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Inhibition of Durum Wheat Lipoxidase with L-Ascorbic Acid[1]

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ABSTRACT

Semolina from five pure durum wheat varieties was mixed with 0 to 200 p.p.m. L-ascorbic acid and processed into spaghetti. Spaghetti pigment content and color data showed that addition of L-ascorbic acid decreased the destruction of semolina pigments during processing and increased the yellowness of spaghetti. Moreover, the greatest color improvement appeared in samples from durum varieties showing high lipoxidase activity. Two separate enzyme kinetic studies were made and both showed L-ascorbic acid to be a fully competitive inhibitor of lipoxidase.

The bright yellow color of good-quality pasta products is attributed to the natural pigments of amber durum wheat. However, these pigments consist mainly of the xanthophylls lutein and taraxanthin, and are subject to oxidation to colorless or off-color pigments during processing of pasta (1,2).

Several factors are known to affect the oxidative stability of durum pigments (2). One of the prime factors influencing the rate of pigment oxidation during pasta processing is the enzyme lipoxidase (2,3). This enzyme is specific for the cis,cis-1,4-pentadiene system and catalyzes the oxidation of these unsaturated bonds to hydroperoxides (4,5). Probably these hydroperoxides oxidize the pigments and cause a loss of yellow color in finished pasta products.

Attempts to reduce pigment oxidation in pasta processing have met with various degrees of success. The exclusion of air by the use of vacuum mixers and continuous extruders has been shown to reduce pigment destruction (6). Because it was not practical to exclude air during drying, pigment oxidation was only partially arrested.

Selection and breeding of amber durum varieties have been highly successful in aiding the production of bright-yellow pasta products. However, some pigment oxidation persists in semolina made from durum varieties having low lipoxidase activity.

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Attempts to reduce pigment oxidation through addition of L-ascrobic to pasta have been made. Menger (7) added lecithin and L-ascorbic acid to pasta at several levels in various combinations. She found that samples which contained L-ascorbic acid showed less pigment oxidation than similar samples which contained no L-ascorbic acid. Recently, Milatovic and Martinec (8) studied the effect of adding L-ascorbic acid to pasta with and without eggs. Their data showed that adding 15 mg. % of L-ascorbic acid to pasta without eggs lowered pigment loss by half. Schmalz and Risdal (9) reported that addition of ultra-fine L-ascorbic acid powder to semolina improved the semolina color stability during storage, reduced color loss during pasta processing, and decreased stickiness in cooked pasta products.

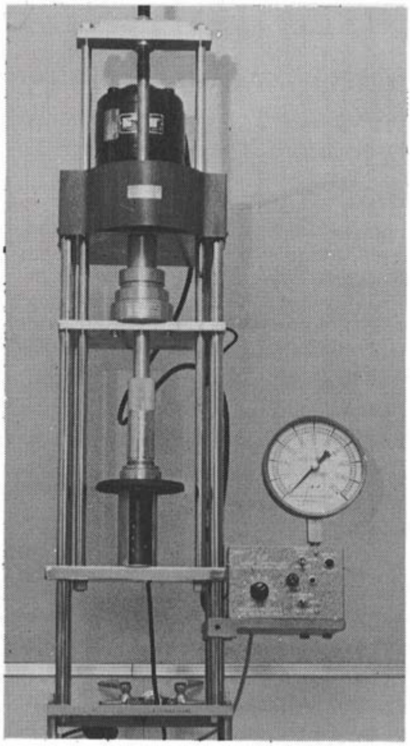


Fig. 1. Experimental press used in extrusion of 40-g. pasta through three-strand, 1/16-in. spaghetti die.

In the present study, the effect of adding L-ascorbic acid at various levels to pasta made with semolina from pure durum wheat varieties was observed. The varieties covered a wide range of pigment content, lipoxidase activity, and pasta quality. Enzyme kinetic studies were conducted in an effort to explain the mechanism of the action of L-ascorbic acid on pasta pigments.

MATERIALS AND METHODS

Samples of five pure durum wheat varieties representing a spectrum of spaghetti colors were selected for study. Included were Leeds and Wells, varieties known for their bright-yellow color; Mindum, a variety of pale-yellow color; and Golden Ball and Peliss, varieties known for gray and brown color, respectively.

