CHAPTER 3

Antioxidant activity and phenolic content during growth and ripening in *Syzygium samarangense* fruits

3.1 Introduction

As mentioned earlier in the first chapter, there is an increasing awareness among consumers of the importance of natural antioxidants, especially from fresh fruits and vegetables since it has been well reported in the literature, that studies have shown that frequent consumption of natural antioxidants is related with to a lower risk of cardiovascular disease and cancer (Renaud *et al.*, 1998; Temple, 2000). Antioxidants in fruits and vegetables can be classified into three major groups, namely, vitamins, phenolics, and carotenoids. Of these, the hydrophilic antioxidants are ascorbic acid and the phenolics, whilst the carotenoids and the tocopherols are classified as lipophilic antioxidants (Halliwell and Gutteridge, 1995). In addition to this, there are also several antioxidant enzymes, including catalase, superoxide dismutase, glutathione peroxidase, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase and monodehydroascorbate reductase that neutralize many types of free radicals (Foyer *et al.*, 1994).

Phenolic compounds and flavonoids, derived from phenylalanine, are widely distributed throughout the Plant Kingdom. Although they typically comprise less than 2% of the fresh weight basis of the plant, these compounds serve such diverse functions as imparting colour to leaves and fruits, attracting or repelling insects, antimicrobial action, antiviral activity, protection from harmful ultraviolet radiation and protection from herbivores (Harborne, 1967a; Harborne and Williams, 2000). Chemically, they are defined as compounds possessing an aromatic ring bearing one or more hydroxyl groups, including their derivatives (Harborne, 1967b). More than 8,000 phenolic

compounds have been identified in plants (Wrolstad *et al.*, 2005) and the major phenolic compounds in fruits include the phenolic acids and polyphenols (flavonoids) (Macheix *et al.*, 1990; Robbins, 2003). Plant phenols and flavonoids are natural antioxidants and are candidates in exerting the protective effects of vegetables and fruit against some forms of cancer and cardiovascular diseases (Arts and Hollman, 2005).

Amongst local fruits, guava (*Psidium guajava* L.), also known locally as *jambu batu*, is rich in ascorbic acid, at levels far higher than most imported and local fruits. The fruit, in particular the pink flesh cultivar, has a high amount of beta-carotene (vitamin A) in addition to having also a high quantity of other antioxidants such as phenols(Lim *et al.*, 2006). Leong (Leong and Shui, 2002) in an experiment investigating antioxidant capacity of fruits in the Singapore markets reported that ciku (*Manilkara zapota*), a local fruit in this region, had the highest antioxidant capacity among Singapore markets fruits. Other fruits such as banana (Mokbel and Hashinaga, 2005), pomegranate (Kulkarni and Aradhya, 2005), black caraway, carrot, cranberry (Yu *et al.*, 2005), tomato (Javanmardi and Kubota, 2006; Toor and Savage, 2006), apple (Maffei *et al.*, 2007), cocoa beans (Othman *et al.*, 2007), blood orange (Kelebek *et al.*, 2008), Chinese bayberry fruit (Zhang *et al.*, 2008), and *Phyllenthus emblica* L. fruit (Luo *et al.*, 2009), have also been reported to be good sources of natural antioxidants.

As a result of the above, several quantitative studies investigating the phenolic content and antioxidant potential of edible fruits have been carried out and widely reported, since the role these factors play in health and disease chemoprevention are important for human well being. It has led to an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. From the literature, previous phytochemical studies of the leaves of *S. samarangense* have shown the presence of ellagitannins (Lee *et al.*, 1992), volatile terpenoids (Wong and Lai, 1996); flavanones (Liu *et al.*, 2005; Maurya and Yadav, 2005), flavonol glycosides (Ross *et al.*, 2005),

proanthocyanidins (Hosseinian and Mazza, 2009), anthocyanidins (El Gharras, 2009; Molan *et al.*, 2009), triterpenoids and chalcones (Srivastava *et al.*, 1995; Resurreccion-Magno *et al.*, 2005).

In this study the antioxidant activity of three varieties of *Syzygium samarangense* fruits were determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,20-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid)] assays and to assess the correlations between antioxidant activity and the total phenolic content and total flavonoids content. In addition to this the activity of the PAL enzyme, responsible for the synthesis of the phenolic compounds were also determined during ripening.

