JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Gingerol Content of Diploid and Tetraploid Clones of Ginger (*Zingiber officinale* Roscoe)

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Ginger (*Zingiber officinale* Roscoe), a monocotyledonous, sterile cultigen, is widely used as a spice, flavoring agent, and herbal medicine. The pungency of fresh ginger is due to a series of homologous phenolic ketones of which [6]-gingerol is the major one. The gingerols are thermally unstable and can be converted to their corresponding shogaols, which are present in dried ginger. Fresh rhizomes of 17 clones of Australian ginger, including commercial cultivars and experimental tetraploid clones, were assayed by HPLC for gingerols and shogaols. [6]-Gingerol was identified as the major pungent phenolic compound in all samples, while [8]- and [10]-gingerol occurred in lower concentrations. One cultivar known as "Jamaican" contained the highest concentrations of all three gingerols and was the most pungent of the clones analyzed. Gingerols were stable in ethanolic solution over a 5-month period when stored at 4 °C. Shogaols were not identified in the extracts prepared from fresh rhizomes at ambient temperature, confirming that these compounds are not native constituents of fresh ginger. In contrast to previous findings, this study did not find significant differences in gingerol concentrations between the tetraploid clones and their parent diploid cultivar.

KEYWORDS: Ginger; *Zingiber officinale*; gingerols; shogaols; tetraploidy

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe; family Zingiberaceae) is a monocotyledonous, sterile cultigen thought to have originated in India or Southeast Asia, from where it was introduced to other parts of the world (*1*, *2*). Both fresh and dried ginger rhizomes are used worldwide as a spice, and ginger and ginger extracts are used extensively in the food, beverage, and confectionary industries in the production of products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, biscuits, and other bakery products. The unique flavor properties of ginger arise from the combination of pungency and aromatic essential oil.

Ginger is also widely used in both traditional and contemporary natural medicine. It has been used medicinally in India since ancient times and is mentioned in Vedic texts dating back to around 4000 BCE (*3*). Ginger is included in the British (*4*), European (*5*), Chinese (*6*), and Japanese (*7*) pharmacopoeias, as well as in many other national pharmacopoeias, and the World Health Organization has published a monograph for *Rhizoma Zingiberis* (*8*). The medicinal uses of ginger are diverse and include the treatment of dyspepsia, colic, diarrhoea, colds and flu, and poor appetite. It is also recommended as an anti-inflammatory agent in rheumatic and muscular disorders and to increase longevity (*8*). Clinical trials support the use of ginger preparations in the prevention of motion sickness (*9*, *10*) and vomiting in pregnancy (*11*−*14*), while the evidence is more ambiguous in the case of musculoskeletal disorders (*15*−*17*).

The main pungent compounds in fresh ginger are a series of homologous phenolic ketones known as gingerols (**Figure 1**). The major gingerol is [6]-gingerol, while [8]- and [10]-gingerol occur in smaller quantities. The gingerols are thermally unstable and are converted under high temperature to [6]-, [8]-, and [10]-shogaol (after *shoga*, the Japanese word for ginger; **Figure 1**) (*18*). Shogaols, which are more pungent than gingerols, are the major pungent compounds in dried ginger rhizome.

In Australia, the most widely grown ginger cultivar is "Queensland", and it is estimated that 40% of the world's

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10.1021/jf050435b CCC: $30.25
Published on Web 06/11/2005

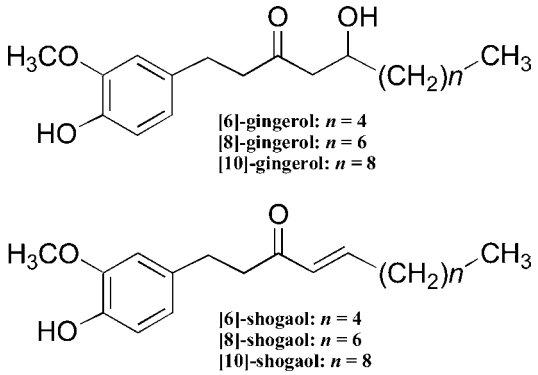

Figure 1. Structures of the major pungent compounds in ginger, gingerols, and shogaols.

