Estimation of Color of Durum Wheat. Comparison of WSB, HPLC, and Reflectance Colorimeter Measurements

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Color is an important parameter involved in the definition of semolina and pasta quality. This character is mainly due to natural pigments (carotenoids) that are present at different levels in cereals and cereal products, due to botanical origin, growing conditions, distribution in the kernel, and technological processes. In food industries, color measurements are usually performed by means of automatic instruments that are rapid and safe, as alternatives to the chemical extraction methods. In this study, automatic measurements (CIE, color-space system L^*, a^*, b^*), water-saturated butanol (WSB), and HPLC determinations have been applied to evaluate the carotenoid content in whole meals and respective semolina samples produced from wheat cultivated in the years 2001 and 2002. In whole meals, total carotenoids, determined by HPLC, were about 3.0 μg/g (2001) and 3.5 μg/g (2002) calculated on dry weight (dw) and about 3.0 and 3.2 μg/g dw in corresponding semolina samples. The b^* values for the same period were 19.78 and 15.75, respectively, in raw materials and 20.03−21.67 in semolina. Results have confirmed lutein and β-carotene as the main components mainly responsible for the yellow color in wheat grains. The ability of the index b^* to express natural dyeing was dependent on sample characteristics as demonstrated by the relationships found between this index and pigments, although the best correlation resulted between HPLC and WSB.

KEYWORDS: Carotenoids; HPLC; cereals; yellow index; WSB

INTRODUCTION

Pasta color is a property that is well accepted by consumers and, for this reason, grain color is a characteristic taken into account by constitutors and, more in general, grain dealers. Semolina color is due to the natural dyeing pigments, which are xanthophyll (lutein type), carotene, flavones, and cryptoxanthin, although carotenoids in flour are mainly represented by xanthophyll (*1*). β-Carotene and zeaxanthin are located in the outer layers of the kernel where they are present at decreasing levels in the embryo, bran, and endosperm; on the contrary, lutein seems to be evenly distributed (*2*). Although semolina color is mainly due to natural carotenoids in seeds and their residual content after the storage of grain (*3*), it is also affected by the milling process applied (*4*). With regard to pasta, yellowness of end products is related to processing conditions applied during manufacturing (*5*); it is the result of carotenoids that are more or less oxidized by lipoxygenase (*6*, *7*). Some authors have emphasized that levels of lipoxygenase activity in durum wheat are cultivar-related and depend on the environment; consequently, the genotypic control of this character should be considered in breeding programs in order to reduce

the enzyme levels (*8*). More recently, some authors have demonstrated that lipoxygenase activity may be inhibited by β-carotene itself and α-tocopherol, and they suggested increasing the endogenous carotenoid content to prevent bleaching during pasta processing (*9*).

In consideration of the important role played by color in the definition of pasta quality, several analytical techniques have been developed over the years to evaluate this parameter in semolina and pasta. Among them, the main methods have involved visual comparison with standard references (*10*), chemical pigment extraction (*10*−*13*), and light reflectance measurements (*10*). In food industries, the most popular color measurement instruments are based on the color-space system L^*, a^*, b^* as defined by the Commission Internationale de l'Eclairage (CIE, 1986) (*14*). The success of these automatic techniques can be found in the rapidity and safety of the procedure in addition to the good correlation found between the chemical extraction and the reflectance measurements (*15*, *16*). In recent years, near-infrared reflectance (NIR) and near-infrared transmittance (NIT) and, more recently, UV−vis spectrophotometers have been used to determine color as a parameter useful to assess food quality (*17*, *18*).

