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Comparison of Trigonelline Content in Three Philippine Coffee Varieties

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Abstract: As the international market for fine flavor and single origin coffee grows, it would be important to evaluate compounds responsible for aroma and health benefits in Philippine coffee. Trigonelline is one of the biomolecules responsible for these and has been widely studied in coffees from other countries. In this study, trigonelline content was compared among Philippine coffee varieties to evaluate the variable contribution of coffee variety type and environment to one of the compounds responsible for quality—trigonelline. Trigonelline was extracted from eighteen samples of *Coffea arabica* (Arabica), *Coffea canephora* (Robusta), and *Coffea liberica* var. Liberica (Liberica) from different sites across the Philippines using washing the precipitate method. Concentrations were obtained spectrophotometrically with methanol as the solvent. Trigonelline content in Liberica and Robusta coffee was not significantly different from each other but were significantly higher than trigonelline content in Arabica. These results were very different from what was known in coffee of other countries. Pairwise comparison of trigonelline content across sites for the same variety type suggests that environment compared to coffee variety type was more important in determining differences among Robusta. In contrast, variety type rather than environment was more significant in determining differences in trigonelline among Arabica. Trigonelline in Liberica samples across sites were not significantly different. These results highlight the possible difference of Philippine coffee from other coffees globally, as well as the variation in the contribution of environment and type in determining coffee quality for different varieties.

Key Words: trigonelline; Philippine coffee; coffee quality; coffee varieties

1. INTRODUCTION

Coffee, produced from *Coffea* species, is one of the most commercialized commodities in the Philippines. In 1880, Philippines became the fourth largest coffee producer worldwide especially when coffee rust hit various countries such as Brazil, Africa and Java (Philippine Coffee Board, n.d.). However up to the present, Philippines became the 27th largest coffee producer primarily because of the inability to supply good quality coffee from overall increasing demand of consumers. Coffee plantations in the country have shrunk and the systems being executed remained conventional as opposed to other big coffee producers namely Brazil, Columbia, and Vietnam whose systems became more advanced from farm planning, nursery management, coffee harvest to post-harvest practices. As a result, the Philippines relied on imports from its neighbors.

Coffee sensorial quality is affected by three factors—environment, genetics, and processing (Avelino et al., 2005; Bertrand et al., 2012, 2006; Borém et al., 2013; Decazy et al., 2003; Figueiredo et al., 2013; Ribeiro et al., 2016). These factors actually cause variations in flavor profiles of coffee. Flavor is composed of taste, texture, and aroma. The four

Philippine coffee varieties (*Coffea arabica, Coffea canephora, Coffea liberica* var. Liberica, and *Coffea liberica* var. Dewevrei) differ in terms of aroma and taste as attributed by alkaloid compounds. Trigonelline is the second most important alkaloid next to caffeine (Allred et al. 2009). Since it is an alkaloid which is derived from amino acids, then it is related to proteins. Trigonelline acts as aroma precursors that also give rise to flavor products (Van Cuong et al., 2014; De Maria et al., 1996). Trigonelline content is found to be higher in green beans than in roasted beans (Farah, 2012). Thus, the researchers used green beans to extract trigonelline which were expected to contain larger amounts of trigonelline.

This study determines trigonelline in Philippine coffee to check the status of Philippine coffee varieties in terms of aroma and quality in general. This study also evaluates the contributions of variety type and environment across Philippine coffee varieties.

This study involved green Arabica beans from Benguet (Atok, Tublay, and La Trinidad) that were known to be pure while those from Digos and Kidapawan were known to be hybrids particularly Catimor cultivars as confirmed by Ms. Jane Ele, who conducted a molecular genomic study on it. All the possible factors considered namely environment, variety type, and farm management were not further investigated in this study.

2. METHODOLOGY

2.1 Collection of Samples

Green coffee beans of Robusta, Arabica, and Liberica from 18 sites (n=18) in the Philippines were collected and identified by CvSU (Cavite State University) and NCRDEC (National Coffee Research Development and Extension Center). Three extraction replicates were obtained per site of each coffee variety.

2.2 Description of the Study Site

The 18 study sites were shown in figure 1. Robusta coffee samples were obtained from 9 sites while 5 sites for Arabica coffee and 4 sites for Liberica coffee.

