CXXIV. OXIDISING ENZYMES IN THE PEEL OF *CITRUS* FRUITS.

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IN previous papers [Willimott and Wokes, 1925, 1, 2], the view was advanced that an important factor in the production of vitamins in *Citrus* fruits is photosynthetic action in the outer pigmented portion of the peel. Following up this idea, it was thought worth while to investigate the occurrence of oxidising enzymes in the outer pigmented portion, or flavedo, as compared with that of the inner colourless portion, or albedo, of the peels of different species of *Citrus* fruits. By so doing it was possible to ascertain whether these enzymes are located principally in the flavedo, or whether they are distributed evenly throughout the peel. It was also thought useful in the case of the lemon to examine the peel of fruit at different stages of growth, and to correlate the findings with the p_H of the tissues, with the object of obtaining data which might assist further investigations on the production of vitamins in the peel during the growth of the fruit.

EXPERIMENTAL METHODS.

The fruits studied were the orange (bitter and sweet), the lemon and the grape fruit. The fruit was first well washed with cold water, then dried and the flavedo removed with a sharp knife, a small portion of the albedo being taken with it in order to rupture the least possible number of oil glands, thus avoiding as far as possible contamination of the albedo with products of the flavedo. The flavedo was freed from adherent albedo, and cut up into small pieces. The albedo left on the fruit was then removed, care being taken not to break through the peel with liberation of the pulp, and this was also cut up into pieces for experiment. In the case of the water and alcohol extracts, the minced tissue was thoroughly triturated with clean sand to ensure liberation of the juices, and then filtered, the filtrate being examined for oxygenase and for peroxidase and for aromatic catechol compounds by Onslow's method [1920, 1]. In testing for oxidising enzymes divergent results may be obtained according as tests are made on the fresh tissue or upon extracts of the tissue. The material was therefore tested both ways as recommended by Onslow [1920, 2]. The 10 % guaiacum tincture had previously been boiled with Merck's charcoal to remove peroxides. The catechol solution

had been tested with starch, iodide, and acetic acid and found free from peroxides. Whenever positive results were obtained with the extract of enzymes, a control experiment was done with a boiled extract which always gave a negative result.

In estimating the p_H of the lemon tissues, grinding with sand was found to produce an extract in which it was difficult to reach a sharp null point electrometrically; the tissue was therefore pounded with about an equal quantity of distilled water, and the resulting extract strained through washed cotton wool. The p_H of this strained liquid was estimated colorimetrically and also electrometrically, using a quinhydrone electrode.

The immature lemons used in this investigation had been specially picked for us in the San Dimas orchards by Mr C. P. Wilson, Chief Chemist of the Research Department of the California Fruit Growers Exchange. They were put immediately into cold storage, in which they remained until their arrival in England, where they were at once examined. It was observed that a number of these immature lemons, which after arrival in England had been put into a warm room while being examined botanically, began to develop a yellow colour within a week or so, whereas others kept in the ice chest retained their original green colour. This would seem to indicate that the precautions observed in storage conditions during transit had prevented any serious change in the fruit from the time it was picked off the tree in California to the time it was examined in England.

In recording the results on the immature lemons, as the exact age of each lemon was not known, they were arranged in order of weight. When these results were being plotted, a correction for the irregular growth of the fruit was required. By courtesy of Dr E. T. Bartholomew, of the University of California Graduate School of Tropical Agriculture, who has recently undertaken a comprehensive study of the growth rate of Californian lemons [1923, 1926], we were able to obtain data forming a suitable basis for this correction, which was applied in plotting the curves of p_H values at different ages.

All the other fruit used was fully grown, in ripe and perfect condition, and was purchased in the open market.

RESULTS.

As noted by Onslow [1920, 2], the results of tests for oxidising enzymes may vary according to the ripeness of the fruit used. Thus, one sample of a given fruit may give a strong positive reaction with a certain reagent, while another sample of the same fruit tested similarly gives much weaker results. By careful selection of the fruit we were able independently to obtain results in agreement with each other in a large number of observations, which are summarised in Table I. It will be seen that we found a peroxidase in both flavedo and albedo of the four kinds of fruit tested, but no reaction could be obtained for oxygenase or for catechol aromatic compounds. This agrees with