In addition to methods for the evaluation of food color, several HPLC procedures have been proposed to estimate carotenoid content. The interest in carotenoid pigments is due to their

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Table 1. Particle Size Distribution in Semolina Samples of Sets A and B

granulation (μm)	set A(%)	set B(%)
363	26.9	8.5
183	56.4	69.0
123	5.8	9.8
85	5.8	8.9
44	2.2	2.4
<44	2.8	1.0
total	99.9	99.6

antioxidant properties, which reduce the oxidative damage to biological membranes by scavenging peroxyradicals (*19*). Recently, besides β-carotene, other carotenoids without provitamin A activity have been discovered to be involved in the prevention or protection against serious human disorders such as cancer and cardiovascular disease. Lutein and zeaxanthin are found in the eye and have been associated with reduced risk of cataract development and age-related macular degeneration (AMD) (*20*).

Because of the ability to distinguish between geometrical structures, high-performance liquid chromatography (HPLC) has become the most applied method for the analysis of carotenoids. The majority of the carotenoid separation involves the use of reversed phase HPLC, although normal phase is more efficient in separating lutein, zeaxanthin, and their geometric isomers. Quite a large number of procedures have been reported for the HPLC analysis of carotenoids on C18 columns (*21*), and C30-RP columns have been successfully applied to discriminate several *cis−trans* isomers of the same carotenoids (*22*). With regard to cereals, some authors have developed HPLC procedures to detect carotenoids in durum wheat cultivars and their byproducts (*2, 23−26*).

In this study, results obtained by means of an automatic reflectance instrument have been compared to those found by the official AACC 14-50 method and by a HPLC procedure. The main goal was to acquire more information on the correspondence between color characteristics and the true carotenoid content in whole meal and semolina samples useful for raw material classification and technological applications.

MATERIALS AND METHODS

Chemicals. β-Carotene and lutein were from Sigma (St. Louis, MO); zeaxanthin was from Extrasynthese (Z.I. Lyon-Nord, Genay, France). All other reagents were of analytical or HPLC grade and were purchased from Carlo Erba (Milano, Italy).

Samples. Two sample sets were considered in this study. The first set was constituted by 15 durum wheat samples (*Triticum durum*) (set A), whereas the second set was represented by 16 samples (set B). All 31 samples under examination were pooled samples constituted by two subsamples at least. Seven pooled samples cultivated in the years 2001 (set A) and 2002 (set B) came from the same cultivar and district. The samples, which were provided by the Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN) in Rome (Italy), were chosen among the most representative Italian cultivars periodically screened for quality control. Once harvested, cleaned, and mixed, grains were used to produce whole meal and semolina samples. Before semolina samples were produced, grains were tempered to 15% moisture.

Whole meals were obtained by a laboratory sample mill (0.5 mm sieve) (Cyclotec 1093, Tecator, Hoganas, Sweden), whereas semolina samples were produced by a Buhler MLU 202 (Uzwill, Switzerland), equipped with three break and three reduction rolls and six steel screens. Semolina characteristics are illustrated in **Table 1**.

Determination of Yellow Index and Pigment Content. The reflectance colorimeter was the Chroma Meter CR-200 (Minolta)
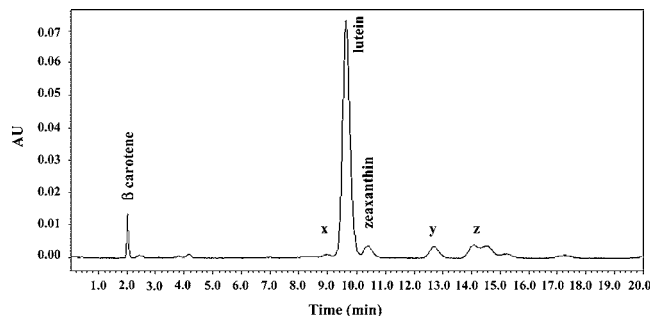
Figure 1. Chromatogram of carotenoids in a semolina sample: x, y, z, compounds tentatively identified as isomers of lutein (*23*).

equipped with a pulsed xenon arc lamp. Absolute measurements in L^*, a^*, b^* (CIE 1976) coordinates in the Munsell color system were taken using D65 lightning. Samples to be analyzed were placed into a granular material attachment. Results are the average of five determinations.

