

OXYDASE IN WHEAT GRAINS.

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In many seeds the embryo is provided with a store of reserve food formed by the parent plant before the separation of the seed. In the dormant seeds the enzymes are usually in minute quantities as they are not needed, but when the food is needed during the germinative process, the enzymes are more strongly developed, the embryo developing itself, and also developing enzymes to provide food in suitable form.

A series of experiments were conducted to determine the enzymes present in dormant wheat seed, and its parts, and also in the germinated seed. The material for the experiments was obtained from a flour mill, and consisted of whole wheat flour, ordinary white flour, bran, shorts, and the unground grain.

Water extracts were made, 60 grams of flour being used with 100 cubic centimeters of distilled water, with the whole flour, and the white flour, 150 cubic centimeters of water with the shorts, and 240 cubic centimeters of water with the bran. The amount of water was varied in order to make them of as nearly as possible equal moisture, the shorts and the bran requiring more than the others. The mixtures were allowed to stand for 12 hours, and were then filtered. Powdered thymol was used to prevent decomposition. Glycerine extracts were also made, but were so much weaker in their action than the water extracts, that they were abandoned.

To determine the changes in enzymic action due to the germinative process, wheat was germinated for different periods, the grain being placed on moist paper under a bell jar, and kept at room temperature. At the end of the given period the grain was pounded in a mortar, then the enzymes were extracted for three days with water to which chloroform was added, after which the extracts were filtered. The periods of germination were three, five, six, and ten days respectively. Fifty grams of wheat grains were used in each case, and for the extraction 200 cubic centimeters of distilled water.

The extracts were slightly acid from the ungerminated grain, as shown by litmus, and more strongly acid from the germinated grains.

In obtaining the extracts from the various parts of the grain, the resulting liquids showed differences in color. The extract from the white flour was colorless, while that from the whole wheat was a straw color, that from the bran was slightly darker, and from the shorts darker still, so that one could recognize the extracts from their colors. The extracts from the germinated wheat grain were a pronounced brown color, the colors varying in depth with the time of germination up to six days, the six days being the darkest, beyond that no differences were appreciable. This was uniform in all the extracts made, so that like the extracts from the parts of the grain, the extracts from the germinated grains could be separated from one another by the degree of discoloration. Then again, sections of the wheat seeds, of the water lily petiole, and of the castor bean stem showed similar degrees of discoloration when placed in the solutions.

To test the oxidation, 5 cubic centimeters of each of the extracts from the different parts of the grain were taken and a few drops of guaiac tincture added, after which they were allowed to stand for some hours. In all there was a blue discoloration, but varying in degree. The white flour extracts showed a faint blue color, the whole wheat extract had a deeper tint of blue, while the bran and shorts extracts showed a decided blue color.

In testing the extracts from the germinated grain, 25 cubic centimeters of each were taken and precipitated with 85 cubic centimeters of 96 per cent. alcohol, then allowed to stand 36 hours, after which the precipitate was filtered off. The precipitate was dried on the filter at 35 degrees C., then redissolved in 25 cubic centimeters of distilled water. These solutions were then tested with the guaiac tincture, and all gave a decided blue color throughout the whole liquid.

The solutions were then tested with hydroquinone and pyrocatechin, polyphenols which are readily oxidized. At the same time for control purposes, equal quantities of the solutions but without the addition of the phenols, and also equal quantities of distilled water plus the phenols, respectively, were kept under similar conditions. The results are shown in the following table:

Extract.	Phenol.	Time.	Color.
Ungerminated.....	Hydroquinon	12 hours.....	Light reddish brown.
Germinated 3 days	Hydroquinon	12 hours.....	Reddish brown.
Germinated 6 days	Hydroquinon	12 hours.....	Deep reddish brown.
Ungerminated.....	Pyrocatechin	12 hours.....	Reddish brown.
Germinated 3 days	Pyrocatechin	12 hours.....	Deep reddish brown.
Germinated 6 days	Pyrocatechin	12 hours.....	Deeper reddish brown.
Ungerminated.....	12 hours.....	Pale straw.
Germinated 3 days	12 hours.....	Deeper shade.
Germinated 6 days	12 hours.....	Still deeper shade.
Dissolved in water	Hydroquinon	12 hours.....	Colorless.
Dissolved in water	Pyrocatechin	12 hours.....	Colorless.

The discoloration became apparent in the solutions with pyrocatechin in about 15 minutes, while the same extent of discoloration was not apparent in the solutions with hydroquinon for an hour. The table gives the results at the end of twelve hours, but the solutions were kept for a week. The longer they stood, the darker they became, the hydroquinon being of a wine red color, while the pyrocatechin solutions were of a brown color. At the end of four days, the six days extract with pyrocatechin was almost black. The experiments were carried on at room temperature. The solutions remained clear, no precipitates forming.

TEMPERATURE TESTS.

To determine the temperature at which the enzyme was destroyed, the three extracts were heated to 60 degrees C. The tubes containing the extracts were placed in the steam sterilizer, a corresponding amount of water being placed in another tube in which was placed a thermometer. The extracts were kept in the sterilizer for one minute after the thermometer registered 60 degrees C.

Another set was tested but the temperature raised to 100 degrees C. Pyrocatechin was used with the enzyme, as, in the other tests, it gave a more rapid response than the hydroquinon.

