

## Special Article - HPLC

# HPLC Quantification and MS/NMR Confirmation of Javamide-I/-II in Arabica and Robusta Coffee Beans from Different Regions for Finding Better Bean Sources for Javamide-I/-II

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Received: December 17, 2018; Accepted: January 10, 2019; Published: January 17, 2019

## Abstract

Coffee is a popular drink with several positive health effects and javamide-I/-II are bioactive phenolic amides found in coffee. However, there is little information about the quantities of javamide-I/-II in Arabica and Robusta beans from different geographical regions, impossible to categorize better bean sources for javamide-I/-II. Therefore, in this paper, javamide-I/-II were first extracted from twelve coffee beans (eight Arabica beans from Brazil, Colombia, Costa Rica, Ethiopia, Hawaii, Papua New Guinea, Puerto Rico and four Robusta beans from India and Vietnam), and their amounts were quantified using the developed HPLC method with MS/MS and NMR confirmation. In eight Arabica beans, javamide-I/-II were detected at levels ranging from 0.04-0.05 and 0.08-0.29 mg/g, meanwhile 0.4-0.76 mg/g of javamide-I and 3.5-4.9 mg/g of javamide-II were detected in four Robusta beans. The data showed that the amounts of javamide-I/-II in Robusta beans were much higher than those of javamide-I/-II in Arabica beans with little geographical impact ( $P < 0.05$ ). To validate these data and to explore better coffee beans for javamide-I/-II, eight additional beans (four Arabica and four Robusta beans) were extracted to examine the amounts of javamide-I/-II. The data of the eight additional beans confirmed the data of the twelve coffee beans in the levels of javamide-I/II ( $P < 0.05$ ), suggesting that Robusta beans may be better sources for javamide-I/-II than Arabica beans. In summary, javamide-I/-II may be found at higher levels in Robusta beans than Arabica beans, and Robusta beans are likely to be better bean sources for javamide-I/-II.

**Keywords:** Javamide-I/-II; MS/MS; NMR; HPLC quantification; Unroasted coffee beans

## Introduction

Coffee is a tropical evergreen tree grown around the globe and coffee beans are typically used to produce a popular drink [1-3]. In fact, *Coffea arabica* (Arabica coffee) and *C. canephora* (Robusta coffee) are two most commercially important coffee species, primarily cultivated in the equatorial regions of the Americas, Southeast Asia, India, and Africa [3-7]. Arabica and Robusta beans are also known to contain numerous chemicals (e.g., caffeine, chlorogenic acids, javamide-I/-II, others) [7-9]. Recently, javamide-I/-II and their derivatives were reported to contain several biological activities related to human health [10-12]. However, there is little information about the amounts of javamide-I/-II in Arabica and Robusta beans from different geographical regions, although there are increasing inquiries about coffee beans with high amounts of javamide-I/-II and geographical impacts on them [7,8]. Therefore, in this paper, javamide-I/-II in twelve coffee beans (eight Arabica and four Robusta) grown in Brazil, Colombia, Costa Rica, Ethiopia, Hawaii, Papua New Guinea, Puerto Rico, India and Vietnam were first extracted and their identities were confirmed by MS/MS and NMR spectroscopic method. Then, javamide-I/-II were quantified using a HPLC method

to gauge the influences of species and geographical influences on javamide-I/-II. Also, to validate the data of the twelve beans and to identify better bean sources for javamide-I/-II, eight additional coffee beans (four Arabica and four Robusta beans) from different regions were purchased and the amounts of javamide-I/-II were further quantified using the developed HPLC method.

