

Characterisation of einkorn (*Triticum monococcum* L. subsp. *monococcum*) wheat oil

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RESEARCH ARTICLE

Abstract

Einkorn (*Triticum monococcum* L.) is one of the ancient wheat varieties which is rich in various functional components. The present research evaluated peroxide, refractive index, fatty acids composition, total carotenoids, chlorophyll content and colour values of einkorn grain and its oil. Total phenolic content and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity analyses were performed with whole wheat, defatted wheat and wheat oil. Whole wheat extract showed a higher total phenolic content and radical scavenging activity than defatted wheat and wheat oil extracts. The results showed that almost all of the phenolic compounds do not transfer during extraction from whole wheat to the wheat oil. Total oil yield of einkorn wheat was 1.58%. A major part of the composition of fatty acids in einkorn wheat oil was linoleic (49.43%), oleic (34.34%) and palmitic acid (10.27%). Total carotenoids in einkorn wheat oil was averaged as 12.33 mg/kg, total chlorophyll content was 0.73 mg/kg.

Keywords: bioactive compounds, einkorn wheat, fatty acids, wheat oil

1. Introduction

Ancient wheat, the earliest domesticated wheat by mankind and the ancestors of current wheat, have shown promise in certain markets in the form of organic/healthy foods and multi-grain food products. Einkorn is also an excellent source of useful traits in breeding programmes for durum and bread wheat improvement (Sharma *et al.*, 1981). Einkorn (*Triticum monococcum* L. subsp. *monococcum*) is one of the ancient wheat varieties, and a potential crop for environmentally friendly organic farming (Løje *et al.*, 2003). It was widely cultivated in the Neolithic Age, but during the Bronze Age it was gradually replaced by tetraploid and hexaploid wheat. Today, einkorn is grown in marginal farmlands in western Turkey, the Balkan countries, Italy, Spain, Switzerland and Germany (Wieser *et al.*, 2009).

Einkorn is a diploid hulled wheat appreciated for its excellent nutritional properties, including high protein, lutein, phytosterol, tocopherols, tocotrienols, some other antioxidants, minerals particularly calcium, manganese and sulphur which are found at high levels compared to common bread and durum wheat. Also, einkorn possesses much higher levels of yellow pigments and carotenoids

than common wheat (Brandolini *et al.*, 2008; Hidalgo and Brandolini, 2008; Lavelli *et al.*, 2009; Wieser *et al.*, 2009). Several studies conclude 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 fibre, carotenoids and tocopherols (Ünal, 2009).

Milling of wheat efficiently separates the bran, germ and endosperm to produce wheat flour (Venn and Mann, 2004). Especially, wheat germ contains various nutrient substances and active ingredients such as lipase and lipoxygenase which readily lead to rancidity. As a result, these fractions are most commonly used as feed (Xu *et al.*, 2013). But, dietary fibre, antioxidant and phenolic components like most nutritive ingredients are chiefly present in the bran layers and germ of the wheat kernel (Beta *et al.*, 2005). Wheat germ contains highly concentrated nutrients: three times as much protein, seven times as much fat, fifteen times as much sugar, six times as much thiamine, and fifteen times as much tocopherol, when compared to wheat flour (Sudha *et al.*, 2007).

Wheat germ oil is well known for its beneficial health effects due to its high content of vitamin E and polyunsaturated

fatty acids, mainly linoleic acid (omega 6, between 44 and 65%) and linolenic acid (omega 3, in a lower proportion, 4-11%) (Megahad and El Kinawy, 2002; Wang and Johnson, 2001). Along with sunflower oil, wheat germ oil is one of the oils with the highest concentration of α -tocopherol. Valued in the food industry, it comes as a dietary product (e.g. capsules, soft gelatin), and plays a role on the safety of edible oils (Gustone *et al.*, 1994; Nolasco *et al.*, 2004, 2006). Other benefits of wheat germ oil include its antioxidant properties and its effect in strengthening the immune system and restoring overall health (Chignola *et al.*, 2002). Wheat germ oils are usually incorporated in cosmetic preparations such as hand and body creams, soaps and shampoos (Rabasco Alvarez and González Rodríguez, 2000).

