TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Implications of Inappropriate Storage Temperatures in Harvested Green Coffee (*Robusta Sp.*) Beans to Antioxidant Properties and Polyphenol Oxidase Activity

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Abstract
The effects of various storage temperatures on antioxidant properties and enzyme activity of green coffee beans were evaluated during storage. The green coffee beans were harvested and stored at different storage temperatures of 35 ˚C, 26 ˚C and 9 ˚C. Lower storage temperature (9 ˚C) greatly preserved the reduction of phenolics and antioxidant capacity detected using FRAP (Ferric Reducing Antioxidant Power) which was 38.20 mg TE/g, total phenolic content (TPC) which was 38.20 mg GAE/g and DPPH (Radical Scavenging Effect) which was 82.56%. Temperature 9 ˚C also retained the moisture of the green coffee beans up to 54.31% at day 5. For enzyme activity of polyphenol oxidase (PPO), the lower temperature inactivated the enzyme which resulted in lower activity of the enzyme stored at temperature 9 ˚C which was 0.82 U/g followed by 0.90 U/g and 1.03 U/g for 26 ˚C and 35 ˚C at day 1 of storage. However, green coffee beans stored at 9 ˚C showed some external chilling injury symptoms starting from day 7 of the storage. The phenolics and antioxidants activity in green coffee beans during storage decreased with increase of storage temperature starting from 9 ˚C, 26 ˚C and 35 ˚C. While the PPO activity increased with increase of storage temperature.

Keywords:
Coffee Bean; Storage; Antioxidant; Polyphenol Oxidase

Introduction

Coffee is one of the world's most popular beverages and also a rich source of dietary antioxidants. Green coffee beans are simply harvested beans that have not been roasted. Green coffee bean has a mild, green, bean-like aroma, in which the desirable characteristic aroma of coffee develops during the roasting process (Liang & Kitts, 2014). The green coffee beans can be classified into legumes group which has its own suitable storage temperature. Like other plant product, green coffee beans contain enzymes including lipase, protease, amylase, catalase, polyphenol oxidase (PPO) and peroxidase. The most likely functions for PPO are its involvement in plant resistance against disease and against insect herbivores (Mazzafera & Robinson, 2000). Upon wounding, the quinines formed by PPO induced oxidation of phenols can modify the plant proteins.

Phenolic compounds are secondary metabolites generally involved in coffee or plants in general adaptation to environmental stress conditions. Phenolic compounds are ubiquitous constituents of higher plants found in a wide range of commonly consumed plant foods such as fruits, vegetables,

cereals, and legumes, and in beverages of plant origin, wine, tea, beer and coffee (Farah & Donangelo, 2006). Chlorogenic acids (CGA) are the main phenolic compounds in coffee and coffee contains the highest concentration of CGA among all plant constituents (Farah *et al.*, 2005). Coffee pulp mainly contains condensed tannins while CGA are found abundantly in the seeds. Other phenolic compounds, such as tannins, lignans and anthocyanins are also present in coffee seeds although in minor amounts. The coffee quality can be influenced by a high concentration of chlorogenic acid present in green coffee seeds (up to 14%) which also plays an important role in the formation of coffee flavour (Farah & Donangelo, 2006). These compounds have a number of beneficial health properties related to their potent antioxidant activity as well as hepatoprotective, hypoglycemic, anti-bacterial, antiviral, anti-inflammatory and anti-carcinogenic activities (Farah & Donangelo, 2006).

Nowadays, there are increasing researches of phenolic compounds and CGA from various types of plant sources due to its health benefits to human that derived attention from various industries. However, postharvest temperatures might fluctuate due to the changes in condition during storage, transportation and distribution. Thus, the objectives of this study were to determine the antioxidant and PPO enzyme activity in green coffee beans during postharvest storage up to 5 consecutive days. The green coffee beans were stored at chilled temperature (9 ˚C), room temperature (26 ˚C) and elevated temperature (35 ˚C). These temperatures were chosen as to simulate chill storage commonly practices by beverage industry (9 ˚C), room temperature (26 ˚C) as practiced during distribution and normal temperature at tropical climate countries (35 ˚C) which justifies the beans could be exposed immediately after harvest prior to being distributed. This research was also carried out in an attempt to identify whether chill storage (9 ˚C) is the most suitable storage temperature for green coffee beans after harvesting. Additionally, the optimum extraction yield of antioxidant obtained from the harvested green coffee beans can also be utilized in medicine and chemical industries.

