

Influence of Genetic and Environmental Factors on Selected Nutritional Traits of *Triticum monococcum*

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To evaluate the effect of genotype, year, and location on protein, lipid, tocol, and lutein content and on fatty acids composition of wholemeal flour, five einkorns (*Triticum monococcum* L. ssp. *monococcum*) and one control bread wheat were cropped in Italy for two years in four different locations. Genotype and year exerted major effects on protein, tocotrienol, and lutein contents, while tocopherol and lipid contents were influenced only by the genotype. The concentrations of linoleic, oleic, and palmitic acids (the most abundant among the 14 fatty acids identified), as well as of saturated (SFA), monosaturated (MUFA), and polyunsaturated (PUFA) fatty acids, differed between einkorns and control and, to some extent, years were constant across locations. Notwithstanding the environmental variation, all einkorns consistently showed higher protein (on average, +59%), lipid (+50%), tocotrienol (+88%), total tocol (+46%), lutein (+483%), and MUFA (+53%) content, along with lower SFA (−21%) and PUFA (−8%) than the bread wheat control.

KEYWORDS: Einkorn; wheat; fatty acids; lutein; protein; tocots

INTRODUCTION

The quality of cereals and derived products is deeply influenced not only by genetic factors but also by the cropping environment, i.e., climate, soil composition, light intensity, fertilization, pests, and diseases, etc. Stress conditions, such as water deficit and high temperatures, stimulate the formation, in plant cells, of chemical compounds which trigger destructive oxidizing processes like chlorophyll decoloring, lipids peroxidation, and nucleic acids damage (1). As a reaction mechanism, the plant synthesizes antioxidant protectants such as α -tocopherol (2). Changes in the quantity and composition of antioxidants and protective pigments, therefore, reflect the impact of environmental stresses on plant metabolism (3). Experiments carried out in canola indicate that tocots are strongly influenced by temperature (4, 5) and solar radiation (4). In wheat, temperatures above 32 °C depress phenol content, iron chelation, and antioxidant activity (6–8); conversely, cooler years increase tocol content (9) while cooler and wetter seasons improve lutein quantity (10).

Fatty acids are compounds important from nutritional and technological perspectives; their composition varies among cereals (11) and has been proposed as a useful discriminant not only for durum and bread wheat (12) but also for varieties, geographical origin, and crop years (13).

Einkorn (*Triticum monococcum* L. subsp. *monococcum*), a diploid hulled wheat closely related to durum and bread wheat, is a cereal with high protein (14), carotenoid (15, 16), and tocol (16)

contents. A lower toxicity toward celiac patients than other *Triticum* species has often been reported (17–20). These considerations suggest a possible utilization of einkorn flour for the development of new or special foods such as bakery products, baby food, or products with superior nutritional quality (14–16, 21, 22).

The aim of this research was therefore to evaluate the influence of genotype, year, and cropping location on proteins, lipids, and lipophilic antioxidants (tocols and lutein) content, as well as on fatty acids composition of einkorn and bread wheat whole meal flour.

MATERIALS AND METHODS

Samples. Five einkorn accessions were cropped during the 2005–2006 and 2006–2007 growing seasons in four different environments, using a randomized complete block design with three replications. Einkorns Monlis and ID331 were selected because of their good breadmaking properties, ID1395 is a high yielding accession, while SAL9832 and SAL9838 are free-threshing breeding lines. One well adapted, broadly cropped local bread wheat cultivar, Blasco, was cultivated as the control. The four environments were: (1) Sant'Angelo Lodigiano (Po plain, northern Italy), standard cultural practices; (2) Sant'Angelo Lodigiano, organic farm and organic cultural practices; (3) Leno (Po plain, northern Italy), organic farm and organic cultural practices; (4) Montelibretti (near Rome, central Italy) standard cultural practices. More information on the four environments and on crop management are presented in **Table 1**; mean temperature and total rainfall during the crucial flowering and seed-setting months (April, May, and June, 2006 and 2007) are depicted in **Figure 1**.

Grain and Flour Characteristics. Recently harvested seeds of einkorns Monlis, ID331, and ID1395 were dehulled with an Otake FC4S thresher (Satake, Japan); dehulling was not required for the

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Table 1. Cropping Locations, Agronomic Management, and Yield Information

	Sant'Angelo Lodigiano	Sant'Angelo Lodigiano	Leno	Montelibretti
latitude	45°13' N	45°13' N	45°20' N	42°07' N
longitude	09°25' E	09°26' E	10°11' E	12°41' E
altitude (m)	73	73	66	232
soil	sandy	sandy	sandy loam	sandy loam
agrotechnique	traditional	organic	organic	traditional
		2005–2006		
planting date	10 Nov 2005	28 Oct 2005	17 Nov 2005	18 Dec 2005
harvesting date	17 July 2006	15 July 2006	16 July 2006	11 July 2006
fertilization	13 Mar. 2006	15 Mar. 2006	17 Mar. 2006	7 Mar. 2006
N (kg/ha)	64	80	80	80
fertilizer	ammonium nitrate	Endurance N8 ^a	Endurance N8 ^a	ammonium nitrate
yield (t/ha ± se)				
einkorns	1.584 ± 0.140	1.288 ± 0.073	0.679 ± 0.133	1.792 ± 0.150
Blasco	6.463 ± 0.166	6.536 ± 0.185	3.316 ± 0.097	5.773 ± 0.354
		2006–2007		
planting date	4 Nov 2006	26 Oct 2006	6 Oct 2006	15 Nov 2006
harvesting date	17 July 2007	18 July 2007	11 July 2007	26 June 2007
fertilization	22 Feb 2007	29 Jan 2007	1 Feb 2007	6 Mar 2007
N (kg/ha)	80	80	80	54
fertilizer	ammonium nitrate	Endurance N8 ^a	Endurance N8 ^a	ammonium nitrate
yield (t/ha ± se)				
einkorns	2.497 ± 0.085	1.668 ± 0.048	0.751 ± 0.087	2.108 ± 0.154
Blasco	6.738 ± 0.317	6.561 ± 0.239	3.505 ± 0.122	4.990 ± 0.370

^a Organic fertilizer (Unimer, Vidor, TV, Italy).