Total carotenoids were extracted by means of water-saturated butanol and determined according to the AACC 14-50 method. Final readings were made at 450 nm, and a standard solution of β-carotene was used for the calculation.

For the HPLC determination, pigments were extracted using hot saponification followed by extraction with specific solvents according to the methods given in refs *25* and *27*. The extracts were dissolved in *n*-hexane/isopropyl alcohol (10%) and were chromatographically determined by means of a Waters (Milford, MA) HPLC analytical system equipped with a solvent delivery system (model 510) and a photodiode array detector (model 991). A Kromasil Phenomenex Si column (250 mm × 4.6 mm i.d., 5 μm; Torrance, CA) was used for the separation. Carotenoids were detected at 450 nm. The mobile phase was *n*-hexane/isopropyl alcohol (5%) with a flow rate of 1.5 mL/min.

Results, which are the average of three determinations, are expressed as micrograms per gram of dry weight (dw).

Statistical Analysis. Data from this study are reported as mean and standard deviation for at least three replications for each sample. Differences between samples were statistically evaluated by means of Student's *t* test. The correlation was expressed with the *r* Pearson coefficient of correlation.

RESULTS AND DISCUSSION

Figure 1 shows a typical HPLC chromatogram of a semolina sample obtained by applying the above-reported HPLC method. Although lutein is the main compound followed by zeaxanthin and β-carotene, others pigments have been detected. The identification of these minor compounds, because commercial standards are not available, is still under investigation.

The comparison between the yellow index (b^*) and the carotenoid content, as determined by the HPLC method in whole meals included in sample set A (year 2001), is reported in **Table 2**. Because the technological importance of colorimetric indices is mainly related to materials used in pasta production, most information on pigments that is available in the literature is referred to semolina. However, information about kernel appearance is useful to determine color classes of wheat, and it is important for millers and bakers to meet customer specifications. The b^* values found ranged between 16.01 and 22.29 absolute value, those for β-carotene between 0.06 and 0.29 μg/g dw, those for lutein between 1.64 and 4.00 μg/g dw, and those for zeaxanthin between 0.14 and 0.33 μg/g dw. These results are in agreement with those found by other authors who described in wheat grains a range of carotenoids between 2.95 and 4.11 μg/g (*28*). The yellow index (b^*) was included in a narrow range of values, and this means that the reflectance method used is not sensitive enough to distinguish whole meals having the same genetic origin (cultivar) but from different locations. For the

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Table 2. Comparison between the Yellow Index (*b**) and the Carotenoid Content (Micrograms per Gram of Dry Weight) As Determined by the HPLC Method in Whole Meals (Year 2001) (Mean and SD[a])

sample	location	*b**	SD		β-carotene	SD	lutein	SD	zeaxanthin	SD	total
									carotenoids (HPLC)		
Ciccio	CA	21.10	0.02		0.16	0.000	3.54	0.108	0.28	0.007	3.98
	FI	18.49	1.04		0.11	0.020	2.53	0.070	0.14	0.014	2.78
	MN	19.99	1.00		0.13	0.004	2.52	0.024	0.27	0.014	2.92
	EN	18.56	1.34		0.14	0.008	2.79	0.120	0.27	0.011	3.20
	CL	19.40	1.02		0.16	0.000	2.79	0.060	0.30	0.015	3.25
Creso	PE	16.01	0.70		0.07	0.002	1.64	0.040	0.28	0.006	1.99
	AN	20.82	0.31		0.18	0.004	3.23	0.029	0.26	0.001	3.67
	VT	17.00	0.90		0.06	0.001	1.71	0.019	0.27	0.004	2.04
	CH	20.43	0.99		0.10	0.007	2.17	0.133	0.26	0.001	2.53
	SI	18.99	1.03		0.06	0.007	1.95	0.260	0.29	0.040	2.30
Giemme	GR	22.29	1.09		0.12	0.000	2.60	0.020	0.24	0.000	2.96
Grazia	GR	19.95	0.99		0.15	0.006	2.31	0.090	0.21	0.004	2.67
	AP	21.74	1.00		0.23	0.001	2.91	0.043	0.26	0.004	3.40
Simeto	BN	19.90	0.14		0.15	0.000	3.06	0.075	0.33	0.008	3.54
Orobel	PZ	21.98	1.03		0.29	0.005	4.00	0.018	0.29	0.003	4.58
mean		19.78			0.14		2.25		0.26		3.05
minimum		16.01			0.06		1.64		0.14		1.99
maximum		22.29			0.29		4.00		0.33		4.58

