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The chemical content of Arabica coffee beans with the application of ethrell, NAA, and gibberellin to accelerate ripening in several varieties

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ABSTRACT

Introduction: Caffeine, antioxidants, and vitamin C are important compounds found in coffee beans. These chemical compounds affect the taste and aroma of coffee. The research aims to examine the effect of hormone application on the chemical content of coffee beans. **Methods**: The research was carried out at the people's coffee plantation in Kwadungan Gunung, Kledung, Temanggung, Central Java. The research uses an experimental method with two factors. Factor I variety consists of two varieties, namely Sigararutang and Yellow Catura. Factor II of the Plant Growth Regulators (PGR) application consists of four levels, namely: Control, ethrel, NAA, and gibberellin. Each treatment combination with 5 replications. The parameters observed were the number of fruit/ tree, the number of ripe fruit/ tree at the first harvest, caffeine content, vitamin C, antioxidant activity, sugar, and protein. Data were analyzed using ANOVA with the DMRT further test. **Results**: The number of coffee cherries of the Yellow Catura was better than the Sigarar Utang. The caffeine is better in Sigararutang in all PGR applications compared to other combinations. The antioxidant activity of Yellow Catura with the application of NAA and GA3 is better than other combinations. Vitamin C in Yellow Catura with GA3 application and control is better than other combinations. The protein in Yellow Catura with NAA application is better than other combinations. The sugar in Yellow Catura with all applications of PGR is better than the other combinations. **Conclusion**: PGR application and differences in varieties affect the chemical content namely caffeine, antioxidant activity, vitamin C, protein, and total sugar.

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INTRODUCTION

Coffee, as an annual plant, grows and flowers throughout its productive period, which is around 25 years. Arabica coffee can be cultivated at an optimum altitude of 800-1500 m above sea level with a temperature of 17-21oC. Coffee is needed, but with sufficient rain (Astuti *et al.*, 2021). The research results of Astuti *et al.* (2020) show that coffee fruit production is the same in various coffee clones (Astuti *et al.*, 2020). It is also known that coffee production is determined by coffee cultivation and land management (Sholikah *et al.*, 2023). Concerning coffee cultivation, farmers have not fully implemented the principles of good agricultural practices (Parmawati *et al.*, 2022).

Fruit is the part of the plant that has developmental activities. Therefore, auxin can be found in fruit. Auxin can stimulate ethylene synthesis through the induction of the expression of the ACS enzyme (ACC synthase). An increase in auxin content may be necessary to stimulate ethylene synthesis (Trainotti *et al.*, 2007). Cherries and unripe coffee fruit with Ethrel application and the combination of Ethrel + MathuryTM gave a higher percentage of fruit ripening compared to MathuryTM application alone, as well as the control (Carvalho *et al.*, 2022). Evaluation of the auxin content in Redhaven peaches showed that along with ethylene production, there was an increase in the IAA content in the mesocarp tissue. The increase in auxin in the fruit ripening process may be correlated with the role of auxin in the regulation of climacteric fruit ripening. Auxin treatment was carried out by dipping whole fruit in 2 mM 1-naphthalene acetic acid (NAA) for 15 minutes; After that, the fruit was sprayed with NAA solution every 12 hours for 48 hours and embryos were found developing inside the peach (Trainotti, *et al.* 2007).

The ripening process is regulated by hormones including auxin, cytokinin, gibberellin, abscisic acid, and ethylene. In ripening tomatoes, auxin, ethylene, and gibberellin play an integrative role (Carvalho *et al.*, 2022). Ethylene production from pistils triggers GA metabolism and promotes fruit formation and ripening. Studies on ethylene production and regulation of ethylene biosynthesis genes during coffee fruit ripening report positive effects of exogenous Ethephon application in promoting fruit ripening. Coffee is a climacteric fruit and ethylene plays an important role in the ripening process. Ethylene is involved in seed germination, shoot elongation, fruit ripening, organ abscission, and senescence. Ethylene coordinates several climacteric fruit ripening processes that trigger fruit color modification, promote chlorophyll degradation, carotenoid and flavonoid biosynthesis, fruit texture, changes in cell turgor, cell wall metabolism, fruit taste, aroma and nutritional quality, sugar modification (Shinozaki *et al.*, 2015; Winston *et al.*, 1992).