3.2 Materials and Methods

3.2.1 Plant Materials

The material used for the study was freshly harvested reddish pink, red and deep red *jambu air* fruits (*Syzygium samarangense*). The pink and red *jambu air* fruits (*Syzygium samarangense)* were collected from a farm in Shah Alam, Selangor, Malaysia whilst the deep red fruits (*Syzygium samarangense*) were collected from a farm in Raub, Pahang, Malaysia. The fruits were wrapped in paper to prevent injury to the fruits and brought to the laboratory for experimentation.

3.2.2 Preparation of extracts

3.2.2.1 Fruit extraction

Fruit extracts for total phenolics, total flavonoids and antioxidant activity determinations were prepared using the method of Swain (Swain and Hillis, 1959), with some modifications. Around 1.5 grams of fruit tissue were mixed with methanol and homogenized in a mortar and pestle and then transferred into polypropylene tubes. The tubes were vortexed and allowed to stand for 1 hour at room temperature to allow for

complete solvent extraction. The extracts were then centrifuged at 2000 g for 15 minutes at 20°C. The supernatant was filtered through a Whatman No. 1 filter, after which the filtrate samples were diluted to 25 ml with distilled water.

3.2.2.2 Protein extraction

Proteins were extracted by adding 4 g of fruit pulp to 8 ml of a modified Kanellis protein extraction buffer containing 50mM Tris-HCl, 0.5 M NaCl, 10 mM 2-mercaptoethanol, 1mM DTT, 1 mM EDTA, 10% glycerol and 0.5% triton X-100, with a tissue to volume ratio of 4:8 (Kanellis *et al.*, 1989). The mixture was then vortexed thoroughly, left on ice for 10 minutes and then centrifuged at 25,000 g for 30 minutes at 4ºC in a refrigerated centrifuge.

3.2.3 Total phenolic content (TPC)

The level of total phenolic content was determined using Folin–Ciocalteau (F&C) colorimetric reaction method of Singleton (Singleton and Rossi, 1965). One milliliter of the fruit extract was added to 10 ml water and 0.5 ml F&C reagent. After 5 minutes, 2 ml of 7.5% sodium carbonate solution was added. The solution was allowed to sit for 2 hour. Readings were taken at 765 nm using a Novaspec II Pharmacia Biotech spectrophotometer. A calibration curve was prepared with gallic acid and the results were expressed as mg gallic acid equivalents (GAE)/100 g fresh weight. A range of gallic acid concentrations from 0.25 to 0.005 mg/ml were used to prepare the calibration curve.

3.2.4 Total flavonoids content (TFC)

The total flavonoid content was determined according to the aluminum chloride colorimetric method of Kim (Kim *et al.*, 2003). To 0.3 ml sodium nitrite solution (5%) was added 1 ml of fruit extract, followed by 10% aluminum chloride solution (0.3 ml). The test tubes were incubated at ambient temperature for 5 minutes, and then 2 ml of 1 M sodium hydroxide were added to the mixture. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CE)/100 g sample. A range of catechin concentrations from 10 to 100 μg /ml were used to prepare the calibration curve.

3.2.5 ABTS free radical decolorization assay

Antioxidant activity (AA) was measured using an improved ABTS method as described by Cai (Cai *et al.*, 2004) and Re (Re *et al.*, 1999). The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared through the reaction of 7.4 mM ABTS and 2.6 mM potassium persulphate, after incubation at room temperature in the dark for 16 hours. The ABTS solution was then diluted with 80% methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. $ABTS^{\bullet+}$ solution (3 ml; absorbance of 0.700 ± 0.02) was added to 0.1 ml of the test sample and mixed vigorously. The reaction mixture was allowed to stand at room temperature for 60 minutes and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using ascorbic acid standard solution at various concentrations (ranging from 1 to10 mg/l) in 80% methanol. The absorbance of the reaction samples was compared to that of the ascorbic acid (AA) standard and the results were expressed as mg of ascorbic acid equivalents antioxidant capacity (AEAC) (Leong and Shui, 2002) per 100 g of homogenate using either one of the following equations:

$$AEAC = \frac{\Delta A}{\Delta A_{AA}} \times C_{AA} \times V \times \frac{100}{W}$$

Where, ΔA is the change of absorbance after addition of fruit extract, C_{AA} is the concentration of AA standard solution (mg/ml), ΔA_{AA} is the change of absorbance obtained from a calibration curve when the same volume of AA standard solution as that of fruit extract was added, V is the volume of filtrate (ml) and W is the weight of sample (g).