Methodology

Preparation of coffee

Selected samples used in this study were green coffee (*Robusta sp.*) beans at maturity stage 3. The samples were selected based on various sizes and free from external defects obtained from supplier in Teluk Gong, Klang, Malaysia. The green coffee beans were placed in HDPE plastic bag before reaching laboratory within 2 hours. Upon arrival at the laboratory, the green coffee beans were stored at chilled temperature (9 ˚C), room temperature (26 ˚C) and elevated temperature (35 ˚C).

Determination of moisture (air oven method)

An aluminum dish with cover was dried for 3 hours in oven at 105 ˚C. The dish was cooled in desiccator and weighed after it attained room temperature. About 5 g of homogenised sample was weighed into the aluminum dish. The sample was placed, uncovered, in a 60 ˚C oven overnight. The lid was replaced while the dish is still in oven, then the dish was remove from oven. The dish was

cooled in desiccator and was weighed soon after attaining room temperature. The processed was replaced until the weighed become constant. The calculation for % of moisture is:

$$\%\ \text{moisture} = \frac{(\text{weight of wet sample} + \text{pan}) - (\text{weight of dried sample} + \text{pan})}{(\text{weight of wet sample} + \text{pan}) - (\text{weight of pan})} \times 100$$

Antioxidant analysis

Extraction

A mixture of ground green coffee beans (35 g) and distilled water (700 mL) were magnetically stirred for 30 min at 80 ˚C, in the dark. After cooling, the solutions were vacuum filtered through celite (1 cm).

Determination total phenolic content (TPC) using Folin-Ciocalteu method

This method is based on Singleton and Rossie (1995) with some modifications. Total phenolic compounds in extract were determined using Folin-Ciocalteu method. The extracts at the concentration of 2 mg/ml were transferred into a test tube and mixed thoroughly with 2.4 ml distilled water and 200 µl Folin-Ciocalteu reagent. After 30 seconds, 600 µl sodium carbonate (20% Na_2CO_3) and 760 µl distilled water were added and mixed. The absorbance of reaction mixture were allowed to stand for 2 hours at room temperature in the dark. The result of mean of the three readings was expressed as mg of gallic acid equivalents (GAE)/g extract.

Determination of antioxidant activity by the Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was conducted in accordance with Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared by mixing acetate buffer (0.3 mol/l), TPTZ (10 mmol/l) and $FeCl_3 \cdot 6H_2O$ (20 mmol/l) in the ratio 10:1:1. For a volume of 100 µl of the coffee extract, 3.0 ml of the FRAP reagent was added to the test tubes with stoppers and the mixture was left at rest in the dark for 30 min at 37 ^{0}C. The absorbance of the samples was measured in comparison to a blank at a wavelength of 595 nm, using a Varian, Cary 50 model spectrophotometer. The calibration curve was prepared using standard solutions of ferrous sulphate at concentrations of 0.5-2.5 mmol/l. All of the measurements were conducted in triplicate and the results were expressed in mmol/l Fe^{3+} per litre sample.

Free radical scavenging by the DPPH assay

The method is modified method of Amin *et al*. (2006). Sample was prepared by mixing 4 ml of ascorbic acid (0.05 mg/ml) and 1 ml of DPPH (0.4 mg/ml), whereas control was prepared by mixing 4 ml of distilled water and 1 ml of DPPH. The mixture was gently homogenised and left to stand at room temperature for 30 minutes. Absorbances were read using a spectrophotometer at 520 nm. The ability of extract to scavenge DPPH free radical was calculated using the following equation:

$$\% \text{ scavenging activity} = \frac{\text{absorbance(control)} - \text{absorbance (sample)}}{\text{absorbance (control)}} \times 100$$

Polyphenol oxidase (PPO) enzyme activity measurement

PPO extraction

The PPO extract was obtained by mixing 10 g of ground coffee beans with 10 ml of Mellvaine buffer (pH 6.6) at 5 ^{0}C and centrifuged at 107.52 x g for 40 minutes at 4 ^{0}C. The extract was filtered with Whatman paper No. 1.

