# Effects of Modified Processing Conditions on Oxidative Properties of Semolina Dough and Pasta

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## ABSTRACT

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During pasta making, semolina is subject to various modifications that are mainly related to oxidative activities with relative effects on some of its components. To evaluate the involvement of hydroperoxidation and bleaching of lipoxygenase (LOX) and peroxidase (POD) activities on loss of pigments and -SH groups, their behavior in semolina and during processing was analyzed. Processing was done in standard and four experimental conditions, applying chemical (pH 5.0 and 8.0) and physical (10 and 40°C) treatments, during the mixing and extrusion phases, to study their effects on components. Results pointed out that treatments principally affected hydroperoxidation and bleaching activities of LOX rather than the POD. During pasta making, enzymatic activities showed the same trend in all cultivars, and this was reproducible in all the experimental conditions. Temperature effects on preservation of components were modest, whereas pH 8.0 improved the residual pigment and –SH group content in pasta, probably because of the concomitant reduction of oxidative enzyme levels. Finally, out of the four investigated wheat cultivars, Cosmodur showed the best performance in the experimental conditions applied. In fact, high pigment content and yellow index associated with low oxidative activity levels in semolina and in processing samples resulted in better pasta color. Such findings have confirmed that breeding and technological approaches may play an important role in the control of oxidative activities during pasta making, preserving the constituents that positively influence the final pasta color.

Production of high-quality pasta depends on semolina characteristics and optimal processing conditions. The effects of drying conditions on cooking and color of pasta are widely investigated (Baroni 1988; De Stefanis and Sgrulletta 1990) but few studies about the influence of mixing and extrusion parameters on biochemical properties of pasta dough were conducted. During pasta processing, semolina is subject to various physical and chemical modifications. The addition of water during the mixing phase induces molecular mobility of semolina components, causing biochemical modifications of proteins and of some other compounds, including carotenoid pigments and oxidoreductase enzymes. These changes allow further transformations of hydrated material into dough (Icard and Feillet 1997). The oxidation-reduction reactions are responsible for most of biochemical changes in dough. An important role is ascribable to lipoxygenase (LOX) that catalyzes the oxidation of polyunsaturated fatty acids by molecular oxygen (Siedow 1991). In addition, the LOX by a coupled oxidation mechanism induces the carotenoid pigment degradation, resulting in dough and pasta bleaching, and the loss of sulfhydryl groups (Siedow 1991). Pigment degradation, especially β-carotene and lutein oxidation, is also affected by peroxidase activity (Iori et al 1995; Fraignier et al 2000). Peroxidases (POD) oxidize a large number of compounds at the expense of hydrogen peroxide (Dunford and Stillman 1976). They are widely distributed in higher plants (Welinder 1992) and are involved in several physiological and genetic functions (Sembdner et al 1980; Espelie et al 1986; Kay and Basile 1987; Lagrimini and Rothstein 1987; Abeles et al 1988; Moerschbacher 1992; Zheng and van Huystee 1992). In food products, deteriorative changes in flavor, texture, and nutritional value could be caused by POD action (Burnette 1977). Furthermore, POD activity seems to induce the development of undesirable pasta brownness (Kobrehel et al 1972, 1974; Taha and Sagi 1987; Fraignier et al 2000).

Carotenoids may have an antioxidant function, and protect cell membrane from injuries by singlet oxygen quenching and free peroxyl radical trapping abilities (Palozza and Krinsky 1992), with a consequent possible role in the treatment of human diseases (Olson 1992). In addition, some carotenoids are the major dietary precursors of vitamin A in mammalians (Olson 1989). Hence, a breeding activity, aimed at selecting genotypes with higher endogenous pigment content and lower oxidative activities, and suitable processing conditions may be useful for improved carotenoid preservation in the end-use products.

The oxidation of –SH groups induces important changes in rheological properties of dough (Hoseney et al 1980; Shiiba et al 1990). The –SH groups and the S-S bonds contribute to the stability of the native conformation of proteins affecting their structure and functional properties (Syenowiecki and Shahidi 1991). During dough development, new protein aggregates, particularly glutenin aggregates of increased molecular size produced by intermolecular hydrophobic interactions, can originate from the loss of –SH groups and the formation of new S-S bonds between proteins (Icard-Vernière and Feillet 1999). The –SH loss induces an increase of free lipids in dough (Daniels et al 1970), of mixing tolerance (Hoseney et al 1980), and of relaxation times of dough (Shiiba et al 1991; Cumbee et al 1997), all of them being particularly important in the improvement of the rheological property of flour dough. The effects observed in flour dough might underlie also changes in the rheological properties of semolina dough.