Fig. 1. Philippine map showing Benguet, Cavite and Batangas regions in larger scale (Google map image- https://www.google.com.ph/maps/)

2.3 Procedure

Various amounts of trigonelline hydrochloride powder was dissolved in methanol to yield concentration ranges from 0.1 mg/mL to 0.6 mg/mL. The absorbance of standard trigonelline solution was quantified using a UV-Vis spectrophotometer at 265 nm against a blank solution of methanol (Caprioli et al., 2014).

Washing the precipitate method by Sridevi and Giridhar (2013) and Taguchi, Sakaguchi & Shimabayashi (1985) for extracting trigonelline was used. However, the values were adjusted in proportions to avoid utilizing too many coffee beans and reagents. The green coffee beans were milled using a coffee grinder, followed by heating using a digital dry bath then centrifugation. Heating the samples at 100°C does not induce the conversion of trigonelline to its derivatives since its degradation to volatile aromatic compounds happens as temperature approaches 160°C or 320°F during Maillard reaction and caramelization phases (Rivera, 2015). The supernatant was discarded while the pellet was consequently added with water and centrifuged again before collecting the final supernatant. The final supernatant was subjected to

spectrophotometric analysis at 265 nm (Caprioli et al., 2014). The prepared solutions were analyzed against a blank solution containing methanol.

3. RESULTS AND DISCUSSION

3.1 Comparison of Trigonelline Content among Coffee Varieties

Among the 3 coffee varieties, mean trigonelline content of Philippine Liberica coffee yielded the highest value followed by Robusta and Arabica coffee (refer to table 1) and confirmed to be significant after performing single factor ANOVA with a p-value of 0.0313. This resulting trend was observed to be opposite to a previous study performed by Stennert and Maier (1994) on trigonelline of coffee varieties wherein Arabica coffee yielded the highest trigonelline content followed by Robusta and Nigeria Liberica coffee. Geographically speaking, trigonelline of African coffee and Philippine coffee is totally different. Liberica is known to have a very strong aroma and earthy taste thus locally calling it as "Barako coffee". Since trigonelline is a bitter alkaloid that produces aromatic compounds and even contributes a unique bitter-sweet and earthy taste to coffee (Trigonelline in Coffee, 2015), then the data is not surprising and in fact unique to Philippine coffee. Similarly, Robusta has also an earthy taste. On the other hand, Arabica coffee has a sweet taste and aroma thus justified to have the lowest trigonelline content. The study of Philippe et al. (2005) showed that trigonelline content increases with decreasing altitude. Since Robusta and Liberica are mostly cultivated in lower altitudes of around 0 to 800 meters while Arabica in higher altitudes of around 600 to 2200 meters, then the trend of trigonelline content in Philippine coffee is reasonable. However, the study of Sridevi and Giridhar (2013) suggested that higher altitude increases trigonelline in Robusta while no significant effects in Arabica. These various effects of altitude to trigonelline suggest that other factors may be involved aside from altitude. This can be the interplay between genetics and other environmental parameters as well as cultivation and processing of coffee.

Although Arabica coffee is generally known to have the highest coffee quality because of its sweet taste, high acidity and worldwide production yet it yielded the lowest trigonelline content. Thus, trigonelline might not be the most important constituent of Philippine coffee quality or might be better analyzed with other biochemical components of coffee that will reflect coffee flavor. It is also possible that varieties of Liberica, Robusta, and Arabica are wider in the Philippines. Thus, site per site in each variety was studied in the next section.

Table 1. Summary of mean trigonelline content and corresponding standard deviation per coffee variety

Coffee Variety	Mean ($\bar{x}$) in µg/µL	Standard deviation (s)
Robusta	1.865	0.410
Arabica	1.274	0.413
Liberica	1.910	0.276

Among the 3 coffee varieties, only Arabica was found to be significantly different to Robusta and Liberica after performing Tukey's pairwise comparison (refer to table 2). On the other hand, Robusta and Liberica were found not to be significantly different to each other. Thus, it was interesting to check the most plausible factors affecting trigonelline per coffee variety.

