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Analytical Methods

Estimation of aroma precursors in radiation processed fenugreek

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ABSTRACT

Bound volatile compounds of fenugreek were isolated and identified. The glycoside profile was dominated by phenyl glucopyranoside accounting for 90% of the total glycosides. The content of this glycoside as estimated both by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC)-densitometry was found to be approximately 0.7 mg gm^{-1} of fenugreek. Gamma-radiation processing resulted in dose dependent break down of phenyl glucopyranoside with a reduction by almost 30% at a dose of 10 kGy. A corresponding increase in phenol content with dose was also observed in the steam volatile oil of the irradiated spice. Based on pulse radiolytic studies the mechanism of radiolytic cleavage was shown to occur via a carbon centred radical. Estimation of absorbed dose based on phenol released during radiation processing is proposed herein. The method is simple and rapid and could estimate absorbed dose in the range of 2.5–10 kGy within an error of 15%.

1. Introduction

Bound volatile constituents such as aroma glycosides are an important class of nonvolatile precursors that are ubiquitous in plant kingdom (Sarry & Günata, 2004; Winterhalter & Skouroumounis, 1997). They are currently gaining increased interest and attention for their role in imparting unique aroma to plant derived foods (López, Ezpeleta, Sánchez, Cacho, & Ferreira, 2004; Sarry & Günata, 2004; Sánchez Palomo, Pérez-Coello, Díaz-Maroto, González Viñas, & Cabezudo, 2006; Winterhalter & Skouroumounis, 1997). They exist mainly as mono and diglycoside derivatives (Winterhalter & Skouroumounis, 1997) and rarely as trisaccharide glycoconjugates. These compounds have been isolated and characterised in several food stuffs where their contribution to the characteristic aroma has been established. Most of the studies on this class of compounds relate to the isolation and characterisation of hydrolytically released aglycones. Very few reports exist on the stability of these compounds during post-harvest processing and storage.

Amongst the newer non-thermal methods for post-harvest hygienization of food, radiation processing using gamma radiation/electron beam occupies a unique position (Raso & Barbosa-Canovas, 2003). Extensive studies have established the efficacy of this process as a safe method for preservation of food without producing any organoleptic changes at the recommended doses (Diehl, 1995). With a ban on chemical fumigation world over due to its adverse effects on human health and environment, processing of food by gamma radiation/electron beam has attained greater significance in recent years (Thakur & Singh, 1994).

Amongst the food products that are amenable to radiation processing, spices occupy a prime position. They are an important class of food commodity widely traded for their unique aroma and flavour. Fenugreek is an important commercial spice extensively used for its flavouring and pharmacological properties. The aroma of the spice is mainly contributed by its steam volatile essential oils. Sotolon (3-hydroxy-4, 5-dimethyl-2(5H)-furanone) a hemiterpenoid γ-lactone has been identified as the character impact compound of the spice (Sauvare, Petit, Baissac, & Ribes, 2000). Bound aroma precursors in several spices have been extensively investigated. However, no report exists on the aroma precursors of fenugreek.

Changes induced by ionising radiation at the doses approved for food applications are minute and rarely specific to the treatment. This makes identification of the treatment in the processed product a challenging task. A significant amount of research work in this field has been carried out (Delincee, 1998). Methods proposed, however, cannot be applied for routine field application. Development of a simple and rapid method for detection of radiation processed food is thus an urgent need. We have earlier reported a dose dependent decrease in glycosidic precursors of nutmeg during radiation processing. Monitoring decrease in these constituents was proposed as a method for detection of the irradiated spice (Arul, Variyar, & Sharma, 2006).

The present study thus aims at isolation, identification and estimation of major glycosidic precursors in fenugreek. Effect of radiation processing on the stability of this important class of precursor molecules will be investigated. Possibility of monitoring

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specific compounds released from bound glycosidic conjugates into the steam volatile constituents as an aid in identifying radiation processed fenugreek will be examined.

2. Experimental

2.1. Materials

Three separate lots of dry fenugreek seeds were procured from a local market. Each lot was divided into two sets. One set was kept as non-irradiated control sample. The other set was subjected to gamma radiation in air in a cobalt-60 Food Package Irradiator, MDS Nordion Int. (Kanata, Ontario, Canada) at a dose rate of 10 Gy min^{-1}. Absorbed doses between 2 and 10 kGy were given to the samples. Dosimetry was performed by a Fricke dosimeter (Fricke & Hart, 1966, Chapter 12). The seeds were powdered before analysis. Both the control and radiation processed samples in each lot were analysed in triplicate for volatile oils and aroma glycosides within one week of storage as described in the following sections. In a separate set of experiment three different samples of fenugreek were subjected to radiation doses (not revealed to the authors) as above and the samples were then analysed in triplicate for essential oil composition. The content of phenol estimated was used for determining absorbed dose. This experiment was designated as blind trial. All solvents (analytical reagent grade) including HPLC grade solvents were redistilled before use. High purity (>99.9%) N_2O was procured from British Oxygen Co. (Mumbai, Maharashtra, India).

2.2. Methods

2.2.1. Isolation and analysis of aroma glycosides of fenugreek

Aroma glycosides were extracted from fenugreek (50 g) according to the procedure reported earlier (Arul et al., 2006). Aroma glycosides present in the extract were then recovered from this solution by passing through a column of Amberlite XAD-16 essentially according to the procedure of Gunata, Bayonove, Baumes, and Cordonnier (1985). The isolate thus obtained was made to 1% solution in methanol.