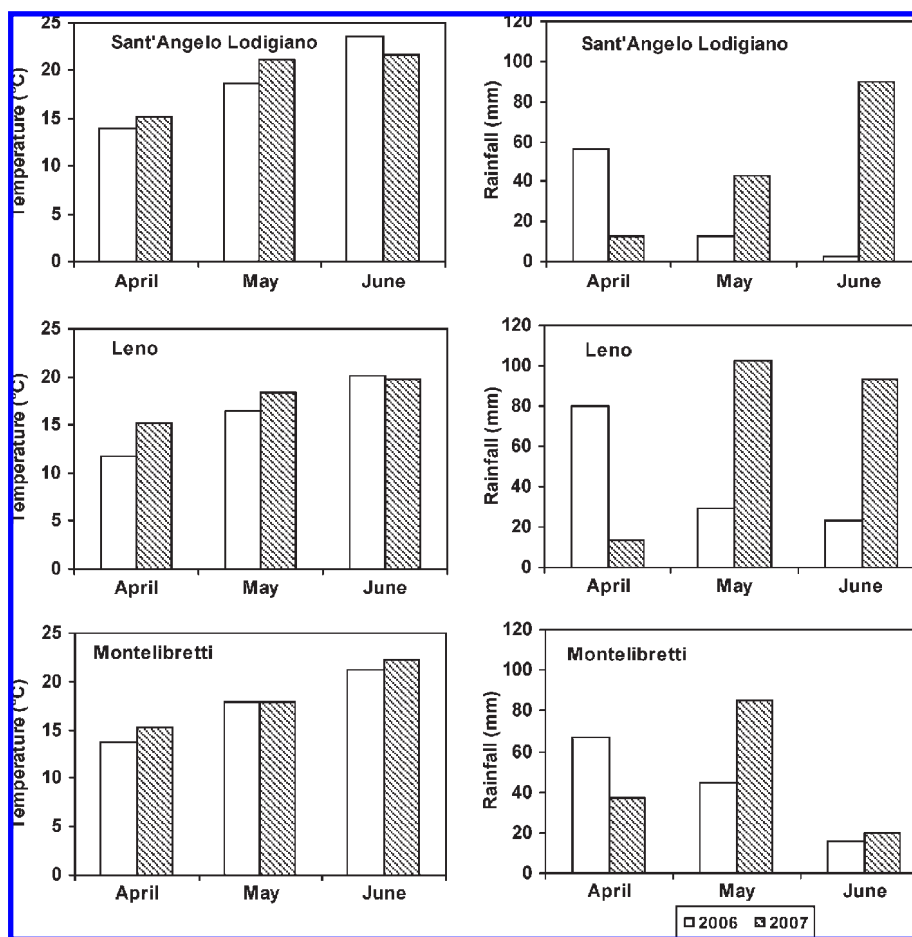


Figure 1. Mean temperature and total rainfall during the months of April, May, and June 2006 and 2007 at S. Angelo Lodigiano, Leno, and Montelibretti.

free-threshing einkorns SAL9832 and SAL9838 or for the bread wheat cv Blasco. The seeds were ground with a Cyclotec 1093 lab mill (Foss Tecator,

Denmark), obtaining a whole meal flour with particle size < 200 μm. The samples were stored under vacuum at -20 °C until analysis.

Table 2. Mean Square Values and Significance for Mixed-Model (Year Random, Location and Genotype Fixed) ANOVA of Proteins, Lipids, Tocols, and Lutein Content in Whole Meal Flour of Five Einkorn Accessions and of Bread Wheat cv Blasco, Cropped in Two Years and Four Locations

source of variation	df	proteins	lipids	α -tocopherol	α -tocotrienol	β -tocopherol	β -tocotrienol	total tocots	lutein
year (Y)	1	163.54 ^c	0.07	0.00	47.90 ^c	0.50	1491.76 ^c	2143.18 ^c	15.71 ^c
location (L)	3	34.04	1.11	24.99	33.60	3.91	165.97	612.21	6.21
Y×L	3	23.85 ^c	0.38 ^c	7.87 ^c	23.44 ^c	2.38 ^c	21.25 ^c	155.86 ^c	0.70 ^b
repetition (YL)	8	0.02	0.02	0.27	0.22	0.15	3.46	7.83	0.05
genotype (G)	5	155.27 ^c	5.61 ^c	82.41 ^c	410.87 ^c	27.49 ^c	945.48 ^c	2150.83 ^c	105.93 ^c
einkorn vs control	(1)	670.43 ^c	25.16 ^c	0.79	1256.63 ^c	99.99 ^c	3644.47 ^c	7212.91 ^c	436.37 ^c
within einkorns	(4)	26.48 ^a	0.72	102.81 ^c	199.43 ^c	9.36 ^a	285.73	885.30 ^a	23.32 ^a
Y×G	5	2.69 ^c	0.21 ^b	2.70 ^c	5.59 ^c	1.03 ^b	77.49 ^c	112.91 ^c	3.68 ^c
L×G	15	3.76 ^a	0.13	2.67	5.75	0.58	20.97	71.71 ^a	0.87
Y×L×G	15	1.34 ^c	0.18 ^c	2.05 ^c	3.88 ^c	0.37	11.25 ^c	26.28 ^c	1.27 ^c
pooled error	40	0.01	0.05	0.23	0.28	0.23	2.50	7.01	0.04

^a* $p \leq 0.05$. ^b** $p \leq 0.01$. ^c*** $p \leq 0.001$.

