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Tocol and β-glucan levels in barley varieties and in pearling by-products

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Abstract

In order to verify a different genotype potential in barley varieties, in terms of tocols and β-glucans, and to demonstrate that pearling by-products have interesting amounts of these bioactive compounds, their content was investigated in 36 barley varieties and in the pearling by-products of a commercial hulled barley stock. The β-glucan content ranged from 2.64 g/100 g dw (dry weight) for Vanessa to 8.05 g/100 g dw for Ludine, with an average value of 3.95 g/100 g dw and 50% of the compounds were in the range between 3.45 and 4.36 g/100 g dw. The total tocol amount ranged from 50.3 mg/kg dw (Ladoga) to 88.6 mg/kg dw (Maggiodoro), with a mean value of 69.1 mg/kg dw and with most genotypes (50%) having a content between 62 and 75 mg/kg dw. Adagio and Sabel were the best source of vitamin E activity, expressed as Tocopherol Equivalents. In the pearling by-products there was no enrichment of β-glucans, on the contrary, a seven and a fivefold increase was observed for tocopherols and tocotrienols, respectively.

Keywords: Barley; Tocopherols; Tocotrienols; β-Glucans; Pearling by-products

1. Introduction

Cereal grains contribute significant amounts of energy, protein, selected micronutrients to the human diet and contain a large variety of biologically active substances, including antioxidants, dietary fibre, phytoestrogens and lignans (Hill & Path, 1998). In particular they are the major sources of tocols (tocopherols and tocotrienols) for humans and barley is one of the best sources, containing both a high concentration of total tocols and a favourable distribution of the major biologically active forms. Moreover, barley is gaining renewed interest as food component also for its soluble dietary fibre and β-glucans in particular.

Tocopherols and tocotrienols, grouped as tocols and recognized generically as vitamin E, are a class of lipid-soluble antioxidants only synthesised by plants and other photosynthetic organisms, composed of eight chemical forms: α-tocopherol (α-T), β-tocopherol (β-T), γ-tocopherol (γ-T), δ-tocopherol (δ-T), and their four corresponding unsaturated tocotrienols: α-tocotrienol (α-T3), β-tocotrienol (β-T3), γ-tocotrienol (γ-T3), δ-tocotrienol (δ-T3).

Tocols have well known antioxidative properties for their effective inhibition of lipid peroxidation in biological membranes and each homologue shows, to a different extent, vitamin E activity depending on chemical structure and physiological factors (αT > βT > αT3 > γT > βT3 > δT) or no activity (γT3 and δT3) (Sheppard, Pennington, & Weihrauch, 1993). α-Tocopherol has been labelled as the most efficient antioxidant for breaking free radical-driven chain reactions *in vivo* even if the role of other forms of vitamin E has received renewed attention and it is still controversial. In particular, some studies (Packer, 1995; Packer, Weber, & Rimbach, 2001; Suzuki et al., 1993; Theriault, Chao, & Gapor, 2002) indicate that α-tocotrienol is more efficient as a scavenger of peroxyl radicals than α-tocopherol while in a recent review (Yoshida, Niki, & Noguchi, 2003) the corresponding tocopherols and tocotrienols have been found to exert the same reactivities toward radicals and the same antioxidant activities against lipid peroxidation in solution and liposomial membranes. Tocotrienols have been the focus of a growing research interest for their hypocholesterolemic action in various

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animal models and in humans (Qureshi, Bradlow, Salser, & Brace, 1997; Qureshi, Burger, Peterson, & Elson, 1986), even if, also in this claim, there is controversy between different authors and researchers (Kerckhoffs, Brouns, Hornstra, & Mensink, 2002). The number and localization of the methyl groups of their chroman rings seem to influence their biological activities, the δ-tocotrienol (8-methyl) being the most potent cholesterol inhibitor, followed by γ-tocotrienol (7,8-dimethyl) and α-tocotrienol (5,7,8-trimethyl) (Qureshi & Qureshi, 1993). Furthermore, the tocotrienols may suppress tumour cell proliferation (He, Mo, Hadisusilo, Qureshi, & Elson, 1997) and may also inhibit atherosclerotic lesions (Qureshi, Salser, Permar, & Emerson, 2001).