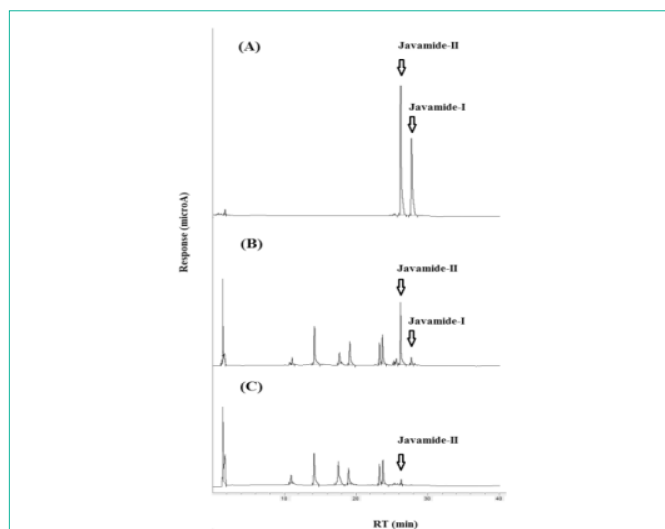
## Materials and Methods

### Chemicals and reagents

Javamide-I/-II standards were prepared as reported previously [9]. All other reagents and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

### Preparation and extraction of coffee beans samples

Eight Arabica beans grown in Brazil, Colombia, Costa Rica, Ethiopia, Hawaii, Papua New Guinea and Puerto Rico and four Robusta beans grown in India and Vietnam were obtained from the Sustainable Perennial Crops Laboratory coffee collection (USDA, ARS). The species identity of all twelve coffee beans were verified using SNP markers as described previously [13]. Additionally, four Arabica and four Robusta beans were separately purchased from



**Figure 1:** HPLC chromatograms of javamide-I/-II standards and the extract prepared from Arabica and Robusta samples. (A) Javamide-I/-II standards (6 n mole for javamide-I/-II). (B) The mixture of four Robusta (S9-12) extracted samples. (C) The mixture of ten Arabica (S1-8) extracted samples. The chromatograms were monitored using an electrochemical detector.

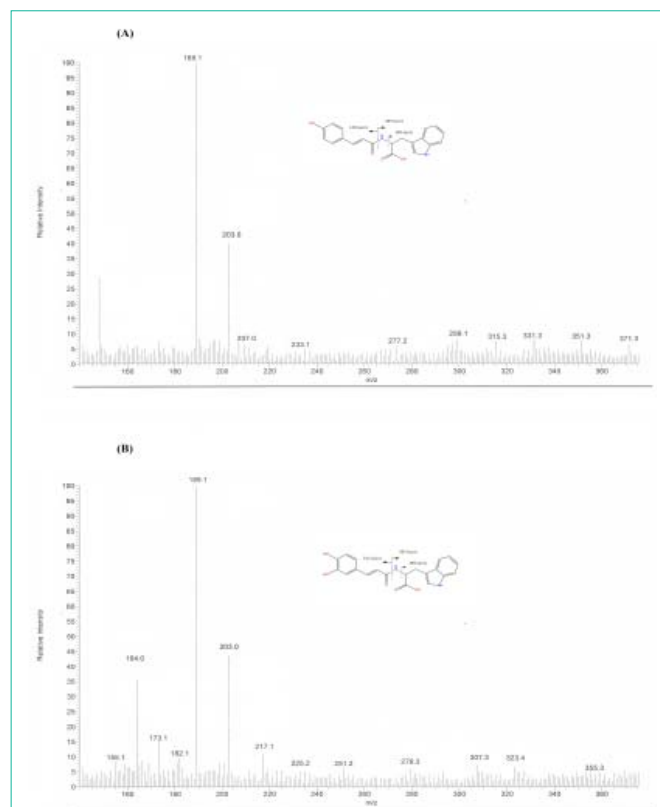


**Figure 2:** The pictures of twelve coffee beans used in the study. Coffee beans with regions and sample names: S1 (C. arabica; Brazil, Bourbon), S2 (C. arabica; Colombia, Gigante Maragogype), S3 (C. arabica; Costa Rica, Tarrazu), S4 (C. arabica; Ethiopian, Harrar), S5 (C. arabica; Ethiopian, Kaffa Forest Tega & Tula), S6 (C. arabica; Guatemala, Typica), S7 (C. arabica; Papua New Guinea, Gumancha), S8 (C. arabica; Puerto Rico, Pergamino), S9 (C. canephora; India, Robusta bulk), S10 (C. canephora; India, Robusta), S11 (C. canephora; India, Sufia Robusta), S12 (C. canephora; Vietnam, Mocha Robusta).

online stores to validate the data for javamide-I/-II. All unroasted coffee beans were grinded with a coffee grinder, and then pulverized using a mortar and pestle. The resulting fine powder was extracted with 80% methanol (50ml per 1g coffee bean) with a gentle shaking at room temperatures for 24 hr. Then, the samples were extracted three times, providing 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> extraction samples. The extraction samples were utilized to isolate javamide-I/-II for HPLC, MS/MS and NMR analysis and determining the extraction recovery.