Table 3. Mean Square Values and Significance for Mixed-Model (Year Random, Location and Genotype Fixed) Factorial ANOVA of Fatty Acids Composition in Whole Meal Flour of Five Einkorn Accessions and of Bread Wheat cv Blasco, Cropped in Two Years and Four Locations

source of variation	df	palmitic	stearic	oleic ^a	linoleic	gadoleic	linolenic	SFA	MUFA	PUFA
year (Y)	1	15.16 ^d	0.52 ^d	138.56 ^d	18.99 ^d	5.14 ^d	0.01	27.44 ^d	89.71 ^d	18.30 ^d
location (L)	3	0.25	0.31	4.44	3.2	0.64 ^d	0.14	1.41	2.02	4.40
Y×L	3	1.18 ^c	0.04 ^d	5.05 ^d	1.45 ^c	0.02	0.02	1.92 ^c	4.69 ^d	1.21 ^c
repetition (YL)	8	0.12	0.01	0.18	0.12	0.03	0.01	0.14	0.27	0.16
genotype (G)	5	66.51 ^d	0.12	254.36 ^d	60.58 ^d	0.62	0.22	80.45 ^d	258.94 ^d	55.84 ^d
einkorn vs control	(1)	291.45 ^d	0.31	1173.98 ^d	278.81 ^d	0.14	0.35	352.46 ^d	1213.97 ^d	259.35 ^d
within einkorns	(4)	10.27 ^c	0.08	24.46 ^b	6.03	0.73	0.19	12.45 ^b	20.18 ^b	4.97
Y×G	5	0.88 ^d	0.13 ^d	3.54 ^d	3.36 ^d	0.25 ^d	0.07 ^d	1.15 ^d	3.51 ^d	4.08 ^d
L×G	15	0.41	0.03	1.68	1.18 ^c	0.19 ^b	0.05	0.73	1.07	1.56 ^d
Y×L×G	15	0.44 ^d	0.02 ^c	0.78 ^d	0.29 ^b	0.08 ^d	0.03 ^c	0.78 ^d	0.69 ^d	0.29
pooled error	40	0.13	0.01	0.10	0.15	0.01	0.01	0.15	0.12	0.18

^a(C18:1n9 + C18:1n7). ^b* $p \leq 0.05$. ^c** $p \leq 0.01$. ^d*** $p \leq 0.001$.

The following determinations were performed: dry matter (method 44–15) (23); protein (N×5.7; method 46–10) (23); total lipids (g/100 g), measured gravimetrically after Soxhlet extraction (using ethyl ether as the solvent) of the acid hydrolyzed sample (method 136) (24); tocol content by NP-HPLC (16, 25); carotenoid content by RP-HPLC (16). Fatty acids composition was determined by gas chromatography of fatty acid methyl esters prepared by following the procedure reported by Liebich et al. (26). Briefly, 0.1–0.2 g of fat extract were weighed in a 10 mL screw-cap Pyrex tube, 2 mL of methanol–toluene (4:1, v/v), and 200 μ L of acetyl chloride were added, the mix was methylated at 100 °C for 1 h in the closed tube and cooled for 5 min at –20 °C. Afterward, 5 mL of K₂CO₃ at 6% (w/v) were added and the mix was centrifuged at 1006g (3000 rpm) for 5 min with a Centrikon T-42K centrifuge (Kontron Instruments, Betchley, UK); the supernatant was collected in a 2 mL vial using a Pasteur pipet. Gas chromatographic analysis of methyl esters was performed using a HRGC 5160 instrument (Carlo Erba Instruments, Milan, Italy). The capillary column used was a 60 m × 0.25 mm i.d., 0.25 μ m, SP-2340 (Supelco, Bellefonte, PA). The operative conditions were as follows: carrier, H₂ at 90 kPa; oven temperature, 177 °C for 18 min, increased to 205 °C (2.3 °C/min), 205 °C for 2 min, increased to 210 °C (2 °C/min), 210 °C for 30 min; injection temperature 240 °C; flame ionization detector temperature, 250 °C.

All measurements were performed twice; the results are presented as means on a dry matter base (DM); fatty acids are reported as relative percentage (%).

Statistical Analysis. Data from all locations were combined and evaluated by analysis of variance (ANOVA). The ANOVA was performed with the software MSTAT-C v. 2.1 (Michigan State University, East Lansing, MI) following a mixed-model factorial approach where year was random while location and genotype were fixed (27). The genotypic variance was further split into “einkorn vs control” and “within einkorns”, to ascertain differences between *T. monococcum* and *T. aestivum*, as well as to assess intraeinkorn variation. Pearson’s correlations of the means were determined using the software SYSTAT 5.03 for Windows (SYSTAT Inc., Evanston, IL).

The fatty acid ANOVAs were performed on actual values as well as on arc sin transformed data; the two approaches gave almost identical results; therefore, to avoid too many decimals, the ANOVA from the original results is presented.

RESULTS AND DISCUSSION

The results of the mixed-model factorial ANOVA for protein, lipid, tocots, and lutein content are presented in **Table 2**, while for the six most abundant fatty acids as well as for the saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid groups are shown in **Table 3**. Genotype (G) and year (Y) had significant effects on the contents of protein, tocotrienols, total tocots, lutein, and the most abundant fatty acids (palmitic, oleic, and linoleic), while lipids and tocopherols were influenced merely by the genotype. The cropping location (L) influenced only gadoleic acid content. No differences were detected between traditional and organic management techniques (data not shown).

