

## Carcass Compositions in Three Different Breeds of Chicken and Their Correlation with Growth Performance

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### ABSTRACT

This study was undertaken with the aim to determine the carcass composition of three breeds of chicken and their correlation with growth performance. For this purpose, fifty Red Jungle Fowl (*Gallus gallus Spadiceus*), fifty Malaysian indigenous chickens (*Gallus gallus Domesticus*) and fifty broiler chickens (ROSS) were used in this study. The chickens in each group were sacrificed at 1, 10, 20, 56, and 120 days post-hatching. The results showed that there were significant differences in the parameters measured between the high performance breed (commercial broilers), and the lower performance breeds (Red Jungle Fowl and Malaysian Indigenous chickens), although they were reared under the same environment and received the same feed, management and other facilities. Meanwhile, relative whole carcass weight, bone and fat weights in the commercial broiler were the highest compared to indigenous chickens and red jungle fowl at (p<0.05).

**Keywords:** Red Jungle Fowl, Malaysian indigenous chicken, commercial broiler, carcass composition

### INTRODUCTION

The red jungle fowl is known to be the ancestor of all domestic fowl. It is classified as omnivorous, slow growth rate and the range of the species stretches from northeast India eastwards across Southern China and down into Malaysia and Indonesia (Condon, 2006). The Malaysian indigenous chicken or known as village chicken arrived from crossbreeding between red jungle fowl and mixed exotic domestics breeds that has been brought by the Europeans, mainly British (Petersen *et al.*, 1991). Current commercial broiler chicken strains are a result of successful selection programs for rapid growth and body

conformation, especially favouring the breast muscles which could have significant economic impact (Scheurmann *et al.*, 2003).

In general, chicken growth is well described as a sigmoid curve with an initial exponential development phase, an intermediate or transitory phase, and a final phase of inhibited growth that consists of a gradual reduction in the growth rate, following an asymptotic increase in the body weight (Aguilar *et al.*, 1983). Meanwhile, the differences in the selection criteria among the primary breeders, favouring specific environments or genotypes, may affect the chronology of events during the growth process (Scheurmann *et al.*, 2003).

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Up to this date, there have been very few data on the carcass composition of the red jungle fowl and the Malaysian indigenous chickens documented elsewhere. Thus, this study was undertaken with the aim to define the carcass composition of the Red Jungle Fowl (*Gallus gallus Spadiceus*) and Malaysian Indigenous Chicken (*Gallus gallus Domesticus*). The results were compared with the commercial broiler chicken.

## MATERIALS AND METHODS

### *Animals*

Three breeds of chicken, known to differ greatly in the growth rates, were used in this study. These breeds were commercial broiler breed (ROSS), Malaysian indigenous chicken (*Gallus gallus Domesticus*) and Red jungle fowl (*Gallus gallus Spadiceous*). A total of 150 chickens, consisting of 50 chickens in each breed, were used in this study. The three breeds of chicken were reared separately in three different cages in an experimental house from their DOC till end of the experiment. The chickens were given commercial feed and drinking water *ad libitum*. For the Malaysian indigenous chickens, the eggs were obtained from the same sources in Jenderam Hulu, Sepang, Selangor, and were hatched in the laboratory using a hatchery. This is similar for the Red jungle fowl, whereby the eggs were obtained from the Centre of Animal Conservation in Jenderam Hulu, Sepang, Selangor, and were hatched in the laboratory using a hatchery. The commercial broiler chickens were obtained from CP (M) Private Limited hatchery in Taiping, Perak. All the chickens for each breed were sacrificed by intravenous (cutaneous ulnar vein) administration of sodium pentobarbitone (80mg/ kg) (Michell & Smith, 1991) at days 1, 10, 20, 56, and 120 post-hatching.

### *Carcass Composition Measurement*

The live weight of each chicken was taken and recorded prior to euthanasation. Upon euthanasation, the skin, viscera, head and legs were removed leaving the whole carcass. The

legs were cut at the end of tibiotarsal bone, while the head and neck were removed at the point of 3<sup>rd</sup> cervical vertebra, and the wings were cut at the point of humerocarpal band. The whole carcass for each chicken was weighted and recorded. The breast circumference of each chicken was taken by using a nylon string and then matching it to the ruler scale, and recorded. This was followed by removing the fat from the carcass, weighted and recorded. After removing the fat, the meat and bone were separated, weighted and recorded.

### *Statistical Analysis*

In this study, the collected data were analyzed statistically using SPSS 17.0 software. The results were illustrated using tables, bar graphs and line chart.

## RESULTS

The result of the mean whole carcass weight for the three breeds of chickens showed that CB has the highest whole carcass weight at all evaluated ages, followed by CV and RJ (*Fig. 1A*). Although the mean weights of the whole carcass at different ages were different, the result showed that there was no significant difference ( $p < 0.05$ ) at the early age (between day 1 and day 10 post-hatching) for all the three breeds. In general, the mean relative weight for all the three breeds showed almost similar increment pattern from day 1 to day 120 post-hatch (*Fig. 1B*). It is important to note that the relative whole carcass weight in all the three breeds increased with age, while the increments were significantly different ( $P < 0.05$ ) between the age intervals.

