

XIII. OXIDISING ENZYMES. V. FURTHER OBSERVATIONS ON THE OXIDISING ENZYMES OF FRUITS.

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THE following is an account of the examination for oxidising enzymes of a further selection of fruits.

The results are in general accordance with those already obtained [Onslow, 1920]. Of the fruits examined, the following were found to contain the components of an oxidase: cherry, peach, apricot, medlar, strawberry, grape, fig and mulberry. In others, the blackberry, red currant, black currant, gooseberry, grape fruit, pomegranate, pineapple, melon and tomato, only a peroxidase could be detected. These results are not contrary to what may be expected on the basis of natural classification; they also exemplify a point mentioned in the former paper, that the enzyme content of the fruit is not always the same as that of the plant itself. Blackberry and tomato leaves, for instance, contain an oxidase, whereas the ripe fruit does not.

The experimental methods employed were those described in the author's previous paper [Onslow, 1920]. Pieces of fresh tissue of the fruits were placed in guaiacum tincture, both with and without hydrogen peroxide; also in solutions of benzidine and of α -naphthol, both with hydrogen peroxide. Water extracts of the tissues (after neutralisation, if acid) were also tested with the same reagents. To remove inhibitors, fresh tissue was pounded thoroughly with 96-98 % alcohol, sucked dry on a filter funnel, and the process repeated. The residue ("tissue residue") was extracted with water; the filtrate ("enzyme extract") was tested for peroxidase with guaiacum and hydrogen peroxide (and if this test were negative, with benzidine and hydrogen peroxide), and for oxygenase (peroxidase being present) with catechol solution and guaiacum. If the extract failed to give any reaction, the tissue residue itself was suspended in the reagents. Finally, a hot alcoholic extract of the fruit was made, filtered, and the alcohol distilled off *in vacuo*. The residue was precipitated with solid lead acetate, and the precipitate formed filtered off. The latter was decomposed with the minimum amount of dilute sulphuric acid, filtered from lead sulphate and neutralised. This extract ("aromatic extract") was tested for the catechol reaction, and, if positive, was treated with enzyme extract (or tissue residue if the extract gave no result) and guaiacum. If organic acids and tannins were present, the former were precipitated with calcium chloride and alcohol, the latter with gelatin.

Some points which have already been mentioned in previous papers may be again emphasised. First, discoloration of tissues cannot be relied upon in every case for an indication of the presence of the system. The simultaneous presence of a certain amount of various substances, such as tannins, organic acids, sugars, etc., may inhibit the discoloration, though the components for its production are present. This is true, for instance, of the apricot, of which the flesh does not discolour on injury, though the enzyme system present is similar to that in the peach, plum, cherry and many others, all of which do discolour on injury.

A second point to be emphasised is that inhibitors may, in the same way, render water extracts of tissues, or even the tissue itself, quite unreliable material with which to test for enzymes. The tissues should be extracted with alcohol which removes the inhibiting substances and precipitates the enzymes in the tissue residue.

A third point is that in some fruits it is difficult or impossible to extract the enzymes from the tissue residue; hence the action of the peroxidase on guaiacum, and of the oxygenase on catechol or the plant aromatic substance, can only be demonstrated by placing the tissue residue itself in the solution. Fruits which show this phenomenon contain tannin, and it is suggested that the tannin may cause precipitation and adsorption of the enzymes, thereby rendering them less soluble. This phenomenon is shown in various degrees by the medlar, quince, cherry and apple.

A similar phenomenon was also observed in another type of fruit, namely that in which the tissues are much disintegrated on ripening, and in which there is frequently much pectin substance. Such are the strawberry, gooseberry, red and black currants and, to some extent, the grape. From the tissue residue consisting chiefly of pectinogen, either no or very little extraction of enzymes could be obtained with water in the case of the gooseberry and currant; neither peroxidase nor oxygenase could be extracted from the strawberry and only peroxidase from the grape.

