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Effect on the physico-chemical properties of liberica green coffee beans under ambient storage

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Abstract

Green coffee beans are stored for a certain period and under certain conditions until they are finally utilized. The storage period may depend on customer demand while the storage conditions depend on where the coffee beans are stored. Thus, this research emphasizes the physico-chemical changes that occur in Liberica coffee beans during storage under the Malaysian climate (average temperature and relative humidity of 29.33ºC and 71.75% respectively). The changes in the physico-chemical (coffee size, mass, densities, colour, proximate analysis, sucrose, chlorogenic acid content) and microbiological (yeast and mould count) properties were evaluated during eight months of storage. After the storage, the physical properties of the coffee changed as the coffee beans expanded in size, reduced in mass and density and became brighter in colour. Changes in the chemical properties were also detected where the moisture decreased and the ash content increased. In addition, the sucrose level was found to decrease with a corresponding increase in chlorogenic acid. During storage, the counts of yeast and mould were reduced. Model equations describing the changes in the properties were developed. The overall conclusion was that the coffee beans reduced in quality during storage.

Introduction

Coffee consumption takes place all year round. However, coffee production is seasonal. Therefore, long term storage of coffee is necessary so that better prices can be achieved. Storage functions to maintain the commercial value of coffee as long as possible by preserving the coffee integrity with all of its characteristics. Since the coffee price is based on its sensorial value, adequate storage considerations such as avoiding close proximity storage of the coffee near to fragrant spices or chemicals with a pervading odour (Rojas, 2009). There are numerous methods of coffee storage used around the world. However, in Malaysia the storage is straightforward and simple whereby coffee bean bags (60 kg each) are stacked on wooden crates with no control over the conditions (temperature or relative humidity) of the warehouse.

Bucheli *et al*. (1998) stated that coffee beans should be dried well, with a moisture content of between 10-12% before the storage process can begin, since a moisture content exceeding 14.5% will favour the growth of mould. Relative humidity in the range of 50-70% and a temperature below 26ºC can also minimize deterioration in the quantity and quality of coffee beans during storage. Coffee quality is also lowered due to the hydrolysis of triacylglycerols (the major constituents of the coffee lipid) releasing free fatty acids which are then oxidized. Multon *et al*. (1973) reported that free amino acids and sugars are degraded while lipids are oxidized to produce an off flavour at the end of one year of storage, leading to a loss of quality (Damyanova *et al*., 1999). However, this is in contrast with a more recent study by Jham *et al*. (2008) conducted in Brazil where they reported that there were no significant effects of storage time on the lipid (triacylglycerol) composition and also on the cup quality or the sensory characteristics of the coffee (Jham *et al*., 2001). In Thailand, researchers (Bucheli *et al*., 2008) stored Robusta beans in silos and bags for 8 months under the tropical environment. During the storage, the moisture content reached up to 15.4 %. They also observed a decrease in microbiological (yeast and mould) presence upon storage.

Nevertheless, some of these facts cannot be used solely and the results could be different when dealing with coffee in Malaysia since the climate in other countries is different from Malaysia. The

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climate of Malaysia is uniformly hot and humid throughout the year. The average temperature is 27.5ºC with range of 1ºC every month (Anonymous, 2011). The temperature is always high with only slight changes in the monthly average temperature but with abundant rainfall (250 cm of rainfall per year) according to Swee-Hock (2007). In addition, the coffee species which the other researchers were studying were mainly Arabica and Robusta which contrasts with the *Liberica* species found in Malaysia. Therefore, the storage test in this study was done under Malaysian climatic conditions by using Liberica beans and stored using the Malaysian coffee warehouse storage method to study the changes in the physical (size, mass, density, colour) chemical (proximate composition, sucrose, chlorogenic acid) and microbiological properties (yeast and mould).

Materials and Methods

Coffee bean sample

Liberica green or crude coffee beans were obtained from Kilang Kopi FAMA, Banting, Selangor, Malaysia, in May 2010. The beans were then subjected to sorting and selection processes manually at the laboratory of the Process and Food Engineering Department in the Faculty of Engineering of Universiti Putra Malaysia (UPM). The coffee beans used consisted of mixed beans (may contain sour or immature beans) but defective beans (black, partly black, broken, infested) were discarded. The coffee beans were processed by the dry method (by the coffee farmers) whereby in this method (Figure 1), the berries were initially crushed by a crusher to facilitate drying. They were then left to dry under the sun on a concrete floor for 7-21 days depending upon the climatic conditions. Hulling and winnowing takes place after that to separate the beans from the dried pulp and parchment.

