Durum wheat by-products as natural sources of valuable nutrients

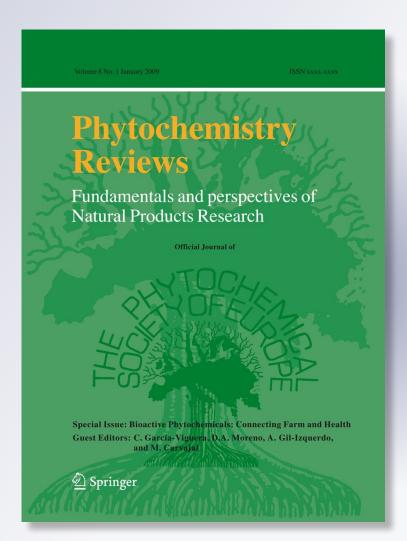
Miriana Durante, Marcello S. Lenucci, Leonardo Rescio, Giovanni Mita & Sofia Caretto

Phytochemistry Reviews

Fundamentals and Perspectives of Natural Products Research

ISSN 1568-7767

Phytochem Rev DOI 10.1007/s11101-012-9232-x





Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media B.V.. This e-offprint is for personal use only and shall not be selfarchived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.



Durum wheat by-products as natural sources of valuable nutrients

Miriana Durante · Marcello S. Lenucci · Leonardo Rescio · Giovanni Mita · Sofia Caretto



Received: 14 December 2011/Accepted: 21 April 2012 © Springer Science+Business Media B.V. 2012

Abstract This review reports the use of wheat milling by-products for the extraction of high quality oil and vitamin E including our results on the exploitation of durum wheat bran as a valuable source of important healthful compounds. Wheat oil can be used as an ingredient in food, pharmaceutical or cosmetic preparations because it contains important bioactive compounds such as vitamin E, carotenoids and unsaturated fatty acids. Different methods are used for oil recovery from plant materials, such as solvent extraction, mechanical pressing or the ecofriendly supercritical carbon dioxide (SC-CO₂) extraction technology. By using SC-CO₂, we obtained an oil from durum wheat (Triticum durum Desf.) bran and optimized the extraction conditions to increase oil and vitamin E yields. Wheat bran, which is composed of pericarp, aleurone layer and germ, is discarded during the early stages of durum wheat milling processes to obtain a final product (semolina) that is stable over

M. Durante · G. Mita · S. Caretto (🖂) Istituto di Scienze delle Produzioni Alimentari—CNR, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy e-mail: sofia.caretto@ispa.cnr.it

M. S. Lenucci

Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (Di.S.Te.B.A.), Università del Salento, Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy

L. Rescio

Pierre S.r.l., s.s. 476 km 17,650 Zona Industriale, 73013 Galatina, LE, Italy

time. Maximum oil and vitamin E yields were obtained when a durum wheat bran matrix with particle size of ~30 mesh and a moisture content of 2.6 % was used. The optimal conditions for oil extraction were: 300–350 bar, 60–70 °C, and 4 1 min⁻¹ gaseous CO₂ flow rate for 1 h. The chemical composition (vitamin E forms, carotenoids, quinones, lipids and fatty acids) of the SC-CO₂ extracted oil was analyzed and compared to that of the oil extracted by Soxhlet using hexane as solvent. The findings here reported highlight the importance of durum wheat bran as a rich source of valuable natural nutrients.

Abbreviations

FAME	Fatty acid methyl esther
FFA	Free fatty acid
SC-CO ₂	Supercritical carbon dioxide

Introduction

Among the several components of the daily human diet, wheat products are present in almost all countries. Wheat is the world's most important cereal crop both in terms of cultivated area and kernel yield. It is cultivated throughout the temperate zones and in some tropical and sub-tropical areas. Two main species of wheat can be distinguished: bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.). The first is used to produce most of wheat-based foods (bread, cookies, etc.) while durum wheat is used in the manufacture of pasta.

Apulia is the leading Italian region in durum wheat production: in 2010, 700,000 tons of *Triticum durum*, and \sim 140,000 tons of milling by-products, mainly wheat bran, were produced (www.agri.istat.it).