3.2.6 DPPH free radical-scavenging assay

The DPPH free radical scavenging activity was determined by the method of Yang (Yang *et al.*, 2008) with some modifications. Each sample of the three varieties *jambu air* fruits at different concentrations in methanol (0.25 ml) was mixed with 2 ml of methanolic solution containing 4×10^{-4} M DPPH. The mixture was mixed vigorously, and then left to stand for 30 minutes in the dark. The absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. DPPH radical scavenging activity of the sample was calculated as follows (Kulkarni and Aradhya, 2005):

$$\text{DPPH radical scavenging activity (\%)} = \frac{1 - A_s}{A_c} \times 100$$

Where, A_s= absorbance of sample, A_c= absorbance of control

IC50 value was determined to be the effective concentration at which DPPH radical was scavenged by 50%. The IC50 value was obtained by interpolation from linear regression analysis. All tests were performed in triplicates.

3.2.7 Assay of PAL enzyme activity

PAL activity was assayed using the modified procedure of Prusky (Prusky *et al.*, 1996). The above protein extract was used to measure PAL activity. The reaction

mixture consisted of 200 µL of the extract and 1.2 ml of 50 mM Tris–HCl buffer, pH 8.0, containing 6 mM of L-phenylalanine. The incubation was performed at 37 °C for 60 minutes and the reaction was stopped by adding 100 µL of 500 mM HCl. PAL was assayed by measuring the amount of trans-cinnamic acid formed at 290 nm and the specific enzyme activity expressed in µmol cinnamic acid min^{-1} mg^{-1} protein, with the protein content determined by the Bradford method (below). Alternatively, the enzyme activity was expressed as units per mg protein.

3.2.8 Determination of total protein (TP)

Protein in the fruit extracts was assayed colorimetrically according to the method of Bradford (Bradford, 1976). Bovine serum albumin protein (Sigma) was used as standard protein.

3.3 Results

3.3.1 Total Phenolic Content (TPC)

The phenolic content recorded in the three different varieties of *Syzygium samarangense*, during storage is shown in Fig. 3.1.

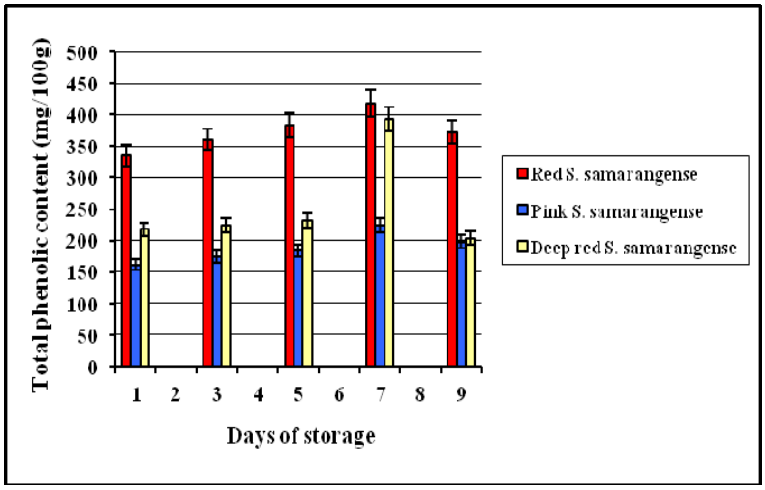

Figure 3.1 Total phenolic content (TPC) changes in *S. samarangense* fruits during storage. (■ Red *S. samarangense*, ■Pink *S. samarangense* and ■ Deep red *S. samarangense*). Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different (P≤0.05, t-test).

The results showed that the red variety of *S. samarangense* possessed the highest amount of phenolic content, which peaked at 7 days after storage. It decreased slightly after that. The other two varieties recorded lesser amounts of phenolic content. The amount of total phenolic content (TPC) in the red variety was 335.48 ± 32.87 mg GAE / 100g on the first day and 372.37 ± 35.04 mg GAE / 100g on day 9. The average TPC was 372.62 ± 69.64 mg GAE / 100g.