The genotype was often the most important factor of variation, as shown by its frequently large mean square estimates as well as by the substantial percentage of total variability explained (not shown). For kernel quality traits such as proteins, pigmentation, and rheological parameters, a similar conclusion was reached by Souza et al. (28) after cropping seven bread wheats for three years in four locations and under two nitrogen fertilization levels. On the other hand, Bergman and Xu (29) tested seven rice cultivars in four states during two years and noticed that growing environment had a greater effect on tocopherols, tocotrienols, and γ -oryzanol levels than did genotype.

The year effect was of primary importance for proteins, β -tocotrienol, total tocots, stearic, and gadoleic acids, while the significant Y×L, G×Y, G×L, and G×Y×L interactions were

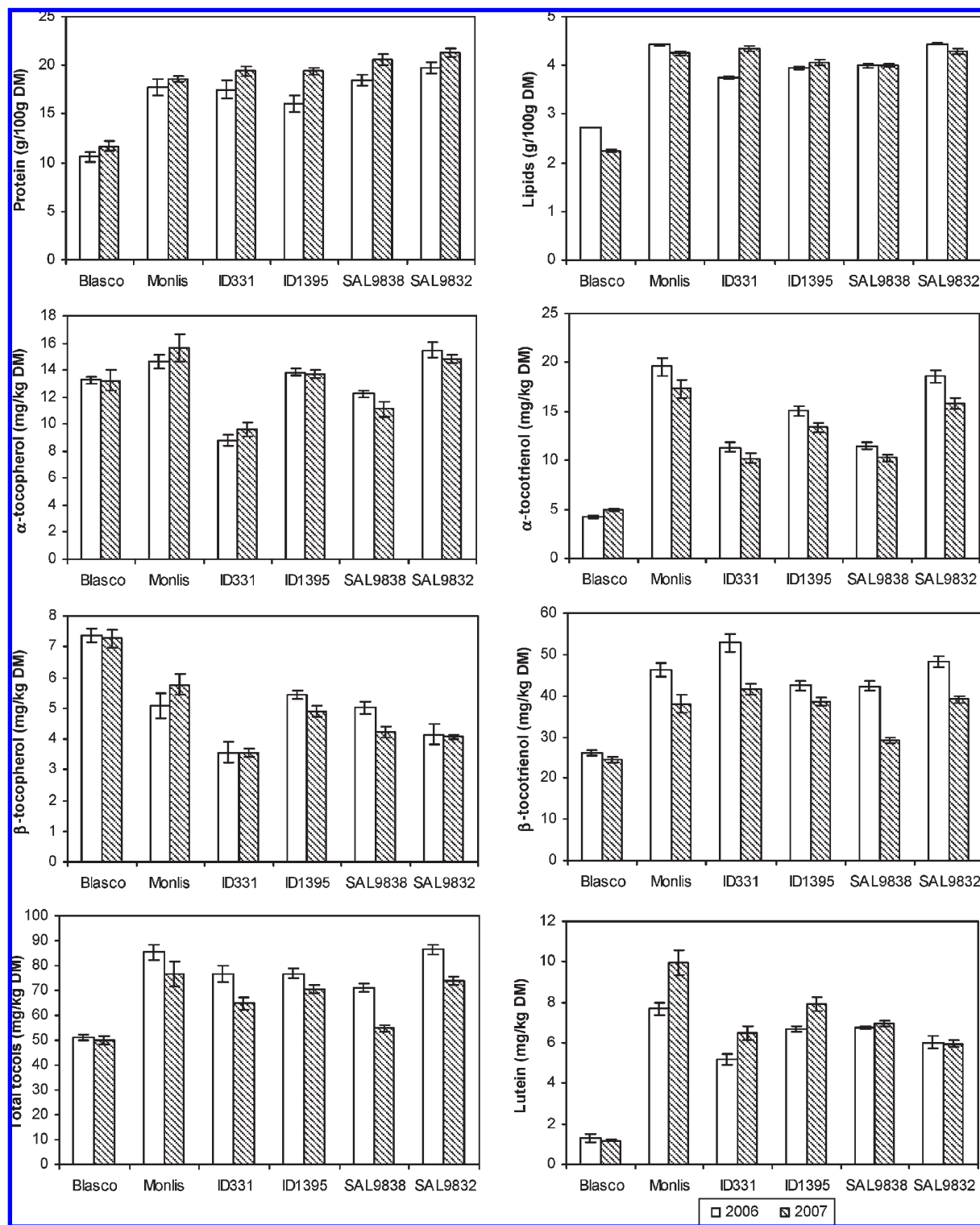


Figure 2. Mean contents of total proteins, total lipids, tocopherols, and lutein in wholemeal flours of one breadwheat cv Blasco and five einkorn accessions cropped in two years and four locations. Error bars represent standard errors.

always much lower in magnitude in comparison to the main effects. The partitioning in an “einkorn vs control” component and a “within einkorns” component showed that most of the genetic variance was attributable to the differences existing between the species *T. monococcum* and the *T. aestivum* control; nevertheless, the “within einkorns” variation was significant for protein, tocopherols (excluding β-tocopherol), lutein, palmitic, oleic, SFA, and MUFA, therefore indicating the existence of sizable differences for these traits among the five einkorns studied.

Because the influence of the location was not significant for almost all the variables, **Figure 2** reports the mean content over the four locations of total proteins, total lipids, tocopherols, and lutein for each year and all the genotypes. Einkorns showed higher protein (19.2 vs 12.1 g/100 g) and lipid (4.2 vs 2.8 g/100 g) contents than the bread wheat Blasco. Analogous results for protein concentrations are reported in literature for einkorn as well as for different *T. aestivum* samples (14, 21, 30). With regards to lipid content, in einkorn values ranging from 2.4 to 3.0 g/100 g

are described, significantly superior to those of bread wheat (1.7 g/100 g) (30) but much lower than the results reported in this research. This discrepancy may be a consequence of their different lipids extraction method because the acid hydrolysis of the sample, as applied in the current research, achieves higher fat yields than a straight extraction with solvents; furthermore, the acid hydrolysis step leads to a more complete extraction of the fatty acids from the cereal matrix, thus allowing their better characterization by gas chromatography (31). For this reason, the official method (24) requires a preliminary acid hydrolysis of the samples.

