Lipoxygenase Inactivation in Wheat Protein Concentrate by Heat-Moisture Treatments[1]

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ABSTRACT

The inactivation of lipoxygenase by heat treatments at various moisture contents was studied in wheat protein concentrate (WPC), a high-protein, high-fat flour obtained by remilling and sieving selected millfeeds. Small increases in moisture content markedly increased the sensitivity of the enzyme to heat. At 16% moisture, 99% of the lipoxygenase activity was destroyed in 30 min. at 65°C. At the same time, the flour blend, Blend A, made with the heat-treated WPC, performed well in baking tests.

Wheat protein concentrate (WPC), prepared by remilling selected by-product fractions (1), is blended with straight-grade flour to produce a high protein flour, Blend A, for use in the Food for Peace Program. The prolonged high-temperature storage to which Blend A may be subjected in overseas shipments, however, can lead to the development of off-flavors and a deterioration of baking quality (2).

Whole-wheat flour, millfeed fractions, and high-protein, high-fat flours derived from millfeeds contain high levels of lipoxygenase. One function of this enzyme (or isozymes) is the catalysis of the peroxidation by molecular oxygen of linoleic acid and its mono- and triglycerides (3,4). These peroxides can in turn give rise to other products, such as volatile carbonyls and alcohols, which in soybeans and unblanched peas, for example, cause off-flavors and odors (5,6). Reviews on lipoxygenase and reactions of hydroperoxides are available (7,8,9).

To determine if lipoxygenase is at least partially responsible for off-flavors in and poor baking quality of Blend A mentioned above, we have attempted to inactivate it by heat-moisture treatments of WPC without affecting the baking quality of Blend A derived from the treated WPC.

MATERIALS AND METHODS

Materials

A single lot of WPC obtained from one commercial source was used throughout this work. Linoleic acid was obtained from Applied Science Laboratories in sealed 1-g. vials.

WPC Moisture Content

The moisture content of WPC was raised by adding the calculated amount of powdered ice by grinding and mixing under liquid nitrogen (10). The WPC-ice mixture was stored at -18°C. for 48 hr. to allow the mixture to reach equilibrium by sublimation. (This treatment does not affect the lipoxygenase activity of WPC.) When low moistures were desired, the WPC was stored over sulfuric acid of the appropriate concentration. Moistures were determined by the AACC vacuum oven method (11).

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Heating WPC

After equilibration of moisture, the WPC samples for enzyme assay were packed into capillary tubes (1.2 mm. I.D. X 60 mm.) in a -18°C. cold room, sealed, and then heated in a constant-temperature bath for the appropriate time. The treated tubes of WPC were stored in dry ice until lipoxygenase activity was measured.

For baking, WPC was packed into 7 mm. I.D. X 60-cm. Pyrex tubes, which were heated in a hot-air oven for 30 min., and then cooled in ice water. The WPC removed from the tubes was air-equilibrated before blending with flour for baking tests.

Lipoxygenase Activity Measurements

Substrate preparation. Sodium linoleate substrate, pH 6.9, was prepared according to a modification of Surrey's method (12). Linoleic acid (1 g.) was added dropwise under a stream of nitrogen to a solution of 1.11 g. of Tween 20 and 20 ml. of 0.1M sodium phosphate buffer, pH 6.9. Then 4 ml. of N sodium hydroxide was added dropwise and the mixture brought to 200 ml. with the phosphate buffer. After 4 ml. of 10 mM. EDTA was added, the pH was adjusted to 6.9 with hydrochloric acid. Finally, the mixture was brought to 400 ml. with deionized water and transferred to 30-ml. screw-cap polyethylene bottles. After being flushed three times with nitrogen and capped tightly, the bottles were stored in a refrigerator. We found that this substrate would last several months before becoming oxidized sufficiently to interfere with a linear rate of reaction.

Assay procedure. A modification of a polarographic method (13) was used to measure the activity of lipoxygenase. Quadruplicate samples from a WPC treatment, the quantity governed by the activity (60 to 130 mg.), were weighed and each mixed for about 30 sec. with 1.0 ml. 0.1M sodium phosphate buffer, pH 6.9. One 0.20-ml. aliquot of the sample was then added to 3 ml. of air-equilibrated sodium linoleate substrate stirred at 25°C. The oxygen concentration in the reaction mixture was continuously measured with a Clark oxygen electrode in conjunction with an oxygen-monitoring system (Yellow Springs Instrument Co.). Under the conditions used, the rate of oxygen uptake was directly proportional to the enzyme concentration, and oxygen consumption could be calculated from the value for oxygen concentration (0.07 μmole O_2 per 3.2 ml. reaction mixture) determined for the air-equilibrated substrate (14).