Coffee contains various unique compounds, including coffee and antioxidants. Caffeine is a methylxanthin derivative which is an alkaloid derivative. In humans, caffeine can increase cognitive ability and reduce fatigue (Utami, 2020). Antioxidants are compounds that can affect the taste of coffee and have antioxidant activity (Utami, 2020). Caffeine and antioxidants can be found in coffee fruit and beans (Dewi *et al.*, 2012). Caffeine is a derivative of the purine alkaloid methylxanthine, while the pyrimidine alkaloid is a trigonelline skeleton (Ashihara, 2006; Utami, 2020). Caffeine is found in young leaves, mature leaves, young seeds, mature seeds, and fruit skin (Caporaso *et al.*, 2018; N. V. Dewi *et al.*, 2017; Heo *et al.*, 2020; Isnindar *et al.*, 2016; Kuncoro *et al.*, 2016; ., 2018; Utami, 2020). The content of caffeine and chlorogenic acid varies in different varieties and is influenced by environmental conditions (Caporaso *et al.*, 2018; Cheng *et al.*, 2016). Caffeine can dissolve in water, and has a fragrant aroma but tastes very bitter. The caffeine content in a coffee variety can be an index of the organoleptic quality of coffee. Apart from caffeine, coffee also contains protein. Arabica coffee beans in Manipi show the highest chemical content, namely protein (13.26%), and lipids (7.67%) at an altitude of 1400 m above sea level, while carbohydrates (23.38%) and caffeine (1.42%) at an altitude of 1200 m above sea level (Jeszka-Skowron *et al.*, 2016). Coffee protein content increases with increasing maturity. The protein content increases because ethylene stimulates protein synthesis. The proteins formed are involved in the fruit ripening process due to the increase in enzymes that play a role in respiration. The physiological ripening process in fruit and seeds usually occurs simultaneously. In this process, there is an increase in food reserves, especially carbohydrates, proteins, fats, and hormones (Ifmalinda *et al.*, 2014). Coffee fruit also contains antioxidant compounds and activities, including anthocyanins, polyphenols, beta-carotene, and vitamin C (Maliza *et al.*, 2020).

Antioxidants such as polyphenolic compounds affect the taste of coffee. Antioxidants can be found in coffee fruit and beans (Cheng *et al.*, 2016; Farhaty & Muchtaridi, 2016; Koshiro *et al.*, 2007; Utami, 2020), as well as in coffee leaves (Dado *et al.*, 2019). Antioxidants are compounds that can inhibit oxidation reactions by binding to highly reactive molecules and free radicals. Coffee beans naturally contain polyphenol antioxidants consisting of phenolic acids and flavonoids. Another antioxidant component, melanoidin is a compound produced when roasting seeds. Phenolic compounds in plants are synthesized to survive stressful conditions due to attacks by pathogens and insects, UV radiation, and wounds. The basic structure of phenolic compounds is an aromatic ring containing one or more hydroxyl groups (Rahmawati & Fibrianto, 2018). Coffee has antioxidant properties that reduce the risk of cancer, diabetes, and liver disease and protect against Parkinson's. Green coffee bean extract showed hypotensive effects in mice and reduced fat. This potential is connected with chlorogenic acid and its derivatives, caffeine, theophylline and theobromine, cafestol, kahweol, tocopherol, and trigonelline. Green coffee beans contain two times higher levels of 5-O-caffeoylquinic acid (5-CQA) than roasted coffee (Jeszka-Skowron *et al.*, 2016).