Several reports demonstrate that tocopherols are preferentially localized in the germ, whereas tocotrienols are concentrated in the other parts of the kernel (Falk, Krahnstöver, van der Kooij, Schlensog, & Krupinska, 2004; Peterson, 1995). A wide variation of tocol amounts in barley has been reported, depending on genotype, different growing conditions and location (Cavallero, Gianinetti, Finocchiaro, Delogu, & Stanca, 2004; Peterson & Qureshi, 1993; Peterson, 1995).

The mixed-linked (1 → 3), (1 → 4) β-D-glucans are characteristic components of cell wall of cereal endosperm which are reported to lower cholesterol levels, blunt the postprandial rise in blood glucose, increase mineral and vitamin bio-availability and control colon cancer (Khalon & Chow, 1997; Klopfenstein, 1988; McIntosh, Whyte, McArthur, & Nestel, 1991; Newman, Klopfenstein, Newman, Guritno, & Hofer, 1992). Barley kernel contains significant amount of β-glucans, about 2–11% dw (MacGregor & Fincher, 1993) but in some barleys as much as 12% or more (Newman et al., 1992). Its content is primarily associated with genotype and it is significantly affected by environmental factors (Güler, 2003; Stuart, Loi, & Fincher, 1988).

In the light of the role of β-glucans and tocols in human health, several technological processes have been employed during years to enrich their content in flour, including extraction with supercritical CO_2 (Colombo, Corsini, Mossa, Sala, & Stanca, 1998; Panfili, Cinquanta, Fratianni, & Cubadda, 2003a) and mechanical means, such as air classification, sieving and pearling (Jadhav, Lutz, Ghorpade, & Salunkhe, 1998; Wang, Xue, Newman, & Newman, 1993), in order to use them as valuable ingredients or additives in food (Brennan & Cleary, 2005; Knuckles, Hudson, Chiu, & Sayre, 1997; Marconi, Graziano, & Cubadda, 2000) and as cosmetic products. The pearling process, in particular, is an abrasive technique that gradually removes the seed coat (testa and pericarp), aleurone and sub-aleurone layers and the germ to obtain polished grain and by-products which contain interesting amounts of bioactive compounds like β-glucans, tocopherols and tocotrienols (Peterson, 1994; Zheng, Rossnagel, Tyler, & Bhatty, 2000).

The objective of this study was to determine both the β-glucan and tocol content in different barley cultivars grown in Italy and in pearling by products, in order to find commercial genotypes rich in these compounds and enriched fractions to be used as potential ingredients in functional foods (Knuckles et al., 1997; Marconi et al., 2000). Tocol analysis was made by applying a method expressly developed for cereals (Panfili, Fratianni, & Irano, 2003b), which, in contrast with other reported methods, proved to give a more accurate quali/quantitative determination of all the eight tocol forms in barley samples.

2. Materials and methods

2.1. Samples

Thirty-one hulled, one hull-less and four experimental barley genotypes (namely BA, BB, BC and BD) (Table 1) were provided by GEA S.r.l. (Acquapendente, VT, Italy). The barley genotypes were grown during 2003/2004 in an experimental field located in Melfi (southern Italy) under

Table 1
Barley genotypes and their characteristics

Sample	2-Rowed/6-rowed	Hulled/hull-less	Malting/feeding
Adagio	2	H	Malting
Alexis	2	H	Malting
Aspen	2	H	Malting
Astoria	2	H	Malting
Barke	2	H	Malting
Bodega	2	H	Malting
Bombay	2	H	Malting
Cellar	2	H	Malting
Clarine	2	H	Malting
County	2	H	Malting
Digersano	2	H-L	Malting/feeding
Esterel	6	H	Malting
Hanka	2	H	Malting
Jersey	2	H	Malting
Labea	2	H	Malting
Ladoga	6	H	Malting
Leonie	2	H	Malting
Ludine	2	H	Malting
Madou	2	H	Malting
Maggiodoro	2	H	Feeding
Meteor	2	H	Malting
Otis	2	H	Malting
Orchidea	2	H	Malting
Regina	2	H	Malting
Romina	2	H	Malting
Riviera	2	H	Malting
Sabel	2	H	Malting
Scarlett	2	H	Malting
Silvana	2	H	Malting
Svenja	2	H	Malting
Tiffany	2	H	Malting
Vanessa	2	H	Malting
BA	2	H	Malting
BB	2	H	Malting
BC	2	H	Malting
BD	2	H	Malting

the same agronomic conditions (plot area 15 m^2, ammonium phosphate dibasic 250 kg/ha, urea 46 kg/ha and ammonium nitrate 100 kg/ha).

