# Lipoxygenase and Lutein Bleaching Activity of Durum Wheat Semolina[1]

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## ABSTRACT

Durum semolina contains lipoxygenase and lutein bleaching activities but no linoleic acid hydroperoxide isomerase activity. Lipoxygenase activity was determined on linoleic acid by light absorption at 234 nm and bleaching activity on purified lutein by light absorption at 452 nm. The activity optimum for lipoxygenase was at pH 4.8, but lower peaks of activity occurred at pH 6.0 and 7.0. The optimum for bleaching was at pH 9.0 with a lower peak of activity at pH 6.0. Heating at 69° C for 1 min inactivated most but not all of both activities. Some activators and inhibitors also were tested. Results with a sulfhydryl blocking reagent and cysteine did not indicate an essential sulfhydryl group in either the bleaching or lipoxygenase enzymes. Ascorbic acid was much more effective as an inhibitor of bleaching activity than of lipoxygenase activity. Calcium chloride had little effect except at the highest pH levels of the assay where both bleaching and lipoxygenase activities were inhibited. Both bleaching and lipoxygenase activities varied widely between different varieties of durum, and bleaching activity was highly correlated with lipoxygenase activity. Compared with the potential for linoleic acid, the lutein oxidizing potential was low but evidently still high enough to cause marked color loss in spaghetti made from poor varieties of durum.


Uncooked spaghetti and macaroni made from durum wheat are yellow, and the color is one of the main criteria for assessing quality. Plant breeders continuously try to maintain in new varieties a bright yellow color that is due to a lipid carotenoid pigment called lutein.

Enzymatic bleaching of carotene by coupled oxidation with unsaturated fats was observed more than 30 years ago (Sumner and Sumner 1940). Loss of yellow lutein pigment in relation to lipoxygenase activity in durum wheat was investigated by Irvine and co-workers (1950, 1953). They concluded that the loss of yellow color during pasta processing was due to bleaching by lipoxygenase enzymes.

The substrate for durum wheat lipoxygenase appears to be free linoleic acid. Bread wheat lipoxygenase rapidly oxidized free linoleic acid, but the methyl ester and monoglycerides, diglycerides, and triglycerides were poor substrates (Guss et al 1968). Dahle (1965) reported that the addition of linoleic acid caused a more rapid loss of yellow pigment in durum flour slurry. Matsuo et al (1970) reported that the addition of linoleic acid caused more rapid loss of lutein pigment in durum semolina dough than did trilinolein, methyl linolein, or methyl linolenate.

This study used a direct spectrophotometric method of enzyme assay and free linoleic acid as substrate to evaluate both the lipoxygenase activity and lutein bleaching activity of durum semolina. Some properties of both enzyme activities were determined, and the relation between bleaching and lipoxygenase activity found in different varieties supports the earlier conclusion that the bleaching of carotenoids in semolina is due to lipoxygenase activity.

## MATERIALS AND METHODS

### Materials

For this study nine varieties of durum wheat (*Triticum durum*) and one variety of hard red spring wheat (*Triticum aestivum*) were comparably grown in Minot, ND, in 1974. The durum wheats were milled to semolina on a durum laboratory mill as described by Seyam et al (1974) and the hard red spring wheat on a Buhler Laboratory Mill (AACC method 26-20, first approval 1961). Analyses of the wheat and semolina or flour are given in Table I.

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Linoleic acid 99+% pure was purchased from Applied Science Laboratories (State College, PA), Tween 20 from Fisher Scientific Co., and Tween 80 from Matheson Coleman & Bell. Hexanes, ethyl ether, acetone, and chloroform were distilled before use.

### Methods

*Analytical Methods.* Approved AACC methods were used for ash content (Method 08-01, approved April 1961), Kjeldahl protein (Method 46-11, N × 5.7), and semolina moisture (Method 44-15A, approved October 1975). Lutein content of semolina was determined by AACC Method 14-50, (approved April 1961) as modified by Sims and LePage (1968).

*Lutein Purification for Bleaching Substrate.* All solutions were placed under $N_2$ by applying a vacuum with a water pump three times and refilling with nitrogen each time. A solution of 125 g of vegetable xanthophyll (Nutritional Biochemical Corp., Cleveland, OH; 0.55% lutein) dissolved in 500 ml of absolute ethanol and 100 ml of 60% (w/v) KOH was allowed to stand in the dark at room temperature for 20 hr. After diluting with 1,500 ml of distilled water, the mixture was divided into five 420-ml aliquots. Each aliquot was extracted twice with 400 ml of freshly distilled ethyl ether, and the two ether extracts from each aliquot were combined. These combined extracts were each washed with five 400-ml aliquots of distilled water, and the washed extract was dried with 60 g of anhydrous $Na_2SO_4$. Ether was removed under vacuum at 25° C on a flash evaporator.