PPO assay

PPO activity was determined according to the method of Guo *et al*. (2009). The catechol was used as a substrate, and the quinone formation was measured on a spectrophotometer at 410 nm. The sample contained 0.5 ml of 0.1 M catechol, 2.0 ml of 0.1 M phosphate buffer (pH 6.8) and 0.5 ml of the enzyme solution. The blank sample contained only 3.0 ml of substrate solution. The sample and blank were kept at ambient temperature for 30 min. PPO activity was assayed in triplicate measurements. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 per min. The initial rate was calculated from the slope of the absorbance - time curve. The enzyme activity was expressed as unit enzyme per gram of sample (U/g sample).

Statistical analysis

All data were expressed as mean ± standard deviation. Data were analysed using two-way ANOVA using IBM SPSS Statistic 20. Duncan's multiple-range test was used to access difference between means. A significant difference is considered at the level of $p < 0.05$.

Result and discussion

Moisture content of green coffee beans

Figure 1 shows that the moisture content of green coffee beans stored at different storage temperatures. The percentage weight loss of green coffee beans was significantly affected by storage temperature after 3 days of storage. Its weight change was positively related to the storage temperature, and weight loss of green coffee beans increased with storage time which was more prominent at 35 ˚C. At day 1, the percentage of moisture content was 61.70% at 9 ˚C, 61.62% at 26 ˚C and 59.9% at 35 ˚C. Meanwhile, all samples indicated decreasing moisture content during storage days, irrespective of storage temperature. Green coffee beans stored at 35 ˚C showed slight peel shriveling at day 5 and were mostly infected by fungus and then rotted after a week of storage. Green coffee beans held at 9 ˚C had lower weight loss followed by 26 ˚C and 35 ˚C. Although the sample placed in 9 ˚C can retain moisture, some abnormal features of chilling injury were observed at the end of storage.

The results is supported by Ismail *et al*. (2013) who reported that the moisture content of green coffee beans stored at 29 ˚C significantly decreased during storage whereby the coffee beans stored at 4 ˚C showed lower weight loss which was only 6.77% of their initial weight followed by coffee held at 10 ˚C and 13 ˚C. The highest weight loss percentage was observed in gac fruit stored at 25 ˚C, when about 22.03% of its initial weight was lost on 20th day of storage. The lower temperature maintained the fruit quality, in particular high fruit firmness, which is an important factor for transportation and logistics (Wins *et al.*, 2015). Postharvest weight losses are usually due to the loss of water through respirations. Water loss during storage is a major cause of food commodity deterioration. Lower weight loss that coincided with a decrease in storage temperature is in agreement with the findings of Ramana Rao *et al*. (2011).