Before milling, the barley grains (1 kg) were de-hulled by passing them through a FC2K – Otake dehuller.

Hulled grains (200 g) from a Scarlett barley stock (HK) were pearled according to a flow chart consisting of six steps using a TM – 05 Taka – Yama Testing Mill fitted with 40 P abrasive roller. Pearling by-products were collected at about 15, 15, 30, 30, 30, 60 s time intervals, in order to obtain 3–7% of kernel weight removed, by successively abrading kernels to about 70% of their original weight. For simplicity, the pearling by-products were designated as fractions I–VI and the residual pearled kernel was designed as fraction PK.

The dehulled barley grains and the residual pearled kernel, together with the original sample (50 g each), were separately milled in a Cyclotec 1093 laboratory mill (0.5 mm sieve; FOSS Italia, Padova, Italy).

2.2. Analytical procedures

Whole-meals, pearling flour, pearling by-products were analyzed using standard procedures (ICC, 1995) for moisture (method 110/1), crude protein ($N \times 6.25$) (method 105/2) and ash (method 104/1). Lipids were determined by the Soxhlet method AOAC 14.088-14.089 (AOAC, 1980). β-glucans were analyzed according to the Approved Method AACC 32.23 (AACC, 2000).

Tocols were extracted, after saponification, and determined as reported by Panfili et al. (2003b). This method allows, mostly of tocotrienols, better recoveries from cereal matrices in comparison with other methods that use methanol extraction.

Briefly, samples (2 g) were saponified under nitrogen in a screw-capped tube with 5 ml of ethanolic pyrogallol (60 g/l), 2 ml of ethanol (95%), 2 ml of sodium chloride (10 g/l) and 2 ml of potassium hydroxide (600 g/l). After alkaline digestion at 70 °C for 45 min, the tubes were cooled in an ice bath and 15 ml of sodium chloride (10 g/l) were added. The suspension was then extracted twice with 15 ml portions of *n*-hexane/ethyl acetate (9/1 v/v). The organic layer was collected and evaporated to dryness, the dry residue was dissolved in isopropyl alcohol (1%) in *n*-hexane. Extracted samples were analysed by high-performance liquid chromatography, under normal phase conditions, using a 250 mm × 4.6 mm i.d., 5 μm particle size, Kromasil Phenomenex Si column (Torrance, CA, USA) and a HPLC analytical system comprising a Waters Model 510 solvent delivery system (Milford, MA, USA) equipped with an injector with a 50 μL loop (Rheodyne, Cotati, CA) and a programmable Model 470 spectrofluorimeter (exc wavelength 290 nm, em wavelength 325 nm). The mobile phase was *n*-hexane/ethyl acetate/acetic acid (97.3/1.8/0.9 v/v/v) at a flow rate of 1.6 ml/min. Results were evaluated by a Waters Millennium Chromatography system.

α, β, γ, δ-tocopherol standards were from Merck (Darmstadt, Germany), while α, β, γ, δ-tocotrienol standards were purified according to Panfili et al. (2003b). All other reagents were of analytical or HPLC grade and were purchased from Carlo Erba (Milano, Italy). Vitamin E activity was expressed as Tocopherol Equivalents (T.E.), calculated as reported by Sheppard et al. (1993).

For tocol and β-glucan analysis the assay precision was confirmed by a RSD% lower than 4% and 2.5%, respectively. The analytical performance of the adopted methodology for tocol determination was previously reported (Fratianni, Caboni, Irano, & Panfili, 2002; Panfili et al., 2003b).

2.3. Statistical analysis

Data are reported as mean and standard deviation for at least three separate determinations for each sample. Results were statistically evaluated by means of the Student's *t*-test.

3. Results and discussion

The main tocols of the investigated 36 barley varieties were, in the following order: α-tocotrienol (α-T3), α-tocopherol (α-T), γ- and β-tocotrienol (γ-T3 and β-T3), γ- and β-tocopherol (γ-T and β-T), δ-tocotrienol and δ-tocopherol (δ-T3 and δ-T) (Table 2). α-Tocotrienol was the main homologue accounting for about 50% of total tocols (range 42%–63%) and for 65% of total tocotrienols (range 49%–83%). These results are in good accordance with Ehrenbergerovà, Belcrediovà, Prýma, Vaculovà, and Newman (2006) who reported, on the average, 54.2% of α-T3 of all tocols in barley.