The lutein dissolved in 400 ml of 4% (v/v) acetone in hexanes was applied to a 3.1 × 22.0 cm column of 1:1 (w/w) Kieselgel-analytical celite. The column was developed with 300 ml of 4% (v/v) acetone in hexanes and then with 300 ml of 10% (v/v) acetone in hexanes. The orange band of lutein was then eluted with 20% (v/v) acetone in hexanes.

The solids in the eluted band contained 54.2% lutein as determined by light absorption at 456 nm in chloroform using an absorption of 2.12 per milligram of lutein in 100 ml of solvent for the calculation (Sims and Lepage 1968). Aliquots that contained 5.3 mg of lutein were evaporated to dryness under vacuum in 16 × 105-mm screw-cap culture tubes, and the dried lutein was stored under nitrogen in a freezer. The spectrum of the lutein in chloroform and buffer (solubilized with Tween 80) is given in Fig. 1.

*Crude Enzyme Extract.* Enzymes were extracted with cold (3° C) 0.1 *M* sodium phosphate buffer, pH 7.0, in an ice bath for 1 hr with stirring by a magnetic bar for 2 min, initially after adding cold buffer and 1 min every 15 min thereafter. For lipoxygenase and hydroperoxide isomerase 10 ml of buffer was used to extract 2 g of semolina, and for bleaching enzymes 11 ml of buffer to extract 5 g of semolina. The maximum amount of lipoxygenase or bleaching

activity was extracted in 30 min and did not increase on extending the extraction time to 90 min. After centrifugation at 34,800 × *g* at 5°C for 15 min, the supernatant was recentrifuged to remove some suspended light material. The extracts in test tubes were stored in an ice-water bath at 0°C and used within two days. Extracts of semolina from Golden Ball variety durum wheat were used except in the study of different varieties.

*Enzyme Assay Procedures.* Linoleic acid substrate for lipoxygenase and hydroperoxide isomerase assay was prepared under nitrogen with Tween 20 according to Surrey (1964). Small aliquots of linoleate in screw-cap culture tubes were placed under nitrogen by evacuating and refilling them with $N_2$ four times, and they were stored in a freezer. The activities for both enzymes at 25°C were determined by measuring conjugated diene absorption at 234 nm by the procedures described by Zimmerman and Vick (1970) in a spectrophotometer fitted with a constant temperature water-jacketed cell holder and a strip chart recorder. For lipoxygenase assay, 0.025 ml of linoleate substrate ($7.5 \times 10^{-3}$M) containing Tween 20 (0.25%), 2.96 ml buffer, and 0.04 ml extract was used in the reaction cell. Buffers used routinely for assay were 0.1*M* sodium acetate, pH 4.8, and 0.05*M* sodium phosphate, pH 6.0 and 7.0, to give pH 4.84, 6.05, and 7.00 in the cell, respectively. For isomerase the procedure of Zimmerman and Vick (1970) was used with 0.04 ml of semolina extract.

The procedure of Ben Aziz et al (1971) for carotene bleaching activity was modified by diluting cold (0°C) linoleic acid and lutein with buffer in the spectrophotometer cell just before assay to reduce the danger of oxidation instead of diluting earlier in 10-ml volumes and holding at 25°C.

For bleaching, linoleic acid emulsion (0.020*M*) containing Tween 80 (9.23 μl/ml) was prepared under nitgrogen with distilled water. A 0.925-ml aliquot of linoleic acid dispersed in 5.54 ml of 25% (v/v) Tween 80 and 100 ml of distilled water was dissolved by adding dropwise 3.5 ml of 1.0*N* NaOH while stirring with a magnetic bar stirrer. The pH was then lowered from 11.6 to 8.6 by adding dropwise 1.5 ml of 1.0*N* hydrochloric acid. Distilled water (38.5 ml) was added to make a total volume of 150 ml. About 8-ml aliquots in screw-cap culture tubes were placed under nitrogen and stored in a freezer until use.

A stock solution of lutein was prepared by dissolving 5.3 mg of purified lutein in 16.1 ml $CHCl_3$. This solution was stored under nitrogen in a freezer. A mixture of 1-ml chloroform lutein solution and 1-ml Tween 80 in $CHCl_3$ (6.6 μl/ml) was evaporated to dryness in a flash evaporator using a 30°C water bath. The residue was dissolved in 6-ml 0.25% aqueous solution of disodium versene previously placed under nitrogen. The lutein concentration in this solution was adjusted with 0.25% aqueous disodium versene solution containing 1.1 μl of Tween 80/ml so that when 0.1 ml was diluted to 2.2 ml with 0.14 ml of Tween 80 solution (6.6 μl/ml) and 1.96 ml of 0.05*M* sodium borate (pH 9.2), the absorbance was 0.40 ± 0.01 at 452 nm in a 1-cm cell. The lutein solution was stored in an ice-water bath and kept under nitrogen until use.