The higher protein and lipid contents of einkorn are partially attributable to the smaller size of *T. monococcum* seeds, which increases the ratio (bran + germ)/endosperm (32); nevertheless, protein concentration is significantly higher than that of bread wheat even in the endosperm (32). A sizable variation for protein content (on average, from 17.7 to 20.5 g/100 g) was observed among einkorn accessions; lipid content, instead, showed a more limited range (from 4.0 to 4.4 g/100 g).

With regard to antioxidants, α and β homologues of tocopherol and tocotrienol were detected in all accessions but their proportions varied (Figure 2). The α -tocopherol content was similar among samples, but Blasco had more β -tocopherol while the einkorns were richer in tocotrienols, the most abundant homologues, preferentially located in the outer layers of the seed (32) and therefore in total tocols, confirming the results reported in literature (16, 33). A relevant within-einkorns variation for all homologues and total tocols was recorded, with cv Monlis and free-threshing line SAL9832 showing the highest total values every year. The carotenoid lutein was more abundant in einkorn than in the control bread wheat (7.0 vs 1.1 mg/kg DM): a superior lutein content in *T. monococcum* than in *T. aestivum* has been described by different authors (10, 16). A high within-einkorn variability (from 5.9 to 8.9 mg/kg DM on average) was observed, with cv Monlis showing the highest value in both years.

The fatty acids analysis showed 14 compounds. Table 4 presents, for each year, the mean values over locations for Blasco and over genotypes and locations for einkorn. Even if the ANOVA sometimes showed significant differences among einkorn accessions, years, and locations, the standard errors indicated variations around the average that, from an analytical point of view, were of little relevance; the only really important factor of variation was therefore einkorn vs Blasco. Minimal albeit significant differences in fatty acids profiles were detected between spring and winter wheats (12), while cropping year was the discriminating factor (13).

In einkorn, linoleic (C18:2n6), oleic (C18:1n9 + C18:1n7), and palmitic (C16:0) acids were by far the most abundant (on average, 50.9, 24.8, and 16.6%, respectively). A survey of minor cereals detected 12 different fatty acids in einkorn (11), while an analogous assessment of hulled wheats spotted 10 fatty acids in *T. monococcum* (30); in both studies, linoleic, oleic, and palmitic were the most plentiful fatty acids, with percentages similar to our results. Linoleic acid was also the most abundant fatty acid in Blasco (55.4%), followed by palmitic (21.3%) and oleic (15.4%) acids; this ranking, with small variations in the relative percentages, is in perfect accordance with previous bread wheat results (12, 34–37). In einkorns and Blasco, similar concentrations were observed for gadoleic (C20:1n11) and linolenic (C18:3n3) acids, while stearic acid (C18:0) was slightly more abundant in the control bread wheat (1.3 vs 1.2%). The remaining eight fatty acids showed minimal concentrations (in total, less than 1.85%); their ANOVAs are not presented.

The different relative compositions were reflected in the higher MUFA (27.8 vs 18.2%) and lower PUFA (52.8 vs 57.2%) and

Table 4. Mean Value (\pm Standard Error) of Fatty Acids Composition in Whole Meal Flour of Bread Wheat cv Blasco and of Five Einkorn Accessions, Cropped in Two Years and Four Locations

fatty acid	bread wheat (Blasco)		einkorn	
	2006	2007	2006	2007
myristic C14:0	0.31 \pm 0.036	1.11 \pm 0.061	0.25 \pm 0.008	0.81 \pm 0.024
	0.12 \pm 0.007	0.15 \pm 0.006	0.11 \pm 0.002	0.14 \pm 0.003
palmitic C16:0	21.1 \pm 0.25	21.5 \pm 0.17	16.2 \pm 0.14	17.1 \pm 0.14
	0.09 \pm 0.005	0.09 \pm 0.003	0.10 \pm 0.002	0.09 \pm 0.002
palmitoleic C16:1n7	0.12 \pm 0.003	0.14 \pm 0.006	0.17 \pm 0.004	0.18 \pm 0.004
	0.11 \pm 0.004	0.11 \pm 0.002	0.11 \pm 0.003	0.11 \pm 0.003
margaric C18:0	1.3 \pm 0.02	1.3 \pm 0.04	1.3 \pm 0.02	1.1 \pm 0.04
oleic C18:1n9 ^a	15.7 \pm 0.46	15.1 \pm 0.24	26.2 \pm 0.21	23.4 \pm 0.23
linoleic C18:2n6	55.8 \pm 0.25	55.1 \pm 0.29	50.2 \pm 0.13	51.5 \pm 0.15
arachidic C20:0	0.21 \pm 0.008	0.20 \pm 0.005	0.19 \pm 0.005	0.16 \pm 0.005
gadoleic C20:1n11	2.4 \pm 0.06	2.9 \pm 0.11	2.5 \pm 0.04	3.0 \pm 0.07
linolenic C18:3n3	1.9 \pm 0.02	1.7 \pm 0.07	1.9 \pm 0.03	2.0 \pm 0.03
behenic C22:0	0.27 \pm 0.019	0.24 \pm 0.008	0.26 \pm 0.010	0.19 \pm 0.005
lignoceric C24:0	0.52 \pm 0.018	0.42 \pm 0.036	0.47 \pm 0.012	0.35 \pm 0.011
	24.0 \pm 0.23	25.1 \pm 0.22	18.9 \pm 0.15	19.9 \pm 0.19
SFA	18.3 \pm 0.40	18.2 \pm 0.32	28.9 \pm 0.19	26.6 \pm 0.21
MUFA	57.7 \pm 0.25	56.8 \pm 0.32	52.2 \pm 0.13	53.4 \pm 0.16

