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ScienceDirect
Food Chemistry 107 (2008) 444–448
Food Chemistry
www.elsevier.com/locate/foodchem

Protein, ash, lutein and tocols distribution in einkorn (*Triticum monococcum* L. subsp. *monococcum*) seed fractions

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Received 27 March 2007; received in revised form 12 June 2007; accepted 1 August 2007

Abstract

The distribution of protein, ash, lutein, tocopherols and tocotrienols in the germ, bran and endosperm portions was studied in seeds of two einkorn accessions and one bread wheat. The two einkorns showed a higher content of most compounds, but the distribution within the kernel was similar in both species. The germ fraction showed the highest concentration of protein, lutein, α-tocopherol, β-tocopherol and total tocols. Ash, α-tocotrienol and β-tocotrienol levels were highest in the bran fraction, although significant quantities were detected also in the germ and, for tocotrienols, in the flour.

Notwithstanding the lower concentrations, the endosperm contributed most protein and lutein, as well as one-third of tocotrienols to the whole kernel; this suggests that, after the milling process, the white flour still retains most of the nutritional value of the whole kernel.

Keywords: Einkorn; Lutein; Tocopherols; Tocotrienols; Seed fractions; HPLC

1. Introduction

Wheat seeds (also dubbed kernels) are monocarpellate indehiscent dry fruits, called caryopses. The milling process separates the ground seeds into three main fractions: embryo, endosperm and bran. Embryo and endosperm originate from the fertilisation process, while the seed outer layers, that constitute most of the bran, are of maternal origin. The endosperm consists of two tissues, the aleurone (an external layer of cells, removed during the milling process) and the starchy endosperm. The embryo (germ) represents ca. 2.5–3.8% of total seed weight, the starchy endosperm 80.1–86.5% and the bran (including pericarp, seed coat, hyaline layer and aleurone) 10.4–17.9% (Pomeranz, 1988).

The distribution of nutrients in these wheat fractions varies considerably: the bran has high levels of ash, minerals, proteins (Pomeranz, 1988), and of some antioxidants, such as tocotrienols and phenols (Beta, Nam, Dexter, & Sapirstein, 2005; Ko et al., 2003; Piironen, Syväoja, Varo, Salminen, & Koivistoinen, 1986); the germ is particularly rich in proteins, lipids and tocopherols (Ko et al., 2003; Pomeranz, 1988); while the starchy endosperm is abundant in storage proteins (mostly gliadins and glutenins) and carbohydrates (Pomeranz, 1988).

The kernel fractions composition of durum and bread wheats is well characterised, but no comparable information is available on einkorn (*Triticum monococcum* subsp. *monococcum*). Einkorn is a diploid hulled wheat appreciated for its excellent nutritional properties, including high protein, carotenoids and tocols contents (Abdel-Aal et al., 2002; Corbellini et al., 1999; Hidalgo, Brandolini, Pompei, & Piscozzi, 2006), and good adaptation to low-input cropping. Current trends towards low-impact and sustainable agriculture, as well as an increase in the utilisation of "biological" and "functional" products, suggest that this cereal may still play a role in human consumption.

The aim of this research was the evaluation of protein, ash, lutein and tocols distribution in the germ, bran and endosperm fractions of the einkorn seed.

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doi:10.1016/j.foodchem.2007.08.009

2. Materials and methods

Two einkorn accessions, the variety Monlis and the free-threshing breeding line SAL 98-32, and the bread wheat cultivar Blasco (as control) were cropped during the 2005-06 growing season in the Po plain, at S. Angelo Lodigiano (Italy), following standard cultural practices (Castagna, Borghi, Di Fonzo, Heun, & Salamini, 1995).

After manual harvesting, the samples were stored at −70 °C and, immediately, before the analyses, were de-hulled in a M3B micro-thresher (Co.Mi.L, Rome, Italy).

Kernel weight was determined with four independent replications of 50 seeds each, and corrected to 15% humidity. To determine germ-to-seed ratio, the germs from four 50-seed samples per accession were manually dissected under a 10× magnifying lens: to this end, after enucleating the germs with the tip of a scalpel blade, the adhering pericarp layers as well as any endosperm remains were carefully removed.

The whole flours were prepared with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark). The bran and endosperm flour were prepared from 150 g of manually de-germinated seeds (ca. 1/3 of each seed, including germ and scutellum, was removed with a scalpel), milled with a Bona-GBR lab mill (Bona, Monza, Italy). Three fractions were obtained, bran, shorts and flour; bran and shorts were carefully sieved using a 200 μm mesh to separate the adhering flour, and then finely ground with the Cyclotec 1093 lab mill. The fractions were weighed and stored under vacuum at −20 °C for a maximum of 24 h before analysis.