For the pink variety of *S. samarangense* fruits, TPC increased from the first day till day 7 and ranged between 161.67 - 224.73 mg GAE / 100g. On day 9, the amount of TPC was 198.28 ± 18.65 mg GAE / 100g. The average TPC value for this variety was 188.59 ± 24.22 mg GAE / 100g.

The results for the deep red variety also showed that the TPC values increased from the first day till day 7 and then decreased slightly on day 9. The amount of TPC was 218 mg GAE / 100g on the first day and 204 mg GAE / 100g on day 9. The highest of amount of TPC was recorded (393 mg GAE / 100g) on day 7. The mean of TPC was 254.27 ± 78.20 mg GAE / 100g.

The results showed that total phenolic content increased during storage in the red and deep red varieties. This result was in agreement with ripening in tomatoes (Periago *et al.*, 2008) who reported that total phenolics and flavonoids increased significantly during ripening. The highest total phenolic content was observed in red variety.

3.3.2 Total Flavonoids Content (TFC)

The total flavonoids contents in the three varieties of *Syzygium samarangense* fruit extracts, determined colorimetrically are presented in Fig. 3.2.

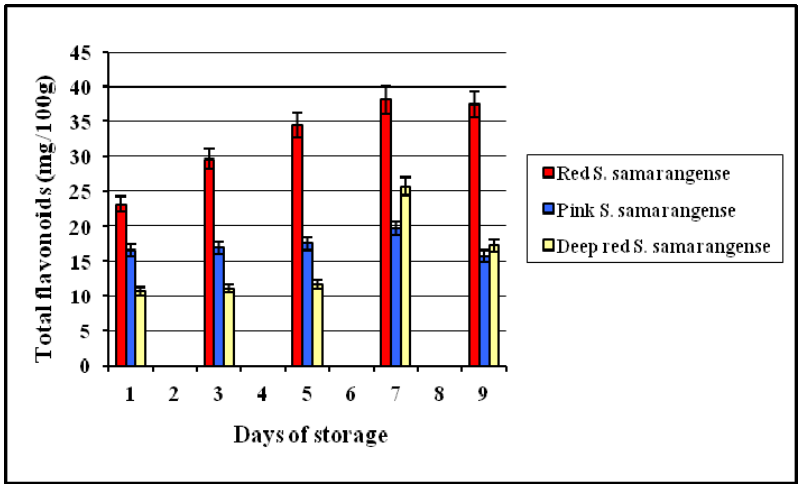

Figure 3.2 Changes in total flavonoids content (TFC) in *S. samarangense* fruits during storage (■ Red *S. samarangense*, ■ Pink *S. samarangense* and ■ Deep red *S. samarangense*).Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different (P≤0.05, t-test).

The results showed that, total flavonoids content (TFC) in the flesh of the three types of fruits were similar in pattern compared to each other and with the results of TPC experiment shown in Fig. 3.1. In all, the three varieties of *jambu* fruits, TFC increased gradually during storage until day 7 and then it decreased slightly on day 9 (Fig. 3.2). The amount of TFC in the red *S. samarangense jambu* fruits was 23.14 mg

CE / 100g on the first day while the average TFC on day 9 was 37.47 mg CE / 100g. The average TFC was 33.01 ± 10.68 mg CE / 100g fresh fruit.

The amount of TFC in the pink *S. samarangense jambu* fruits was16.55 mg CE / 100g on the first day and 15.71 mg CE / 100g on day 9. The average of TFC was 17.46 ± 4.78 mg CE / 100g.

The TFC results for the deep red *S. samarangense* fruits were similar to that for the pink and red *S. samarangense* fruits. The results showed that the TFC of the deep red *S. samarangense* fruits increased slightly until day 5, followed by a sharp increase on day 7 and then a decrease on day 9. The highest TFC recorded in deep red *S. samarangense* fruits was 25.69 ± 6.38 mg CE / 100g on day 7. The mean TFC of deep red *S. samarangense* fruits was 15.23 ± 6.41 mg CE / 100g.

3.3.3 Total Antioxidant Capacity of Fruits using the ABTS ◌̇ * Decolorization Assay

The results showed that the ABTS radical scavenging activities in the pink and red colored varieties were similar with that obtained by the DPPH assay (Figures 3.3 and 3.4).

