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Short communication

Gas chromatographic determination of eugenol in ethanol extract of cloves

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Abstract

An ethanolic extract of cloves was analyzed by gas chromatography directly to identify eugenol and other major phenolic compounds without previous separation of other components. Separation was performed on a fused-silica capillary column of 30 m×0.53 mm I.D., 0.53 μm film thickness. The detector was a flame ionization detector. Helium gas at a flow-rate of 3 ml/min was used as a carrier gas. The analysis were performed with linear temperature programming. Nine components were detected and special attention was given to the major phenolic compound, eugenol.

Keywords: Eugenol

1. Introduction

It is well known that cloves possess a phenolic compound, 4-allyl-2-methoxyphenol, commonly called eugenol ($C_{10}H_{12}O_2$). Eugenol acts as an antioxidant on oleaginous foods, as an anticarminative, an antispasmodic, an antiseptic in pharmacology and as an antimicrobial agent [1–3].

El-Beeb et al. [4] reported that by gas–liquid partition chromatography, clove oil had peaks of eugenol, methyl salicylate, limonene, carvone, α-pinene and eucalyptol. Deyama and Horiguchi [5] investigated the steam-volatile constituents of air-dried cloves by using gas chromatography (GC), infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometry (MS). They identified eugenol (80.87%), β-caryophyllene (9.12%), acetyleugenol (7.33%) and nine other major components. Application of direct ^{13}C NMR spectroscopy in the analysis of clove oil was reported by Kubeczka and Formacek [6,7]. Direct mass spectrometry measurement of a small piece of clove was mentioned in Ref. [8]. Kramer [9] identified eugenol and gallic acid as the two major antioxidants in cloves through the use of thin-layer chromatography (TLC), UV, IR, MS and high-performance liquid chromatography (HPLC). Smith and Beck [10] developed an HPLC method for eugenol in pimento spice using UV and electrochemical detection.

The aim of the present work was to identify eugenol and other major phenolic compounds in an ethanol extract of cloves directly, using GC, without

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previous separation of other components in the extract.

2. Experimental

2.1. Materials

Fresh green cloves were obtained from Kampung Batu Laut (Banting, Selangor, Malaysia). Analytical-grade reagent, Univar, 95% ethanol was used as the extraction solvent. HPLC grade, 99.9% methanol from Lab-Scan (Dublin, Ireland) was used as the sample solvent in GC analysis. Eugenol with purity of 99.9% and isoeugenol with purity greater than 98% were purchased from Merck (Darmstadt, Germany). Eugenol acetate (acetyl eugenol) and β-caryophyllene were purchased from TCI (Kasei, Japan).

2.2. Extraction method

Ground cloves (5 g) of particle size 250 μm were mixed with 25 ml 95% ethanol in a 50-ml conical flask and shaken for 2 h at 50°C. The temperature during extraction was controlled by immersing the flask in a thermostatically controlled water bath (Lab-Line Dubnoff Incu-Shaker Model 3575/3575-1, Lab-Line Plaza, Melrose Park, IL, USA). The extract was filtered through a Whatman No. 1 filter paper followed by filtration of the aliquot twice, through Whatman No. 540 filter paper, prior to GC analysis.

2.3. Sample preparation for analysis

Individual standard solutions of eugenol, iso-eugenol, β-caryophyllene and eugenol acetate were prepared by dissolving 0.1 ml of each compound in 10 ml methanol. A standard mixture of the four compounds was prepared by dissolving 0.1 ml of each compound in 40 ml of methanol. The clove ethanol-extract solution sample was prepared by dissolving 1 ml ethanol extract in 10 ml of methanol.

2.4. Analysis method

GC analysis was performed on a Hewlett-Packard HP 5890 fitted with a fused-silica capillary column (SPB™-1) of 30 m×0.53 mm I.D., 0.53 μm film thickness (Supelco, Bellefonte, PA, USA). A flame ionization detector was used. The sample (0.2 μl) was injected directly onto the column with a Hewlett-Packard 10-μl syringe. Preliminary analysis was performed on reference samples to determine the optimum operating conditions such as sample sizes, temperature parameters, flow-rates and attenuation according to chromatography operating hints described in Ref. [11]. The optimum operating conditions for the most satisfactory elution profile, composition and spaced peaks was attained with the GC oven temperature programmed at a rate of 6 C°/min from 80°C to 230°C. Detector and injector temperatures were set at 230°C and helium gas flow-rate at 3 ml/min. The same data acquisition was used for the clove ethanol-extract sample.

