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Note

High-performance liquid chromatographic separation of pungency components of ginger*

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The pungency of fresh ginger is primarily due to the presence of phenolic ketones. These were first isolated in a crude mixture termed gingerol[1]. Much later, thin-layer chromatography (TLC) was used to separate the components of ginger oleoresin[2]. Three major pungent components were isolated: gingerols, homologues of 1-(3-methoxy-4-hydroxyphenyl)-3-keto-5-hydroxyhexane; shogaols, dehydration products of the gingerols; and zingerone, 3-methoxy-4 hydroxyphenyl-2-butanone resulting from a retro-aldol degradation of the gingerols. By measurement of the resultant aldehydes produced from a hot alkali treatment of the gingerol fraction, a ratio of 53:17:30 for the (6)-, (8)- and (10)-gingerols, respectively, was found (6, 8, and 10 refer to the aldehyde released from the parent gingerol, hence the decane, dodecane and tetradecane homologues, respectively).

Quantitation of the gingerols has historically been made on the basis of TLC or gas–liquid chromatography (GLC). The TLC systems used are those of Connell and Sutherland[2], with detection adaptations made using Folin-Ciocalteau reagent for visualization[3]. GLC analysis has been more difficult as the gingerols partially decompose under the high temperatures required. This reaction has been exploited and estimates of individual gingerol concentrations have been made based on the measurements of the aliphatic aldehydes produced by the reaction of gingerols with hot alkali[2] or by direct pyrolysis of the sample[4,5]. These techniques are less than ideal due to incomplete retro-aldol degradation in the GLC methods, and poor TLC separations. A method based on high-performance liquid chromatography (HPLC) was developed for the direct detection of the major ginger pungency compounds to overcome these problems.

MATERIALS AND METHODS

HPLC was used to separate the pungent components of ginger. This was accomplished on a Whatman PXS 10/25 ODS column (25 × 4.25 mm I.D.) preceeded by a 7.5 cm guard column of the same material. The chromatographic system used

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was a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 3 liquid chromatograph equipped with a Rheodyne (Berkeley, CA, U.S.A.) injector, and a Perkin-Elmer LC-65T detecter oven. Elution was isocratic using acetonitrile–2% aq. acetic acid (2:3) at 2.5 ml/min. Detection was based on UV absorption at 270 nm, and visualization was accomplished with a Linear (Linear Instruments, Irvine, CA, U.S.A.) strip chart recorder.

Using this system, the major peak in the chromatogram was isolated from a portion of "crude gingerols"[2] prepared from 400 g freeze-dried fresh ginger. The fractions collected were evaporated at 35°C under vacuum, resuspended in ethanol and refractionated to increase purity. The fraction of interest was again evaporated under vacuum. To ensure that all solvent was removed, water in small portions was added and the material was evaporated under vacuum until no trace of solvent odor was detected. The material was dissolved in 95% ethanol, flushed with nitrogen and stored at −10°C.

For identification, UV (ethanol) and IR (thin-film, Perkin-Elmer 397 infrared spectrophotometer) spectra were prepared, as was a mass spectrum [solid probe introduction, Finnigan (Sunnyvale, CA, U.S.A.) Series 3000 GC peak identifier, 0–320 mass range, 69.8 eV]. These data were compared to spectra for authentic (6)-gingerol for identification[2,6]. The only identity determinate inconsistent with published data was $E=3341$ in 95% ethanol at 282 nm. This is somewhat higher that the $E=2560$ reported earlier for this compound[2], however the latter was based on a mixture of gingerols. A small portion of this preparation was diluted (1:1) with 0.1 *M* hydrochloric acid and heated at 125°C for 1 h to produce the corresponding shogaol.