^a*(C18:1n9 + C18:1n7)

SFA (19.4 vs 24.5%) of einkorn with respect to Blasco, suggesting an interesting fatty acids composition for *T. monococcum*. From a nutritional point of view, a high MUFA concentration in the foods, coupled with a low SFA content, contributes to the prevention of cardiovascular diseases (38) because the MUFA influence lipids and cholesterol synthesis, reducing thrombosis and atherosclerosis risks (39), while from a technological perspective, the high MUFA and low PUFA contents provide better stability to oxidation (40) and longer shelf life.

The 2006 spring was characterized by high temperatures and drought throughout the late growth stages of wheat (Figure 1), including seed setting and maturation; these conditions limited pathogen diffusion and led to sound seeds but low yields. The 2007 spring, on the other hand, was characterized, during the late growth and ripening stage, by high temperatures coupled with persistent rain (Figure 1). As a result, while the early maturing Blasco showed similar production levels in both years, einkorn yields were generally higher in 2007 (Table 1) but kernels quality was poorer because of diseases and pregermination bouts. In terms of quality, in 2007, there was a significant protein increase in the seeds, along with a higher lutein concentration in three einkorns out of five (Figure 2). Significant lutein variation among years, with exceptionally high values in the wettest year, is described in literature (10). Total tocols content in einkorn showed a marked decrease between 2006 and 2007 (on average, from 79.3 \pm 1.97 to 68.1 \pm 2.42 mg/kg DM) as a result of a significant decrease in both tocotrienol homologues; Blasco, instead, did not present any significant variation. Cropping temperature is the most important single factor for tocols content in canola (5) as well as in soybean, where a 1 °C temperature variation already modifies the metabolism of tocols (41). In our research, however, the temperatures were similar in the two years (Figure 1) and the only major climatic difference was rainfall quantity (Figure 1); interestingly, a reduction in barley tocals concentration during humid, hot years is described (9). The general opposite behavior of lutein and tocotrienols in einkorn (Figure 3) could be linked to the synthesis pathways of both compounds (42). Tocotrienols are synthesized by the condensation of homogentistic acid and geranylgeranyl-PP, while tocopherols derive from the condensation of homogentistic acid and

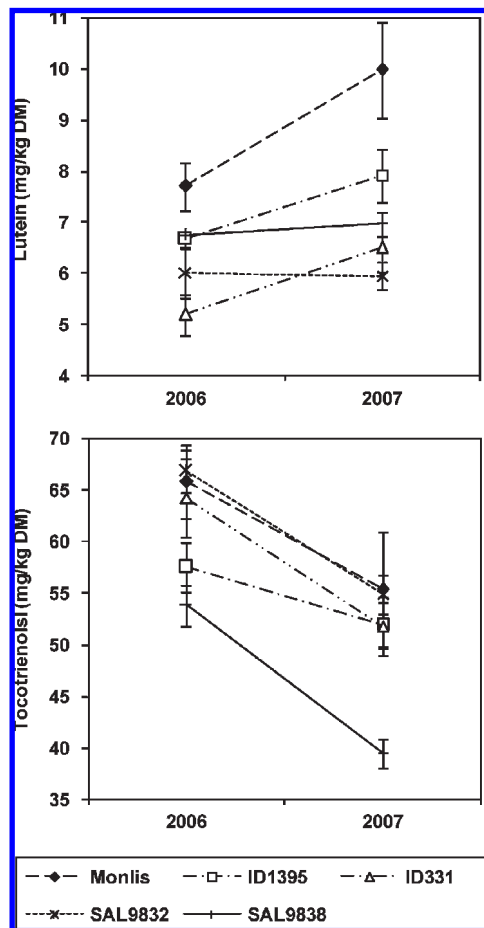


Figure 3. Variation across years of lutein and tocotrienols content for the five einkorn accessions.

phytyl-PP. Geranylgeranyl-PP is also a precursor of carotenoids, therefore environmental conditions that stimulate lutein synthesis might contribute to hinder tocotrienols production. However, not all einkorns showed a significant lutein increase in 2007 (Figures 2 and 3), suggesting that other and more complex pathways might be implicated.

Only small differences were observed for fatty acid percentages between 2006 and 2007 (Table 3); overall, the relative MUFA percentage diminished slightly (on average, from 28.9 to 27.2%), with the concomitant increase of SFA (from 18.9 to 19.6%) and PUFA (from 52.2 to 53.2%).

The analysis of the correlations among variables, performed only with the five einkorns in order to avoid species bias, showed many significant, but modest, values. Among the few correlations with $r \geq 0.6$, the most interesting were those between lipids and α -tocopherol ($r = 0.62$), total tocols and MUFA ($r = 0.66$), and total tocols and SFA ($r = -0.64$). These results are justified by the biochemical activity of tocols in plants, which is the protection of unsaturated fatty acids from oxidation. A positive correlation between MUFA (C16:1 and C18:1) and tocols in several seed oils was reported (43); however, no relationship between SFA and tocols was noticed (43).