Semolina was experimentally milled from each variety and stored at 5°C. in dark sealed containers prior to testing. Spaghetti was processed in duplicate from each semolina by extruding the pasta through a 0.073-in. brass spaghetti die using a modification of the method described by Martin et al. (10). Whereas in the method of Martin et al. (10) pasta was extruded with a hand-pumped hydraulic press, a new type of mechanical press (Research Products TC-800DC press shown in Fig. 1) was used. To extrude at a uniform driving speed of 0.70 in. per min., the press was modified by fitting it with special drive gears (6 and 2 in. diameter) and a 1-in.-diameter extruding piston was attached to the upper press plate; thus, the pasta extrusion cylinder and stand could be raised, forcing the pasta uniformly through the die. During hand pumping in the method described by Martin et al. (10), the extrusion pressure fluctuated drastically and resulted in a product with an irregular surface. The new press, on the other hand, extruded pasta at a fixed rate at a constant pressure; consequently, a more uniform product was obtained.

Spaghetti color scores were determined by the light reflectance method of Walsh et al. (11), with a Hunter color-difference meter, Model D25, equipped with a D25A optical unit (12). The entire 2-in.-diameter specimen area was covered with spaghetti strands, and readings were taken against a black background having 0% reflectance. The pigment content of the semolina and spaghetti samples was analyzed according to AACC Approved Methods (13).

Lipoxidase activities were analyzed by a modification of the method described by Surrey (14). The substrate was prepared in an atmosphere of nitrogen by dissolving 100 μl. of 99+% pure linoleic acid in a mixture of 0.12 ml. Tween 20, 2.5 ml. 0.05M phosphate buffer, pH 7, and 0.32 ml. 1.0M sodium hydroxide. After the linoleic acid was dissolved, the mixture was diluted to 50 ml. with the 0.05M phosphate buffer. The substrate was sealed under nitrogen in a syringe bottle and stored at 5°C. in the dark prior to use as a stock solution. The substrate was used until 90 μl. in 2.5 ml. 0.1M phosphate buffer, pH 5.9, showed an absorbance greater than 0.4 at 234 mμ.

Lipoxidase was extracted from ground grain, semolina, or spaghetti (Wiley mill, 30-mesh screen) by gently mixing 1 g. of sample with 20 ml. phosphate buffer, pH 5.9, allowing the mixture to stand 1 hr. at 5°C., and centrifuging under refrigeration at 6,000 $\times$ g. Reaction mixtures consisted of 2.5 ml. phosphate buffer, pH 5.9 90 μl. stock substrate, and 5 μl. of lipoxidase extract. The progress of the reaction was recorded continuously against a blank containing no enzyme in a double-beam spectrophotometer at 234 mμ. The slope of the line, showing an increase in absorbance with time, was used as a measure of lipoxidase activity. The lipoxidase activity of each sample was converted into lipoxidase units with the use of standard curves of samples of known lipoxidase activity. A unit of lipoxidase was defined as that activity which increases the absorbance by 1 O.D., at 234 mμ in 1 min., of a 2.5 ml. substrate solution, pH 5.9 (25°C.) with the use of a 1-cm. light path cell. Whereas pH 6.5 has been reported as the optimum for wheat lipoxidase by the manometric assay (15), in our system the optimum was found at pH 5.9. This apparent lowering of the optimum pH was similar to the effect reported by Ames and King (16), who found that the pH optimum for lipoxidase varied with the ionic strength of the buffers used in the substrates.

RESULTS AND DISCUSSION

The durum wheat varieties, Leeds, Wells, Mindum, Peliss, and Golden Ball, were grown under comparable conditions, milled, and analyzed for semolina pigment content, semolina lipoxidase activity, and spaghetti color. Table I summarizes the data for the five varieties. Leeds showed the highest pigment content and low lipoxidase activity, and produced bright-yellow spaghetti with the highest color score, 9.4, of the varieties tested. Wells was second highest in pigment content, had low lipoxidase activity, and produced yellow spaghetti with a color score of 9.2. Mindum had a medium pigment content and the lowest lipoxidase activity of the varieties tested, and produced pale-yellow spaghetti with a color score of 8.6. Apparently, the low lipoxidase activity compensated in part for the lack of pigments in Mindum and resulted in pale-yellow rather than gray or white spaghetti.