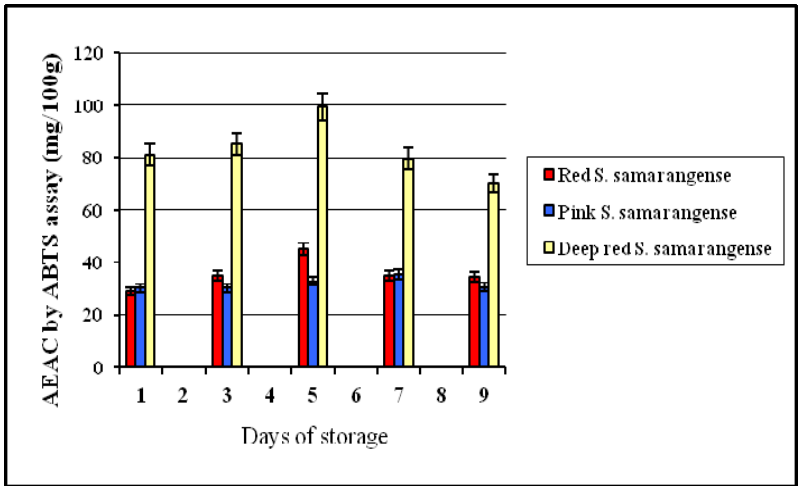

Figure 3.3 AEAC by ABTS assay in *S. samarangense* fruits during storage. (■ Red *S. samarangense*, ■Pink *S. samarangense* and ■ Deep red *S. samarangense*). Values are means of five replications ±S.D (n = 5). Mean

values at the same day, are significantly different (P≤0.05, t-test).

In the deep red color *S. samarangense* fruits, AEAC increased or peaked at day 5 and then decreased on days 7 and 9. The results for the deep red colored *S. samarangense jambu* were similar with red colored fruits (Fig. 3.3). However the ABTS radical scavenging activities of the pink colored *S. samarangense* fruits were different compared to the two other varieties. The AEAC values peaked at day 7 and decreased slightly on day 9.

The average AEAC value was 35.50 ± 5.80 mg /100g fresh fruit in the red *S. samarangense jambu* and 31.86 ± 2.30 mg /100g fresh fruit in pink *S. samarangense jambu*. The mean AEAC value in deep red varieties was 69.47 ± 14.26 mg /100g fresh fruit.

3.3.4 Scavenging DPPH Free Radicals

The DPPH radical scavenging activities of the *jambu air* fruits are shown in Figures 3.4 and 3.5.

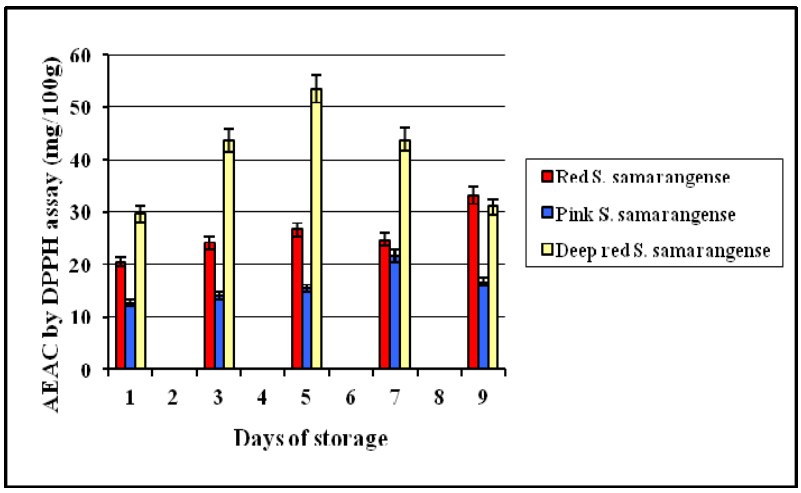

Figure 3.4 AEAC by DPPH assay in *S. samarangense* fruits during storage. (■ Red *S. samarangense*, ■Pink *S. samarangense* and ■ deep red *S. samarangense*). Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different (P≤0.05, t-test).

As shown in Fig. 3.4, the AEAC values in the red color *S. samarangense jambus* generally showed an increasing trend overall although it increased up to day 5 and then decreased slightly on day 7 and then increased on day 9. The average AEAC was 25.80 ± 4.64 mg / 100g fresh fruit.