The mean meat weight and bone weight showed almost similar patterns as the whole carcass weight, whereby CB had the highest content of meat and bones at all the ages evaluated, followed by CV and RJ (*Figs. 2A and 3A*). Although the meat content increased according to age, the differences were not significant at day 1 and day 10 post-hatching. However, a significant difference ( $p < 0.05$ ) was

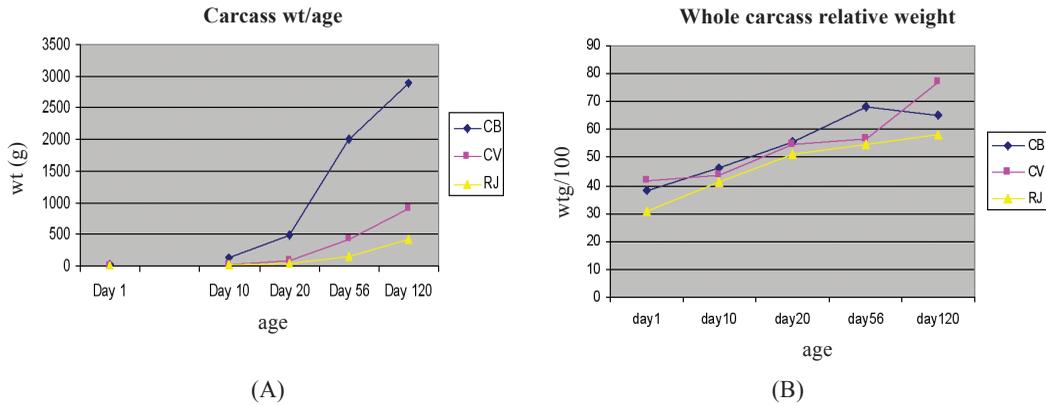


Fig. 1: The mean whole carcass weight (A), and the mean relative whole carcass weight (B) vs. age at post-hatching for the three breeds of chicken

shown at the later ages. As for the mean relative weight, all the three breeds generally showed almost a similar increment pattern of mean relative weight from day 1 to day 120 post-hatch (Fig. 2B). Meanwhile, the CB showed the highest mean meat relative weight, except at days 1 and 120 of post-hatch, whereby the CV was the highest at these points. The mean bone relative weight revealed a significant difference between the breeds at all ages, except at day 10 post-hatch. In general, the bone relative weight for the CB showed a decreasing pattern from day 1 to day 120, with the CV showing an increasing pattern, and RJ showing almost a constant pattern (Fig. 3B).

The result for the mean fat weight showed that the CB had the highest content of fat at all different ages evaluated, followed by CV and RJ (Fig. 4A). Within the breeds, although the fat content was increased when the age increased, the differences were not significant between day 1 and day 10 post-hatching for CB and CV. Nonetheless, the significant difference ( $p < 0.05$ ) was observed at the later ages. The content of fat in the RJ, on the contrary, remained insignificantly different until day 56 of post-hatching. In general, the RJ showed the lowest fat relative weight among the three breeds (Fig. 4B), while the CV had the highest fat relative weight at days 10 to 56, but lowest at

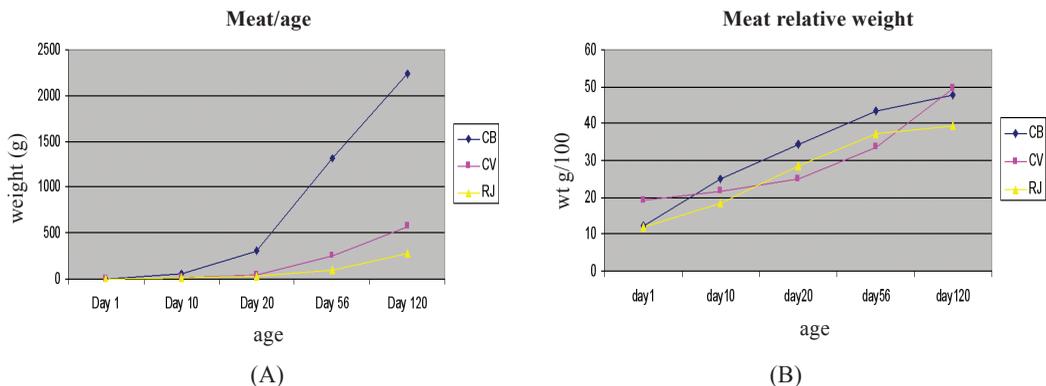


Fig. 2: The mean weight of meat (A) and the mean relative weight of meat (B) vs. age post-hatching for the three breeds of chicken