### High-Performance Liquid Chromatography (HPLC) analysis

HPLC analysis was performed with a C18 column and a mixed step and linear gradient condition. Briefly, a 150 mm × 4.6 mm i.d., 5 μm, Eclipse Plus C18 (Agilent, Santa Clara, CA) was used as the stationary



**Figure 3:** LC MS/MS Mass Spectra. The mass spectra were measured with Electrospray Ionization (ESI) on a Water MicroMass TQD Tandem Mass Spectrometer running under Waters Empower 3 Chromatography Data Software. The javamide-I (A)/-II (B) were analyzed using the following mass transitions given in parentheses: (m/z).

phase to analyze the standards and coffee samples. The samples were separated using a mobile-phase condition; buffer A (5 mM phosphate, pH 5) for 0-2 min, 5% buffer B (60% acetonitrile) for 2-5 min, a linear gradient from 5%-100% buffer B (60% acetonitrile) for 5-35 min, and buffer B for 5 min (1 mL/min). The samples were injected by an auto-sampler into an Alliance 2690 HPLC system (Waters, Milford, MA), and were monitored by a CoulArray electrochemical detector with four electrode channels (ESA, Chelmsford, MA).

### Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

The LC MS/MS mass spectra were measured with Electrospray Ionization (ESI) on a Water MicroMass TQD Tandem Mass Spectrometer running under Waters Empower 3 Chromatography Data Software.

### Nuclear Magnetic Resonance (NMR) analysis

The peaks of javamide-I/-II were isolated from the coffee samples using the method described previously [8,9]. The chemical structures of javamide-I/-II were verified using NMR spectroscopic methods. NMR samples were prepared by dissolving javamide-I/-II (20 mg) in d<sub>6</sub>-DMSO (0.75 mL). <sup>1</sup>H and <sup>13</sup>C spectra were acquired at ambient temperature on the JEOL BCX-400 NMR spectrometer operating 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C.

### Quantification of javamide-I/-II in coffee beans

Twelve coffee beans obtained from the Sustainable Perennial

**Table 1:** Regions and sample names for the Arabica and Robusta coffee beans used in the study. S1-8 samples from Arabica beans and S9-12 samples from Robusta beans.

Sample	Species	Regions	Sample Names
S1	<i>C. arabica</i>	Brazil	Bourbon
S2	<i>C. arabica</i>	Colombia	Gigante Maragogyne
S3	<i>C. arabica</i>	Costa Rica	Tarrazu
S4	<i>C. arabica</i>	Ethiopian	Harrar
S5	<i>C. arabica</i>	Ethiopian	Kaffa Forest Tega & Tula
S6	<i>C. arabica</i>	Guatemala	Typica
S7	<i>C. arabica</i>	Papua New Guinea	Gumancha
S8	<i>C. arabica</i>	Puerto Rico	Pergamino
S9	<i>C. canephora</i>	India	Robusta bulk
S10	<i>C. canephora</i>	India	Robusta
S11	<i>C. canephora</i>	India	Sufia Robusta
S12	<i>C. canephora</i>	Vietnam	Mocha Robusta

Crops Laboratory coffee collection (USDA, ARS) were individually analyzed using the HPLC method as described in "Materials and Method". Since the extraction was performed three times (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>), the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> extraction samples were individually analyzed to determine the amounts of javamide-I/-II in each extraction. In addition, additional eight coffee beans were separately purchased from online stores and the extraction was prepared as described in "Materials and Methods". The amounts of javamide-I/-II were quantified using Coul Array electrochemical detector with four electrode channels (ESA, Chelmsford, MA).