Recent reports have given useful pieces of information on the oxidative enzyme behavior in some physical and chemical conditions applied during mixing (Delcros et al 1998; Icard-Vernière and Feillet 1999). On the basis of that, we investigated 1) the relationship between LOX and the POD activities during pasta making and the loss of carotenoids and –SH groups in pasta; and 2) the effect of the variation of physical and chemical conditions applied during mixing and extrusion to identify those treatments that could reduce the oxidative activities during processing.

## MATERIALS AND METHODS

### Plant Materials

Four durum wheat cultivars, Cosmodur, Ofanto, Tresor, and Varano, widely cultivated in South Italian environment and with good grain quality and different pigment content and oxidative enzyme levels, were grown in the 1998-99 growing seasons at the farm of the Experimental Institute for Cereal Research located in Foggia (Southern Italy). The experimental design was a randomized complete block with three replicates. Experimental plots were 10.2 $m^2$ in size, with eight rows of a length of 7.5 m and a spacing of 0.17 m. After harvesting, the cleaned seeds were bulked and stored at 4°C until analysis.

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## Semolina Production

Cleaned seeds were conditioned overnight to 16.5% moisture content and processed in an MLU 202 laboratory mill (Bühler Brothers, Uzwill, Switzerland) fitted with three breaking and three sizing passages with an attached semolina purifier. The cultivar extraction rate was 65.0% for Cosmodur, 65.5% for Varano, 67.3% for Ofanto, and 56.3% for Tresor due to the high presence of shrunken kernels. The semolina was stored at 4°C until analysis and processing.

## Pasta Making Process

Semolina samples were divided into five aliquots maintained overnight at room temperature and then processed as follows: standard conditions (deionized water, 25°C and pH 7.0) (St); deionized water, 10°C and pH 7.0 (Aa); deionized water, 40°C and pH 7.0 (Ab); acetate buffer 0.05*M*, 25°C and pH 5.0 (Ba); phosphate buffer 0.05*M*, 25°C and pH 8.0 (Bb).

The processing temperatures were selected to reduce oxidative reactions (10°C), to reproduce the room temperature (25°C) and the temperature of the water used in the industrial process (40°C). The 40°C corresponded also to the optimal temperature for hydroperoxidation activity (Barone et al 1999). Variations of pH were induced to promote (pH 5.0) and to reduce (pH 8.0) all enzyme activities (Iori et al 1995; Barone et al 1999). Semolina was mixed with deionized water or different buffers, at the experimental temperature, to obtain total dough moisture content of 33–34% db. Dough was processed into spaghetti (1.7 mm diameter) using 2-kg capacity laboratory press (Namad, Rome, Italy). The mixer and the vacuum press were thermostated at the experimental temperature by copper coils placed around both the devices and connected to a circulating ultra-thermostat (Haake F3). They were both covered with insulating material. The mixing times were 10 min at both 10 and 25°C, and 5 min at 40°C. The mixing speed remained constant. The extrusion conditions were a pressure of 90–120 atm and a vacuum of 700 mmHg. A circulating ultra-thermostat (Neslab RTE-111) maintained the extrusion head with a shape for spaghetti at a constant temperature of 30°C. A low-temperature drying procedure (50°C for 18 hr) was applied in the pilot plant (Giussani, Fara D'Adda, Bergamo, Italy). Dried pasta was analyzed after milling in a laboratory mill (Cyclotec 1093, Tecator, Höganäs, Sweden) (1-mm screen, 60 mesh).

## Enzyme Assay

LOX and POD activities on crude extracts of semolina, hydrated material (HM), taken at the end of mixing, and dough products (DP) corresponding to the compressed and extruded product were determined in triplicate. Each value was expressed as µmol of changed substrate/min/g.

*Preparation of crude extract.* The HM and DP were briefly stored at 4°C and analyzed in the same day of pasta processing. Crude extracts were obtained by homogenizing samples as described by Delcros et al (1998) and Icard-Vernière and Feillet (1999) using the Ultra Turrax homogenizer (T45 Ika-Werk, Staufen, Germany). LOX evaluation samples (2 g) were extracted in 10 mL of phosphate buffer (0.1*M*, pH 7.0), whereas POD evaluation samples (1 g) were extracted in 20 mL of acetate buffer (0.1*M*, pH 4.2). The homogenates were gently mixed for 15 min in ice and centrifuged at 35,000 × *g* for 20 min at 4°C. The supernatants were stored at 4°C and analyzed the same day. Extract protein content was evaluated by the Lowry et al (1951) method using crystalline bovine serum albumin as a standard.