Table 1. Ginger Clones Studied, Their Genotype, and Their Origin

ID	genotype	cultivar name	origin
Z22	tetraploid[c]	unnamed	derived from "Queensland" (selection 1) by colchicine treatment
Z23	tetraploid[c]	unnamed	
Z24	tetraploid[a]	unnamed	
Z25	tetraploid[c]	unnamed	
Z26	tetraploid[a]	"Buderim Gold"	
Z27	tetraploid[a]	unnamed	
Z28	tetraploid[c]	unnamed	
Z29	tetraploid[c]	unnamed	
Z30	tetraploid[b]	unnamed	
Z31	tetraploid[a]	unnamed	
Z32	tetraploid[c]	unnamed	
Z33	tetraploid[a]	unnamed	
Z44	diploid	"Queensland" (selection 1)	selected by J. Roscoe, BGL[e]
Z45	diploid	"Queensland" (selection 2)	selected by L. Palmer, BGL[e]
Z46	diploid	"Jamaican"	imported from Jamaica
Z47	diploid[d]	"Brazilian"	imported from Brazil
Z58	diploid	"Canton"	imported from China

[a] Confirmed as solid tetraploids by flow cytometry. [b] Chimera with both diploid and tetraploid tissue sectors. [c] Presumed to be tetraploid from stomatal measurements. [d] Unknown but presumed to be diploid. [e] BGL = Buderim Ginger Ltd.

confectionary ginger products are prepared from this cultivar (*19*). The origin of this cultivar remains uncertain. Various authors have suggested that it arrived in Australia from the Cochin coast of India (*20*), from Fiji (*21*), or from China in the early 1900s (*22*).

We report here on the analysis of 17 ginger clones grown in Eastern Australia, including commercial cultivars and 12 experimental tetraploid clones derived from "Queensland", and the quantification by HPLC of the major pungent phenolic compounds, gingerols and shogaols. The objectives of the study were to explore the variability of Australian ginger clones in terms of their content of pungent phenolic compounds with a view to identify one or more high-yielding clones as candidates for commercial cultivation for flavor or pharmaceutical use.

MATERIALS AND METHODS

Plant Materials. Seventeen clones of ginger (**Table 1**) were obtained from the Queensland Department of Primary Industries & Fisheries, Maroochy Research Station at Nambour, Queensland. They included two selections of the cultivar "Queensland", which is grown commercially in Queensland, the cultivars "Jamaican", "Brazilian", and "Canton", which were introduced to Queensland between 1970 and 1972 for cultivar evaluation studies, and 12 experimental clones developed at the Maroochy Research Station at Nambour, including the newly released cultivar "Buderim Gold" (*23*). The experimental clones were obtained after in vitro colchicine treatment of shoots of diploid ($2n = 22$) "Queensland" parent material provided by J. Roscoe. They were confirmed as solid tetraploids except for one (Z30), which proved to be a periclinal chimera with both diploid and tetraploid tissues (*19*).

The clones were grown from rhizome stock in raised outdoor beds under uniform irrigated conditions at Southern Cross University, Lismore, New South Wales (latitude 28° 49′ S, longitude 153° 18′ E) for approximately 8 months.

Sample Preparation. Fresh rhizomes were washed, and a cylindrical sample was taken from the thickest part of the rhizome using an apple corer. The epidermis was removed, and the sample was cut into cubes approximately $1.5 \times 1.5 \times 1.5$ mm^3. A 5-g sample was placed in a large centrifuge tube to which twice the sample mass of 99% ethanol was added. The preparation was sonicated for 20 min (Ultrasonic Cleaner 50 Hz, Unisonics Pty Ltd, Manly Vale, Australia) and subsequently centrifuged for 5 min at 4000 rpm (Hettich Universal 16A Centrifuge, Tuttlingen, Germany). The supernatant was transferred to a brown glass vial using a transfer pipet, stored at 4 °C and filtered through a Zymark/Millipore Automation Certified Filter (Glassfiber APFB 1.0 μm prefilter, hydrophilic PTFE membrane 0.45 μm) before being injected onto the HPLC column.