Hoseney (1994), stated that the highest fat concentration in wheat kernels is observed in the germ that contains over 28% fat. While, linoleic (C18:2), palmitic (C16:0) and oleic acid (C18:1) were reported as major fatty acids in spring wheat, the minors were α -linolenic (C18:3) and stearic acids (C18:0) (Davis *et al.*, 1980; Ruibal-Mendieta *et al.*, 2004; Suchowilska *et al.*, 2009). Suchowilska *et al.* (2009) studied some chemical properties of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta*. They reported the major fatty acids of the samples in decreasing order as: linoleic, oleic, palmitic, α -linolenic and stearic acid, and the percentages were reported as 51.9, 24.8, 16.9, 4.0 and 1.3% for *T. dicoccum* variety, 55.9, 19.4, 18.77, 3.5 and 1.1% for *T. spelta* variety, respectively. The percentages of major fatty acids in *T. monococcum*, in the same order, was reported as 52.79, 26.35, 14.85, 3.76 and 0.96%, respectively.

It is important to know the level of phenolics and antioxidant activity of wheat germ oil as this oil has a high nutritional and biological value, and is truly unique in its biochemical composition and curative properties of natural plant product. It is a natural antioxidant that helps to keep other vegetable oils. There is a growing interest in the world for wheat germ and oil production that should not be underestimated. For instance, the application in modern mill complexes allows a wheat germ flour production of over 100 thousand tons annually, placing Kazakhstan in the first place in the world in production of wheat germ (Tultabayeva *et al.*, 2013).

Chlorophylls and carotenoids are important group of compounds from the nutritional and technological point of view. In this study we examined some characteristics of einkorn wheat oil which should be taken in account in the view of its potential use in the food industry due to its physical and chemical properties and some antioxidant compounds.

2. Materials and methods

Materials

Einkorn samples were provided by Kastamonu, İhsangazi Provincial Directorate of Republic of Turkey Ministry of Food, Agriculture and Livestock, which were grown in 2011-2012 at Çatalyazi, İhsangazi district of city of Kastamonu, Turkey. Wheat samples were obtained from three different field locations, each containing 3 kg of wheat kernel. After manual harvesting, einkorn kernels were cleaned manually and stones, straw and dirt were removed. Thus, de-hulled einkorn wheat was obtained. Then, the einkorn wheat samples were stored at 5 °C and analysed within one month.

Extraction of oil from wheat and determination of oil content

Wheat samples were milled into fine material on a hammer mill (FN-3100 Laboratory Mill, Perten Instruments AB, Huddinge, Sweden) with 500-micron-sieve opening. Then, oil samples were extracted by petroleum ether of boiling range between 40-60 °C using the Soxhlet procedure (Barthet *et al.*, 2002). Oil from milled wheat was extracted by repeated washing (percolation) with petroleum ether. 200 ml of petroleum ether was poured into round bottom flask. 25 g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated to 40 °C. After 6 h, the flask was removed, the hot oil dissolved in petroleum ether, the solvent filtered on filter paper and the solvent evaporated under vacuum using a rotary evaporator. The remaining solvent traces were removed by applying nitrogen flush. The oil obtained was thereafter stored in hermetically closed bottles and kept in a refrigerator till further analysis. The percentage of oil in the initial sample was calculated using the following formula:

$$\text{Oil\%} = \frac{P}{M} \times 100 \quad (1)$$

Where P = mass of obtained oil, and M = mass of seed flour used.

Oil samples were stored at 4 °C, in darkness, using amber glass bottles without headspace until analysis.

Peroxide value of the oil

Method Cd 8-53 of the American Oil Chemists Society (AOCS, 1993) was used for the determination of peroxide values of the samples. Results were expressed as meq O₂/kg oil.