*Hydroperoxidation assay.* LOX hydroperoxidation activity (Hp) was determined at pH 6.6 (Barone et al 1999) by measuring conjugate diene absorption at 234 nm and at 25°C, using a λ 18 UV/VIS spectrometer (Perkin-Elmer, Norwalk, CT) equipped with a water-jacketed cell holder. The linoleic acid substrate for the assay was prepared under $N_2$ with Tween 20 and $O_2$ free-water according to Surrey (1964). For the assay, the reaction mixture (3 mL) contained nondeoxigenated sodium phosphate buffer (0.05*M*, pH 6.6) 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*). The autoxidation 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 conjugate hydroperoxydienoic/min using a molar extinction coefficient of 28 m*M*/cm (Privett et al 1955).

*Bleaching assay.* β-carotene bleaching activity (Bl) was evaluated at pH 5.2 (Barone et al 1999) by measuring the decrease in absorbance at 460 nm and at 25°C, according to the modified method of Ben-Aziz et al (1971), with a λ 18 UV/VIS spectrometer equipped with a water-jacketed cell holder. β-carotene content, linoleic acid, and enzyme extract corresponded to 7 µ*M*, 15 m*M*, and 0.7 mg of protein, respectively, in 2 mL of reaction mixture. One unit of enzyme activity corresponded to destruction of 1 µ*M* of β-carotene/min using a molar extinction coefficient of 123.5 m*M*/cm. β-carotene solution was prepared by dissolving 25 mg of β-carotene in 25 mL of chloroform and 900 µL of Tween 80. Solution (1 mL) was evaporated in a vacuum flask and the residue was dissolved in 10 mL of EDTA, disodium salt 0.25%, w/v. The solution was stored in an ice bath and used the same day.

*Peroxidase assay.* Peroxidase (POD) activity was determined spectrophotometrically at 30°C by measuring the slope from the linear increase in absorbance at 470 nm and at pH 4.2 (Delcros et al 1998) as described by Iori et al (1995) with minor modifications, using a spectrometer. The assay mixture contained guaiacol (40 m*M*), hydrogen peroxide (10 m*M*), and $CaCl_2$ (20 m*M*) in acetate buffer (0.1 m*M*, pH 4.2) and a suitable aliquot of sample in a total volume of 2.5 mL. One unit of activity is defined as the change of one absorbance unit/min with an ε value of 26.6 m*M*/cm.

*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).

## Laboratory Analyses

Laboratory analyses were made on semolina and ground dried pasta. Moisture (%) was determined by oven drying for 3 hr at 130°C on 5 g of sample in duplicate (Approved Method 44-19, AACC 2000). The protein content (N × 5.7) was determined by Kjeldhal analysis in duplicate (Approved Method 46-13). The ash content (% db) was determined in duplicate on 5 g of sample by dry combustion for 16 hr at 580°C (Approved Method 08-01). A chromameter (CR200, Minolta, Osaka, Japan) was used to determine yellow ($b^*$) and brown (100-$L^*$) indices. Each index was the average of three measurements.

Contents of β-carotene and lutein were measured by HPLC with an isocratic solvent program. Sample (1 g) was extracted with 5 mL of hexane and ethyl alcohol (3:4) for 5 hr in the dark and at room temperature with continuous shaking. The extract was filtered through a 0.22-µm filter (Millex-GS, Millipore, Molsheim, France). Crude extract (20 µL) was used the same day for the evaluation. The determination was performed on an HPLC system (LKB, Bromma) with an HPLC pump model 2150, equipped with a detector (UV-VIS model 2141) and a recorder (model 2221). Separation was made using Supelcosil LC-SI column (250 × 4.6 mm, 5 µm particle size of the packing) (Supelco, Supelco Park, Bellefonte, PA). The mobile phase consisted of hexane and ethyl alcohol (85:15) at a flow rate of 0.8 mL/min with peak detection at 454 nm. The column and the mobile phase were maintained at constant temperature (25°C). The quantification was performed referring to the calibration curve obtained by using a relative standard. Reagents for HPLC were purchased by J. T. Baker (Deventer, Holland).

Free linoleic acid content was determined in semolina and pasta by gas chromatography. Sample (1 g) was extracted with 5 mL of diethyl ether for 5 hr with continuous shaking. The extract was filtered through a 0.22-µm. Solution (1 mL) was evaporated under a nitrogen flow and methylated with 20 µL of methyl acetate and

150 μL of 2*N* NaOH in methanol and then mixed with a vortex mixer for 60 sec at room temperature. Hexane (5 mL) was added and the solution was shaken vigorously. The extracts were centrifuged at 2000 × *g* for 5 min, and 0.5 μL of supernatant was used for gas chromatography analysis on a splitless system, (HRGC 5300, Mega Series, Carlo Erba Instruments, Rodano Italy) equipped with a Supelcowax capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (Supelco) and a flame-ionization detector (FID). The injector and the detector were set at 280°C. The oven was programmed at 100°C for 10 min; from 100 to 240°C at a rate of 20°C/min; 240°C for 20 min. The flow rate of the carrier gas (N at 70 kPa pressure) was set at 2 mL/min. Analysis of the chromatograms was performed by measuring the areas of peak versus calibration curve. Reagents for gas chromatography were purchased by J. T. Baker (Deventer, Holland).