The analyses were performed on intact and degerminated seed whole flour, endosperm flour and bran. Germ composition was assessed by comparing the results of the intact and degerminated seed whole flours.

Dry matter, protein ($N \times 5.7$) and ash contents were determined following methods 44–15, 46–10 and 08–03 (AACC, 1994), respectively. Tocopherols and tocotrienols quantification was performed by normal-phase HPLC, following Panfili, Fratianni, and Irano (2003), while lutein content was determined by reverse-phase HPLC, as suggested by Hidalgo et al. (2006). All data, expressed on dry matter (DM) basis, are averages of two independent measurements.

3. Results and discussion

Precise separation of the structural parts of the wheat caryopsis is a difficult and laborious task. Seed fractions evaluation therefore often relies on kernel milling and subsequent recovery of germ, bran and different flour types. In this research we tried to combine the accuracy of manual separation with the simplicity of milling. Therefore, germ composition was assessed by comparing the whole flour results from intact and degerminated kernels, while bran and endosperm compositions were determined from milled de-germinated kernels.

Germ proportion, evaluated by accurate manual recovery (see Section 2), in einkorn was only slightly superior to bread wheat (3.0 ± 0.06 and 3.2 ± 0.04% for Monlis and SAL 98–32, vs. 2.9 ± 0.02% for Blasco). Sharp differences were observed instead for bran (22.0 and 23.8%, vs. 16%) and endosperm (74.9 and 73.1%, vs. 81.2%). The results of Blasco fit nicely into the variation reported for wheat germ (2.5–3.8%), bran (10.4–17.9%) and endosperm (80.1–86.5%) by Pomeranz (1988), implicitly corroborating the efficiency of fractions separation and, consequently, the validity of our results. The higher bran fraction of einkorn is presumably related to its smaller kernels (22.0 ± 0.2, 24.7 ± 0.2 and 46.2 ± 0.1 mg/seed for Monlis, SAL 98-32 and Blasco, respectively), that, as a consequence, have a higher proportion of external surface (i.e., the bran part) than bread wheat.

The concentration of protein, ash and lutein in whole flour and the three different seed fractions are presented in Table 1. The contents of all three compounds are higher in the two einkorn samples than in the bread wheat, as reported also by D'Egidio, Nardi, and Vallega (1993), Corbellini et al. (1999) and Hidalgo et al. (2006), but their distribution in the three fractions follows a similar pattern. Protein concentration is at a maximum in the germ, with values 2–3 times higher than in the two other fractions. Ash, on the other hand, is low in the endosperm and high in the bran, as reported for bread wheat by Pomeranz (1988). Lutein, a lipophilic antioxidant, in Blasco is mainly restricted to the germ; Adom, Sorrells, and Liu (2003) observed that the lutein content of the bran + germ milled fraction of bread wheat was about 4-fold higher than that of the flour fraction. The *T. monococcum* samples however show a different pattern: lutein concentration is indeed highest in the germ (38.0 and 26.3 μg/g DM for Monlis and SAL 98–32, respectively), but high values are also observed in the endosperm (7.4 and 5.1 μg/g DM) and bran (4.0 and 4.5 μg/g DM).

The tocopherols and tocotrienols concentration in the whole flour and in the three different seed fractions are

Table 1
Protein (g/100 g DM), ash (g/100 g expressed as dry matter [DM]) and lutein (μg/g DM) content (± s.e.) of whole flour, endosperm, bran and germ fractions of einkorns Monlis and SAL 98–32, and bread wheat Blasco

	Protein			Ash			Lutein		
	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco
Whole flour	15.8 ± 0.05	24.2 ± 0.45	11.1 ± 0.02	2.2 ± 0.01	2.5 ± 0.01	1.5 ± 0.00	7.5 ± 0.02	5.8 ± 0.19	0.9 ± 0.09
Endosperm	13.8 ± 0.02	22.3 ± 0.11	10.1 ± 0.07	0.4 ± 0.01	0.7 ± 0.03	0.4 ± 0.00	7.4 ± 0.15	5.1 ± 0.03	0.8 ± 0.01
Bran	18.2 ± 0.18	26.2 ± 0.54	14.2 ± 0.10	7.5 ± 0.03	6.8 ± 0.01	6.8 ± 0.01	4.0 ± 0.08	4.5 ± 0.01	0.7 ± 0.01
Germ	48.5 ± 2.26	45.4 ± 2.75	22.4 ± 2.32	4.8 ± 0.11	4.4 ± 0.15	2.7 ± 0.11	38.0 ± 1.06	26.3 ± 3.83	6.3 ± 2.88

reported in Table 2. The normal-phase HPLC analysis detected four tocols: α-tocopherol, β-tocopherol, α-tocotrienol and β-tocotrienol; their whole flour content was higher in the einkorns than in the control bread wheat, with the exception of β-tocopherol, as reported also by Hidalgo et al. (2006).