High contents were also observed for α-tocopherol, about 11%–19% of total tocols, γ-tocotrienol (10%–22%) and β-tocotrienol (7%–20%). The last one was the fourth isomer for abundance, except for some genotypes (Maggiodoro, Meteor, Otis), where its amount was higher than γ-tocotrienol. Lowest amounts were found for γ-tocopherol (2%–10%) and β-tocopherol (0.6%–2%). In particular, tocopherols account for about 23% of total tocols, with a range from 18% for Madou to 29% for Scarlett, which, therefore, have the highest (82%) and the lowest (71%) percentages of tocotrienols.

These findings support those of Bhatty (1999a) and those of Ehrenbergerovà et al. (2006) while Holasova, Velisek, and Davidek (1998) reported 63% tocotrienols and 37% tocopherols of total tocols of barley.

In some genotypes all the eight forms were present, δ-tocopherol was detected in small quantities in almost all samples, while several varieties lacked δ-tocotrienol.

A great variability for tocol amounts was observed between varieties. Silvana, as reported in Table 2, showed the highest α-tocopherol amount (12.7 mg/kg dw), while β-tocotrienol and γ-tocotrienol reached the highest values respectively in Maggiodoro (18.1 mg/kg dw) and Hanka

Table 2
Tocol content in barley genotypes (mg/kg dw)

Sample	α-T[a]	α-T3[b]	β-T	γ-T	β-T3	γ-T3	δ-T	δ-T3
Adagio	11.9	34.4	1.7	5.6	11.9	11.5	1.1	n.d.
Alexis	8.4	29.4	0.5	3.3	7.1	9.7	0.8	n.d.
Aspen	9.0	33.2	0.9	2.3	9.9	10.4	0.8	0.8
Astoria	10.2	33.7	1.0	4.9	8.3	14.3	0.8	n.d.
Barke	8.3	27.7	0.6	3.9	9.5	9.7	0.4	n.d.
Bodega	10.9	38.1	1.0	7.2	9.9	9.7	0.8	n.d.
Bombay	9.5	32.7	0.8	1.7	5.4	7.7	0.5	n.d.
Cellar	11.1	30.3	0.9	5.5	9.6	10.3	0.9	n.d.
Clarive	7.8	32.0	0.6	2.3	8.2	8.0	0.4	1.3
County	8.8	35.1	1.0	5.6	10.7	12.8	1.0	1.2
Digersano	10.9	39.3	1.1	4.8	7.9	11.2	1.1	1.2
Esterel	8.2	36.8	0.9	2.2	7.4	9.9	0.9	n.d.
Hanka	11.3	43.5	1.0	4.7	6.5	14.4	1.1	n.d.
Jersey	10.1	34.3	1.0	5.3	6.4	10.4	0.5	n.d.
Labea	8.9	28.9	0.7	3.8	6.9	8.9	0.8	n.d.
Ladoga	9.7	26.0	0.6	2.2	3.6	7.1	0.6	0.4
Leonie	10.1	29.2	0.8	2.3	7.3	10.9	0.6	n.d.
Ludine	12.4	40.5	1.0	5.2	10.9	11.7	0.8	n.d.
Madou	8.8	42.2	0.5	1.8	6.3	7.6	1.1	n.d.
Maggiodoro	10.4	40.3	1.1	4.4	18.1	11.7	0.8	1.7
Meteor	11.8	34.3	1.4	4.5	10.9	7.9	0.6	n.d.
Otis	10.6	41.9	1.5	4.5	16.2	11.4	1.2	n.d.
Orchidea	10.9	42.1	1.0	6.0	6.7	7.3	0.6	0.7
Regina	10.8	35.6	1.0	3.8	7.1	8.7	0.5	n.d.
Romina	8.2	26.1	0.5	2.6	3.8	11.9	0.6	n.d.
Riviera	8.5	30.7	1.1	3.4	10.3	9.4	0.6	n.d.
Sabel	11.8	44.6	1.2	8.5	10.6	9.5	n.d.	0.7
Scarlett	10.7	26.6	1.5	5.3	5.8	12.2	0.5	n.d.
Silvana	12.7	34.1	1.1	3.1	10.6	9.7	0.7	n.d.
Sventa	9.1	38.2	0.5	1.3	7.4	7.4	0.8	n.d.
Tiffany	10.0	35.0	0.9	3.1	6.4	7.6	0.8	n.d.
Vanessa	10.3	35.6	0.8	2.6	6.2	7.2	n.d.	n.d.
BA	8.4	26.3	1.1	2.7	3.9	12.0	0.6	0.8
BB	10.0	40.5	0.4	1.5	8.4	7.7	0.5	n.d.
BC	9.3	30.5	0.8	4.6	7.5	9.6	n.d.	1.2
BD	10.7	36.6	0.9	1.9	6.8	8.8	0.5	n.d.
Min	7.8	26.0	0.4	1.3	3.6	7.1	0.4	0.4
Max	12.7	44.6	1.7	8.5	18.1	14.4	1.2	1.7
Mean	10.0	34.6	0.9	3.8	8.3	9.9	0.7	1.0
C.V.%	13.0	15.3	31.2	43.9	36.5	19.9	28.4	38.4