For the assay 0.1 ml of cold lutein solution and 0.1 ml of cold linoleic acid emulsion were added to a 1-cm cell and mixed. Then 1.92 ml of pH 9.2 sodium borate buffer (0.05*M*) or 1.95 ml of pH 6.0 sodium phosphate buffer (0.05*M*) or pH 4.0 sodium acetate buffer (0.1*M*) was added. The reaction was started by adding 0.08 ml of extract for pH 9.2 buffer and 0.05 ml of extract for pH 6.0 and 4.0 buffers for a total volume of 2.2 ml with the final pH in the cell being, respectively, pH 9.2, 6.2, and 4.1. The cell was immediately capped and inverted three times, and the change in absorbance at 452 nm was recorded. The blank contained, in place of the lutein, 0.1 ml of 0.25% disodium versene containing 1.1 μl Tween 80/ml. At pH 9.2 the induction period was about 1 min, and at pH 6.2 and 4.1, about 0.25–0.5 min before the bleaching occurred.

For lutein the molar absorptivity in the presence of linoleate used to calculate activities was 97,800 with pH 9.2 borate buffer, 136,000 with pH 6.0 phosphate buffer, and 131,000 with pH 4.0 acetate buffer. A molar absorptivity at 234 nm of 21,600 was used to calculate diene conjugation of linoleic acid by lipoxygenase (MacGee 1959). Linoleate for both assays was always less than 4% oxidized, as estimated by light absorption at 234 nm. The standard deviation of the activity of extracts as estimated from the range of duplicates (Grant and Leavenworth 1972) were with lipoxygenase 0.19, 0.11, and 0.12 μmol/min/g semolina for pH levels of 4.8, 6.0, and 7.0, respectively, and with bleaching 0.05, 0.12, and 0.09 $\times 10^{-2}$ μmol/min/g for pH levels of 9.2, 6.2, and 4.1, respectively. Bleaching and lipoxygenase activities, calculated as μmoles substrate changed per minute per gram of semolina extracted, are the mean of duplicate extracts assayed in duplicate or triplicate.

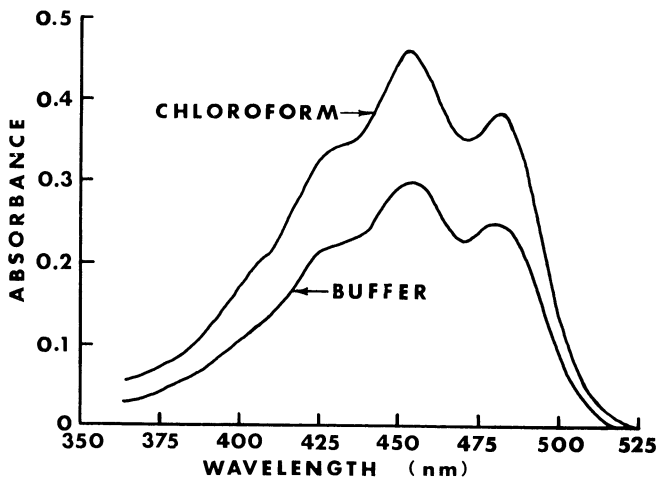


Fig. 1 Light absorption spectrum of purified lutein in chloroform and in 0.05*M* sodium phosphate buffer, pH 6.0, with 0.47 μl/ml of Tween 80.

## RESULTS

### Enzyme Assay Conditions

*Variation of Activity with pH.* Extracts of the durum variety Golden Ball, which has high activity, were used to study enzyme properties. An activity optimum occurred at pH 4.8 for lipoxygenase, but smaller peaks of activity also occurred at pH 6.0 and 7.0 (Fig. 2). Walsh et al (1970) reported the optimum at pH 5.9; they used a similar assay with Tween 20 at a higher linoleate concentration of $2.3 \times 10^{-4}$*M* as compared with $6.3 \times 10^{-5}$*M* used here. For lipoxygenase of wheat germ, Wallace and Wheeler (1975) found that the pH optimum usually shifted to lower pH values at lower substrate concentrations. Bleaching activity optimum occurred at pH 9.0 with a smaller peak of activity at pH 6.0, and activity also occurred down to much lower pH. The wide range of pH in which both enzyme activities occurred suggests there may be more than one lipoxygenase or bleaching enzyme in durum semolina. For this reason, lipoxygenase was assayed at pH 4.8, 6.0,

