Durum Wheat Lipoxygenase Activity and Other Quality Parameters that Affect Pasta Color

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ABSTRACT

Pasta yellowness is affected by different factors, the most important of which are intrinsic to the quality of semolina (natural carotenoid pigments, protein, ash, and lipoxygenase [LOX] activity) and processing conditions. Because all the parameters involved in pasta color are under the control of varietal and environmental factors, the role of the genotype, environment, and the interaction between genotype and environment on color expression were studied. Although the analysis of variance showed the genotype-by-environment interaction to be significant, a nonorthogonal analysis attributed a higher weight to genotype on parameters directly involved in color expression: β-carotene content, yellow index, and LOX activity. Furthermore, the loss of pigments and yellow index after milling and processing was evaluated and correlated with all the parameters involved in the determination of final pasta color. The phase mainly responsible for pigment loss was pasta processing. A decrease of 16.3% in semolina β-carotene content during pasta processing versus a 7.9% loss during milling was determined. The isoenzymatic forms LOX-2 and LOX-3, active at the pH of dough, were responsible for the loss of color in pasta products. Simple correlations and the linear multiple regression corroborated this finding. Hydroperoxidation activity at pH 6.6, bleaching activity, and ash content were responsible for 87% (R^2 adjusted) of total variance, with each variable accounting for 57, 61, and 22% of the variation, respectively. This confirms that LOX activity is the main factor involved in the loss of color, while a secondary and lesser role can be seen for ash content. Therefore, a high pigment content, located in the interior of the whole grain, and a lower LOX activity in semolina must be the selection characteristics by which breeding programs obtain a bright yellow pasta.

The genetic improvement of durum wheat (*Triticum durum* Desf.) involves developing cultivars that combine the desired agronomic traits together with the capability to yield products (semolina or pasta) with a higher commercial, technological, or nutritional value. In recent years, the attention of consumers has focused on pasta color, particularly on yellow-amber products, in addition to cooking quality.

Pasta yellowness is affected by various factors: the inherent carotenoid pigment content of seeds, which is largely a varietal characteristic; the residual pigment content after the storage of grain and semolina; the extraction rate during milling; the oxidative degradation of pigments by lipoxygenase (LOX) during pasta processing; and the processing conditions (Irvine and Winkler 1950, Irvine and Anderson 1953).

The nature and the relative role of different variables involved in pasta color have already been defined (Irvine 1971, McDonald 1979). Xanthophills (free lutein) and their esters are the principal wheat carotenoid pigments (Laignelet 1983). Carotenoids are natural compounds that reduce the oxidative damage to biological membranes by scavenging peroxy-radicals such as those involved in certain human diseases and in the aging processes (Olson et al 1992, Rousseau et al 1992, Van Poppel et al 1993). Furthermore, carotenoids protect cells and organisms against the harmful effects of light and air (Krinsky 1989). Natural antioxidants in human foods may help to maintain the quality of products by inhibiting free radicals and oxidative processes (Frankel 1989). Therefore, an improvement in the endogenous quantity of these compounds should enhance the nutritional value of pasta products.

Pigment loss during processing and the subsequent yellow color of pasta are affected by LOX activity (Irvine and Winkler 1950, Irvine and Anderson 1953), peroxidase (PO) and polyphenoloxidase (PPO) activities (Kobrehel et al 1972, 1974; Taha and Sagi 1987), and semolina ash content (Kobrehel et al 1974, Taha and Sagi 1987). Furthermore, the protein content affected the semolina brownness (Walsh and Gilles 1971, Matsuo et al 1972, Dexter and Matsuo 1977, Taha and Sagi 1986), reducing the final pasta color.

LOX catalyzes the addition of molecular oxygen to polyunsaturated fatty acids containing *cis,cis*-1,4 pentadiene systems to produce conjugated *cis,trans*-diene hydroperoxide. Fatty acid radicals produced during the intermediate steps of substrate peroxidation are responsible for oxidative degradation of pigments such as β-carotene, xantophylls, and chlorophylls (Siedow 1991).

It is generally thought that all the factors involved in the color of pasta are largely varietal characteristics, although they can also be affected by environmental factors (Irvine and Anderson 1953). Therefore, to describe and delimit the role of genotype, environment, and genotype-by-environment interaction on expression, we have conducted a study on semolina and pasta obtained from six wheat cultivars grown in Southern Italy. Moreover, we have also evaluated the loss of the pigments and the yellow index after pasta making and verified its degree of correlation with all parameters analyzed.

