chromatographic method yet devised for studies of amino acid composition of proteins and peptides. In all likelihood, HPLC techniques will continue to gain popularity for the routine analysis of amino acids.

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Registry No. Asp, 56-84-8; Glu, 56-86-0; Asn, 70-47-3; Ser, 56-45-1; Gln, 56-85-9; His, 71-00-1; Ala, 56-41-7; Arg, 74-79-3; Tyr, 60-18-4; Tau, 107-35-7; Val, 72-18-4; Met, 63-68-3; Ile, 73-32-5; Trp, 73-22-3; Leu, 61-90-5; Phe, 63-91-2; Lys, 56-87-1.

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Characterization of Mango-like Aroma in *Curcuma amada* Roxb.

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The essential oil of the rhizomes of *Curcuma amada* Roxb. was isolated by steam distillation, distillation–extraction, and low-temperature–high-vacuum distillation techniques, and its composition with respect to mango aromatic principles was determined by gas chromatography and spectrometry. The oil was primarily composed of terpene hydrocarbons tentatively identified as α-pinene, car-3-ene, and *cis*-ocimene, where the latter two compounds contributed essentially the characteristic mango odor of this rhizome.

Curcuma amada Roxb. is a rhizome cultivated mostly in India and Malaysia, where it is known as *Curcuma mangga valet* and having vegetative characteristics similar to those of *Curcuma longa*. The rhizomes of *C. amada* are called "Amada" in Bengali because of the characteristics odor of mangoes, superimposed over the mild turmeric and ginger odor, and hence they are popularly called as "mango ginger". Besides the medicinal use of this rhizome as a healing agent for sprains and bruises, it finds an extensive application in the preparation of sweetmeats, chutneys, and pickles because of its exotic mango aroma. However, little attention has been given to studying the volatile aroma components responsible for its characteristic mango aroma. Dutt and Tayal (1941) initially examined the chemical composition of steam volatile oil of mango ginger and reported ocimene as the major constituent besides linalool, linalyl acetate, and safrol. Ahuja and Nigam (1971), on the other hand, reported curcumene as the main component of the essential oil of this rhizome and failed to detect the above constituents. Recently Govindarajan (1980) reviewed the chemistry and technology of this rhizome along with other species and indicated that terpene compounds are presumably essential components of the steam volatile oil of *C. amada*. It appears that the mango aromatic principle of this rhizome has so far not been convincingly demonstrated. The purpose of the present paper was to reevaluate the essential oil composition of *C. amada* with a view to characterize the components contributing to the mango aroma of the rhizome.

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EXPERIMENTAL SECTION

Isolation of Essential Oil. Freshly harvested rhizomes of *C. amada* procured from a local market were cut into small pieces and blended with distilled water (1:2 w/v) in a Waring blender for 2 min. The pulp (200 g each) was subjected separately to conventional steam distillation, simultaneously distillation–extraction using isopentane as the extracting solvent according to the method of Nickerson and Likens (1966), and low-temperature–high-vacuum distillation techniques as described by Bandyopadhyay et al. (1973) for the isolation of the essential oil. The distillate obtained by conventional and vacuum distillation was extracted with peroxide-free diethyl ether (3 times, 200 mL each). The solvent extract was dried over sodium sulfate and the oil was recovered after removal of solvent in a flash evaporator at room temperature.

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Analytical Methods. *Thin-Layer Chromatography (TLC).* Silica gel G (E. Merck) spread 250 μm thick on 20 × 20 cm glass plates was activated by heating at 110 °C for 1/2 h before use. The essential oil in chloroform solution (200 μg) was spotted on the plate and the plate was developed by using petroleum ether (bp 40–60 °C)–diethyl ether (70:30 v/v) as the solvent system. A preliminary odor assessment of the separated components was done directly on the plate after complete removal of the solvent by flushing the plate with a slow stream of nitrogen according to the method of Bandyopadhyay et al. (1970). The chromatograms were then visualized by spraying the plate with 50% sulfuric acid followed by charring at 140 °C for 20 min. Some authentic reference samples (Aldrich Chemicals), e.g., linalool, linalyl acetate, geraniol, myrcene, and car-3-ene, were used for identification of the TLC-separated components of the essential oil.