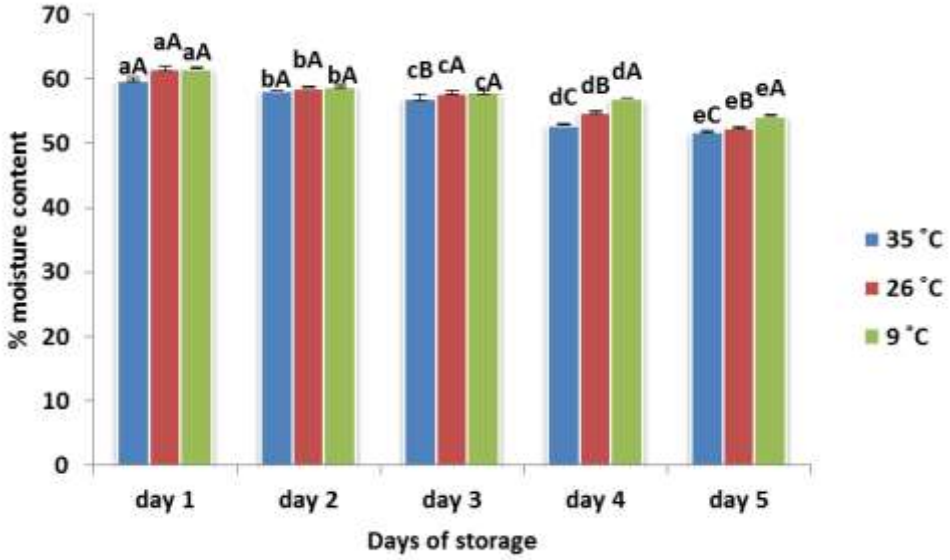

Figure 1. Percentage moisture content of green coffee (*Robusta sp.*) beans at different storage temperatures. Capital letter shows the significant difference between temperature at $p<0.05$. Small letter shows the significant difference between days of storage at $p<0.05$

Antioxidant activity of green coffee beans

Figure 2 shows that the total phenolic contents decreasing with increasing of storage temperature and storage time. Overall, green coffee beans stored at chill temperature (9 ˚C) is consistently higher than the ones stored at elevated temperature (35 ˚C). Among the three storage temperatures, chill temperature (9 ˚C) showed higher total phenolic content which was 38.20 mg GAE/g on day 1 compared to 32.70 mg GAE/g at 35 ˚C. Similarly, total phenolic content was 26.37 mg GAE/g and 21.53 mg GAE/g for green coffee beans stored at 9 ˚C and 35 ˚C, respectively, after 5 days of storage. Previous study carried out by Fawole and Opara (2013) found that the decline in total phenolic concentration in pomegranate fruit may be related to the breakdown of phenolic compounds as a result in enzymatic activity occurring during storage. Another study using gac fruit subjected to postharvest treatments with storage temperature by Win *et al*. (2015) also resulted in decrease of total

phenolic contents. It was found that the high temperature (25 ˚C) decreased the phenolic contents from 266.50 mg GAE/g at day 0 to 81.50 mg GAE/g at day 30 of storage. A higher phenolic content was observed in fruit stored at 10 ˚C which was 122.17 mg GAE/g compared to 25 ˚C which was 74.83 mg GAE/g. They stated that the activity of enzymes responsible for polyphenol degradation where polyphenol oxidase was inhibited at lower temperature. Leja *et al*. (2003) stated that higher in phenol content could due to lower activity of polyphenol oxidase at lower temperature, so oxidation processes were minimised. Degradation of phenolic contents in green coffee beans also caused by other factors and not limited to enzymatic activity. The individual phenolic compounds normally depend on the maturity of coffee beans and to a smaller degree on the composition of soil, climate conditions and agricultural practices related to the coffee plant (Chu, 2012; Farah & Danangelo, 2006). Extraction solvents also affect the value of phenolic content. Mussatto *et al*. (2011) found that methanol solvent gave better extraction results than the use of only water. This could be explained by the fact that the phenolic compounds are often more soluble in organic solvents which is less polar than water.