Table 2. Tukey's pairwise comparison of trigonelline content among coffee varieties (highlighted portions indicate significant difference because of p-value <0.05)

	Arabica	Liberica	Robusta
Arabica		0.002	0.001
Liberica	0.002		0.957
Robusta	0.001	0.957	

3.2 Comparison of Trigonelline Content within Coffee Varieties

3.2.1 Comparison among sites of Robusta coffee

Trigonelline content was significantly different among sites for Robusta based on single factor ANOVA with p-value of 0.003 but not much differences were observed across sites except for Amadeo, Cavite. Amadeo, Cavite yielded the highest trigonelline content while Silang, Cavite yielded the lowest (refer to figure 2). Amadeo, Cavite is found at the highest altitude of around 1521.50 feet to 1742.01 feet as compared to the rest of the sites. Alzo et al. (2016) mentioned that total protein content in 9 sites of Nestle Company in the Philippines (exactly the same 9 sites in this study) cultivating the same kind of Robusta still showed significant difference thus attributing Robusta coffee quality to environmental factor, neither variety nor farm management. Since

trigonelline trend is related to protein trend, then this significant difference might be attributed to environmental factor specifically altitude since Sridevi and Giridhar (2013) and Philippe et al. (2005) confirmed that trigonelline content varies with altitude but this was not investigated in this study.

Fig. 2. Comparison of trigonelline content of Robusta across sites

Table 3. Tukey's pairwise comparison of trigonelline content within Robusta (highlighted portions indicate significant difference because of p-value <0.05)

	AC	AMC	GEA	SC	BS	SL	SK	KA	LB
AC		0.243	0.998	0.318	0.991	0.691	1.000	1.000	0.978
AMC	0.243		0.067	0.002	0.049	0.994	0.470	0.273	0.037
GEA	0.998	0.067		0.729	1.000	0.288	0.947	0.995	1.000
SC	0.318	0.002	0.729		0.813	0.012	0.150	0.284	0.876
BS	0.991	0.049	1.000	0.813		0.225	0.900	0.985	1.000
SL	0.691	0.994	0.288	0.012	0.225		0.914	0.735	0.179
SK	1.000	0.470	0.947	0.150	0.900	0.914		1.000	0.844
KA	1.000	0.273	0.995	0.284	0.985	0.735	1.000		0.966
LB	0.978	0.037	1.000	0.876	1.000	0.179	0.844	0.966	

3.2.2 Comparison among sites of Arabica coffee

Trigonelline content was significantly different among sites for Arabica based on single factor ANOVA with p-value of 0.014 but not much differences were observed across sites except for Atok, Benguet. Atok, Benguet yielded the highest trigonelline content while Digos, Davao Del Sur yielded the lowest (refer to figure 3). Although Atok, Benguet is located nearby Tublay, Benguet which both have very high altitudes at around 2,500 feet to 3,500 feet and both located at the foot of Mt. Pulag, yet significant difference was still observed. Although all Arabica coffee from Benguet were known pure breeds while Arabica coffee from Digos was a known hybrid, significant difference was indeed observed. Van Der Vossen (2009) mentioned that even though Catimors (hybrid) have higher resistance to diseases and pests such as coffee berry borers, they still often impart undesirable flavor to the cup. Thus, trigonelline and other coffee flavor constituents might greatly vary between pure and hybrid Arabica in parallel to those found in Atok, Benguet and Digos. The difference might be attributed to variety type specifically to different levels of purity and hybridization but this was not investigated in this study.

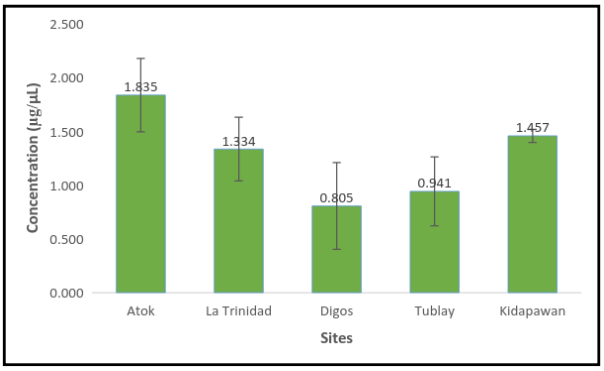

Fig. 3. Comparison of trigonelline content of Arabica across sites

Table 4. Tukey's pairwise comparison of trigonelline content within Arabica (highlighted portions indicate significant difference because of p-value<0.05)