### Statistical analysis

All statistic analyses were performed with the SigmaPlot 11.0 (Chicago, IL). Data points in all figures were represented as the mean  $\pm$  SD of five measurements for each sample. *P* value was calculated using one-way ANOVA with Holm-Sidak method and *P*<0.05 was considered as statistically significant.

## Results

### Development and validation of HPLC method

The HPLC, mobile-phase and detector conditions were developed for optimizing the detection, separation, resolution and reproducibility of the peaks of javamide-I/-II in the chromatography as described in "Materials and Methods". Using the developed HPLC method, javamide-I/-II standards were reliably separated and detected with fine separation and resolution; total HPLC running time for the assay was 40 min, and javamide-I/-II were detected at a retention time of 27.7 and 26.5 min, respectively (Figure 1A). To validate the HPLC method, standard samples of javamide-I/-II (0-400 pmol) were prepared and HPLC assay was conducted. The detection limit was approximately <5 pmol with SD lower than 15%. In this standard curve, satisfactory linear responses (n=4) for the amides were obtained at the concentrations between 5 to 400 pmol (correlation coefficient (R) = 0.998). Also, intra-assay and inter-assay precision of the method was evaluated as described previously [8] (Data not shown here).

**Table 2:** The amounts and recovery of javamide-I/-II in twelve coffee samples. The amounts and recovery of javamide-I (A) and javamide-II (B). Coffee extract samples (S1-12) were individually extracted twice with 80% MeOH extracts (50 mL) as described in "Materials and Methods", providing the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> extracts. The amounts of javamide-I were presented as mg per 1g unroasted coffee sample (n=5) and recovery ratio (%) =  $(1^{st} + 2^{nd} / 1^{st} + 2^{nd} + 3^{rd}) \times 100$ . (A)

Sample	1 <sup>st</sup> (mg/g)	2 <sup>nd</sup> (mg/g)	3 <sup>rd</sup> (mg/g)	Recovery ratio (%)
S1	ND	ND	-	-
S2	ND	ND	-	-
S3	0.04 $\pm$ 0.01	ND	-	100
S4	ND	ND	-	-
S5	0.05 $\pm$ 0.01	ND	-	100
S6	0.04 $\pm$ 0.02	ND	-	100
S7	0.04 $\pm$ 0.01	ND	-	100
S8	ND	ND	-	-
S9	0.72 $\pm$ 0.08	0.04 $\pm$ 0.01	ND	100
S10	0.77 $\pm$ 0.09	0.04 $\pm$ 0.01	ND	100
S11	0.76 $\pm$ 0.08	0.04 $\pm$ 0.01	ND	100
S12	0.38 $\pm$ 0.06	0.02 $\pm$ 0.01	ND	100

(B)

Sample	1 <sup>st</sup> (mg/g)	2 <sup>nd</sup> (mg/g)	3 <sup>rd</sup> (mg/g)	Recovery ratio (%)
S1	0.16 $\pm$ 0.02	ND	ND	100
S2	0.14 $\pm$ 0.02	ND	ND	100
S3	0.15 $\pm$ 0.02	ND	ND	100
S4	0.14 $\pm$ 0.01	ND	ND	100
S5	0.27 $\pm$ 0.03	0.02 $\pm$ 0.003	ND	100
S6	0.17 $\pm$ 0.02	ND	ND	100
S7	0.16 $\pm$ 0.02	ND	ND	100
S8	0.08 $\pm$ 0.01	ND	ND	100
S9	4.56 $\pm$ 0.51	0.37 $\pm$ 0.07	ND	100
S10	4.26 $\pm$ 0.56	0.25 $\pm$ 0.07	ND	100
S11	4.25 $\pm$ 0.50	0.25 $\pm$ 0.07	ND	100
S12	3.31 $\pm$ 0.48	0.19 $\pm$ 0.06	ND	100

### Extraction and recovery of javamide-I/-II in coffee bean samples

The detail description of twelve coffee samples is shown in (Table 1) and their pictures are in (Figure 2). The samples were extracted as described in "Materials and Methods". After several preparations of coffee samples, 1g coffee beans per 50mL 80% methanol was found optimal for extraction and HPLC separation of javamide-I/-II in the samples. To determine the extraction recovery, the extraction was performed twice. The amounts of javamide-I in the 1<sup>st</sup> extraction were 95% higher than those of the 2<sup>nd</sup> extraction, high enough for no subsequent 3<sup>rd</sup> extraction (Table 2A). The recovery was determined by measuring the amounts following the addition of javamide-I standard (0.2 mg) and found to be more than 98% (Table 2A). Similarly, the amounts of javamide-II in the 1<sup>st</sup> extraction were 96% higher than those of the 2<sup>nd</sup> extraction (Table 2B) and the recovery was found to be more than 98% (Table 2B).

