Food ScienTech Journal Vol. 6 (2) 2024 ISSN : 2685-4279 DOI:10.33512/fsj.v6i2.25143

The Effects of Different Roasting Degrees on Antioxidant of Coffee From West Sumatra

Dwijatmoko, M. I., Fadri, R.A.*, Syahrul, S., Harni, M., and Muchrida, Y.

Department of Food Technology, Politeknik Pertanian Negeri Payakumbuh, Indonesia

* E-mail: rince.alfia@gmail.com

Submitted: 06.05.2024; Revised: 05.10.2024; Accepted: 23.10.2024

ABSTRACT

Coffee has several benefits for human health because it contains bioactive components and one of which is polyphenol components. Regional differences in coffee cultivation and roasting processes influence the polyphenol content. The aim of this research was to explain the effect of roasting temperature and the area of region of coffee. Coffee beans were obtained from three regions, which are Solok, Situjuh and Pasaman, West Sumatra. Coffee beans were roasted with three temperatures, 191-200^{o}C (light), 201-210^{o}C (medium), and 211-220^{o}C (dark) for ±20 min, and other factors were considered as constant. Next, the coffee beans were analyzed for air content, color, flavonoids, total phenols and antioxidant activity. Results of the research analysis used SPSS. The results of the research showed that during the roasting process, coffee beans experienced a decrease in the attributes of color, water content, total phenols, total flavonoids and antioxidant activity. Overall, coffee beans at the light roast level had high attribute values of color, water content, total phenols, total flavonoids and antioxidant activity, whereas the highest in Solok coffee bean samples were antioxidant activity 27.45 ± 13.37 % and flavonoids 6.93 ± 0.33 mgEQ/g, the highest in Situjuh were total phenols 85.57 ± 3.84 mg GAE/ g and water content 3.17 ± 0.21 %, and the highest in Pasaman was color 24.49 ± 1.32 (L). Meanwhile, coffee beans at the dark roasting level had low attribute values of color, water content, total phenols, total flavonoids and antioxidant activity, whereas the lowest in Solok coffee bean samples were water content 1.44 ± 0.48 % and color 16.08 ± 0.19 (L), the lowest in Situjuh were antioxidant activity 14.82 ± 1.15 % and total flavonoids 4.47 ± 0.36 mgEQ/g, and the lowest in Pasaman was total phenol 50.35 ± 4.63 mg GAE/ g.

Keywords: Coffee, Polyphenol, Roasting, West Sumatra.

INTRODUCTION

Coffee beans are one of the plantation crops in Indonesia that are widely available and have high exports in economic activities. Coffee production in Indonesia reached 762,000 tons, the largest was from smallholder plantations at 99.33%, from large state plantations with 0.49%, and from large private plantations at around 0.18%. West Sumatra is a region that produces quite a lot of coffee. The area of coffee-producing plantations in West Sumatra province reached 25,538 hectares, with coffee production of 12,528 tons (BPS, 2022).

Coffee has several benefits for human health because it contains bioactive

components. That coffee contains hundreds of other biologically active phytochemicals, including polyphenols such as chlorogenic acid and lignans, the alkaloid trigonelline, and melanoidins formed during roasting (Ludwig et al., 2014). Bioactive components in coffee include caffeine, chlorogenic acid, trigoneline, melanoidin, cafestol, kahweol, volatile components, and various other types of polyphenols. Polyphenol components are reported to have various properties such as anti-inflammatory, detoxification, antibacterial, anticancer, antidiabetic and it can also increase immunity, and prevent cardiovascular disease. The polyphenol content in coffee also plays an important role as antioxidant activity. Antioxidant activity functions to inactivate oxidation reactions with the formation of free radicals. The presence of bioactive components and antioxidant activity makes coffee a functional product (Febrianto & Zhu, 2023).

Roasting is one of the stages of processing coffee products. Roasting coffee beans is a process that will shape the taste and aroma, control the uniformity of texture size, specific gravity, water content and chemical structure (Edvan et al., 2016). The roasting process also produces melanoidin compounds which come from the Maillard reaction. Roasting temperature and roasting time are factors that need to be considered for product quality (Fadri et al., 2019). Roasting will cause color changes and increase the aroma and taste of the coffee due to the chemical reactions that occur. However, this process will cause a decrease in the degradation of secondary metabolites due to relatively high heating. Roasting carried out at high temperatures and long time can cause changes in chemical components amino acids (Santosa et al. 2020).

