

**Summary.**—A study has been made of the sodium borohydride-hydrochloric acid reaction of flavanones. This reaction is given by chalcones and aurones also. These results provide support for the explanation offered by Geissman and Clinton for the colour reactions of flavanones with magnesium-hydrochloric acid and sodium amalgam-hydrochloric acid. In all these cases the same type of chromophore  $\text{Ar}-\overset{+}{\text{C}}\text{H}-\text{CH}=\text{CH}-$  is formed. Borohydride reduction products of flavanones and 2'-hydroxy chalcones when heated in alcoholic hydrochloric acid produce flavylum salts. A study of the acetic anhydride sulphuric acid test for chalcones, flavanones and aurones shows that the chromogen involved is  $\text{Ar}-\text{CO}-\text{CH}=\text{CH}-\text{Ar}$  and that the flavanone ring should open in order to develop colour. The reaction involves the attack by acetylium ions at the carbonyl of the above chromogen giving  $-\overset{+}{\text{C}}(\text{OAc})-\text{CH}=\text{CH}-$

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## THE INFLUENCE OF SOME AGRICULTURAL PRACTICES ON THE ENZYME ACTIVITY OF COCONUT WATER

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**I**T is now well established that a particular plant itself is the best indicator of the nature and quantity of nutrients it can absorb and utilise from the soil. Thus, the use of the plant tissue analysis, particularly foliar composition studies for diagnosing mineral deficiencies of crop plants such as rubber, oil palms, groundnuts, tea, cocoa, etc., has now become very popular. Suitable methods have been standardised to overcome the usual difficulties experienced in the case of perennial plants in the matter of leaf sampling from inaccessible heights from the ground level, availability of samples of comparable physiological age, possible nutrient level fluctuations on account of climatic changes and so on. In his studies on the potash content of nut water as a guide to the manuring of coconut palm, Salgado<sup>1</sup> found that potash content of nut water affords a relatively simple method of assessing potash availability in the soil in relation to the needs of the palm. He claimed superiority for this method over the conventional methods of soil

analysis assuming coconut water to be analogous to plant sap in indicating the physiological status of the palm and of the soil conditions on which it grows. We examined the pattern of enzyme activity in the nut water in relation to cultural and manurial practices received by the palm to see whether this could be more useful and reliable for diagnostic purposes. Indeed the determination of enzyme activity in plants has been shown to provide information about the effect fertilizers have on plant by Hofmann<sup>2</sup> who concluded that the amount and the activity of enzyme depends on the nature of the soil, on the fertilizers used and the method of cultivation during the vegetative period. He also stressed that enzyme activity orientates crop quality, stability on storage, germinating power and certain other properties and that the determination of enzyme activity of plants is also simple to carry out and exact and comparable values are usually obtained. The activity of the enzymes catalase and saccharase in estimating the biological activity

and fertility of soils has been stressed by Kozlov.<sup>3</sup>

To obtain representative average samples nut water of mature nuts from palms of comparable age were collected in dry weather in the early morning when all the assimilation products of previous day have been transported from the leaves and before renewed assimilation commenced. The analysis was carried out immediately and directly as the samples needed no preparation or pre-treatment. Selected trees belonged to medium yield groups, standing in the permanent observation plots of this Research Station, where three sister plots are maintained, the first receiving absolutely no cultivation or manuring, the second receiving cultivation operations only while the third receives both cultivation and manurial treatments. These three plots under three entirely different experimental conditions afforded the ideal sample for this study. The activity of the three oxidising enzymes catalase, peroxidase and polyphenolase usually found active in all growing plants, were determined by the methods described by Sumner and Somers<sup>4</sup> and Hawk *et al.*<sup>5</sup> Suitable modifications wherever found necessary were standardised and adopted. For catalase assay, the reaction mixture consisted of 5.0 ml. of phosphate buffer (pH 6.8), 1.0 ml. of 0.2 N.  $H_2O_2$ , 2.0 ml. of fresh coconut water (treated with  $CaCO_3$  and filtered through glass wool) and incubated for exactly 30 minutes in the ice-bath. The reaction is stopped by the addition of 5% sulphuric acid and the remaining  $H_2O_2$  is determined using 0.01 N.  $KMnO_4$ . For peroxidase assay the reaction mixture consisted of 10.0 ml. of 0.25% pyrogallol, 7.0 ml. of phosphate buffer (pH 5.3), 1.0 ml. 0.025%  $H_2O_2$  and 1.0 ml. of fresh centrifuged nut water. 1.0 ml. of 5%  $H_2SO_4$  was added after 5 minutes to arrest the reaction and the optical density was directly measured in the photovolt colorimeter using 420 filter. A reaction mixture consisting of 10.0 ml. of 0.25% pyrogallol, 8.0 ml. of phosphate buffer (pH 6.5), 1.0 ml. of centrifuged nut water served for the assay of polyphenolase activity. After allowing the reaction for exactly 10 minutes, 1.0 ml. of 5%  $H_2SO_4$  was added and the optical density was directly measured as above. Suitable controls were run with distilled water in place of the nut water as well as by initial inhibition of the enzyme by addition of sulphuric acid. Typical

results showing the pattern of enzyme activity in the nut water samples are given in Table I

TABLE I

Enzyme activity	Not cultivated or manured	Cultivated only	Cultivated and manured
<b>Catalase</b> (Micromoles of $H_2O_2$ decomposed per milligram of nitrogen in 30 minutes)	130.50	98.74	62.89
<b>Peroxidase</b> (Optical density of purpurogallin formed per milligram of nitrogen in 5 minutes in 20 ml. reaction mixture)	1.6070	0.8971	0.1285
<b>Polyphenolase</b> (Optical density of purpurogallin formed per milligram of nitrogen in 10 minutes in 20 ml. reaction mixture)	0.7142	0.3781	0.1072

These show that the nut water sampled from the trees receiving no attention had the maximum activity in the case of all the three enzymes, those from the trees given cultivation only being comparatively less while the samples from the trees receiving manuring and cultivation showed the least enzyme activity indicating that agricultural practices do exert measurable influence on the oxidising enzymes present and active in nut water. The possible scope for this type of studies to serve as guides in the selection and adoption of the usual agricultural practices, using carbohydrate, fat and protein splitting enzymes in coconut water is receiving careful attention.

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