Gas–Liquid Chromatography (GLC). The essential oil was analyzed by GLC using two gas chromatographs, e.g., the BARC model and Shimadzu GC4A. The former one was equipped with a flame ionization detector and a glass column (6 ft × 1/4 in. o.d.) packed with 10% Carbowax 20 M on Chromosorb W (AW), 60–80 mesh (Field Instruments, United Kingdom), and nitrogen at a flow rate of 30 mL/min was used as the carrier gas. The latter one was equipped with thermal conductivity detector and a dual column of stainless steel (3 m × 3 mm o.d.) packed with the same material as above, and helium at a flow rate of 40 mL/min was used as the carrier gas. Chromatographic separation in both cases was achieved at column temperature of 96 °C. The composition of the separated components was determined from peak areas measured by multiplying peak height by peak width at half-height. Identification of the essential oil components was followed by comparison of their retention times on the chromatogram with that of authentic standards (Aldrich Chemicals) myrcene, car-3-ene, α-pinene, *cis*-ocimene [isolated from latex of raw Alphonso mango (Gholap and Bandyopadhyay, 1977)], camphor, linalool, and linalyl acetate.

Subjective odor evaluation of GLC-separated components at the exit port of the Shimadzu GC 4A gas chromatograph was carried out by three expert judges, who were familiar with mango aroma.

Spectrometry. Ultraviolet (UV) and infrared (IR) spectra of the essential oil were recorded on a Shimadzu UV 240 graphical recording spectrophotometer with Graphic Printer PR-1 and Shimadzu IR 400, respectively. For IR the sample was used as a thin film between sodium chloride windows.

RESULTS AND DISCUSSION

The yield of the volatile oil of *C. amada* appeared to vary depending upon the isolation technique. Both conventional steam distillation and distillation–extraction methods resulted in a 0.3% yield of volatile oil as compared to 0.2% in case of the low-temperature–high-vacuum distillation method. However, the oil obtained by the former two techniques gave rise to the characteristic mango ginger odor superimposed with a cooked odor note, and this cooked odor note was virtually absent in the high-vacuum distillate.

The essential oil on TLC separation resolved into few minor components besides the major ones being detected at the solvent front, which seemed to be terpene hydrocarbons. Among the minor components separated on the TLC plate, none of their R_f values resembled that of authentic linalool and linalyl acetate, indicating that their presence could not be confirmed in this rhizome. On odor evaluation of the separated components on the TLC plate, it revealed that the major spot at the solvent front had characteristic raw mango odor. Thus, raw mango odorous principles appeared to be essentially terpene hydrocarbons.

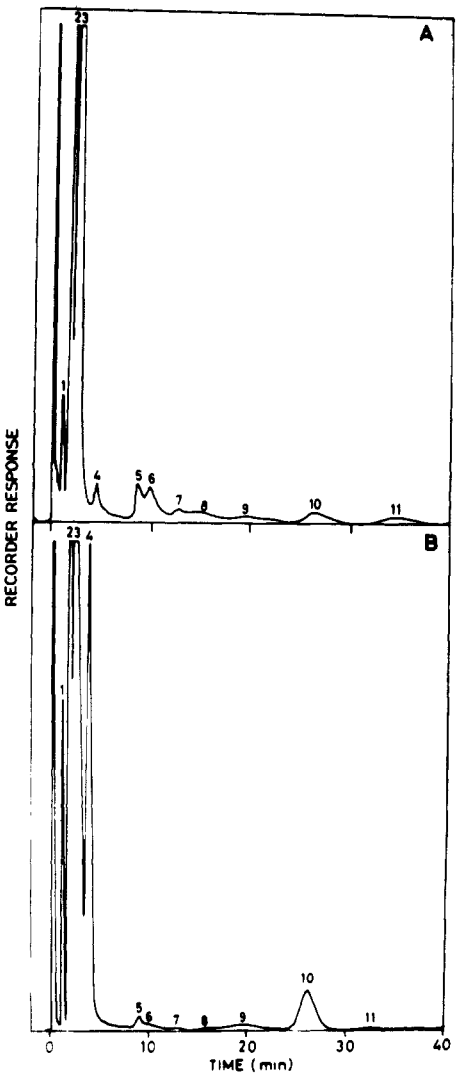

Figure 1. Gas chromatogram of the essential oil isolated from *C. amada* by high-vacuum distillation (A) and steam distillation (B). Peaks: 1, α-pinene; 2, car-3-ene; 3, *cis*-ocimene.