Storage conditions

The dried coffee beans were stored in the Laboratory of Agricultural Process Engineering of the Process and Food Engineering Department in the Faculty of Engineering of Universiti Putra Malaysia (UPM). The coffee beans were stored for eight months (six months storage is the common practice by the coffee mixture industries in Malaysia) from the beginning of June 2010 until the end of January 2011 in open laboratory ambient conditions. Some 30 kg of coffee beans were stored in jute fibre bags with 5 kg per bag for a total of 6 bags. Data was sampled after every two months of storage. The temperature and relative humidity of the coffee storage in the laboratory was recorded by a data logger (EBI-20TH, Ebro, Germany) every hour.

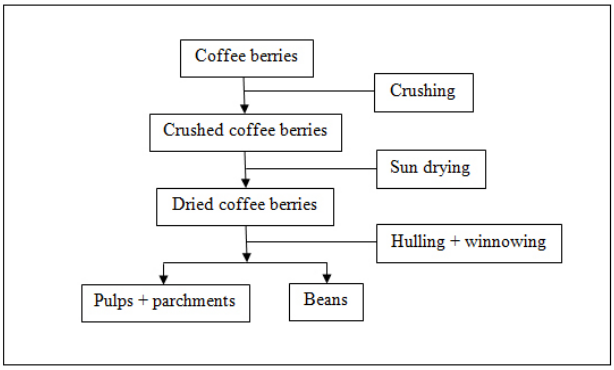

Figure 1. Dry processing of coffee in Malaysia

Size and dimension

A digital vernier calliper with 0.01 mm accuracy (Series 500, Mitutoyo, Japan) was used to measure the dimensions of the 100 coffee bean samples. The length (L), width (W) and thickness (T) measurements were taken. The L, W and T can also be referred as the major diameter, intermediate diameter and minor diameter.

Mass

The mass was determined by a digital balance (ER-120A, AND, Japan) with an accuracy of 0.0001 g. Some 100 samples were weighed and then the sum of the weights was divided by 100 to obtain the average sample weight. The test was repeated three times and the resultant three averages were themselves averaged to obtain a single value for the mass (Bart-Plange and Baryeh, 2003).

Volume

Following Dutra *et al*. (2001), the shape of the bean can be assumed as half a triaxial ellipsoid. The bean volume was calculated using Equation (1).

$$V = \frac{2}{3}\pi abc \qquad (1)$$

where 2a, 2b and c are the length, width and thickness of the bean respectively.

Density

For the true bean density, 100 beans were first weighed and each bean volume was calculated by using Equation (1). True bean density was calculated by dividing the weight of 100 beans by the total volume of the 100 beans (Franca *et al*., 2005). The bulk density was determined by filling a sample in a 500 ml measuring cylinder. By dividing the weight of the filled sample by the cylinder volume, the bulk density was obtained. The test was done in triplicate and the results averaged (Chandrasekar and Viswanathan, 1999).

Colour

The colour of samples was observed using a colour meter (CR-10, Konica Minolta, Japan). The values of L*, a* and b* so obtained were used to determine the chroma and hue angle by using Equation (2) and Equation (3) (Leite da Silveira *et al*., 2007). The test was repeated five times and the results averaged.

Chroma, $c^* = [(a^*)^2 + (b^*)^2]^{1/2}$ (2)

Hue angle, $h^* = \tan^{-1}(b^*/a^*)$ (3)

Moisture

Each sample of whole coffee beans (3-5 g) was oven-dried (UNB400, Memmert, Germany) at 105ºC for 24 h (in triplicate) (Reh *et al*., 2006). The moisture content was calculated by dividing the mass changes of the beans by the initial mass and then times by 100 to obtain the percentage. The test was done in triplicate and the results averaged.

Extraction

The extraction process was carried out after the green coffee beans were dried at 63°C for two days by using an oven (UNB400, Memmert, Germany) in order to facilitate grinding. The dried sample was then ground before it was sieved to 710 μm particle size (the maximum permissible size of Liberica coffee powder in the Malaysian market). The extraction method employed was based on Mazzafera (1999). A water bath was maintained at 80°C. Then, a conical flask was filled with 5 g of powdered sample and 100 mL of 80% methanol placed inside. The extraction was slightly modified as it was undertaken for 30 minutes at a time with gentle agitation. The solvent was changed three times (every 30 min for a total of 90 min). Afterwards, it was filtered through Whatman filter paper No 541. All the extracts were then placed in one conical flask and evaporated until approximately 10 mL was left. The extract was then used in the phenolic, sucrose and chlorogenic acid analyses.

Lipid

The lipid was determined by the Soxhlet method. Some 5 g of powdered sample (in triplicate) was refluxed with petroleum ether for 8 h in round bottom flasks. The extracts were then evaporated by a rotary evaporator at 60ºC until all the petroleum ether was evaporated. The flasks containing the lipid were dried in an oven until a constant weight was achieved. (Pomeranz and Meloan, 1978).