TABLE I
Analyses of Wheat and Semolina or Flour

| Variety | Wheat Protein[a] (%) | Semolina or Flour Protein[a] (%) | Semolina or Flour Ash[a] (%) | Semolina or Flour Extraction (%) |
|---|---|---|---|---|
| Durum | | | | |
| Mindum | 13.7 | 12.9 | 0.55 | 59.5 |
| Steward 63 | 13.6 | 12.7 | 0.56 | 62.7 |
| Yuma | 14.1 | 13.2 | 0.57 | 62.1 |
| Golden Ball | 13.6 | 11.9 | 0.58 | 60.9 |
| Cocorit | 12.0 | 11.0 | 0.67 | 62.5 |
| Rugby | 14.7 | 13.8 | 0.59 | 61.0 |
| Wakooma | 15.2 | 14.2 | 0.58 | 60.7 |
| Pelissier | 12.7 | 11.7 | 0.59 | 63.6 |
| Leeds | 15.3 | 14.3 | 0.61 | 59.6 |
| Hard spring | | | | |
| Waldron | 16.2 | 15.3 | 0.36 | 68.4 |

[a] 14% mb.

and 7.0 and bleaching activity at pH 4.1, 6.2, and 9.2.

Linoleic acid hydroperoxide isomerase was reported to be necessary for lipoxygenase bleaching of chlorophyll (Zimmerman and Vick 1970); it might also be needed for lutein bleaching. There does not appear to be any hydroperoxide isomerase activity in durum semolina, however. No activity was detected over a wide pH range using pH 3.8, 4.3, 5.6, 6.0, 7.0, 7.5, 8.5, and 9.5 buffers by the procedure of Zimmerman and Vick (1970). Also, there should have been no interference in our lipoxygenase assay by decomposition of hydroperoxide by isomerase or reducing agents as observed by Berkeley and Galliard (1976).

*Variation of Activity with Linoleate and Extract.* Maximum bleaching activity occurred when more than $6.8–7.9 \times 10^{-4} M$ linoleate was used for the assay (Table II). For our bleaching assay $9.1 \times 10^{-4} M$ linoleate was used. Bleaching activity was nearly linear to 0.05 ml of extract with assays at pH 4.1 and 6.2 and linear to 0.1 ml of extract with assays at pH 9.2 (Fig. 3). In bleaching 0.05 ml of semolina extract was used for pH 4.1 and 6.2 assays and 0.08 ml for pH 9.2 assay. Lipoxygenase activity at pH 4.8 was linear up to the 0.06 ml of extract, and 0.04 ml was used in the lipoxygenase assay.

*Variation of Activity with Tween 80.* Tween 80 used for emulsifying linoleic acid substrate had an inhibiting effect on bleaching activity (Table III). For assays a final Tween 80 concentration of 0.46 $\mu$l/ml was used because at lower concentration a turbidity formed in the reaction cell.

**TABLE II**
**Effect of Linoleate on Bleaching Activity**

| Concentration Linoleate (Molarity) | Bleaching Activity[a] pH 9.2 | pH 6.2 | pH 4.1 |
|---|---|---|---|
| | ($\mu$mol/min/g Semolina $\times 10^2$) | | |
| $4.5 \times 10^{-4}$ | 2.90 | 0.36 | 0.56 |
| $5.6 \times 10^{-4}$ | 3.56 | ... | 1.37 |
| $6.8 \times 10^{-4}$ | 5.77 | 1.99 | 1.98 |
| $7.9 \times 10^{-4}$ | 5.65 | 2.54 | ... |
| $9.1 \times 10^{-4}$ | ... | 2.59 | 1.83 |

[a]Dry basis.

**TABLE III**
**Inhibition of Bleaching Activity**

| Tween 80 Concn. ($\mu$l/ml) | Bleaching—$\mu$mol/min/g Semolina $\times 10^2$ pH 4.1 | pH 6.2 | pH 9.2 |
|---|---|---|---|
| 0.46 | 2.8 | 3.3 | 5.1 |
| 0.54 | 2.5 | 2.9 | 5.0 |
| 0.61 | 2.2 | 2.6 | 4.5 |
| 0.76 | 1.3 | 1.5 | 3.7 |