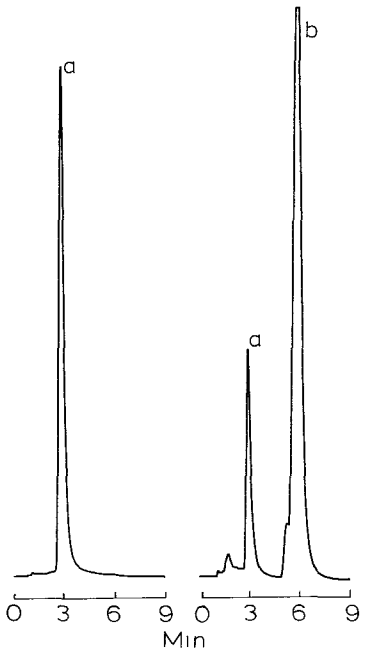

Fig. 1. HPLC traces of isolated (6)-gingerol (a) and (6)-shogaol (b) prepared by hot acid treatment of (a).

Zingerone was prepared by heating a 1:1 dilution of the (6)-gingerol with 2 *M* sodium hydroxide in a boiling water bath.

Samples of commercially dried ginger were purchased at a local market for pungency analysis. A sample of 5 g was blended with 170 ml methanol for 30 sec at both low and medium speed in a Waring blender. This was allowed to stand for 20 min prior to being filtered under vacuum, using a small amount of methanol to transfer the blended sample. The filter mat was washed with 50 ml methanol and re-extracted using the above procedure. The combined extracts and washings were made to volume (500 ml) with methanol, filtered, and stored under nitrogen at −10°C prior to analysis. A further extraction of the filter mat yielded only 1.5% of the (6)-gingerol extracted in the above scheme.

RESULTS AND DISCUSSION

Chromatograms of isolated (6)-gingerol and its dehydration product, (6)-shogoal, prepared by acid treatment of the parent compounds are shown in Fig. 1. The longer retention time for the shogaol (5.9 *vs.* 3.0 min) is consistent with separation on an ODS column, as dehydration should produce a compound with lower polarity than its parent. Breakdown of (6)-gingerol to zingerone occurred rapidly (90% breakdown in 10 min), with the product eluting at 1.6 min in the chromatogram.

For simplicity, an isocratic elution system was chosen for routine analysis. A programmed elution of a linear gradient from 25 to 80% methanol over 15 min, with the remainder of the solvent methanol–2% aq. acetic acid (1:3) gave a superior separation, but gradient elutions are difficult to consistently reproduce. There were a number of unidentified late eluting peaks found with the gradient elution, for which the use of a gradient system may be preferred.

By using the technique, samples of three commercially dried gingers from different sources were analyzed (Fig. 2). For each, the (6)-gingerol peak is inversely

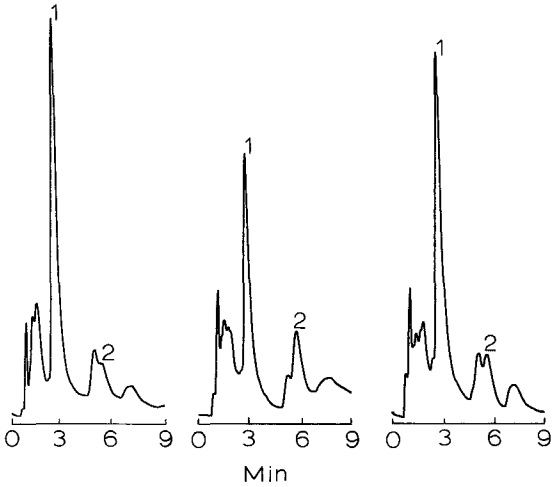

Fig. 2. HPLC traces of three commercial dried ground ginger samples: 1 = (6)-gingerol, 2 = (6)-shogaol.

proportional in size to the (6)-shogoal peak. Shogaols are found at very low concentrations in fresh ginger, forming during storage of either dried ginger or ginger oleoresin[2,7] and a ratio of their concentrations may be a useful index of the freshness of the sample. Zingerone eluted concurrently with a number of other components in the sample, so again a gradient elution may be preferred.

This simple isocratic HPLC technique for gingerols should provide a rapid method for analysis of ginger pungency. The technique described could be easily expanded to include other ginger pungency components by the use of a gradient elution system. This technique should make possible a simple method of analysis for use in determining changes during drying and other processing, and possibly for monitoring adulteration of dried ginger.

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