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EFFECT OF GAMMA-RADIATION ON MAJOR AROMA COMPOUNDS AND VANILLIN GLUCOSIDE OF CURED VANILLA BEANS (*VANILLA PLANIFOLIA*)

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ABSTRACT

Radiation processing of food materials by gamma-radiation is a well-established method for microbial decontamination and insect disinfestation. Irradiation of spices at doses ranging from 10 to 30 kGy has been reported to result in complete elimination of microorganisms with negligible changes in the flavour quality. The effect of gamma-radiation on microflora and vanillin content of cured vanilla beans in the dose range of 5 - 50 kGy has been investigated, but its effect on other major aroma compounds and vanillin glucoside (vanillin aroma precursor) remaining after curing have not been studied so far. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one such compound used as a flavouring agent and as a dietary component. It is the major component of natural vanilla, which is one of the most widely used and important flavouring materials throughout the world. Vanillin is an antioxidant capable of protecting membrane against lipid peroxidation and DNA against strand breaks induced by reactive oxygen species. The present work was aimed to study the effect of gamma-radiation processing on the major aroma compounds of cured vanilla beans and also to investigate possible enhancement in vanillin content by the radiolytic breakdown of vanillin glucoside present already. Cured vanilla beans were irradiated (5, 10, 15, 20 and 30 kGy) and the vanillin content of control and irradiated samples were analysed, respectively for possible enhancement of vanillin content by radiolysis of vanillin glucoside. Radiolytic breakdown of glycosidic precursors of aroma constituents and consequent release of free aroma was shown to result in the enhancement of aroma quality of these products. Since a considerable amount of vanillin exists as its glycosidic precursor in cured vanilla pods, a possible enhancement in yield of vanillin by radiation processing is thus expected. Hence the highly stable oxygen–carbon linkage between vanillin and glucose limits the possible enhancement of aroma quality of irradiated beans.

Keywords: Aroma glycosides, gamma irradiation, *Vanilla planifolia*, vanillin, vanillin glucoside

INTRODUCTION

The characteristic aroma of vanilla is only obtained after a curing process of green fruits of the orchid *Vanilla planifolia*. The harvested fruits, called beans or pods, contain many different glucosidic compounds. The curing process is meant to hydrolyse the glucosides and to release the aroma compounds. β-Glucosidases are believed to play an important role in this process (Arana, 1943). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is one of the most widely used and important flavouring materials worldwide (Ranadive, 1994). Worldwide total demand for vanillin is more than 10,000 tons per year, of which less than 0.5% is met out by vanillin isolated from vanilla beans (Priefert et al., 2001). The rest is produced synthetically, mostly from petrochemicals, such as guaiacol and lignin (Clark, 1990).

Cured vanilla beans have moisture content in the range of 25 - 30%. They are generally kept in cartons or wooden boxes for conditioning (3 - 6 month's period) and subsequent storage. The conditions prevailing in these boxes are conducive for fungal growth. Thomas and Bindumol (2005) reported microbial spoilage of cured vanilla beans by fungal species belonging to the genera Aspergillus and Penicillium. Radiation processing of food materials by gamma-radiation is a well-established method for microbial decontamination and insect disinfestation. Irradiation of spices at doses ranging from 10 to 30 kGy has been reported to result in complete elimination of microorganisms with negligible changes in the flavour quality (Farag et al., 1995). The effect of gamma-radiation on micro flora and vanillin content of cured vanilla beans in the dose range of 5 - 50 kGy has been investigated by Bachman et al. (1995), but its effect on other major aroma compounds and vanillin glucoside (vanillin aroma precursor) remaining after curing have not been studied so far.

Recent studies have demonstrated the role of radiation processing in improving the sensory quality of spices, such as saffron (Zareena et al., 2001) and nutmeg (Ananthakumar et al., 2006). Radiolytic breakdown of glycosidic precursors of aroma constituents and consequent release of free aroma was shown to result in the enhancement of aroma quality of these products. Since a considerable amount of vanillin exists as its glycosidic precursor (7.74 ± 0.20 mg/g) in cured vanilla pods, a possible enhancement in yield of vanillin by radiation processing is thus expected. Results with enzymatic preparations have demonstrated that as much as half of the amount of vanillin sequestered in the complex cellulose matrix is in either free or bound glycosidic forms, and can be liberated by enzyme assisted procedures (Ovando et al., 2005; Ruiz-Teran et al., 2001). Hence, the present work was aimed to study the effect of gamma- radiation processing on the major aroma compounds of cured vanilla beans and also to investigate possible enhancement in vanillin content by the radiolytic breakdown of vanillin glucoside present already.