Genotype and year exerted major effects on almost all the variables studied. Notwithstanding the environmental variation, all einkorns consistently showed greater proteins (on average, 59%), lipids (50%), tocotrienols (88%), total tocols (46%), lutein (483%), and MUFA (53%) content, along with lower PUFA (−8%) and SFA (−21%) than the bread wheat control. Interestingly, while the year-to-year variation for endosperm-abundant

compounds (protein, tocotrienols, and lutein) was relevant, for germ-abundant compounds it was limited (fatty acids) or non-significant (lipid and tocopherol). These results open the door to the selection of einkorn lines rich in lipids and monosaturated fatty acids, with steady composition profiles. The examination of a larger number of einkorn accessions is required to identify accessions with even higher mono- and polyunsaturated fatty acids contents.

ACKNOWLEDGMENT

We gratefully thank Francesca Gusmini and Elisa Dioni for their technical help and Dr. Norberto Pogna for supervising the Montelibretti trials.

LITERATURE CITED

- (1) Herbinger, K.; Tausz, M.; Wonisch, A.; Soja, G.; Sorger, A.; Grill, D. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Phys. Biochem.* **2002**, *40*, 691–696.
- (2) Smirnoff, N. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* **1993**, *125*, 27–58.
- (3) Polle, A.; Rennenberg, H. Causes of photooxidative stress and amelioration of defence systems in plant. In *Photoprotective Stress in Trees*; Foyer, C. H., Mullineaux, P. M., Eds.; CRC Press: Boca Raton, FL; 1994, pp 199–218.
- (4) Marquard, R. Untersuchungen über den Einfluss von Sorte und Standort auf den Tocopherolgehalt verschiedener Pflanzenöle. *Fat. Sci. Technol.* **1990**, *92*, 452–455.
- (5) Marwede, V.; Schierholt, A.; Mollers, C.; Becker, H. C. Genotype \times environment interactions and heritability of tocopherol contents in canola. *Crop Sci.* **2004**, *44*, 728–731.
- (6) Moore, J.; Liu, J.; Zhou, K.; Yu, L. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J. Agric. Food Chem.* **2006**, *54*, 5313–5322.
- (7) Yu, L.; Haley, S.; Perret, J.; Harris, M. Comparison of wheat flours grown at different location for their antioxidant properties. *Food Chem.* **2003**, *90*, 311–316.
- (8) Yu, L.; Zhou, K. Antioxidant properties of bran extracts from “Platte” wheat grown at different location. *Food Chem.* **2004**, *86*, 11–16.
- (9) Ehrenbergerová, J.; Belcrediová, N.; Pryma, J.; Vaculová, K.; Newman, C. W. Effect of cultivar, year grown, and cropping system on the content of tocopherols and tocotrienols in grains of hulled and hullless barley plant. *Foods Hum. Nutr.* **2006**, *61*, 145–150.
- (10) Abdel-Aal, E.-S. M.; Young, J. C.; Rabalski, I.; Hucl, P.; Frégeau-Reid, J. Identification and quantification of seed carotenoids in selected wheat species. *J. Agric. Food Chem.* **2007**, *55*, 787–794.
- (11) Gabrovská, D.; Fiedlerová, V.; Holasová, M.; Mascková, E.; Smrcinová, H.; Winterová, R.; Michalová, A.; Hutar, M. The nutritional evaluation of underutilized cereals and buckwheat. *Food Nutr. Bull.* **2002**, *23*, 246–253.
- (12) Konopka, I.; Czaplicki, S.; Rotkiewicz, D. Differences in content and composition of free lipids and carotenoids of spring and winter wheat cultivated in Poland. *Food Chem.* **2006**, *95*, 290–300.
- (13) Armanino, C.; De Acutis, R.; Festa, M. R. Wheat lipids to discriminate species, variety, geographical origins and crop years. *Anal. Chim. Acta* **2002**, *454*, 315–326.
- (14) Borghi, B.; Castagna, R.; Corbellini, M.; Heun, M.; Salamini, F. Breading quality of einkorn wheat (*Triticum monococcum* ssp. *monococcum*). *Cereal Chem.* **1996**, *73*, 208–214.
- (15) Abdel-Aal, E.-S. M.; Young, J. C.; Wood, P. J.; Rabalski, I.; Hucl, P.; Falk, D.; Frégeau-Reid, J. Einkorn: a potential candidate for developing high lutein wheat. *Cereal Chem.* **2002**, *79*, 455–457.
- (16) Hidalgo, A.; Brandolini, A.; Pompei, C.; Piscozzi, R. Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *J. Cereal Sci.* **2006**, *44*, 182–193.
- (17) De Vincenzi, M.; Luchetti, R.; Dal Belin Peruffo, A.; Curioni, A.; Pogna, N. E.; Gasbarrini, G. In vitro assessment of acetic-acid-soluble proteins (glutenin) toxicity in celiac disease. *J. Biochem. Toxicol.* **1996**, *11*, 205–210.