MATERIALS AND METHODS

Durum Wheat Samples

Twenty-five Italian cultivars, grown in three different locations in Southern Italy (Foggia, Matera, Ururi) as part of the National Network of Durum Wheat Performance Test in the 1994-1995 and 1995-1996 growing seasons, were analyzed for whole grain β-carotene content. Subsets of three cultivars with a higher β-carotene content (Trinakria, Ofanto, and Simeto [Group-1]) and a lower content (Messapia, Creso, and Duilio [Group-2]) were selected from these samples.

Laboratory Analysis

Analytical tests on whole grain, semolina, and ground pasta samples were performed in duplicate. Protein content (%N × 5.7) was determined by Approved Method 46-12 (AACC 1995). Ash content was determined by Approved Method 08-12 (AACC 1995). β-Carotene content was determined according to Approved Method 14-50 (1995) as modified by Fares et al (1991). A chromameter (CR200, Minolta, Osaka, Japan) was used to determine yellow index. Each index was the average of three measurements.

Enzyme Activity

Enzyme activity was evaluated on semolina and ground pasta at pH 4.8 and pH 6.6 corresponding to LOX-2 and LOX-3 isoenzymatic forms of LOX of durum wheat (Hsieh and McDonald 1984) active during pasta making. They show an optimal hydroperoxidation activity at pH 4.8 (Hp-1) and a smaller peak of activity at pH ≈6.6 (Hp-2), along with an optimal bleaching (Bl) activity at pH 6.6 (McDonald 1979). Hence, while Hp-1 and Hp-2 activities

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are both innate expressions of a particular variety, Hp-2 activity reflects to a greater extent the enzymatic level present in the dough. Enzymatic activities of extracts of semolina or ground pasta were calculated as μmol of substrate changed min^{-1} g^{-1} and were determined in duplicate.

Hp activity assay. LOX activity was determined by measuring conjugate diene absorption at 234 nm and 25°C with a λ18 UV/VIS spectrometer (Perkin-Elmer, Norwalk, CT) equipped with a water-jacketed cell holder (Grossman and Zakut 1979). The linoleic acid substrate for the assay was prepared under N with Tween 20 and O_2 free-water according to Surrey (1964). Crude extracts were prepared as described by McDonald (1979), stored in an ice-water bath at 0°C, and used the same day. Protein content of extracts was evaluated by the method of Lowry et al (1951) with crystalline bovine serum albumin (BSA) as a standard.

For the assay, the reaction mixture (3 mL) contained nondeoxygenated buffer of desired pH level and 0.1 mg of protein of enzyme extract. The reaction was started by adding 0.15 mL of linoleic acid substrate solution (≈5 m*M*). Buffers used were 0.1*M* sodium acetate (pH 4.8) and 0.05*M* sodium phosphate (pH 6.6). The auto-oxidation of the substrate was corrected with a control assay to eliminate the nonenzymatic increase in absorbance. One unit of enzymatic activity corresponded to the production of 1 μmol of conjugated hydroperoxydienoic min^{-1}, using a molar absorptivity of 28 mM^{-1} cm^{-1} (Privett et al 1955).

Carotene Bl activity. The β-carotene Bl activity at 25°C was evaluated by measuring the decrease in absorbance at 460 nm according to the modified method of Ben-Aziz et al (1971). β-Carotene content, linoleic acid, and enzyme extract corresponded to 7 μ*M*, 15 m*M*, and 0.7 mg of protein, respectively. One unit of enzyme activity corresponded to the destruction of 1 μmol of β-carotene min^{-1}, using a molar absorptivity of 123.5 mM^{-1} cm^{-1}. The β-carotene solution was prepared by dissolving 25 mg of β-carotene in 25 mL of chloroform and 900 μL of Tween 80. One milliliter of this solution was evaporated in a vacuum flask and the residue was dissolved in 10 mL of sodium and ethylenediaminetetraacetic acid (EDTA) 0.25% (w/v). The solution was stored in an ice-water bath and used the same day. The concentration of β-carotene was spectrophotometrically determined at 453 nm using an ε value of 140.6 mM^{-1} cm^{-1} (Davies 1976).