MATERIALS AND METHODS

Materials

Cured vanilla beans were procured from Indonesia. Beans were stored at 4^{o}C until analysis. Standard compounds were procured from Sigma–Aldrich Chemical Company. All solvents including HPLC grade were purchased from E. Merck Sing Ltd., Singapore. Solvents were distilled before use. Other chemicals and reagents of AR grade were obtained from local supplier in Malaysia.

Irradiation of the Vanilla Beans

Five different doses (5, 10, 15, 20 and 30 kGy) of gamma-radiation were given to cured vanilla beans samples of 250 g each. All the treatments were performed in triplicate. Irradiation was carried out at MINTec-Sinagama, Bangi (Cobalt-60 Irradiator JS10000 serial no. IR-219), with dose rate of 2.14 kGy/h.

Extraction of Vanilla Beans

The procedure developed by Ranadive (1992) was followed with some modifications. Cured vanilla bean samples (3 g) from each treatment were dried and ground using a pestle and mortar. 2 g of the powdered cured vanilla were weighed and placed in a thimble and extracted using a Soxhlet

Extractor for 8 hours using ethanol as the solvent. The resultant extract were concentrated using a rotary evaporator. Exactly 50 ml of the supernatant were taken and dried completely under vacuum and the residue was made up to 10 ml using hexane. These solutions were analysed by GC-MS for qualitative and quantitative changes in aroma profile of irradiated vanilla beans.

LC-MS Analysis

An Agilent 1200 series LC–MSD Trap G2445 VL (Agilent Technologies, Palo Alto, CA, USA) was used for the analysis. The system consists of an auto sampler, a degasser unit and a quaternary pump, coupled in series with an ion trap mass selective detector (MS). The MS consisted of an electrospray ionization (ESI) interface. The LC-MS system was controlled by LC-MS Trap control software, version 5.2 (Agilent Technologies, Palo Alto, CA, USA), and data acquisition was analyzed, on a computer equipped with Agilent Chemstation software, version A. 10.02. For optimization of LC conditions, an Eclipse XDBC-18 column of 4.6 mm × 150 mm and 5 µm particle size was used. The injection volume was 1 µL, the flow-rate was 10 µL/min and the run-time was 35 min using ESI interface. Chemical compounds were identified by matching their retention indices with retention times of standards.

GC-MS Analysis

Gas Chromatography - Mass Spectrometry (GC-MS) analysis was carried out on a Finnigan Trace GC Ultra (Thermo Scientific Corp), Finnigan, equipped with a Polaris Q mass spectrometer and provided with a DB-5 (J&W Scientific, Folsom, CA) capillary column (length: 30 m; i.d.: 0.25 mm and film thickness: 0.25l m). The operating conditions were: column temperature programmed from 60 to 200^{o}C at the rate of 4^{o}C/min, held at initial temperature and at 200^{o}C for 5 min and further to 280^{o}C at the rate of 10^{o}C/min, held at final temperature for 20 min; injector and interface temperatures, 210 and 230^{o}C, respectively; carrier gas helium (flow rate: 0.9 ml/min); ionization voltage: 70 eV; electron multiplier voltage: 1 kV. Samples (0.5 µl) were injected in splitless mode. Peaks were identified by comparing their mass fragmentation pattern with that of standard compounds as well as with the data available in the spectral library (Wiley/NIST Libraries) of the instrument.

RESULTS AND DISCSSION

Effect of Radiation Processing on Aroma Profile of Irradiated Cured Beans

Natural vanilla aroma is a mixture of more than 200 volatile compounds. Among these, four major compounds collectively constitute 97% w/w of the total aroma compounds reported. They include vanillin (86% w/w), vanillic acid (6% w/w), p-hydroxybenzaldehyde (4% w/w) and p-hydroxybenzoic acid (1% w/w) (Perez-Silva et al., 2006). The aqueous ethanol extracts from treated samples (control, 5, 10, 15, 20 and 30 kGy) were analysed by LC-MS as per the procedure described earlier. Bachman et al. (1995) studied the effect of gamma-radiation on vanillin content. No change in the vanillin content was reported, even at a dose of 50 kGy (Figure 1). Analysis of the same extract with GC-MS also did not show any major difference in the content of the control and irradiated beans (Figure 2 and 3). Presence of the four major compounds was confirmed using authentic standards. The quantitative distribution of the four major aroma compounds in samples subjected to different radiation doses are presented in Table 1. The content of the four major aroma compounds identified and reported in this study also showed no significant quantitative changes up to a dose of 30 kGy. Hence, gamma radiation may be the method of choice for hygienisation of cured vanilla beans without any significant effect on the aroma profile.