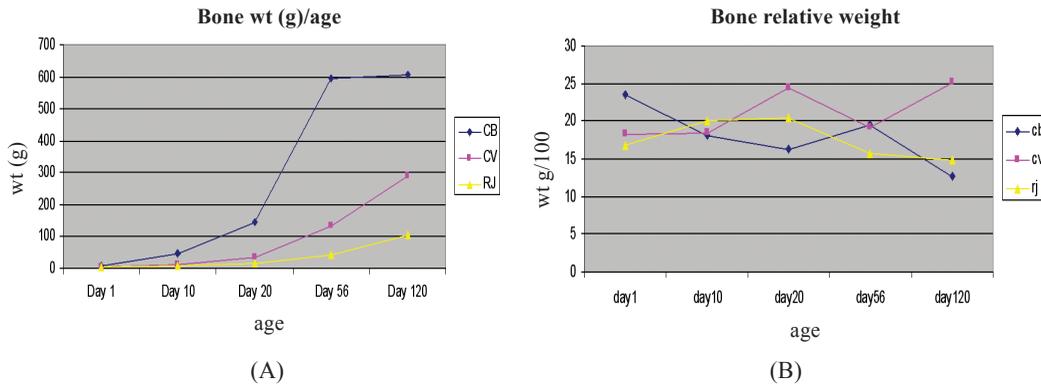


Fig. 3: The mean weight of the bones (A) and the mean relative weight of the bones (B) vs. age at post-hatching for the three breeds of chicken

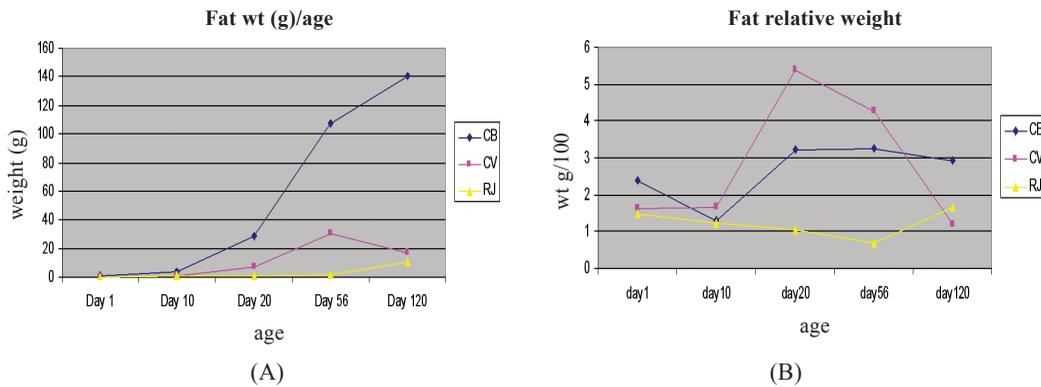


Fig. 4: The mean weight of fat (A) and the mean relative fat weight (B) vs. age post-hatching for the three breeds of chicken

day 120 post-hatch. The CB had the highest mean fat relative weight at day 1 and day 120 of post-hatching. Both the CV and RJ showed no significant difference at days 1, 10 and 120, while CV showed higher mean fat relative weight than the RJ, except at day 120 of post-hatching.

The results for the mean breast circumference showed that the CB was the highest at all different ages evaluated, and this was followed by the CV and RJ. The differences between the three breeds were significantly different ( $p < 0.05$ ), except at days 1 and 20 post-hatching for the CV and RJ (Fig. 5A). Even though the breast circumference was higher in the CV as compared to RJ at days 1 and 20 post-hatching, the differences between

them were not significant ( $p > 0.05$ ). Within the breeds, the breast circumference was found to increase with age, while the increments between the age evaluated were significantly different ( $p < 0.05$ ). As for the mean breast circumference relative weight, all the three breeds generally showed almost a similar decrement pattern from days 1 till 120 of post-hatching, with the RJ showing the highest, followed by CV and CB (Fig. 5B). There were significant differences ( $P < 0.05$ ) between the three breeds at all ages evaluated, except at day 1, whereby the CV and RJ showed no significant difference even though the RJ was found to have a higher mean breast circumference relative weight.

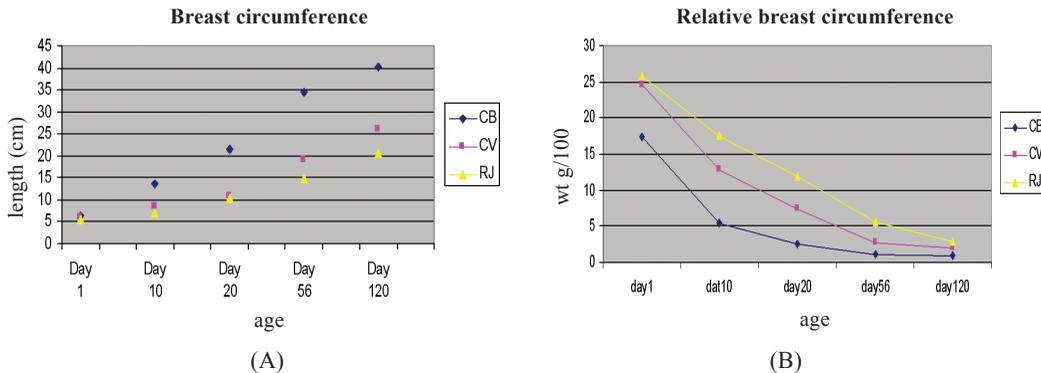


Fig. 5: The mean breast circumference (A), the mean relative breast circumference (B) vs. age at post-hatching for the three breeds of chicken

### DISCUSSION

The findings of the present study showed that broiler had significantly higher means for the whole carcass weight, meat weight, bone weight, and fat weight, and this was followed by village chicken and red jungle fowl, although they were reared under the same environment and received the same feed, management and facilities. Meanwhile, skeletal muscle growth and muscle fibre size in animals selected for large body size or rapid growth rate have been reported for several species (Chen *et al.*, 2004). The Red Jungle Fowl and Malaysian Indigenous chicken have been known to have had slow growth performances compared to the commercial broiler chickens. The development of the organs, which include skeletal muscle system, seems to be slower and leads to smaller body size. This is why they can produce good quality meat at considerable cost because of the low growth rate and perhaps of low food efficiency. Broilers have been selected and genetically programmed for rapid growth rate, larger body weight and meat production (William & Goldspink, 1978).

