yeast (Jones, & Pierce, 1964). Tyrosine is absorbed only during the latter stages of fermentation, because of competition for the permease sites in the yeast cell wall (Jones & Pierce, 1964), so the excess present in the syrup should not effect the yeast metabolism. Lysine could block the transport of several amino acids, as it is preferentially absorbed by the yeast permease system (Jones & Pierce, 1964). Consequently the excess of lysine might affect the quality of the beer if a major percentage of the wort was derived from the wheat syrup. However, small-scale brewing trials have shown that the use of a wheat-based syrup to replace 30% of a malt wort produced beers that could not be distinguished organoleptically from the all-malt wort control beers.

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The Reaction of Malonaldehyde with Wheat Flour Proteins

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Recent studies on lipid-protein interaction in dry systems (Roubal & Tappel, 1966; Roubal, 1970) have demonstrated that the free radicals derived from oxidizing lipids are the dominant cause of reactions leading to protein polymerization and amino acid destruction. However, there is some evidence that the difunctional aldehydes malonaldehyde and glutaraldehyde, products of the oxidation of polyunsaturated fatty acids, are able to react with proteins (Menzel, 1967; Ewart, 1968) and amino acids (Buttkus, 1968) directly. This communication confirms previous findings and demonstrates the reaction mechanism of malonaldehyde-protein in wheat flour systems.

Malonaldehyde was prepared from 1,1,3,3-tetraethoxypropane (Kwon & Watts, 1963) and allowed to react with wheat flour gliadin under the conditions described by Ewart (1968) for gliadin-glutaraldehyde reaction. The modified protein was hydrolysed with 6M-hydrochloric acid for 18h at 105°C, and amino acid analyses were carried out as described by Ewart (1967). Results demonstrate that the treatment of gliadin with malonaldehyde results in the destruction of all the detectable tyrosine, in contrast with other workers, who have reported losses of lysine and tyrosine (Ewart, 1968), of cysteine and methionine (Buttkus, 1968) and of methionine (Tannenbaum, Barth & Le Roux, 1969).

Glycerol tri[U-¹⁴C]linolenate was synthesized (Daubert & Baldwin, 1944) via the acyl chloride (Wood, Jackson, Baldwin & Longenecker, 1944) and stored with a mixture of wheat flour proteins for 35 days at 30°C (80% relative humidity). Portions of the mixture were withdrawn at intervals, exhaustively defatted and fractionated by polyacrylamide discontinuous gel electrophoresis. The developed gels were sliced into 1mm sections and the radioactivities counted. During storage significant amounts of radioactivity became associated with the more mobile proteins. After storage the mixture was slurried with water and the more volatile components, containing 70% of the residual radioactivity, were isolated by steam-distillation. All of the radioactivity in this isolate was associated with a compound whose properties were identical with those of malonaldehyde. A sample of [U-¹⁴C]-malonaldehyde was prepared from [U-¹⁴C]linolenic acid and dispersed with unlabelled aldehyde on to a similar protein mixture. Subsequent isolation and counting of radioactivity showed the pattern of radioactivity-protein combination to be identical with that observed in stored glycerol tri-linolenate-protein mixtures.

This evidence supports the view that the aldehydes released from oxidizing lipids combine directly with proteins.

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The Isolation of Plant Root Protoplasts and their Use in the Separation of Subcellular Components

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Power & Cocking (1968) described a method for the isolation of large numbers of protoplasts from fully expanded tobacco leaves. This method has been adapted for use on roots, either for the production of intact protoplasts or for the release of

subcellular components without recourse to the customary high shear forces.

Root tips (1–2 cm) were excised from 4-day-old seedlings of maize (*Zea mays*; Crow Hybrid WF9 × M14) and incubated for 12–18 h in a slightly hyperosmotic medium containing cellulase (5%, w/v, Onozuka SS) and Pectinase (0.5%, w/v, Macerozyme) obtained from R. W. Unwin and Co. Ltd., Welwyn, Herts., U.K. Protoplasts were produced in media based on sucrose, mannitol, a complete tissue-culture medium (Straus, 1960) or in a mixture of potassium chloride and calcium chloride of suitable osmolality.