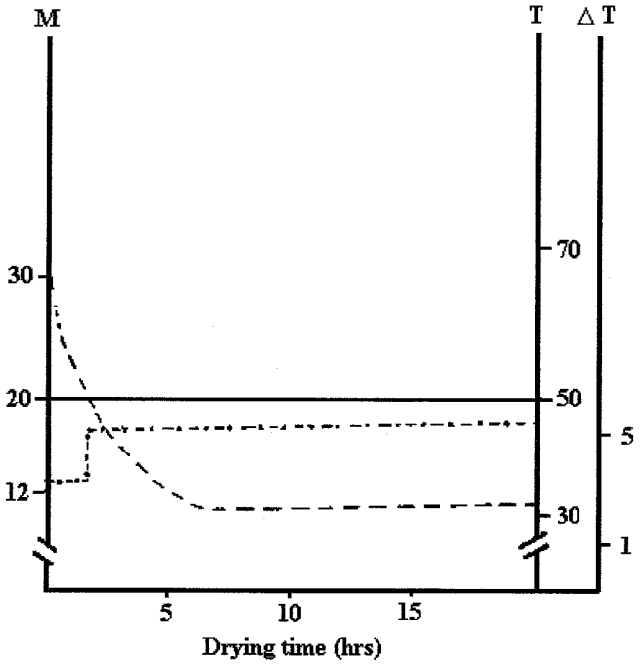

Fig. 1. Processing diagram for low-temperature drying cycle. M = % pasta moisture (– – – –), *T* = dryer temperature (°C) (——), Δ*T* = difference between temperatures (°C) inside dryer on dry and wet bulb thermometers (– · – · –).

Free Unesterified Lipid Extraction

Linoleic acid content was determined only on semolina from the second year of the study. Free phospholipid extraction was performed by suspending the semolina in 15 mL of methanol and chloroform (2:1, v/v) for 1.5 hr at room temperature according to Bligh and Dyer (1959). The homogenate was centrifuged for 5 min at 4,000 × *g*, and 10 mL of chloroform and water (1:1, v/v) were added to the supernatant. The chloroform phase was separated by centrifugation and evaporated to dryness under vacuum at room temperature. The crude lipid extract was dissolved in 4 mL of absolute ethyl alcohol. The total free unesterified lipid content was determined by measuring conjugate diene absorption at 234 nm.

For the assay, the reaction mixture (2 mL) contained 0.1*M* borate buffer (pH 9.0), 1 μL of soybean LOX, and 10 μL of lipid extract. To determine the linoleic acid content, the difference in absorbance from LOX addition to kinetic curve flattening was used with a molar absorptivity of 28 mM^{-1} cm^{-1} evaluated experimentally. The linoleic acid content was expressed as μmol of substrate g^{-1} of semolina.

Semolina and Pasta Processing

Cleaned durum wheat (10 kg for each sample) was conditioned overnight to 16.5% moisture content and processed in a laboratory mill (MLU 202, Bühler Brothers, Uzwill, Switzerland), fitted with three breaking and three sizing passages. The cultivars' semolina extraction rate range was 60–70%. The semolina was mixed with tap water to obtain a total dough water content of 43–44%. The dough was processed into spaghetti (1.7 mm diameter) using a 2-kg capacity laboratory press (NAMAD, Rome, Italy). A tem-

TABLE I
Analysis of Variance for All Parameters Analyzed in Semolina[a,b]

Source of Variation	DF	AC	PC	β-CC	YI	Hp-1	Hp-2	Bl
Replicate (R)	1	0.000	0.011	0.000	0.001	0.000	0.022	4.590
Year (Y)	1	0.000	50.083**	3.641**	24.804**	3.397	158.005**	2.442
Error a	1	0.000	0.004	0.001	0.004	0.880	0.018	2.268
Environment (E)	2	0.033***	88.502***	0.582***	10.854***	1.780	1.799	471.711***
Y × E	2	0.003*	72.646***	0.432***	14.263***	19.056***	10.320**	534.432***
Error b	4	0.000	0.042	0.000	0.003	0.266	0.438	2.667
Genotype (G)	5	0.062***	21.714***	16.738***	88.047***	43.218***	19.852***	2702.139***
Y × G	5	0.012***	5.530***	0.644***	5.851***	7.643***	0.732	148.482***
E × G	10	0.005***	1.343***	0.180***	3.375***	6.817***	3.153***	164.925***
Y × E × G	10	0.002***	1.425***	0.189***	1.796***	6.495***	4.697***	153.431***
Residual	30	0.000	0.034	0.000	0.002	0.437	0.349	4.858

