Determination of Acrylamide Monomer in Mushrooms Grown on Polyacrylamide Gel

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Mushrooms (*Agaricus bisporus*) grown on a casing mixture containing polyacrylamide gel have been analyzed to assess the possible uptake of acrylamide monomer from the polymer gel to the fruit during cultivation. The polymer was found to contain 220 mg/kg residual monomer. At the application rate employed for cultivation this would have given an initial monomer level of 220 μg/kg in the casing mix. Mushrooms were analyzed by extraction, bromination, and quantification of acrylamide as 2-bromopropenamide using gas chromatography–mass spectrometry in the selected ion mode. No acrylamide monomer could be detected at a limit of detection of 0.5 μg/kg. This demonstrates that acrylamide either does not translocate significantly from the mycelia to the mushroom fruit or that the monomer does not bioaccumulate, due perhaps to a high water solubility or chemical reactivity.

INTRODUCTION

The production of edible mushrooms is a major industry and is one of the most important examples of microorganisms cultured for human consumption. Of the estimated 1.5–2.0 megatonnes of edible mushrooms cultivated worldwide per annum (Gry and Kirsten, 1991; O'Brian, 1989), more than 85% are the variety *Agaricus bisporus* with an estimated value of U.S. $4 billion. Horticultural "value-added" is significantly higher than for other agricultural sectors, and crop protection, increased efficiency of production, and quality are priorities (MAFF, 1992).

A general requirement for successful mushroom cultivation is a composting substrate along with a second substrate, a capping or casing soil, which overlays the composting substrate and in which the fruits form. The traditional substrate is composted stable manure, although chicken manure is now more readily available in quantity and also finds widespread commercial use. The function of the upper casing is to provide support for the developing fruits and maintain a constantly moist culture. The casing has traditionally been peat neutralized with chalk or limestone.

Incorporation of water-imbibing polyacrylamide into the casing mix is a recent development and is claimed to offer two major advantages to growers. First, it is said to encourage mycelia of the fruiting bodies to form and colonize the casing mix. Second, the polymer should reduce the need to apply water due to its large water capacity once hydrated to a gel. Since watering is considered to be a major route by which disease is introduced in mushroom cultivation (Hudson, 1986), extended intervals between watering should help reduce the incidence and severity of disease.

Although polyacrylamide is itself an inert polymer, it can contain residual acrylamide monomer as a result of incomplete polymerization. Monomer levels in the agricultural grade polymer are typically around 200–500 mg/kg on a dry weight basis. The toxicity of acrylamide monomer is well established (Le Quesne, 1980; WHO, 1985), and this is reflected, for example, in the strict limit of 0.25 μg/L set by many countries for drinking water (WHO, 1985). There is a need therefore to ensure that this water-soluble monomer is not taken up and bioaccumulated by crops grown using polyacrylamide gel and intended for human consumption.

We have reported earlier on the analysis of tomatoes grown hydroponically on polyacrylamide gel, where no monomer was detected in the fruits at the 1 μg/kg (ppb) level (Castle *et al.*, 1991). The work reported here extends these studies to the analysis of mushrooms, where a quite different set of translocation and accumulation parameters may be expected to operate.

EXPERIMENTAL PROCEDURES

Materials. Mushrooms were grown in Germany and supplied for analysis by Polyplant GmbH (Xanten, D). The mushroom cultivar was Somycel 208. The polyacrylamide Hydratex 2 (Agricultural Polymers Ltd., Gloucester, U.K.) was added at 73 g/m^2 to the casing mix giving 1.8 kg/m^3 calculated for polyacrylamide on a dry weight basis. The polyacrylamide was added to the casing mix on April 2, 1992, and the day of harvest was 26 days later. Mushrooms for analytical controls were cultivated in the normal fashion, without the use of polyacrylamide. Immediately following harvest, the mushrooms were dispatched to the laboratory in polyethylene bags and were received the following day. They were stored frozen at −18 °C between receipt and analysis.

A sample of polyacrylamide typical of that incorporated into the mushroom casing mix was supplied by Agricultural Polymers Ltd.