2.2.2. Thin layer chromatography (TLC) analysis

TLC of the glycosidic conjugates was carried out on silicagel G plates using ethyl acetate:iso-propanol:water (65:30:15) as developing solvent system (Arul et al., 2006). Separated bands were identified either by exposing the plate to iodine vapour or by heating the plate at 180 °C for 30 min after spraying with 50% sulphuric acid. The major band at R_f 0.85 isolated from prep TLC was eluted with methanol, evaporated to dryness and dissolved in the same solvent to make a 1% solution. Quantitative estimation of glycosidic conjugates was performed on a dual wavelength flying spot scanning densitometer, CS-9301PC, Shimadzu, (Kyoto, Japan). The density of the spots of interest was determined in the reflectance mode at a wavelength of 529 nm. Aliquots of suitably diluted samples were spotted on the plate and the concentration of the individual spots in the sample (control as well as irradiated) was obtained from the standard curve (correlation coefficient 0.99) prepared using phenyl-β-D-glucoside (linear in the range 2–25 μg) and expressed as μg g^{-1} of food sample.

2.2.3. High performance liquid chromatography (HPLC) analysis

HPLC analysis was carried out on a Jasco HPLC system, Jasco Corporation (Tokyo, Japan) equipped with a C-18 reverse phase stainless steel column (30 cm × 0.46 cm) and a PDA detector set at a wavelength of 275 nm. Samples of the above total glycosides as well as those isolated from TLC (10 μl, 0.01% solution in methanol each) were injected on to the column and then eluted with water as solvent A and acetonitrile as solvent B. A gradient elution from 0% to 100% B in A over a period of 30 min, at a flow rate of 1.0 ml min^{-1} was carried out (Ly, Yamauchi, Shimoyamada, & Kato, 2002). Peaks were identified by comparing their retention times with that of authentic standards injected under identical conditions. Content of glycosidic conjugates was estimated from a standard curve (correlation coefficient 0.99) prepared using phenyl-β-D-glucopyranoside (linear in the range of 2–20 μg). Content of the phenyl-β-D-glucopyranoside present in each of the samples was expressed as μg g^{-1} of food sample.

2.2.4. Acid hydrolysis of aroma glycosides

A part of the aroma glycoside (1% (w v^{-1}) solution) from the control and irradiated samples as well as TLC isolated band at R_f 0.85 were subjected to acid hydrolysis (1 M HCl, 1 h, 80 °C). The free aroma was extracted with diethyl ether as reported earlier (Arul et al., 2006) and then subjected to Gas chromatography-Mass spectrometry (GC/MS) analysis. In case of the TLC isolated band the remaining aqueous solution was neutralised with 1 N KOH, dried under vacuum and the residue dissolved in dry methanol. This methanol solution was subjected to TLC in order to identify the sugar residue. The acetylated sugar residue was also analysed by GC/MS to confirm the nature of the carbohydrate moiety. Glucose was the only sugar moiety identified in the isolated band.

2.2.5. Pulse radiolysis

The pulse radiolysis system using 7 MeV electrons, generation and studies on the free radical reactions have been described earlier (Adhikari & Mukherjee, 2001). Dosimetry was carried out using an air-saturated aqueous solution containing 5×10^{-2} M KSCN assuming $G\epsilon$ for $(SCN)_2^-$ = 23,889 M^{-1} cm^{-1} per 100 eV at 500 nm (Buxton & Stuart, 1995). The width of the electron pulse was 50 ns and the dose per pulse was 15 Gy. The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The bimolecular rate constant was calculated by plotting the pseudo-first order rates of formation of the transient species against the corresponding solute (reactant) concentrations. The uncertainty in the measurement in bimolecular rate constant is less than ±10%.

2.2.6. Isolation of volatile oil

Ground fenugreek (50 g) samples were subjected to steam distillation using simultaneous distillation–extraction technique according to the procedure described earlier (Variyar, Ahmad, Bhat, Niyas, & Sharma, 2003). Peroxide free diethyl ether, S.D. Fine-Chem. Ltd., (Mumbai, Maharashtra, India) was used as extracting solvent. The essential oils thus obtained (2% in ether) were then analysed by gas liquid chromatography (GLC) as described below. Each sample lot as described above was analysed in triplicate making a total of nine repetitions for each food material.

2.2.7. Analysis of essential oil

Analysis of essential oil was carried out using a GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph and provided with a DB-5, J&W Scientific (Folsom, CA, USA) capillary column ((5%-phenyl)-methylpolysiloxane, length, 30 m; id., 0.25 mm and film thickness, 0.25 μm). The operating conditions were: column temperature programmed from 60 to 200 °C at the rate of 4 °C min^{-1}, held at initial temperature and at 200 °C for 5 min. and further to 280 °C at the rate of 10 °C min^{-1}, held at final temperature for 20 min; Injector and interface temperatures, 210 and 230 °C, respectively; carrier gas helium (flow rate, 0.9 ml min^{-1}); ionisation voltage, 70 ev; electron multiplier voltage, 1 kV. Samples were analysed in scan mode in the mass range of m z^{-1} 50–600. Peaks were tentatively identified