[a] *, **, *** significant at *P* = 0.05, 0.01, 0.001, respectively.
[b] DF = degrees of freedom; AC = ash content; PC = protein content; β-CC = β-carotene content; YI = yellow index; Hp-1 = hydroperoxidation activity at pH 4.8; Hp-2 = hydroperoxidation activity at pH 6.6; Bl = bleaching activity.

perature of 50 ± 5°C, a pressure of 90–100 atm, and a vacuum of 700 mmHg were the conditions of extrusion. A low-temperature drying procedure (50°C for 18 hr) was applied in the pilot plant (Fig. 1).

Statistical Analysis

For the six cultivars selected in this study, an analysis of variance (ANOVA) of semolina quality characteristics, considering year and environment as random factors and the cultivar as a fixed factor, was calculated with the MSTAT-C program (Michigan State University). For year, environment, and year-by-environment effects, tests of significance were conducted considering "error a" for year, "error b" for remaining effects, and the "residual" for cultivar and other interactions. To isolate the relative weight of genotype and environment from the genotype-by-environment interaction on all of the parameters analyzed, a nonorthogonal analysis was also computed. Differences between means of genotype-by-environment were assessed using the least significant difference ($P < 0.01$). In addition, Pearson's simple correlations between semolina β-carotene content and yellow index, or their loss in the pasta, with other characteristics were considered. A linear multiple regression with a backward stepwise selection procedure using all of variables analyzed was performed with the Statistica program v. 5.1/G (StatSoft Inc., Tulsa, OK) to isolate those mainly involved in color loss during pasta making.

Chemicals. Linoleic acid, β-carotene, Tween 20, and LOX soybean (type IV) were purchased from Sigma Chemical (St. Louis, MO). Tween 80 was purchased from Merck (Darmstadt, Germany).

RESULTS AND DISCUSSION

ANOVA

Table I shows the ANOVA for semolina quality characteristics: β-carotene, ash and protein content, yellow index, and LOX activity. As expected, environment and year factors played a determinant role on all of the qualitative parameters such as protein, β-carotene content, and yellow index, with ash content being influenced by the environment only. In LOX activities, Hp-2 was affected significantly by year, while Bl activity was influenced by the environment only. With regard to genotypic influence, all of the parameters studied were affected significantly. Moreover, for each of the interaction levels, all the characteristics were significantly affected, except Hp-2, for the year-by-genotype interaction.

Because the genotype-by-environment interaction was highly significant for each parameter considered in this study, a nonorthogonal analysis was computed to verify the specific importance of genotype and environment. The results are reported in Table II. A dominant role was ascribable to the genotypic effect because the mean squares of all parameters were higher than the environmental effect. Protein content and, to a lesser extent, ash content were influenced by the environment, while only ash content displayed a considerable genotype-by-environment interaction, which is in agreement with the literature (Fowler and de la Rote 1975, Abacuses and Alause 1979, Rousset et al 1985, Mariani et al 1995). The particular effect of genotype for β-carotene content in whole grain and semolina across locations is shown in Fig. 2. Across environments, the cultivars' rank within each group and the differences between groups was maintained in both whole grain (Fig. 2A) and semolina (Fig. 2B).

Factors Involved in Loss of Color

The pigments and the LOX enzyme are not homogeneously distributed in the wheat kernel. Embryo, bran, and endosperm contain decreasing levels of β-carotene and LOX activity (Quaglia 1988). During milling, depending on extraction rate, a variable amount of these components are lost initially (Chen and Geddes 1945). In addition, pasta processing results in a large decrease in β-carotene content and LOX activity levels (Taha and Sagi 1987, Irvine 1971, Kobrehel et al 1974).