In both *Triticum* species, the two tocopherols were limited almost exclusively to the germ fraction, where the concentrations were extremely high (on average, 390 μg/g DM for α-tocopherol and 141 μg/g DM for β-tocopherol); only traces were detected in the two remaining fractions. A similar trend was observed, in millstream fractions, by Morrison, Coventry, and Barnes (1982) and Piironen et al. (1986) for bread wheat, and by Ko et al. (2003) for rice, barley, wheat and maize, leading Morrison et al. (1982) to propose tocopherols as markers for germ lipids in flour.

The tocotrienols were located mainly in the bran, but significant quantities of α-tocotrienol were observed also in the germ, and of β-tocotrienol in the germ and in the flour. Morrison et al. (1982) and Piironen et al. (1986) reported that the tocotrienols were almost all concentrated in the wheat bran; a significant β-tocotrienol content (13.5 μg/g DM) was however observed in the endosperm by Morrison et al. (1982). Ko et al. (2003), on the other hand, did not recognise β-tocotrienol in wheat, but reported significant concentrations of α-tocotrienol, not only in the bran but also in the other two fractions.

Overall, the concentration of total tocols in the germ (on average, 574 μg/g DM) was about four times higher than in the bran and 24 times higher than in the endosperm.

As previously mentioned, the endosperm constitutes the vast majority of wheat and einkorn seed, while the germ is only ca. 3%. Therefore, the relevance of each fraction to whole kernel composition is a consequence of nutrients concentration and fraction weight. The contribution of endosperm, bran and germ to whole seed content of protein, ash, lutein and tocols is depicted in Fig. 1. For both species, the endosperm was by and large the major contributor for both protein (66.4% and 73.6% for einkorn and bread wheat, respectively) and lutein contents (74.6% and 69.4%), while the other two fractions had only a marginal role; the ash, instead, came mostly from the kernel bran (71.0% and 72.0%). Pomeranz (1988) reported that germ and bran account for 3.4% and 14.1% of the total wheat seed proteins, while the different flour fractions make up the remaining portion.

The germ contained the vast majority (>90%) of the tocopherols. Most of the α-tocotrienol (about 75% in einkorn) was in the bran, but the endosperm showed also a significant contribution; β-tocotrienol, on the other hand, was stored in similar quantities in the bran and the endosperm. The contribution of the germ fraction to the total tocotrienols content was almost negligible (on average <5%). A similar contribution was observed by Morrison et al. (1982) in bread wheat flours millstreams, and by Falk, Krahnstover, van de Kooij, Schlensog, and Krupinska (2004), studying manually-prepared fractions of barley kernels.

Table 2
Content (± s.e.) of α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol, and total tocols (μg/g DM) in whole flour, endosperm, bran and germ fractions of einkorns Monlis and SAL 98–32, and bread wheat Blasco

	α-Tocopherol			α-Tocotrienol			β-Tocopherol			β-Tocotrienol			Total tocols		
	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco	Monlis	SAL 98–32	Blasco
Whole flour	14.7 ± 0.30	13.5 ± 0.53	9.9 ± 0.12	17.6 ± 0.06	17.9 ± 0.26	3.5 ± 0.06	5.6 ± 0.40	3.0 ± 0.30	5.6 ± 0.04	45.3 ± 0.75	48.0 ± 0.48	21.4 ± 0.15	83.3 ± 1.39	82.5 ± 0.60	40.5 ± 0.37
Flour	0.5 ± 0.03	0.4 ± 0.18	0.4 ± 0.08	4.4 ± 0.05	5.8 ± 0.04	1.2 ± 0.07	0.3 ± 0.01	0.3 ± 0.00	0.5 ± 0.04	21.0 ± 0.36	23.9 ± 0.30	13.3 ± 1.00	26.2 ± 0.48	30.4 ± 0.52	15.4 ± 1.18
Bran	1.5 ± 0.27	1.4 ± 0.04	1.7 ± 0.01	54.4 ± 2.18	57.5 ± 0.61	14.2 ± 0.32	0.6 ± 0.06	1.2 ± 0.18	0.8 ± 0.34	130 ± 8.89	125 ± 1.78	62.5 ± 2.62	187 ± 11.40	184 ± 1.03	79.6 ± 4.16
Germ	448 ± 10.73	403 ± 19.37	321 ± 3.52	25.1 ± 0.55	8.7 ± 4.52	9.3 ± 1.65	167 ± 12.98	77.9 ± 9.92	177 ± 2.32	31.7 ± 0.57	29.5 ± 5.07	23.4 ± 8.35	671 ± 23.73	519 ± 38.87	531 ± 2.68