Refractive index of the oil

The refractivity index was determined according to AOAC official method 921.08 (AOAC, 1999) at 25 ± 0.05 °C with a Carl Zeiss Abbé refractometer (32-G 110e; Carl Zeiss AG, Oberkochen, Germany) with a precision of 1×10^{-4} at 589 nm. The measurement was repeated ten times.

Carotenoids and chlorophylls

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values, according to the method of Minguez-Mosquera *et al.* (1991). About 2.5 g of oil was added to 25 ml of isooctane for spectrophotometry. The specific absorptions were compared to the curves obtained measuring the specific absorptions of solutions at known concentrations of β -carotene and chlorophylls soluble in oil measured in isooctane. Data were expressed as milligram of β -carotene per kilogram of oil (mg/kg), and milligram of chlorophylls per kilogram of oil (mg/kg).

Assessment of instrumental colour

A colourimeter (Minolta Chroma meter CR 400; Minolta Co., Osaka, Japan) was used to assess the oil colour and the CIELAB colourimetric system was applied. The colour meter was calibrated against a standard calibration plate of a white surface and set to CIE standard illuminant C. Each time, 20 ml of a sample was put into a glass Petri dish, and the liquid probe of the instrument was immersed into the dish sitting on the white tile, and readings of the CIE lab coordinates were recorded. The colour brightness coordinate L^* represents the whiteness value of a colour and ranges from black at 0 to white at 100. The chromaticity coordinate a^* represents red when positive and green when negative, and chromaticity coordinate b^* represents yellow when positive and blue when negative. The L^* , a^* , b^* values are averages of ten readings (Criado *et al.*, 2004).

Extraction of phenolics from whole and defatted wheat

The samples remained after the n-hexane extraction procedure in the Soxhlet apparatus as defatted wheat were analysed for phenolics. 10 g of ground whole wheat and defatted wheat were weighed and mixed with 50.0 ml of methanol-water (80:20, v/v). The mixture was kept in a shaking water bath for 1 h. After filtering, the extract was evaporated under reduced pressure at a temperature not exceeding 35 °C. Then the extract was concentrated to a volume of 6 ml under nitrogen flush. The same extraction procedure was applied for defatted wheat samples.

Extraction of phenolics from wheat oil

The extraction was performed according to the procedure described by Pirisi *et al.* (2000). Briefly, 5 g of oil were weighed in a centrifuge tube, after which 2.5 ml of n-hexane and 5 ml of methanol-water (80:20, v/v) were added. The mixture was stirred for 2 min in a vortex apparatus, and the tube was centrifuged at 3,000 rev/min (30 cm diameter) for 5 min. The methanol layer was separated and the extraction repeated three times. The methanolic extracts were combined and evaporated under reduced pressure at a temperature not exceeding 35 °C. The extract was concentrated to a volume of 6 ml under nitrogen flush. Samples were filtered through a 0.45 μ m nylon filter and used for determination of total phenols and for antioxidant activity assays.

DPPH radical scavenging activity

0.1 ml methanolic extract was placed in a test tube. 0.9 ml buffer solution and 2.0 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution were added into the tube. The absorbance was measured at 517 nm after 30 min of reaction. The buffer solution was prepared by Tris-HCl (3.0276 g in 350 ml distilled water). The pH of the buffer solution was calibrated to 7.4 by addition of 2 M NaOH. Then, the flask was filled up to 500 ml final volume by the additional distilled water. The percentages of DPPH decolouration of the samples were calculated according to the formula (Lee *et al.*, 1998):

$$\text{Anti-radical activity} = 100 \times \frac{1 - \text{absorbance of sample}}{\text{absorbance of control}} \quad (2)$$

Where the sample has been replaced by the same amount of methanol, for control.

Determination of total phenolics content

The concentrations of phenolic compounds in the extracts were determined by the Folin-Ciocalteu's colourimetric method (Singleton and Rossi, 1965). Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolics were expressed as gallic acid equivalents (mg GAE/g extract).

