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Effect of post harvest radiation processing and storage on the volatile oil composition and glucosinolate profile of cabbage

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ABSTRACT

Effect of radiation processing (0.5–2 kGy) and storage on the volatile oil constituents and glucosinolate profile of cabbage was investigated. Among the volatile oil constituents, an enhancement in *trans*-hex-2-enal was noted on irradiation that was attributed to the increased liberation of precursor linolenic acid mainly from monogalactosyl diacyl glycerol (MGDG). Irradiation also enhanced sinigrin, the major glucosinolate of cabbage that accounted for the enhanced allyl isothiocyanate (AITC) in the volatile oils of the irradiated vegetable. During storage the content of *trans*-hex-2-enal increased immediately after irradiation and then returned to the basal value within 24 h while the content of sinigrin and AITC increased post irradiation and thereafter remained constant during storage. Our findings on the enhancement in potentially important health promoting compounds such as sinigrin and AITC demonstrates that besides extending shelf life and safety, radiation processing can have an additional advantage in improving the nutritional quality of cabbage.

1. Introduction

Leaves of *Brassicaceae* family are recognised for their nutritional value and are familiar components of salads around the world. Fresh leaves of cabbage (*Brassica oleracea*), a vegetable of the *Brassica* family are used for preparation of a wide variety of recipes including delicacies like sauerkraut and kimchi. They possess a typical flavor and odor attributed to volatile sulfur compounds (Eskin, 2012). Isothiocyanates have been shown to be the major compounds that impart pungent flavor and sulfurous aroma to these vegetables. Cruciferous vegetables including cabbage have also been extensively investigated recently for their contribution to the anticarcinogenic compounds in the diet (Traka & Mithen, 2009). Isothiocyanates have been reported to be mainly responsible for the observed chemoprotective activity of these vegetables (Traka & Mithen, 2009). The isothiocyanates are the hydrolytic products of sulfur containing glucosides namely glucosinolates. Cleavage of the glucose moiety from glucosinolates by enzyme myrosinase in the presence of water results in an unstable aglycone that gets converted to a thiocyanate, an isothiocyanate or a nitrile (Traka & Mithen, 2009). These hydrolytic products are the active substances produced by plant as defence against pathogens. Due to their anticarcinogenic properties, glucosinolates and their hydrolysed products have generated considerable interest as nutraceuticals. Fresh vegetables possess a green odor that also contributes to their organoleptic quality. These odors are attributed to the release of C_6 aldehydes and alcohols and their corresponding esters, collectively termed as green leaf volatiles (GLVs) (Hatanaka, 1996). Unsaturated fatty acids liberated from galactolipids, phospholipids and triglycerides of plastid membranes have been demonstrated to be the precursors in the formation of these compounds (Hatanaka, 1996). A group of lipid hydrolysing enzyme called lipases release fatty acids from the membrane lipids. The released fatty acids are acted upon by enzymes such as a non-heme iron dioxygenase called lipoxygenase (LOX) and further by hydroperoxide lyase (HPL) of the lipoxygenase pathway to form C6 aldehydes and alcohols (Hatanaka, 1996).

Widespread outbreak of food borne illness worldwide in recent years has been associated to the consumption of fresh leafy vegetables. Traditional methods for elimination of food borne pathogens from these vegetables such as blanching and mild heat treatment can result in lowering their sensory quality. This necessitates the use of non thermal methods for reducing health risks. In this regard, use of radiation processing for elimination of food borne pathogens as a viable alternative, while maintaining fresh attributes of the produce, has been recognised (Arvanitoyannis, 2010; Arvanitoyannis, Stratakos, & Tsarouhas, 2009). However, the effect of such a processing on the flavor, aroma and bioactive constituents of cruciferous vegetables has not been extensively investigated. We report here the effect of radiation processing at recommended doses on the content of GLVs and isothiocyanates in cabbage. The impact of radiation processing on the enzymes of the lipoxygenase pathway and on the content of GLV as well as

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on isothiocyanate precursor, glucosinolates, is of specific interest and will be investigated.

2. Materials and methods

2.1. Materials

Cabbage (*B. oleracea*) samples of BC-79 and NS-22 varieties were obtained from farmers of Akola district, Maharashtra, India. The samples were authenticated at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola as belonging to the above varieties. Harvesting was done 65 days after planting when the vegetable was known to be mature. A variety of unknown origin was also obtained from a local market in Mumbai for comparison and was designated as market sample.

Chemicals were purchased from various suppliers: *trans*-hex-2-enal, sinigrin, tripalmitylglycerol, linoleic acid and linolenic acid from Sigma–Aldrich (USA); allyl isothiocyanate from Fluka, Sigma–Aldrich (USA); Monogalactosyldiacylglycerol (MGDG), Digalactosyldiacylglycerol (DGDG) from Avanti polar lipids (India); lipoxygenase, and sulfatase from Sigma–Aldrich (USA). All solvents were procured from Merck (India) and redistilled before use.

2.2. Irradiation of cabbage samples

Cabbage samples were subjected to gamma irradiation using a ^{60}Co gamma irradiator (GC-5000, BRIT, India, dose rate 4.1 kGy/h) in air to an average absorbed dose of 0.5, 1, and 2 kGy. Dosimetry was carried out using Fricke dosimeter.