[a] Standard deviation.

Table 3. Comparison between the Yellow Index (*b**) and the Carotenoid Content (Micrograms per Gram of Dry Weight) As Determined by the HPLC Method in Whole Meals (Year 2002) (Mean and SD[a])

sample	location	*b**	SD		β-carotene	SD	lutein	SD	zeaxanthin	SD	total
									carotenoids (HPLC)		
Ciccio	MT	16.20	0.17		0.17	0.002	3.65	0.020	0.29	0.018	4.11
	AG	16.40	0.50		0.15	0.002	3.43	0.006	0.18	0.006	3.76
	CL	15.00	0.13		0.13	0.009	3.26	0.006	0.30	0.016	3.69
Creso	PE	14.76	0.31		0.08	0.003	1.99	0.028	0.25	0.014	2.32
	AN	14.13	0.40		0.07	0.003	2.00	0.036	0.28	0.003	2.35
	VT	14.62	0.26		0.07	0.000	1.75	0.013	0.27	0.003	2.09
	CH	14.72	0.31		0.07	0.008	2.09	0.081	0.23	0.081	2.39
	BN	14.31	0.16		0.07	0.000	1.95	0.006	0.22	0.003	2.24
Giemme	GR	16.70	0.10		0.26	0.000	3.59	0.072	0.26	0.021	4.11
Duilio	FG	14.80	0.15		0.13	0.002	2.37	0.072	0.19	0.004	2.69
Simeto	BN	18.80	0.20		0.21	0.014	4.02	0.024	0.28	0.014	4.51
Orobel	AR	18.79	0.15		0.44	0.009	5.50	0.101	0.39	0.016	6.33
Colosseo	FG	15.38	0.49		0.10	0.003	2.93	0.013	0.28	0.001	3.31
Appulo	CB	15.62	0.21		0.18	0.002	4.86	0.015	0.15	0.005	5.19
Latino	VT	14.01	0.10		0.09	0.002	1.69	0.006	0.17	0.006	1.95
Cirillo	PI	16.14	0.17		0.21	0.000	3.40	0.001	0.25	0.002	3.86
mean		15.65			0.15		3.03		0.25		3.03
minimum		14.01			0.07		1.69		0.15		1.95
maximum		18.80			0.44		5.50		0.39		6.33

[a] Standard deviation.

pigment content, a good correlation was found between the *b** values and β-carotene ($r = 0.70$, $p \leq 0.01$), lutein content ($r = 0.72$, $p \leq 0.01$), and total content ($r = 0.72$, $p \leq 0.01$) as determined by the HPLC procedure. On the contrary, no significant correlation was found between the *b** index and the zeaxanthin content. It must be emphasized that the WSB method was found not to be applicable to whole meals (data not shown); in fact, in this case, total carotenoid content can be wrongly increased due to interfering pigments present on the seed coat.