3. Results and discussion

The chromatogram for the standard mixture is shown in Fig. 1. The gas chromatogram of the clove ethanol-extract in Fig. 2 shows three major peaks

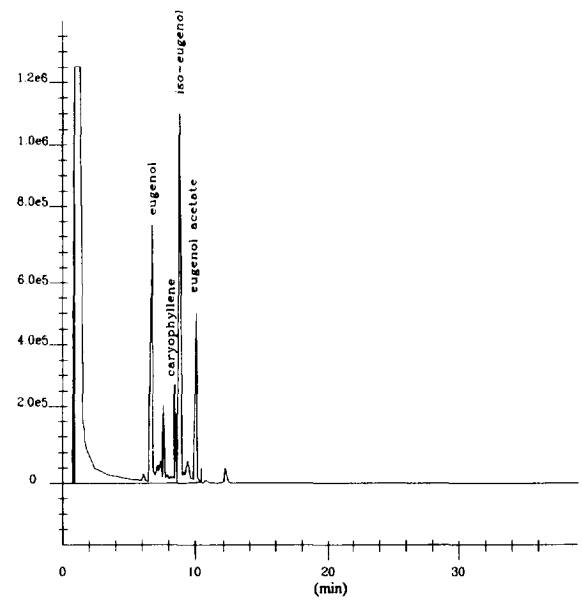

Fig. 1. Chromatogram of standard mixture. Conditions: fused-silica capillary column (30 m×0.53 mm I.D.), 0.53 μm film thickness. Column temperature programmed from 80°C (2 min) to 230°C at 6 C°/min. Helium gas flow-rate, 3 ml/min. Detection: flame ionization.

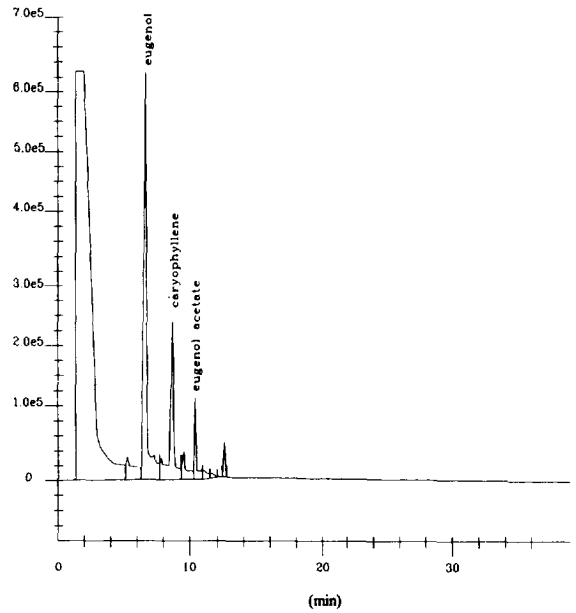

Fig. 2. Chromatogram of clove ethanol-extract. Conditions: fused-silica capillary column (30 m×0.53 mm I.D.), 0.53 μm film thickness. Column temperature programmed from 80°C (2 min) to 230°C at 6 C°/min. Helium gas flow-rate, 3 ml/min. Detection: flame ionization.

and six minor peaks. Identification of eugenol and other major phenolic compounds was made by comparing the retention time and peak appearance in the clove ethanol-extract in Fig. 2 with the standard mixture chromatogram in Fig. 1. The retention times and the chromatogram peak areas were reproducible for several different aliquots at 0.2 μl injection of the sample.

The three major peaks were identified as eugenol, caryophyllene and eugenol acetate. The eugenol peak, the main constituent and high proportion of the extract is quite significant. The three major phenolic compounds in the clove ethanol-extract are similar to the compounds in the steam volatile extract of air-dried clove [5] but the ethanol extract chromatogram shows less compounds as it displays, in particular, ethanol-soluble compounds of the cloves. Isoeugenol also naturally occurs in cloves and, on gentle oxidation, yields vanillin [12] but the isomer compound is not detected in this ethanol extract. No identification of the minor peaks has been done as this is outside the scope of this work.

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