- (18) Mølberg, O.; Uhlen, A. K.; Jensen, T.; Flaete, N. S.; Fleckenstein, B.; Arentz-Hansen, H.; Raki, M.; Lundin, K. E.; Sollid, L. M. Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. *Gastroenterol.* **2005**, *128*, 393–401.
- (19) Pizzuti, D.; Buda, A.; D'Odorico, A.; D'Inca, R.; Chiarelli, S.; Curioni, A.; Martines, D. Lack of intestinal mucosal toxicity of *Triticum monococcum* in celiac disease patients. *Scand. J. Gastroenterology* **2006**, *41*, 1305–1311.
- (20) Vincentini, O.; Maialetti, F.; Gazza, L.; Silano, M.; Dessi, M.; De Vincenzi, M.; Pogna, N. E. Environmental factors of celiac disease: cytotoxicity of hulled wheat species *Triticum monococcum*, *T. turgidum* ssp. *dicoccum*, and *T. aestivum* ssp. *spelta*. *J. Gastroenterol. Hepatol.* **2007**, *22*, 1816–22.
- (21) Corbellini, M.; Empilli, S.; Vaccino, P.; Brandolini, A.; Borghi, B.; Heun, M.; Salamini, F. Einkorn characterization for bread and cookie production in relation to protein subunit composition. *Cereal Chem.* **1999**, *76*, 727–733.
- (22) Løje, H.; Møller, B.; Laustsen, A. M.; Hansen, Å. Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *J. Cereal Sci.* **2003**, *37*, 231–240.
- (23) AACC (American Association of Cereal Chemists). AACC Official Methods 44–15, 46–10. In *Approved Methods of the American Association of Cereal Chemists*, AACC: Minneapolis, MN, 1994.
- (24) ICC (International Association for Cereal Science and Technology). ICC Standard method 136. In *ICC Standard Methods*, 2nd supplement; International Association for Cereal Science and Technology: Vienna, Austria, 1995.
- (25) Panfili, G.; Fratianni, A.; Irano, M. Normal-phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *J. Agric. Food Chem.* **2003**, *51*, 3940–3944.
- (26) Liebich, H. M.; Jakober, B.; Wirth, C.; Pukrop, A.; Eggstein, M. Analysis of the fatty acid composition of the lipid classes in human blood serum under normal diet and when supplemented with fish oil. *J. High Resol. Chromatogr.* **1991**, *14*, 433–437.
- (27) Annicchiarico P. *Genotype × Environment Interactions. FAO Plant Production and Protection Paper 174*. Food and Agriculture Organization of the United Nations: Rome, Italy, **1998**; 115 pp.
- (28) Souza, E. J.; Martin, J. M.; Guttieri, M. J.; O'Brien, K. M.; Habernicht, S. P.; Lanning, R.; McLean, R.; Carlson, G. R.; Talbert, L. E. Influence of genotype, environment, and nitrogen management on spring wheat quality. *Crop Sci.* **2004**, *44*, 425–432.
- (29) Bergman, C. J.; Xu, Z. Genotype and environment effects on tocopherol, tocotrienol and γ -oryzanol contents of southern U.S. Rice. *Cereal Chem.* **2003**, *80*, 446–449.
- (30) Suchowilska, E.; Wiwart, M.; Borejszo, Z.; Packa, D.; Kandler, W.; Krska, R. Discriminant analysis of selected yield components and fatty acid composition of chosen *Triticum monococcum*, *Triticum dicoccum*, and *Triticum spelta* accessions. *J. Cereal Sci.* **2009**, *49*, 310–315.
- (31) Zou, W.; Lusk, C.; Messer, D.; Lane, R. Fat contents of cereal foods: comparison of classical with recently developed extraction techniques. *J. AOAC Int.* **1999**, *82*, 141–150.
- (32) Hidalgo, A.; Brandolini, A. Protein, ash, lutein and tocopherols distribution in einkorn (*Triticum monococcum* spp. *monococcum*) seed fractions. *Food Chem.* **2008**, *107*, 444–448.
- (33) Lampi, A.-M.; Nurmi, T.; Ollilainen, V.; Piironen, V. Tocopherols and tocotrienols in wheat genotypes in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9716–9721.
- (34) Chung, O. K. Cereal Lipids. In *Handbook of Cereals Science and Technology*; Lorenz, K. J., Kulp, K., Eds.; Marcel Dekker: New York, 1991; pp 497–553.
- (35) Grela, E. R. Nutrient composition and content of antinutritional factors in Spelt (*Triticum spelta* L.) cultivars. *J. Agric. Food Chem.* **1996**, *71*, 399–404.
- (36) Kashlan, N. B.; Hassan, A. S.; Srivastava, V. P.; Mohanna, N. A. Fatty acids composition and distribution in the free lipid fraction of major types of wheat flour and bread consumed in Kuwait. *Food Chem.* **1992**, *43*, 199–201.
- (37) Marconi, E.; Panfili, G.; Iafelice, G.; Fratianni, A.; Caboni, M. F.; Cubadda, R. Qualitative and quantitative evaluation of lipidic fraction in *T. dicoccum* Schrank and *T. spelta* L. *Tec. Molitoria* **2001**, *52*, 826–838.
- (38) Kris-Etherton, P. M. Monounsaturated fatty acids and risk of cardiovascular disease. *Circulation* **1999**, *100*, 1253–1258.
- (39) Spady, D. K.; Woollett, L. A.; Dietschy, J. Regulation of Plasma LDL-Cholesterol levels by dietary cholesterol and fatty acids. *Ann. Rev. Nutr.* **1993**, *13*, 355–381.
- (40) Min, D. B.; Boff, J. M. Lipid oxidation of edible oil. In *Food Lipids. Chemistry, Nutrition and Biotechnology*, Akoh, C. C., Min, D. B., Eds.; Marcel Dekker, Inc.: New York, 2002; pp 335–363.
- (41) Britz, S.; Kremer, F. Warm temperature or drought during seed maturation increase free α -tocopherol in seed soybean. *J. Agric. Food Chem.* **2002**, *50*, 6058–6063.
- (42) Jube, S.; Borthakur, D. Recent advances in food biotechnology research. In *Food Biochemistry and Food Processing*, Hui, Y. H., Nip, W.-K., Nollet, L. M. L., Paliyath, G., Sahlström, S., Simpson, B. K., Eds.; Blackwell Publishing: Oxford, U.K., 2006; pp 35–70.
- (43) Kamal-Eldin, A.; Andersson, R. A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. *J. Am. Oil Chem. Soc.* **1997**, *74*, 375–380.

Received April 8, 2009. Revised manuscript received June 11, 2009. Accepted June 11, 2009. This research was financially supported by project no. 1018 “MonICA-Monococco per l’innovazione cerealicola e alimentare”, sponsored by the Regione Lombardia, Italy.