The comparison between the colorimetric values obtained on the whole meals included in set B (year 2002) and the corresponding HPLC values is illustrated in **Table 3**. The *b** values ranged between 14.01 and 18.80 absolute value, those of β-carotene between 0.07 and 0.44 μg/g dw, those of lutein between 1.69 and 5.50 μg/g dw, and those of zeaxanthin between 0.15 and 0.39 μg/g dw. The correlation between the yellow index (*b**) and β-carotene ($r = 0.85$, $p \leq 0.01$), lutein ($r = 0.83$, $p \leq 0.01$), and total carotenoids content ($r = 0.85$,

$p \leq 0.01$) improved with respect to the results found for set A. This result could be due to the intrinsic characteristics of the colorimetric measurements that are affected by the physical appearance of the sample. In fact, defects due to the irregular development of the granule, such as lightlt stained kernels, are also visible in milling products, and the *b** value can lower independently from the pigment content because of the appearance of the sample surface.

To get more information on the *b** index, seven samples, originated from the same cultivars and locations grown in the years 2001 and 2002, were compared, and the results are illustrated in **Table 4**. Looking at the *b** values, set B always showed values lower than those of sample set A ($p \leq 0.01$). However, the reduced *b** values observed in sample set B did not always correspond to a comparable decrease in the carotenoid content determined by the HPLC method. Therefore, reduction in the yellowness, as measured through the *b** index, has to be attributed to other factors apart from the pigment

Table 4. Comparison between the Yellow Index (*b**) and the Carotenoid Content (Micrograms per Gram of Dry Weight) As Determined by the HPLC Method in Whole Meals (Sets A and B) Originated from the Same Cultivar and Location

| | | | | | carotenoids (HPLC) | | | | |
| | | *b** | | β-carotene | | lutein | | zeaxanthin | |
sample	location	A	B	A	B	A	B	A	B
Ciccio	CL	19.40	15.00	0.16	0.13	2.79	3.26	0.30	0.30
Creso	PE	16.01	14.76	0.07	0.08	1.63	1.99	0.28	0.25
	AN	20.82	14.13	0.18	0.07	3.23	2.00	0.26	0.28
	VT	17.00	14.62	0.06	0.07	1.71	1.75	0.27	0.27
	CH	20.43	14.72	0.10	0.07	2.17	2.09	0.26	0.23
Giemme	GR	22.29	16.70	0.12	0.26	2.60	3.59	0.24	0.26
Simeto	BN	19.90	18.80	0.15	0.21	3.05	4.02	0.33	0.28

Table 5. Comparison between the Yellow Index (*b**) and the Carotenoid Content (Micrograms per Gram of Dry Weight) As Determined by the HPLC and Water-Saturated Butanol (WSB) Method in Semolina Samples (Year 2001) (Mean and SD[a])

| | | | | carotenoids (HPLC) | | | | | | | | |
sample	location	*b**	SD	β-carotene	SD	lutein	SD	zeaxanthin	SD	total	WSB	SD
Ciccio	CA	26.54	0.25	0.13	0.008	3.24	0.072	0.14	0.001	3.51	4.10	0.002
	FI	19.89	0.47	0.10	0.004	2.56	0.053	0.09	0.001	2.74	3.03	0.000
	MN	22.82	0.21	0.08	0.000	2.38	0.001	0.11	0.001	2.57	2.88	0.001
	EN	20.77	0.25	0.10	0.002	2.69	0.079	0.11	0.001	2.90	3.17	0.002
	CL	20.61	0.11	0.11	0.000	2.58	0.010	0.13	0.001	2.82	3.25	0.002
Creso	PE	18.97	0.12	0.05	0.002	1.54	0.023	0.10	0.001	1.69	2.01	0.002
	AN	25.25	0.10	0.14	0.001	3.13	0.049	0.11	0.001	3.38	3.70	0.000
	VT	18.12	0.20	0.04	0.001	1.61	0.069	0.09	0.001	1.74	2.06	0.000
	CH	21.64	0.16	0.07	0.001	2.19	0.000	0.13	0.001	2.39	2.63	0.002
	SI	20.60	0.41	0.05	0.001	2.05	0.023	0.11	0.001	2.21	2.41	0.002
Giemme	GR	24.50	0.15	0.09	0.003	2.65	0.169	0.08	0.001	2.82	2.90	0.004
Grazia	GR	22.69	0.08	0.13	0.003	2.54	0.077	0.08	0.001	2.75	3.05	0.002
	AP	22.83	0.09	0.14	0.002	2.69	0.004	0.11	0.001	2.94	3.25	0.000
Simeto	BN	18.80	0.11	0.08	0.005	2.33	0.081	0.10	0.001	2.51	2.85	0.002
Orobel	PZ	21.00	0.22	0.13	0.001	2.97	0.039	0.14	0.001	3.24	3.57	0.002
mean		21.67		0.10		2.47		0.11		2.68	2.99	
minimum		18.12		0.04		1.54		0.08		1.69	2.01	
maximum		26.54		0.14		3.24		0.14		3.51	4.10	