The –SH group content was determined in semolina and pasta according to the Ellmann's method (1959), as modified by Icard-Vernière and Feillet (1999). Samples (500 mg) were carefully stirred, first with 1.5 mL of purified water, then with 2.5 mL of a 5 m*M* solution of 5-5'-dithiobis (2-nitrobenzoic acid) (DNTB), deoxygenated under N, containing 3 m*M* EDTA, disodium salt, and 0.2*M* Tris at pH 8.0. Sample solutions were incubated in the dark at 20°C for 20 min with continuous shaking, and centrifuged for 20 min at 14,600 × *g* at 20°C. The absorbance of supernatant was immediately read at 412 nm and thiol content was calculated using the molar extinction coefficient of $NTB^{2-}$ (2-nitro-5 thio benzoate) (13,600*M*/cm). The 5-5'-dithiobis(2-nitrobenzoic acid) was purchased from Sigma.

### Statistical Analysis

To separate the effects due to genotypes, treatments, and samples (semolina, mixing and extruded material, and pasta) an analysis of variance (ANOVA) on all the investigated parameters was made. When differences among means occurred, they were separated by the least significant difference test ($P = 0.01$).

## RESULTS

### Semolina Characteristics

Significant differences were found for protein, ash, linoleic acid (substrate of LOX), carotenoid pigment (evaluated as lutein and β-carotene), and –SH group content, as well as for moisture %, and yellow and brown indices (Table I). In particular, Cosmodur had the highest pigment content and yellow index and the lowest brown index and ash content values; on the contrary, Varano, notwithstanding a good pigment content, had the lowest yellow index and the highest brown index together with the highest ash, protein, and –SH group content. Really, Varano had a markedly grey semolina color as varietal characteristic. With regard to linoleic acid content, only Tresor significantly differed from the others with the highest value of this component.

Significant differences among cultivars also were observed for enzyme activities (Table I). The highest hydroperoxidation (Hp) and bleaching (Bl) activities of LOX were found in Tresor, while the lowest Hp, together with high Bl, and the highest POD activities were detected in Varano, confirming the known association between POD and high brownness.

### Pasta Processing

Standard and modified processing conditions were performed to evaluate whether changes in pasta components occurred, and to check which chemical and physical parameters were able to reduce oxidative activities in dough. Based on the dependence of LOX and POD activities on pH and temperature profiles (Iori et al 1995; Barone et al 1999), four experimental conditions (EC) were chosen. Results are reported in Table II.

As for pigment content, a marked variability in β-carotene loss with respect to lutein one in standard conditions was observed among cultivars, with Varano and Tresor showing the highest and the lowest losses of carotenoids, respectively. These cultivars behaved in the same manner also with regard to the –SH group decrease that was, on average, ≈50% in standard conditions. The loss of free linoleic acid ranged from 68.3% of Cosmodur to 85.2% of Ofanto, with a substantial decrease of this component in all cultivars. Yellow and brown indices in pasta reflected the same trend as in semolina.

With regard to the experimental conditions applied during pasta processing, the pH 8.0 treatment was the only condition where an improved preservation of all the analyzed components was univocally detected in all cultivars. The other experimental conditions did not always have the same effects on all the analyzed factors because they were widely variable both on components and cultivars, with the exception of –SH groups, where the experimental treatments had shown a similar trend in all cultivars, with the highest losses at pH 5.0.

The yellow index and, in particular, the brown index were affected to a smaller extent by treatments. It is important to point out that yellow index values in pasta did not always reflect the residual pigment content, thus confirming that this parameter is the result of concurrent physical and chemical factors (De Stefanis and Sgrulletta 1990). Varano maintained the grey color in pasta previously observed in semolina.