[a] T = tocopherol.
[b] T3 = tocotrienol.

and Astoria (about 14 mg/kg dw). The mean tocopherol amount (Table 3) was 15.5 mg/kg dw (range 11.1–21.5 mg/kg dw) while that of tocotrienols showed a range of 41.8–71.9 mg/kg dw, with a mean value of 53.6 mg/kg dw.

The total tocol amount ranged from 50.3 mg/kg dw (Ladoga) to 88.6 mg/kg dw (Maggiodoro), with a mean value of 69.1 mg/kg dw. These findings are in good agreement with those previously found by Peterson and Qureshi (1993), Cavallero et al. (2004), and Ehrenbergerovà et al. (2006) (52 mg/kg).

Looking at the percentage distribution of tocols among the different varieties, expressed in quartile ranges, most genotypes (50%) showed a content between 62 mg/kg dw and 75 mg/kg dw, the 25% lowest between 50 mg/kg dw and 62 mg/kg and the 25% highest between 75 mg/kg dw and 88 mg/kg dw. Without considering environmental factors, which have not been investigated in this study, our data reported a different genotype potential in the different varieties, in terms of tocol productivity, which could be applied for the production of food ingredients and for molecular biology approaches (i.e., breeding programs).

Table 3 also provides vitamin E activity, expressed by the Tocopherol Equivalents (T.E.), and the tocotrienols/tocopherols ratio (T3/T), which is an index of the different distribution of tocols. Adagio and Sabel were the best source of vitamin E activity (T.E.), with an average of about 27.2 mg/kg dw versus a mean value of 21.9 mg/kg dw. Hundred grams of whole barley grains provide from about 16% to 27% of vitamin E RDA (10 mg/die) (EC, 1990). The average value of the T3/T ratio was 3.5, with a range from 2.5 (Scarlett) to 4.6 (Madou).

Table 3
Tocopherol (T) and tocotrienol (T3) content and vitamin E activity (T.E.) in barley genotypes (mg/kg dw)