Protein

Nitrogen was determined by the Kjeldahl method after digesting a sample (0.15 g) with sulphuric acid and the protein was calculated as nitrogen × 6.25 (Pearson, 1970).

Ash

Ash was obtained by heating 3 g of powdered sample at 550°C until the ash was free from black particles (Pomeranz and Meloan, 1978).

Sucrose

Sucrose was determined by using the method employed by Ramalakshmi *et al*. (2007). High-performance liquid chromatography (HPLC) with RI detector (410 Differential refractometer, Waters) and a column of NH_2 Bondapak (3.9 x 300 mm) were used. The mobile phase consisted of acetonitrile (HPLC grade) and deionized water in the ratio of 80:20 at 1 mL/min flow rate. A sucrose standard solution was employed for the peak identification and quantitation.

Chlorogenic acid

The chlorogenic acid (5-caffeoyilquinic acid) content was analyzed by high-performance liquid chromatography (HPLC) equipped with a photodiode array (PDA) detector (MD-2010 Plus, Jasco) and the column used was C18 Crestpak, Jasco (4.6 x 150 mm). The mobile phase consisted of deionized water, methanol (HPLC grade) and acetic acid in the ratio of 85:15:1 at 1 mL/min flow rate (Franca *et al*., 2005). The wavelength used was 254 nm. A chlorogenic acid standard (Acros Organics, USA) solution was employed for the peak identification and quantitation.

Microbiological properties

Coffee beans (25 g) were diluted into 225 ml of 0.1% peptone water. 10-fold dilutions in peptone water were made and 0.1 ml was spread into the Plate Count Agar (PCA) and Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates. The plates were then incubated at 35-37°C (PCA) and at 25°C (DRBC) for 24 to 48 hours prior to enumeration.

Statistical analysis

The data obtained was analysed by ANOVA at 5% probability using SPSS 16 (Statistical Package for Social Sciences) software in determining the significance correlated with the error in the parameter estimations.

Regression analysis

From the results of the statistical analysis, the properties of the coffee beans which differed significantly throughout the storage period were curve-fitted with related properties by linear (Equation 4) and non-linear regression (Equation 5 and Equation 6). Equation (5) and Equation (6) are the hypothesis polynomial and power law equation. Linear regression was done by using Microsoft Excel 2010 software whereas NLREG software was used in curve fitting the data with the non-linear regressions.

$$y = mx + c \quad (4)$$

$$y = ax^3 + bx^2 + cx + d \quad (5)$$

$$y = ax^n \quad (6)$$

Results and Discussion

Relationship between temperature and relative humidity of storage.

The average relative humidity (RH) of the coffee beans and the storage temperature are presented in Table 1. The minimum and maximum RH was 66.13% (in June) and 76.56% (in December). The minimum and maximum temperature was 28°C (in December) and 30.65°C (in June) respectively. The standard deviation of the storage temperature shows that there was not much difference between the months. These conditions similarly match with the article by McGinley *et al*. (2010) with reference to the climate of Peninsular Malaysia. They stated in their article that the driest months were June and July which correspond to the higher temperature and lower relative humidity due to the lowest rainfall period. Throughout this study, the storage temperature showed a decreasing trend while the RH showed an increasing trend. The RH in this study was much higher than the recommended level of below 60% by Rojas (2009) since the combination of temperature variations and a high RH may lead to fungi and insect proliferation. Further, the storage temperature should be maintained as low as possible to reduce the coffee metabolism and respiration that could speed up deterioration of the beans. Hence, as stated in Bucheli *et al*. (1998), the ideal relative humidity and temperature of the storage should be in the range of 50-70% with a temperature of below 26ºC.

Effects of storage on the moisture content of Liberica coffee beans.

Figure 2 illustrates the moisture content of the coffee beans and the RH of the storage. The moisture content of the coffee beans plays an important part in determining the coffee storage stability against deterioration (Clarke, 1989). Other than the RH and temperature of the storage being the main factors leading to coffee deterioration, the moisture content of the coffee beans also depends on the type of coffee, the origin and the form in which the coffee is stored (berry, parchment, or bean). In this research, the storage periods were separated into four phases as denoted by the numbers in the figure to make the explanation easier. From the figure, the moisture of the beans ranged from the minimum 11.14% (at month 8) to the maximum 12.54% (at month 0) and it significantly decreased during storage. Based on the trendlines, it was found that the RH increased did not increase the moisture content overall. The moisture content of the beans only increased in phase II (months 2-4) and III (months 4-6) while it decreased in the other phases. However, a Tukey test indicated that the trend in phase II and III was not significant while phase I and IV were significant ($P < 0.05$). Hence, the statement from Rojas (2009) stating that the coffee beans are hygroscopic in nature cannot be used for Liberica beans since they do not appear to absorb moisture from their surroundings during storage. Therefore, this result is in contrast with the findings of Bucheli *et al*. (1998) where in their study, a high RH lead to a high moisture content in Robusta coffee beans. Hence, Liberica beans are found to be independent of RH or temperature during storage.