Wheat kernel is composed of a number of tissues with specific composition and structure (as illustrated in Fig. 1). It is composed of 80-85 % mealy endosperm, 2-3 % germ and 13-17 % bran (on a dry matter basis, Belderok et al. 2000). Although human consumption of whole grains is associated with a reduced risk of health disorders, such as cancers and diabetes (Meyer et al. 2000), the traditional milling process aims at isolating and only using the endosperm that is mainly composed of starch and storage proteins, discarding the outer teguments and germ. In fact, after an initial pre-cleaning step that eliminates most of the major impurities and foreign seeds based on the shape, dimension, color, density and weight, mechanical methods such as kernel debranning (or decortication) and degerming are routinely used as milling pretreatments in semolina production. Germ is rich in lipids and in oxidative and hydrolytic enzymes: lipase, lipoxidase and protease. Thus germ removal reduces oxidation and enhances flour storage stability (Dawe et al. 2000). Debranning and degerming are carried out by a combination of friction and abrasion (Dexter and Wood 1996), producing the so-called "wheat bran" consisting of the outer and inner pericarp, seed coat (or testa), hyaline layer, aleurone layer and germ. These tissues contain insoluble fibers, lipids, minerals, B vitamins and vitamin E. Particularly, germ is an important source of nutrients; it contains 26 % proteins, 17 % sugars, about 10 % oil with highly valuable ω -6 and ω -3 fatty acids (Wang and Johnson 2001). It is also the most abundant source of vitamin E as tocopherols and tocotrienols (Atwell 2001). These are lipophilic molecules (Fig. 2), differing by the degree of saturation in their side chains and possessing an essential role in human nutrition and health. They consist of a hydrophilic chromanol head and a hydrophobic isoprenoid side chain. Each group is composed of four members differing in the number

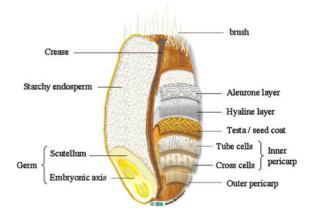


Fig. 1 Histological composition of wheat grain. From Surget and Barron (2005)

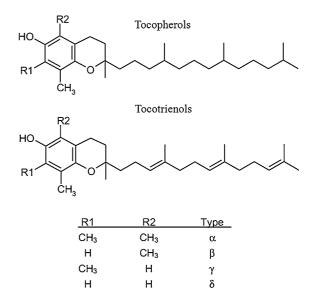


Fig. 2 Naturally occurring forms of vitamin E: tocopherols and tocotrienols

and position of methylation on the aromatic ring, named α , β , γ , δ - forms (Brigelius-Flohe and Traber 1999). Natural α -tocopherol occurs as a single stereoisomer RRR- α -tocopherol, while synthetic vitamin E is a mixture of all eight stereoisomers (all-*racemic*, allrac) with lower biopotency (Lodge 2005). This raises interest in establishing new vitamin E production systems from natural sources (Caretto et al. 2010).

Wheat bran is currently applied in the livestock industry to formulate animal feeds. It is also used to extract an oil that is a valuable ingredient for food, nutraceutical, pharmaceutical and cosmetic formulations. Several studies have been carried out to optimize oil extraction methods from wheat milling by-products.

Here we review the employment of wheat milling by-products for the extraction of high quality oil and vitamin E molecules, including our results on the exploitation of durum wheat bran as a valuable source of important healthful compounds.

Extraction of wheat bran oil

Wheat bran oil has been obtained by either mechanical pressing or chemical solvent extraction. Hexane extraction in Soxhlet apparatus resulted in a higher percentage (90 %) of wheat bran oil compared to pressing (50 %) (Singh and Rice 1979). Currently, the oil obtained by these methods needs a refining step using conventional degumming, neutralization, bleaching and deodorization processes (Wang and Johnson 2001).