Acrylamide (99%), methacrylamide (98%), hydrobromic acid (ACS grade, 48% w/v), and bromine (99.99%) were from Aldrich Chemical Co. (Gillingham, U.K.). Potassium bromide (AnalaR) was from BDH Chemicals (Poole, U.K.). Methanol, ethyl acetate, and hexane were of glass-distilled grade from Rathburn (Walkerburn, U.K.). A bromination reagent was prepared from potassium bromide (200 g), hydrobromic acid (10 mL), and bromine-saturated water (saturated at 4 °C, 160 mL) made to a total volume of 1000 mL with distilled water. This reagent was stored at 4 °C.

Extraction of Mushrooms. Mushrooms were taken from frozen storage and allowed to warm to room temperature over 1 h and then subsampled. They were analyzed both "as received" and after washing by hand under cold running tap water to remove surface dirt. Randomly selected mushrooms (caps and stalks, 200 g) were cut into small pieces using a knife, and the pieces were mixed to further randomize. An accurately weighed subsample (50 g) was added to water (200 g) in a 400-mL tall-form beaker and homogenized using an Ultra Turrax homogenizer fitted with a 25N shaft and operated at full speed for 1 min. The resulting slurry was allowed to stand for 5 min and then the top layer of "foam" poured off and discarded (*ca.* 100 mL). An aliquot (20 mL) of the remaining mixture was poured into a vial and

0021-8561/93/1441-1261$04.00/0 Published 1993 by the American Chemical Society

centrifuged at 3500 rpm for 15 min. This gave a solid pellet of *ca.* 3 cm^3 and a supernatant that was slightly turbid and pale yellow/pink in color.

Bromination of Extracts. The supernatant was decanted from the pellet into a screw-top vial. Bromination reagent was added (15 mL), and the tube was inverted to mix and then set aside in a dark incubator overnight at 4 °C. Excess bromine was then neutralized by the addition of sodium thiosulfate solution (0.7 M, *ca.* 7 drops required with a noticeable loss of bromine color resulting), and the mixture was then extracted with ethyl acetate (8 mL) using a mechanical shaker for 10 min. Phase separation was effected by centrifugation (2000 rpm). It was noted that the smallest interface layer of debris was obtained if the sample was shaken immediately prior to centrifugation. The organic extract was pipetted into a screw-cap tube containing anhydrous sodium sulfate (*ca.* 0.5 g) and shaken for 15 min to dry the solvent. The sample was centrifuged to "pellet" the desiccant and the organic solution decanted into a fresh tube and evaporated to *ca.* 30 μL under a stream of nitrogen using a hot block at 40 °C. Hexane (200 μL) was added, and this was evaporated just to dryness to remove the last ethyl acetate. At this stage, a small quantity (*ca.* 3–7 mg) of brown residue (coextractives) was present. This residue was redissolved in 20% v/v ethyl acetate/hexane (250 μL) prior to cleanup.

Cleanup of Derivatized Extract. A BondElut cleanup cartridge (silica, 500-mg size) was preconditioned with 20% ethyl acetate/hexane (7 mL). The sample was applied and washed onto the cartridge with small aliquots taken from 3 mL of 20% ethyl acetate/hexane. The cartridge was eluted with the remainder of this 3 mL of solvent mixture. The cartridge was then eluted with 30% ethyl acetate/hexane, and the first 2 mL was discarded. The second 2 mL of eluate was collected in an amber vial.

2,3-Dibromo-2-methylpropionamide was added to the fraction at this stage to act as a chromatographic standard. This had been prepared by bromination of methacrylamide to give a 0.5 μg/mL solution in ethyl acetate (concentration expressed as methacrylamide) of which 50 μL was added. The fraction was then evaporated to a small volume under a stream of nitrogen using a hot block at 30 °C, transferred quantitatively to an amber autosampler vial, and finally evaporated to a volume of *ca.* 30 μL ready for GC–MS analysis.

Quantification. For standard addition, the mushroom subsample (50 g) received 2 or 10 μg/kg acrylamide added before homogenization (10 or 50 μL of a 10 μg/mL solution in water). To determine the extraction recovery, a set of comparison samples was fortified with 10 μg/kg acrylamide at the bromination stage.