### MS/MS and NMR data of javamides-I/-II

For MS/MS and NMR analysis, each peak of javamide-I/-II was purified from the Arabica (S1-10) and Robusta (S11-14) extracted samples using the HPLC method as described in "Materials and Methods". The MS/MS spectra of javamide-I/-II from Arabica and Robusta samples were measured in the ESI+ mode (Figure 3). The daughter ions ( $m/z$  189 and  $m/z$  203) corresponding to 3-(2-carboxyethyl)-1H-indol-1-ium and L-tryptophanate were observed as the base peaks in javamide-I and -II. However, the daughter ion ( $m/z$  148.1; (E)-3-(4-hydroxyphenyl) acrylaldehyde) showed up in the spectrum of javamide-I (Robusta only) and the daughter ion ( $m/z$  164.0; (E)-3-(3,4-dihydroxyphenyl)acrylaldehyde ion) appeared in the spectrum of javamide-II (Robusta and Arabica). All LC-MS/MS data supported that the compound was indeed javamide-I/-II. These MS/MS data were also confirmed by NMR data. The NMR data of javamide-I were the following: <sup>1</sup>H NMR (d<sub>6</sub>-DMSO, 400 MHz)  $\delta$  7.58 ((1H, d, J) 8.2 Hz, H-18), 7.45 ((1H, d, J) 8.2 Hz, H-1/H-5), 7.37 ((1H, d, J) 15.6 Hz, H-7), 7.33 ((1H, d, J) 7.8 Hz, H-15), 7.20 (1H, s, H-13), 7.06 ((1H, t, J) 7.3 Hz, H-16), 6.98 ((1H, t, J) 7.3 Hz, H-17), 6.59 ((1H, d, J) 8.2 Hz, H-2/H-4), 6.46 ((1H, d, J) 15.6 Hz, H-8), 4.72 ((1H, t, J) 7.3 Hz, H-10), 3.24 ((1H, dt, J) 6.0, 6.9 Hz, H-11), 9.68 (1H, br s, OH-a), 12.89 (1H, br s, OH-a'), 10.79 (1H, br s, NH-beta), 8.38 (1H, br s, NH-alpha); <sup>13</sup>C NMR (d<sub>6</sub>-DMSO, 100 MHz) 174.7 (C, C-20), 166.8 (C, C-9), 157.5 (C, C-3), 141.7 (C, C-7), 136.4 (C, C-14), 130.6 (C, C-1), 130.6 (C, C-5), 127.9 (C, C-6), 127.4 (C, C-19), 124.0 (C, C-8), 123.0 (C, C-13), 121.7 (C, C-16), 119.8 (C, C-17), 118.8 (C, C-18), 115.7 (C, C-2), 115.7 (C, C-4), 111.5 (C, C-15), 109.3 (C, C-12), 60.3 (C, C-10), 28.2 (C, C-11). Based on the NMR data, the structure of the isolated compound was determined as being coumaroyl-L-tryptophan (N-coumaroyltryptophan; javamide-I). Likewise, the NMR data for javamide-II were the following: <sup>1</sup>H NMR (d<sub>6</sub>-DMSO, 400 MHz)  $\delta$  7.58 ((1H, d, J) 8.2 Hz, H-18), 7.33 ((1H, d, J) 7.8 Hz, H-15), 7.32 ((1H, d, J) 15.6 Hz, H-7), 7.20 (1H, s, H-13), 7.06 ((1H, t, J) 7.3 Hz, H-16), 7.06 (1H, s, H-5), 6.98 ((1H, t, J) 7.3 Hz, H-17), 6.82 ((1H, d, J) 8.7 Hz, H-2), 6.67 ((1H, dd, J) 8.2, 1.4 Hz, H-1), 6.46 ((1H, d, J) 15.6 Hz, H-8), 4.72 ((1H, t, J) 7.3 Hz, H-10), 3.24 ((1H, dt, J) 6.0, 6.9 Hz, H-11), 9.48 (1H, br s, OH-a, b), 12.89 (1H, br s, OH-a'), 10.79 (1H, br s, NH-beta), 8.38 (1H, br s, NH-alpha); <sup>13</sup>C NMR (d<sub>6</sub>-DMSO, 100 MHz) 174.7 (C, C-20), 166.8 (C, C-9), 146.5 (C, C-3), 145.9 (C, C-4), 141.7 (C, C-7), 136.4 (C, C-14), 128.0 (C, C-6), 127.4 (C, C-19), 124.0 (C, C-8), 123.2 (C, C-1), 123.0 (C, C-13), 121.7 (C, C-16), 119.8 (C, C-17), 118.8 (C, C-18), 117.2 (C, C-2), 115.2 (C, C-5), 111.1 (C, C-15), 109.7 (C, C-12), 60.3 (C, C-10), 28.2 (C, C-11). Based on the NMR data, the structure of the isolated compound was determined as being (E)-(3-(3,4-dihydroxyphenyl)acryloyl)tryptophan (N-caffeoyltryptophan; javamide-II).

