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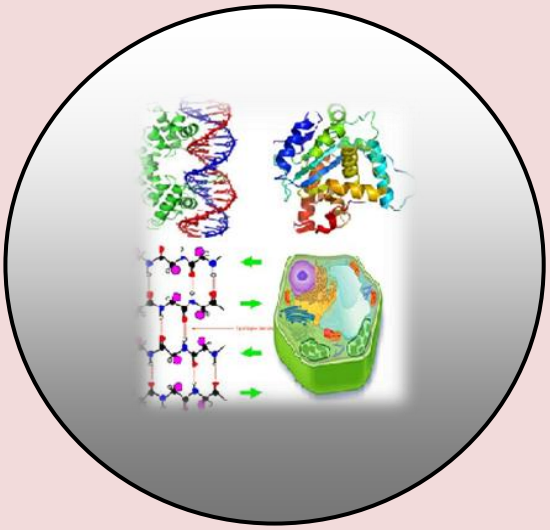

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The Bioactive Compounds Formation of "Kintamani" *Arabica* Coffee Bean during Dry Fermentation

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ABSTRACT

Green coffee bean contain many bioactive compounds, especially caffeine and chlorogenic acid. The research aimed was to evaluated and determined of the temperature and fermentation time on the formation of bioactive compounds of "Kintamani" arabica coffee bean S 795 cultivar. This research consists of two factors. The first factor is the temperature of fermentation consists of 20 ± 1°C, 30 ± 1°C and 40 ± 1°C. The second factor of fermentation times consists of 20, 30 and 40 hours with. The results showed that interaction of temperature and fermentation time gave very significantly effect on the formation of chlorogenic acid content but not gave significantly effect on caffeine content. The treatment produced of chlorogenic acid content ranged 4,69-11,21 %. The highest chlorogenic acid content 11,21% was produced by fermentation temperature 40 ± 1°C and fermentation time of 20 hours. The caffeine content of the treatment ranged 0,98-1,20 %. The highest content of caffeine 1,20 % was produced at fermentation temperature of 30 ± 1°C for 20 hours but this treatment was not significant effect with treatment of 40 ± 1°C and fermentation time of 20 hours with chlorogenic acid content 1,19 %. The fermentation of 40 ± 1°C for 20 hours can increase of the bioactive compounds are chlorogenic acid and caffeine content.

Keywords: Arabica coffee, Bioactive Compound, Dry Fermentation and Kintamani-Bali.

INTRODUCTION

Indonesia is the fourth largest coffee exporter after Brazil, Vietnam and Colombia (ICO, 2017). Fujioka and Shibamoto (2008) stated that coffee is the second ranks in all food commodities consumed and traded throughout the world. Bali is one of the provinces in Indonesia which is also known as a coffee producer. Kintamani District-Bangli is one of the largest arabica coffee producer areas in Bali. *Kintamani arabica* coffee is well-known internationally because of its high quality and orange flavor. Based on the data from the Statistics Central Bureau (BPS) of Bali Province in 2016 that the total amount of arabica coffee production in 2015 was 4,153.97 tons, and the highest *arabica* coffee production was produced by Bangli Regency at 2,456 tons, followed by Buleleng Regency 859.20 tons, and Tabanan 666.58 tons. Coffee is known as a functional food because of its high content of antioxidant compounds and beneficial bioactive properties (Farah, 2012), rich in antioxidants and has radical scavenger activity (Shan, *et al* 2015), and contains antioxidants in particular high content of phenol and caffeine compounds (Emma, 2016).

Furthermore, Nuhu (2014) four important bioactive compounds present in coffee are caffeine, chlorogenic acid, diterpene, and trigonelin. The main antioxidant compounds found in coffee beans are chlorogenic acid. The benefits of chlorogenic acid for humans are as antioxidants, antiviral, hapatoprotective, and contribute to antispasmodic activities (Farhaty and Muchtaridi, 2012). Farah and Donangelo (2006), chlorogenic acid is an important compound that influences the formation of taste and flavor during coffee roasting and is a parameter commonly used to determine of coffee quality. Widagdyo, *et al* (2013), stated that the most important components contained in coffee are caffeine and cafeol. Caffeine is an active ingredient that stimulates nerve work. Caffeine has a clinically beneficial pharmacological effect (Maramis, *et al* 2013). Rosita, *et al* (2016), caffeine is one of the most important quality indicators of coffee.