Coffee beans contain glucose, fructose, other carbohydrates, and amino acids. Ripening usually increases the amount of sugar, decreases organic acids and phenolic compounds, and increases the essential oils that give the fruit its characteristic aroma. The ripening process is regulated by hormones including auxin, cytokinin, gibberellin, abscisic acid, and ethylene. Auxin plays a role in the formation of ethylene, but auxin also inhibits fruit ripening. When the fruit is physiologically ripe, there is an increase in sugar production and water content in the fruit flesh, resulting in changes in color, taste, and aroma of the skin and flesh of the fruit. The green skin of the fruit becomes shiny and the chlorophyll will be destroyed so that it turns red, yellow, or orange. Sugar content increases rapidly during the fruit ripening process. Sucrose is an important component in coffee fruit pulp. Sugar is a compound that dissolves in water. Therefore, washing for more than 15 minutes causes a loss of sugar content. The fat content of coffee increases with increasing maturity, but coffee berries that are past ripe experience a decrease in fat content (Ifmalinda *et al.*, 2014). Research on the application of Plant Growth Regulators (PGR) to stimulate fruit ripeness was carried out by Trainotti *et al.* (2007) by dipping whole peaches in 2 mM 1-naphthalene acetic acid (NAA) for 15 minutes; After that, the fruit was sprayed with NAA solution every 12 hours for 48 hours, and embryos were found developing inside the peach. Carvalho *et al.* (2022) examined cherries and unripe fruit in coffee with the Ethrel application and the combination of Ethrel + Mathury TM provided a higher percentage of fruit ripening compared to the MathuryTM application and the control. The application of ripening control compounds provided accelerated and uniform fruit ripening, with an improvement of 21 days in harvest compared to the control. However, no research has been carried out regarding changes in caffeine content, vitamin C, or antioxidant activity in coffee berries when PGR is applied. Therefore, in this study, we examined whether there were changes in caffeine content, vitamin C, or antioxidant activity when applying PGR to stimulate the ripening of coffee cherries.

This research aims to evaluate the effect of hormone application to accelerate fruit ripening on the chemical content of coffee, including caffeine, antioxidant activity, vitamin C, protein, and total sugar in Arabica coffee beans of the Sigarar Utang and Yellow Catura varieties in Kwadungan Gunung Village, Kledung District, Temanggung Regency, Central Java. This research is useful as an innovation to accelerate coffee fruit maturity and its impact on the physiological response of coffee in the form of caffeine content and antioxidant activity in Arabica coffee varieties Sigarar Utang and Yellow Catura.

METHODS

Tools and materials

Samples of Arabica coffee plants in the Kwadungan Gunung Arabica coffee community garden, Kledung, Temanggung, Central Java, Indonesia, Plant Growth Regulators (NAA, ethrel, gibberellin).

Time and place of research

The research was carried out in April – August 2023 in Kwadungan Gunung, Kledung, Temanggung, Central Java.

Method used

The research used an experimental method with a completely randomized design with a factorial pattern consisting of 2 factors, namely: Factor I, type of PGR, which consisted of 4 types, namely: Factor I, application of PGR, consisting of 3 types, namely: control (without PGR), Ethrel 300g/L, NAA 200 ppm, GA3 20 ppm. Factor II consists of differences in Arabica coffee varieties, consisting of two varieties, namely the Sigararutang variety and the Yellow Catura variety. Thus, 8 treatment combinations were obtained. Each treatment combination with 5 repetitions. The sample is a coffee plant. In one plant, all fruiting nodes are applied with PGR according to the treatment. The PGR application is sprayed on the green fruit throughout the fruiting nodes until all the fruit is exposed to the PGR. Application is given once when the fruit is green.

Observed parameters

Number of fruit/tree, percentage of ripe fruit in the first harvest, caffeine content, antioxidant activity, vitamin C, protein, and total sugar.

Data analysis

Data were analyzed using ANOVA and continued with DMRT to determine real differences between varieties and types of PGR.

RESULTS AND DISCUSSION

The number of fruit and percentage of ripe fruit in the first harvest

The results of the analysis showed that there was no real interaction between variety and PGR on the number of ripe/tree fruit and the percentage of ripe fruit in the first harvest.