Temperature and pH are parameters that influence phenolic components and antioxidant activity. Polyphenols and flavonoid compounds that contribute to antioxidant activity will decrease due to high heating temperatures. Apart from that, regional differences in coffee cultivation affect the polyphenol content that is present. In research conducted by Parras et al. (2007) with samples of arabica and robusta coffee from 12 regions (Uganda, Papua, Jamaica, Ethiopia, Kenya, Puerto Rico, "Caracolillo" Puerto Rico, Nicaragua, Colombia, Vietnam, Brazil and Guatemala) showed different antioxidant values, but they were not significantly different. On the other hand, there has not been much research on coffee region in Indonesia. Therefore, this study aims to describe the effect of roasting temperature and the region of the coffee on the phenolic and antioxidant characteristics of coffee beans.

MATERIALS AND METHODS

Materials

Coffee beans were obtained from three regions, namely Solok, Situjuh and Pasaman, West Sumatra. The other materials were aquades, concentrated HCL, Folin Ciocalteu reagent, Na_2CO_3 solution, gallic acid, acid buffer, ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, absolute ethanol, acetic acid buffer, and ascorbic acid standard.

Preparation of coffee

The coffee beans were preparation method as described by Yusbani et al. (2023) with slight modification. The coffee beans were washed and dried for 2 – 3 days. They were then roasted using a roaster with three different temperatures namely 191-200^{o}C (light roast), 201-210^{o}C (medium roast), and 211-220^{o}C (dark roast) for ±20 min. The roasted seeds were then dried and ground into powder using a blender and then they were stored in packaging for 3 days prior to analysis.

Water content (AOAC, 2005)

The cup was dried in an oven at 105^{o}C for 15 min and cooled in a desiccator for 10 min. Then, they were weighed 3 g sample of coffee powder was weighed and placed in a cup that had been dried. Then, the sample and cup were dried in an oven at 105^{o}C for 6 hours. Next, the cup was removed from the oven, cooled in a desiccator and weighed. Following that, it was dried again until a constant weight was obtained.

Colour analysis (Hutching, 1999)

Color measurements of coffee grounds were carried out using a Minolta CR-300 Chromameter (Konica Minolta Camera, Co. Japan 82281029). The principle of measuring color using this tool is measuring color differences through the reflection of light by the sample surface. The sample was placed in a special place and after pressing the start button, the values L, a, and b were obtained. These three parameters are the characteristics of Hunter notation. The L notation ranges between 0 (black) to ± 100 (white). The notation a states the chromatic value of a red-green mixture with a value of +a (positive) from 0 to +100 for red and –a (negative) from 0 to -80 for green. The notation b states the chromatic color of a blue-yellow mixture with a +b (positive) value from 0 to +70 for yellow and a –b (negative) value from 0 to -80 for blue.

Total flavonoid (Chang, 2002)

The sample was diluted with a ratio between samples of 1:10 g/mL. Then, 1 mL of sample was added by 3 mL of methanol, 0.2 AlCl3 2%, 0.2 mL of 1 M glacial acetic acid, and 5.6 mL of distilled water. Next, the solution mixture was allowed to stand for 30 min and the absorbance was measured using a spectrophotometer at an absorbance of 370 nm. To create a calibration curve, quercitin was used. The total flavonoid content in the ethanol extract is described in mg quercetin/g.

Total Phenol (Asmira et al. 2020)

1 gram of sample was weighed, and 10 mL of methanol was added and sonicated until it was dissolved, and then vortexed. 1 mL of the sample solution was taken for analysis and 1 mL of distilled water was added. Next, 1 mL of folin reagent (1: 10 mL of distilled water) and 1 mL of 7.5% Na_2CO_3 solution were added. The solution was vortexed and incubated in the dark for 60 min and then the absorbance was determined at a wavelength of 725 nm using a UV-VIS spectrophotometer.

