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Identification and quantification of methyl nicotinate in rice (*Oryza sativa* L.) by gas chromatography–mass spectrometry ☆

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Abstract

During the course of analysis of popular rice varieties cultivated in India towards identification of their aroma compounds, methyl nicotinate (MN), a medicinal and flavour additive compound, was identified for the first time in rice samples. A simple direct solvent extraction method using 300 mg of the sample is developed to extract MN in rice samples and detected by capillary gas chromatography–mass spectrometry analysis. Quantitative analysis of MN is performed for polished rice, brown rice and rice bran samples from five rice varieties that are widely produced in India by using GC–MS operating under SIM mode (*m/z* 106). The quantity of MN is in the range of 0.63–1.30 μg/g, 1.37–3.99 μg/g and 1.87–12.04 μg/g for polished rice, brown rice and rice bran samples, respectively. Breeding programmes for rice with high concentrations of MN can be greatly facilitated by establishing the concentrations of the MN in new cultivars. This method is more economic with less time consumption and enables fast screening of a large number of samples.

Keywords: Methyl nicotinate; Rice; *Oryza sativa* L.; Solvent extraction; Gas chromatography–mass spectrometry; Flavoring agent; Medicinal compound

1. Introduction

Rice (*Oryza sativa* L.) is the most important food crop of the world and has a great influence on human nutrition and food security. Rice contains a number of bioactive compounds and ingredients that can be used in medicinal formulations. The medicinal values of rice have been described in ancient Indian books and rice is considered to be ocrid, oleaginous, tonic, aphrodisiac, fattening, diuretic and useful in biliousness (Caius, 1986). Rice bran is an important source of minerals, amino acids, proteins, carbohydrates, lipids, tocopherols, anthocyanins and tocotrienols (Escribano-Bailon, Santos-Buelga, & Rivas-Gonzalo, 2004; Jariwalla, 2001; Orthoefer, 1996; Qureshi, Mo, Packer, & Peterson, 2000; Qureshi, Qureshi, & Wright, 1991; Saunders, 1985). More than 100 compounds have been identified in the aroma of rice, among them 2-acetyl-1-pyrroline was identified as the principal aroma compound (Buttery, Ling, Juliano, & Turnbaugh, 1983; Tanchotikul & Hsieh, 1991; Tsugita, 1986; Widjaja, Craske, & Wootton, 1996). 2-Acetylpyridine was also established, for the first time, as the characteristic aroma compound of Xiangjing-8618 rice (a scented rice, *Oryza sativa* L.) (Jianming, 2002). Among the compounds that have high medicinal values, Oryzanol has been extensively studied (Fang, Yu, & Badger, 2003; Juliano, Cossu, Alamanni, & Piu, 2005; Miller, Frenzel, Schmarr, & Engel, 2003; Xu & Godber, 1999).

During the analysis of rice samples for aroma and bioactive compounds in rice, we observed the presence of

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methyl nicotinate (MN) for the first time. It is an active ingredient in a number of medicinal products as a rubifacient and vasodilator for the treatment of a variety of diseases such as respiratory, vascular and rheumatoid disorders (Koivukangas, Oikarinen, Salmela, & Lahti, 2000; Wilkin et al., 1985). MN is a non-immunological compound and it helps in removing wrinkles, rejuvenates the skin (Caselli et al., 2003), induces local skin vasodilation (http://www.eudra.org//emea.html), and dilates the blood vessels for quicker, better absorption and it gives a warming sensation. It has been widely used in cosmetics as an ingredient in skin products like soaps, shampoo and creams due to its rubefacient nature (Riviere, Qiao, Baynes, Brooks, & Mumtaz, 2001). *Aenictus* species utilizes MN in its chemical communication system (Oldham, Morgan, Gobin, & Billen, 1994). MN is also an active flavour compound in fruits like Cupuacu (*Theobroma grandiflora*) (Franco & Janzantti, 2005; Pelissier et al., 1994), Soursop (*Annona muricata*) (Wong & Khoo, 1993), Mammee apple (*Mammea americana*) (Morales & Duque, 2002), Strawberries (*Fragraria Vesca*) (Pyysalo, Honkanen, & Hirvi, 1979), Papaya, Guava, headspace of the living orchids Calanthe izu-insularis Ohwi et Satomi (Orcidaceae), *Calanthe sieboldii* Decne (Orcidaceae) and Tuberose and flowers like calanthe (Orchidaceae) (Awano, Ichikawa, Tokuda, & Kuraoka, 1997). In the 63rd meeting of the Joint FAO/WHO experts committee on food additives (JECFA), MN has been accepted as a flavouring agent and there is no safety concern for intake (FAO/WHO Expert Committee on Food & Additives, 2004).