GC–MS Analysis. Mushroom extracts were analyzed on a VG 7070 EQ mass spectrometer coupled to a Carlo Erba Mega Series gas chromatograph and operated in the selected ion monitoring mode. The GC column was a J&W 30 m × 0.25 mm fused silica capillary with a 0.2-μm DB17 phase. The column was programmed from 65 to 250 °C at 15 °C/min. Ions monitored were *m/z* 106, 108, 150, and 152 for the acrylamide derivative and *m/z* 120 and 122 for the chromatographic standard 2,3-dibromo-2-methylpropionamide. Quantification was on the basis of peak areas normalized against the chromatographic standard. The VG data system was employed, but baselines were defined manually.

Extraction of Polyacrylamide. An accurately weighed portion of polymer granules (0.1 g) was swollen in distilled water (25 mL) containing methacrylamide (internal standard, 100 μg) on standing overnight at ambient temperature. Methanol (250 mL) was then added to dehydrate the gel (one day/ambient temperature) and thus draw out any free residual monomer along with the internal standard.

Derivatization of Polymer Extract. An aliquot of the polymer extract (1 mL) was added to bromination reagent (14 mL) and allowed to stand overnight at 4 °C in the dark to derivatize. Extraction into ethyl acetate (2 mL) was followed by GC-ECD analysis. Calibration was by standard addition of acrylamide (15, 25, and 40 μg) corresponding to levels of 150, 250, and 400 mg/kg added. Samples were prepared in duplicate.

GC-ECD Analysis. Polymer extracts were analyzed by GC-ECD on a Chrompack 17 m × 0.25 mm × 0.2 μm CPSIL 19CB capillary column operated isothermally at 175 °C in a Carlo Erba 4160 gas chromatograph. The injector and detector ports were held at 230 and 250 °C, respectively. The Carlo Erba ECD 400 detector was held at 280 °C and operated in the constant current mode. Injections of 1 μL were split *ca.* 20:1. Quantification employed peak area ratios of derivatized acrylamide *vs* methacrylamide (internal standard).

RESULTS AND DISCUSSION

Level of Interest Selected. While there seem to be no specific regulations on the use of polyacrylamide as a water retention agent in agriculture, a relevant area that does have specific regulations is that of migration from food contact plastics such as packaging materials and plastics used in food processing industries. European Community Directive 90/128 (EEC, 1990) as ammended by Directive 92/39 (EEC, 1992) permits the use of polyacrylamides, or copolymers prepared from the acrylamide monomer, in direct contact with food provided acrylamide migration is not detectable using an analytical method with a limit of detection of 10 μg/kg. Taking this limit as a guideline, the present work was undertaken with a level of interest set at 1 μg/kg and the analytical procedure was designed accordingly.

Analytical Method Adopted. The GC–MS method employed was based on that used previously for the analysis of tomatoes for acrylamide (Castle *et al.*, 1991) with modifications to allow for the different food matrix. The concentration of bromine in the bromination reagent was increased approximately 2-fold since it was considered that the slightly higher content of unsaturated fatty acids in mushrooms compared with tomatoes (Holland *et al.*, 1991) might lead to exhaustion of the standard charge of reagent. A second modification was a minor change to the elution scheme for the silica cleanup cartridge to obtain a cleaner fraction.

The most important variation from this earlier work was the identity of the acrylamide derivative quantified by GC–MS. GC–MS analysis of a brominated acrylamide standard showed that the retention time of the major peak was much less than expected for the dibromo derivative of acrylamide, 2,3-dibromopropionamide (I) (Tenkel *et al.*, 1989; Castle *et al.*, 1991). Scanned spectra of a strong standard solution revealed that the major derivative detected was in fact bromopropenamide (II). Bromopropenamide gave a good response in the *m/z* 106/108 channels set up to monitor the expected dibromopropionamide, and thus both derivatives could be monitored as the $CH_2{=}CHBr^{\bullet+}$ fragment.

$$CH_2{=}CH{-}CONH_2 \xrightarrow{\text{bromination}} CH_2Br{-}CHBr{-}CONH_2 \text{ (I)}$$

$$I \xrightarrow{\text{GC-MS conditions, -HBr}} CH_2{=}CBr{-}CONH_2 \text{ (II)}$$

$$II \xrightarrow{\text{EI fragmentation, -HNCO}} CH_2{=}CHBr^{\bullet+}$$

Following the GC–MS analysis (performed over one night), an acrylamide standard was taken from the GC–MS samples and analyzed using the GC-ECD system used for polymer analysis. This revealed that the major product in the vial was the dibromo derivative as expected, and this in turn suggested that dehydrobromination had occurred either in the injection port of the GC–MS or on column. Dibromination of acrylamide followed by dehydrobromination to 2-bromopropenamide is an analytical strategy that has been employed by others for acrylamide