In general, chicken growth is well described as a sigmoid curve with an initial exponential development phase, an intermediate or transitory phase, and a final phase of inhibited growth that consists of a gradual reduction in the growth rate following an asymptotic increase in the body weight (Aguilar *et al.*, 1983). Therefore,

at the early age (day 1 and day 10), there were no significant differences in terms of the whole carcass weight, meat weight, bone weight, and fat weight for all three breeds.

The results showed that the relative whole carcass weight in all the three breeds increased when the age increased with a significant difference at  $P < 0.05$ . In particular, the CB had highest relative whole carcass weight, followed by CV and RJ at days 10, 20 and 56 post-hatch. The findings also showed increased relative weight as in the initial exponential development phase and intermediate or transitory phase but it decreased at later ages as it reached the final growing phase.

CV and RJ also showed a slower meat relative weight compared to the CB which was found to grow slower although there was an increase in the relative meat weight. Thus, further study is still needed to determine the best weight for marketing the CV and RJ. The results in this study revealed that the CB had significantly ( $P < 0.05$ ) higher bone content compared to the CV and RJ. The relative bone weight for the CB showed a gradual decrease as the body weight increased, which is best condition for the meat-type as it is designed for fast growing birds. Meanwhile, the CB had the highest content of fat at all ages evaluated, followed by the CV and RJ which had very little fat contents, respectively.

Genetically, the CB has gone through successful selections for meat-type breed as well as utilization of feed and converted it into muscle mass and deposition of fat as compared to the CV and RJ, even though all the three breeds had received similar quantity and quality of feed. The fat content in RJ remained the lowest, except at day 120 of post-hatch. This might be due to their nature, i.e. more active, alert and some general behaviour like exploring and anti-predator. Thus, this finding seems to suggest that the utilization of fat for the energy is higher in the RJ.

As for the carcass composition evaluation, the results generally showed that the CB had the best quality in terms of its relative whole carcass and meat content. However, it was also found to be higher in bone and fat contents. Thus, the CB had higher meat yield as compared to CV and RJ. On the contrary, the RJ had the lowest relative fat content, lowest relative whole carcass weight, lowest meat content, but a higher relative bone weight. Therefore, the CV is always in between the CB and RJ.

### CONCLUSION

There were significant differences in the parameters measured between the high performance breed (commercial broilers), and lower performance breeds (namely Red Jungle Fowl and Malaysian Indigenous chickens), although they were reared in the same environment and received the same feed, management and other facilities. Relative to their body weight, commercial broilers had the highest quality in terms of the whole carcass and meat content, but they also had higher bone and fat contents compared to indigenous chickens and red jungle fowl. Thus, the commercial broiler had a higher meat yield compared to the other two breeds; however, this may not necessarily be high quality meat. Red jungle fowl was found to have the lowest relative fat

content, lowest relative whole carcass weight, lowest meat content, but higher relative bone weight. Indigenous chickens are always in between the commercial broiler and Red jungle fowl. Thus, this finding revealed that the red jungle fowl possesses more healthy meat as it is low in carcass fat content compared to the other two breeds. Conversely, less meat yield due to small size.

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## Antioxidant Study of Garlic and Red Onion: A Comparative Study

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### ABSTRACT

Garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.) are among the most common ingredients in Malaysian cuisines. These two *Allium* species are believed to possess medicinal properties including antioxidants. Accordingly, the aim of this study was to compare antioxidant level and activities (i.e. at primary and secondary levels) in both the *Allium* species collected from markets around Kuantan, Pahang Darul Makmur, Malaysia. Current results of total phenolic content (TPC) assay indicate that TPC is higher in red onion (i.e.  $53.43 \pm 1.72$  mg GAE/100g) as compared to garlic (i.e.  $37.60 \pm 2.31$  mg GAE/100g). In addition, EC<sub>50</sub> value of garlic is lower than that of the red onion, showing a higher free radical scavenging activity in garlic than in red onion. However, the primary antioxidant activities of both the samples are lower than the standard antioxidant, BHA. Therefore, there is a poor relationship between the TPCs and the primary antioxidant activities, indicating that the primary antioxidant activities of both the *Allium* species are not solely due to the phenolic compounds. For secondary antioxidant activity, FIC assay shows that at the highest sample concentration of 1.0 mg/mL, red onion has higher ferrous ion chelating effect (i.e.  $45.00 \pm 1.73\%$ ) as compared to garlic (i.e.  $43.29 \pm 3.89\%$ ). Furthermore, both the *Allium* samples show slightly higher ion chelating effect than BHA (i.e.  $43.14 \pm 1.07\%$ ) but lower than EDTA (i.e.  $97.9 \pm 0.07\%$ ). Overall, the findings of the present study show a negative relationship between the results of TPC assay, DPPH radical scavenging activity assay, and FIC assay. To strengthen the validity of the present results and to further assess the potential of both the *Allium* species as natural antioxidant sources, more different assays need to be considered for future work.

