Lipid distribution in potato tubers

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Summary

To elaborate on off-flavour development in dehydrated potato granules, lipids in subcellular particles and membrane systems of the tuber were investigated. Lipid acyl-hydrolase and lipoxygenase activities were suppressed in tuber homogenates by a buffer isolation medium of pH 7.8 containing nupercaine which minimized the breakdown of phospho- and galactolipids. Phospholipid, glycolipid and neutral lipid constituents, their fatty acid composition, and unsaturation ratios were reported for amyloplasts, cell wall, microsomes, mitochondria, peroxisomes, and plasmalemma.

Introduction

The high unsaturation of tuber fatty acids and evidence that their oxidation is largely responsible for off-flavours in dehydrated potato granules has prompted research into potato lipids and their composition and spatial distribution within the tuber. However, such research is hampered by lipid-degrading enzymes in the tuber. Galliard and co-workers (Galliard, 1970; Galliard & Matthew, 1973; Galliard & Rayward-Smith, 1977) were among the first to demonstrate the presence of phospho- and galactolipases and lipoxygenase (LOX) in tubers and the rapidity with which lipids were hydrolysed and peroxidized in tuber homogenates, even at 0 °C. Hasson & Laties (1976a, b) claimed that the American potato cv. Russet Burbank, once sliced, might lose as much as 20 % of its galacto- and phospholipids in a matter of seconds, while up to 40 % might be lost in just a minute.

Separation and characterization of potato lipid acyl-hydrolases (LAH) by Hasson & Laties (1976a, b) and potato LOX (Berkeley & Galliard, 1976a; Sekiya et al., 1977) have also been reported.

In light of these results, the aim of this study was to reinvestigate the lipid distribution within the major tuber subcellular particles and membranes. For analysis, advantage was taken of the differential rates of breakdown of free and

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membrane-bound lipids, and also of nupercaine, a novel and potent LAH suppressor. Nupercaine has been shown to effectively suppress phospholipase A₂ activity in rats (Scarpa & Lindsay, 1972), and in cauliflower bud mitochondria (Moreau et al., 1974).

Results of this study, relative to those reported in a review by Mazliak (1977), differed somewhat for lipid composition in plasmalemma and peroxisomes, but were quite similar for other membranes. The lipid composition of amyloplasts and the pattern of fatty acid unsaturation ratios within the potato cell were also determined.

Experimental

Potato tubers
Tubers of cv. Netted Gem (Russet Burbank; specific gravity 1.098), commercially grown under irrigation in Southern Alberta, were used, along with Irish Cobbler, Norgold Russet, Norland, and Warba cultivars.

Isolation of membranes and subcellular particles
Potato tubers (200 g - 2 kg) prechilled to 4 °C were peeled, diced and added in a 1:2 w/v ratio to a 0.1 M Tris-Cl buffer of pH 7.8, containing 0.4 M sucrose, 0.5 mM Na-mercaptobenzothiazole (Eastman Kodak Co., Rochester, NY), and 50 μM nupercaine hydrochloride (dibucaine hydrochloride; Ciba, Basel, Switzerland). The mixture was homogenized at medium speed for 1 min at 4 °C in a type 45 Virtis homogenizer (Virtis Co. Inc., Gardiner, N.Y.). The homogenate was filtered through two layers of Miracloth and then centrifuged at 2000 g for 7 min to sediment the starch granules. The supernatant was centrifuged at 21 000 g for 15 min. The pellet obtained was carefully washed with buffer and used as crude mitochondria, while the supernatant was centrifuged at 105 000 g for 90 min in order to collect the crude microsomes. The purification of crude microsomes and mitochondria and recovery of plasmalemma and peroxisomes were done according to Ben Abdelkader & Mazliak (1970). Viability of isolated mitochondria was examined by measuring their respiratory control ratio (r.c.r.) and ADP/0 values (Haydar & Hadziyev, 1974), while transmission electron microscopy examinations of all the preparations were carried out as described elsewhere (Chung & Hadziyev, 1979).

The tuber cell wall material was isolated from a separate batch of diced tubers. These were mechanically disintegrated with the Virtis homogenizer at full speed in ice-cold 0.5 M Tris-Cl buffer pH 7.8 containing 50 μM nupercaine and 0.5 % sodium sulphite. The slurry was transferred to several layers of a 200 mesh polyester sieve cloth and washed with deionized water until the residue was free of starch when examined under a polarized-light microscope.

Lipid analysis
Isolated membranes and organelles were treated immediately with boiling isopropanol. The TL, purified from non-lipid contaminants, were separated on Silicic acid-Celite columns into NL, GL and PL fractions. The NL were eluted with diethyl ether, GL with acetone, and PL with chloroform:methanol (1:1 v/v) followed by methanol. Collected fractions were further separated into individual components on thin-layer plates (TLC) coated with MN Kieselgel N (Macherey & Nagel