[a] Standard deviation.

amount. Environmental conditions and agricultural practices could influence the composition and the structure of the grain, in a different way, varying the bran-to-endosperm ratio, thus influencing the *b** value. As an example of the effect of environmental conditions on kernel characteristics, ash content, referred to sample set B reported in **Table 4**, increased by ∼12% compared to the same samples of set A, according to ref *29*.

The results obtained by applying the different analytical procedures on semolina samples grouped in sets A and B are summarized in **Tables 5** and **6**. With regard to set A, significant correlations were found between the yellow index (*b**) and the pigment content as determined by WSB method ($r = 0.72, p \le 0.01$) and lutein ($r = 0.75, p \le 0.01$) and total carotenoid amount ($r = 0.75, p \le 0.01$), determined by HPLC, whereas a low correlation was found between the *b** index and β-carotene ($r = 0.65, p \le 0.01$). However, the best correlation was found between WSB and the total pigment amount determined by the HPLC method ($r = 0.98, p \le 0.01$). For sample set B, all correlations between the parameters under investigation increased with respect to set A. In particular, a good relationship was found between the yellow index and β-carotene ($r = 0.82, p \le 0.01$), lutein ($r = 0.89, p \le 0.01$), and total pigments determined by WSB ($r = 0.94, p \le 0.01$) and HPLC methods ($r = 0.89, p \le 0.01$). A good correlation was also found between WSB and HPLC methods ($r = 0.89, p \le 0.01$). Compared to the raw materials, the *b** data always increased as a consequence of the straight-grade of flours that appears yellowish owing to the virtual absence of bran and germ particles.

One of the main parameters that plays an important role in determining semolina color is particle size. Nowadays, pasta producers prefer fine semolina that guarantees a rapid and homogeneous mixing process and the absence of white specks in the pasta (*30*); therefore, in set B (2002) the milling process was changed to produce semolina size closer to the market requirement. In sample set A, ∼30% of the particles were >250 μm, whereas in set B particles of this size constituted only 9% (**Table 1**). This explains why *b** values in set A showed a trend higher than that observed in set B. On the other hand, different correlation coefficients have been found by other authors depending on semolina characteristics (*15*, *31*).

A comparison of carotenoid amounts of sets A and B, determined by the HPLC procedure, was made between whole and respective semolina samples (**Tables 2** and **3** and **Tables 5** and **6**, respectively). A general good correlation between whole meals and semolina was found for both sample sets ($r = 0.87$, $p \le 0.01$, and $r = 0.85, p \le 0.01$, for 2001 and 2002, respectively).