**Keywords:** *Allium cepa* L., *Allium sativum* L., total phenolic content, antioxidant activity

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## INTRODUCTION

Modern consumers are becoming more health conscious and more aware of food nutritional value. Among the nutrients, antioxidants are popular due to their ability to prevent many physiological diseases or illnesses. Antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Li *et al.*, 2007). Antioxidants are believed to play a very important role in the body defence system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells. Increasing the intake of dietary antioxidant is believed to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system. According to Tepe *et al.* (2005), antioxidants have great importance in terms of preventing oxidative stress that may cause several generative diseases. Many fruits and vegetables are potentials for decreasing risk effect of several chronic diseases, such as cancer, coronary heart disease and many more.

The *Allium* family has over 700 members; each with different tastes, forms and colours; nonetheless, they are close in biochemical, phytochemical, and nutraceutical contents (Tepe *et al.*, 2005). Red onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are among the important parts of diet in many world populations, and there is also a long-held belief in their health enhancing properties. Among the oldest cultivated plants, garlic and red onion are used as food and for medicinal application as they have been proven to convey many benefits to human due to their long storage and portability. One of the advantages of these *Allium* species is that they could be dried and preserved for several months. Garlic, for instance, has been applied as culinary spice and medicinal herb, and it is an important constituent of the traditional Chinese medicine. On the other hand, onions (including red onions) are native to Eurasia but now grow all over the world. The

bulb of onion is used medicinally and onion has been consumed as food for many centuries. In Malaysia, these two *Allium* species are widely used and they are becoming very important components in the preparation of almost all Malaysian cuisines and delicacies.

According to Benkeblia (2005), *Allium* species are revered to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries. During the last 20 years, *Allium* spices have been among the most studied vegetables and aroused great interest. In previous studies, garlic is found to exhibit antioxidants activity (Tepe *et al.*, 2005) and this fact is set as the foundation for possibilities on the presence of antioxidant activities in other *Allium* species. Apart from that, according to Li *et al.* (2006), synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) need to be replaced with natural antioxidants as several studies showed that a number of synthetic antioxidants were toxic and carcinogenic in animals. Consumers are quite sceptical on the production of any synthetic antioxidant products, and there has always been a good public acceptance when it comes to natural antioxidants. For that reason, the aim of this project was to compare the antioxidant level and activities (i.e. at primary and secondary levels) in garlic and red onion that are available in Malaysian markets in Kuantan, Pahang Darul Makmur.

## MATERIALS AND METHODS

### *Sample Collection*

Samples of garlic (*A. sativum* L.) and red onion (*A. cepa* L.) were purchased from the local markets and supermarkets in Kuantan, Pahang Darul Makmur, Malaysia. The samples were randomly selected off the shelves based on their freshness.

*Sample Preparation and Extraction*

The samples were cut into smaller pieces to ease the drying process. Following the suggestion by Khamsah *et al.* (2006), the drying process was done in a warm room at 45°C (not exceeding 50°C) until all the moisture was gone. However, the findings of our previous antioxidant studies suggest no significant detrimental effects on the total phenolic compounds when drying the samples at 60°C and 70°C (Norshazila *et al.*, 2010; Nurliyana *et al.*, 2010). Extraction was done using the Soxhlet method (Siddhuraju *et al.*, 2002). The samples were weighed at 100 g and inserted into an extraction tube of Soxhlet apparatus. The extracting solvent, i.e. 70% ethanol, was then added into the round flask. The Soxhlet apparatus was then assembled, the heat was set at 60°C and left running for 12 hours. After the extraction process, the samples in the round flask were subjected to rotary evaporation to remove the extracting solvent from the extracts. Finally, the extracts were subjected to freeze drying to remove water from the extracts. The extracts were kept in the dark at 4°C until further uses.

*Total Phenolic Content (TPC) Assay*

TPC was determined by using Folin Ciocalteu's reagent (Lim *et al.*, 2006). 0.3 mL of the extract was introduced into the test tubes, followed by 1.5 mL of Folin Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min in the dark. The absorption of the samples was taken at 765 nm using Perkin Elmer Lambda 25 UV/Vis spectrophotometer. The TPCs were expressed in gallic acid equivalents (GAE). The gallic acid calibration line has the equation of  $y = 9.2402x + 0.0149$  ( $R^2 = 0.9971$ ), where  $y$  is the absorbance at 765 nm and  $x$  is the concentration of phenolic compounds in mg/g of the sample (the graph is not shown).