Table 1. Coffee bean storage average relative humidity (RH) and temperature

	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
RH (%)	66.13	67.97	69.04	72.03	73.01	75.18	76.56	74.11
Std.dev.	3.56	4.93	3.46	3.47	4.05	4.56	3.83	4.11
Temperature (ºC)	30.65	29.48	30.19	29.54	29.54	29.15	28.00	28.12
Std. dev.	0.66	0.89	0.92	0.87	0.71	0.74	0.55	0.77

Table 2. Effect of storage on the size and dimension of Liberica coffee beans

Properties	Month					Average
	0	2	4	6	8	
Length (mm)	11.99ª ±1.03	12.07ª ± 1.08	12.12ª ± 1.02	12.14ª ± 1.02	12.14ª ± 1.06	12.09
Width (mm)	7.67ª ±0.57	7.73ª ± 0.59	7.76ª ± 0.60	7.81ª ± 0.60	7.82ª ± 0.62	7.76
Thickness (mm)	4.67ᶜ ± 0.51	4.75ª ± 0.48	4.78ª ± 0.50	4.81ª ± 0.48	4.87ᵈ ± 0.53	4.76
Volume(10^{-9} m^3)	225.96ᶜ±41.95	233.1ª ± 42.28	236.2ª ± 43.24	239.7ª ± 43.61	243.3ᵈ ± 45.95	235.68

* Group means with the same letters in a row are not significantly different at a 5 % level of significance by the Tukey test

Effect of storage on the physical properties of Liberica coffee beans

Effect on size and dimensions

According to Table 2, the length, width, thickness, aspect ratio, sphericity, surface area and volume of Liberica beans increased after 8 months of storage. However, these increments were not significant by the Tukey test. The minimum and maximum values of the size and dimensions were at 0 and 8 months respectively without any value fluctuation during storage. Only the thickness, surface area and volume of the Liberica beans between 0 and 8 months of storage differed significantly based on a Tukey test ($P < 0.05$). Therefore, the size of the Liberica beans (thickness, surface area, volume) increased with

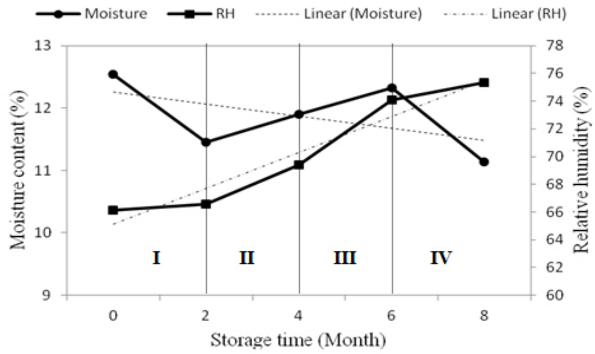

Figure 2. Coffee bean moisture content and relative humidity of coffee storage

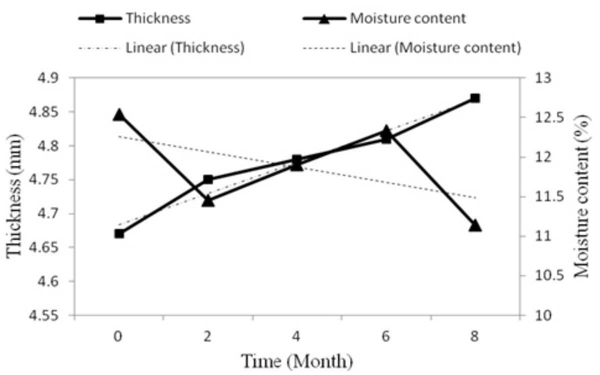

Figure 3. Effect of storage on the thickness and moisture content of Liberica coffee beans

increasing time. Some of the researchers have related the size and dimension changes as a function of moisture content since size expansion is probably due to moisture absorption. Amin *et al*. (2004) reported that the thickness of lentil seeds increased linearly with an increase of moisture content. A similar trend was also reported for the thickness and surface area of pistachio nut (Razavi *et al*., 2007a) and caper seeds (Dursun and Dursun, 2005). However, Chandrasekar and Viswanathan (1999) reported a different trend where the coffee parchment size does not increase with an increase in moisture content even though the mass increased. The hard nature of the coffee parchment contributed to its size being independent of moisture absorption. In another study, coffee beans undergoing a monsooning process in India could increase 1.5 times their original size due to moisture absorption and could have a moisture content of up to 15% (Tharappan and Ahmad, 2006). However, as discussed earlier and illustrated in Figure 3, the moisture content of the Liberica beans decreased, but the size of the beans increased. Therefore, the size expansion of the Liberica coffee beans was probably not due to moisture absorption and probably some unknown factors could have contributed to their expansion.