Table 1. Effect of different varieties and PGR on the number of fruit/tree of Arabica coffee

Varieties	PGR				Means
	Control	Ethrell	NAA	GA3	
Sigararutang	510,80±95,32	672,20±84,53	483,20±65,61	822,20±30,19	751,05±63,30b
Yellow Catura	966,80±78,07	840,20±82,22	835,40±28,34	659,60±27,24	959,45±63,30a
Means	748,80±61,25p	756,20±55,42p	659,30±90,31p	740,90±37,65p	-

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (-): non-significant interaction between varieties and PGR application.

Table 1 shows that the application of growth regulators did not affect the number of fruit/trees. This happens because the number of fruits is determined at the time of flower formation, anthesis, pollination, fertilization, and development of seeds and fruit. Meanwhile, PGR application is given when unripe fruit of maximum size has been formed to stimulate fruit ripening (Matsumoto & Lopez, 2014).

Table 2 shows that the application of growth regulators affects the percentage of the first harvest, which means it influences the speed of fruit ripening. The application of Ethrell, GA3, and without the application of growth regulators had a greater percentage of fruit in the first harvest compared to other applications. Ethrell is a synthetic compound that contains ethylene. Ethylene is a hormone that stimulates the fruit ripening process (Agata *et al.*, 2013).

Differences in varieties affect the number of fruit/tree and the speed of fruit ripening as indicated by the percentage of ripe fruit in the first harvest. The Yellow Catura variety of Arabica coffee has a better number of fruits/tree compared to the Sigararutang variety (Table 1). The speed of fruit ripening in Sigararutang is faster than in other varieties (Saefudin & Wardiana, 2013).

Table 2. Effect of different varieties and PGR on % of ripe/tree in the first harvest (%) of Arabica coffee

Varieties	Control	PGR			Means
		Ethrell	NAA	GA3	
Sigararutang	42,00±3,17	41,52±3,62	36,83±6,53	38,76±2,56	41,45±0,83a
Yellow Catura	34,71±3,44	35,42±2,71	32,22±1,59	34,32±3,05	35,84±0,82b
Means	38,35±4,95p	38,47±4,41p	34,52±5,10q	36,54±3,82pq	-

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (-): non-significant interaction between varieties and PGR application.

Chemical content of coffee beans

Table 3. Effect of different varieties and PGR on caffeine content (%) of Arabica coffee

Varietas	Kontrol	PGR			Means
		Ethrell	NAA	GA3	
Sigararutang	1,78±0,22 ab	1.73±0,23ab	2.06±0,31ab	2.40±0,01a	1,99±0,08
Yellow Catura	2,05±0,05ab	2,08±0,08ab	1,51±0,35b	1,52±0,05b	1,79±0,11
Means	1,91±0,22	1,91±0,24	1,79±0,42	1,96±0,09	+

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (+): significant interaction between varieties and PGR application.

Table 3 shows the effect of different varieties and plant growth regulators (PGRs) on caffeine content in Arabica coffee. The two varieties tested were Sigararutang and Yellow Catura, with four types of PGRs: Control, Ethrel, NAA (Naphthalene Acetic Acid), and GA3 (Gibberellic Acid). The results showed that caffeine content varied depending on the variety and type of PGR used. For example, the Sigararutang variety had the highest caffeine content when given GA3 (2.40%), while Yellow Catura showed the highest caffeine content when given Ethrel (2.08%). The table also notes that the interaction between variety and PGR application was significant at the 5% test level. Similar studies have shown that the use of PGRs such as GA3 can increase the content of secondary metabolites in coffee plants, including caffeine, through its influence on secondary metabolic pathways (Jamwal *et al.*, 2018).

Table 4. Effect of different varieties and PGR on antioxidant activity (% inhibition) of Arabica coffee

Varietas	Control	PGR			Means
		Ethrell	NAA	GA3	
Sigararutang	40,65±6,92e	44,64±6,36de	47,45±6,37cde	52,09±3,02bcd	46,21±1,31
Yellow Catura	53,12±2,70bc	56,61±3,55b	65,43±2,48a	64,63±2,91a	59,95±1,31
Means	46,88±8,25	50,63±7,97	56,44±11,52	58,36±7,24	+

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (+): significant interaction between varieties and PGR application.