Antioxidant activity (Asmira et al. 2020)

1 gram of sample was weighed, and 10 mL of methanol was added and sonicated until it was dissolved and then vortexed. A sample of 2 mL was put into a microplate and 1 mL of DPPH was added. The DPPH solution was made by dissolving 5 mg in methanol and measuring to a volume of 50 mL in a measuring flask. Then, the measuring flask was covered with aluminum foil. Blanks were prepared with 2 mL methanol and the addition of 1 mL DPPH. Samples were incubated at room temperature in the dark for 15 min and measured using an ELISA reader at a wavelength of 517 nm.

Data analysis

All experiments were performed in duplicate, and data were presented as mean ± standard deviation (SD). Comparison of multiple samples was conducted by one-way analysis of variance (ANOVA) with SPSS 19 software (IBM). $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Colour

Table 1 shows the differences in roasting treatments for various coffee bean variants.

The L* value states the brightness level of the product. The results of the research show that various coffee bean variants experienced a decrease in the L* value during the roasting process, with levels from high to low, light roast, medium roast and dark roast. This shows that coffee beans from various variants will become darker when roasted at a longer temperature and time. The samples also show differences between the region variants. Solok coffee beans had a lower L* value than Situjuh and Pasaman (light roast). However, it was not significantly different from Situjuh coffee beans, but it was significantly different from Pasaman. The b* and a* coordinates also decreased during the roasting process. This shows that the color became dark yellow and dark red during the roasting process. The brightness of each treatment was significantly different from each other in each sample. The longer the roasting process causes the brightness level of the coffee to become smaller or the color to become darker.

This is in accordance with research conducted on the light roast for each origin having the highest L* values, with L* near 31 for light roasts, near 28 for medium roasts, and near 23 for dark roasts, again regardless of origin (Yeager, et al. 2022).

Water content

The water content of coffee beans in West Sumatra has different values. The lowest water content was 1.44 ± 0.48% for Solok coffee beans with a dark roasting level and the highest water content was 3.17 ± 0.21% for Situjuh coffee beans with a light roasting level. During the roasting process, the water content decreased significantly, where the lowest was at the dark roast level. The Solok area produced water content of 1.44 – 2.83%, Situjuh ranges between 2.36 – 3.17%, and Pasaman with 1.76 – 2.50%. From the analysis results, all water content was below 10%, where the safe water content to prevent the growth of microorganisms is 12.5%, and reducing coffee water content can reduce the growth of microorganisms (Reh et al., 2006). Several countries have standards for coffee water content. Vietnam and Indonesia have a water content requirement of 13%.

Total Phenol

The results of measuring the total phenol content can be seen in Table 2. It was concluded that the light roasting treatment coffee beans had the highest total phenol. These findings were consistent with previous reports suggesting the superiority of green and light roasted coffee as a rich source of free polyphenols (Król et al. 2020). Table 2 shows that each treatment was significantly different. Solok coffee grounds had total phenol levels of 85.15 ± 5.92 mgGAE/gr (light roast), 60.65 ± 0.65 mgGAE/gr (medium roast), and 56.11 ± 3.16 mgGAE/gr (dark roast). Situjuh coffee powder had levels of 85.57 ± 3.84 mgGAE/gr (light roast), 83.96 ± 4.16 mgGAE/gr (medium roast), and 79.85 ± 9.75 mgGAE/gr (dark roast). Pasaman coffee powder had levels of 56.49 ± 9.18 mgGAE/gr (light roast), 51.47 ± 1.37 mgGAE/gr (medium roast), and 50.35 ± 4.63 mgGAE/gr (dark roast). In research conducted under light roasting conditions, Solok coffee powder had a high total polyphenol content compared to Situjuh and Pasaman, amounting to 85.15 ± 5.92 mgGAE/gr, while in medium and dark roasting, Situjuh coffee powder had a high total polyphenol content compared to Solok and Pasaman, amounting to 83.96. ± 4.16 mgGAE/gr and 79.85 ± 9.75 mgGAE/gr. Different content reductions are possible due to differences in geographic location and the characteristics of the content they contain and differences between roasted samples also may be attributed to the degradation of chlorogenic acids and their contribution to the development of Maillard reaction

products (Corso et al. 2016). Meanwhile, phenolic compounds, such as caffeoylquinic acid, were more crucial than melanoidins. In addition, dicaffeoyl quinolactone had a lower impact on the antioxidant effect of coffee products compared to dicaffeoylquinic acid (El-Hawary et al., 2022). Heat treatment of food ingredients usually has a destructive effect on flavonoids and phenolics because both are unstable components (Saika 2013). Other studies have shown that coffee bean contains a total phenol content of 95.32 mgGAE/g for the soxhlet method (Hilma et al., 2020). So, the research coffee is lower compared to previous research.