The quality and flavour of coffee is influenced by a number of factors, one of those is processing method. Most of coffee processing by farmers in Bali were done by dried process because it was easier to done but the quality is lower. Improving of the coffee quality can be done by using of a wet method with fermentation. This method is more difficult but in terms of time is faster and the quality of the coffee produced is better. Avallone, *et al* (2002): Wamuyu, *et al* (2017), the processing method used in production green coffee affects the chemical composition and cup quality of the coffee bean and wet coffee processing with fermentation produces better quality than other methods without fermentation. Murthy and Naidu (2011); Yusianto and Widyotomo (2013), the most important conditions of fermentation are temperature and time of fermentation, thickness of mucilage, enzyme concentration and microbiology. Many chemical compounds formed during the fermentation process, namely, organic acids, amino acids, reducing sugars (Lin, 2010). A well-done fermentation process will increase the formation of bioactive compounds such as caffeine and chlorogenic acid. However the bioactive compounds in coffee beans are very useful for the health of the human body as an antioxidant, the availability of these bioactive compounds needs to be improved through the proper fermentation process. Therefore, it is necessary to conduct a research with the aim to measure of caffeine and chlorogenic acid formation in Kintamaniarabicacoffee beans during the dry fermentation.

MATERIAL AND METHODS

Full rife of arabica coffee cherries S 795 cultivar was used in this research and its obtained from a farmer in Kintamani Bangli-Bali. This research and analysis were conducted at Laboratory of Agricultural Faculty, Warmadewa University Denpasar, Food Analysis Laboratory Faculty of Agricultural Technology Udayana University and Biosain Laboratory of Polytechnic Jember-East Java. This research has been conducted from June to October 2018.

Separation Condition Chlorogenic Acid		
Instrument	Shimadzu LC-MS 2020	
Column	C 18 Waters	
Mobile Phase A	Water / acetic acid 0,1 %	80 %
Mobile Phase B	Methanol	20 %
Flow rate	0,8 mL/min	
Column Temperature	40˚C	
Injection Volume	10 µL	
Interface	ESI	
Total Runtime	5 min	
SIM	Positive mode 355 m/z Positive mode 353 m/z	

The research procedure consists of sortation, pulping, fermentation, washing, drying, hulling, packaging and analysis. This research was designed with a Completely Randomized Design (CRD) with two treatment factors. The first factor is the temperatures of fermentation which consists of three levels including of 20 ± 1^{o}C, 30 ± 1^{o}C and 40 ± 1^{o}C. The second factor of fermentation times consists of three levels, namely, 20, 30 and 40 hours with replicated 3 times.

Two bioactive compounds, caffeine and chlogenenic acids content of *Kintamani arabica* coffee bean were evaluated after fermentation processed. The caffeine was analyzed with method of spectrophotometer UV-vis double beam at wave length 275 nm (Maramis, *et al* 2013) and chlorogenic acids content was analyzed with LC-MS method (Wanika, *et al* 2010; Ayelign and Sabally, 2013). The procedure condition of separation chlorogenic acid instrument as shown below. The dates were analyzed with ANOV Ausing Microsoft Excel program. If the F test shows a significant effect on the 5% test level, then proceed with a Duncan›s Multiple Range Test (DMRT) 5% (Steel and Torrie, 1991).

RESULTS AND DISCUSSIONS

A. Chlorogenic Acids

Based on the statitical analysis found that the treatment of temperature and fermentation time and their interactions gave a very significant effect ($P<0.01$) on the chlorogenic acid content of the *Kintamani arabica* coffee bean cultivar S 795 produced. The average value of chlorogenic acid *Kintamani arabica* coffee beans due to the influence of temperature and fermentation time as presented in Table 3.1.

Table 3.1. Chlorogenic acid content (%) of *Kintamani arabica* coffee bean due to influenced of Temperature and Fermentation Time.

Fermentation		Chlorogenic acid (%)
Temperature (°C)	Time (hours)	
	20	5,18 g
20 ±1	30	4,69 h
	40	5,31 f
	20	6,82 e
30 ±1	30	7,72 c
	40	7,02 d
	20	11,21 a
40 ±1	30	10,88 b
	40	11,14 a
Coefficient of Diversity = 0,74%		

Note: Mean with the same letter in column are not significantly different at P≤0.05