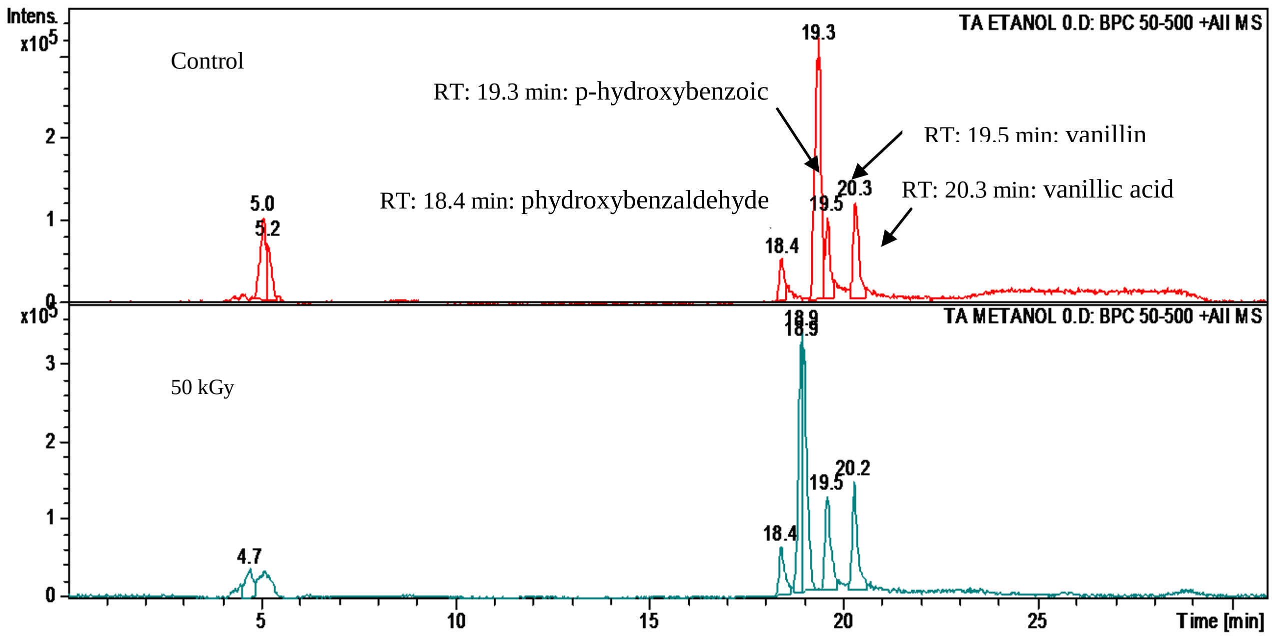

Figure 1: LC-MS chromatogram of aroma glycosides in control and irradiated beans

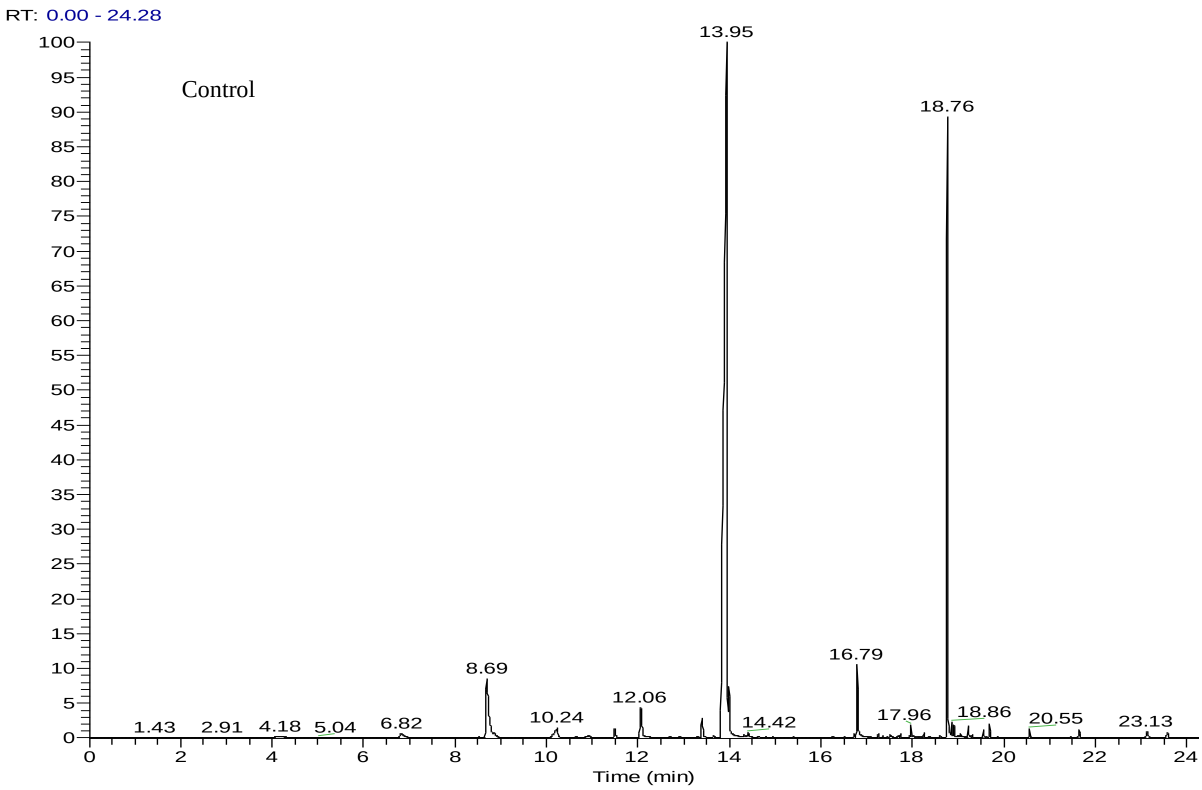

Figure 2: GC-MS chromatogram of vanillin glucosides in control beans

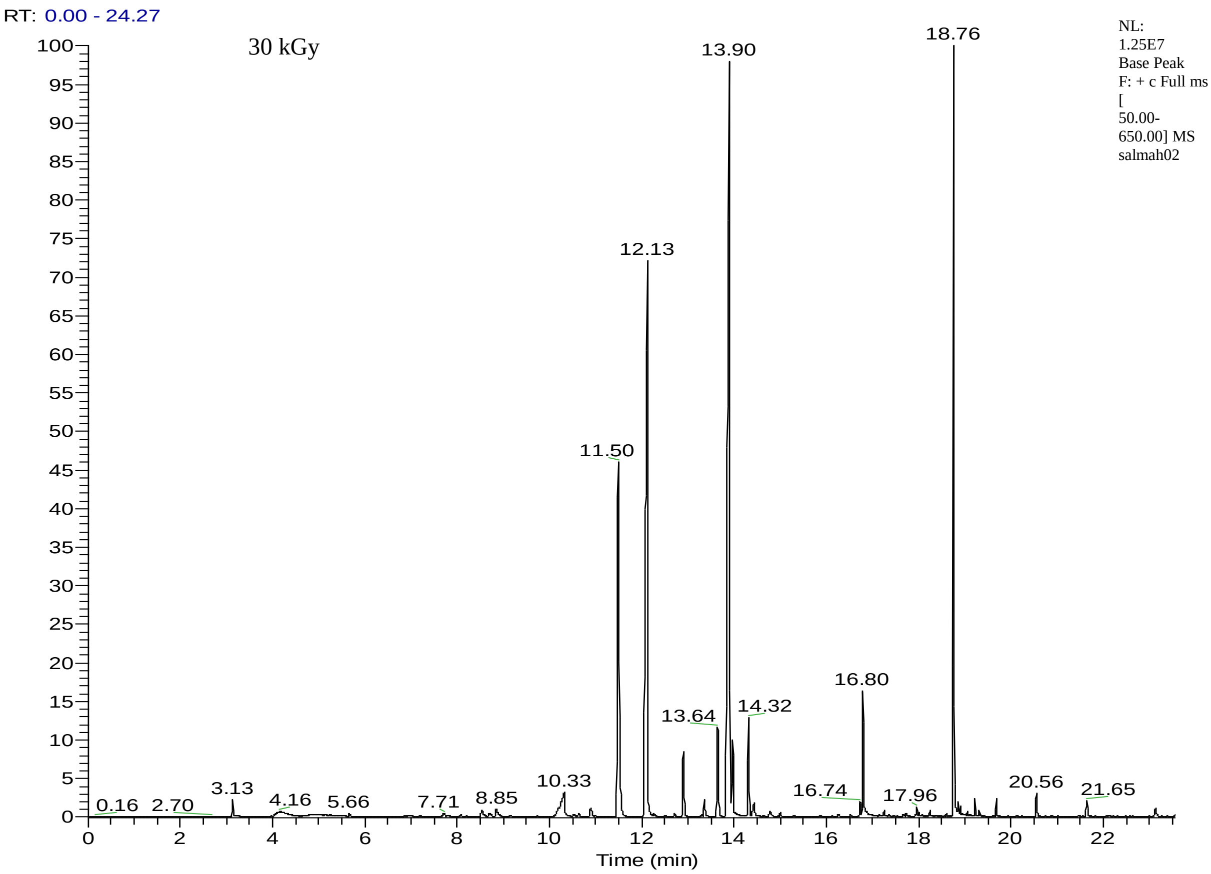

Figure 3: GC-MS chromatogram of vanillin glucosides in irradiated beans

Table 1: Contents of major aroma compounds and vanillin glucoside of cured vanilla beans irradiated at different doses of gamma-radiation (mg/g)

Aroma Constituents	**Irradiation Dose (kGy)**					
	0	**5**	**10**	**15**	**20**	**30**
Vanillin	26.60	26.50	25.80	25.62	26.20	26.91
Vanillic acid	0.93	0.91	0.90	0.95	0.94	0.95
p-hydroxybenzaldehyde	1.32	1.30	1.28	1.31	1.29	1.33
p-hydroxybenzoic acid	0.45	0.40	0.43	0.44	0.41	0.42
Vanillin glucoside	7.74	7.40	7.35	7.45	7.50	7.35

Effect of Radiation Processing on Vanillin Glucoside Content

Irradiation has been successfully used for the quality improvement of many agricultural products. Ananthakumar et al. (2006) reported the radiolytic breakdown of aroma glycosides in nutmeg. A