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TESTING FOR ENZYME ACTIVITY IN COMMERCIAL
FRUIT AND VEGETABLE PROCESSING

by
Earl Litwiller, Assistant Food Technologist
Food Industries Department

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Oregon Agricultural Experiment Station

Enzymes are complex chemical compounds which are present in all living plants and animals. Unless inactivated by heat or other means these substances are capable of starting or hastening changes which ultimately result in various degrees of alteration or decomposition of food products. While many such transformations are destructive others, such as occur in the manufacture of cheese, sauer kraut or wine, are regarded as highly desirable. There are many kinds of enzymes but all of them have one characteristic in common. They may be rather easily inactivated by means of heat. There are, however, some differences in this respect, with the enzyme peroxidase being less easily inactivated than most others. If the activity of this enzyme is destroyed, therefore, it is generally accepted that the inactivation treatment is sufficient to destroy the activity of all other enzymes.

As the best means for the inactivation of enzymes, which speed up unwanted chemical changes, blanching has been demonstrated as necessary in the successful preparation of foods for both freezing and dehydration. These changes become more apparent the longer the foods are in storage and are characterized by such undesirable features as loss of natural flavors, destructive chemical breakdown, which often results in the rancidification of fats that may be present, and a general deterioration of the food. To judge how to blanch sufficiently to protect the foods without overblanching them and causing physical injury frequently becomes a real problem. Fortunately there are simple chemical tests which aid the operator in determining when adequate blanching has been attained.

A well-known test, which anyone with little or no previous experience can apply and interpret, utilizes benzidine and hydrogen peroxide. This test indicates whether or not the enzyme peroxidase is present.

Benzidine Test:

To perform the benzidine test one should first select some of the raw food material to make certain that peroxidase is present and to be sure the reagents are active. Because stale chemicals may not give a positive test when they should it is important to make them up fresh at least once a week. If after breaking or

cutting a piece of the raw food (never touch the surface to be treated with the bare fingers), apply a drop or two of one per cent solution of benzidine hydrochloride in 95 per cent ethyl alcohol. If another enzyme named oxidase is present a blue color will develop in a few seconds. If it does, one need not test further for peroxidase is certain to be present. However, oxidase frequently is absent so that no blue coloration appears. In that case, in addition to the benzidine solution, apply a drop or two of three per cent hydrogen peroxide solution (prepared by mixing one part of 30 per cent hydrogen peroxide with nine parts of distilled water). If a high content of peroxidase is present the blue color will appear usually within two or three minutes. A negligible amount of enzyme activity is denoted if the color does not appear until after 10 to 15 minutes.

After blanching the fruit or vegetable according to standard procedures recommended for freezing or dehydration, select the largest piece of such food and break or cut it to expose a fresh surface. Be careful to test material that is taken from those spots or areas on the trays or blanching belt where there has been the least penetration of heat, such as the center of thick masses of pieces. If several tests on this type of material are negative one concludes the blanching has been adequate.

A one per cent alcoholic solution of guaiacol or a one per cent solution of gum guaiac in 95 per cent ethyl alcohol may be substituted for the benzidine solution. The guaiacol solution gives a rose red to red-brown color when a positive test is registered. On certain colored products, such as beets or carrots this color is not as easy to distinguish as is the blue produced by benzidine or gum guaiac.

Improved Benzidine Test:

In the case of some vegetables, beets for example, it is not always possible to apply the above simple test and be certain that all the peroxidase has been inactivated. Therefore, a more sensitive test may be used, such as the one recommended by the Western Regional Research Laboratory of the U. S. Department of Agriculture, Bureau of Agricultural and Industrial Chemistry.

For this test the reagent is prepared by mixing just before using, two drops of 30% hydrogen peroxide (acetanilid-free) with 10 ml. (1/3 fluid ounce) of a one per cent solution of benzidine hydrochloride in 95% ethyl alcohol.

To 20 grams (one ounce) of blanched material add 50 ml. (two fluid ounces) of distilled water and one ml. (20 drops) of the mixture of hydrogen peroxide and benzidine; upon stirring, allow the mixture to stand 30 minutes. If after that time no blue-black color appears on the solid pieces of material the test can be considered negative. It is also recommended that a duplicate sample of blanched material be boiled ten minutes, cooled and then tested in the same way as suggested above. If a blue color develops in this it will be due to other substances than peroxidase. Comparison can be made between the color of the boiled material and that of the unboiled material and any necessary correction can be made in the interpretation of the results.

If dehydrated materials are to be tested, use a five gram (one-fifth ounce) sample, add 15 to 20 ml. (one-half to two-thirds of a fluid ounce) of distilled water, allow soaking for 30 minutes and then apply the test as suggested above.

Source of Reagents:

Because ordinary hydrogen peroxide sold by druggists contains acetalid, a substance which interferes with the peroxidase tests, it is necessary to use chemically pure (C.P.) reagent. This and any of the other chemicals suggested may be obtained from Scientific Supplies Company, 122 Jackson Street, Seattle, Washington, or from any other dealer in equipment and chemicals used in research.

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