TABLE I. SEMOLINA PIGMENT CONTENT, LIPOXIDASE ACTIVITY, AND SPAGHETTI COLOR FOR FIVE VARIETIES OF DURUM WHEAT

	Semolina		Spaghetti	
Variety	Pigment p.p.m.	Lipoxidase units/g.	Color Score	Appearance
Leeds	5.88	8.93	9.4	Bright yellow
Wells	5.08	9.15	9.2	Yellow
Mindum	4.07	7.35	8.6	Pale yellow
Peliss	4.01	14.70	7.5	Tan
Golden Ball	4.12	18.53	7.1	Gray

TABLE II. COLOR SCORES AND PIGMENT CONTENTS OF SPAGHETTI MADE WITH SEVEN LEVELS OF ASCORBIC ACID FROM FIVE DURUM VARIETIES

Variety	L-Ascorbic Acid Added p.p.m.	Color Score	Pigment p.p.m.	Variety	L-Ascorbic Acid Added p.p.m.	Color Score	Pigment p.p.m.
Leeds	0	9.4	4.28	Golden Ball (contd.)	80	8.1	2.54
	10	9.4	4.54		100	8.3	2.80
	20	9.5	4.81		150	8.5	3.07
	40	9.6	4.81		200	8.5	3.07
	60	10.0	4.94				
	80	9.7	5.08	Wells	0	9.2	4.01
	100	9.8	5.08		10	9.2	4.14
	150	9.9	5.07		20	9.2	4.14
	200	10.0	5.10		40	9.3	4.14
Mindum	0	8.6	3.07		60	9.3	4.28
	10	8.7	3.21		80	9.4	4.54
	20	8.6	3.21		100	9.5	4.54
	40	8.6	3.35		120	9.4	4.54
	60	8.7	3.34		150	9.4	4.54
	80	8.7	3.47		200	9.6	4.68
	100	8.8	3.47	Peliss	0	7.5	2.27
	150	8.9	3.48		10	7.8	2.54
	200	9.2	3.47		20	8.1	2.80
Golden Ball	0	7.1	1.60		40	8.2	2.80
	10	7.2	1.73		60	8.4	3.07
	20	7.3	1.87		80	8.3	3.21
	40	7.4	2.12		100	8.6	3.34
	60	7.5	2.27		150	8.7	3.34
					200	8.7	3.47

Peliss displayed a medium pigment content and high lipoxidase activity, and produced tan, off-color spaghetti which had a color score of 7.5. Golden Ball showed a medium pigment content and high lipoxidase activity, and produced gray spaghetti with a color score of 7.1.

Thus, it was apparent that the color of spaghetti varied with both semolina pigment content and lipoxidase activity. If a chemical such as L-ascorbic acid were added to the pasta to block the pigment oxidation, it would have the greatest effect on those varieties which showed a medium semolina pigment content and high lipoxidase activity. Of the five varieties tested, only Golden Ball and Peliss met the above conditions.

Semolina from each of the five varieties was treated with eight different levels of L-ascorbic acid and processed into spaghetti. Table II presents average data obtained when duplicate spaghetti samples were analyzed for color and pigment content. Spaghetti processed from Leeds semolina showed little change in color score but increased slightly in pigment content from 4.28 to 5.10 p.p.m. as the ascorbic acid content was increased to 200 p.p.m. Wells spaghetti increased in color score from 9.2 for the control to 9.6 for the sample with 200 p.p.m. L-ascorbic acid, but showed little change in the spaghetti pigment content. Mindum showed little response to L-ascorbic acid in either spaghetti color score or pigment content. Peliss, however, increased in spaghetti color score from 7.5 for the control to 8.7 for the sample with 200 p.p.m. L-ascorbic acid, and increased over 50% in spaghetti pigment content. An even greater improvement was observed when L-ascorbic acid was added to Golden Ball. Spaghetti color score increased from 7.2 for the control to 8.5 for the sample with 200 p.p.m.; the spaghetti pigment content increased over 90%.

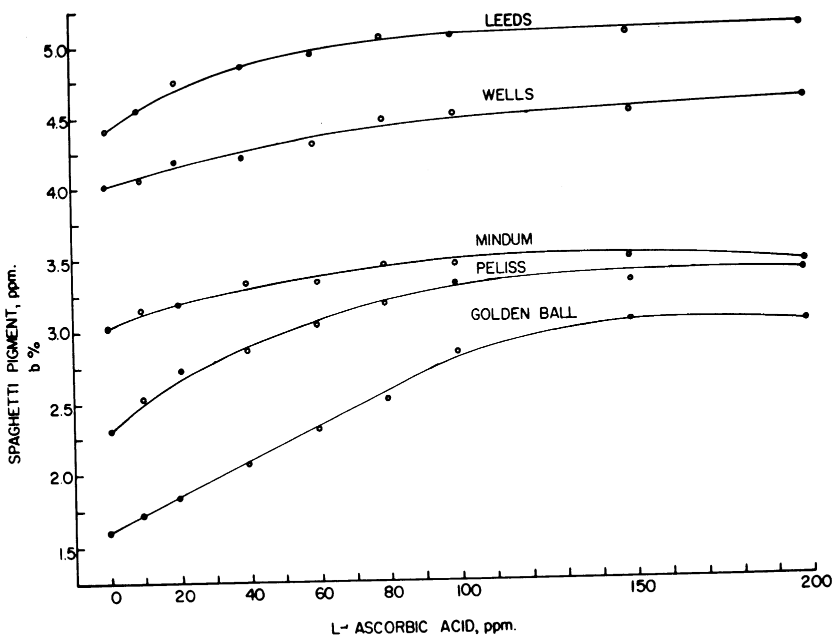


Fig. 2. Spaghetti pigment content vs. L-ascorbic acid added to semolina for five durum varieties.