To take account of variation between individual plants, three samples from different rhizomes were prepared for each clone.

HPLC Methods. The same extracts were analyzed by HPLC on two occasions, approximately 5 months apart, during which period they were stored at 4 °C. Measurement reproducibility was determined by repeated analyses of a mixed standard solution (see below).

Method 1. The first (baseline) reverse-phase HPLC analysis of the extracts was performed on an Agilent (Palo Alto, CA) 1100 HPLC fitted with a HP LiChrospher 100 RP-18e (5 μm) column. Mobile phase A consisted of HPLC-grade water obtained from an in-house Milli-Q system (Waters, Milford, MA); mobile phase B consisted of HPLC-grade acetonitrile (EM Science, Gibbstown, NJ); both contained 0.05% trifluoroacetic acid (Sigma-Aldrich, St. Louis, MO). The gradient eluting mobile phase was A/B (70:30, v/v) to A/B (10:90, v/v) over 20 min followed by A/B (10:90, v/v) for 10 min. Mobile phase was pumped at 1.00 mL/min, the column temperature was 40 °C, and the injection volume was 10.0 μL. Data were collected using a UV/visible diode array detector collecting absorption spectra from 200 to 400 nm with quantification performed at 228 nm.

Method 2. The second analysis (at 5 months) was carried out on an Agilent 1100 Series LC/MSD system fitted with a Phenomenex (Torrance, CA) Luna 5μ (150×4.6 mm^2) C-18 column. Mobile phase A and mobile phase B were as described above. The gradient eluting mobile phase was A/B (70:30, v/v) to A/B (10:90, v/v) over 20 min followed by A/B (10:90, v/v) for 5 min. Mobile phase was pumped at 0.40 mL/min, the column temperature was 40 °C, and the injection volume was 10.0 μL. Data were collected using a UV/visible diode array detector collecting absorption spectra from 200 to 400 nm with quantification performed at 228 nm.

Standards. Synthetic standards of [6]-gingerol (99%), [8]-gingerol (99%), [10]-gingerol (98%), [6]-shogaol (99%), and [8]-shogaol (99%) were obtained from the Department of Pharmacy, University of Sydney (Sydney, NSW). A known quantity of each standard was dissolved in 99% ethanol and made up to 10 mL in a volumetric flask. A mixed standard solution was prepared by combining 100 μL of each of the individual standard solutions of [6]-, [8]-, and [10]-gingerol and [6]- and [8]-shogaol. A 10-fold dilution of this mixed standard was employed in the analyses. It contained the standard compounds in concentrations ranging from 15 to 25 μg/mL. Identification of gingerols and shogaols in samples was based on comparisons of retention time and UV-spectra with the standards. Quantification was based on standard curves prepared with pure standards.

Statistical Analysis. Statistical analyses were performed using SPSS (Chicago, IL) for Windows Release 11.0.0. The mean, standard deviation, and coefficient of variance were calculated for repeated measurement of the standard solution. The mean and standard error were calculated for the analyses of the samples. Two-factor repeated measure analysis of variance was used to compare the content of the three gingerols ("gingerol" = the within factor) from the 17 clones ("clone" = the between factor). The analysis was based on three