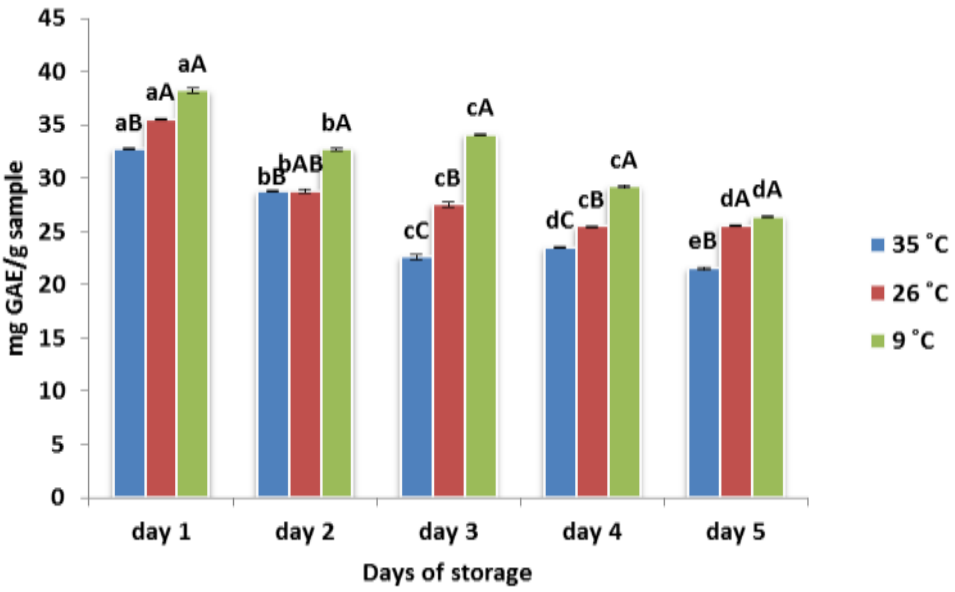

Figure 2. Total phenolic content (TPC) in green coffee beans at different storage temperatures. Capital letter shows the significant difference between temperature at $p<0.05$. Small letter shows the significant difference between days of storage at $p<0.05$

In Figure 3, the ferric reducing antioxidant power (FRAP) was found to decrease significantly with increasing storage temperatures and storage time. Similar to TPC results, green coffee beans stored at chill temperature (9 ˚C) is consistently higher than the ones stored at elevated temperature (35 ˚C) which decreased from 38.20 mg TE/g at 9 ˚C to 32.70 mg TE/g at 35 ˚C and decreasing from 38.20 mg TE/g at day 1 to 26.57 mg TE/g at day 5. At storage temperature of 9 ˚C, the FRAP activity of the green coffee beans is higher than at storage temperature of 26 ˚C and 35 ˚C and significantly decreased with time. Win *et al*. (2015) found that gac fruit stored at lower temperature maintained its

high antioxidant activity than those in high temperature. It was also found that the decrease in antioxidant activity may be due to the decreasing content of phenolic compounds during storage at high temperature. At day 0 of storage, the antioxidant activity was 380.83 mg TEA/g. There was a large decreased in antioxidant activity at 25 ˚C which was 121.33 mg TEA/g compared to 4 ˚C which was 237.67 mg TEA/g at day 30 of storage. Similar results was found in blood orange (*Citrus sinensis* cv. Tarocco) by Hamedani *et al*. (2012) who reported that an increase in temperature and storage time reduced the antioxidant capacity. At 22 ˚C, the phenol decreased from 521.60 mg/L to 361.5 mg/L after 85 days of storage. Additionally, FRAP does not measure potentially important coffee antioxidant components that contain thiol groups, due to a redox potential threshold that is below FRAP detection. Some examples of sulphur containing compounds present in coffee that exhibit antioxidant activity include thiazole and related derivatives (Yanagimoto *et al*., 2002).

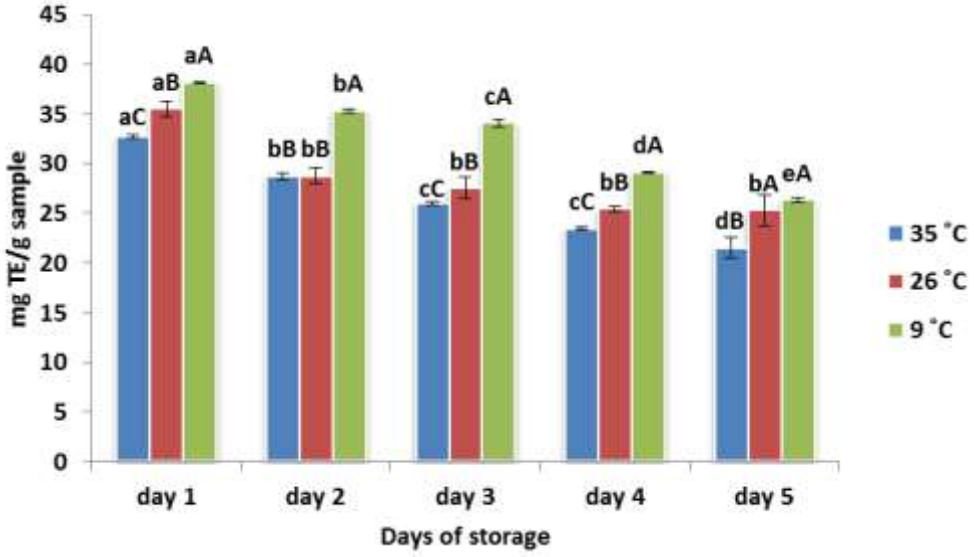

Figure 3. Ferric reducing antioxidant power in green coffee beans at different storage temperatures. Capital letter shows the significant difference between temperature at $p<0.05$. Small letter shows the significant difference between days of storage at $p<0.05$