The results of DPPH radical scavenging activities of the pink color *S. samarangense jambus* was a little different but generally showed an increasing trend up to day 7 (Fig. 3.4). The AEAC values was increased up to day 7 and then decreased on day 9. However the results of AEAC activity determined by DPPH radical scavenging assay in deep red varieties was significantly different compared to the two other varieties *S. samarangense jambus*. The AEAC values were high originally and increased till day 5 after which it decreased up to day 9.

The average AEAC values in the pink color *S. samarangense jambus* was 16.04 ± 3.46 mg / 100g, 25.78 ± 4.60 mg / 100g in the red color *S. samarangense jambus* and 39.53 ± 11.32 mg / 100g fresh fruit in deep red *S. samarangense*. The AEAC value in the red color *S. samarangense jambus* and deep red color *S. samarangense* was higher than in the pink color *S. samarangense jambus*.

The IC50 of the DPPH-radical scavenging activity is shown in Fig. 3.5. The IC50 of the DPPH-radical scavenging activity was determined as the midpoint between zero and full inhibition of the reaction in solution, of the sample and DPPH. The DPPH-radical scavenging activity in the pink *S. samarangense jambus* fruit and deep red *S. samarangense* was significantly ($P<0.05$) highest on day 7. In the red *S. samarangense jambus* fruit, it was highest on day 9.

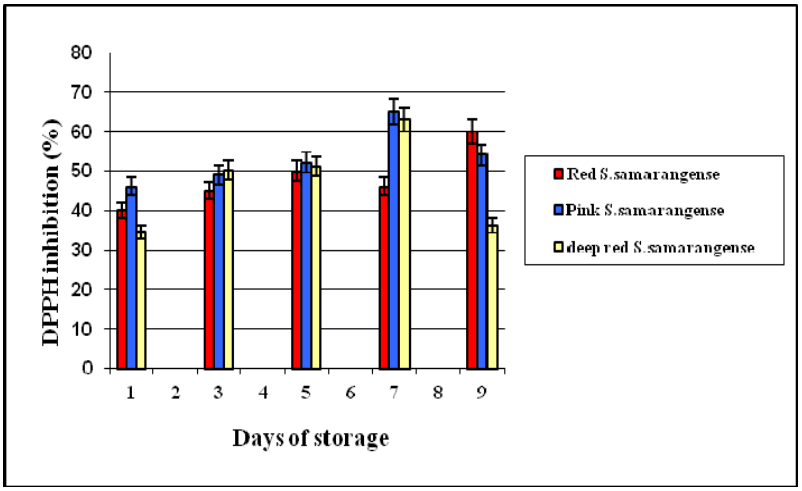

Figure 3.5 Inhibition (%) of DPPH radical scavenging activity by methanolic extract of sample fruits (■ Red *S. samarangense*, ■Pink *S. samarangense* and ■ deep red *S. samarangense*).Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different (P≤0.05, t-test).

The IC50 value of the DPPH-radical scavenging activity in relation to different concentrations of the red and pink *S. samarangense* and deep red *S. samarangense jambus* fruit extracts are shown in Figures 3.6, 3.7 and 3.8 Results showed that scavenging activity increased as the concentration of extract increased until a plateau was reached between 0.75 to 1.5 mg/ml for all the different storage times.

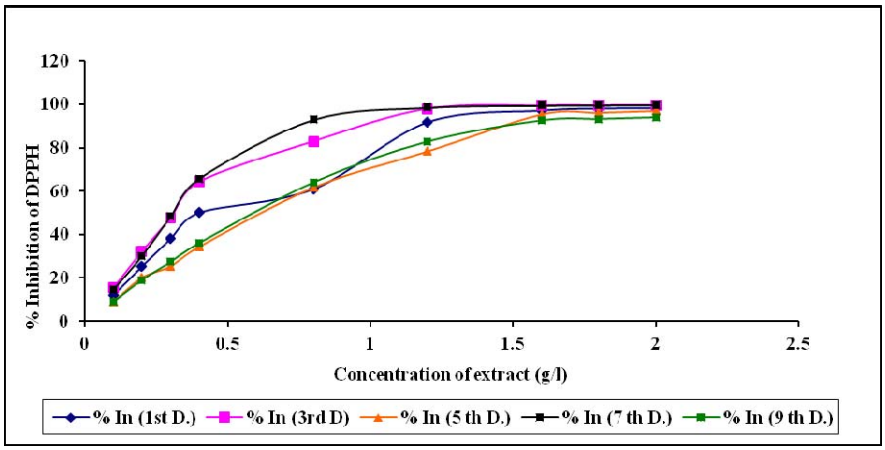

Figure 3.6 Effect of different concentrations of red *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