Sample	∑T[a]	∑T3[b]	Totals[c]	T.E[d]	T3/T
Adagio	20.7	57.9	78.6	26.7	2.8
Alexis	13.1	46.2	59.3	19.6	3.5
Aspen	13.2	54.3	67.5	20.2	4.1
Astoria	16.9	56.3	72.3	23.7	3.3
Barke	13.3	46.8	60.1	17.9	3.5
Bodega	19.9	57.7	77.6	24.0	2.9
Bombay	12.7	45.8	58.5	20.2	3.6
Cellar	18.4	50.2	68.6	21.6	2.7
Clarive	11.1	49.6	60.7	18.4	4.5
County	16.4	59.8	76.2	23.1	3.6
Digersano	17.9	59.5	77.4	24.1	3.3
Esterel	12.2	54.1	66.3	20.3	4.4
Hanka	18.1	64.4	82.5	25.7	3.5
Jersey	16.9	51.1	68.0	21.8	3.0
Labea	14.2	44.7	58.8	18.7	3.1
Ladoga	13.1	37.2	50.3	18.2	2.8
Leonie	13.8	47.4	61.2	19.9	3.4
Ludine	19.3	63.1	82.4	26.1	3.3
Madou	12.3	56.1	68.4	23.0	4.6
Maggiodoro	16.7	71.9	88.6	24.4	4.3
Meteor	18.3	52.3	70.6	23.8	2.8
Otis	17.8	69.5	87.4	25.2	3.9
Orchidea	18.5	56.8	75.3	25.0	3.1
Regina	16.1	51.4	67.5	22.7	3.2
Romina	11.9	41.8	53.7	16.7	3.5
Riviera	13.6	50.3	64.0	19.1	3.7
Sabel	21.5	65.5	87.1	27.2	3.0
Scarlett	17.9	44.6	62.5	20.2	2.5
Silvana	17.7	54.5	72.1	24.3	3.1
Sventa	11.8	53.1	64.9	21.4	4.5
Tiffany	14.8	49.1	64.0	21.7	3.3
Vanessa	13.6	48.3	61.9	21.7	3.6
BA	12.9	43.1	56.0	17.3	3.3
BB	12.5	56.6	69.1	22.9	4.5
BC	14.7	48.7	63.4	19.6	3.3
BD	14.1	52.2	66.3	22.7	3.7
Min	11.1	41.8	50.3	16.7	2.5
Max	21.5	71.9	88.6	27.2	4.6
Mean	15.5	53.6	69.1	21.9	3.5
C.V.%	18.5	13.6	13.4	12.7	16.2

[a] ∑T = Sum of tocopherols.
[b] ∑T3 = Sum of tocotrienols.
[c] Totals = Sum of tocopherols and tocotrienols.
[d] T.E. = Tocopherol Equivalents.

Table 4
β-Glucan content (g/100 g dw) in barley genotypes

Sample	Mean ± S.D.
Adagio	3.56 ± 0.04
Alexis	3.07 ± 0.06
Aspen	3.76 ± 0.01
Astoria	3.62 ± 0.01
Barke	3.96 ± 0.05
Bodega	3.23 ± 0.06
Bombay	4.71 ± 0.01
Cellar	4.70 ± 0.06
Clarive	3.40 ± 0.01
County	4.50 ± 0.03
Digersano	3.68 ± 0.03
Esterel	3.42 ± 0.06
Hanka	3.73 ± 0.02
Jersey	4.34 ± 0.08
Labea	4.71 ± 0.06
Ladoga	3.49 ± 0.05
Leonie	3.46 ± 0.08
Ludine	8.05 ± 0.18
Madour	3.60 ± 0.06
Maggiodoro	3.53 ± 0.10
Meteor	3.03 ± 0.04
Otis	3.94 ± 0.00
Orchidea	2.98 ± 0.01
Regina	3.32 ± 0.02
Romina	4.03 ± 0.02
Riviera	3.94 ± 0.08
Sabel	3.26 ± 0.01
Scarlett	4.72 ± 0.08
Silvana	3.81 ± 0.06
Sventa	4.44 ± 0.01
Tiffany	4.03 ± 0.10
Vanessa	2.64 ± 0.04
BA	3.78 ± 0.10
BB	4.69 ± 0.01
BC	4.98 ± 0.01
BD	4.23 ± 0.12
Min	2.64
Max	8.05
Mean	3.95
C.V.%	23.0

Through literature data (Ajjawi & Shintani, 2004; Grusak & DellaPenna, 1999) a clear picture has emerged that identifies enzymatic steps regulating quantitative and qualitative changes in plant-tissue tocol pools, therefore the different T3/T ratio could be explained by a different biosynthetic flux regulation leading to a different tocol composition.

Also β-glucan content shows differences among samples (Table 4). β-Glucan amount ranged from 2.64 g/100 g dw for Vanessa to 8.05 g/100 g dw for Ludine, which is the only cultivar showing an amount higher than 5 g/100 g dw. It is worth noticing that, among the different genotypes under investigation, Ludine has also one of the highest tocol amount (82.4 mg/kg dw). In terms of β-glucan distribution the mean of all 36 cultivars was 3.95 g/100 g dw and 50% of the samples had a content between 3.45 and 4.36 g/100 g dw. The lowest 25% showed an amount between 2.64 g/100 g dw and 3.45 g/100 g dw and the highest 25% between 4.36 g/100 g dw and 8.05 g/100 g dw. β-Glucan amount in barley cultivars has been reported to range between 3% and 8% (Zhang, Junmei, & Jinxin, 2002), 4% and 8% (Bhatty, 1999b) and from 2% to 11% (MacGregor & Fincher, 1993). The wide difference between Ludine and the other samples may account for the influence of genotype since the different samples grew under the same conditions. Moreover genetic factors have been found to be more important than others by several authors (Cavallero et al., 2004; Henry, 1987; Molina-Cano et al., 1997). The low β-glucan content found in most varieties accounts for the fact that, during years, commercial low β-glucan genotypes have been selected according to the primary use of barley in stock-feed and in beer indus-