Figure 4 shows that the percentage of radical scavenging activity (DPPH) decreased with increasing storage temperature from 9 ˚C to 35 ˚C and storage time from day 1 to day 5. Higher DPPH activity was at temperature 9 ˚C on day 1 which was 82.56% followed by 26 ˚C (62.72%) and 35 ˚C (53.17). DPPH activity results were similar to TPC and FRAP results. The results show that storage temperature of 9 ˚C has higher antioxidant potential than other storage temperatures which was 59.09% compared to 26 ˚C and 35 ˚C which were 48.08% and 29.80% after 5 days of storage. DPPH activity of the pomegranate fruit declined at all storage regimes with storage time which was over 56% in fruits stored at 5 ˚C, 7.5 ˚C and 10 ˚C (Arendse *et al*., 2014). Meanwhile, Perez-Hernandez *et al*. (2012) reported that the antioxidative effectiveness of coffee beans is due to the presence of polyphenols, whereby the main component is chlorogenic acid. This means that the chlorogenic acid is higher and not affected much when stored at lower temperature compared to the higher storage temperatures.

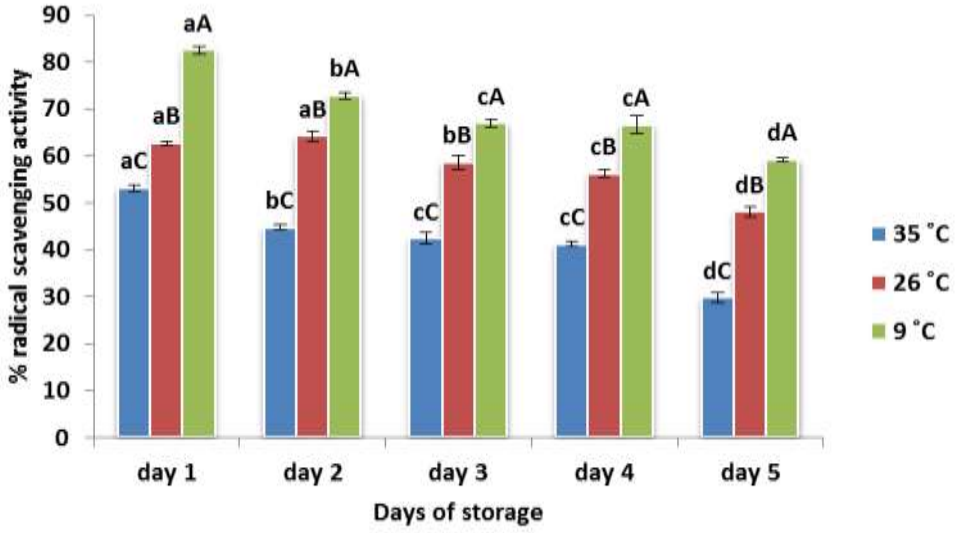

Figure 4. Radical scavenging activity (DPPH) of green coffee beans at different storage temperatures. Capital letter shows the significant difference between temperature at $p<0.05$. Small letter shows the significant difference between days of storage at $p<0.05$

Polyphenol oxidase (PPO) enzyme activity

Enzymatic browning occurs in fruits and vegetables after bruising, cutting or during storage. This results from oxidation of phenolic compounds by PPO. The PPO is a copper containing enzyme which can undergo reversible oxidation and reduction in the process of hydroxylation and oxidation (Othman, 2012). Figure 5 shows higher PPO activity was at elevated temperature (35 ˚C) which was 1.03 U/g at day 1 and decreasing to 0.24 U/g at day 5. Mazzafera & Robinson (2000) in their study on PPO activity of coffee found that the optimum PPO activity was observed between pH 6 and 7 and the optimum temperature was between 25 ˚C and 30 ˚C for both leaf and endosperm PPO of coffee. As stated by Wins *et al*. (2014), the activity of enzymes responsible for polyphenol degradation, PPO was inhibited at lower temperature. This is also indicated in Figure 5. The results obtained can also be supported by the results of TPC, FRAP and DPPH where at higher temperature the antioxidant and phenolic content decreases while PPO is higher meaning that they are correlated with each other and the previous studies that reported the degradations of antioxidant due to PPO activity can be applied.