Total Flavonoid

The results of measuring the total flavonoid content in Table 2 show that each treatment was significantly different and some were not significantly different. Solok coffee powder had total flavonoid levels of 6.93 ± 0.33 mgEQ/gr (light roast), 5.93 ± 0.34 mgEQ/gr (medium roast), and 5.02 ± 0.36 mgEQ/gr (dark roast). Situjuh coffee powder had levels of 5.60 ± 0.33 mgEQ/gr (light roast), 5.17 ± 0.18 mgEQ/gr (medium roast), and 4.47 ± 0.36 mgEQ/gr (dark roast). Pasaman coffee powder had levels of 6.57 ± 0.24 mgEQ/gr (light roast), 6.29 ± 0.06 mgEQ/gr (medium roast), and 5.56 ± 0.16 mgEQ/gr (dark roast). In research conducted in light roasting conditions, Solok coffee powder had a high total flavonoid content compared to Situjuh and Pasaman, amounting to 6.93 ± 0.33 mgEQ/gr, while in medium and dark roasting Pasaman coffee powder, it had a high total flavonoid content compared to Solok and Situjuh, amounting to, 6.29 ± 0.06 mgEQ/gr and 5.56 ± 0.16 mgEQ/gr. Different content reductions are possible due to differences in geographic location and the characteristics of the content they contain, this is in accordance with research conducted by Adzkiya and Hidayat (2022) with Arabica coffee from various regions of Aceh, Bogor, Bandung, Situbondo and Temanggung which produced different values between one coffee and another.

Antioxidant Activity

Phenolic compounds have been shown to have antioxidant activity. Antioxidants are nutraceutical compounds that have many health benefits. Antioxidant activity in coffee extracts was carried out using antioxidant analysis with the DPPH method. The DPPH radical neutralization reaction causes a color change from purple to yellow to colorless. This color change was analyzed quantitatively by using spectrophotometry at a wavelength of 517 nm (Vignoli et al. 2011). The results of measuring the antioxidant activity of the DPPH method are presented in Table 3. The DPPH method shows that each treatment was significantly different and some were not significantly different starting from light, medium and dark roasting levels at a significance level of 0.05. Research on the effect of processing on antioxidant activity in food ingredients shows significant different results between samples. This is in line with research conducted by Mei (2022) showing that different processing methods affect the phytochemical components of dried coffee leaves, resulting in unique antioxidant and anti-inflammatory abilities. From the nine samples and each treatment, it was concluded that the light roasting treatment with Solok coffee beans had the highest antioxidant power to reduce free radicals with a value of 27.45 ± 13.37 %, while the lowest antioxidant activity was in the dark roasting treatment with Situjuh coffee beans with a value of 14.82 ± 1.15 %. The differences in antioxidant activity have also been reported by Adzkiya and Hidayat (2022) in their research on coffee beans from various regions. The longer the roasting process lasts, the lower the antioxidant activity value will be. The highest antioxidant

capacity in lightly roasted coffee is consistent with previous reports (Bobková et al., 2020).

CONCLUSION

Coffee beans with 3 different roasting treatments and 3 different samples showed that during the roasting process, the coffee beans experienced a decrease in the attributes of color, water content, total phenols, total flavonoids and antioxidant activity. Overall, coffee beans at the light roast level have high attribute values of color, water content, total phenols, total flavonoids and antioxidant activity. The highest in Solok coffee bean samples were antioxidant activity and flavonoids, the highest in Situjuh were total phenols and water content, and the highest in Pasaman was color. Meanwhile, coffee beans at the dark roasting level had low attribute values of color, water content, total phenols, total flavonoids and antioxidant activity, whereas the lowest in Solok coffee bean samples were water content and color, the lowest in Situjuh were antioxidant activity and total flavonoids, and the lowest in Pasaman was total phenol.

ACKNOWLEDGEMENT

This research was funded by DIPA 2023 Politeknik Pertanian Negeri Payakumbuh.