*1, 1-diphenyl-2-picrylhydrazyl (DPPH)**Radical Scavenging Activity Assay*

The free radical scavenging activity of each sample was measured using Perkin Elmer Lambda 25 UV/Vis spectrophotometer, based on the decrease absorbance of ethanolic DPPH solution at 517 nm (Lim *et al.*, 2006). Different dilution extracts (0.2 – 1.0 mg/mL), amounting to 1.0 mL, were added to 2.0 mL of the DPPH solution. The samples were then vortexed to thoroughly mix it. The samples were then left to stand in the dark for 30 min. The absorbance readings of the samples were taken at 528 nm using Perkin Elmer Lambda 25 UV/Vis spectrophotometer. Synthetic antioxidant, butylated hydroxyanisole (BHA), was used as a positive control for this assay. The antioxidant activity was expressed as:

$$\% \text{ disappearance} = \frac{[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100}$$

$A_{\text{control}}$  = Absorbance reading of the control

$A_{\text{sample}}$  = Absorbance reading of the sample

$EC_{50}$ , effective concentration of the extract required for 50% scavenging of DPPH radicals were calculated from the plotted graph of scavenging activity against sample concentration.

*Ferrous Ion Chelating (FIC) Assay*

Chelating effects of the samples were measured using the FIC assay. Serial dilutions of the samples were prepared (0.02 mg/mL - 0.1 mg/mL). Next, 50  $\mu$ L of Ferum chloride ( $FeCl_2$ , 2 mM) and 1.65 mL of 70% ethanol were added to 500  $\mu$ L of the sample. The samples were vortexed to mix it thoroughly and were left to stand for 5 min in the dark. After that, 100  $\mu$ L of ferrozine (5 mM, dissolved in 70% ethanol) was added, and subjected to vortex to mix the samples thoroughly. The samples were once again left to stand in the dark for another 5 min. Finally, the absorbance readings of the samples were measured at 562 nm using Perkin Elmer

Lambda 25 UV/Vis spectrophotometer. Both ethylenediaminetetraacetic acid (EDTA) and BHA were used as the controls. The ability of each sample to chelate ferrous ion was calculated relative to the control consisting of only iron ferrozine, using the following formula:

$$\text{Chelating effect \%} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

$A_{\text{control}}$  = Absorbance reading of the control

$A_{\text{sample}}$  = Absorbance reading of the sample

#### Statistical Analysis

All the samples and readings were prepared and measured in triplicate. The results were presented in mean  $\pm$  standard deviation. As for the data and graphs, they were subjected to analyses using Microsoft® Office Excel 2003.

## RESULTS AND DISCUSSION

#### TPC Assay

Phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of the compounds is partly due to one electron reduction potential that is the ability to act as hydrogen or electron donors (Chan *et al.*, 2007). Atoui *et al.* (2005) mention that the antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents,

hydrogen donors, and singlet oxygen quenchers. Determination of these compounds is usually performed by reacting phenolic compounds with Folin-Ciocalteu's reagent. The Folin-Ciocalteu reagent, Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants. Upon this reaction, the two classes of compounds will form a complex known as the phosphomolybdic-phosphotungstic-phenol complex which triggers the formation of a blue colour solution. According to Ajila *et al.* (2007), the more intense the formation of blue colour indicates a higher phenolic content inside the samples. The present study shows that red onion possessed higher TPC (i.e.  $53.43 \pm 1.72$  mg GAE/100 g) compared to garlic (i.e.  $37.60 \pm 2.31$  mg GAE/100 g), whereby red onion exerted an intense blue solution than the sample solution of garlic (Fig. 1). However, Benkeblia (2005) found out that the methanolic extract of garlic (*A. sativum* L. var. Cristo) shows higher TPC than the methanolic extract of red onion (*A. cepa* var. Rouge Amposta), and the difference could be due to the different types of species variants and extracting solvents used in both the studies.

#### DPPH Assay

DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. Primary antioxidant involves the mechanism, whereby

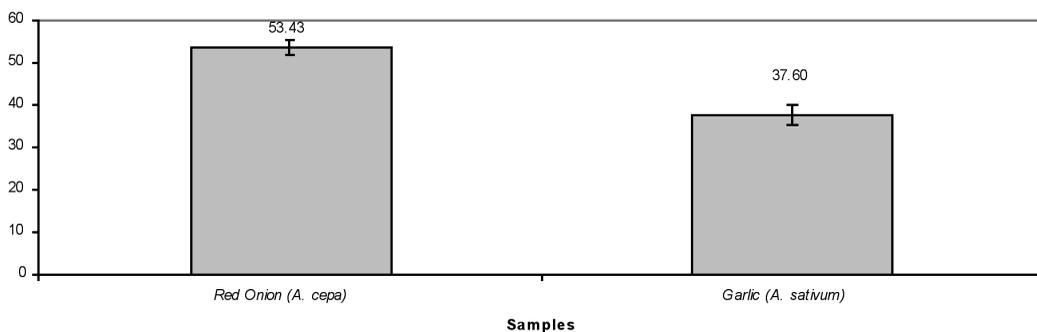


Fig. 1: Level of the total phenolic content in each sample. The results were expressed as gallic acid equivalents (GAE)

it inhibits the oxidation reaction by combining it with the free radicals or reacting hydrogen peroxides. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to the reduced form of the DPPH compound, leading to the reduction of the violet colour.