Determination of fatty acids by gas chromatography-mass spectrometry

Fatty acid composition for einkorn wheat samples was determined using a modified fatty acid methyl ester method as described by Hışıl (1998). Gas chromatographic analysis was performed using an Agilent 6890 N GC gas chromatograph fitted with an auto-sampler AS 2000 and with a split/splitless injector for HP-88 fused silica capillary column (100 m \times 0.20 mm i.d. \times 0.25 μ m film thickness) supplied by Agilent Technologies Inc. (St. Clara, CA,

USA). Detection was carried out with a mass selective single quadrupole detector (Agilent). The NIST-library software (NIST/EPA/NIH Mass Spectral Library with Search Program; data version NIST 14, software version 2.2g; NIST, Gaithersburg, MD, USA) was used for acquiring and handling the data. The carrier gas was helium (purity 99.999%) used with a 1 ml/min flow rate (~30 cm/s constant flow). Samples were injected in the split mode with a split ratio 1:10. The sample volume in the direct injection mode was 1 µl. The injector temperature was set at 260 °C. The transfer line and ion source temperatures were respectively of 300 and 230 °C. The column head pressure of helium gas as carrier gas was set to 10 kPa. The compounds were ionised by electron impact in the positive ion mode, using electron energy of 70 eV and the mass range scanned was 33–450 m/z. The detector was operated in full scan mode. The analysis programme was 4 °C/min to 240 °C and maintained at that temperature for 5 min.

Statistical analysis

The experiments were carried out in duplicate. The number of samples per repetition was two. Standard deviations were calculated using the statistical functions included in Microsoft Excel, Office 2000 software (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

Physical and chemical properties of wheat oil

Some physical and chemical properties of einkorn wheat oil are given in Table 1. The overall mean wheat oil content was 1.58% with a range of 1.45–1.74%. Suchowilska *et al.* (2009) reported that the crude fat content of *T. monococcum* grain (2.7%) was significantly higher compared with *T. spelta* (2.4%) and *T. dicoccum* (2.3%). In another study, the crude fat content of *T. monococcum* grain was reported as 2.48% (Abdel-Aal *et al.*, 1995).

Table 1. Some physical and chemical properties of einkorn wheat oil (mean values ± standard deviation).

Properties ¹	Quantity
Total oil yield (%)	1.58±0.12
Peroxide (meq O ₂ /kg)	2.43±0.15
Total carotenoids (mg/kg)	12.33±0.71
Total chlorophyll (mg/kg)	0.73±0.08
Refractive index (nD20)	1.4644±0.01
L*	22.59±0.30
a*	5.65±0.14
b*	10.98±0.40

¹ L* = whiteness; a* = redness; b* = yellowness.

The value for the refractive index of wheat oil was 1.464±0.01 nD20. Gómez and De la Ossa (2000) reported the refractive index values of wheat germ oil as 1.472 nD25. This difference may be attributed to sampling, as in the present study the oil was extracted from whole wheat instead of wheat germ.

The peroxide value of einkorn wheat oil was 2.43±0.15 meq O₂/kg. This result indicates that the oil in seed was not oxidised too much up to analyses. And also, the reason for the obtained value may be attributed to the time, starting from harvest till the analyses.

Einkorn is a high nutritional-value cereal having elevated levels of carotenoid and tocol content (Hidalgo *et al.*, 2006). The average value of total carotenoids in einkorn wheat oil was determined as 12.33±0.71 mg/kg. Abdel-Aal and Rabalski (2008) reported the content of lutein, the primary carotenoid in wheat, significantly differed among wheat species ranging from 1.0 to 8.1 mg/kg.

In a previous study, the total carotenoid contents of the einkorn accessions were reported 5.33 (Italy) and 13.64 (Greece) mg/g dry matter (dm), with an average of 8.41 mg/g dm (Hidalgo *et al.*, 2006). It is well known that the carotenoids are affected by geographic location (Yu, 2008). In bread wheat, however, the concentration of carotenoids is low (from 0.1 to 2.4 mg/g dm) but they are more abundant in durum wheat (1.5 to 4.0 mg/g dm) (Panfili *et al.*, 2004; Zandomeneghi *et al.*, 2000).