In the present work, we report the presence of MN, a medicinal and flavour additive compound, for the first time in the rice samples. MN was extracted by using direct solvent extraction technique from the polished rice, brown rice and rice bran using less amount of the sample, and its quantity has been estimated by GC–MS in selected ion monitoring (SIM) mode.

2. Materials and methods

2.1. Rice samples

Five rice varieties viz., Taroari basmati, Vasumathi, Yamini, Krishna hamsa, IR-64, which are popular rice varieties in India, were grown during kharif 2004 at Directorate of Rice Research, Hyderabad, India. The rice grains were stored at 4 °C in sealed polypropylene bags prior to analysis.

2.2. Chemicals

Methanol, acetone, acetonitrile, hexane, isopropyl alcohol, toluene, ethanol, tetrahydrofuran and dichloromethane used in this work are of HPLC grade and were procured from Merck (Mumbai, India). Methyl nicotinate (99%) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.3. Equipments

Ultrasonic bath Sonarex super 10P (Bandelin, Germany) was used for the extraction of MN from rice and rice bran samples. The generator of the ultrasonic bath has an output of 150 W and a frequency of 35 kHz. Standards and samples were weighed on BP1215 Sartorius analytical MIC balance (Sartorius, Germany).

2.4. Preparation of standard MN solutions

A 1000 mg/L standard solution of MN was prepared in methanol by dissolving 10 mg of MN in 10 mL of methanol. This solution was diluted with methanol to obtain the necessary concentrations (10 μg/mL to 100 ng/mL) to draw a calibration curve for quantification of MN in the samples.

2.5. Sample preparation

The rice samples collected from five varieties were hulled by a Class 35A rice machine (Satake, Japan) to get brown rice samples. The brown rice samples were milled for 90 s using a Class 05 grain-testing mill (Satake, Japan) to obtain polished rice samples and rice bran samples. Both brown rice and polished rice samples were grounded using disc grinder (Swantech International, France) with a 0.5 mm mesh screen prior to solvent extraction.

2.6. Solvent extraction

Solvent extraction was carried out initially by taking 10 g of brown rice sample and extracted with 10 mL of solvent (methanol). The mixture was sonicated for 60 min at room temperature and filtered through 0.45 μm membrane filter. The filtrate was concentrated by using a gentle stream of nitrogen at room temperature. In order to reduce total weight of sample, the method was later optimized by decreasing the amount of rice sample. Finally the method was optimized with 300 mg of rice sample and 500 μL of methanol for extraction. Powdered (polished and brown rice)/bran samples (300 mg) were transferred into 2 mL crimp cap vials (12 × 32 mm) and 500 μL methanol was added to each vial. The vials were crimp sealed tightly by using PTFE septa and aluminum seals. The vials were placed in the ultrasonic water bath and sonicated at various temperatures (30, 40, 50, 55, 60, 70 and 80 °C) for different time periods (30–150 min) to extract maximum MN. The optimized extraction temperature and time is 50 °C for 120 min. The vials were removed from the sonicator and kept at 4 °C for 10 min. The cooled vials were centrifuged at 5000*g* rpm for 20 min and 1 μL of the supernatant was injected into GC–MS system using an autosampler.