REFERENCES

AOAC. 2005. Official method of analysis. Washington: The Association of Official Analytical Chemist.

Adzkiya, M., A., Z., and Hidayat, A., P. 2022. Uji Fitokimia, Kandungan Total Fenol dan Aktivitas Antioksidan Kopi Arabika (*Coffee arabica)* pada Tingkat Penyangraian Sama. Jurnal Sains Terapan: Wahana Informasi dan Alih Teknologi Pertanian 12 (1).

Asmira S., Nurhamidah, and Analdi A. 2020. Aktivitas antioksidan dan total fenol pada kopi kawa daun yang berpotensi sebagai alternatif pangan fungsional. Jurnal Farmasi Kesehatan SCIENTIA 10 (2).

BPS. 2020. Statistik kopi Indonesia. ISSN: 2714-8505. Jakarta (ID): BPS.

Bobková, A., Hudáˇcek, M., Jakabová, S., Belej, L'., Capcarová, M., Curlej, J., Bobko, M., Árvay, J., Jakab, I., Capla, J. and Demianová, A. 2020. The effect of roasting on the total polyphenols and antioxidant activity of coffee. Journal of Environmental Science and Health 55: 495–500.

Corso, M.P., Vignoli, J.A., and Benassi, M.d.T. 2016. Development of an instant coffee enriched with chlorogenic acids. Journal of Food Science and Technology 53: 1380–1388.

Chang, C. -C., Yang, M. -H., Wen, H. -M., and Chern, J. -C. 2002. Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis 10(3): 178 – 182.

Ding, J., Mei, S., Gao, L., Wang, Q., Ma, H., and Chen, X. (2022). Tea processing steps affect chemical compositions, enzyme activities, and antioxidant and anti-inflammatory activities of coffee leaves. Food Frontiers, 3, 505–516. https://doi.org/10.1002/fft2.136

Edvan, B. T., Edison, R., and Same, M. 2016. Pengaruh jenis dan lama penyangraian pada mutu kopi robusta (coffea robusta). Jurnal Agro Industri Perkebunan 4 (1).

El-Hawary, E. A., Zayed, A., Laub, A., Modolo, L.V., Wessjohann, L., and Farag, M.A. 2022. How does LC/MS compare to UV in coffee authentication and determination of antioxidant effects? Brazilian and Middle Eastern coffee as case studies. Antioxidants 11(1): 131.

Febrianto, N. A., and Zhu, F. 2023. Coffee bean processing: emerging methods and

their effects on chemical, biological and sensory properties. Food Chemistry 412.

Fadri, R. A., Sayuti, K., Nazir, N., and Suliansyah, I. 2019. Review proses penyangraian kopi dan terbentuknya akrilamida yang berhubungan dengan kesehatan. Jurnal of Applied Science and Technology 3(1): 129 – 145.

Hilma Hutching, J. B. 1999. Food Color and Appearance. London (UK): Academic Press

Król, K., Gantner, M., Tatarak, A., and Hallmann, E. 2020. The content of polyphenols in coffee beans as roasting, origin and storage effect. European Food Research and Technology 246: 33–39.

Ludwig, I. A., Clifford, M. N., Lean, M. E. J., Ashihara, H., and Crozier, A. 2014. Coffee: biochemistry and potential impact on health. Food and Function Journal 5(8):1695-717.

Parras, P., Martineztome, M., Jimenez, A., and Murcia, M. 2007. Antioxidant capacity of coffees of several origins brewed following three different procedures. Food Chemistry 102(3): 582–592.

Pomeranz, K., and Topik, S. 2006. The world that trade created: society, culture, and the world economy, 1400 to the present. 2nd ed. New York: Routledge.

Reh, C. T., Gerber, A., Prodolliet, J., and Vuataz, G. 2006. Water content determination in green coffee method comparison to study specificity and accuracy. Food Chemistry 96: 423–430.

Saika, S., and Mahanta, C. l. 2013. Effect of steaming, boiling, and microwaves cooking on the total phenolics, flavonoids, and antioxidant properties of different vegetables of Assam, India. International Journal of Food and Nutritional Science 2(3): 47-53

Santosa, K. M., Supriyadi, Anggrahini, S., and Rahmadian, Y. 2020. Sensory Analysis, Caffeine, Chlorogenic Acid and Non-Volatile Taste Compounds of Arabica Coffee (Coffea arabica) Fermented with Sugar Addition for Brew Taste. Indonesian Food and Nutrition Progress.