 $SC-CO_2$ is an alternative technique to the conventional extraction methods. For many not-polar compounds, it allows extraction yields similar to those obtained using organic solvents, with the additional benefits that CO₂ is nontoxic, nonflammable, noncorrosive, cheap, recyclable and is a gas under normal conditions of temperature and pressure, so that the extracts do not require any further refining (Eisenmenger and Dunford 2008). In addition, CO_2 has a low critical temperature and pressure (31 °C and 74 bar, respectively) which makes it the ideal solvent for the extraction of thermo-sensitive molecules (Reverchon et al. 1993; Lenucci et al. 2010). Above its critical point, CO₂ possesses physical properties (density, viscosity and diffusivity) that are intermediate between liquid and gas in a single phase "the supercritical fluid" that has a strong solvent power suitable to extract lipophilic molecules. The SC-CO₂ extraction technology gives totally solvent free extracts, inactivates microorganisms, decreases lipoxygenase activities and reduces the development of rancidity avoiding contact with atmospheric oxygen (Haas et al. 1989; Tedjo et al. 2000).

 $SC-CO_2$ has already been used to extract high quality oil and vitamin E from wheat bran. Various authors reported the chemical composition of oil obtained by $SC-CO_2$ compared to classical solvent extraction, Soxhlet extraction and mechanical pressing (Table 1).

Extraction of durum wheat bran oil by SC-CO₂

In spite of several studies carried out on wheat bran obtained as a by-product of bread wheat chain, no data have been reported so far on wheat bran deriving from durum wheat milling process.

The treatments most frequently reported for wheat bran stabilization include: toasting, defatting and steaming, infrared heating, microwave treatment, lowering the moisture content by different drying methods. In order to increase the stability of durum wheat bran and to inactivate enzymatic activities that can reduce shelf life, we used heating by far-infrared rays for 8 min at 105 °C.

It is known that the presence of water, in wheat bran, could interfere with the extraction process by SC-CO₂. Ge et al. (2002), using wheat germ from bread wheat (Triticum aestivum) at different degrees of dehydration, reported that a 5 % moisture content allowed optimal extraction of vitamin E. On this basis, the initial moisture of durum wheat bran samples was progressively reduced by oven drying (T = 60 °C) from 24 to 96 h, and the effects of residual moisture on oil and vitamin E yields by SC-CO₂ extraction were evaluated by using a laboratory scale SPE (Solid Phase Extraction) extractor (Spe-ed SFE, Applied Separation, Allentown, PA, USA) (Table 2). Under the applied operative conditions (25 g matrix samples, pressure 300 bar, temperature 60 °C, CO_2 flux 4 l min⁻¹ and extraction time 60 min), oil and vitamin E yields increased as water content decreased. However, while the increase in vitamin E yield was statistically significant (P < 0.05), that of oil was not. Maximum oil and vitamin E yields were about 79 and 72 %, respectively, when a matrix with a < 3 % moisture content was used. This durum wheat bran matrix was used in all the subsequent extractions by SC-CO₂.

The particle size of the matrix could be important to allow optimal flow of CO_2 through the plant material packed into the extraction vessel, to increase contact surface area with the solvent and to minimize the path length that bioactives have to diffuse through to reach the bulk phase. To evaluate the effects of granulometry on oil and vitamin E yields, the low moisture wheat bran was ground in a laboratory scale ultra centrifugal mill (model ZM200–Retsch, Haan, Germany) through a 30 mesh (0.505 mm) or 100 mesh (0.130 mm) sieve. The bran, as such and milled (30 and 100 mesh), was extracted by SC-CO₂ as described