In Table 3.1 above were showed that the chlorogenic acid content of *Kintamani arabica* coffee beans of the S 795 cultivar range from 4.69-11.21%. The highest average of chlorogenic acid content 11.21% was produced by the combination treatment of 40 ± 1°C fermentation temperature for 20 hours fermentation time and this result was significant difference ($P<0.05$) with all fermentation treatments but this treatment was not significant difference ($P>0.05$) with fermentation temperature of 40 ±1°C for 40 hours was 11.14%. The lowest average value of chlorogenic acid was 4.69 % produced by fermentation temperature of 20 ± 1°C for 30 hours and this treatment showed a significantly differences ($P<0.05$) for all treatments. The analysis of chlorogenic acid with LC-MS method of *Kintamani arabica* coffee bean S795 cultivar as shown on standard chromatogram (Fig. 3.1). The treatment of fermentation temperature 40 ± 1°C for 20 hours has the largest peak area of chromatogram therefore gave the highest of chlorogenic acids content of *Kintamani arabica* coffee bean (Fig. 3.2). In Figure 3.3, showed that increased of chlorogenic acid concentration during dried fermentation due to the higher of temperature and the longer of fermentation time. It is indicate that the fermentation process carried out at a temperature of 40 ± 1°C for 20 hours is better than at are temperatures of 20 ± 1°C and 30 ± 1°C. Increasing of chlorogenic acid content is probably due to the fermentation carried out at 40 ± 1°C for 20 hours is the right temperature and time for the fermentation process in *Kintamani arabica* coffee beans, therefore the degradation of the chlorogenic potassium caffeine complex into chlorogenic acid will increase the concentration of chlorogenic acid in the coffee beans produced. According to Febriyani, *et al* (2013), chlorogenic acid was formed from degradation of chlorogenic potassium caffeine complex during fermentation. During the fermentation process many bioactive compounds are formed, one of these is chlorogenic acid (Lin, 2010).

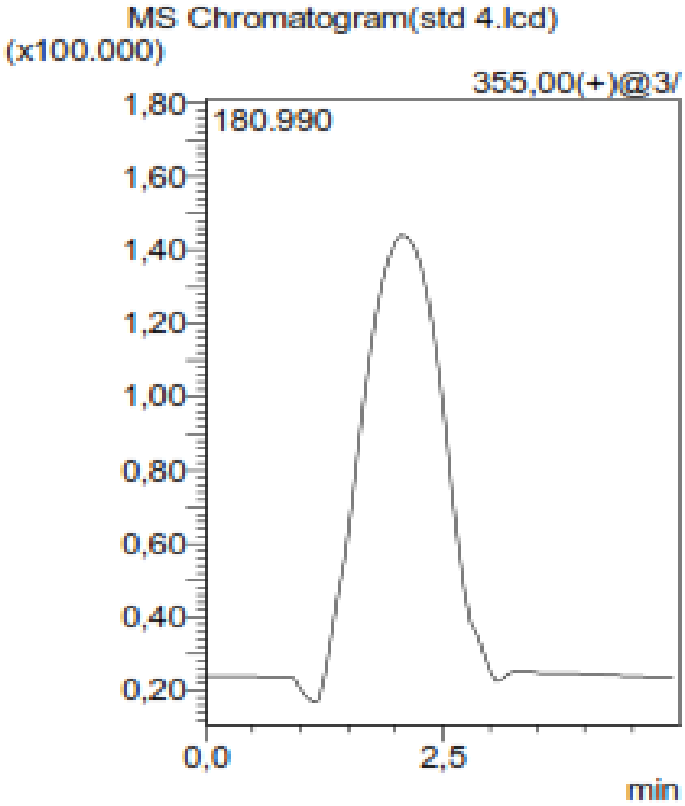

Figure 3.1. Chromatogram standard of Chlorogenic acid.