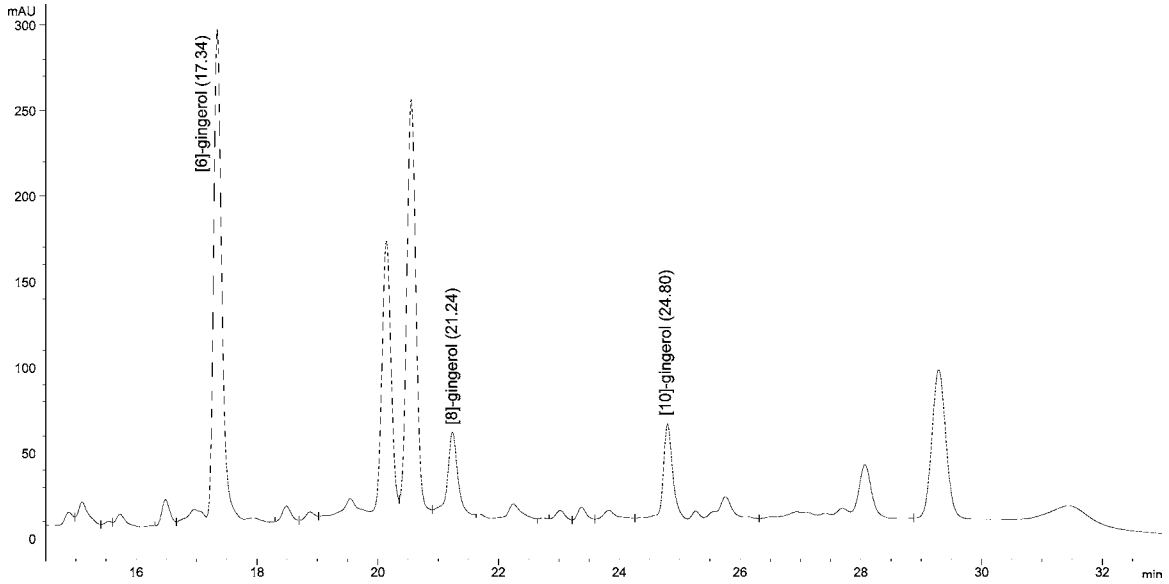

Figure 2. Typical HPLC chromatogram of ethanolic extract of fresh ginger rhizome (Z44) obtained with HPLC method 2.

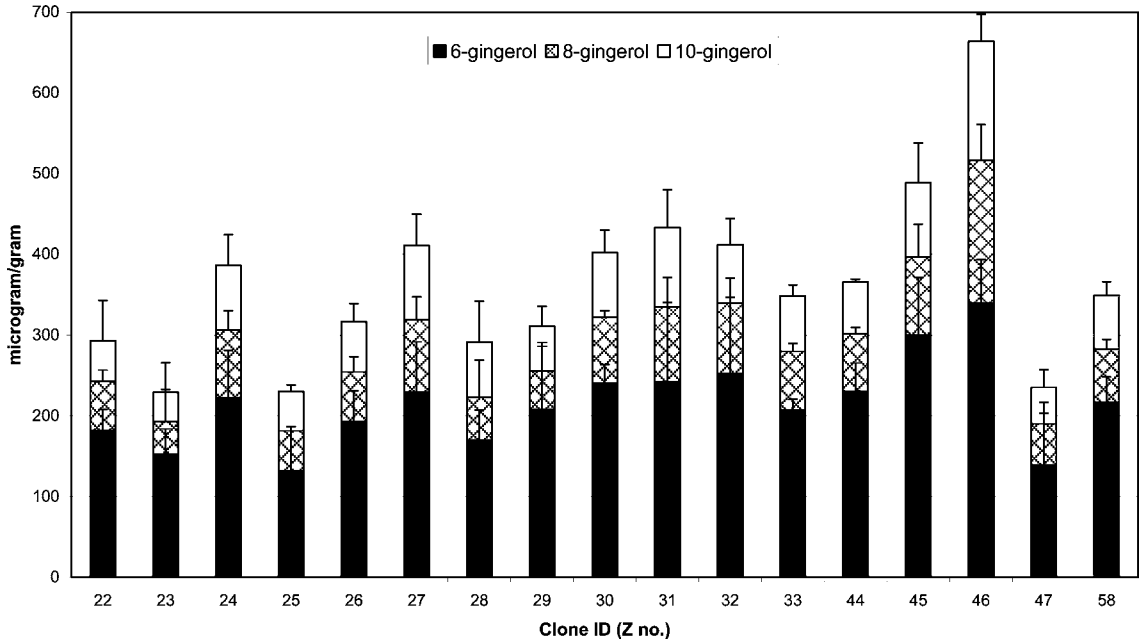

Figure 3. Concentrations of [6]-, [8]-, and [10]-gingerol in fresh rhizomes of 17 ginger clones. Values are means of three separate rhizomes. Error bars indicate standard deviation (only positive half shown).

replicates for each clone. The assumption of compound symmetry was not met, so tests of effects were adjusted using the Greenhouse−Geisser method. Pairwise comparisons were also employed to compare the gingerol content across the clones. The correlation between the different gingerols was examined by way of scatter plots and Pearson product−moment correlations. The stability over time of the gingerols was explored by repeated measure *t*-tests. The mean, standard error, and range were calculated for the gingerol content of diploid and tetraploid clones.