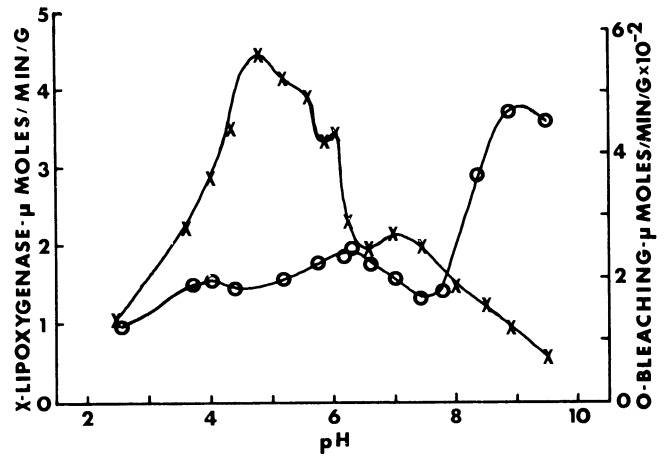


Fig. 2. Durum semolina bleaching and lipoxygenase activity with pH (Golden Ball variety of durum). Here 0.60 $\mu$l/ml concentration of Tween 80 and 0.060 ml of enzyme extract were used in the bleaching assays. Also for lutein a molar absorptivity of 133,000 was used for calculating results at all pH levels.

## Inactivation by Heat

The bleaching and lipoxygenase enzymes are rather unstable to heat (Table IV). The bleaching and lipoxygenase activities in extracts were rather quickly inactivated at 69°C, but some activity remained after 1-min heating. Heating to 69°C (in 45 sec) inactivated more than 50% of the activity in most cases.

Stability toward heat is similar to soybean lipoxygenase 2, which is 50% inactivated in 0.7 min or less at 69°C, in contrast to soybean lipoxygenase 1 with a half-time survival of 25 min at 69°C (Christopher et al 1970).

## Activators and Inhibitors

The sulfhydryl group blocking reagent *p*-chloromercuriphenyl sulfonic acid did not inhibit lipoxygenase or bleaching activity but

**TABLE IV**
**Heat Inactivation at 69°C**

| Activity | Minutes at 69°C[a] | Activity—% of Control | | |
|---|---|---|---|---|
| Lipoxygenase | | pH 4.8 | pH 6.0 | pH 7.0 |
| | 0 | 37 | 51 | 36 |
| | 0.5 | 11 | 27 | 0 |
| | 1.0 | 6 | 21 | 0 |
| Bleaching | | pH 4.1 | pH 6.2 | pH 9.0 |
| | 0 | 37 | 43 | 62 |
| | 0.5 | 20 | 0 | 17 |
| | 1.0 | 7 | 0 | 10 |

[a]45 sec required to bring extract to 69°C.

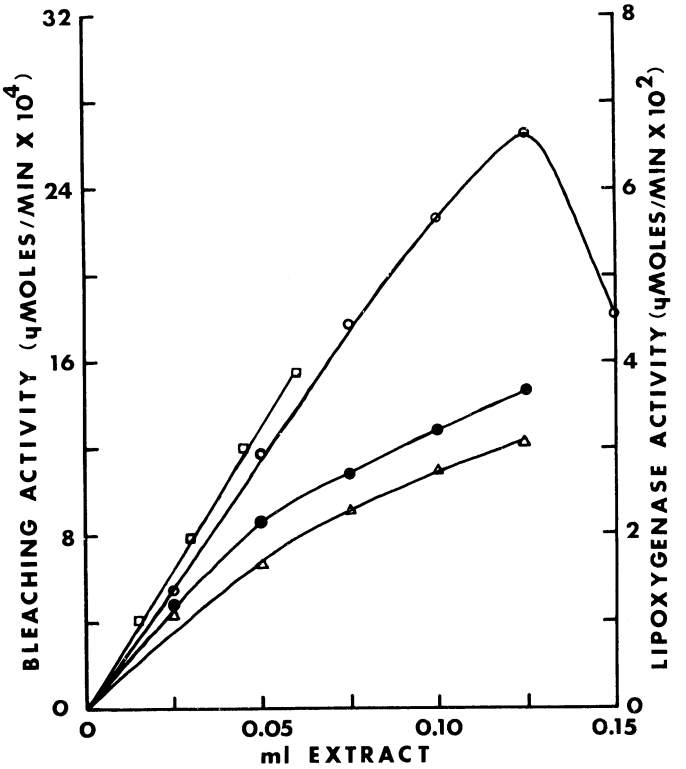


Fig. 3. Variation of extract quantity and enzyme activity. o = pH 9.2, • = pH 6.2, △ = pH 4.1 bleaching activity, □ = pH 4.8 lipoxygenase activity.

caused an activation of bleaching at pH 9.2 (Table V). The small sulfhydryl compound cysteine did not cause activation of either enzyme activity. Thus, there does not appear to be an essential sulfhydryl group in the active site of the bleaching or lipoxygenase enzymes of durum endosperm.