All enzyme activities are expressed as a percent of the activity of an untreated sample of WPC at the same moisture as the heat-treated WPC. The absolute value for the untreated WPC, 12.6 to 12.8% moisture, stored at 4°C. was constant (17 units per gram WPC) throughout these experiments. One unit is defined as the uptake of 1 μmole of oxygen per min. in the peroxidation of linoleic acid.

Baking Tests

The Finney and Barmore formula (15) was followed (with omission of potassium bromate) to bake two pup loaves from a 200-g. batch of flour. Dough handling and baking were according to Finney and Barmore, except that the baking was for 25 min. at 425°F.

WPC was heated at adjusted moisture levels prior to blending with untreated white flour (30% WPC/70% flour) to make Blend A (not fortified with vitamin A or calcium carbonate for these tests). The protein contents were 11.9% for flour (N X 5.7) and 25% for WPC (N X 6.25). The Blend A thus contained 15.8% protein.

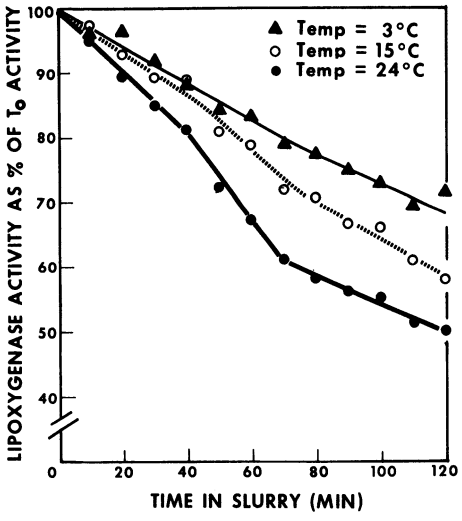
RESULTS AND DISCUSSION

Enzyme Stability in Slurries

Early in our work we found that after buffer was added to the WPC before assay, lipoxygenase activity would immediately begin to drop at rates dependent upon the temperature of the slurry (Fig. 1). Since a single assay required 3 to 8 min., subsequent assays on the same slurry were affected by decreasing lipoxygenase activity, resulting in poor replication. Any or all of the following may effect this lipoxygenase inactivation in slurries: 1) enzyme adsorption on glass surfaces (16); 2) destructive association with substrate under aerobic conditions (17); and 3) reaction with proteases present in the WPC. An aryl esterase in WPC, which we have monitored in similar treatments, does not show this rapid deactivation[2].

Enzyme Stability at Elevated Moisture Levels

We also found that WPC samples at moisture levels greater than 13.1% lost enzyme activity on standing, even in the refrigerator, at 3°C., although at lower moisture levels the activity was indefinitely stable, even at room temperature. This effect is seen in Fig. 2 for samples containing 14.3 and 16.2% moisture. The activity dropped slowly for the first few days in the refrigerator, then leveled off and thereafter remained constant. If the temperature, moisture, or both, was again raised, the activity dropped to a new plateau of stability. To eliminate any possibility that the loss in activity was caused by the treatment with liquid nitrogen, we moistened WPC to 14.1% in a humidity chamber at 30°C. and noted a similar loss of activity.



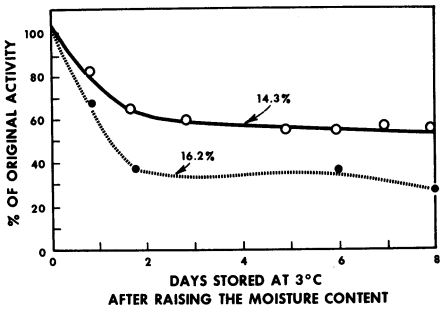


Fig. 1 (left). Loss of lipoxygenase activity in slurries of WPC buffered at pH 6.9 and held at three temperatures.

Fig. 2 (right). Instability of lipoxygenase in WPC stored at 3°C. with a 14.3 or 16.2% moisture content.

[2]Wallace, J. M. Unpublished results.

To avoid these activity changes, we handle the WPC at -18°C. or colder (where activity is stable at all moistures) except for the few minutes required to weigh samples and extract with buffer.

Heat Treatments

The same sort of activity plateau shown in Fig. 2 was found, but with a reduced time scale, when WPC was heated at a given temperature and moisture for different lengths of time (Fig. 3). After a given heating time, which varies with moisture and temperature, an activity plateau was reached (unless, of course, all activity was destroyed first) where longer heating times caused only very small activity changes. Figures 2 and 3 are similar if we consider Fig. 2 as showing the results of a 3°C. heat treatment over a period of several days. This sort of activity behavior as a function of temperature and moisture (actually water activity) is discussed in detail by Acker (18).

We tested many combinations of temperature, moisture, and heating times to find the combination that would most effectively inactivate lipoxygenase without altering baking quality. Figure 4 shows a plot of lipoxygenase activity vs. temperature for 30-min. heating periods at four moisture levels. Enzyme activity is given as the percent of activity remaining after heat treatment relative to the unheated control. We see that virtually all the activity was lost over a very narrow temperature range and was a function of moisture content (water activity).