RESULTS

Measurement Reproducibility. To determine the reproducibility of the HPLC measurements, repeated analyses of the mixed standard solution were carried out by both methods. The analysis by method 1 showed a high degree of reproducibility when the same sample was injected nine times over a 5-day period. The coefficient of variance ranged from 2.2% to 6.4% for the five standard compounds. The reproducibility of the measurements by method 2 was assessed by comparing 11 injections of the mixed standard over a 37-hour period. This analysis showed a very high degree of reproducibility with the coefficient of variance being less than 2% for all compounds.

Quantification of Gingerols in Test Samples. Rhizome extracts of 17 clones of ginger were analyzed twice, approximately 5 months apart. For each clone, three extracts were prepared from different rhizomes. The mean concentrations of [6]-gingerol, [8]-gingerol, and [10]-gingerol in three rhizomes of each clone were calculated from the peak areas obtained at 228 nm. A typical HPLC trace is shown in **Figure 2**.

The concentrations of gingerols in freshly extracted ginger are shown in **Figure 3**. The most abundant gingerol in all clones was [6]-gingerol, which occurred at concentrations ranging from 132 to 339 μg per gram of fresh rhizome (mean 215 $\pm$ 54 μg/g). [8]-Gingerol and [10]-gingerol occurred in lower concentrations; [8]-gingerol ranged from 40 to 177 μg/g (mean 75 $\pm$

Table 2. Results of Pairwise Analyses of 17 Ginger Clones in Terms of [6]-, [8]-, and [10]-Gingerol Content

	clone (Z no.)																
gingerol	22	23	24	25	26	27	28	29	30	31	32	33	44	45	46	47	58
[6]	2	2		7	2		4	2	1	1	2	2	1	8	8	2	2
[8]	1	5	1	3	2		1		1	6		2		2	9	2	2
[10]	1	4	1	2	1	2	1	1	1	2	1	1	1	1	15	1	1
total no.	4	11	2	12	5	2	6	3	3	9	1	5	2	11	32	5	5

Each clone is compared with all other clones and the number of significantly ($p<0.05$) different cases (max. 16) is shown for each gingerol. Note that Clone Z46 ("Jamaican") showed 32 significantly different comparisons, distinguishing it from all other clones.

31), while [10]-gingerol ranged from 37 to 148 μg/g (mean 73 $\pm$ 26). The cultivar "Jamaican" (Z46) had the highest total gingerol content of any clone (664 μg/g). Neither [6]- nor [8]-shogaol was identified in any of the samples (freshly prepared or stored 5 months).

Two-factor repeated measure analysis of variance (Greenhouse−Geisser test) of the clone by gingerol interaction effect showed that the mean values of [6]-, [8]-, and [10]-gingerol varied significantly across the 17 clones ($F = 2.335$, $p = 0.01$). When the mean total gingerol content of each clone was compared with the mean value for all clones, only two clones showed a statistically significant difference from the overall mean. These were the cultivar "Jamaican" (Z46), which contained a significantly higher concentration of gingerols ($p = 0.002$), and one of the experimental tetraploid clones (Z25), which had a gingerol content that was significantly lower than the overall mean ($p = 0.028$).

The difference in gingerol content between the clones was also explored by way of pairwise comparisons on each of the three gingerols. This analysis is summarized in **Table 2**, which shows the number of significantly ($p < 0.05$) different cases between each clone and the other 16 clones for each of the three gingerol compounds. The pairwise analysis confirms that the cultivar "Jamaican" (Z46) is the most outstanding clone in terms of gingerol content. This is particularly true in terms of [10]-gingerol concentration, which is significantly higher in "Jamaican" than in all but one other clone (Z31).