Under standard conditions, in all cultivars the Hp activity of LOX increased in hydrated material (HM) and subsequently decreased in dough product (DP) (Fig. 1A–D), whereas Bl activity was never

**TABLE I**
**Mean Values of Different Components in Semolina[a,b]**

| Cultivar | Hp (EU/g) | Bl (EU/g) | POD (EU/g) | Protein (%, db) | Ash (%, db) | Moisture (%) |
|---|---|---|---|---|---|---|
| Cosmodur | 1.35b | 0.009c | 26.8d | 14.0b | 0.91d | 13.5c |
| Ofanto | 1.14b | 0.006d | 180.8b | 13.6c | 0.98c | 13.8b |
| Tresor | 1.92a | 0.029a | 75.7c | 14.1b | 0.99b | 13.9b |
| Varano | 0.70c | 0.020b | 269.4a | 15.1a | 1.08a | 14.2a |

[a] Hp = hydroperoxidation activity; Bl = bleaching activity; POD = peroxidase activity; –SH = sulphydryl groups.
[b] Values followed by same letter in the same column are not significantly different ($P = 0.01$).

**TABLE I (continued)**
**Mean Values of Different Components in Semolina[a,b]**

| Cultivar | Lutein (μg/g, db) | β-Carotene (μg/g, db) | Linoleic (μg/g, db) | –SH (μ*M*/g) | Yellow Index | Brown Index |
|---|---|---|---|---|---|---|
| Cosmodur | 7.24a | 1.78a | 2,575b | 0.54c | 26.6a | 13.9c |
| Ofanto | 4.11c | 1.07b | 2,924b | 0.72b | 22.3b | 14.8b |
| Tresor | 3.62d | 0.50c | 3,404a | 0.49c | 22.0b | 13.8c |
| Varano | 4.53b | 1.28b | 2,646b | 0.87a | 19.6c | 15.4a |

[a] Hp = hydroperoxidation activity; Bl = bleaching activity; POD = peroxidase activity; –SH = sulphydryl groups.
[b] Values followed by the same letter in the same column are not significantly different ($P = 0.01$).

detectable in HM and showed very low values in DP (*data not shown*). Finally, POD activity appeared more stable during mixing and extrusion stages (Fig. 2A–D), which agrees with Icard-Vernière and Feillet (1999).

Experimental conditions (EC) applied in mixing and extrusion phases resulted in changes of enzymatic activity levels. As for Hp activity, a progressive increase in HM and DP was found only at pH 5.0, up to the highest values, while in the other conditions the trend was similar to the standard one (Fig. 1A–D). In addition, a higher Hp activity at 10°C with respect to other experimental temperatures was detected in HM; on the contrary, in DP its values were close to those found at 40°C. At 40°C, no difference between HM and DP, with the exception of Ofanto, was found. Bleaching activity showed in all experimental conditions the same trend already observed in standard one, with values generally higher in DP with respect to HM and with HM not always measurable. The effects of experimental conditions on POD activity were very low in all cultivars, with Ofanto and Varano confirming higher values with respect to Cosmodur and Tresor (Fig. 2A–D). As in standard, in these conditions POD activity remained stable during the mixing and extrusion phases.

## DISCUSSION

The implication of LOX and POD activities on the loss of some components is already known (Ben-Aziz et al 1971; McDonald 1979; Siedow 1991; Iori et al 1995; Gelinas et al 1998; Borrelli et al 1999). With a view to define a possible relationship between oxidative enzymes and component preservation in pasta, we have studied the behavior of the former in semolina and during different phases of pasta processing.

Our data denote that in the considered samples, Hp and Bl activities of LOX are not related, confirming that they are quite distinct from each other. In fact, bleaching resulted from the co-oxidative reaction due to free radicals and other reactive oxygen species that have originated during fatty acid oxidation and not directly from LOX action. In fact, cooxidizing ability varies among wheat isoenzymes and this variation does not show parallel differences in the lipid-oxidizing activity (Hsieh and McDonald 1984).

During mixing, we have constantly observed an activation of Hp reaction, probably related to the concurrence of a greater availability of oxygen provided with water and of a mechanical action that promoted the substrate-enzyme-$O_2$ contact. In addition, because the LOX is bound to the hydrophobic sites of glutenin, we supposed that a modification of glutenin surface hydrophobicity during dough development might result in the release and the activation of the enzyme, according to Shiiba et al (1990). In addition, the progressive decrease of Hp activity observed in extruded materials might derive, at least partly, from enzyme destruction or inhibition due to its reaction products, according to Delcros et al (1998). On the other hand, protein physical and chemical modifications during mixing and extrusion phases (Camire et al 1990; Camire 1991) might irreversibly denature the enzyme, as for catalase (Delcros et al 1998). Finally, the vacuum imposed in the press by reducing available $O_2$ might result in the Hp reaction decrease. Disagreeing with the findings of Delcros et al (1998), our results at pH 5.0 might be explained on the ground of the different species and the different devices and analytical methods utilized.