In the present study, the mechanism of the radical scavenging activity was observed based on the reducing purple colour of DPPH solution. *Fig. 2* shows that free radical scavenging activities of both garlic and red onion were lower than the positive control, BHA; indicating their weak free radical scavenging activities. In terms of  $IC_{50}$ , the lowest value is shown by the positive control, BHA ( $0.16 \pm 0.01$  mg/mL), followed by garlic ( $0.95 \pm 0.01$  mg/mL) and red onion. However,  $IC_{50}$  for red onion could not be directly determined from the graph due to the low percentage of the radical scavenging activities over the measured extract concentrations. Nonetheless, *Fig. 2* clearly suggests that their  $IC_{50}$  could be more than 1.0 mg/mL. The results clearly show that garlic has more capability to scavenge the free radicals as compared to red onion, although the primary antioxidant activities of both samples are lower than the standard antioxidant, BHA. Likewise, the findings from Benkeblia (2005), garlic shows higher free radical scavenging activity than red onion over the increasing sample concentrations.

Similar research conducted in other plants and fruit have shown that high radical scavenging activities are usually associated with high TPC. For instance, Lim *et al.* (2006) described that high radical scavenging activity was contributed by the presence of high phenolic content in guava extracts. There are several other studies that share similar results on the contribution of phenolic compounds to the high radical scavenging activity. However, the present study does not support the findings. Therefore, it is suggested that apart from phenolic compounds, there could be other organic compounds contributing to the high radical scavenging effect in garlic, even though its TPC is lower than red onion. Khamsah *et al.* (2006) suggested that free radical scavenging activity is not due

to the phenolics only because they found that the antioxidant activity of methanol extract of *Orthosiphon stamineus* was not solely caused by phenolic compounds.  $IC_{50}$  data further support that garlic has higher radical scavenging activity (i.e.  $IC_{50}$  of garlic = 0.95 mg/mL) than red onion, but the  $IC_{50}$  of red onion could not be determined directly from *Fig. 2* due to the low activity over the increasing concentrations. On the contrary, *Fig. 2* clearly suggests that its  $IC_{50}$  could be more than 1.0 mg/mL.  $IC_{50}$  of both the *Allium* samples are higher than BHA (i.e.  $IC_{50}$  of BHA = 0.16 mg/mL), showing their low and weak free radical scavenging activities.

#### FIC Assay

FIC assay is a common test used to determine the secondary antioxidant activity by observing the reducing purple colour of the reaction solution. The assay mechanism is based on the decrease in the absorbance of iron (II)-ferrozine complex. Meanwhile, secondary antioxidants are also known as the peroxide decomposers, where it inhibits polypropylene oxidation by decomposing hydroperoxide. Secondary antioxidants are responsible for suppressing the formation of radicals and protecting against oxidative damage (Lim *et al.*, 2006). Iron-ferrozine complex has the maximum absorbance at 562 nm and large decrease in absorbance indicates strong chelating power. By forming a stable iron (II) chelate, an extract with a high chelating power reduces free ferrous ion concentration, which leads to decrease the extent of Fenton reaction that are implicated in many diseases (Lim *et al.*, 2006). The assay determines the effectiveness of the chemical compound in the sample extract in competing ferrous ion with ferrozine.

Iron is known to generate free radicals through the Fenton and Haber-Weiss reaction. Fenton Weiss reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases (Llyod *et al.*, 1997). Metal ion-chelating activity of an antioxidant molecule prevents oxy-radical generation and

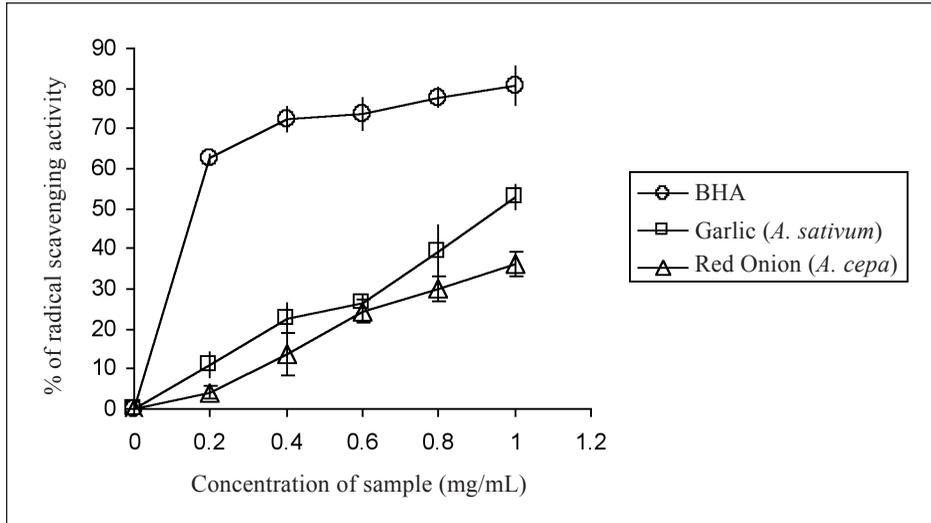


Fig. 2: Comparison of the free radical scavenging activity between the positive control - BHA and samples.  $IC_{50}$  value (in mg/mL) for each sample was derived from the graph at 50% free radical scavenging activity

the consequent oxidative damage (Kumar *et al.*, 2008). Metal ion-chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in lipid oxidation.

