Qualitative and Quantitative Analyses of Free Neutral Carbohydrates in Mushroom Tissue by Gas–Liquid Chromatography and Mass Spectrometry

The analysis of the free carbohydrates in *Agaricus bisporus*, the cultivated mushroom, has been accomplished using gas–liquid chromatography and mass spectrometry. Trimethylsilyl derivatives and hexa-*O*-acetyl derivatives were used. The qualitative and quantitative analysis of fructose, glucose, mannitol, and sucrose is reported. Mannitol was present in the largest amount, accounting for over 12% of the dry weight of the mushroom.

Gas–liquid chromatography of volatile derivatives of carbohydrates provides rapid analyses of free sugars and products of polysaccharide hydrolysates. Of these, trimethylsilyl derivatives have proven most effective in quantitative and qualitative analyses. The existence of mannitol in the sporophore tissue of *Agaricus campestris* was established by paper chromatographic and colorimetric methods (Hughes *et al.*, 1958). Hughes (1961) reported that, depending on time of harvest, mannitol accounted for 11 to 19% of the dry weight of mushroom sporophores. Recent interest in the postharvest changes in mushrooms has focused on changes in carbohydrate content (Lee, 1969; Dommel, 1964). This report describes the qualitative and quantitative analysis of free sugars in mushroom tissue as their trimethylsilyl derivatives and hexa-*O*-acetyl derivatives by gas chromatography and mass spectrometry.

MATERIALS AND METHODS

The mushrooms harvested were strain 310 from the culture collection at The Pennsylvania State University. The mushrooms were grown under carefully controlled environmental conditions, as described by Schisler (1967). The sporophores were harvested from a first "break" or "flush" of the crop cycle. Three samplings of each of three crops in a first break were fractionated. Fresh mushrooms (20 g) were extracted with 100 ml of 20% aqueous methanol by grinding in a Waring blendor for 5 min. *N*-Acetylglucosamine (40 mg) was added as an internal standard for the quantitation of sugars other than mannitol, since it was not found to be present in the free carbohydrate fraction. *N*-Acetylglucosamine (200 mg) was added to another similar extraction to quantitate mannitol. The extracts were filtered through Whatman No. 1 filter paper by suction. The filtrate was brought to a volume of 100 ml by addition of water. A 1-ml aliquot was pipetted into a 7-ml vial and frozen in Dry Ice/acetone for 10 min. After freezing, the sample was vacuum dried in a Stokes Freeze Drier for 24 hr.

The dried sample was dissolved in 1 ml of pyridine (stored over KOH pellets). The TMS derivatives of sugars were formed by the addition of 0.5 ml of hexamethyldisilazane and 0.3 ml of trimethylchlorosilane. The TMS sugar derivatives were then chromatographed on a Hewlett-Packard 5750B gas chromatograph, equipped with a hydrogen flame detector, within 4 hr after silylation. Separation of TMS derivatives was accomplished using a 6-ft × 1/8-in. stainless steel column packed with 5% SE-30 on 80/100 mesh Hi-Performance AWDMCS treated Chromosorb B. The column temperature was programmed from 150 to 250° C at 4°/min with a carrier gas (N_2) flow of 30 ml/min and an injection port temperature of 280° C.

Identification of the sugars was accomplished using an LKB-9000 glc–mass spectrometer according to the procedure of Reineccius *et al.* (1970). A standard curve was established for each of the four sugars. The standard curves were generated by running equivalent amounts of each sugar and internal standard through the procedure and plotting peak area, relative to the internal standard, against concentration. Therefore, quantitative data were obtained by comparing the relative peak areas of the mushroom sugars to the internal standard.

TMS–mannitol cannot be distinguished from TMS–glucitol or TMS–galactitol by mass spectra glc retention time. To identify mannitol, the hexa-*O*-acetyl derivatives of the mushroom alditols were prepared according to the method of Shaw and Moss (1969). The alditol acetates were chromatographed on a Hewlett-Packard 5750B gas chromatograph as previously described. A 6-ft × 1/8-in. stainless steel column packed with 3% OV-225 on 80/100 mesh Gas Chrom W was used to separate the alditol acetates. The column temperature was maintained at 160° C.

RESULTS AND DISCUSSION

Only four sugars could be identified by glc: fructose, glucose, mannitol, and sucrose (Figure 1). Only one hexa-*O*-acetyl derivative was formed in the mushroom sugars having a retention time equal to hexa-*O*-acetyl mannitol. By comparing relative peak areas of fructose, glucose, sucrose, and mannitol to *N*-acetylglucosamine the concentrations were calculated for mushroom tissue (Table I). The standard deviation for the replicate samples was not greater than ±2.1%.

Concentrations are given on a fresh weight basis since studies pertaining to carbohydrate changes in postharvest storage of mushrooms have been performed using fresh weights as a basis for analysis (Dommel, 1964). Under the environmentally controlled growing conditions used, the

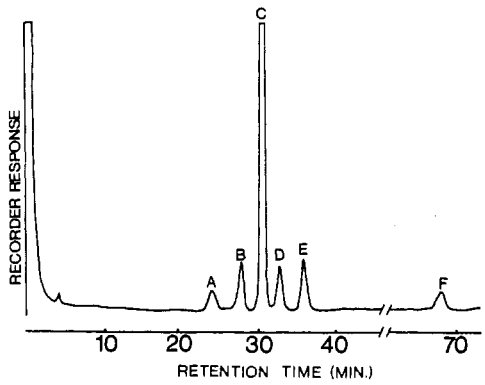

Figure 1. A typical gas chromatogram of the TMS sugars from *Agaricus bisporus:* (A) fructose; (B) α-glucose; (C) mannitol; (D) β-glucose; (E) *N*-acetylglucosamine (internal standard); (F) sucrose

fluctuation of dry weight of first "break" mushroom tissue is almost nil. The average moisture percentage is 92%. Using this figure it can be calculated that the mannitol concentration is 12.8% of the dry weight, which is within the range described by Hughes *et al.* (1958). Variations in mannitol concentration were noted from samples taken from other breaks or flushes of the crop cycle. No detailed study of these variations was completed at this time.

Mushroom tissue is approximately 92% water. It may be suggested that the high amounts of free carbohydrate, especially mannitol, may provide the osmotic potential necessary to maintain this concentration of water.

Table I

	mg/g fresh weight
Fructose	0.48
Glucose	2.20
Sucrose	0.52
Mannitol	11.50

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