Higher Hp activity values, observed in HM at 10°C, are in contrast with the expected ones based on temperature-dependence profile found on the purified enzyme (Barone et al 1999). An explanation of this phenomenon might be related, on the one hand, to the increase of $O_2$ solubility at lower temperature and, on the other hand, to a different aggregation protein status that might induce the LOX release and its extraction. Indeed, we have seen that HM produced in this condition exhibited a very loose appearance. On

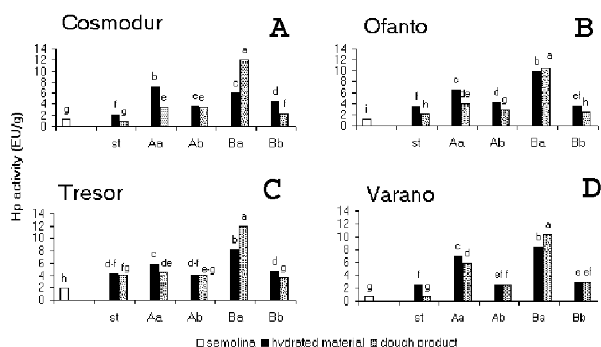


**Fig. 1.** Hydroperoxidation activity of lipoxygenase (LOX) analyzed in hydrated material and dough product obtained during pasta making process in experimental conditions and in semolina of cultivars Cosmodur (**A**), Ofanto (**B**), Tresor (**C**), Varano (**D**). St = pH 7.0, 25°C; Aa = pH 7.0, 10°C; Ab = pH 7.0, 40°C; Ba = pH 5.0, 25°C; Bb = pH 8.0, 25°C. Means with the same letters are not significantly different at $P = 0.01$.

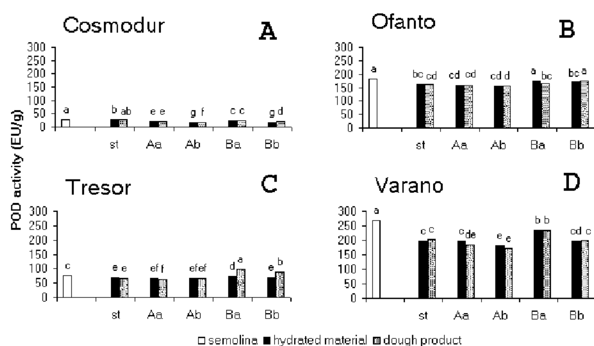


**Fig. 2.** Peroxidase (POD) activity analyzed in hydrated material and dough product obtained during pasta making process in experimental conditions and in semolina of cultivars Cosmodur (**A**), Ofanto (**B**), Tresor (**C**), Varano (**D**). St = pH 7.0, 25°C; Aa = pH 7.0, 10°C; Ab = pH 7.0, 40°C; Ba = pH 5.0, 25°C; Bb = pH 8.0, 25°C. Means with the same letters are not significantly different at $P = 0.01$.

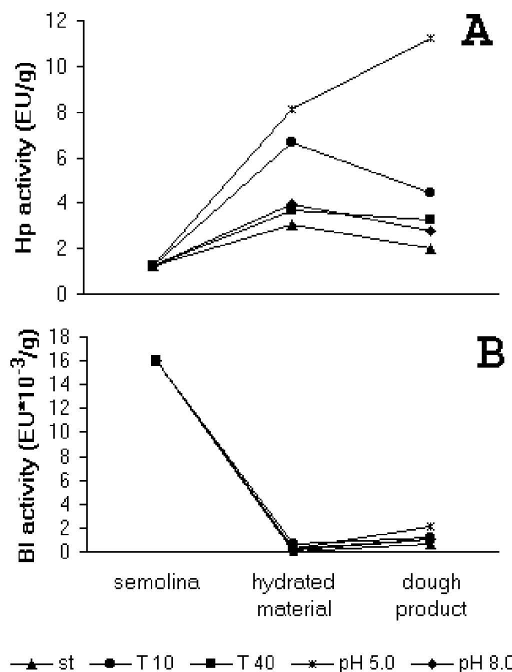


**Fig. 3.** Average values of all cultivars for hydroperoxidation (**A**) and bleaching (**B**) activities of lipoxygenase (LOX) recorded during pasta making in St and other experimental conditions.

the contrary, vacuum application during extrusion reduced the contribution of $O_2$ and nullified the associated effect. Unlike Matsuo et al (1970), we generally observed that higher Hp activity was not necessarily related to higher availability of linoleic acid in semolina.