Correlation between Gingerols. The correlations between the mean concentrations of [6]-, [8]-, and [10]-gingerol in the freshly prepared extracts of 17 clones are illustrated by way of scatter plots in **Figure 4**. A linear relationship between the concentrations of [6]-, [8]-, and [10]-gingerol is apparent. The cultivar "Jamaican" (Z46) stands out from the others by containing higher concentrations of [8]- and [10]-gingerol relative to [6]-gingerol (**Figure 4A,B**).

The Pearson product−moment correlations between the concentrations of [6]-, [8]-, and [10]-gingerol in the rhizomes at zero and five months were calculated (**Table 3**). Since the scatter plots clearly show a positive correlation between the different gingerols a one-tailed test for significance was chosen. The highly significant correlations confirm the strong positive correlation among the three gingerols.

Gingerol Ratios. The ratio of [6]-gingerol/[8]-gingerol/[10]-gingerol was calculated for the 17 clones on the basis of the HPLC data. The mean ratio of [6]-gingerol/[8]-gingerol/[10]-gingerol was 3:1:1 across the clones, but some clones deviated considerably from this ratio, for example, Z29 (4.4:1:1.2) and Z46 ("Jamaican") (1.9:1:0.8). Since both [8]- and [10]-gingerol possess considerable pungency (albeit less than [6]-gingerol) (*24*), the relatively high levels of [8]- and [10]-gingerol present in "Jamaican" in addition to the high concentration of [6]-gingerol make this clone by far the most pungent of the 17 clones assayed. This was confirmed organoleptically.