Mean values of β-carotene content in whole grain, semolina, and pasta, and the relative loss of this component in semolina versus whole grain, and in pasta versus semolina for six cultivars in both crop years are reported in Table III. For each year, cultivars of Group-1 and Group-2 were always significantly different for whole grain β-carotene content. The overall mean loss of the latter in

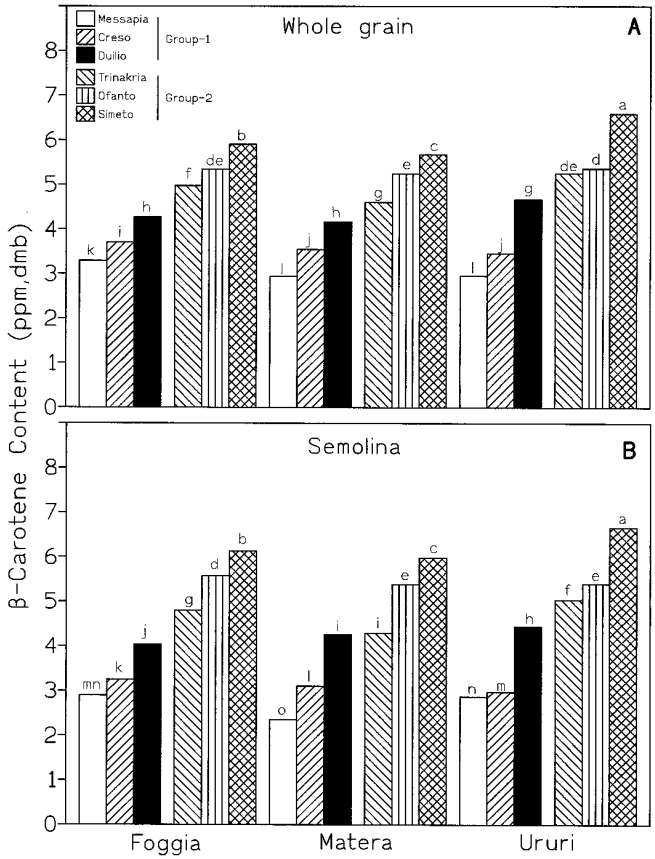

Fig. 2. Mean values of genotype-by-environment interaction ($n = 18$) for β-carotene content in whole grain (**A**) and semolina (**B**) at Foggia, Matera, and Ururi locations. Means with the same letters are not significantly different at $P < 0.01$ based on least significant difference test.

TABLE II
Nonorthogonal Analysis Computed on Genotype-by-Environment Interaction for All Parameters Analyzed[a,b]

Source of Variation	DF	*F* Ratios	AC	PC	β-CC	YI	Hp-1	Hp-2	Bl
Genotype (G)	5	s^2_G/s^2_{GE}	13.54***	16.17***	92.88***	26.08***	6.34**	6.30**	16.38***
Environment (E)	2	s^2_E/s^2_{GE}	7.14*	65.90***	3.23	3.22	0.26	0.57	2.86
Interaction (G × E)	10	s^2_{GE}/s^2_e	2.74**	0.30	1.01	1.81	2.30*	0.70	2.54*
Error	54	s^2_e	0.01	4.42	0.18	1.86	2.96	4.47	65.02

[a] *, **, *** significant at $P < 0.05$, 0.01, 0.001, respectively.
[b] DF = degrees of freedom; AC = ash content; PC = protein content; β-CC = β-carotene content; YI = yellow index; Hp-1 = hydroperoxidation activity at pH 4.8; Hp-2 = hydroperoxidation activity at pH 6.6; Bl = bleaching activity.

two years was 7.9 and 16.3% for semolina and pasta, respectively. With regard to β-carotene loss in semolina, although the cultivars of each group showed different values in the two years, mean values for each year were similar for Group-2 only. Furthermore, Group-2 cultivars had a lower β-carotene loss than Group-1. In Group-1, Duilio had the highest β-carotene content in whole grain, and during milling it showed a minimal decrease in this component in comparison to the other two cultivars. This behavior may be explained by a distribution of β-carotene in the inner kernel layers in Duilio (Group-1), and Trinakria, Ofanto, and Simeto (Group-2).

During pasta processing, Trinakria (Group-2) showed the highest loss of β-carotene for both years. In Group-1, Duilio showed the highest pigment loss, most evident in the second year, in spite of a minimal decrease during milling.

With exception of Messapia (1994-95 and 1995-96) and Creso (1994-95), data for the other cultivars confirmed that pasta processing was the phase most responsible for pigment loss.