The hump at 76°C. for the 12.8% moisture sample was reproducible, and may have been caused by the destruction at 75° to 76°C. of a heat-labile inhibitor or a protease at a faster rate than lipoxygenase.

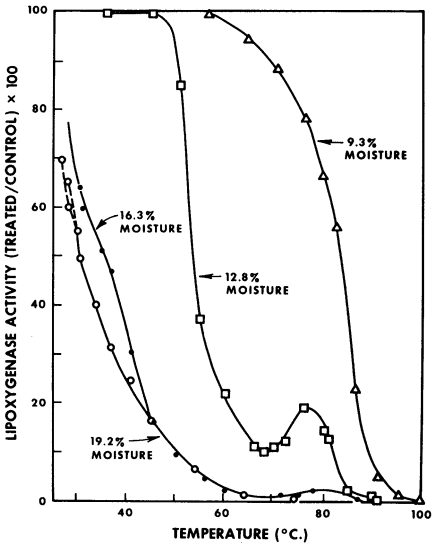


Fig. 3 (left). Loss of lipoxygenase activity in WPC while heated at 54° and 75°C. and a constant moisture content.

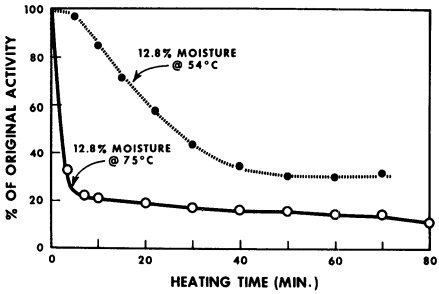


Fig. 4 (right). Effects of moisture and temperature on inactivation of lipoxygenase in WPC. All samples were heated for 30 min.

TABLE I. EFFECT OF HEAT TREATMENT OF WPC ON LIPOXYGENASE ACTIVITY AND FLOUR BLEND A LOAF VOLUME[a]

	Moisture Content %	Temperature °C.	Lipoxygenase Activity Remaining %	Loaf Volume cc.
	12.8	0	100	735[b]
Experiment	13.1	55	4.2	735
1	16.4	65	about 1.5	770
	12.8	0	100	680[b]
Experiment	13.9	55	4.1	700
2	16.7	65	<1	715

[a]WPC samples heated in Pyrex tubes for 30 min. at moisture content and temperature shown. WPC then air-equilibrated, during which time some of the residual activity was lost.

[b]Different batches of flour were used in experiments 1 and 2 for making Blend A.

The denaturation of gluten by heating at elevated moisture contents was empirically judged by whether the centrifuged residue from a slurry of WPC in water would form a dough ball. We found that in samples of less than 12% moisture, lipoxygenase activity was destroyed at those temperatures at which dough balls could no longer be made from WPC. At higher moisture levels lipoxygenase activity could be destroyed while still retaining the capacity for dough-ball formation. Experience with baked samples has indicated that the baking quality is definitely impaired when a dough ball cannot be formed from a sample of the WPC.

Two temperature-moisture treatments were selected and tested for residual lipoxygenase activity and baking quality (Table I). In 16 to 17% moisture samples heated at 65°C., 99% of the original lipoxygenase activity was eliminated, and baking tests indicated that the loaf-volume and dough-handling characteristics of the Blend A made with these samples were acceptable. The 65°C.-heated WPC in both experiments produced loaves averaging 35 cc. greater in volume than the controls. Although this apparent increase in loaf volume may not be significant, it is consistent with similar results of heating flour and whole wheat discussed by Kent-Jones and Amos (19). In subsequent storage studies of these samples, as yet unpublished, this apparent difference in volume between the 65°C.-heated WPC and the control has continued to manifest itself.

The lipoxygenase level in WPC is affected by moisture content and temperature, temperatures as low as 3°C. (Fig. 2) affecting the stability. This suggests that for valid varietal comparisons on enzyme activity (20,21,22) the wheats to be compared should be handled from harvest on through milling and storage under heat and moisture conditions as much alike as possible.

As reported above, we can state that lipoxygenase was almost completely inactivated in one lot of WPC by heat-moisture treatments that do not adversely affect the dough-handling characteristics and the baking quality of Blend A made from the treated WPC. To ascertain whether WPC from other commercial sources would respond to heat and moisture in the same way, we treated WPC from a

second source and duplicated the lipoxygenase-inactivation results, again without detriment to baking quality.

Other enzymes present in the WPC were undoubtedly inactivated to some degree by the heat-moisture treatments. Study is in progress on another enzyme involved with lipid components of wheat, a fatty acid monoesterase. The assignment of specific flour-baking and -storage responses to inactivation of a particular enzyme system is difficult. Correlation between the effects on these different enzymes and responses in the flours will be considered further in a study on storage of WPC and Blend A.

Acknowledgment

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