### HPLC quantification of javamide-I/-II in Arabica and Robusta beans

For the quantification of javamide-I/-II, each coffee bean was individually extracted and prepared for HPLC analyses using the same extraction method described in "Materials and Methods". As shown in (Table 3), javamide-II was detected in all twelve samples (0.08 to 4.93 mg/g), while javamide-I was detected in all four Robusta beans (0.40 -0.81 mg/g), and only four Arabica beans (0.04 -0.05 mg/g). The data showed that the amounts of javamide-II in Robusta beans were more than 20 times higher than the average amount of

**Table 3:** The amounts of javamide-I/-II in the samples. The amounts were presented as mg/g coffee sample (n=5). P value was calculated using one-way ANOVA with Bonferroni method and the marks (\*) indicate statistical significance (P<0.05) verse Arabica samples (S1-8). The marks (\*\*) indicate statistical significance (P<0.05) between Robusta sample (S9) and Robusta sample (S12).

Sample	Javamide-I (mg/g)	Javamide-II (mg/g)
S1	ND	0.16±0.02
S2	ND	0.14±0.02
S3	0.04±0.01	0.15±0.02
S4	ND	0.14±0.02
S5	0.05±0.01	0.29±0.04
S6	0.04±0.02	0.17±0.02
S7	0.04±0.01	0.16±0.02
S8	ND	0.08±0.01
S9	0.76±0.08*	4.93±0.59/**
S10	0.81±0.11*	4.51±0.63*
S11	0.80±0.09*	4.50±0.57*
S12	0.40±0.07*	3.50±0.55*

**Table 4:** The amounts of javamide-I/-II were presented as mg /g coffee sample (n=4). The bold values indicate Robusta samples.

Sample	Javamide-I/-II (mg/g)	Designation	Identities/Name
A1	<b>0.23±0.03/3.35±0.45</b>	Robusta	Philippine Robusta
A2	0.04±0.01/0.11±0.03	Arabica	Colombia Arabica
A3	0.08±0.02/0.31±0.06	Arabica	Indian Arabica
A4	<b>0.27±0.04/2.31±0.32</b>	Robusta	Madagascar Robusta
A5	<b>0.45±0.06/3.16±0.49</b>	Robusta	Vietnam Robusta
A6	0.04±0.01/0.19±0.04	Arabica	Mexico Arabica
A7	0.03±0.01/0.12±0.03	Arabica	Nicaragua Arabica
A8	1.12±0.12/4.50±0.62	Robusta	Indian Robusta

javamide-II in Arabica beans (P<0.05).