	Atok	Digos	Kidapawan	La Trinidad	Tublay
Atok		0.014	0.580	0.332	0.033
Digos	0.014		0.144	0.288	0.980
Kidapawan	0.580	0.144		0.986	0.308
La Trinidad	0.332	0.288	0.986		0.548
Tublay	0.033	0.980	0.308	0.548	

3.2.3 Comparison among sites of Liberica coffee

Trigonelline content was not significantly different among sites for Liberica based on single factor ANOVA with p-value of 0.225. No differences were actually observed across sites although Liberica coffee in Indang, Cavite yielded the highest trigonelline content while Magallanes, Cavite yielded the lowest (see figure 4). Considering that Philippine Liberica coffee is very limited in variety and geography because it usually grows in Batangas and

Cavite areas only (STRIDE 2017), then it is indeed expected that Liberica coffee quality will not cause significant differences. Since all these sites were located nearby from Southern part of Luzon with altitude that ranges from 500 feet to 2,000 feet, then environmental conditions are less likely to differ for long periods of time. Liberica coffee plants are known to be diploid so they are not expected to hybridize and produce plants that are polyploid unlike the case of Arabica coffee plants (Sy et al. 2016). Thus, the trigonelline content in Liberica coffee might not be variable because of its limited variety and distribution.

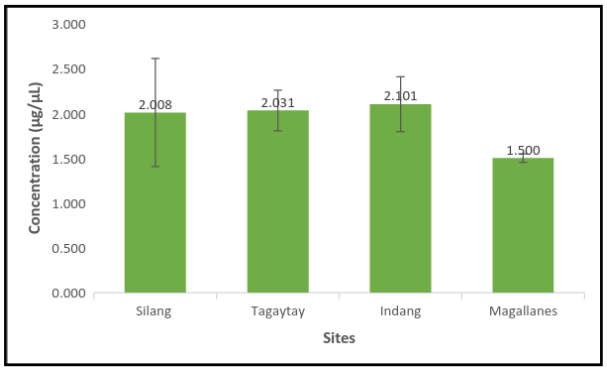

Fig. 4. Comparison of trigonelline content of Liberica across sites

3.3 PCA of Trigonelline and Protein Content across Sites

When comparing Robusta and Liberica coffee, figure 5 showed that both Robusta and Liberica sites were closely similar to each other at one side of the factor-plane while very different from Arabica sites at the other side of the factor-plane. Thus, this result suggests that both Robusta and Liberica coffee were not significantly different not just in trigonelline but also in protein contents. The distinction among these 3 coffee varieties was supported by a total of 99.99% variance explained in this factor-plane.

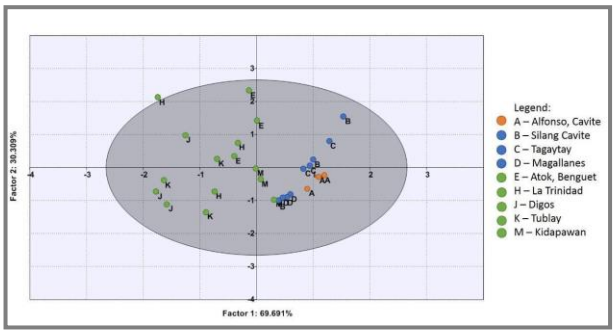

Fig. 5. Principal component analysis of sites among each coffee variety

4. CONCLUSIONS

The highest trigonelline content was observed in Liberica, followed by Robusta then Arabica, which was the opposite trend found in coffee from other countries. Arabica has generally the highest coffee quality yet with the lowest trigonelline content. Thus, trigonelline might not be one of the most important constituents of coffee quality or might be better analyzed together with other flavor constituents. Trigonelline of Arabica coffee quality might be mostly attributed to variety type particularly to different levels of purity and hybridization while environment specifically altitude for Robusta coffee quality in reference to the study of Alzo et al. (2016). Trigonelline of Liberica coffee quality was neither attributed to variety type nor environment or might also be equally attributed to these two. These results highlight the possible difference of Philippine coffee from other coffees globally, as well as the interplay contribution of genetics, environment, and farm management to Philippine coffee quality. These results highlight the importance of site and variety type selection in determining one of the chemicals reflecting coffee quality and help improve strategies in producing better Philippine coffee across sites.

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