Table 1 Analysis of oil fr	Table 1 Analysis of oil from wheat milling by-products extracted with different methods	
Extraction method	Compounds (mg g ⁻¹ oil)	References
Commercial	Σ Ts and Σ T3 (α T > β T > β T3 > α T3 > γ T > γ T3) (2.68)	Schwartz et al. (2008)
Solvent extraction		
Soxhlet (petroleum ether)	$\Sigma Ts (\alpha T > \beta T > \gamma T) (2.60); FFA (88)$	Zacchi et al. (2006)
Soxhlet (petroleum ether, 9 h)	Σ Ts and Σ T3 (α T > β T > β T3 > α T3) (2.29); FAME (C18:2 ω 6 > c C18:1 ω 9 > C16:0 > C18:3 ω 3 > C16:1 ω 7 = C18:0); Carotenoids (lutein > zeaxanthin > β -carotene) (0.05)	Panfili et al. (2003)
Soxhlet (hexane, 16 h)	$\Sigma Ts (\alpha T > \beta T) (21.54)$	Ge et al. (2002)
Soxhlet (chloroform/ methanol, 2.3 h)	Σ Ts (α T > β T) (18.27)	Ge et al. (2002)
Soxhlet (hexane, 16 h)	Σ Ts (α T > β T) (232.60); FFA (270.30); FAME (C18:2 ω 6 > C16:0 > c C18:1 ω 9 > C18:3 ω 3 > C16:1 ω 7 > C18:0	Gomez and de la Ossa (2000)
Commercial (hexane extracted, crude)	Σ Ts (α T > β T > γ T) (15.08); Phospholipids (ND); FAME (C18:2 $\omega 6 > C16:0 > cC18:1\omega 9 > C18:3\omega 3 > C20:1 > C22:0 > C18:0 > C22:1 > C20:0 > C16:1\omega 7 > C24:0 > C14:0); FFA (79)$	Eisenmenger and Dunford (2008)
NS	Σ Ts (α T > β T) (2.68); FFA (157); FAME (C18:2 $\omega 6 > c$ C18:1 $\omega 9 > C16:0 > C18:3 \ \omega 3 > C20:1 > C18:0 > C20:0$)	Wang and Johnson (2001)
Mechanical pressing		
Pressed	Σ Ts (α T > β T > γ T) (1.72); FFA (100.2)	Zacchi et al. (2006)
Commercial (pressed)	$\Sigma Ts (\alpha T > \beta T > \gamma T) (2.58);$ FFA (70)	Zacchi et al. (2006)
$SC-CO_2$		
380 bar, 55 °C, 75 min	Σ Ts and Σ T3 (α T > β T > β T3 > α T3) (2.93); Carotenoids (lutein > zeaxanthin > β -carotene) (0.01); FAME (C18:2 $\omega 6 > c$ C18:1 $\omega 9 > C16:0 > C18:3 \ \omega 3 > C18:0 > C16:1\omega 7)$	Panfili et al. (2003)
400 bar, 40 °C	Σ Ts (α T > β T > γ T) (2.13); FFA (64)	Zacchi et al. (2006)
164 bar, 40 °C, 10 min	$\Sigma Ts (\alpha T > \beta T > \gamma T) (9.62)$	Gelmez et al. (2009)
690 bar, 80 °C, 45 min	$ \sum_{\omega \in S} T_{S} (\alpha T > \beta T > \gamma T); FFA (62); FAME (C18:2) \omega 6 > C16:0 > cC18:1\omega9 > C18:3\omega3 > C20:1 > C22:0 > C18:0 > C22:1 > C20:0 > C16:1\omega7 > C14:0 > C24:0); Phospholipids (PI + PA > PE > PS > PC) (19.80) $	Eisenmenger and Dunford (2008)
150 bar, 40 °C, 180 min	Σ Ts (α T > β T) (416.70); FFA (124); FAME (C18:2 $\omega 6$ > C16:0 > c C18:1 $\omega 9$ > C18:3 $\omega 3$ > C16:1 $\omega 7$ > C18:0	Gomez and de la Ossa (2000)
276 bar, 40 °C, 90 min	$\Sigma Ts (\alpha T > \beta T > \gamma T > \delta T) (21.79)$	Ge et al. (2002)
300 bar, 60 °C	Σ Ts (α T > β T) (2.22); FAME (C18:2 $\omega 6$ > C16:0 > c C18:1 $\omega 9$ > C18:3 $\omega 3$)	Kwon et al. (2010)
Σ Ts sum of tocopherols, Σ phosphatidylinositol, PA pl specificated	ΣTs sum of tocopherols, ΣTs sum of tocotrienols, $\alpha T \alpha$ tocopherol, $\beta T\beta$ tocopherol, $\beta T3 \beta$ tocotrienol; $\gamma T3 \gamma$ tocotrienol. FAME fatty acid methyl ester, Pl phosphatidylinositol, PA phosphatic acid, PE phosphatidylethanolamine, PS phosphatidylserine, PC phosphatidylcholine, ND not detected. FFA free fatty acid, NS not specificated	y acid methyl ester, <i>PI</i> ee fatty acid, <i>NS</i> not

extracted with different methods nroducto Table 1 Analysis of oil from wheat milling hy-

Table 2	Effect	of	moisture	content	on	oil	and	vitamin	Е
extracted	by SC	-CC	D_2 from du	irum whe	eat ł	oran			