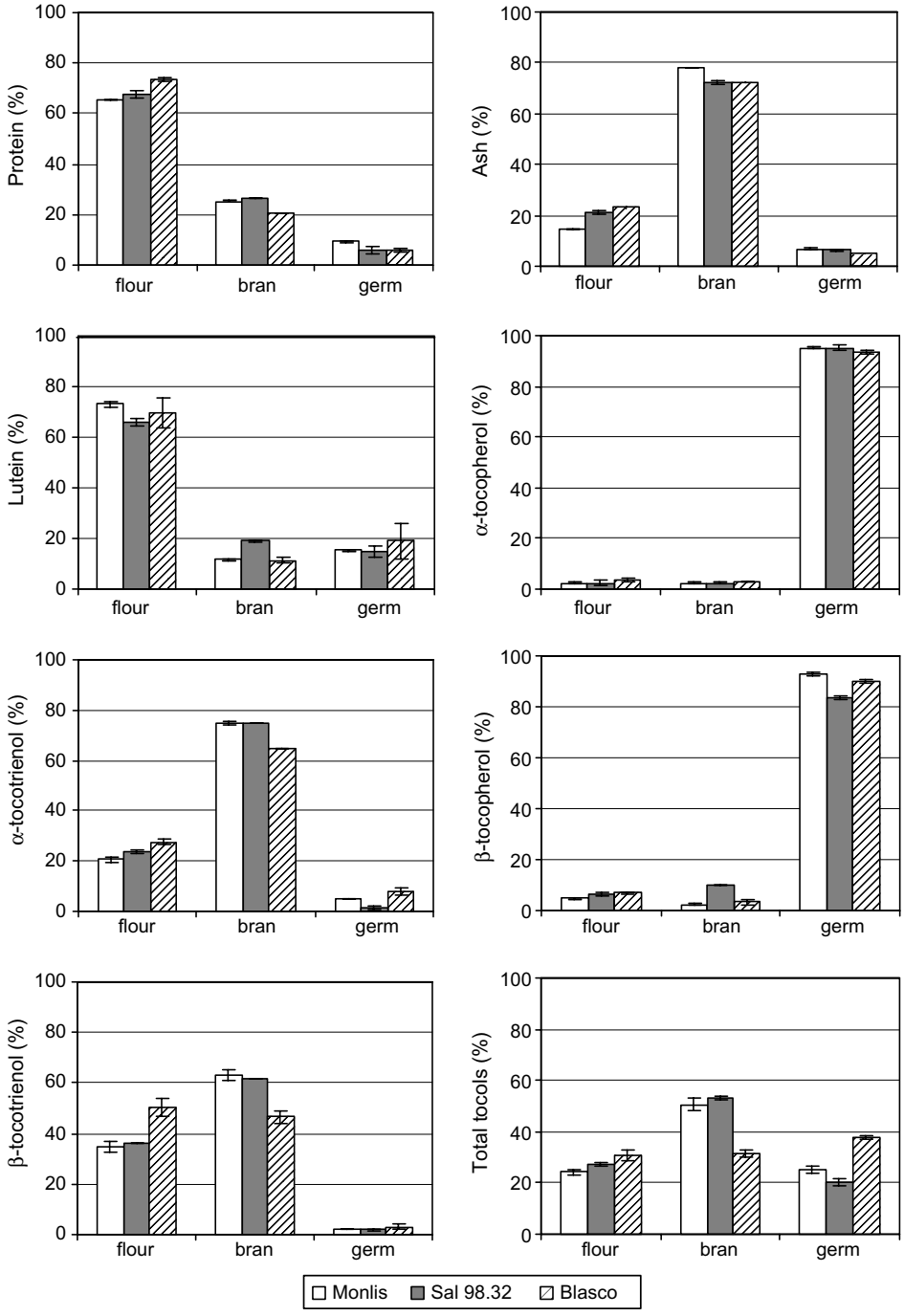

Fig. 1. Endosperm, bran and germ contribution (%) to the total kernel composition of protein, ash, lutein, α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol and total tocols of einkorn variety Monlis, einkorn line SAL 98-32 and bread wheat cultivar Blasco. Error bars indicate the standard error of the mean ($n = 2$).