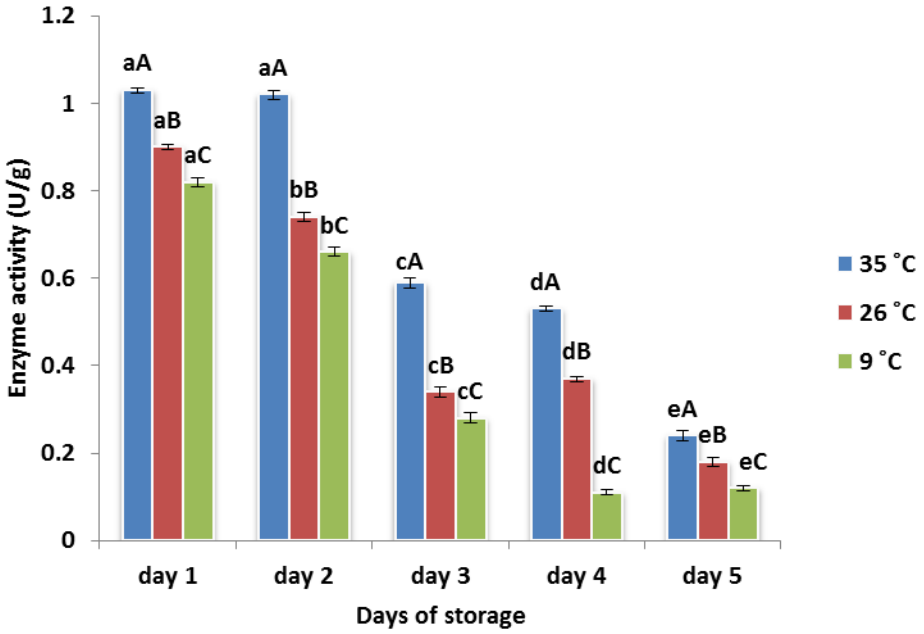

Figure 5. PPO enzyme activities of green coffee beans at different storage temperatures. Capital letter shows the significant difference between temperature at $p<0.05$. Small letter shows the significant difference between days of storage at $p<0.05$

Conclusion

As a conclusion, chill temperature (9 ˚C) is the most suitable storage temperature for the green coffee beans after harvesting. Temperature 9 ˚C can reduce the moisture loss. The moisture content of the green coffee beans plays an important part in determining the coffee storage stability against deterioration. At day 1, the percentage of moisture content was 61.70% at 9 ˚C, 61.62% at 26 ˚C and 59.9% at 35 ˚C. After 5 days of storage, the moisture content was 54.31%, 52.46% and 51.87 at 9 ˚C, 26 ˚C and 35 ˚C, respectively. Chill temperature storage also has the ability to preserve the antioxidant reduction compared to the other storage temperatures which are 26 ˚C and 35 ˚C. For antioxidant analysis, TPC in green coffee beans was higher at 9 ˚C which was 38.20 mg GAE/g than 26 ˚C and 35 ˚C which were 35.52 mg GAE/g and 32.70 mg GAE/g, respectively. In FRAP analysis, the mg TE/g sample also shown the higher value at 9 ˚C than 26 ˚C and 35 ˚C. At 9 ˚C the value was 38.20 mg TE/g and for 26 ˚C and 35 ˚C were 35.52 and 32.7 mg TE/g. For DPPH analysis, the percentage of radical scavenging activity was 82.56% at 9 ˚C, 62.72% at 26 ˚C and 53.17% at 35 ˚C. Chill temperature storage also inactivated the enzyme of polyphenol oxidase (PPO) which is 0.82 U/g at 9 ˚C compared to 26 ˚C and 35 ˚C which are 0.90 U/g and 1.03 U/g at day 1 and also decreased during storage days. Further studies should be carried out to delay the deterioration of green coffee beans during postharvest storage as the deteriorated products can cause the products cannot be sold and have no values leading to increased environmental wastage.