Inactivation of LOX activity by temperature has been reported (McDonald 1979, Taha and Sagi 1987). We have investigated whether varietal differences using a drying cycle at 50°C for 18 hr could be related to differences in pigment degradation. The activity levels of LOX in semolina and ground pasta, and the enzyme inactivation values for each cultivar and year, expressed as a percentage of semolina LOX activities, are reported in Table IV. For LOX activity in semolina, only Trinakria showed significant differences when compared to the other cultivars and years. Trinakria consistently had the highest LOX values. Furthermore, the Hp activity levels of each cultivar, excluding Messapia for Hp-1, were markedly affected by year; they were higher in the 1995-96 growing season, except Trinakria for Hp-1. A similar phenomena was observed in the pasta, although in general, not all of the cultivars showed strong differences in the enzyme activity levels for each year. Bl activity in semolina was also influenced by year-to-year variability.

A wide variability among cultivars was observed for enzyme inactivation. The highest Hp-2 inactivation values were for Trinakria and Duilio in 1994-95 and Messapia in 1995-96. Simeto displayed the lowest loss of Hp-2 for both years, while variable behaviors were observed in the other cultivars. Bl activity inactivation offers an occasion to clarify the role of LOX in the decrease of carotenoid pigment during pasta making.

With exception of Ofanto in 1994-95, we observed that where the level of Bl activity in semolina was $\leq 1.0 \times 10^{-2}$ EU/g, no inactivation of B1 in pasta was found. On the contrary, when the value in semolina was higher, as for Trinakria and Duilio, the inactivation rate following processing increased. Our study showed that, independent of Bl activity levels, the β-carotene content in

TABLE III
Mean Values of β-Carotene Content in Whole Grain, Semolina, and Pasta and Loss of β-Carotene in Semolina vs. Whole Grain and Pasta vs. Semolina for Group-1 and Group-2 Cultivars Grown in Three Locations of Southern Italy During 1994-95 and 1995-96[a]

		β-Carotene Content (ppm, dmb)						Loss of β-Carotene (%)			
		1994-95			1995-96			1994-95		1995-96	
Group	Cultivar	Whole Grain	Semolina	Pasta	Whole Grain	Semolina	Pasta	Semolina	Pasta	Semolina	Pasta
1	Messapia	3.23d	2.76	2.55	2.89f	2.50	2.38	14.7	7.6	13.6	4.8
	Creso	3.64cd	3.03	2.72	3.54e	3.21	2.81	16.2	10.4	9.5	12.5
	Duilio	3.89c	3.60	3.17	4.85d	4.66	3.64	7.3	11.9	4.0	21.8
	Mean	3.58	3.13	2.81	3.76	3.46	2.94	12.7	10.0	9.0	13.0
2	Trinakria	4.77b	4.48	2.59	5.11c	4.91	2.78	6.0	42.2	4.0	43.2
	Ofanto	5.01ab	4.78	4.39	5.62b	5.28	4.85	4.6	8.1	6.0	8.2
	Simeto	5.54a	5.36	4.55	6.58a	6.15	5.56	3.3	15.1	6.6	9.6
	Mean	5.11	4.87	3.84	5.77	5.45	4.40	4.6	21.8	5.5	20.3

[a] Means followed by same letters are not significantly different at $P < 0.01$ based on least significant difference test.

TABLE IV
Mean Values of Lipoxygenase (LOX) Activity in Semolina and Pasta and Loss of LOX Activity in Pasta for Group-1 and Group-2 Cultivars Grown in Three Locations of Southern Italy During 1994-95 and 1995-96 [a,b]