Table I. *Reactions for oxidising enzymes given by peels of ripe Citrus fruits.*

	Bitter orange (<i>Citrus vulgaris</i>)		Sweet orange (<i>Citrus aurantium</i>)		Lemon (<i>Citrus medica</i> var. <i>Limonum</i>)		Grape fruit (<i>Citrus decumana</i>)	
Flavedo (mm.)	2		1		1		2	
Albedo (mm.)	5		2		2		6	
Browns on injury	—		—		—		—	
	Flavedo	Albedo	Flavedo	Albedo	Flavedo	Albedo	Flavedo	Albedo
A. Reactions of tissues								
Guaiacum	—	—	—	—	—	—	—	—
Guaiacum + H ₂ O ₂	+	+	+	+	+	+	++	±
Benidine + H ₂ O ₂	+	+	+	+	+	+	++	+
α-Naphthol + H ₂ O ₂	+	±	+	+	+	+	++	+
B. Reactions of water extract								
Guaiacum	—	—	—	—	—	—	—	—
Guaiacum + H ₂ O ₂	—	—	±	—	—	—	+	—
Benidine + H ₂ O ₂	+	—	+	±	—	—	+	±
α-Naphthol + H ₂ O ₂	+	—	—	—	+	±	++	+
C. Reactions of extract of enzymes								
Guaiacum	—	—	—	—	—	—	—	—
Guaiacum + H ₂ O ₂	—	—	—	—	+	±	+	±
Benidine + H ₂ O ₂	—	—	+	+	+	—	++	—
α-Naphthol + H ₂ O ₂	—	—	±	—	±	—	+	+
Guaiacum + catechol	—	—	—	—	—	—	—	—
D. Reactions of aromatic extract								
FeCl ₃ + Na ₂ CO ₃	—	—	—	—	—	—	—	—

the results obtained by Onslow on the whole peel of the orange and lemon. Tests applied direct to the tissues showed practically no difference in peroxidase content of flavedo as compared with albedo in the case of the orange and the lemon, but in the grape fruit there is a markedly higher concentration in the flavedo. Tests applied to the extracts showed that the enzyme can be extracted from the flavedo much more readily than from the albedo. To avoid the inhibitory effect of tannins on oxidising enzymes, the extracts were treated with strong solution (about 25 %) of gelatin, filtered and tests applied to the filtrate. To ensure that the negative results obtained with extracts of the albedo were not due to adsorption of the enzyme by the spongy tissue, fragments of the alcohol-extracted tissue were added to the test-tubes containing negative tests, which in the case of the grape fruit produced a positive result.

A series of immature lemons of weights ranging from 1 g. to nearly full-grown was also examined, and the same result obtained, *i.e.* strong positive reactions for peroxidase in both flavedo and albedo, and negative reactions for oxygenase and aromatic catechol compound. In the smallest lemons (less than 5 g.) the freshly cut tissue gradually discoloured, but did not give the typical brown.

The p_H of the tissues in lemons of different stages of growth has been plotted in Fig. 1. The continuous line represents the average p_H of the peel at different ages, and the broken line the p_H of the juice. Little difference could be detected in p_H between flavedo and albedo at the same age. It will

be seen that both the peel and the juice gradually become more acid as the fruit matures until about four months old, when each reaches a fairly constant level. In the smallest lemons (less than 1 g.) the p_H of all the tissues would appear to be the same (about 5.8), but as there was insufficient material for electrometric determinations, and only colorimetric estimations could be made, these sections of the curves have not been completed. It is interesting to notice that the rapid increase of acidity which takes place between the ages of one and three months corresponds closely with the commencement of growth. Up to the age of one month the lemon increases very little in size. It then increases almost in geometric ratio up to about two months old, when it reaches a rate of growth which remains constant until the age of eight or nine months [Bartholomew, 1926].

Fig. 1. Reaction of peel and of juice of Californian lemons at different ages.

SUMMARY.

The localisation of oxidising enzymes in the flavedo as compared with the albedo has been studied in the orange (bitter and sweet), the lemon and the grape fruit. Peroxidase only could be found, and appeared to be fairly uniformly distributed throughout the peel. The concentration in the flavedo was a little higher than in the albedo, but quite insufficient to explain the preponderance of vitamin in the flavedo. It must be borne in mind, however, that the whole of the peel is traversed by an elaborate conducting system, which we have found by microscopic examination to be well marked at all stages of growth.

The reaction of both peel and juice is acid at all stages of growth. In mature lemons we have previously found the peel to contain considerable amounts of vitamin B but very little C, while it is known that the juice contains much C but little B. The data now given on the increase of acidity with growth of the fruit should make it possible to plan further experiments

on the vitamin content of the peel and juice of immature fruit, in order to trace possible relationships between the p_H of plant tissues and the nature of the vitamins found therein.

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