A surface adenosine triphosphatase activity was found in protoplasts by biochemical assay and histochemical techniques, confirming the observations made by Hall (1970) on whole roots.

Digested roots retain cohesion, and the digestive enzymes can be washed away easily. The washed roots are then susceptible to homogenization by ten manual strokes in a Jencons Teflon-in-glass tissue grinder (150–200 μ m clearance).

The homogenate has been fractionated by differential and density-gradient centrifugation on sucrose and Ficoll (Pharmacia Ltd., London W.13, U.K.) gradients in 25 mM-tris-HCl buffer, pH 7.2, into eight discrete bands.

Electron-microscopical examination with differential phosphotungstic acid staining techniques (Glauert, 1965; Rambourg, 1967) localized a plasma-membrane-rich band (cf. Lembi & Morre, 1970). A K^+ -stimulated adenosine triphosphatase activity was found to be associated with this fraction. In addition, the distribution of univalent ion- and bivalent ion-stimulated adenosine triphosphatase activities in all the fractions was determined. Separate Mg^{2+} -stimulated, Mg^{2+} -inhibited and further K^+ -stimulated activities have been recognized. The highest specific activity of adenosine triphosphatase was associated with a very light vesicular fraction (cf. Matile, 1968).

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L-Tyrosine Decarboxylase from Barley Roots

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Hordenine (*NN*-dimethyltyramine) is synthesized in barley roots over a 5–8-day period after germination (Raoul, 1937). Isotope experiments have shown that tyrosine is decarboxylated to tyramine, which is methylated successively to *N*-methyltyramine and hordenine (Leete, Kirkwood & Marion, 1952; Leete & Marion, 1953). Only one enzyme in the sequence, *S*-adenosylmethionine-tyramine *N*-methyltransferase, which catalyses the methylation of tyramine to *N*-methyltyramine, has hitherto been isolated (Mann & Mudd, 1963).

L-Tyrosine decarboxylase has now been extracted from roots of 5-day barley seedlings and purified up to 80-fold by successive ammonium sulphate precipitation, passage through Sephadex G-50 and elution from DEAE-cellulose columns. Alternatively, a rapid 20-fold purification has been achieved by adsorption from the crude extract on DEAE-cellulose and subsequent elution. The enzyme was specific for the L-isomer and the reaction shown to produce equivalent quantities of tyramine and CO_2 . Although the preparations also showed L-glutamate decarboxylase activity (Beever, 1951), tyrosine decarboxylase was distinguished by its different pH optimum, stability and sensitivity to *p*-hydroxyphenylpyruvate and 2-oxoglytarate.

Inhibition by *p*-hydroxyphenylpyruvate was shown to be competitive, giving K_i 0.07 mM. Similar inhibitions by *p*-coumarate (K_i 0.07 mM), ferulate (K_i 0.06 mM) and chlorogenic acid (K_i 0.06 mM) were found, but caffeate and sinapate were relatively ineffective.

The enzyme was stimulated by *S*-adenosylmethionine over a concentration range from 0.1 μ M to 1 mM with an optimum at 0.1 mM. The products were then found to include *N*-methyltyramine and hordenine, which accumulated as the reaction progressed.

The inhibition of enzyme activity by *p*-hydroxyphenylpyruvate suggests that tyrosine decarboxylation may take place only while the roots are dependent on tyrosine from the reserve protein and is inhibited when this has been exhausted and tyrosine is synthesized *de novo*. It may also be inhibited in cells producing lignin precursors or other phenolic substances by the hydroxylated cinnamic acids. Stimulation by *S*-adenosylmethionine may be held to support the view that alkaloids can act as pools for methyl groups (Mothes, 1955), but there is no evidence that these are subsequently

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