Moisture content (% w/w)	Oil yield (%)	Vitamin E yield (%)
13.1 ± 1.2	72.7 ± 2.6^{a}	36.2 ± 0.9^{b}
5.5 ± 1.1	73.9 ± 0.9^{a}	$38.0 \pm 0.2^{\mathrm{b}}$
3.5 ± 1.1	$77.6\pm2.5^{\rm a}$	$41.2\pm6.7^{\rm b}$
2.6 ± 1.1	78.9 ± 14.5^a	71.8 ± 11.1^{a}
2.4 ± 1.1	79.5 ± 14.8^a	72.0 ± 12.0^a

Values represent the mean of three independent experiments \pm standard error (SE). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple Comparison Procedures (Tukey Test), different letters indicate significant differences (P < 0.05)

above. No significant differences were observed in oil and vitamin E yields, thus indicating that, in this case, the particle size was irrelevant (data not shown).

Various authors have investigated the effects of pressure (150-690 bar), temperature (40-80 °C) and extraction time (10-180 min) on tocopherols, tocotrienols, carotenoids, phospholipids, FFA and FAME contents of SC-CO₂ extracts from wheat milling byproducts (Table 1), indicating the optimal operative parameters within more restricted ranges. On the basis of this information, we searched for optimal extraction parameters for oil and vitamin E from durum wheat bran in the range between 200 and 400 bar, 30-70 °C and 15-75 min, for pressure, temperature and extraction time, respectively. Furthermore, in order to compare $SC-CO_2$ to the conventional extraction processes using liquid solvents, the matrix (25 g) was extracted by a Soxhlet-type apparatus using hexane as solvent (200 ml) for 8 h, time required to obtain the maximum oil yield (data not shown).

Pressure is the main parameter influencing the SC-CO₂ solvent power so that it has a strong effect on extraction efficiency. Figure 3 shows the influence of operating pressure on the extraction yields of oil and vitamin E from durum wheat bran by SC-CO₂. To determine the optimal pressure for vitamin E extraction, total oil and vitamin E were evaluated in wheat bran extracted at a constant temperature (T = 60 °C) at different pressure values. In terms of oil and vitamin E yields, the optimal extraction pressure was in the range between 300 and 350 bar. Within this range the oil yield was not significantly different from that obtained by Soxhlet using hexane as solvent, while the

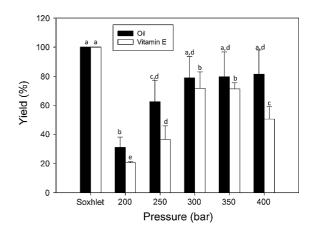


Fig. 3 Effect of pressure on the extraction yields of oil and vitamin E from durum wheat bran by SC-CO₂. *Data* are expressed as percentage of total oil and vitamin E obtained with Soxhlet extraction. *Data* were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple Comparison Procedures (Tukey Test), *different letters* indicate significant differences (P < 0.05)

vitamin E yield was significantly lower, likely due to the stronger solvent power of hexane to extract vitamin E.

To determine an optimal temperature range for vitamin E extraction, durum wheat bran was extracted at constant pressure (300 bar) and increasing temperatures (Fig. 4). Temperatures between 60 and 70 °C proved optimal for the extraction of oil and vitamin E. Maximum oil and vitamin E yields were about 78 and 72 %, respectively, compared to Soxhlet extraction. These pressure and temperature conditions likely improved the interaction between oil and SC-CO₂ and resulted in a greater oil and vitamin E solubility (Gomez and de la Ossa 2000).

The time course of oil and vitamin E extraction by SC-CO2 showed that the kinetics of extraction of oil and vitamin E were slightly different (Fig. 5). After 15 min, ~570 mg oil containing ~32 % total vitamin E were extracted from 25 g wheat bran;, the amount of oil extracted in the next interval of time (from 15 to 30 min) slightly decreased (~510 mg), while the vitamin E extraction reached its maximum (~35 % of the total extracted vitamin E). Afterwards, both the amounts of oil and vitamin E progressively decreased. Between 60 and 75 min only ~5 % (80 mg) of the total recovered oil was extracted and the amount of vitamin E was negligible (< 2 mg). Oil yield (7.6 % with respect to the matrix weight) obtained at 300 bar and 60 °C was only slightly below