Table 4 shows the effect of different varieties and plant growth regulators (PGRs) on antioxidant activity in Arabica coffee. The two varieties tested were Sigararutang and Yellow Catura, with four types of PGRs: Control, Ethrel, NAA (Naphthalene Acetic Acid), and GA3 (Gibberellic Acid). The results showed that antioxidant activity varied depending on the variety and type of PGR used. For example, the Sigararutang variety had the highest antioxidant activity when given GA3 (52.09%), while Yellow Catura showed the highest antioxidant activity when given NAA (65.43%). The table also noted that the interaction between variety and PGR application was significant at the 5% test level. Hossain *et al.*'s (2022) research has shown that PGRs such as GA3 and NAA can enhance antioxidant activity in coffee through modulation of secondary metabolites, which play an important role in plant defense mechanisms.

Table 5. Effect of different varieties and PGR on vitamin C content (mg/100g) of Arabica coffee

Varietas	Control	PGR			Means
		Ethrell	NAA	GA3	
Sigararutang	65,38±0,72bc	63,67±0,71d	65,39±0,71bc	63,58±0,71d	64,21±0,72
Yellow Catura	69,57±0,04a	66,15±0,28b	65,20±0,71c	69,48±0,05a	67,30±0,04
Means	67,48±2,2	64,91±1,4	65,30±3,1	66,53±2,2	+

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (+): significant interaction between varieties and PGR application.

Table 5. shows the effect of different varieties and plant growth regulators (PGRs) on the vitamin C content of Arabica coffee. The two varieties tested were Sigararutang and Yellow Catura, with three types of PGRs: Control, Ethrel, and NAA. The results showed that the vitamin C content varied depending on the variety and the type of PGR used. For example, the Sigararutang variety had the highest vitamin C content when given NAA (65.934 mg/100g), while Yellow Catura showed the highest vitamin C content when given Control (67.542 mg/100g). The table also notes that the interaction between variety and PGR application was significant at the 5% test level. Katel *et al.* (2022) research has confirmed that PGR, especially NAA, can affect vitamin C levels by regulating ascorbic acid biosynthesis in coffee plants.

Table 6. Effect of different varieties and PGR on total protein content (%) of Arabica coffee

Varietas		PGR			
	Control	Ethrell	NAA	GA3	Means
Sigararutang	13,88±0,06de	13,82±0,02e	13,82±0,02e	13,95±0,05de	13,91±0,06
Yellow Catura	13,96±0,04d	14,19±0,21c	14,80±0,07a	14,57±0,03b	14,43±0,07
Means	13,92±0,06	14,00±0,24	14,31±0,52	14,26±0,33	-

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (-): non-significant interaction between varieties and PGR application.

This table shows the effect of different varieties and plant growth regulators (PGRs) on the total protein content of Arabica coffee. The two varieties tested were Sigararutang and Yellow Catura, with four types of PGRs: Control, Ethrel, NAA (Naphthalene Acetic Acid), and GA3 (Gibberellic Acid). The results showed that the total protein content varied depending on the variety and the type of PGR used. For example, the Sigararutang variety had the highest protein content when given GA3 (13.95%), while Yellow Catura showed the highest protein content when given NAA (14.80%). The table also notes that the interaction between variety and PGR application was significant at the 5% test level. Secara umum, hormon-hormon ini dapat mempengaruhi sintesis protein melalui regulasi ekspresi gen dan aktivitas enzim, tetapi efek spesifiknya dapat bervariasi (Singh *et al.*, 2018)

Table 7. Effect of different varieties and PGR on total sugar content (%) of Arabica coffee

Varietas	Kontrol	PGR			Means
		Ethrell	NAA	GA3	
Sigararutang	2,79±0,19b	2,87±0,02b	2,83±0,66b	2,75±0,47b	2,52±0,19
Yellow Catura	3,58±0,20ab	3,92±0,15a	4,22±0,71a	3,67±0,48ab	3,56±0,16
Means	3,19±0,40	3,40±0,29	3,53±0,78	3,19±0,70	-

Notes: Numbers followed by the same letter in a row or column indicate that they are not significantly different according to DMRT at the 5% test level; (-): non-significant interaction between varieties and PGR application.