Comparing the pigment amount of raw materials with that found in semolina, the two sample sets showed an opposite trend. In fact, as to whole samples, in semolinas, the total carotenoid content, expressed as mean value in micrograms per gram dw, generally decreased in set A (3.05 vs 2.68) and tended to increase in set B (3.03 vs 3.53). This behavior is mainly evident when the seven semolina samples originated from the same cultivars and locations grown in the years 2001 and 2002 are compared (**Figure 2**). As shown in **Figure 2** in six samples

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Table 6. Comparison between the Yellow Index (*b**) and the Carotenoid Content (Micrograms per Gram of Dry Weight) As Determined by the HPLC and Water-Saturated Butanol (WSB) Method in Semolina Samples (Year 2002) (Mean and SD[a])

| sample	location	*b**	SD	β-carotene	SD	lutein	SD	zeaxanthin	SD	total	WSB	SD
						carotenoids (HPLC)						
Ciccio	MT	20.03	0.88	0.14	0.002	4.34	0.052	0.15	0.007	4.63	2.79	0.000
	AG	18.79	0.61	0.07	0.000	2.81	0.130	0.10	0.004	2.99	3.23	0.001
	CL	20.15	0.24	0.08	0.002	2.99	0.080	0.15	0.003	3.22	3.23	0.000
Creso	PE	17.19	0.44	0.08	0.007	2.51	0.011	0.14	0.006	2.73	2.46	0.009
	AN	19.77	0.14	0.17	0.002	3.28	0.009	0.14	0.010	3.59	3.45	0.001
	VT	18.13	0.21	0.07	0.003	2.48	0.062	0.14	0.009	2.70	2.70	0.002
	CH	16.80	0.42	0.07	0.000	2.14	0.006	0.13	0.002	2.34	2.53	0.015
	BN	16.99	0.26	0.08	0.002	2.23	0.015	0.12	0.013	2.43	2.47	0.002
Giemme	GR	21.90	0.46	0.13	0.004	3.66	0.011	0.17	0.001	3.96	3.87	0.003
Duilio	FG	20.22	0.21	0.07	0.003	2.31	0.108	0.06	0.003	2.44	2.76	0.000
Simeto	BN	20.73	0.18	0.17	0.009	3.80	0.170	0.14	0.009	4.11	3.67	0.001
Orobel	AR	23.73	0.33	0.31	0.001	4.57	0.068	0.18	0.015	5.06	4.97	0.001
Colosseo	FG	22.30	0.54	0.14	0.002	3.60	0.013	0.17	0.003	3.91	3.81	0.001
Appulo	CB	23.58	0.21	0.19	0.008	5.04	0.188	0.16	0.005	5.39	4.81	0.000
Latino	VT	16.07	0.29	0.07	0.000	1.96	0.016	0.07	0.006	2.10	2.11	0.002
Cirillo	PI	24.07	0.34	0.27	0.004	4.53	0.112	0.15	0.002	4.95	4.60	0.005
mean		20.03		0.13		3.26		0.14		3.53	3.17	
minimum		17.19		0.07		1.96		0.06		2.10	2.11	
maximum		24.07		0.31		5.04		0.18		5.06	4.97	

[a] Standard deviation.
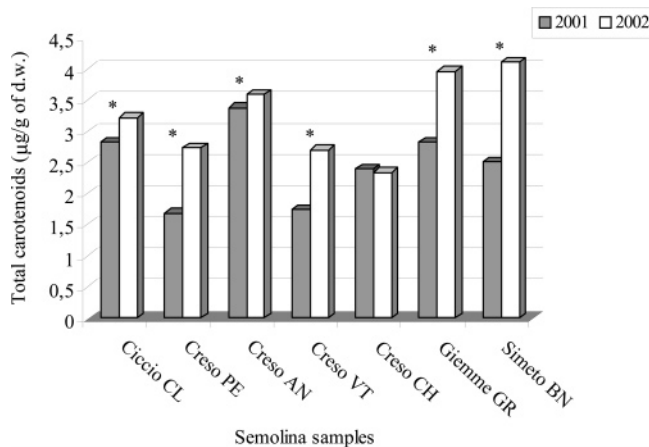
Figure 2. Comparison between the carotenoid content as determined by the HPLC method in semolina samples originated from the same cultivar and location growth in the years 2001 (set A) and 2002 (set B). (Flagged samples are statistically different (*p* ≤ 0.02).)

of set B the carotenoid content significantly increases (*p* ≤ 0.02). It is worthwhile to emphasize that the carotenoid levels are not dependent on different analytical recoveries on samples of different size (**Table 1**). In fact, a test performed to assess the reliability of the HPLC procedure showed no statistical difference of carotenoid recoveries (*p* ≥ 0.05) in coarse and fine semolina coming from the same raw material (data not reported).