		LOX Activity (EU/g)						Loss of LOX Activity (%)		
		Semolina			Pasta			Pasta		
Year and Group	Cultivar	Hp-1	Hp-2	Bl ($\times 10^{-2}$)	Hp-1	Hp-2	Bl ($\times 10^{-2}$)	Hp-1	Hp-2	Bl
1994-95										
1	Messapia	3.8b	1.7c	1.6bc	1.5a	1.3b	0.6c	60	23	64
	Creso	1.4c	1.9c	1.2cd	1.3a	1.5ab	0.7bc	7	21	42
	Duilio	2.9b	2.5b	1.7b	1.5a	1.5ab	0.6c	48	40	68
	Mean	2.7	2.0	1.5	1.4	1.4	0.6	38	28	58
2	Trinakria	8.7a	4.6a	4.3a	1.6a	2.0a	1.2a	81	56	72
	Ofanto	1.6c	1.2d	1.0d	1.6a	1.6ab	0.8bc	...	...	19
	Simeto	2.8b	1.9c	1.1cd	1.5a	1.8ab	0.9ab	46	5	16
	Mean	4.4	2.6	2.1	1.6	1.8	1.0	42	20	36
1995-96										
1	Messapia	3.7bc	5.4b	0.7cd	3.0a	3.3b	0.8bc	19	39	...
	Creso	3.1bc	4.7b	1.0bc	2.7a	3.0b	1.0b	13	36	...
	Duilio	4.2b	5.8ab	1.4d	2.3a	4.0a	0.5c	46	31	64
	Mean	3.7	5.3	1.0	2.7	3.4	0.8	26	35	21
2	Trinakria	6.1a	7.8a	5.4a	2.4a	4.0a	1.5a	61	49	72
	Ofanto	2.7c	3.9b	0.6d	2.5a	3.2b	0.8bc	7	18	...
	Simeto	4.0bc	4.2b	1.5b	2.8a	4.0a	0.9b	30	5	42
	Mean	4.3	5.3	2.5	2.6	3.7	1.1	33	24	38

[a] Hp-1 = hydroperoxidation activity at pH 4.8; Hp-2 = hydroperoxidation activity at pH 6.6; Bl = bleaching activity.
[b] Means followed by same letters are not significantly different at $P < 0.01$ based on least significant difference test.

pasta was not always better preserved in the cultivars with high inactivation levels. In fact, Trinakria, which held the highest Bl activity in semolina and showed the highest inactivation rate, displayed the highest loss of β-carotene (Table III). A similar pattern was observed for Messapia. Simeto and Duilio showed inverse behavior with better preservation of β-carotene content in the pasta, although they had a high enzyme activity and elevated inactivation of Bl activity. In both years, Creso and Ofanto lost similar amounts of β-carotene in the pasta in relation to low Bl activity levels in semolina and different inactivation rates. However, the role of Hp-2 activity in semolina to supply radicals for pigment co-oxidation must not be forgotten. In fact, even though the inactivation of Hp-2 and Bl activities for Duilio and Trinakria were similar (Table IV), their respective absolute values in semolina were very different, with Trinakria having higher activity than Duilio in both years. Since we have demonstrated that the amount of pigment was not preserved due to a high enzyme inactivation rate, drying cycles at high temperature may have an effect on the final color of pasta as suggested by Dexter et al (1981) and De Stefanis and Sgrulletta (1990). In fact, the latter reported that high temperature affected only the yellow index as it inhibited enzymatic reactions that are involved in brownness as well as attenuating the negative effects of the increased Maillard reaction. The best preservation of β-carotene content in the pasta therefore depends upon lower LOX activity levels in semolina because it is in the dough stage in which the LOX-2 and LOX-3 isoenzymatic forms are more active. The advantage of enzyme inactivation offered by high drying temperature becomes relative as it operates after the dough phase.

To verify the degree of involvement of semolina LOX activities and other quality parameters in color loss, simple correlations on the means of genotype-by-environment interaction among all the parameters analyzed were computed (Table V).

Ash and protein content were significantly correlated ($r = 0.50$, $P = 0.05$). Furthermore, they correlated with loss of β-carotene content and loss of yellow index but not with the remaining parameters (Table V). Since ash content is directly implicated in the increase of brownness (Matveef and Alause 1967, Burov et al 1974), its positive correlation with the decrease in β-carotene or yellow index in the pasta could be explained as a consequence of its effect on the brownness and not as a direct action on the pigment destruction (Kobrehel et al 1974, Matsuo and Dexter 1980, Taha and Sagi 1987). This interpretation was supported by the lack of correlation between β-carotene content or yellow index with ash content in semolina.

As expected, LOX activities were all highly correlated with loss of β-carotene content, confirming that the pigment decrease in pasta products must depend on enzymatic activity level in semolina. This statement was corroborated by our laboratory analysis conducted using specific LOX inhibitors such as *n*-propyl gallate (≈500 m*M*) that suppressed LOX activities (data not published).