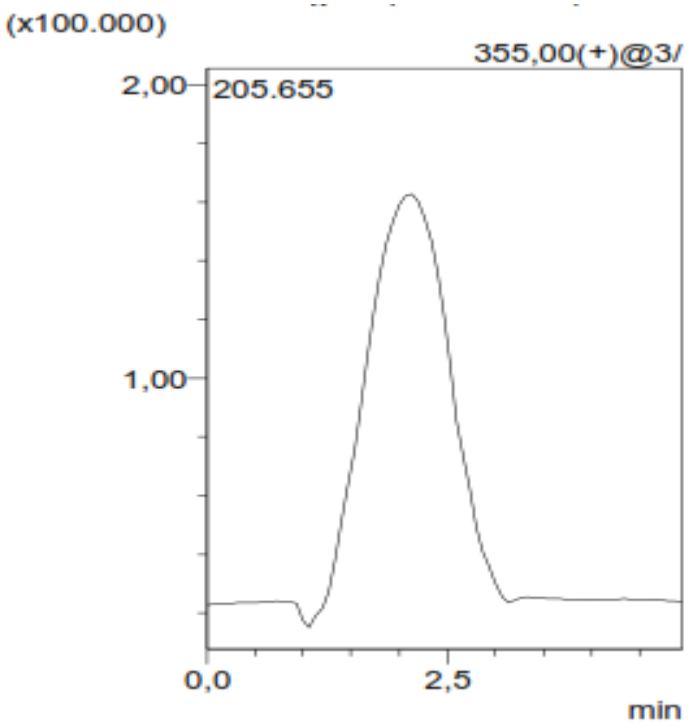

Figure 3.2. Chromatogram Chart of Chlorogenic Acid *arabica* coffee beans due to treatment Fermentation temperature of 40 ± 1°C and fermentation time of 20 hours.

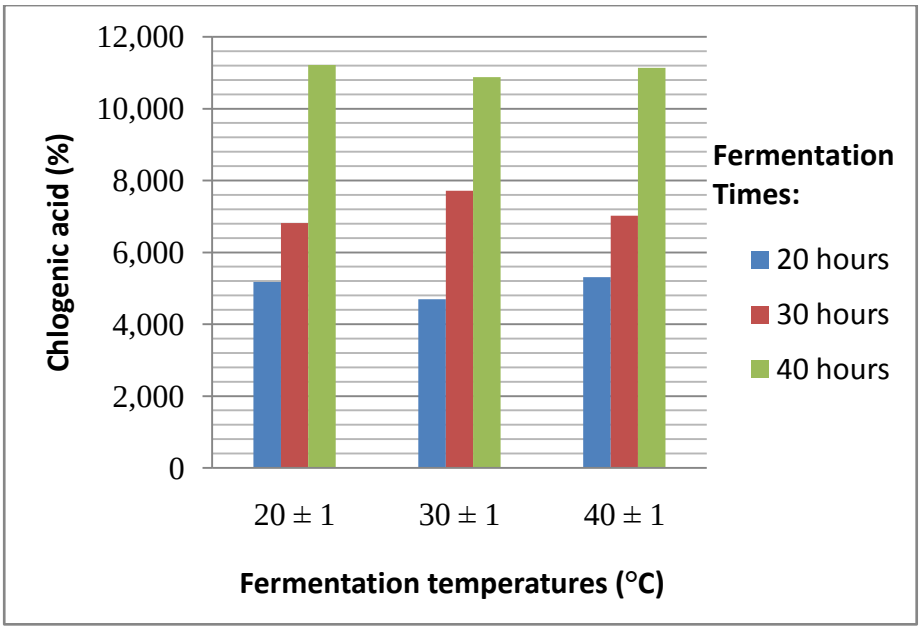

Figure 3.3. Relationship between a Temperature and Fermentation Time on Chlorogenic Acid content.

The results of this research found that the treatment of temperature and fermentation time can increased of chlorogenic acid content of *Kintamani arabica* coffee beans range from 4.69 to 11.21%. High levels of chlorogenic acid in *Kintamani arabica* coffee beans will be very beneficial for the body as an antioxidant. The results of the study by Farhaty and Muchtaridi (2012) showed that the chlorogenic acid content of unfermented *arabica* coffee beans are 4.1-7.9 %. Compared of this chlorogenic acid content, therefore the results of this study were able to increase the concentration of chlorogenic acid by 3.31% after fermentation at a temperature of 40 ± 1°C for 20 hours.

Table 3.2. Caffeine content (%) of *Kintamani arabica* coffee bean due to effect of Temperature and Fermentation Time.

Fermentation		Caffeine (%)
Temperature (°C)	Time (hours)	
	20	1,15 a
20 ±1	30	1,16 a
	40	1,04 a
	20	1.20 a
30 ±1	30	1.16 a
	40	0.98 a
	20	1.19 a
40 ±1	30	1.10 a
	40	1.11 a
Coefficient of Diversity =13,35%		

Note: Mean with the same letter in column are not significantly different at P≤0.05

B. Caffeine Content

The statistical analysis showed that the treatment of temperature and fermentation time and their interactions gave no significant effect (P>0.05) on the caffeine content of the *Kintamani arabica* coffee beans produced. The caffeine content of *Kintamani arabica* coffee beans were treated as shown in Table 3.2.

