Research Article

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Contribution of Enzymic Browning to Color in Sugarcane Juice

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The contribution of polyphenol oxidase (PPO) and peroxidase (POD) to enzymic browning in sugarcane juice was investigated. Inactivation of these enzymes with heat resulted in juice of lower color (absorbance measured at 420 nm), but POD was found to be more heat stable than PPO. Salicylhydroxamic acid (SHAM) completely inhibited PPO activity and markedly reduced juice color but had no effect on POD activity. Removal of oxygen in the presence of the substrate chlorogenic acid also stopped color formation. Upon subsequent addition of oxygen, browning continued, indicating that the process was oxygen dependent. Color development in juice was complete after 20–30 min even though PPO was still active. Addition of chlorogenic acid at this point restarted browning, suggesting that color development was limited by the availability of phenolic substrates. Varietal differences were observed in levels of PPO activity, phenolics, and color. There was a correlation between juice color and phenolic content but not between juice color and PPO activity. The sugarcane PPO enzyme was most active with chlorogenic acid. It was not active with *p*-diphenols and was inhibited by SHAM, suggesting that it is a catechol oxidase-type enzyme (EC 1.10.3.1) and not a laccase (EC 1.10.3.2). It is atypical in that it was inhibited by SDS. The results suggest that enzymic browning contributes significantly to color formation in sugarcane juice and that PPO is the major enzyme involved.

INTRODUCTION

Brown pigments are formed during the processing of sugarcane from the initial juice extraction through to crystallization of the raw sugar. The presence of these colored impurities is of considerable significance to the sugar industry as their presence impedes crystallization and results in lower sugar yields, poorer quality sugars, and increased costs of refining (Jimenez and Samaniego, 1981).

At least four different mechanisms are believed to contribute to color formation during raw sugar production (Kort, 1979): (1) melanoidins formed from sugar–amino acid reactions via the Maillard reaction; (2) thermal degradation and condensation reactions of sugars (caramelization); (3) alkaline degradation and condensation reactions of reducing sugars; and (4) oxidative reactions of phenolic compounds. The first three are nonenzymic reactions, whereas the oxidation of phenolic compounds to the chemically more reactive quinones is enzymic and occurs early in the extraction process, when the cane is first crushed. Previous studies suggest that enzymic browning may contribute significantly to color in cane juice. Smith (1976) found that heating cane to 80–90 °C prior to crushing resulted in a 47% reduction in average juice color, and Tu (1977) observed a similar reduction in both cane juice and raw sugar color when the juice was made alkaline to inhibit enzyme activity.

Both polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7) have been implicated in enzymic browning of plant tissues (Vamos-Vigyazo, 1981). Polyphenol oxidase is widely distributed in the plant kingdom (Mayer and Harel, 1979). It is a copper-containing enzyme which catalyses the ortho-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones (Mayer and Harel, 1979). The highly reactive quinones thus formed can polymerize to form the red, black, and brown pigments associated with the browning of plant tissues. Normally, PPO is separated from its phenolic substrates, which are located in the vacuole, so that browning only occurs when cells are damaged and compartmentation is lost. As yet the physiological function of PPO has not been established, although it has been associated with disease resistance (Vaughn *et al.*, 1988).

Peroxidase is an iron-containing enzyme capable of oxidizing phenolics to quinones in the presence of hydrogen peroxide. Like PPO, the physiological role of peroxidase in plants is not well understood but it has been implicated in a number of primary and secondary metabolic functions including lignin biosynthesis, ripening and senescence, ethylene biosynthesis, hormone balance, and membrane integrity (Vamos-Vigyazo, 1981). Lagrimini (1991) produced transformed tobacco plants overexpressing peroxidase which showed rapid browning in response to wounding, but the role of peroxidase in normal enzymic browning is still not well established.

An active PPO with a high specificity for chlorogenic acid has been isolated from sugarcane leaf tissue (Coombs *et al.*, 1974). Subsequent inhibition and heat inactivation studies suggested PPO contributed significantly to color formation in sugarcane (Gross and Coombs, 1976b; Coombs and Baldry, 1978; Goodacre *et al.*, 1980). The presence of POD in sugarcane and its properties have been studied (Alexander, 1966); however, its contribution to color formation has not been investigated.

The aim of this study was to determine the contribution of enzymic browning to color formation in sugarcane juice and to determine the relative contributions of each of these enzymes to browning.

MATERIALS AND METHODS

Plant Material. Sugarcane varieties Q87 and Q96 were propagated from clonal setts and grown in a heated glasshouse with an average day temperature of 30 °C and a night temperature of 17 °C. The canes 81C236, Q87, H56-752, 81C337, 81C497, Q96, 81C542, 81C558, and 81C509 for the varietal studies (Figures 2 and 3) were grown at the Bureau of Sugar Experiment Station (BSES), Mackay Queensland, under field conditions and harvested Sept 5, 1991.

Tissue Extraction and Enzyme Assays. All experiments were repeated several times with cane tissue showing differing degrees of browning; unless otherwise stated the data shown are representative of a typical situation. Individual measurements were replicated two to three times. Lengths of cane were

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