linear increase in antioxidant isoflavones with radiation dose resulting from a breakdown of glycosidic precursors, was also noted in soybean (Variyar et al., 2004). In a recent work on the stability of phenol-□-D-glucopyranosides in fenugreek, Chatterjee et al. (2009) have demonstrated a radiolytic breakdown of glycosidic linkage, via a carbon-centred radical using pulse radiolysis studies. Thus carbon-oxygen linkages in the glycoside are susceptible to radiolysis. It was of interest to understand the stability of vanillin glucoside during radiation processing, with a view to improve the vanillin content in cured beans. Contents of vanillin and its glycoside as estimated by LC-MS and GC-MS in cured beans samples subjected to various doses of gamma-radiation, is provided in Table 1. No difference in either vanillin or glucovanillin content was noted in the samples subjected to different radiation doses up to 30 kGy. Thus unlike other glycosides reported above, the carbon–oxygen linkage in glucovanillin appears to be quite stable. This observation warranted understanding the mechanism of stability of vanillin glucoside to radiolytic cleavage, so a pulse radiolysis experiment on vanillin glucoside was carried out.

CONCLUSIONS

The gamma-radiation processing (up to 30 kGy) did not cause any significant changes in the aroma profile of cured vanilla beans both qualitatively and quantitatively. This study showed that, gamma-radiation may not be a useful alternate to □-glycosidase enzyme for the release of vanillin from its glycoside precursor, either for the purpose of aroma enhancement or for accelerated curing. But gamma-radiation may be useful in rupturing the cellulose matrix of fresh vanilla beans, thereby enhancing the contact between □-glycosidase enzyme and vanillin glucoside, which are separately located in the outer fruit wall region and the placental region, respectively. However, gamma- radiation is the best tool for the complete hygienisation of vanilla beans from spoilage and toxin-producing microbes without altering its aroma quality and market value.

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