A different situation was evident for total tocols, where the three fractions contributed rather similar quantities to the kernel. In particular, Blasco possessed flour, bran and germ values of 31%, 32% and 37%, respectively; the two einkorns, instead, showed a relatively larger contribution of the bran fraction (on average, 51%), followed by flour (26%) and germ (23%). The results reported by Falk et al. (2004) for barley, in comparison, emphasise the importance of the endosperm (58.6–59.5%), followed by the pericarp (25.8–27.9%) and the germ (6.7–8.5%).

4. Conclusions

Although the absolute values are different, the concentration of protein, ash, lutein and tocols in the kernels of the einkorns Monlis and SAL 98–32, and of the bread wheat Blasco follow a similar pattern. Whole flour enjoys

a nutritional advantage over the de-branned and de-germinated flour, mainly because of the high germ and bran tocols content; however, the relevant concentrations of protein, lutein and tocotrienols observed in the endosperm (particularly in the case of einkorn) imply that most of their nutritional value is still retained by the white flour after the milling process.

Acknowledgement

This research was supported by the project n.1018 "MonICA-Monococco per l'innovazione agricola e colturale", sponsored by the Regione Lombardia, Italy.

References

AACC – American Association of Cereal Chemists. (1994). AACC Official Methods 08–03, 14–50, 44–15, 46–10. In *Approved Methods of the American Association of Cereal Chemists*. Minneapolis, MN, USA.

Abdel-Aal, E.-S. M., Young, J. C., Wood, P. J., Rabalski, I., Hucl, P., Falk, D., & Frégeau-Reid, J. (2002). Einkorn: A potential candidate for developing high lutein wheat. *Cereal Chemistry, 79*, 455–457.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry, 51*, 7825–7834.

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry, 82*, 390–393.

Castagna, R., Borghi, B., Di Fonzo, N., Heun, M., & Salamini, F. (1995). Yield and related traits of einkorn (*Triticum monococcum* ssp. *monococcum*) in different environments. *European Journal of Agronomy, 4*, 371–378.

Corbellini, M., Empilli, S., Vaccino, P., Brandolini, A., Borghi, B., Heun, M., & Salamini, F. (1999). Einkorn characterization for bread and cookie production in relation to protein subunit composition. *Cereal Chemistry, 76*, 727–733.

D'Egidio, M. G., Nardi, S., & Vallega, V. (1993). Grain, flour and dough characteristics of selected strains of diploid wheat *Triticum monococcum* L.. *Cereal Chemistry, 70*, 298–303.

Falk, J., Krahnstover, A., van de Kooij, A. W., Schlensog, M., & Krupinska, K. (2004). Tocopherol and tocotrienol accumulation during development of caryopses from barley (*Hordeum vulgare* L.). *Phytochemistry, 65*, 2977–2985.

Hidalgo, A., Brandolini, A., Pompei, C., & Piscozzi, R. (2006). Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *Journal of Cereal Science, 44*, 182–193.

Ko, S.-N., Kim, C.-J., Kim, H., Kim, C.-T., Chung, S.-H., Tae, B.-S., & Kim, I.-H. (2003). Tocol levels in milling fractions of some cereal grains and soybean. *Journal of the American Oil Chemists Society, 80*, 585–589.

Morrison, W. R., Coventry, A. M., & Barnes, P. J. (1982). The distribution of acyl lipids and tocopherols in flour millstream. *Journal of the Science of Food and Agriculture, 33*, 925–933.

Panfili, G., Fratianni, A., & Irano, M. (2003). Normal-phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *Journal of Agricultural and Food Chemistry, 51*, 3940–3944.

Piironen, V., Syväoja, E. L., Varo, P., Salminen, K., & Koivistoinen, P. (1986). Tocopherols and tocotrienols in cereal products from Finland. *Cereal Chemistry, 63*, 78–81.

Pomeranz, Y. (1988). Chemical compounds of kernel structures. In Y. Pomeranz (Ed.), *Wheat chemistry and technology. American Association of Cereal Chemists Inc.* (pp. 97–158). USA: St. Paul., Minnesota.