The difference in carotenoid content observed in the two semolina sample sets could be due to the fact that in milled samples the bran and the germ of wheat kernel are removed as purification proceeds. Environmental conditions that could have affected the kernel development of set B could have determined an increase in carotenoid concentration in the endosperm, which becomes more evident after the removal of the peripheral parts. The increase in carotenoid content due to environmental conditions is in accordance with results previously reported by other authors who found an increment in the antioxidant levels in plant systems under stress (*32*). However, concerning this topic, the influence of well-defined cultural parameters needs to be investigated.

Table 7. Correlation between *b** and HPLC and WSB Methods on the 31 Grouped Whole Samples and the Corresponding Semolinas

		semolinas			whole meals
		*b**	HPLC	WSB	*b**
whole meals	*b**	**[a]	ns[b]	ns	
	HPLC	**	**	**	ns
semolinas	WSB	**	**		ns
	*b**		**	**	**

[a] *p* ≤ 0.01. [b] Not statistically significant.

Concerning the reliability of the applied methods to verify correspondence between color characteristics and the true carotenoid content in whole meals and semolina samples, a further comparison of *b**, HPLC, and WSB was made on the 31 grouped whole samples and the corresponding semolinas (**Table 7**).

In particular, although a significant correlation between *b** and HPLC method and *b** and WSB method was found for semolinas, respectively *r* = 0.54, *p* ≤ 0.01, and *r* = 0.71, *p* ≤ 0.01, the *b** values of whole meals were not so reliable in predicting the true carotenoid content either in whole meals (*r* = 0.30, *p* ≥ 0.05) or in semolinas, determined by HPLC and WSB (*r* = −0.03, *p* ≥ 0.05; *r* = 0.19, *p* ≥ 0.05, respectively). On the contrary, the carotenoid content, determined by HPLC in whole meals, shows significant correlations with *b**, HPLC, and WSB values in semolinas (*r* = 0.56, *p* ≤ 0.01; *r* = 0.83, *p* ≤ 0.01; *r* = 0.85, *p* ≤ 0.01, respectively).

Data obtained on the 31 grouped samples confirmed the high correlation between WSB and HPLC (*r* = 0.89, *p* ≤ 0.01).

Results obtained in this study, showing the correspondence between the reflectance measurements and the total carotenoid content determined by both the standard WSB method and the HPLC procedure on semolina samples, confirmed that carotenoids, and lutein in particular, are the most important pigments responsible for the yellow color impression of durum wheat. This result is in contrast with that recently published by other authors (*2*), who show that other substances of unknown structure contribute to the yellow color of grain extracts. The two chemical procedures used (WSB and HPLC) led to

comparable results and reconfirm that the WSB method is able to provide measurements of true pigment content in semolina. HPLC, although expensive, resulted in a sensitive, selective, and reliable method for the determination of the qualitative and quantitative distribution of carotenoid compounds in cereals. Moreover, contrarily to the other techniques developed to evaluate cereal color, it is free from interferences of the matrix and therefore applicable to all cereal samples.

The ability of the *b** index to express natural dyeing is dependent on sample characteristics (sample size and presence of peripheral parts), as demonstrated by the variability of the relationships found between this index and pigment content in different whole meals. On the basis of these considerations, reflectance measurements, although fast and safe, provide only relative and not absolute values.

In view of the importance of color in the definition of grain quality, the use of *b** values is still a matter of debate, and further tests are needed to standardize this method; therefore, only by combining *b** index and HPLC methods is it possible to obtain more complete information on the estimation of color in durum wheat and durum wheat products.

ACKNOWLEDGMENT

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