### Influence of coffee bean cultivars on the amounts of javamide-I/-II

The eight Arabica beans showed low levels and limited ranges of javamide-I (0.04 to 0.05 mg/g) and javamide-II (0.08 to 0.29 mg/g) compared to Robusta beans (Table 3). This result supports previous reports showing the lack of genetic diversity in Arabica cultivars [13] and the *Arabica coffee* germplasm in general (World Coffee Research, <https://worldcoffeeresearch.org/work/measuring-genetic-diversity-coffee-arabica/>). Related to geographical effects, the data showed that the production location appears not to have a major influence on the javamide levels especially in Arabica cultivars (Table 3). Compared to the Arabica beans whose uniformity differs greatly from the high heterogeneity of the Robusta samples, the four Robusta samples in this study had relatively high detectable levels of javamide-I (0.40 to 0.81 mg /g) and javamide-II (3.50 to 4.93 mg /g). Furthermore, the particular Robusta sample from India (S9) contains higher levels of javamide-II than the Robusta sample from Vietnam (S12), suggesting that there may be significant disparity in the amounts of javamide-II even in Robusta beans.

## Investigation of Robusta coffee beans for better sources for javamide-I/-II

Because the data showed that Robusta samples had relatively high levels of javamide-I/-II compared to Arabica samples, we explored Robusta species as candidate bean sources for javamide-I/-II. For this study, eight additional coffee beans (four Arabica and four Robusta beans) were purchased and the extraction and HPLC analysis was performed as described in “Materials and Methods”. As expected, Robusta beans (A1, A4, A5 and A8) contained javamide-I/-II significantly higher than Arabica beans (A2, A3, A6 and A7) ( $P < 0.05$ ) (Table 4). Once again, it was noticed that higher levels of javamide-I/-II were found in Robusta beans from India than the Robusta beans from other regions (Vietnam, Philippine, Madagascar). These data were in line with the data from the twelve coffee beans that Robusta beans contain more javamide-I/-II than Arabica beans, suggesting that Robusta beans may be better beans sources for javamide-I/-II than Arabica beans.

## Discussion

Coffee is one of the most widely consumed beverages worldwide. Recent studies showed that coffee consumption is positively associated with potential beneficial effects on several human diseases [14-17]. Arabica and Robusta beans are two highly traded coffee beans cultivated in over 70 countries. There are a great number of Arabica cultivars, but the genetic diversity of this species is very limited due to the genetic bottle neck during its evolution and dispersal, as well as the nature of production by self-fertilization [18]. On the other hand, Robusta has a higher genetic diversity due to the wide geographical distribution, diverse wild populations and the nature of out-crossing [19]. Moreover, Robusta can be cultivated in lower altitudes and warmer climates than Arabica because Robusta is less susceptible to disease than Arabica [19,20]. Due to these differences in their genetic diversity and cultural practice, it is believed that the chemical composition of coffee beans may be influenced by genetic and cultivation factors. Javamide-I/-II are relatively new phenolic amides found in coffee beans [7-9]. Recently, they and derivatives were reported to contain several biological activities related to human health [9-12]. However, the influence of geographical impacts on amounts of javamide-I/-II is currently unknown in Arabica and Robusta beans. Therefore, in this paper, we investigated the quantities of javamide-I/-II in two most traded coffee species (Arabica and Robusta) and potential geographical influences on their amounts. Based on the data herein, javamide-I/-II may be found at higher levels in Robusta than Arabica beans, and the geographical regions (Ethiopia, Hawaii, Costa Rica, Colombia, Brazil, Argentina, Papua New Guinea, Vietnam) seem to have little effect on the javamide levels especially in Arabica varieties (Table 3). However, we noticed that the Robusta beans with India origin contains significantly higher levels of javamide-II than the Robusta beans from other regions (Tables 3,4), suggesting the possible heterogeneity of the Robusta coffee, although growing conditions and limited sample number cannot be discounted in the interpretation of the data. Therefore, in the future, further analysis of more coffee beans grown in different cultivated conditions may be required to reveal how growing conditions influence the levels of javamide-I/-II in coffee beans.

## Conclusion

The data suggest that javamide-I/-II may be present at higher levels in Robusta beans than in Arabica, and Robusta species are likely to be better bean sources for javamide-I/-II than Arabica species.

## Acknowledgment

This study was funded by USDA (project number 1235-51000-054-00D).

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