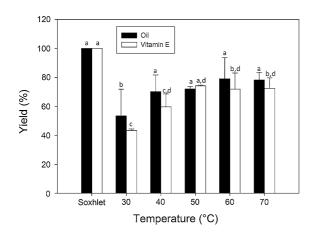


Fig. 4 Effect of temperature on the extraction yields of oil and vitamin E from durum wheat bran by SC-CO₂. *Data* are expressed as percentage of total oil and vitamin E obtained with Soxhlet extraction. *Data* were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple Comparison Procedures (Tukey Test), *different letters* indicate significant differences (P < 0.05)

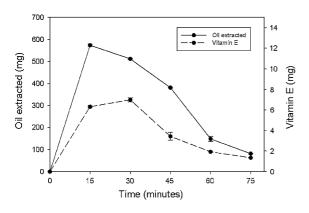


Fig. 5 Time course of the extraction of oil and vitamin E from 25 g durum wheat bran by SC-CO₂ at 300 bar, T = 60 °C, flow of gaseous CO₂ 4 1 min⁻¹. *Values* represent the means of three independent experiments \pm standard error (SE)

the yield achieved after 8 h Soxhlet extraction by hexane (8.2 %). Hence 60 min seem to be the optimal time to balance the yields of oil and vitamin E with SC-CO₂ extraction costs.

Determination of antioxidant capacity

Wheat bran oil samples obtained either after $SC-CO_2$ or hexane were assayed to determine the antioxidant capacity (Table 3) as well as the content of different **Table 3** Antioxidant capacity of durum wheat bran tested by Trolox-equivalent antioxidant capacity (TEAC) and DPPH radical scavenging assay by $SC-CO_2$ and Soxhlet

Antioxidant capacity	SC-CO ₂	Soxhlet
TEAC assay (μ mol trolox g ⁻¹ oil)	2.48 ± 0.19^{a}	3.22 ± 0.25^{a}
DPPH assay (µmol trolox g ⁻¹ oil)	1.90 ± 0.03^{a}	1.93 ± 0.09^{a}

Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple Comparison Procedures (Tukey Test), different letters indicate significant differences (P < 0.05)

Table 4 Chemical composition of oil extracted from durum wheat bran by $SC-CO_2$ and Soxhlet. The analyses were carried out by HPLC and GC-MS

	SC-CO ₂	Soxhlet			
Vitamin E (mg g^{-1} oil)					
α tocopherol	$2.3\pm0.5^{\rm a}$	$3.5\pm0.3^{\rm a}$			
$\beta\gamma$ tocopherol	$2.0\pm0.2^{\mathrm{a}}$	1.6 ± 0.1^{a}			
α tocotrienol	$0.8\pm0.7^{\rm a}$	$0.6 \pm 0.1^{\mathrm{a}}$			
$\beta\gamma$ tocotrienol	$4.4 \pm 0.8^{\mathrm{a}}$	$4.6 \pm 0.9^{\mathrm{a}}$			
Carotenoid ($\mu g g^{-1}$	oil)				
Lutein	$4.1 \pm 1.5^{\mathrm{a}}$	$9.6\pm0.2^{\rm b}$			
Zeaxanthin	1.6 ± 0.2^{a}	$2.1 \pm 0.1^{\mathrm{a}}$			
β carotene	$1.9 \pm 0.4^{\mathrm{a}}$	$2.6\pm0.1^{\rm a}$			
Quinone isoprenoid	$(mg \ g^{-1} \ oil)$				
Coenzyme Q8	$0.2\pm0.1^{\mathrm{a}}$	$0.2\pm0.1^{\mathrm{a}}$			
Coenzyme Q9	$0.4 \pm 0.1^{\mathrm{a}}$	$0.8\pm0.1^{\mathrm{a}}$			
Coenzyme Q10	0.1 ± 0.1^{a}	$0.2\pm0.1^{\mathrm{a}}$			
Lipid classes (mg g ⁻	⁻¹ oil)				
Triglycerides	683.0 ± 52.0^a	576.0 ± 28.0^a			
Diglycerides	127.0 ± 18.0^{a}	198.0 ± 16.0^{a}			
Free fatty acids	$88.8\pm3.3^{\rm a}$	110.6 ± 6.6^{a}			
Fatty acid (mg g^{-1} oil)					
C16:1 ω7	1.1 ± 0.1^{a}	$1.0 \pm 0.3^{\mathrm{a}}$			
C16:0	139.0 ± 2.5^{a}	141.0 ± 2.0^{a}			
C18:2 \u03c6	366.0 ± 8.2^{a}	368.2 ± 4.0^a			
cC18:1 ω9	192.0 ± 4.6^{a}	199.0 ± 3.2^{a}			
tC18:1 ω9	$9.3 \pm 0.1^{\mathrm{a}}$	$8.8\pm0.3^{\rm a}$			
C18:0	$7.6\pm0.7^{\mathrm{a}}$	7.1 ± 1.0^{a}			
C18:3 <i>ω</i> 3	$10.4\pm0.7^{\rm a}$	9.1 ± 0.4^{a}			

