Spot test for lipoxygenase activity

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Summary A qualitative iodometric test for lipoxygenase activity is described. The sensitive method is useful for rapidly screening large numbers of samples, as from a chromatography column or after enzyme inactivation treatment in food processing.

Supplementary key words linoleic acid

A TEST FOR LIPOXYGENASE is described which is adopted from a procedure for the detection of lipoxygenase bands in polyacrylamide gels (1). The test is essentially an iodometric test for peroxides. It is a very simple, rapid method which has been used in locating lipoxygenase activity in the effluent of chromatographic columns (Fig. 1) and from a continuous preparative electrophoresis apparatus during the purification of lipoxygenase from milled wheat products.[1] It also has applicability in the food-processing industry for screening samples of foods after blanching for the purpose of destroying lipoxygenase activity, as in precooked dehydrated legumes (2).

Materials and methods

Materials. The substrate, sodium linoleate, is prepared as for that used in the spectrophotometric method (3), except that sodium phosphate buffer is used. 1 ml (1.11 g) of Tween 20 is dissolved in 20 ml of 0.046 M sodium phosphate buffer (pH 7.0, ionic strength 0.1). 1 g of linoleic acid is added dropwise with stirring, then 4 ml of N NaOH is added dropwise and mixed until a clear solution is obtained.

The solution is diluted to 200 ml with phosphate buffer, adjusted to pH 7.0 with concentrated HCl, then made to a final volume of 400 ml with deionized water. The substrate is purged three times with N_2 and stored under N_2

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.

[1] Wallace, J. M. Unpublished results.

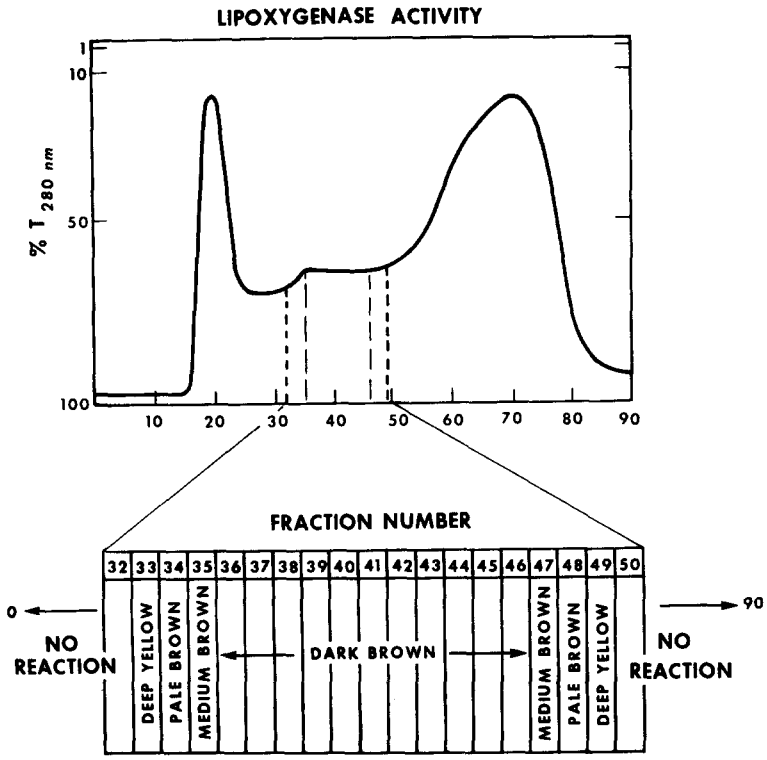

FIG. 1. Sephadex G-200 chromatography of crude wheat bran lipoxygenase. Elution was performed with 0.088 M sodium phosphate buffer, pH 7.0. Lipoxygenase activity is indicated by brown color in the spot test.

at 3°C in screw-capped bottles. The substrate in unopened bottles is stable for at least 3 months, although traces of hydroperoxides do form slowly to interfere with the color reaction as noted below.

Procedure. One drop of the sample to be tested is placed on a clean spot plate and one drop of substrate is added. After a suitable reaction period (usually 5 min), one drop of aqueous KI solution is added to stop the reaction. The KI solution may be prepared according to Guss, Richardson, and Stahmann (1) as acidic KI containing 5 ml of saturated aqueous KI per 100 ml of 15% acetic acid and kept under N_2. We prefer a saturated aqueous solution of KI, which results in a more intense color being formed due to the more concentrated (20 times) KI in the mixture. The color also develops more rapidly than when diluted acidified KI is used. If the sample has considerable activity, the formation of I_2 will be apparent by the appearance of a pale yellow color when the more concentrated nonacidified KI solution is used. The addition of one drop of 1% soluble starch solution (Soluble Starch Merck, Special for Diastatic Determination, prepared by heating to 100°C to solubilize the starch) will result in the development of a brown color with neutral KI or a blue-purple color in the acidified mixture. When using substrate which has been stored longer than a few days, control tests containing no active lipoxygenase must be made simultaneously with sample tests, because traces of peroxides from the stored substrate cause oxidation of iodide, which begins to be visible in the absence of enzyme about 10 min after the addition of aqueous KI to a reaction mixture. Therefore, color comparisons must be made between control tests and sample tests before the color caused by nonenzymatic oxidation develops. If a series of fractions, as from a chromatographic column, are to be tested, a simple procedure is to first spot the samples on the plate and then perform in the whole series simultaneously the digestion, and then the color development reactions. This avoids the complications of nonenzymatic development of color.

Results

A comparison for sensitivity has been made between the spot test and the polarographic procedure of Mitsuda et al. (4). The minimum detectable activity by both methods is the same and corresponds to the quantity of enzyme that will consume 0.006 μmole of oxygen/min/ml of reaction mixture measured by the polarographic procedure. The maximum rate of oxygen consumption measurable with our oxygen monitoring system (Yellow

Springs Instrument Company, Yellow Springs, Ohio) is in the order of 0.22 μmole/min/ml of reaction mixture. Relative activities of this level and less can be distinguished from each other in the spot test by the differences in intensity of color developed 5 min after the addition of KI to the reaction mixture.

Impurities, and enzymes such as catalase and peroxidase, present in wheat bran extract do not interfere with the test for lipoxygenase, nor do bovine liver catalase and horseradish peroxidase (Calbiochem) when added to the bran extract.

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