Fig. 3 shows that BHA, garlic and red onion have not much difference in the chelating effects, whereby all of them reached up to  $43.14 \pm 1.07\%$ ,  $43.29 \pm 3.89\%$ , and  $45.00 \pm 1.73\%$  at the highest sample concentration of 1.0 mg/mL, respectively. The results also indicate that the ferrous ion chelating effects of red onion are higher than BHA over the increasing concentrations. Furthermore, among the two *Allium* species, the chelating activity of red onion is slightly higher than that of garlic. However, EDTA which serves as the positive control shows the highest percentage of the chelating effect ( $97.9 \pm 0.07\%$ ). Besides, red onion can be considered as moderate metal chelator since its activities are approximately two times lesser than EDTA. Overall, the results suggest that both *Allium* species may be regarded incapable of strongly obstructing the generation of  $\bullet OH$  radicals from Fenton reaction (Kosem *et al.*, 2007).

#### The Relationship between the Results of TPC, DPPH and FIC Assays

There are positive relationships between TPC assay and DPPH radical scavenging activity assay, based on the findings of several previous studies (e.g. Ordoñez *et al.*, 2005; Luther *et al.*, 2007; Silva *et al.*, 2007; Tawaha *et al.*, 2007). Most of the researches have mentioned that high phenolic content will lead to high radical scavenging activity. Nonetheless, the present study on garlic and red onion shows a negative relationship between results of TPC assay and DPPH radical scavenging activity assay. Garlic is proven to be better radical scavenger as compared to red onion even though it expresses lower phenolic content. As for FIC assay, most of the previous findings have discovered that even though certain samples possess potent radical scavenging activity, the samples either possess moderate or weak ion chelating activity. For instance, a study conducted by Lim *et al.* (2006) on guava has shown that the samples of guava possess potent radical scavenging activity but they have weak ion chelating effects. Likewise, the current study revealed that garlic and red onion had weak (i.e. for red onion) to

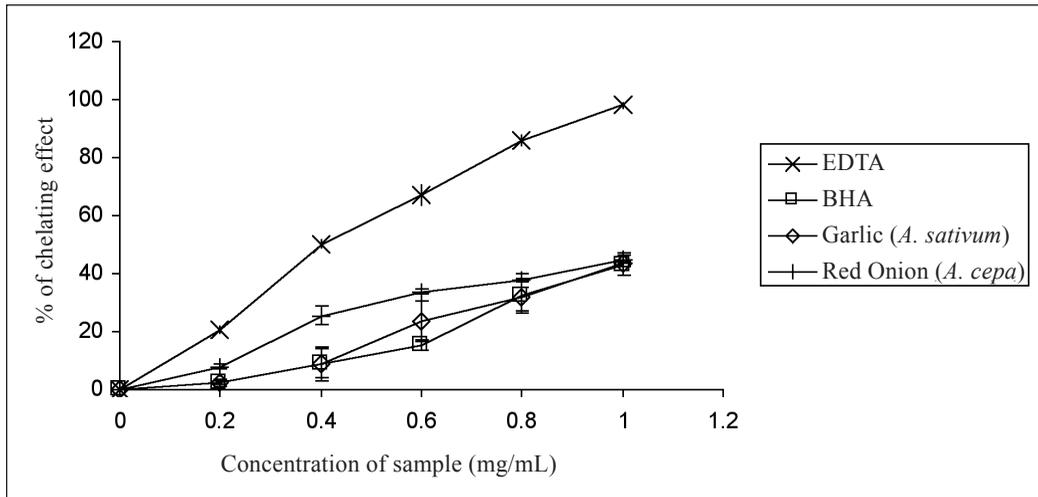


Fig. 3: Comparison of ferrous ion chelating effects between EDTA, BHA and the samples

moderate (i.e. for garlic) ion chelating activities. Overall, the results of the present study do not show good relationship between TPC assay, DPPH radical scavenging activity assay and FIC assay.

### CONCLUSIONS

The findings of the current study have shown that red onion (*A. cepa* L.) possesses higher TPC than garlic (*A. sativum* L.). However, garlic has expressed higher free radical scavenging effect (i.e. the primary antioxidant activity) as compared to red onion. As for the ion chelating effect (i.e. the secondary antioxidant activity) measured by the FIC assay, both *Allium* species have been found to have weak (i.e. for red

onion) to moderate (i.e. for garlic) ion chelating activities compared to the controls - BHA and EDTA. Overall, the current findings reveal a negative relationship between the results of TPC assay, DPPH radical scavenging activity assay and FIC assay (Table 1). Nonetheless, in order to gain better views on the antioxidant levels and activities in red onion and garlic, further studies on purification, identification and quantification of each phenolic compound and other non-phenolic compounds are necessary in future.

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TABLE 1  
Summary of the antioxidant properties for garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.)

Sample	Total phenolic content	Free radical scavenging activity	Metal ion chelating effect
Garlic	37.60 ± 2.31 mg GAE/100g	Lower than BHA	Slightly higher than BHA, lower than EDTA
Red onion	53.43 ± 1.72 mg GAE/100g	Lower than BHA	Higher than BHA, lower than EDTA

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Antioxidant Study of Garlic and Red Onion: A Comparative Study

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