Inhibition of bleaching by cysteine at the higher pH of assay suggests that the products or intermediates of linoleic acid oxidation that normally oxidize lutein are also reacting with sulfhydryl groups. The activation of bleaching at pH 9.2 by the sulfhydryl blocking reagent could be due to removal of the free sulfhydryl group in the enzyme extract that would otherwise react with oxidation products of linoleic acid. Cytochrome c and glutathione peroxidase catalytic oxidation of cysteine by linoleic acid hydroperoxide has been reported in several papers (Little and O'Brien 1967, 1968a,b).

Ascorbic acid at 0.05 m*M* concentration, where Walsh et al (1970) reported competitive inhibition of durum semolina lipoxygenase, caused marked inhibition of bleaching but little inhibition of lipoxygenase. These workers also reported that ascorbic acid helped prevent color loss on processing semolina into spaghetti. Our results indicate that ascorbic acid in low concentrations is much more effective at inhibiting bleaching than lipoxygenase activity, probably being oxidized itself by linoleic acid oxidation products rather than the yellow lutein pigment.

Calcium ions increase the activity of some lipoxygenases (Restrepo et al 1973). Lipoxygenase activity of durum semolina was increased little if any by $Ca^{2+}$ at pH 4.8 and 6.1 but was markedly inhibited at pH 7.0. Also with bleaching there was little if any increase in activity at pH 4.1 but small and marked inhibition, respectively, at pH 6.2 and 9.2. Zimmerman and Synder (1974) reported a similar inhibition of soybean lipoxygenase 2 by $Ca^{2+}$ (pH 6.8) when $1 \times 10^{-3} M$ linoleate and 0.02% Tween 20 were present, but Restrepo et al (1973) reported activation of the same enzyme by $Ca^{2+}$ (pH 8) in the presence of $7 \times 10^{-4} M$ linoleate and 0.004% Tween 20. Evidently the effect of $Ca^{2+}$ depends on the relative amounts of linoleate and Tween 20. Here for durum lipoxygenase $6.3 \times 10^{-5} M$ linoleate and 0.0021% Tween 20 were present. With $1 \times 10^{-3} M$ linoleate and 0.0125% Tween 80, Weber et al (1974) reported no effect by $Ca^{2+}$ for soybean lipoxygenase 2 (pH 6.5) but inhibition of soybean lipoxygenase 3. Here in the bleaching assay $9.1 \times 10^{-4} M$ linoleate and 0.047% Tween 80 were used.

### Lipoxygenase and Bleaching Activities of Durum Varieties

Bleaching and lipoxygenase activities in varieties of durum that were expected to differ in lipoxygenase activity are given in Table VI. These include old and new varieties. Golden Ball, an old variety noted for high lipoxygenase activity, was highest in both bleaching[2] and lipoxygenase activity, and Pelissier, another old high lipoxygenase variety, was next to the highest. The newer high quality varieties Rugby, Wakooma, and Leeds were lowest in both activities. A hard red spring wheat bread variety, Waldron, was higher in both activities than the high quality durum but not as high as Golden Ball.

The bleaching activity correlated highly with lipoxygenase activity (Table VII). On Yuma at pH 9.2 relative high bleaching activity was not accompanied by high lipoxygenase activity (Table VI). The pH 6.2 bleaching correlated especially high with lipoxygenase activity. The significant correlation indicated that bleaching activity is probably caused largely by lipoxygenase enzyme.

The question of bleaching being caused by lipoxygenase enzymes

[2]Bleaching activity here for Golden Ball was somewhat higher than shown in Fig. 2 where some inhibition occurred with use of a higher concentration of Tween 80 and more extract. Also, a lower molar absorptivity for lutein was used for calculating pH 9.2 results in Table VI.

TABLE V
**Bleaching and Lipoxygenase Activitors and Inhibitors**[a]

| Activator or Inhibitor | Concn. | Activity—% of Control | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bleaching | | | Lipoxygenase | | |
| | | pH 4.1 | pH 6.2 | pH 9.2 | pH 4.8 | pH 6.0 | pH 7.0 |
| *p*-Chloromercuriphenyl sulfonic acid | 0.002 m*M* | 100 | 102 | 132 | 96 | 108 | 101 |
| Cysteine + versene | 1.0 m*M* + 0.1 m*M* | 91 | 78 | 0 | 90 | 108 | 93 |
| Ascorbic acid | 0.5 m*M* | ... | ... | ... | 54 | 87 | 87 |
| | 0.05 m*M* | 63 | 66 | 15 | 104 | 95 | 101 |
| $CaCl_2$ | 0.5 m*M* | 104 | 90 | 39 | 98 | 108 | 48 |

[a]The extract was treated 10 min at 25°C with 0.002 m*M* concentration of *p*-chloromercuriphenyl sulfonic acid prior to assay. The other compounds were used at the concentration indicated in the assay cell.