The explanation of Bl activity behavior during mixing and extrusion is more complicated. As described above, the reaction of cooxidation is due to free radicals and peroxy radicals produced during hydroperoxidation. It is also demonstrated that the reaction of carotenoids with peroxy radicals competes with the reaction that produces lipid hydroperoxides (Yanishilieva et al 1998) through a chain reaction mechanism. Our data confirm that the hydroperoxide production rate was higher after mixing (Fig. 1). Subsequently, it decreased, and the peroxy radicals began to react with carotenoids, so bleaching, initially not measurable, became slightly detectable after extrusion. Hence, we agree with Taha and Sagi (1987) that the carotenoid degradation also occurred during drying. In fact, we have found that decreasing carotenoid rate is, on average, lower in DP versus semolina (20%) than in pasta versus DP (54%).

Our experiments confirmed the high stability of POD activity during dough production (Icard-Vernière and Feillet 1999). However, the involvement of POD in final pasta color seems not to be very clear. In fact, during processing, the hydrogen peroxide, its primary substrate, is lacking and perhaps another oxidative enzyme, the polyphenoloxidase, might be involved (Feillet et al 2000).

An interesting aspect that emerges in all the analyzed cultivars is the trend of extractable Hp and Bl activities of LOX during pasta making (Fig. 3A,B). Such a trend was similar in all cultivars and experimental conditions, with the exception of Hp activity at pH 5.0 that always showed a progressive increase up to DP, pointing out that the phenomenon was repeatable and not casual. This evidence could lead to the conclusion that during the in vivo

**TABLE II**
**Mean Values of Lutein, β-Carotene, Linoleic Acid, and –SH Group Content, and Yellow and Brown Indices in Pasta Obtained in Different Experimental Conditions and Relative % Variation Respect to Semolina[a–c]**

| Cultivar | EC | LC (µg/g, db) | % Loss | β-CC (µg/g, db) | % Loss | LAC (µg/g, db) | Loss% |
|---|---|---|---|---|---|---|---|
| Cosmodur | St | 1.43c | 80 | 0.57d | 68 | 817c | 68 |
| | Aa | 2.19a | 70 | 0.85b | 52 | 496e | 81 |
| | Ab | 1.69b | 77 | 0.71c | 60 | 967b | 63 |
| | Ba | 1.64b | 77 | 0.64cd | 64 | 562d | 78 |
| | Bb | 2.24a | 69 | 1.00a | 44 | 1,160a | 55 |
| Ofanto | St | 0.99e | 76 | 0.28c | 74 | 432d | 85 |
| | Aa | 1.12c | 73 | 0.43b | 60 | 578c | 80 |
| | Ab | 1.15b | 72 | 0.43b | 60 | 774b | 74 |
| | Ba | 1.09d | 74 | 0.43b | 60 | 557cd | 81 |
| | Bb | 1.46a | 65 | 0.71a | 34 | 981a | 67 |
| Tresor | St | 0.94a | 74 | 0.43a | 14 | 779b | 77 |
| | Aa | 0.88b | 76 | 0.43a | 14 | 436c | 87 |
| | Ab | 0.60d | 83 | 0.28b | 44 | 743b | 78 |
| | Ba | 0.73c | 80 | 0.28b | 44 | 353d | 90 |
| | Bb | 0.94a | 74 | 0.43a | 14 | 952a | 72 |
| Varano | St | 0.78c | 83 | 0.14b | 89 | 761c | 71 |
| | Aa | 0.68d | 85 | 0.28a | 78 | 551e | 79 |
| | Ab | 0.96a | 79 | 0.36a | 72 | 852b | 68 |
| | Ba | 0.68d | 85 | 0.14b | 89 | 596d | 78 |
| | Bb | 0.86b | 81 | 0.28a | 78 | 975a | 63 |

[a] Experimental conditions (EC); pH 7.0-25°C (St); pH 7.0, 10°C (Aa); pH 7.0, 40°C (Ab); pH 5.0, 25°C (Ba); pH 8.0, 25°C (Bb).
[b] Lutein content (LC); β-carotene content (β-CC); linoleic acid content (LAC); yellow index (YI); brown index (BI).
[c] Values followed by the same letter for the same cultivar are not significantly different ($P = 0.01$).