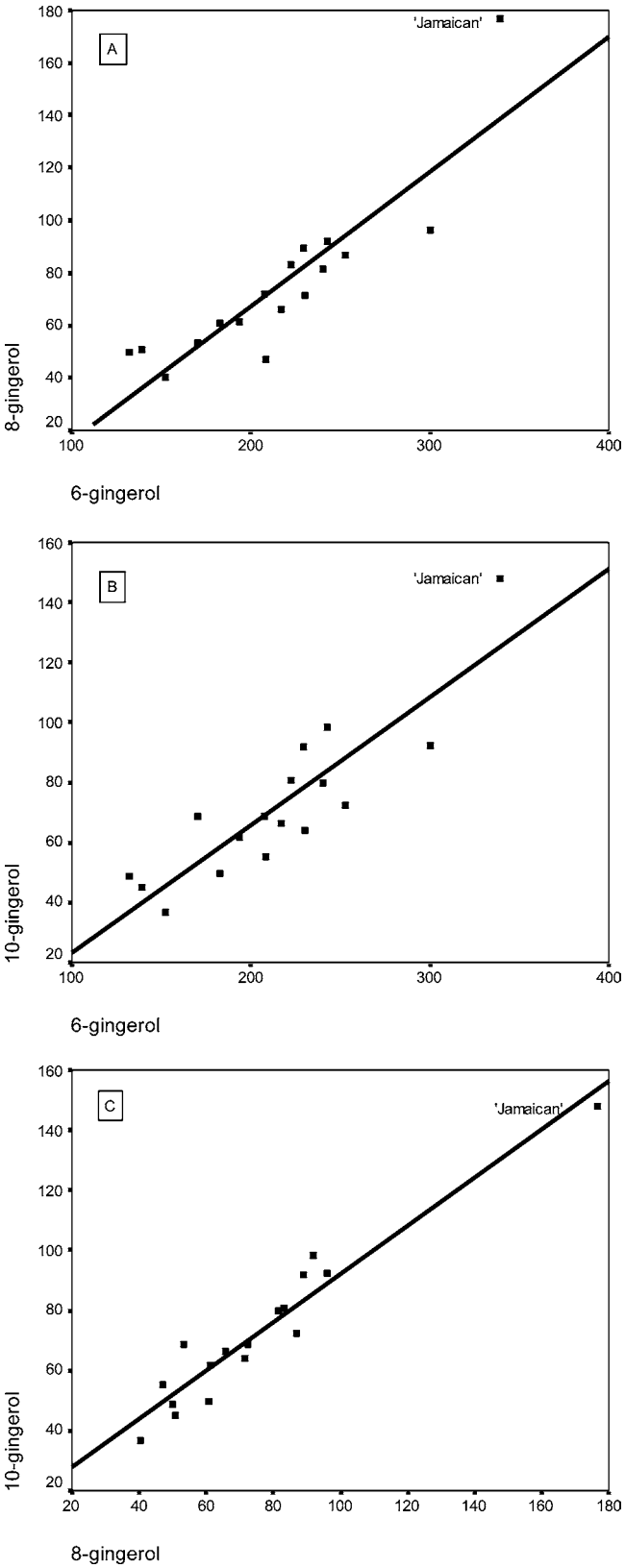

Figure 4. Scatter plots illustrating the correlation between concentrations (μg/g) of [6]- and [8]-gingerol (**A**), [6]- and [10]-gingerol (**B**), and [8]- and [10]-gingerol (**C**) in fresh ginger rhizomes based on HPLC data from 17 different clones.

Stability of Gingerols. Ethanolic extracts were assayed twice approximately 5 months apart. Between analyses, the extracts

Table 3. Pearson Product–Moment Correlations between the Concentration of Gingerols in Fresh Rhizomes of 17 Ginger Clones Assayed by HPLC at Zero and Five Months

	[6]-gingerol		[8]-gingerol	
	0 months	5 months	0 months	5 months
[8]-gingerol	0.882[a]	0.850[a]		
[10]-gingerol	0.880[a]	0.803[a]	0.959[a]	0.914[a]

[a] $p < 0.0005$ (one-tailed).

Table 4. Mean ± SE (Range) Concentrations (μg/g) of Gingerols in Two Commercial "Queensland" Clones and 12 Tetraploid Clones

	[6]-gingerol	[8]-gingerol	[10]-gingerol
"Queensland" clones ($n = 2$)	245.2 ± 77.7 (139.3–339.4)	92.2 ± 49.9 (50.9–176.6)	83.2 ± 39.9 (45.2–147.9)
tetraploid clones ($n = 12$)	202.8 ± 37.9 (131.6–252.5)	68.1 ± 18.2 (40.2–92.0)	67.7 ± 18.4 (36.6–98.5)
p-value (*t*-test)	0.058	0.279	0.471

were refrigerated at 4 °C. The concentrations of [6]-, [8]-, and [10]-gingerol at zero and five months were compared by way of repeated measure (paired samples) *t*-tests. The mean concentrations of [6]- and [8]-gingerol did not change significantly in the 17 clones over the 5-month period, and these compounds therefore appear to be stable in ethanolic solution at 4 °C for this period of time. The concentration of [10]-gingerol, however, showed a small (5%) but statistically significant ($p = 0.01$) decrease over the same period.

Gingerol Content of Tetraploid Clones. The mean, standard error, and ranges for gingerol concentrations in two commercial selections of the diploid "Queensland" cultivar and 12 experimental tetraploid clones are shown in **Table 4**. "Queensland" (selection 1) is the parent clone from which the tetraploids were generated by colchicine treatment. Both diploid and tetraploid clones displayed considerable variation in gingerol concentrations. On average, the diploid "Queensland" clones contained higher levels of all three gingerols, but the differences were not statistically significant.

DISCUSSION

An extensive survey of fresh rhizomes of five diploid ginger cultivars, 11 experimental tetraploid clones, and one recently released tetraploid clone grown in Australia was conducted with respect to their content of pungent gingerols and shogaols. Ethanol was chosen as the extraction solvent, because it is the solvent of choice for herbal medicine preparations.

Gingerols. The three pungent compounds, [6]-, [8]-, and [10]-gingerol, were identified and quantified in all samples. [6]-Gingerol was the most abundant gingerol in all clones, which is in accordance with the literature (*25*−*27*). The mean ratio of [6]-gingerol/[8]-gingerol/[10]-gingerol was 3:1:1 across all 17 clones. A strong positive linear correlation between levels of the three gingerols was found in all clones, reflecting the close biosynthetic relationship among these compounds.