TABLE VI
**Bleaching and Lipoxygenase Activities of Semolina from Varieties of Durum Wheat and Waldron Spring Wheat Flour**

| Variety | Lipoxygenase Activity[a] | | | Bleaching Activity × 10[2] | | |
|---|---|---|---|---|---|---|
| | pH 4.8 | pH 6.0 | pH 7.0 | pH 4.1 | pH 6.2 | pH 9.2 |
| | (μmol Substrate Change/min/g Semolina) | | | | | |
| Mindum | 1.18 | 0.76 | 0.44 | 1.12 | 1.67 | 0.60 |
| Stewart 63 | 1.97 | 1.48 | 0.56 | 0.71 | 1.47 | 0.51 |
| Yuma | 1.10 | 0.54 | 0.42 | 1.27 | 1.37 | 3.20 |
| Golden Ball | 4.74 | 3.36 | 2.35 | 3.44 | 4.25 | 5.83 |
| Cocorit | 1.33 | 0.84 | 0.66 | 1.37 | 1.67 | 0.44 |
| Rugby | 1.40 | 1.04 | 0.58 | 0.76 | 1.42 | 0.54 |
| Wakooma | 1.86 | 1.08 | 0.84 | 0.76 | 1.57 | 0.51 |
| Pelissier | 3.22 | 2.40 | 1.94 | 2.03 | 3.05 | 4.78 |
| Leeds | 1.98 | 1.18 | 0.74 | 0.86 | 1.57 | 0.66 |
| Waldron | 2.11 | 1.58 | 1.18 | 1.62 | 2.06 | 5.10 |

[a]The average activity in duplicate extracts of each variety expressed on a dry basis.

and of one or more lipoxygenase or bleaching enzymes could be resolved by separation, fractionation, and purification of the enzyme activities. Results here on heat inactivation, $Ca^{2+}$ inhibition, and relative bleaching and lipoxygenase activities in different varieties suggest one enzyme and the pH activity curves suggest several enzymes. For bread wheat flour Guss et al (1967) observed only one lipoxygenase isoenzyme by a polyacrylamide electrophoresis method, but two other minor bands of lipoxygenase were observed in wheat shorts.

### Lutein Cooxidizing Potential

A cooxidation potential[3] for lutein and linoleic acid when both activities were assayed under optimum conditions and at pH 6 was calculated, assuming bleaching is caused by lipoxygenase. The cooxidation potential of the durum lipoxygenase to oxidize lutein is not very high (Table VIII). There was a variation of cooxidation potential with a mean of 0.010 μmol lutein bleached per μmole of diene formed at the optimum pH levels of assay and 0.016 at pH 6. Grosch et al (1976) reported a cooxidation potential in wheat seeds (*T. aestivum*) of 0.06 for β-carotene, which was similar to that of flax lipoxygenase and soybean lipoxygenase 1 but much less than soybean lipoxygenases 2 and 3 with a potential of 0.4–0.5. If most of the bleaching is due to lipoxygenase, the lutein cooxidizing potential is still high enough to cause marked loss of color during spaghetti processing of semolina from poor varieties of durum like Golden Ball and Pelissier.

### Pigment Loss on Making Spaghetti

Lutein pigment content in the semolina differs widely between varieties (Table IX), with Cocorit (lowest) and Rugby, Wakooma, and Leeds (highest). Pigment loss on making spaghetti was highest with Golden Ball and Pelissier, the varieties with the highest enzyme activities (Table VI). Yuma, the variety with high enzyme activity only for bleaching at pH 9.2, also was high in color loss. Rugby, Wakooma, and Leeds lost less pigment. The pigment loss is significantly correlated with both bleaching and lipoxygenase activity (Table X), but bleaching activity showed a considerably better correlation. Thus, the loss of pigment on making spaghetti must depend on enzyme activity.

### Spaghetti Pigment Prediction Equations

Equations for predicting spaghetti pigment are given in Table XI. Semolina pigment and bleaching activity in regression equations can be used to predicted spaghetti pigment with a

[3]Bleaching activity divided by the lipoxygenase activity.

**TABLE VII**
**Correlation of Bleaching to Lipoxygenase Activity**

| Enzyme Correlation | Correlation Coefficient |
|---|---|
| pH 4.1 Bleaching vs. pH 4.8 lipoxygenase | 0.87** |
| pH 4.1 Bleaching vs. pH 6.0 lipoxygenase | 0.85** |
| pH 4.1 Bleaching vs. pH 7.0 lipoxygenase | 0.92** |
| pH 6.2 Bleaching vs. pH 4.8 lipoxygenase | 0.96** |
| pH 6.2 Bleaching vs. pH 6.0 lipoxygenase | 0.95** |
| pH 6.2 Bleaching vs. pH 7.0 lipoxygenase | 0.98** |
| pH 9.2 Bleaching vs. pH 4.8 lipoxygenase | 0.80** |
| pH 9.2 Bleaching vs. pH 6.0 lipoxygenase | 0.79** |
| pH 9.2 Bleaching vs. pH 7.0 lipoxygenase | 0.86** |