In addition, the LOX activities were highly correlated. The correlation between Hp-1 and Hp-2 ($r = 0.90$, $P = 0.001$) supports the idea that they are the expression of the same activity carried out by the same isoenzymatic forms. Their association with Bl activity confirms that the latter might be ascribable to a co-oxidative action by LOX enzyme, according to McDonald (1979). In particular, the high correlation between Hp-2 with Bl and the loss of β-carotene content demonstrates that the pH 6.6 of the dough constitutes a favorable condition for pigment degradation. Although loss of β-carotene content was correlated with the loss of yellow index, the lack of an association between yellow index and enzyme activities can be explained. In fact, the expression of pasta color is affected by factors different from LOX activity, such as PO and PPO activities (Kobrehel et al 1972, 1974; Taha and Sagi 1987), ash content (Kobrehel et al 1974, Taha and Sagi 1987), and protein content (Walsh and Gilles 1971, Matsuo et al 1972, Dexter and Matsuo 1977, Taha and Sagi 1986) in semolina.

It has been reported that addition of free linoleic acid caused a more rapid loss of yellow pigments than the addition of purified durum wheat LOX (Matsuo et al 1970, Dahle 1975). Therefore, to verify its influence on LOX activity, we have analyzed, only in the second year, the linoleic acid content in semolina. Our data disagrees with the literature cited as no significant correlations between linoleic acid content and the parameters analyzed were found. However, caution must be taken in interpreting this data because our experimental procedure was not specifically for determining linoleic acid content and can be misleading due to other compounds.

Because β-carotene loss was affected by both LOX activity and other quality parameters, a multiple regression with the backward stepwise selection procedure was computed using the seven variables considered to isolate those significantly involved in color loss during pasta processing. This analysis selected three variables (ash content, Hp-2, and Bl activities) accounting for 87% of total variance (R^2 adjusted). In particular, the relative importance of each variable was 22, 57, and 61% (R^2), respectively. Therefore, this confirms that LOX activity is the main factor involved in the loss of color, as already observed from simple correlations, while a secondary and lower role can be seen for ash content.

CONCLUSIONS

With regard to the interaction between the genotype and the environment, this study has shown that a strong genotypic component affected all parameters directly involved in color expression: β-carotene content, yellow index, and LOX activities. No environmental influence was detected. We have computed that 16.3% of semolina β-carotene content is lost during pasta making as compared to a 7.9% loss during milling.

Another interesting aspect established was that high pigment content in pasta products is not always derived from a higher β-carotene content in whole grain. In fact, we have demonstrated that the LOX activity levels are more important than the β-carotene content in whole grain or semolina. These findings are confirmed by

TABLE V
Summary of Simple Correlations Computed on Means of Environment-by-Genotype Interaction ($n = 18$) Among Traits Analyzed for Two Years[a,b]

	AC	PC	β-CC	β-C loss	YI	YI loss	Hp-1	Hp-2	Bl
PC	0.50*								
β-CC	0.07	–0.09							
β-C loss	0.61**	0.54*	0.28						
YI	0.08	0.12	0.95***	0.28					
YI loss	0.60**	0.59**	0.27	0.49*	0.43				
Hp-1	0.30	0.44	0.10	0.84***	0.12	0.38			
Hp-2	0.35	0.39	–0.01	0.84***	–0.03	0.24	0.90***		
Bl	0.46	0.43	0.21	0.87***	0.21	0.32	0.77***	0.75***	
LAC[c]	0.05	0.11	0.23	0.32	0.11	–0.41	0.04	0.09	0.44

[a] AC = ash content; PC = protein content; β-C = β-carotene; βCC = β-carotene content; YI = yellow index; Hp-1 = hydroperoxidation activity at pH 4.8; Hp-2 = hydroperoxidation activity at pH 6.6; Bl = bleaching activity.
[b] *, ***, correlation significant at $P = 0.05$ and 0.001, respectively.
[c] LAC = linoleic acid content. Data refers to second year only.

simple correlations, and in particular by linear multiple regression where Hp-2 and Bl activities were mainly involved in decreasing β-carotene in pasta. A secondary effect was also found for ash content attributable to its involvement in the brownness.

This study suggests that to obtain a bright yellow pasta, a higher content of pigments, located centrally within the kernel, and lower LOX activity in semolina must be selection characteristics used in breeding programs.

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