Equations (7a) and (8a) were derived from the linear equation while equations (7b) and (8b) were developed from the polynomial equation. Equations (7c) and (8c) were formed from the power law equation. Equations (7a), (7b) and (7c) relate the surface area of the Liberica beans with its thickness while equations (8a), (8b) and (8c) relate the volume to the thickness of the Liberica beans. Table 3 presents the coefficient of determination, r^2 of the equations. In addition to the linear equations, the hypothesis equations (Equation 7b, 7c, 8b and 8c) can also be used to represent the surface area and volume in relation to the thickness of the Liberica beans since the r^2 are near to 1 (A value whereby $r^2 = 1$ indicates the regression line fits the data perfectly).

$$S = 145.6505t - 117.989 \quad (7a)$$

$$S = -4739.7748t^3 + 67695.8968t^2 - 322099.027t + 511125.596 \quad (7b)$$

$$S = 87.9434t^{1.2038} \quad (7c)$$

$$V = 89.1688t - 190.1862 \quad (8a)$$

$$V = -2860.6395t^3 + 40867.3059t^2 - 194495.222t + 308596.826 \quad (8b)$$

$$V = 13.9666t^{1.8072} \quad (8c)$$

where S = bean surface area (10^{-9} m^2), t = bean thickness (mm), V = bean volume (10^{-9} m^3)

Effect on mass and densities

The mass and densities (bulk density and true density) of stored Liberica beans are illustrated in Figure 4 and Figure 5. The range of mass was from 0.23 g (at month 4) and 0.26 g (at month 0). The true density range spread from 992.68 kg/m^3 (at month 4) to 1138.25 kg/m^3 (at month 0). The trends for both mass and true density are approximately the same whereby the values started to decrease after month 0 but increased after month 4 with the minimum and maximum values at month 4 and 0 respectively. The bulk density range was from 632.43 kg/m^3 (at month 8) to 677.79 kg/m3 (at month 0). No fluctuation of bulk density values occurred whereby the trend was decreasing. Through a Tukey test, the bean mass and bulk density decreased significantly ($P < 0.05$) during storage. Therefore, the mass, true density and bulk density of the Liberica beans decreased with increasing time. From the density formula ($\rho = m/v$), the density value was found to be dependent on the value of the mass or volume. For instance, a small density value can be obtained when the volume increased or the mass decreased and vice versa. In Figure 4, it is clearly shown that the true density of the beans is dependent on the bean mass since the trend of the curves is the same and that the volume of the beans has less influence on the bean true density. However, this is different for the bulk density of the beans (Figure 5) where the bean volume has more

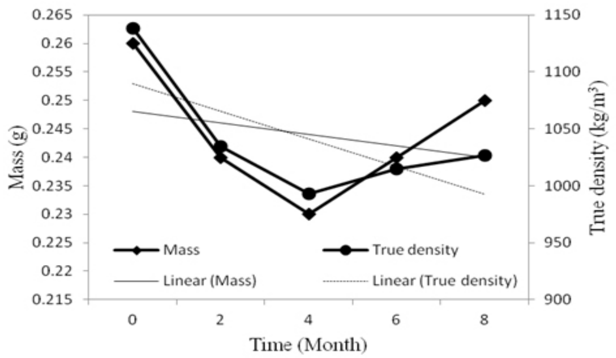

Figure 4. Effect of storage on the true density of Liberica beans and their relation with mass

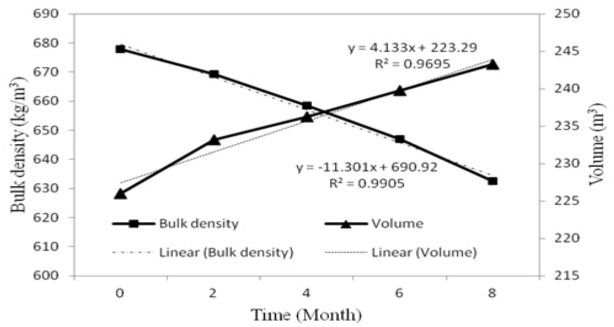

Figure 5. Effect of storage on the bulk density of Liberica beans and the relation with volume

Table 3. Coefficient of determination (r^2) of equations

	Linear	Polynomial	Hypothesis
Surface area vs Thickness	0.9878	0.9994	0.9876
Volume vs Thickness	0.9892	0.9995	0.9884
Bulk density vs Volume	0.9344	0.9986	0.9230
True density vs Mass	0.7840	0.9850	0.7843
Luminosity vs Relative humidity	0.6580	0.9556	0.6574
Sucrose vs Chlorogenic acid	0.8323	0.8547	0.8501