**TABLE VIII**
**Lutein Cooxidation Potential of Durum Lipoxygenase as Lutein Bleached per Micromoles of Linoleate Diene Formed**

| Variety | pH 9.2 Bleaching/pH 4.8 Lipoxygenase (μmol/min Lutein Decrease/μmol/min Diene Formed) | pH 6.2 Bleaching/pH 6.0 Lipoxygenase (μmol/min Lutein Decrease/μmol/min Diene Formed) |
|---|---|---|
| Mindum | 0.0051 | 0.022 |
| Stewart 63 | 0.0026 | 0.0099 |
| Yuma | 0.029 | 0.025 |
| Golden Ball | 0.012 | 0.013 |
| Cocorit | 0.0033 | 0.020 |
| Rugby | 0.0039 | 0.014 |
| Wakooma | 0.0027 | 0.014 |
| Pelissier | 0.015 | 0.013 |
| Leeds | 0.0033 | 0.013 |
| Waldron | 0.024 | 0.013 |
| Range | 0.0026–0.029 | 0.0099–0.025 |
| Mean | 0.0101 | 0.0157 |

**TABLE IX**
**Semolina Pigment Content and Pigment Loss in Making Spaghetti**

| Variety | Lutein Content | | Lutein Loss | |
|---|---|---|---|---|
| | Semolina (μg/g) | Spaghetti (μg/g) | (μg/g) | (%) |
| Mindum | 4.51 | 3.12 | 1.4 | 31 |
| Stewart 63 | 4.80 | 3.82 | 1.0 | 21 |
| Yuma | 5.52 | 3.78 | 1.7 | 31 |
| Golden Ball | 5.15 | 2.54 | 2.6 | 51 |
| Cocorit | 2.74 | 2.20 | 0.5 | 20 |
| Rugby | 6.24 | 4.99 | 1.2 | 20 |
| Wakooma | 6.13 | 4.85 | 1.3 | 21 |
| Pelissier | 4.67 | 3.10 | 1.6 | 34 |
| Leeds | 6.51 | 5.30 | 1.2 | 19 |

**TABLE X**
**Relation of Enzyme Activity to Pigment Loss**

| % Pigment Loss | Correlation Coefficient |
|---|---|
| Bleaching | |
| pH 4.1 | 0.92** |
| pH 6.2 | 0.87** |
| pH 9.2 | 0.89** |
| Lipoxygenase | |
| pH 4.8 | 0.75* |
| pH 6.0 | 0.73* |
| pH 7.0 | 0.79** |

TABLE XI
Equations for Predicting Spaghetti Color

| Enzyme Activity and pH | Predicted vs. Actual Pigment r | Coefficient of Variance (%) |
|---|---|---|
| Bleaching | | |
| pH 4.0 Spaghetti pigment = 0.735 semolina pigment − 58.4 bleaching activity + 0.77 | 0.98** | 6.1 |
| pH 6.0 Spaghetti pigment = 0.787 semolina pigment − 47.4 bleaching activity + 0.65 | 0.97** | 8.5 |
| pH 9.2 Spaghetti pigment = 0.826 semolina pigment − 22.6 bleaching activity − 0.07 | 0.98** | 7.4 |
| Lipoxygenase | | |
| pH 4.8 Spaghetti pigment = 0.852 semolina pigment − 0.34 lipoxygenase activity + 0.08 | 0.95** | 10.8 |
| pH 6.0 Spaghetti pigment = 0.835 semolina pigment − 0.43 lipoxygenase activity + 0.06 | 0.95** | 11.4 |
| pH 7.0 Spaghetti pigment = 0.823 semolina pigment − 0.54 lipoxygenase activity + 0.05 | 0.95** | 10.2 |

correlation coefficient of 0.97–0.98 and coefficient of variance of 6–8% between predicted and actual values. With lipoxygenase activity in place of bleaching activity, the correlation coefficient is 0.95 and coefficient of variance 10–11% between predicted and actual values. Correlations with lipoxygenase are near those reported by Irvine and Anderson (1953) who obtained a correlation coefficient of 0.95; a coefficient of variance of 10.2% was calculated from their data. These workers used oxygen uptake in Warburg monometers to assay lipoxygenase; this study used light absorption at 234 nm by hydroperoxide products.

Prediction of spaghetti pigment from semolina pigment and bleaching activity might be used in place of making spaghetti for color determination. In wheat breeding the prediction would be especially useful in early generation where only small samples are available.

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