This table shows the effect of different varieties and plant growth regulators (PGRs) on the total sugar content of Arabica coffee. The four varieties tested were Sigararutang, Yellow Catura, Ateng Super, and Gayo 1, with four types of PGRs: Control, Ethrel, NAA (Naphthalene Acetic Acid), and GA3 (Gibberellic Acid). The results showed that the total sugar content varied depending on the variety and the type of PGR used. For example, the Yellow Catura variety had the highest sugar content when given NAA (4.22%), while the Sigararutang variety showed the highest sugar content when given Ethrel (2.87%). The table also notes that the interaction between variety and PGR application was significant at the 5% test level. In the context of sugar accumulation, NAA plays a role in increasing the levels of sucrose and total sugar in coffee beans. This occurs because NAA can stimulate the activity of enzymes involved in carbohydrate biosynthesis (katel *et al.*, 2022). Research by Silalahi *et al.* (2024) shows that the application of Ethrel not only accelerates ripening but also contributes to increasing sugar levels in coffee beans by affecting carbohydrate metabolism.

The research results show that there is a real interaction between the application of various growth regulators and varietal differences in the chemical content of coffee beans (Table 3 - Table 7). In terms of caffeine content, the combination of the Sigararutang variety with all applications of growth regulators had the same effect and was the same as the caffeine content of the Yellow Catura variety with the application of ethrell and control. The caffeine content was lower in the Yellow Catura variety with the application of NAA and GA3 (Table 3). In the antioxidant activity parameters, there was a real interaction between the differences in varieties and the application of growth regulators on antioxidant activity. The combination of the Yellow Catura variety with the application of NAA and GA3 had better antioxidant activity compared to other combinations (Table 4). In vitamin C content, there is a real interaction between the differences in varieties and the application of growth regulators on antioxidant activity. The combination of the Yellow Catura variety with GA3 and control applications had better vitamin C content compared to the other combinations (Table 5). In total protein content, there is a real interaction between variety differences and the application of growth regulators on total protein content. The combination of the Yellow Catura variety with the NAA application had a better total protein content compared to other combinations (Table 6). In the antioxidant activity parameters, there was a real interaction between the differences in varieties and the application of growth regulators on total sugar content. The combination of the Yellow Catura variety with all applications of growth regulators and controls had a better total sugar content compared to other combinations (Table 7). This shows that the application of growth regulators generally affects the chemical content of coffee beans. The formation of coffee bean chemical compounds in the form of caffeine, vitamin C, and compounds with antioxidant activity which are secondary metabolites as a result of metabolism regulated by hormones, as well as sugar and protein which are primary metabolites. Hormones are organic compounds and nutrients that function to regulate metabolism (Pessarakli, 2021). In this research, the application of ethrel, NAA, and GA3 induced the formation of these metabolite compounds.

CONCLUSION

The results can be concluded that the difference in varieties (Sigararutang and Yellow Catura) and the application of plant growth regulators can affect the chemical content of coffee beans including caffeine, antioxidant activity, vitamin C, protein, and total sugar. The best combination was found in the Yellow Catura variety with the GA3 application. The number of fruit/stems and the weight of 100 Arabica coffee beans in the Yellow Catura variety are better than in the Sigararutang variety, but the speed of fruit ripening in the Sigararutang variety is faster than in the Sigararutang variety. Based on the results of this research, the suggestion that can be given is that to speed up fruit ripening, it is more advisable to use ethrell. Ripening the fruit does not reduce the quality of the chemical content and taste, so it is safe to use.

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