Table 4. Effect of storage on the colour of Liberica coffee beans

Month	L*	a*	b*	c*	h*
0	39.94^{a}	12.80^{a}	25.08^{a}	28.16^{a}	62.96^{a}
2	38.18^{b}	12.14^{a}	25.46^{a}	28.23^{a}	64.49^{a}
4	39.36^{a}	13.08^{a}	25.70^{a}	28.85^{a}	63.09^{a}
6	42.92^{c}	13.34^{a}	26.32^{a}	29.52^{a}	63.11^{a}
8	41.28^{a}	13.44^{a}	25.88^{a}	29.19^{a}	62.66^{a}

* Group means with the same letters in a column are not significantly different at 5% level of significance by Tukey test

influence on the bulk density value compared to the mass of the beans.

Some biological materials such as the mass of arecanut kernel, lentil seeds and karanja kernel depend on their moisture content where the mass increases with increasing moisture content and vice versa (Kaleemullah and Gunasekar, 2002; Amin *et al*., 2004; Pradhan *et al*., 2008). However, in this study the reduction in mass could not be due solely to the reduction of the moisture content of the beans, as illustrated in Figure 6. The trends were not similar to each other as seen in the figure even though the trends of changes were decreasing. For the reduction in the bulk and true density of the Liberica beans, this could be contributed by the loss of mass and the volume increase of the beans (Figure 4 and Figure 5). This trend is similar to the coffee parchments where the bulk and true density increase with increasing mass (Chandrasekar and Viswanathan, 1999). Nevertheless, this trend is actually against the trend of other biological material such as gram, neem nut and bambara groundnut (Dutta *et al*., 1988; Visvanathan *et al*., 1996; Baryeh, 2001) where the bulk and true densities decreased with increasing mass due to the their size expansion and mass increase compared to the coffee parchment.

Equations (9a), (9b) and (9c) relate the bulk density with the volume of Liberica beans. In addition to the linear equation (Equation 9a), the polynomial and polynomial equation (Equation 9b and 9c) may represent the relationship between bulk density and the volume of Liberica beans due to higher r^2 (Table 3).

$$\rho_b = -2.615v + 1273.3208 \quad (9a)$$

$$\rho_b = 0.005744v^3 - 4.1676v^2 + 1004.0629v - 79675.2201 \quad (9b)$$

$$\rho_b = 99746.1613v^{-0.9196} \quad (9c)$$

where ρ_b = bean bulk density (kg/m^3), v = bean volume (10^{-9} m^3)

Equations (10a), (10b) and (10c) relate the true density with the mass of Liberica coffee beans. The linear equation (Equation 10a) may represent the mass-true density relationship as well as the power law equation (Equation 10c) since the r^2 values are acceptable (Table 3). However, the polynomial equation highly represents the relationship due to higher r^2.

$$\rho_t = 4377.3846m - 26.6238 \quad (10a)$$

$$\rho_t = 23311666.7m^3 - 16935600m^2 + 4100202.83 - 329793.78 \quad (10b)$$

$$\rho_t = 4471.6819m^{1.033} \quad (10c)$$

where ρ_t = bean true density (kg/m^3), m = bean mass (g)

Effect on colour

Table 4 presents the effect of storage time on the colour of the Liberica beans. The L* (luminosity, brightness), a* (greenness-redness), b* (yellowness-blueness) and c* (colour intensity) showed an increasing trend while h* (hue angle) showed a decreasing trend. Nevertheless, a significant change ($P < 0.05$) was only observed in the brightness of the beans. The coffee bean discolouration (bleached or white appearance) could indicate that biochemical changes have taken place in the beans (Vincent, 1989). Throughout the storage, the brightness values fluctuated whereby the values first decreased (Phase