In Table 3.2 above it can be seen that the average caffeine content of the S 795 cultivar of *Kintamani arabica* coffee bean produced ranged from 0.98-1.20 %. The caffeine content of this study is slightly higher than the results of a study conducted by Putri & Latunra (2013) found that caffeine content in arabica coffee is 1%.The caffeine content of *Kintamani arabica* coffee beans produced by each treatment (Table 3.2) showed no significant difference (P>0.05). It is means that the caffeine content of *Kintamani arabica* coffee beans are relatively stable during fermentation and not significantly changed due to the treatment of temperature and time of fermentation given. According to Wamuyu, *et al* (2017), caffeine is less soluble and its strongly bound to the alkaloid and other compounds in the coffee bean.

In Figure 3.4 showed that the longer of fermentation time, the caffeine content of *arabica* coffee beans produced tends to decrease. In fermentation of up to 20 hours the caffeine content of coffee beans is still relatively high then tends to decrease after fermentation for 30 and 40 hours even though the caffeine content produced shows no significant difference (P>0.05). This is probably due to the degradation of caffeine by bacteria during fermentation into xanthin and uric acid, therefore the caffeine content in *Kintamani arabica* coffee beans decreases. The results of this study are supported by the results of a study by Farida, *et al* (2013) and Mubarok *et al* (2014) showed that the longer the fermentation time, the lower caffeine content in *arabica* coffee beans. Furthermore, Gokulakrishnan *et al* (2005) the degradation of caffeine into uric acid began to form at 12-36 hours of fermentation.

The availability of caffeine compounds in coffee beans in a certain amount is needed because caffeine is a bioactive compound and is an antioxidant that can provide physiological effects on the human body. Emma (2016), found that the main compounds found in coffee beans are caffeine and chlorogenic acid, and caffeine is known to have antioxidant properties.

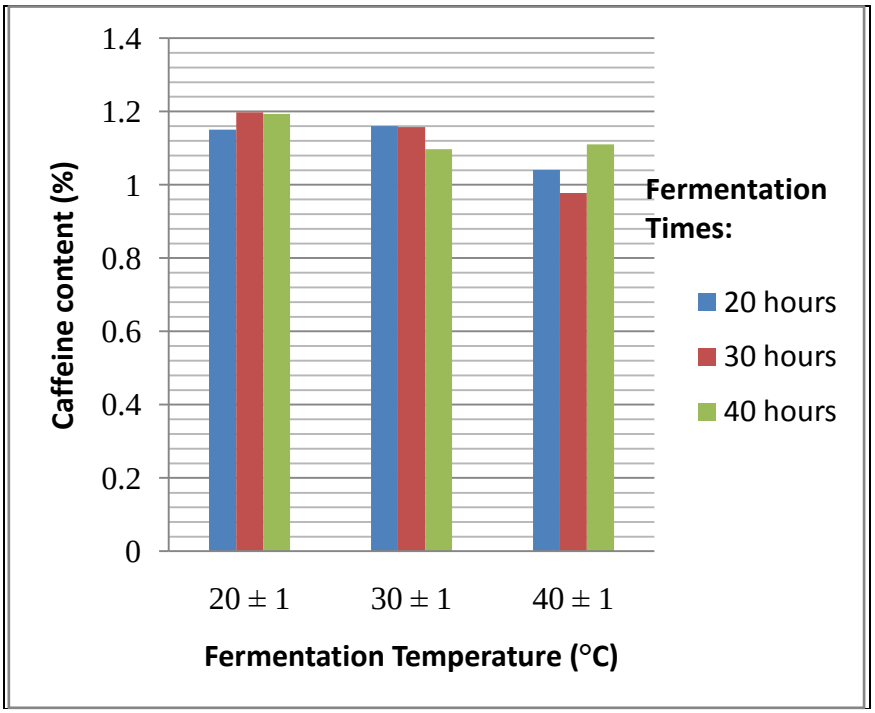

Figure 3.4. Relationship between a Temperature and Fermentation Time on Caffeine content.

CONCLUSION

The interaction between a temperature and fermentation time gave signifficant effect of formation the chlorogenic acids content but was not effect of caffeine content of Kintamani arabica coffee bean. Chlorogenic acid content of *Kintamani arabica* coffee beans of the S 795 cultivar range from 4.69-11.21%. The highest average of chlorogenic acid content 11.21% was produced by the combination treatment of fermentation temperature 40 ± 1°C and fermentation time 20 hours.

The higher of temperature and longer of fermentation time can increase of chlorogenic acid content during dried fermentation. It was indicate that the fermentation process carried out at a temperature of 40 ± 1°C for 20 hours is better than at are temperatures of 20 ± 1°C and 30 ± 1°C. The average caffeine content of the S 795 cultivar of *Kintamani arabica* coffee bean produced ranged from 0.98-1.20 %.

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