try, where β-glucans adversely affect the nutritional intake and the technology, respectively. On the other hand, in view of the positive roles of β-glucans, attempts could be made in order to improve this trait in barley varieties already containing high levels of these compounds and therefore useful as food ingredients.

In order to find enriched fractions to be used as potential ingredients for functional foods, β-glucan amount was investigated on pearling by products together with tocol content. This investigation could widen previous knowledge since most work on abraded barley focused on changes of β-glucan or of tocol contents, separately (Bhatty, 1997; Klamczynski, Baik, & Czuchajowska, 1998; Marconi et al., 2000; Peterson, 1994, 1995; Zheng et al., 2000).

The weight of the successive by-products produced in pearling the barley stock together with their protein, lipid and ash content is shown in Table 5. The lowest amount of protein was found in the first by-product (fraction I), while the highest concentration was found in fractions III and IV, rich in aleurone layer and germ. The lipid content shows that the germ was mainly removed during the III and IV pearling steps. In fact the lipid content of these fractions was 7.4% and 6.3% vs a lipid content of 2.1% of the original kernel. A progressive decrease in the percentage of ash from I (5.7%) to VI (3.1%) was found, since the mineral components are mainly distributed in the outer layers of the kernel (pericarp, aleurone and germ).

Table 5 reports also the composition and levels of tocols, expressed as tocopherols, tocotrienols, total tocols and Tocopherol Equivalents (T.E.), in the removed by-product fractions and in the pearled kernel. In the pearling by-products, in comparison with the hulled grain, there was a progressive increase in tocopherol concentration which reached its maximum level (about 4–5-fold enrichment) in fractions II, III and IV, corresponding to the highest lipidic amount and therefore to the main presence of the germ. Tocotrienols reached their highest level in fraction III and IV (about five- and four-fold enrichment) corresponding to the highest protein amount and to the main presence of the aleurone layer, as previously observed by other authors (Peterson, 1994, 1995).

Considering the distribution of total tocols, their highest concentration was found in fraction III, with about a five-fold increase, with a T.E. enrichment in fraction IV, due to the highest amount of α-tocopherol, which accounts for the maximum vitamin E activity. Hundred grams of removed by-product fractions (from I to VI) provide from 33% to 85% of vitamin E RDA (10 mg/die) (EC, 1990). In the pearled kernel (PK) tocols reached their lowest amount, with a minimum concentration of both tocopherols and tocotrienols.

These results show that tocopherols and tocotrienols are distributed in a tissue specific manner in barley kernels, with tocopherols located in the germ and tocotrienols in the aleurone and sub-aleurone layer, therefore they could fulfil different functions.

Looking at the individual distribution of tocopherols (Table 6), α- and β-tocopherol reached their highest concentration in fraction IV (44.1 and 0.9 mg/kg, respectively), with an enrichment of about seven and five-folds, if compared to the hulled grain (6.9 and 0.2 mg/kg, respectively).

γ-Tocopherol seemed to be mainly localized in fraction I (14.1 versus 2.9 mg/kg), with about five-fold increase, however this different behaviour needs to be further investigated in other varieties.

With regard to tocotrienols the same behaviour was observed for all four homologues, which increased from four-folds (β-tocotrienol) to nine-folds (δ-tocotrienol) in fraction III.

Considering β-glucans (Table 5), their amount in fractions I and II was very low, while it increased during the successive removal of the outer layers in the pearling process, although, in our condition, in the removed by-product fractions no enrichment was observed as to hulled kernel. This behaviour is in accordance to what observed by other authors on by-product fractions at the same degree of pearling (Marconi et al., 2000; Zheng et al., 2000).