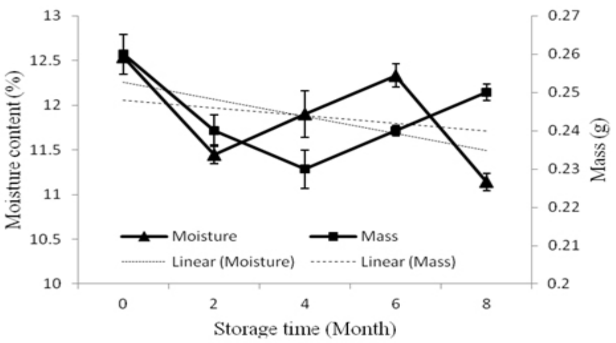

Figure 6. Effect of storage on Liberica beans mass and moisture content

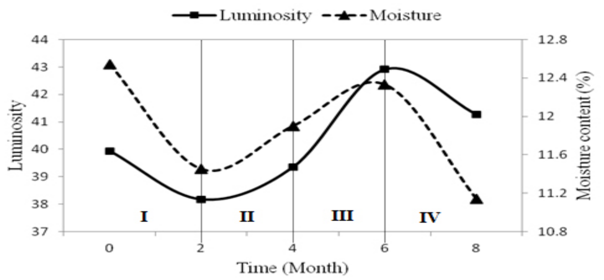

Figure 7. Effect of storage on the luminosity of Liberica coffee beans

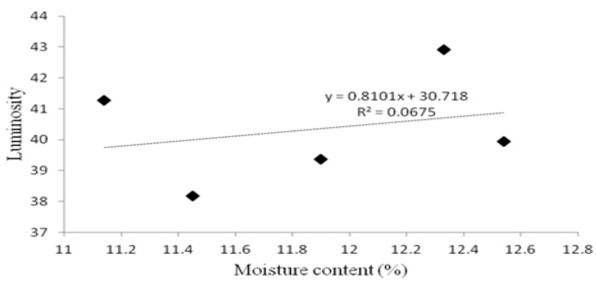

Figure 8. Effect of storage on the luminosity of Liberica coffee beans and the relation to the moisture content

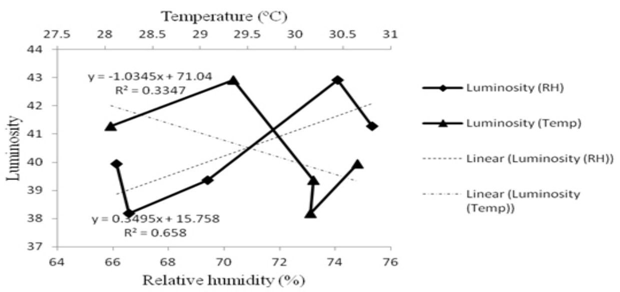

Figure 9. Effect of relative humidity and temperature on the luminosity of the Liberica beans

I), then increased (Phase II and III) and finally decreased again (Phase IV) as in Figure 7. Overall, the brightness of the beans increased with increasing time. This is similar to the Indian monsooned coffee beans which were slightly bleached after being stored 6-7 weeks in an open warehouse. The luminosity range was higher (60 – 63.3) while the chroma range was lower (21.23 - 26) than the stored Liberica beans in this study which indicated paleness and a bleached appearance (Murthy and Manonmani, 2009). Figure 7 also illustrates the trend similarities between the brightness and moisture content of the beans where the brightness value will increase when the moisture content increases and vice versa. This indicates that the moisture content influenced the brightness of the beans. However, the relationship between the moisture content and the brightness of the beans is not accurately presented in Figure 8 due to the low r^2 even though the brightness increased with increasing moisture content. Other than time, bean discoloration also depends on the storage RH or temperature of the air as seen in Figure 9. However, the effect of the RH on the brightness of the beans was more apparent than the temperature due to the higher r^2 of the RH over temperature. Therefore, it can be concluded that the higher the storage relative humidity, the higher the brightness value of the beans.

$$L = 0.3495H + 15.758 \quad (11a)$$

$$L = -0.0555H^3 + 11.7591H^2 - 828.6473H + 19473.0289 \quad (11b)$$

$$L = 3.0086H^{0.6104} \quad (11c)$$

where L = luminosity , H = relative humidity (%)

The linear, polynomial and power law equations are given as equations (11a), (11b) and (11c) respectively. The equations relate the luminosity of the beans to the relative humidity of the storage. According to Table 3, the polynomial equation (Equation 11b) can be used to represent the actual data since the r^2 of Equation (11b) is near to 1 which indicates that it accurately represents the data.

Effect of storage on the chemical properties of Liberica coffee beans.

Effect on the proximate composition

The effect on the proximate composition of Liberica beans due to storage is shown in Table 5. Through a Tukey test, only the fat and protein did not change significantly. The moisture and fibre decreased while the ash increased significantly ($P < 0.05$). The minimum and maximum ash value were 3.71% (at month 0) and 5.45% (at month 2) while the minimum and maximum fibre value were 15.45% (at month 8) and 21.51% (at month 2) respectively. The moisture has been discussed earlier. Hence, this section will discuss the ash and fibre content. However, since these components do not contribute to beverage quality, literature on these components is very limited and comparison cannot be made. However, the mineral content was found to increase, which is good for human health since minerals such as potassium and magnesium aid in the maintenance of bone mineral density which can reduce osteoporosis by reducing the rate of bone attrition and calcium excretion while calcium itself is good for bone health

