **49**, 2657 (2001).
10. M. Nabrzyski, *Mineral Components in Foods*, Taylor & Francis Group, CRC Press, New York, 2007.
11. Agilent Application Catalogue, 5989-3760 EN, www.agilent.com, 2008.
12. U. Sahin, S. Kartal, and A. Ulgen, *Anal. Sci.,* **24**,751 (2008).
13. H. G. Maier, Steinkopff Verlag, Darmstadt, 1990.
14. M. Dogan and M. Soylak, *Water Chemistry*, Erciyes University Press, Turkey, 2006.
15. SAS: SAS/STAT User′s guide (8.0); SAS Institute, Inc.: Cary, North Carolina, 2000.