The increase in β-glucan concentration in the inner layers of the kernels reflects their location, since they are the

Table 5
Protein, lipid, ash (% wb) and β-glucans (g/100 g wb), tocols (mg/kg wb) in successively removed by-product fractions of a barley stock

Stock and fraction[a]	% Kernel weight removed	% Total cumulative weight removed	Protein in fraction	Lipid in fraction	Ash in fraction	β-Glucans	∑T[b]	∑T3[c]	Total tocols	T.E.[d]
HK			8.6	2.1	1.6	4.34[a]	10.1[a]	38.0[a]	48.1[a]	17.2[a]
I	6.8	6.8	5.1	3.0	5.7	0.35[b]	29.3[b]	51.0[b]	80.4[b]	33.4[b]
II	3.2	10.0	15.1	4.0	5.6	0.62[c]	41.0[c]	177.4[c]	218.4[c]	68.7[c]
III	4.3	14.3	16.7	7.4	5.4	2.78[d]	42.0[d]	205.4[d]	247.4[d]	83.9[d]
IV	4.3	18.6	16.8	6.3	5.1	3.46[e]	54.4[e]	155.7[e]	210.1[c]	84.9[c,d]
V	4.2	22.8	15.4	5.1	3.9	3.79[f]	35.1[b]	113.4[f]	148.5[e]	55.9[c]
VI	7.2	30.0	14.0	3.9	3.1	4.50[g]	25.4[b]	65.1[g]	90.6[b]	37.0[b]
PK			6.4	1.0	0.6	4.82[h]	1.8[f]	6.2[h]	8.0[f]	2.7[f]

Values within each column followed by the same letter do not differ significantly at $P > 0.05$.
[a] HK = Hulled kernel, PK = pearled kernel.
[b] ∑T = Sum of tocopherols.
[c] ∑T3 = Sum of tocotrienols.
[d] T.E. = Tocopherol Equivalents.

Table 6
Individual tocols (mg/kg wb) and T3/T ratio in removed by-product fractions of the barley stock

Stock and fraction[a]	Tocols								
	α-T[b]	α-T3[c]	β-T	γ-T	β-T3	γ-T3	δ-T	δ-T3	T3/T
HK	6.9	28.6	0.2	2.9	2.9	6.3	0.1	0.2	3.7
I	14.4	38.6	0.3	14.1	3.1	8.8	0.4	0.5	1.7
II	28.2	136.7	0.8	11.6	9.6	29.6	0.4	1.4	4.3
III	30.9	157.2	0.8	9.7	11.7	35.2	0.5	1.2	4.9
IV	44.1	117.7	0.9	8.9	9.7	27.1	0.5	1.1	2.9
V	26.9	83.0	0.6	6.9	7.3	22.2	0.6	0.9	3.2
VI	18.7	49.6	0.6	5.8	4.1	11.0	0.2	0.4	2.6
PK	1.3	3.9	0.1	0.4	1.2	0.9	0.0	0.1	3.5

[a] HK = Hulled kernel, I–VI = pearling by-products, PK = pearled kernel.
[b] T = Tocopherol.
[c] T3 = Tocotrienol.

main cell wall components of the starchy endosperm and the aleurone. The inner location of β-glucans is confirmed by data on the pearled kernel (PK), where they reached their maximum level (4.82%).

Literature data report a non uniform distribution of β-glucans among the by-products due to the natural shape of the kernel, the cultivar (waxy–non-waxy, low–high β-glucan) and the moisture level (Bhatty, 1997; Klamczynski et al., 1998; Zheng et al., 2000).

Looking at the content of both tocols and β-glucans in the removed by-product fractions (Table 5) their different distribution is evident, with fractions III and IV having the highest tocopherol and tocotrienol content and fraction VI the highest β-glucan amount. From a nutritional point of view fractions II, III and IV, for their highest tocol content (about five-folds the levels of the hulled kernel) could be of interest. Relatively to β-glucans, since no enrichment was observed in the same fractions, use of varieties with higher β-glucan amounts or with their maximum localization in the aleurone and sub aleurone layers is worthwhile.

4. Conclusions

In this study a wide variability of β-glucans and tocols among barley genotypes was found, either for total amount or, as for tocols, for their relative homologue abundance. The obtained results also showed that, as reported by other authors, some barley pearling by-products have interesting amounts of these bio-active compounds.

By means of physical processes, such as milling, sieving, air classification, genotypes with high levels of β-glucans and tocols, or with a favourable distribution of these compounds in the kernel, could be used to produce enriched fractions for the realization of functional foods.

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