Values represent the mean of three independent experiments \pm standard error (SE). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple Comparison Procedures (Tukey Test), different letters indicate significant differences (P < 0.05)

vitamin E forms, carotenoids, quinones, lipids and fatty acids (Table 4).

The antioxidant capacity of the extracts was tested by two different methods: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)-equivalent antioxidant capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Using both methods, the antioxidant capacity of durum wheat bran oil extracted by hexane was only slightly higher than SC-CO₂ extracted oil. The lower values observed in the SC-CO₂ extracts could be due to Maillard-type antioxidants produced when samples were exposed to high temperatures (Krings et al. 2000).

Chemical composition of oil samples

It is known that both bread and durum wheat have high contents of β -tocotrienol, followed by α -tocopherol, β -tocopherol and α -tocotrienol. However, durum wheat contains lower levels of saturated forms, and slightly higher of unsaturated analogs α -tocotrienol and β -tocotrienol (Slover et al. 1969). In durum wheat bran oil β/γ -tocotrienols were the most abundant vitamin E forms being 4.4 mg g⁻¹ oil, followed by α -tocopherol, β/γ -tocopherols and α -tocotrienol. No differences were observed in the tococromanol composition of the oils obtained by SC-CO₂ and Soxhlet extraction processes (Table 4).

In wheat bran oil, lutein was found to be the most abundant carotenoid, followed by zeaxanthin and β -carotene. In the experimental conditions used here, the amount of lutein found in durum wheat bran oil extracted by Soxhlet was 2.3 fold higher than SC-CO₂ extracted samples, likely due to a greater solubility of lutein in hexane.

Interestingly, durum wheat bran oil, composed mainly of triglycerides, contained about 80 % unsaturated fatty acids and 20 % saturated fatty acids. The main fatty acid was linoleic (C18:2 ω 6), representing about 50 % of the total. It was followed by oleic (C18:1 ω 9), palmitic (C16:0) and linolenic (C18:3 ω 3) acids. Fatty acid composition of wheat bran oil extracted by SC-CO₂ was not significantly different from the oil extracted with hexane (Soxhlet). The amounts of other extracted compounds were similar in both extraction processes.

Conclusions

Durum wheat by-products can be a good source of wheat bran oil. The eco-friendly extraction by $SC-CO_2$ resulted to be an effective alternative method compared to conventional ones. The resulting products, being free from organic solvents, are directly suitable for pharmacological and industrial food use.

Durum wheat bran was oven treated to obtain a matrix with a residual moisture content of 2.6 % suitable for SC-CO₂ extraction. The best operative conditions for durum wheat bran oil extraction by this technology were found to be: 300–350 bar, 60–70 °C, 4 1 min⁻¹ gaseous CO₂ flow rate, 1 h extraction time. SC-CO₂ and Soxhlet extraction showed very similar "solvent power" to extract different vitamin E forms, some carotenoids, quinones and lipids from durum wheat bran.

Altogether the findings reported here highlight the importance of by-products of the wheat milling industry as rich sources of valuable natural nutrients.

Acknowledgments The authors are grateful to Molino Tandoi, Corato (Ba) for providing raw wheat bran and Leone D'Amico for his technical assistance. M.D. was supported by a fellowship funded by Regione Puglia, Italy. This work was partially supported by MIUR PON 01_01445 (ISCOCEM).