The mean content of gingerols obtained in the present study is considerably higher than those found by Bartley in a supercritical CO_2 extract of Australian ginger (*26*) but lower than levels reported from other parts of world (**Table 5**). This variability may reflect genetic differences between clones in different regions or physiological responses to environmental factors such as climate, soil characteristics, or predation, but they may also be due to differences in extraction and analytical methodologies.

The cultivar "Jamaican" contained the highest concentration of all three gingerols on a fresh weight basis and was therefore the most pungent of the clones assayed. It also contained higher levels of [8]- and [10]-gingerol relative to [6]-gingerol than any other clone. "Jamaican" may thus be suitable for commercial production of highly pungent ginger rhizomes with potential application in both the pharmaceutical and flavor industries, even though, eventually, the viability of commercial production of this clone will depend on biomass yield.

Repeated analyses of ethanolic extracts five months apart showed that [6]- and [8]-gingerol did not degrade during this period when stored at 4 °C. Concentrations of [10]-gingerol showed a small but statistically significant decrease (5%). It is not known whether [10]-gingerol is in fact less stable than [6]- and [8]-gingerol under these conditions or this finding represents a type 1 error resulting from the small sample size.

Shogaols. Neither [6]- nor [8]-shogaol were identified in our samples, which were prepared at ambient temperature from fresh rhizomes. In contrast, [6]-shogaol was identified in fresh rhizome extracts prepared by hot Soxhlet extraction (data not shown). These findings support the hypothesis that shogaols are not native constituents of fresh ginger rhizomes but form from gingerols by dehydration as a result of heat treatment or alkaline or acidic conditions (*28*, *31*, *32*). Earlier reports of shogaols in fresh ginger extracts analyzed by GC-MS (*26*, *33*) can probably be explained by the high temperatures samples are exposed to during this form of analysis, resulting in the formation of shogaols as artifacts of analysis.

Table 5. Literature Data on Gingerol Content of Fresh Ginger Rhizomes[a]

country (ref)	solvent/ extraction	analytical method	[6]-gingerol	[8]-gingerol	[10]-gingerol
Australia (present study)	ethanol	HPLC	215	75	72
Hawaii (*28*)	methanol	HPLC	2100	288	533
United States (*29*)	methylene chloride	HPLC	880	93	120
Taiwan (*30*)	ground; acetate buffer solution (pH 4.0) added	HPLC	806	*b*	*b*
Australia (*26*)	supercritical CO_2	NIES-MS	120	19	24

[a] Values are μg per gram of fresh rhizome. [b] Not determined.

Ploidy. The mean concentrations of all three gingerols were lower for the tetraploid clones than for the parent diploid "Queensland" cultivar, although the differences were not statistically significant. This observation is in marked contrast to the findings of Nakasone and colleagues who reported that tetraploid clones of three Japanese ginger cultivars contained higher concentrations of total gingerols ([6]-, [8]-, and [10]-gingerol) and in particular of [10]-gingerol than did their parent diploid genotypes (*34*).

Comparative quantitative studies of secondary metabolites in diploid versus polyploid genotypes have been conducted on numerous medicinal plants. Although polyploidy often appears to result in increased expression of secondary metabolites, this is not always the case, and the effects of polyploidy are not predictable (*35*).

Our findings do not therefore preclude the possibility of identifying a tetraploid clone with elevated gingerol biosynthesis. In this context, it would be of particular interest to monitor experimental tetraploid clones derived from the cultivar "Jamaican", which are currently under development at the Maroochy Research Station.

This study has described the variability of gingerol compounds in Australian commercial and experimental ginger clones. When combined with agronomic data, the present information should allow for selection of clones with specific levels of pungency, a characteristic which, along with the aroma produced by the essential oil, determines the flavor characteristics of ginger.

ACKNOWLEDGMENT

The authors thank Don Brushett from the Centre for Phytochemistry and Pharmacology for analytical assistance and Lyndon Brooks from the Research Methodology Unit at Southern Cross University for statistical advice.

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Received for review February 25, 2005. Revised manuscript received May 9, 2005. Accepted May 15, 2005.

JF050435B