Employing the above methods, the following results have been obtained:

X. The cherry, *Prunus Cerasus* (Rosaceae), contains an oxidase (oxygenase, catechol substance and peroxidase).

A slightly pigmented variety was used. Discolours on injury. *Tissues and their water extract*: guaiacum alone and benzidine (H_2O_2), positive; α -naphthol (H_2O_2), negative. *Water extract of tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, positive; added to enzyme extract (or tissue residue) and guaiacum, negative. After precipitation with calcium chloride and alcohol, again added to tissue residue and guaiacum, positive. Tannin present.

XI. The peach, *Prunus Persica* (Rosaceae), contains an oxidase.

Discolours on injury. *Tissues and their water extract*: guaiacum alone and other two tests, positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, positive; added to enzyme extract (or tissue residue) and guaiacum, negative. After precipitation with calcium chloride and alcohol, again added to enzyme extract and guaiacum, positive.

XII. The apricot, *Prunus Armeniaca* (Rosaceae), contains an oxidase.

Does not discolour on injury. *Tissues and their water extract*: guaiacum alone and other two tests, positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, positive; added to enzyme extract and guaiacum, positive.

XIII. The medlar, *Mespilus germanica* (Rosaceae), contains an oxidase. The oxygenase cannot readily be extracted by water from the tissue residue, but reacts as if adsorbed to this residue. Sufficient peroxide is formed in a water extract of the crushed tissues to give a reaction with benzidine alone¹.

Fruit examined in October before it becomes soft. Discolours rapidly on injury. *Tissues and their water extract*: guaiacum alone and other two tests, positive. *Water extract*, positive reaction with benzidine alone. *Water extract of tissue residue*: peroxidase reaction, positive; oxygenase reaction, negative. *Tissue residue*: oxygenase reaction, positive. *Aromatic extract*: catechol reaction, positive; added to tissue residue and guaiacum, negative. Tannin present. Precipitation with calcium chloride and alcohol not tried.

XIV. The strawberry, *Fragaria elatior* (Rosaceae), contains an oxidase, though the reactions are not very readily obtained. Neither the oxygenase nor the peroxidase can be extracted by water from the tissue residue, but both act as if adsorbed to the residue.

Does not discolour on injury. *Tissues*: all three tests negative. *Water extract* (after neutralisation): all three tests negative. *Water extract of tissue residue*. If fruits are smashed and squeezed through muslin to remove seeds, and juice and pulp stirred up with excess of alcohol, a bulky gelatinous precipitate of pectinogen mixed with tissue residue is obtained. Treated again with alcohol. Residue is largely pectinogen and swells up in water, but water extract is negative for peroxidase and oxygenase reactions. *Tissue residue*: peroxidase and oxygenase reactions, positive though not given very readily. *Aromatic extract*: catechol reaction, positive though abnormal; added to tissue residue and guaiacum, negative. After treatment with calcium chloride and alcohol, still negative.

XV. The blackberry, *Rubus fruticosus* (Rosaceae), contains a peroxidase.

All colour reactions difficult on account of anthocyan pigment. *Water extract of tissue residue*: all three tests negative. *Tissue residue*: only benzidine (H_2O_2) positive. *Aromatic extract*: catechol reaction, doubtful owing to the presence of an iron-blueing substance, probably a tannin.

XVI. The red currant, *Ribes rubrum* (Saxifragaceae), contains a peroxidase. This cannot be extracted by water from the tissue residue but behaves as if adsorbed to the residue.

Does not discolour on injury. *Tissues*: guaiacum (H_2O_2), negative; other two tests positive. *Water extract* (after neutralisation): only benzidine (H_2O_2) positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, negative. *Tissue residue*: benzidine (H_2O_2) and α -naphthol (H_2O_2), positive. *Aromatic extract*: catechol reaction, negative.

XVII. The black currant, *Ribes nigrum* (Saxifragaceae), contains a peroxidase. This cannot be extracted by water from the tissue residue but behaves as if adsorbed to the residue.

Colour reactions difficult on account of anthocyan pigment. *Water extract of tissue residue*: peroxidase and oxygenase reactions, negative. *Tissue residue*: only benzidine (H_2O_2) positive.