The GLC analysis of essential oil isolated by different methods showed a similar pattern of separation of the components but with an appreciable difference in their relative proportions, particularly with respect to the major component as shown in Figure 1. In the isolation technique where steam distillation was involved, the major component contributed 48% of the essential oil as compared to 68% of that obtained by high-vacuum distillation method. The major peak (no. 3) along with other two peaks (no. 1 and 2) were tentatively identified as *cis*-ocimene, α-pinene, and car-3-ene, respectively, by comparing their retention times as well as cochromatographs with those of authentic samples. Further odor evaluation of GLC-separated components exhibited different odor notes such as floral, mango leaves, green mango, earthy, spicy, and sweet. *cis*-Ocimene was earlier mentioned by Gholap and Bandyopadhyay (1977) as an important contributor to green odor of raw mango, whereas α-pinene and car-3-ene were reported to impart floral and mango leave odor, respectively, in the essence of ripe mango as shown by Macleod and Troconis (1982). The presence of car-3-ene to the extent of 24% in the high-vacuum distillate and 14% in the steam distillate of the rhizome has not been earlier reported. Here, also, linalool and linalyl acetate

could not be detected in the essential oil. The relatively lower content of *cis*-ocimene in the steam distillate than in the vacuum distillate could be attributed to its susceptibility to thermal change during the isolation procedure. Recently, β-myrcene, structurally analogous to *cis*-ocimene, was reported by Hayashi and Komae (1982) to undergo thermal polymerization, resulting in various monomers and dimers.

The UV spectra of the essential oil isolated by the high-vacuum distillation technique showed λ_{max}^{MeOH} at 222 nm, and its IR characteristics according to the order of strong to weak absorption were as follows: 3.85 (s), 11.01 (s), 10.05 (s), 6.02 (w), 6.25 (m), 6.85 (m), 7.15 μm (m). These analytical data, although determined from crude essential oil, resembled that of *cis*-ocimene identified earlier by Gholap and Bandyopadhyay (1977) in the latex of raw Alphonso mango. The foregoing results suggest that the mango-like aroma in *C. amada* was essentially due to the abundant occurrence of *cis*-ocimene, supplemented by car-3-ene hving a mango leave odor.

Registry No. α-Pinene, 80-56-8; car-3-ene, 13466-78-9; *cis*-ocimene, 27400-71-1.

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Headspace Sampling of Cooked Beef Aroma Using Tenax GC

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A novel technique was developed and optimized for isolating genuine cooked beef aroma volatiles, using a modified headspace sampling procedure involving adsorption of the headspace vapors onto Tenax GC. Rapid heat desorption transferred the volatiles directly into a gas chromatography column for separation. The aroma isolation, heat desorption, and gas chromatographic techniques were validated sensorially. By use of combined gas chromatography/mass spectrometry, 67 identifications were made, including 8 compounds tentatively identified for the first time in cooked beef aroma. The relevance of heterocyclic compounds, especially the thiazoles, is discussed. The isolates obtained can be directly analyzed sensorially, since there is no interference from solvent odor, and this enables complementary instrumental and sensory analyses of the desorbed aromas.

The aroma of cooked beef has been extensively studied, and about 600 volatile compounds from variously heated beef samples have been identified (Ching, 1979; Uralets and Golovnja, 1980; Yamaguchi et al., 1980; Lee et al., 1981; MacLeod and Seyyedain-Ardebili, 1981). Unfortunately, aroma isolation methods have usually involved multistage techniques including distillations, flash evaporation, cold trap condensation, solvent extraction, acid/base fractionation, and/or chemical derivatization. The long and complicated nature of several of these methods adds to the possibility of losses and artifact formation already inherent in them.

Recently indirect headspace methods, involving adsorption and simultaneous concentration of volatiles on porous polymers, have become widely used in the analysis of trace volatiles and food aromas. Surprisingly, only one publication relates to beef aroma, and in this study Bryant (1971) trapped the heat-generated volatiles of beef flavor precursors on Porapak Q. Advantages of adsorptive techniques are that the volatiles are concentrated on the basis of their relative volatilities rather than affinity for, or solubility in, a solvent and that, compared with an isolate obtained from solvent extraction, there is no solvent odor to interfere with sensory analysis (Tassan and Russell, 1974).

The sampling method described in this paper was developed such that it (i) provided mild treatment of the beef throughout cooking and isolation, (ii) was as simple as possible and resembled home cooking, (iii) captured and concentrated the aroma quickly and in one stage (*direct* heat desorption into the gas chromatographic column was also desirable), (iv) minimized artifact formation, (v) achieved an aroma isolate representative of the original sample, and (vi) achieved aroma isolates amenable to direct sensory analysis.

Tenax GC was chosen as the adsorbent for several reasons. It has a very high affinity for organic compounds, which it adsorbs reversibly (Micketts and Lindsay, 1974). It is relatively hydrophobic, which is important in view of the large volume of water vapor produced on heating meat. It is the preferred polymer in applications where relatively high boiling compounds are of interest (Boyko et al., 1978; Cole, 1980), and compared with the Porapaks and Chromosorbs it has the highest temperature limit of 375 °C (Micketts and Lindsay, 1974). When heat desorption is the chosen method for releasing aroma volatiles, Barnes et al. (1981) determined that Tenax (desorbed at 250 °C)

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