2.3. Simultaneous steam distillation extraction and GC–MS analysis

Blended cabbage leaves (200 g) were subjected to steam distillation using simultaneous distillation–extraction technique as described earlier (Variyar, Ahmad, Bhat, Niyas, & Sharma, 2003). The essential oils (mg/wet weight) thus obtained were then subjected to GC–MS analysis using similar parameters as described earlier (Variyar et al., 2003). Peaks were identified by comparing their mass fragmentation pattern (Wiley/NIST Libraries), retention time and Kovats index with standards. The amount of each individual compound present in the sample was calculated by mean of the internal standard, and expressed as mg per kg of dry weight.

2.4. Extraction and analyses of lipids

Cabbage leaves (300 g), ground in liquid nitrogen were extracted in 900 mL of chloroform: methanol (2:1) as reported earlier (Chatterjee, Variyar, & Sharma, 2010). The total lipid extract thus obtained was subjected to silica gel TLC (Kieselgel 60, Merck, Germany). Neutral lipids were analysed using solvent mixture of hexane:diethyl ether:acetic acid (80:20:2) while phospholipids were separated and identified using ethyl acetate:2-propanol: chloroform:methanol:0.25% aq KCl (25:25:25:10:9) as the developing solvent system. Separation of galactolipids was carried out using chloroform: methanol: water (80:18:2) as the solvent system. The individual lipid class was identified from R_f values of standards spotted separately on the same plate. The separated spots were visualised by exposing to iodine vapor and the area of the individual spots was quantified on a TLC-densitometer (CS9301PC, Shimadzu, Japan) from a standard curve of spot area vs. concentration using different concentrations of standard lipid species referred above. Free fatty acids were isolated using 50 mg of lipid extract containing dodecanoic acid (50 μg) as internal standard and analysed by GC/MS after converting to methyl esters using diazomethane under similar parameters (Chatterjee et al., 2010). To analyse fatty acid composition of MGDG and TAG, total lipid extracts were subjected to preparative (0.5 mm thickness) silica gel TLC using solvent system used for neutral lipid and galactolipid separation. The bands corresponding to TAG and MGDG were isolated, hydrolysed, methylated with diazomethane and subjected to GC–MS.

2.5. Lipase assay

Cabbage leaves (20 g) were extracted in 60 mL of ice cold extraction buffer (0.1 M TrisHCl, pH 8) containing 0.1 M KCl, 0.1% Triton X-100 and 2 g PVPP as reported earlier (Pérez, Sanz, Olías, & Olías, 1999). Lipase activity was measured by quantifying spectrophotometrically (410 nm) the *p*-nitrophenol (λ_{max} 410) released following hydrolysis of *p*-nitrophenyl laurate substrate by lipase as described previously (Pisirodom & Parkin, 2001). Reaction was started by the addition of 1 mL enzyme extract to 2.5 mL 420 μM *p*-nitrophenyl laurate in 2.5 mL Tris–HCl buffer (0.1 M, pH 8.2). Absorbance was monitored in a spectrophotometer (UV-2450, Shimadzu, Japan) up to 15 min. p-Nitrophenol standard curve was used to convert absorbance to μM substrate hydrolysed.

2.6. Lipoxygenase assay

Crude enzyme was extracted in sodium phosphate buffer according to Gardner (2001). Lipoxygenase activity was measured as conjugated diene formed (Gardner, 2001). The reaction mixture contained linoleic acid (7.5 mM, 10 μl) and 30 μl crude extract made up to volume (3 mL) with 0.1 M acetate buffer (pH 5). Absorbance was measured for 10 min using a spectrophotometer. An extinction coefficient of 25,000 $M^{-1}cm^{-1}$ was used to convert absorbance values at 234 nm to μmol of conjugated diene.

2.7. Hydroperoxide lyase assay

Extraction procedure followed was same as for lipase assay. HPL was assayed by the loss in absorption at 234 nm by the hydroperoxide (Vick & Zimmerman, 1976). Briefly, linoleic acid substrate (0.6 mL of 7.5 mM) was incubated with 1.12 mg of soybean lipoxygenase (100,000 units/mg) in 30 mL distilled water for 1 h to obtain a solution of hydroperoxide substrate. The final reaction mixture contained 250 μl of the hydroperoxide substrate prepared earlier and 250 μl of enzyme solution made up to a volume of 3 mL with potassium phosphate buffer (0.1 M, pH 6). Readings were taken for 10 min by a spectrophotometer. An extinction coefficient of 25,000 M^{-1} cm^{-1} was used to convert absorbance values at 234 nm to μmol of products formed.

2.8. Analysis of end products of lipid oxidation

Cabbage sample (30 g) was blended in a homogenizer with 100 mL of ice cold phosphate buffer (50 mM, pH 7), containing 0.2 mM EDTA, 0.2% TritonX-100 and 2 g PVPP. Resulting homogenate was vacuum filtered and the residue washed 2 times with 25 mL of buffer. Extracts were centrifuged at 14,000 rpm for 20 min at 4 °C. To 2 mL of supernatant, 2 mL of 10 mM acid substrate (linolenic or linoleic acid) in 10 mL phosphate buffer (100 mM, pH 7) was added and incubated for 30 min. Reaction was stopped by adjusting pH to 3. The mixture was passed through a C18 extraction column (Superclean ENVI-18 SPE, 500 mg) and the products eluted with methanol. The residue after removal of methanol was esterified with 2 M methanolic KOH and subjected to GC–MS analysis.