¹ The experiment of adding a solution of catechol oxidised by an oxygenase, together with a peroxidase, to benzidine has also been tried with a positive result.

XVIII. The gooseberry, *Ribes Grossularia* (Saxifragaceae), contains a peroxidase. As in the case of the currant, it cannot be extracted from the tissue residue.

Both red and green varieties used, and reactions may vary with the variety employed. Does not discolour on injury. *Tissues*: guaiacum (H_2O_2), negative; other two tests, positive. *Water extract of tissues* (from green variety after neutralisation): two latter tests positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, negative. *Tissue residue* (tested only in red variety): all peroxidase tests positive. *Aromatic extract*: catechol reaction, negative.

XIX. The grape fruit, *Citrus decumana* (Rutaceae), contains a peroxidase.

Tissues: (rind), all peroxidase tests positive; (flesh), two latter only positive. *Water extract of tissues* (after neutralisation): almost completely negative results. *Water extract of tissue residue*: only benzidine (H_2O_2) positive. *Tissue residue*: all peroxidase tests positive. *Aromatic extract*: catechol reaction, negative.

XX. The pomegranate, *Punica Granatum* (Punicaceae). The inner tissues contain a peroxidase. A substance giving the catechol reaction is present and is probably a tannin.

Tissues (fleshy part of seed): guaiacum (H_2O_2), negative; other two tests positive. *Water extract* (after neutralisation): only benzidine (H_2O_2) positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, negative. *Aromatic extract*: catechol reaction, positive though abnormal: tannin present.

XXI. The grape, *Vitis vinifera* (Vitaceae), contains an oxidase. Neither the oxygenase nor peroxidase is readily extracted by water from the tissue residue but both act as if adsorbed to this residue.

Both purple and white varieties were used. Results seem to vary in detail with the variety used. Discolour on injury. *Tissues*: guaiacum alone and other two tests, positive. *Water extract* (after neutralisation): only benzidine (H_2O_2) positive (white variety). *Water extract of tissue residue*: only benzidine (H_2O_2) positive; oxygenase reaction, positive in white variety only. *Tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, results on the whole positive, especially in the white variety: added to enzyme extract (or tissue residue) and guaiacum, negative; also after precipitation with calcium chloride and alcohol.

XXII. The pineapple, *Ananas sativus* (Bromeliaceae), contains a peroxidase.

Does not discolour on injury. *Tissues and their water extract*: positive with all three peroxidase tests. *Water extract of tissue residue*: peroxidase reaction, positive; oxygenase reaction, negative. *Aromatic extract*: catechol reaction, negative.

XXIII. The melon, *Cucumis Melo* (Cucurbitaceae), contains a peroxidase.

Does not discolour on injury. *Tissues and water extract*: positive with all three peroxidase tests. *Water extract of tissue residue*: peroxidase reaction, positive; oxygenase reaction, negative. *Aromatic extract*: catechol reaction, negative.

XXIV. The tomato, *Solanum Lycopersicum* (Solanaceae), contains a peroxidase.

Does not discolour on injury. *Tissues and water extract*: positive with all three peroxidase tests. *Water extract of tissues*: peroxidase reaction, positive; oxygenase reaction, negative. *Aromatic extract*: catechol reaction, negative.

XXV. The fig, *Ficus Carica* (Moraceae), contains an oxidase.

Discolours on injury. *Tissues and water extract*: guaiacum alone and other two tests, positive. *Water extract of tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, positive.

XXVI. The mulberry, *Morus nigra* (Moraceae), contains an oxidase.

Discolours somewhat on injury. *Tissues*: guaiacum alone and other two tests, positive. *Water extract* (after neutralisation): no satisfactory reaction, and observation difficult because of anthocyan pigment. *Tissue residue*: peroxidase and oxygenase reactions, positive. *Aromatic extract*: catechol reaction, difficult to detect on account of anthocyan pigment; added to enzyme extract (or tissue residue) and guaiacum, negative. After precipitation with calcium chloride and alcohol, again added to tissue residue and guaiacum, positive.

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REFERENCE.

Onslow (1920). *Biochem. J.* 14, 541.