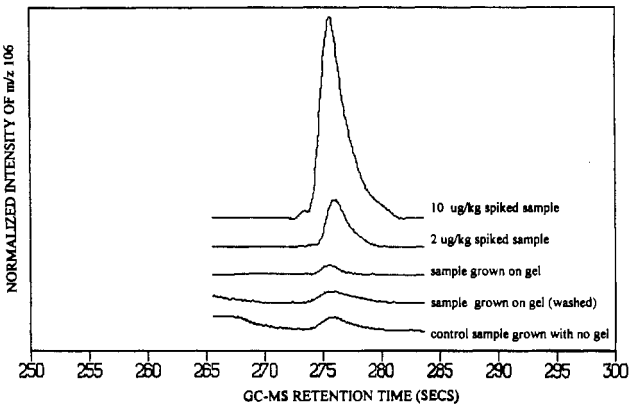

Figure 1. Gas chromatographic–mass spectrometric determination of acrylamide in mushrooms. Acrylamide was determined via ion m/z 106 for the 2-bromopropionamide product resulting from bromination of mushroom extracts. A comparison of traces for samples, spiked samples, and a control sample grown without polyacrylamide gel is shown.

analysis (Takata and Okamoto, 1991), although in that case dehydrobromination was achieved intentionally by the use of triethylamine following sample cleanup on silica. It may be relevant that immediately prior to the present work for acrylamide analysis the GC–MS had been used extensively for the analysis of free amines. The standard addition graph was linear over the range 0–10 μg/kg, indicating that quantification via the 2-bromopropenamide derivative was reliable.

Acrylamide Monomer Levels. A calibration graph from standard addition of acrylamide to mushrooms over the range 0–10 μg/kg intercepted the x axis at 0.4 μg/kg, suggesting this to be the background (contamination) level in the food. Figure 1 shows GC–MS selected ion monitoring traces for a mushroom control (grown without gel), mushrooms grown on polyacrylamide (with and without washing by hand), and samples spiked with monomer at 2 and 10 μg/kg. It can be seen that the samples grown with polyacrylamide are indistinguishable from the controls. The apparent background at 0.4 μg/kg equivalents is attributed to the analytical method *per se*, and transfer of acrylamide monomer to the mushrooms from the growing medium was less than 0.5 μg/kg and not detectable. This figure is within the 1 μg/kg level of interest set for this work and is well within the 10 μg/kg migration level set for food contact plastics (EEC, 1992) and which came into force in all European Community member states on January 1, 1993 (in the U.K. for example; U.K., 1992).

Analysis of the polyacrylamide itself revealed a level of 220 mg/kg residual monomer which was close to the value of 280 mg/kg suggested by the manufacturer. The application rate of the polymer in the casing mixture was approximately 0.1% w/w and would have given an initial concentration of free monomer in the casing of *ca.* 220 μg/kg. The evident lack of bioaccumulation of acrylamide in the mushrooms is presumably due either to a lack of translocation to the fruit via the mycelia or to the high reactivity of the monomer in the composting substrate and/or in the mushroom.

The half-life of acrylamide in aerobic soil is of the order of several days at 20 °C and decreases with increasing temperature (Lande *et al.*, 1979). Biotransformation in mammals and microorganisms (WHO, 1985) typically involves irreversible loss of acrylamide by, for example, reaction through the alkene bond or by formation of acrylic acid and then decarboxylation. For these reasons, it is considered to be unlikely therefore that the monomer is taken up by the mushroom and accumulated as a conjugate which reversibly liberates free acrylamide when the mushroom is consumed. A radiolabeling experiment would be the best means to test for this possibility.

ACKNOWLEDGMENT

We thank Mr. Brian Longhurst of Agricultural Polymers Ltd., Gloucester, U.K., for arranging the supply of mushrooms and polyacrylamide.

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Received for review February 17, 1993. Accepted May 18, 1993.