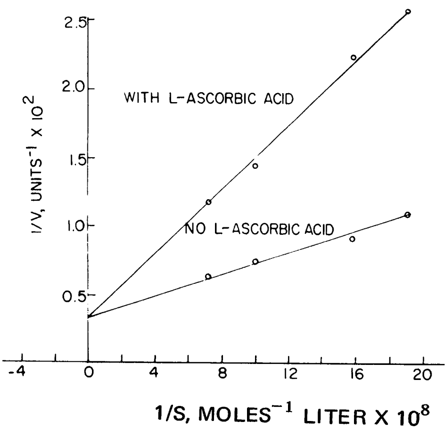
The data show that the additon of L-ascorbic acid to pasta improved spaghetti color. As seen in Fig. 2, the effect of increasing amounts L-ascorbic acid on pigment content was most pronounced up to 100 p.p.m.; above this, only minor improvements were noted. Moreover, the greatest improvement is shown for the high-lipoxidase varieties, Golden Ball and Peliss.

Since the effect was greatest with high-lipoxidase samples, it was thought that L-ascorbic acid might act as an inhibitor of lipoxidase. To determine the presence of inhibition, two enzyme kinetic studies were made. In the first study, reaction rates (v) were measured in two sets of reaction mixtures. One contained 9×10^{-7} g. L-ascorbic acid and the other, no L-ascorbic acid. The enzyme concentrations of each set were held constant and the substrate concentration (S) varied. Figure 3 shows a Lineweaver-Burk plot (1/v vs. 1/S) obtained from these data. The "no L-ascorbic" line intersects the "with L-ascorbic" line practically on the vertical axis. According to Dixon and Webb (17), this occurs in fully competitive inhibition.

In the second kinetic study, reaction rates (v) were measured in two sets of reaction mixtures. The first contained 30 μl. of substrate and the second, 50 μl. The lipoxidase concentration of each set was held constant and the L-ascorbic acid concentration (i) varied. The results were plotted according to the method of Dixon (1/v vs. i) (18) as shown in Fig. 4. The substrate I line intersects the substrate II line to the left of the vertical axis. According to Dixon (18), this occurs in fully competitive inhibition.

CONCLUSIONS

The addition of 10 to 100 p.p.m. of L-ascorbic acid to pasta improved the color



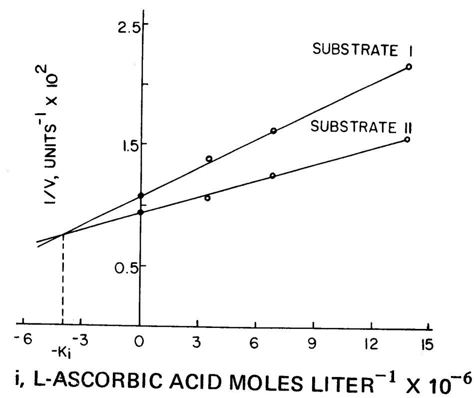


Fig. 3 (left). Lineweaver-Burk plot (1/v vs. 1/S) for wheat lipoxidase. "With L-ascorbic acid" contained 9.0 X 10^{-7} g. L-ascorbic acid in the substrate-enzyme reaction mixture. The points shown are averages of quadruplicate determinations. Standard deviation for the determination was 0.14 lipoxidase unit.

Fig. 4 (right). Inhibitor plot, (1/v) vs. L-ascorbic acid concentration (i) for two levels of substrate. "Substrate I" contained 8.34 X 10^{-6} moles of linoleic acid per liter. "Substrate II" contained 12.9 X 10^{-6} moles of linoleic acid per liter. The points shown are averages of quadruplicate determinations. Standard deviation for the determination was 0.35 lipoxidase unit.

of spaghetti. This color improvement was more pronounced in spaghetti made from durum varieties having high lipoxidase activity. L-ascorbic acid was shown to be a fully competitive inhibitor of wheat lipoxidase.

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