References

- Atwell WA (2001) Wheat flour. AACC, Eagan Press, St Paul, MN
- Belderok B, Mesdag H, Donner DA (2000) Bread-making quality of wheat. Springer, New York
- Brigelius-Flohe R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13:1145–1155
- Caretto S, Nisi R, Paradiso A, De Gara L (2010) Tocopherol production in plant cell cultures. Mol Nutr Food Res 54(5):726–730
- Dawe PR, Kill RC, Turnbull K (2000) Pasta and semolina technology. Blackwell Science, Oxford, pp 119–157
- Dexter JE, Wood PJ (1996) Recent applications of debranning of wheat before milling. Trends Food Sci Tech 7:35–41
- Eisenmenger M, Dunford NT (2008) Bioactive components of commercial and supercritical carbon dioxide processed wheat germ oil. J Am Oil Chem Soc 85:55–61
- Ge Y, Yang H, Hui B, Ni Y, Wang S, Cai T (2002) Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. J Agric Food Chem 50:685–689
- Gelmez N, Kõncal NS, Yener ME (2009) Optimization of supercritical carbon dioxide extraction of antioxidants from roasted wheat germ based on yield, total phenolic and tocopherol contents, and antioxidant activities of the extracts. J Supercrit Fluid 48:217–224

Author's personal copy

- Gomez AM, de la Ossa EM (2000) Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. J Am Oil Chem Soc 77:969–974
- Haas GJ, Prescott HE Jr, Dudley E, Dik R, Hintlian C, Keane L (1989) Inactivation of microorganisms by carbon dioxide under pressure. J Food Saf 9:253–265
- Krings U, El-saharty YS, El-Zeany BA, Pabel B, Berger RG (2000) Antioxidant activity of extracts from roasted wheat germ. Food Chem 71:91–95
- Kwon KT, Uddin MS, Jung GW, Sim JE, Chun BS (2010) Supercritical carbon dioxide extraction of phenolics and tocopherols enriched oil from wheat bran. WASET 64:255–260
- Lenucci MS, Caccioppola A, Durante M, Serrone L, Rescio L, Piro G, Dalessandro G (2010) Optimisation of biological and physical parameters for lycopene supercritical CO₂ extraction from ordinary and high-pigment tomato cultivars. J Sci Food Agric 90:1709–1718
- Lodge JK (2005) Vitamin E bioavailability in humans. J Plant Physiol 162:790–796
- Meyer KA, Kushi LH, Jacobs DR Jr, Slavin J, Sellers TA, Folsom AR (2000) Carbohydrates, dietary fiber, incident type 2 diabetes mellitus in older women. Am J Clin Nutr 71:921–930
- Panfili G, Cinquanta L, Fratianni A, Cubadda R (2003) Extraction of wheat germ oil by supercritical CO2: oil and defatted cake characterisation. J Am Oil Chem Soc 80:157–161

- Reverchon E, Donsi G, Osséo LS (1993) Modeling of supercritical fluid extraction from herbaceous matrices. Ind Eng Chem Res 32:2721–2726
- Schwartz H, Ollilainen V, Piironen V, Lampi AM (2008) Tocopherol, tocotrienol and plant sterol contents of vegetablele oils and industrial fats. J Food Compos Anal 21:152–161
- Singh L, Rice WK (1979) Method for producing wheat germ lipid products. U.S. Patent 4:298–622
- Slover HT, Lehmann J, Valis RJ (1969) Nutrient composition of selected wheats and wheat products. III. Tocopherols. Cereal Chem 46(635):641
- Surget A, Barron C (2005) Histologie du grain de ble'. Ind Cér 145:3–7
- Tedjo W, Eshtiaghi MN, Knorr D (2000) Impact of supercritical carbon dioxide and high pressure on lipoxygenase and peroxidase activity. J Food Sci 65:1284–1287
- Wang T, Johnson L (2001) Refining high-free fatty acid wheat germ oil. J Am Oil Chem Soc 78:71–76
- Zacchi P, Daghero J, Jaeger P, Eggers R (2006) Extraction/ fractionation and deacidification of wheat germ oil using supercritical carbon dioxide. Braz J Chem Eng 23:105–110