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References

[1] Amin, I., Norazidah, Y. & Emmy Hanaida, K. I. (2006). Antioxidant activity and phenolic content of raw and blanced. *Amaranthus* species. *Food Chemistry*, **94**, 47-52.

[2] Arendse, E., Fawole, O. A. & Opara, U. L. (2014). Effect of postharvest storage conditions on phytochemical and radical-scavenging activity of pomegranate fruit (cv. *Wonderful*). *Journal of Scientia Horticulturae*, **169**, 125–129.

[3] Benzie, I. F. & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP Assay. *Analytical Biochemistry*, **239**, 70–76.

[4] Chu, Y. F., Chen, Y., Brown, P.H., Lyle, B. J., Black, R. M., Cheng, I. H., Ou, B. & Prior, R. L. (2012). Bioactivities of crude caffeine: Antioxidant activity, cyclooxygenase-2 inhibition, and enhanced glucose uptake. *Food Chemistry*, **131**, 564–568.

[5] Farah, A. & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazil Journal of Plant Physiology*, **18**(1), 23-36.

[6] Farah, A., De Paulis, T., Trugo, L. C. & Martin, P. R. (2005). Effect of roasting on the formation of chlorogenic acid lactones. *Journal Agriculture of Food Chemistry,* **53**, 1505-1513.

[7] Fawole, O. A., Opara, U. L. & Theron, K. I. (2012). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food Bioprocess Technology*, **5**, 2934-2940.

[8] Hamedani, M., Rabiei, V., Moradi, H., Ghanbari, A. & Azimi, M. R. (2012). Determination of storage duration and temperature effects on fruit quality parameters of blood orange (*Citrus sinensis cv. Tarocco). Biharean Biologist,* **6**(1), 10-13.

[9] Ismail, I., Anuar, M. S. & Shamsudin, R. (2013). Effect on the physico-chemical properties of liberica green coffee beans under ambient storage. *International Food Research Journal*, **20**(1), 255-264.

[10] Leja, M., Marezek, A. & Ben, J. (2003). Antioxidant properties of two apple cultivars during long-term storage. *Food Chemistry*, **80**, 303-307.

[11] Liang, N. & Kitts, D. D. (2014). Antioxidant property of coffee components: assessment of methods that define mechanisms of action. *Molecules*, **19**, 19180-19208.

[12] Mazzafera, P. & Robinson, S. P. (2009). Characterization of polyphenol oxidase in coffee. *Phytochemistry*, **55**, 285 – 296.

[13] Mussatto, S. I., Ballesteros, L. F., Martins, S. & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. *Journal of Separation and Purification Technology,* **83**, 173–179.

[14] Perez-Hernandez, L. M., Chavez-Quiroz, K., Medina-Juarez, L. A. & Meza, G. N. (2012). Phenolic characterization, melanoidins, and antioxidant activity of some commercial coffees from *Coffea arabica* and *Coffea canephora*. *Journal of Mexican Chemical Society,* **56***(4),* 430-435.

[15] Ramana R, T. V., Gol, N. B. & Shah, K. K. (2011). Effect of postharvest treatments and storage temperatures on the quality and shelf life of sweet pepper (*Capsicum annum* L.). *Scientia Horticulturae,* **132**: 18-26.

[16] Singleton, V. L. & Rossi, J. A. (1965). Calorimetry of total phenolics with phosphomoybdic-phosphoungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144-158.

[17] Win, S., Buanong, M., Kanlayanarat, S & Wongs-Aree, C. (2015). Response of gac fruit (*Momordica cochinchinensis* Spreng) to postharvest treatments with storage temperature and 1-MCP. *Journal of International Food Research*, **22**(1): 178 - 189.

[18] Yanagimoto, K., Lee, K. G., Ochi, H. & Shibamoto, T. (2002). Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard reaction. *Journal of Agricultural and Food Chemistry*, **50**, 5480–5484.