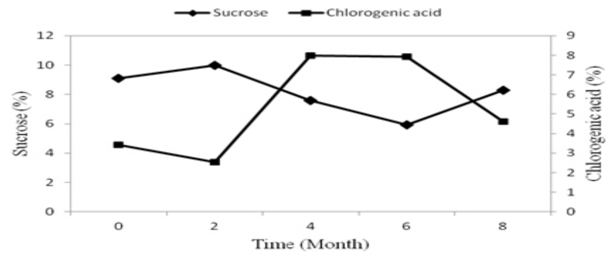

Figure 10. Effect of storage on the sucrose and chlorogenic acid of Liberica coffee beans

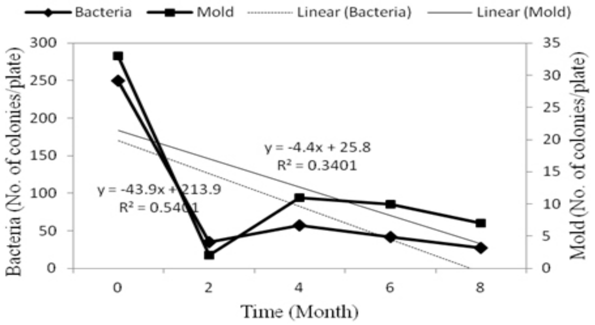

Figure 11. Microbiological growth changes on coffee beans during storage

Table 5. Proximate composition of stored Liberica coffee beans

Properties	Month of storage				
	0	2	4	6	8
Moisture (%)	12.54[a]	11.45[c]	11.90[d]	12.33[e]	11.14[f]
Fat (%)	9.55[b]	9.41[b]	9.48[b]	10.06[b]	10.13[b]
Protein (%)	14.42[b]	14.47[b]	16.15[b]	14.53[b]	15.43[b]
Ash (%)	3.71[a]	5.45[c]	4.30[d]	5.01[e]	5.21[f]
Fibre (%)	19.15[a]	21.51[c]	16.50[d]	17.45[e]	15.45[f]

* Group means with the same letters in a row are not significantly different at a 5 % level of significance by the Tukey test

(Tucker *et al.*, 1999). Since fibre does not contribute to the beverage quality, the changes that occurred will not be a disadvantage.

Effect on the sucrose and chlorogenic acid content

The sucrose and chlorogenic acid content of the Liberica beans during storage are presented in Figure 10. The highest sucrose value (9.09%) was at 2 months storage while the lowest (5.92%) was at 6 months storage. The highest (7.99%) and lowest (2.55%) chlorogenic acid content was at 4 and 2 months storage respectively. Changes from both values differ significantly ($P < 0.05$) based on the Tukey test. From the figure, both curves showed trends which are relatively opposite whereby the sucrose content decreased as the chlorogenic acid increased and vice versa. Therefore, the sucrose value is inversely proportional to the chlorogenic acid and with increasing time, the sucrose content decreased as the chlorogenic acid content increased. Chlorogenic acid has been said to be responsible for bitterness (Ky *et al.*, 2001) while sucrose is responsible for sweetness (Trugo, 1989) in coffee. Hence, the inverse relationship between sucrose and chlorogenic acid can be depicted by equations (12a), (12b) and (12c) whereby each equation represents the linear, polynomial and power law equation respectively. The polynomial equation has the highest r^2 according to Table 3 and may represent the sucrose-chlorogenic acid relationship more accurately compared to the other equations.

$$S = -0.5544C + 11.109 \quad (12a)$$

$$S = 0.041C^3 - 0.541C^2 + 1.4092C + 9.1365 \quad (12b)$$

$$S = 13.8033C^{-0.342} \quad (12c)$$

where S = bean sucrose content (%), C = bean chlorogenic acid content (%).

Effect of storage on microbiological analysis of Liberica coffee beans

Figure 11 shows the changes of bacterial count and mould colony numbers during the storage of Liberica beans. The bacterial count was in the range of 27 (at month 8) to more than 250 (at month 0) colonies per plate while the mould ranged from 2 (at month 2) to 33 (at month 0) colonies per plate. The trend for both curves was similar whereby in the 2nd month of storage, the number of colonies dropped drastically and then the number increased after two months of storage. After four months of storage, both numbers of colonies decreased. These results are similar to Bucheli *et al.* (1998) where the aerobic microorganisms and fungi quantity of Robusta coffee stored under tropical condition is high at the beginning of storage, but decreases during storage. The decreasing trend of bacteria and fungi was expected since the moisture content of beans was also decreasing overall. Therefore, the bacterial and mould count of the Liberica beans decreased with increasing time and the bacterial count was found to be directly proportional to the mould count.

Conclusions

The physico-chemical properties of Liberica green coffee beans were determined during storage. Physically, the coffee beans became larger, lighter with lower bulk and true density and also brighter in colour. Chemically, the coffee beans became drier due to a loss of moisture but increased in ash content. In addition, the sweetness was lowered due to a loss of sucrose and increased in chlorogenic acid content. However, the coffee beans were still safe for consumption after the storage period.

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