Total chlorophyll value of einkorn wheat oil was determined as 0.73±0.08 mg/kg (Table 1). Colour values einkorn wheat oil colour is summarised in Table 1. L*, a* and b* values of einkorn wheat oil was determined as 22.59±0.30, 5.65±0.14 and 10.98±0.40, respectively.

Whole wheat extract showed higher total phenolic content and radical scavenging activity than the defatted wheat and wheat oil extracts (Table 2). As expected, the total phenolic content and radical scavenging activity of wheat oil extract were dramatically lower than those of the extracts from wheat samples. Abdel-Aal and Rabalski (2008) reported

Table 2. DPPH radical scavenging activity and phenolic content of einkorn wheat oil.

Sample	DPPH-RSA ¹ (% inhibition)	Total phenolic content
Whole wheat	88.55	1,349.64 mg phenol/kg sample
Defatted wheat	76.27	1,170.93 mg phenol/kg sample
Wheat oil	14.68	73.48 mg phenol/kg oil

¹ % 2,2-diphenyl-1-picrylhydrazyl (DPPH) - radical scavenging activity.

total phenolics of *T. monococcum* wheat between 2,319-2,355 µg/g. They also reported the antioxidant activity of wheat extracts of *T. monococcum* was higher than those of *Triticum turgidum* and *Triticum aestivum*. Abdel-Aal and Rabalski (2008) reported that the total phenolic content in wheat significantly varied between wheat species and cultivars ranging from 881 to 2,382 µg/g. Fogarasi *et al.* (2015) examined the total phenolic compounds of some grains and they reported that einkorn grain samples showed significantly higher total phenolic values than optional winter and winter cultivation samples. Their values were reported ranging between 349-593 µM GAE/g dm for all grain types examined including barley.

Fatty acids

The fatty acids (% in the whole wheat oil) identified in the studied variety were palmitic, oleic, linoleic, linolenic and eicosenoic acid (Table 3). The major components were the mono-unsaturated oleic acid, the poly-unsaturated linoleic and the saturated palmitic, as found by others (Suchowilska *et al.*, 2009).

In common wheat varieties, the contribution of oleic acid to oil (%) was reported to be 17.22% in *T. aestivum* (cv. Torka) and 19.42% in *T. spelta* (Suchowilska *et al.*, 2009). When compared to *T. monococcum* oil, oleic acid percentage (34.34%) is higher than that reported for *T. aestivum* and *T. spelta* varieties. Linoleic and palmitic acid is generally present at concentrations of 59.6 and 17.45% in *T. aestivum* (cv. Torka) (Suchowilska *et al.*, 2009). The percentages of these fatty acids in the variety *T. monococcum* are lower than those reported for *T. aestivum* varieties.

Fatty acid composition is highly influenced by ecological conditions (Seferoglu *et al.*, 2006). Recently, it was suggested that the geographical origin of some foods such as pistachios (Arena *et al.*, 2007), olive oil (Lanza *et al.*, 1998), and wheat (Armanino *et al.*, 2002) could be discriminated from their fatty acids distribution. However, these findings are valid only provided that the same varieties are studied since differences among varieties may exceed differences between countries (Tsantili *et al.*, 2010).

Table 3. Fatty acid composition of einkorn wheat oil (mean values ± standard deviation).

Fatty acids	% of total fatty acid
palmitic (C16:0)	10.27±0.98
oleic (C18:1n9)	34.34±1.10
linoleic (C18:2n6)	49.43±0.98
linolenic (C18:3n3)	3.59±0.49
eicosenoic (C20:1n9)	2.38±0.46

4. Conclusions

Composition of einkorn wheat oil is interesting from a nutritional point of view which might have a potential use in food industry. In this study the results showed that einkorn wheat oil is particularly rich in unsaturated fatty acids and antioxidants and can be a potential alternative to oils of conventional wheat species. The results showed that most of the phenolic compounds do not transfer to wheat oil from whole wheat during extraction. The data, obtained, may be valuable for the evaluation of the nutritional value and the functional properties of einkorn and also may lead to new research studies.

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