**TABLE II (continued)**
**Mean Values of Lutein, β-Carotene, Linoleic Acid, and –SH Group Content, and Yellow and Brown Indices in Pasta Obtained in Different Experimental Conditions and Relative % Variation Respect to Semolina[a–c]**

| Cultivar | –SH (µM/g) | % Loss | YI | % Loss | BI | % Rise |
|---|---|---|---|---|---|---|
| Cosmodur | 0.28d | 48 | 21.7ab | 18 | 14.8a | 6 |
| | 0.35b | 35 | 21.5b | 19 | 15.1a | 9 |
| | 0.28c | 48 | 21.2b | 20 | 15.3a | 10 |
| | 0.23e | 57 | 21.5b | 19 | 15.1a | 9 |
| | 0.43a | 20 | 22.4a | 16 | 15.3a | 10 |
| Ofanto | 0.34d | 52 | 19.4b | 13 | 14.7ab | . . . |
| | 0.41b | 43 | 19.1b | 14 | 14.8ab | . . . |
| | 0.39c | 46 | 18.5c | 17 | 14.6b | . . . |
| | 0.33e | 54 | 18.4c | 18 | 14.8ab | . . . |
| | 0.43a | 40 | 20.3a | 9 | 15.1a | 2 |
| Tresor | 0.32d | 35 | 17.9bc | 19 | 14.3b | 3 |
| | 0.38b | 23 | 18.3ab | 17 | 14.3b | 3 |
| | 0.32c | 35 | 16.9d | 23 | 14.3b | 3 |
| | 0.18e | 63 | 17.6c | 20 | 14.0b | 1 |
| | 0.47a | 4 | 18.5a | 16 | 15.3a | 11 |
| Varano | 0.29d | 67 | 18.0a | 8 | 16.0a | 4 |
| | 0.39b | 55 | 17.5ab | 11 | 16.3a | 6 |
| | 0.32c | 63 | 17.1b | 13 | 16.2a | 5 |
| | 0.23e | 74 | 17.8ab | 9 | 16.4a | 6 |
| | 0.44a | 49 | 17.8ab | 9 | 16.3a | 6 |

[a] Experimental conditions (EC); pH 7.0-25°C (St); pH 7.0, 10°C (Aa); pH 7.0, 40°C (Ab); pH 5.0, 25°C (Ba); pH 8.0, 25°C (Bb).
[b] Lutein content (LC); β-carotene content (β-CC); linoleic acid content (LAC); yellow index (YI); brown index (BI).
[c] Values followed by the same letter for the same cultivar are not significantly different ($P = 0.01$).

pasta making process, the enzyme activities might undergo the same variations, though to a different extent. However, great care should be given when in vitro results are applied to in vivo phenomena; enzyme activities are recorded on crude extracts and in optimal reaction conditions while real dough conditions are completely different in physical and chemical characteristics. Also, the particular hydrophobic environment due to the presence of gluten might modify the real reaction mechanism, as observed by other authors (Graveland 1970).

The treatment at pH 8.0 is the experimental condition that always allows preserving the highest amount of the analyzed components in relation to lower enzymatic activity levels. However, such a condition that induces negative effects on proteins is not a feasible proposition, but it may be only indicative of the possibility to affect the loss of components by acting on enzymatic levels. In fact, at pH 5.0, we recorded the major decrease of components associated with the highest enzyme levels during processing. With regard to –SH loss, evaluated as an additional measure of cooxidative reaction of LOX, we have found that the decreases of this component and LOX levels in each cultivar were not related. In fact, Varano, which had a lower Hp activity in semolina and during processing (Fig. 1D) but a considerable bleaching activity in semolina (Table I), has shown the highest –SH loss, whereas the Tresor had the least loss, though higher Hp and Bl activities (Table I, Fig. 1C). That might be due to different protein modifications that occurred in dough (Hoseney et al 1986; Icard-Verniére and Feillet 1999) causing free –SH decrease independently of LOX-mediated oxidation. The effects of –SH decrease due to LOX action on the rheological properties of durum dough will be studied later.

Finally, we have observed that among the cultivars analyzed in this study, Cosmodur showed the best performance in all the investigated experimental conditions. In fact, samples in this genotype always had the highest pasta yellowness together with lower levels of oxidative activities in semolina and in processing.

## CONCLUSIONS

The production of yellow-amber pasta is surely due to the concurrence of both genetic and technological factors. The starting point is the identification of cultivars with higher endogenous carotenoid content and lower enzyme levels. In addition, the study of the effects of different processing conditions on the dough extractable LOX and POD activities has been very important to confirm the involvement of these enzymes in color expression, providing remarkable information on their behavior during different phases of processing.

For any univocal relationship between the analyzed enzymatic activities and the losses of pigment and –SH groups, processing conditions at pH 8.0 induced a significant reduction of enzymatic activity levels, especially of Hp and POD, resulting in a greater preservation of various components in pasta. The loss of components might be reduced by acting on enzymatic levels. However, to identify a closer relationship between enzyme activities and the corresponding effects, more precise indication of the effects of enzymes may be drawn by measuring the activities in the same treatment conditions used during processing and not in the optimal conditions for enzymatic analysis.


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