Yeager, S. E., Batali, M. E. Lim, L.X., Liang, J., Han, J., Thompson, A. N., Guinard, J. X., Ristenpart, W. D. 2022. Roast level and brew temperature significantly affect the color of brewed coffee. Journal Food Science 87(4): 1837–1850.

Table 1. Effects of different roasting treatments on L*, a*, b*, a*/b* values of coffee beans

Sample	L*	a*	b*
Solok (light roast)	21.94 ± 0.83^{d}	7.76 ± 0.36^{d}	8.29 ± 0.59^{c}
Solok (medium roast)	18.65 ± 0.14^{bc}	5.73 ± 0.68^{b}	5.56 ± 0.22^{b}
Solok (dark roast)	16.08 ± 0.19^{a}	4.16 ± 0.38^{a}	3.46 ± 0.63^{a}
Situjuh (light roast)	22.74 ± 0.54^{d}	9.04 ± 0.01^{e}	10.04 ± 0.02^{d}
Situjuh (medium roast)	18.67 ± 0.60^{bc}	6.83 ± 0.12^{b}	6.21 ± 0.17^{b}
Situjuh (dark roast)	16.58 ± 0.53^{a}	5.09 ± 0.20^{b}	4.18 ± 0.24^{a}
Pasaman (light roast)	24.49 ± 1.32^{e}	8.53 ± 0.35^{de}	9.80 ± 0.86^{d}
Pasaman (medium roast)	19.51 ± 0.78^{c}	6.77 ± 0.34^{c}	6.43 ± 0.53^{b}
Pasaman (dark roast)	17.35 ± 0.14^{ab}	4.96 ± 0.19^{b}	4.43 ± 0.13^{a}

Note: Value with different notation in the same column has a significant differences at 5% (Tukey test)

Table 2. Water, total phenol, and flavanoid content

Sample	Water content (%)	Total Phenol (mg GAE/ g)	Total Flavonoid (mgEQ/g)
Solok (light roast)	2.83 ± 0.72^{a}	85.15 ± 5.92^{b}	6.93 ± 0.33^{e}
Solok (medium roast)	2.61 ± 0.61^{a}	60.65 ± 0.65^{a}	5.93 ± 0.34^{cd}
Solok (dark roast)	1.44 ± 0.48^{a}	56.11 ± 3.16^{a}	5.02 ± 0.36^{ab}
Situjuh (light roast)	3.17 ± 0.21^{a}	85.57 ± 3.84^{b}	5.60 ± 0.33^{bc}
Situjuh (medium roast)	2.48 ± 1.41^{a}	83.96 ± 4.16^{b}	5.17 ± 0.18^{b}
Situjuh (dark roast)	2.36 ± 0.34^{a}	79.85 ± 9.75^{b}	4.47 ± 0.36^{a}
Pasaman (light roast)	2.50 ± 0.74^{a}	56.49 ± 9.18^{a}	6.57 ± 0.24^{de}
Pasaman (medium roast)	2.13 ± 1.15^{a}	51.47 ± 1.37^{a}	6.29 ± 0.06^{de}
Pasaman (dark roast)	1.76 ± 0.26^{a}	50.35 ± 4.63^{a}	5.56 ± 0.16^{bc}

Note: Value with different notation in the same column has a significant differences at 5% (Tukey test)

Table 3. Antioxidant activity

Sample	Antioxidant activity
Solok (light roast)	27.45 ± 13.37^{b}
Solok (medium roast)	22.45 ± 2.69^{ab}
Solok (dark roast)	18.91 ± 1.28^{ab}
Situjuh (light roast)	15.91 ± 1.15^{ab}
Situjuh (medium roast)	15.64 ± 1.28^{ab}
Situjuh (dark roast)	14.82 ± 1.15^{a}
Pasaman (light roast)	17.72 ± 2.43^{ab}
Pasaman (medium roast)	16.45 ± 1.93^{ab}
Pasaman (dark roast)	16.45 ± 0.89^{ab}

Note: Value with different notation in the same column has a significant differences at 5% (Tukey test)