60 Degrees C.—A slight darkening was apparent in twenty minutes. The longer they were kept, the darker they became, until at the end of four days they had a rich, deep brown color. They also showed the varia-

tions as to the ungerminated extracts being the lightest, the six days germination extract being the darkest, the three days germination extract being a shade between the two.

100 Degrees C.—These solutions were a little slower in responding, the discoloration not being apparent for 30 minutes, but showed similar discolorations to those extracts raised to only 60 degrees C. At the end of four days they were slightly lighter in shade than the corresponding extracts at 60 degrees C.

100 Degrees C.—To avoid any chance of error, the same quantities of the extracts were again taken, but heated over the direct flame of a Bunsen burner until the solutions boiled vigorously. They were then cooled rapidly in the snow, after which the pyrocatechin was added. At the same time two tubes of distilled water were boiled over the flame, and also had the pyrocatechin added, one being cooled before the addition, the other being quite warm.

The extracts plus the pyrocatechin behaved exactly the same as the extracts heated in the steam sterilizer. The solutions of distilled water plus the pyrocatechin remained clear and colorless.

100 Degrees C.—In the first set of experiments with the extracts from the various parts of the flour, the white flour extract gave the weakest color reaction, seeming to indicate either weakest or smallest quantity of enzyme. An equal quantity to that used in the other experiments was boiled over the direct flame for two minutes. Another quantity was taken but not boiled, both had pyrocatechin added to them. The boiling caused a white precipitate to form.

The discoloration was slow in appearing, it being fully three hours before there was a certainty in regard to it. Then it had a reddish appearance, like apple must when exposed to the air. In twenty-four hours there was a decided red color, but the boiled solution was slightly darker than the unboiled. The unboiled solution also formed a precipitate, both precipitates showing the coloration of the liquids. At the end of three days the color remained the peculiar red, but darker, the boiled one being considerably darker.

There was next tried some white flour extract and some six days germination extract, these two extracts being at the extremes of the discolorations, the former showing the lightest, the latter the darkest in the extracts, and in their action on the phenols. The extracts were placed in the autoclav, and kept until the indicator registered a pressure of ten

pounds, the temperature being 112 degrees C. It required thirty minutes to reach this pressure. The solutions were taken out as quickly as possible and cooled in the snow at once, after which pyrocatechin was added to each. The white flour extract had a white precipitate formed by the heat action, but this was an unpurified extract. The six days germination extract remained clear. A slight reddening appeared in the white flour, and a slight darkening in the other. (They were compared with extracts without the pyrocatechin.) This became more marked, the longer the extract stood. At the end of seven days the discoloration was quite marked.

The action of the oxydases is a very interesting and practical subject, as their action explains many puzzling phenomena, which were formerly classed as oxidations, but the cause and conditions of which were unknown. The composition of the oxydases is unknown, and consequently it is impossible to determine the number of oxydases—if there be numbers of them—except by the differences in reactions and conditions. From considering oxidation as a purely physiological process, as exemplified by respiration, and which only took place through vital processes, one has to consider oxidation from the opposite extreme.*

The most common manifestations of the action of oxydases are the discolorations of beets, carrots, apples, and many plant tissues and juices, besides the browning of wines and other liquids. The juice of the plant *Rhus vernicifera* from which lac varnish is made contains an oxydase which is, perhaps, the most widely known. Many of these have been investigated, and have been found to have certain points in common, though differing in others. They are all susceptible to the reaction of the medium, and also the temperatures at which they are rendered inactive vary within certain limits. The browning of wines is prevented by a temperature between 70 and 80 degrees C. or by Pasteurization at 60 to 62 degrees.† A large number of oxydizing enzymes which are found in different plants and animals are mentioned by Oppenheimer‡, the most resistive of which succumb to boiling temperature.

The enzyme which exists in the wheat grain, both in the quiescent and germinated grains, from the differences in degree of discoloration of extracts, and also action on phenols, exists in least amount in the white

*Pozzi-Escot, M. E. *Les Diastases at Leures Applications*, 1900.

†Lafar, F. *Technical Mycology*, p. 401, 1898.

‡Oppenheimer, C. *Ferments and their Actions*, 1901.

flour, and greatest amount in the shorts, that is, the endosperm has least, and the embryo most. This accounts for the discoloration of flour containing the embryo. The enzyme must be increased in amount or intensity during the process of germination, and up to six days germination, the extracts become darker, and the action on phenols also gives the same degrees of difference. The enzyme is more resistive to heat than those already noted. Bertrand and Bourquelot* say the oxydase extracted from *Russula foetens* Pers. is so resistive to heat that it has to be boiled some time before being destroyed.

Boutroux† has separated an oxydase from dough by soaking dough with twice its weight of water for half an hour, extracting by means of a press, then clearing by filtering through a Chamberlain filter. The extract was at first clear, then a precipitate formed, after which it turned brown, becoming black in the course of some weeks. His oxydase, however, lost its activity at 100 degrees C.

That the oxydase extracted by Boutroux is identical with the present one extracted from the various flours and grain is very probable. The difference in its resistance to heat may be due to a different kind of wheat, or to influences of environment.

Whether it be necessary to have a diastase present, as is claimed by Raciborski‡, it is impossible to determine, for the methods of separating the oxydases will also cause the separation of diastase and other ferments, and there is no known method of separating the majority of enzymes from one another.

*Green, J. H. *The Soluble Ferments and Fermentation*, 1901.

†Boutroux, L. *LePain et la Panification*, 1897.

‡Raciborski. *Ber. der deut. bot. Ges.* XVI. 119, 1898.