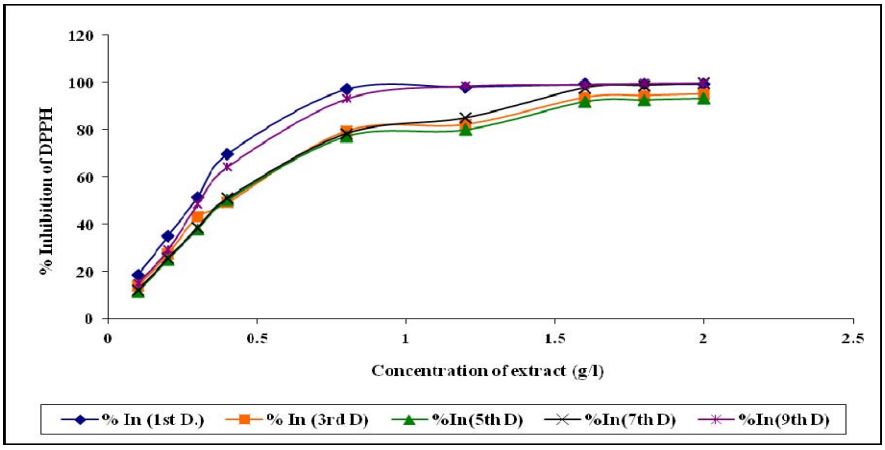

Figure 3.7 Effect of different concentrations of pink *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

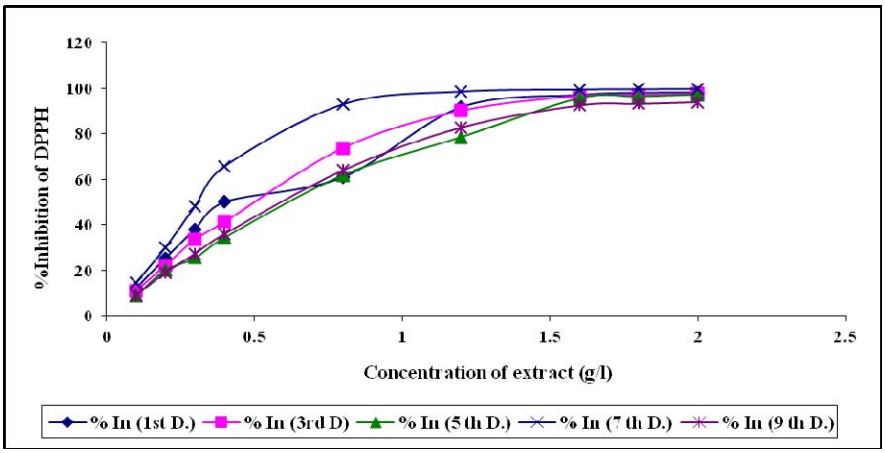

Figure 3.8 Effect of different concentrations of deep red *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

3.3.5 Correlation of Phenolic Compound and Antioxidant

Phenolics can scavenge ROS through direct and enzymatic reactions. Phenolic compounds such as flavonoids and anthocyanins are considered among the most active antioxidant compounds in plant tissues (Llorach *et al.*, 2002; Orak, 2006). The antioxidant properties of phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, oxygen scavengers and metal chelators (Kahkonen *et al.*, 1999).

In this work, the correlation between the radical scavenging activity and phenols content of the *jambu* fruits was studied using a linear regression analysis. As demonstrated in Figures 3.9, to 3.14, the correlation coefficient between total phenolics and ABTS values $R^2 = 0.909$ in red *S. samarangense jambu* fruit, $R^2 = 0.9712$ in pink *S. samarangense jambu* fruit and $R^2 = 0.7554$ in deep red *S. samarangense*. DPPH scavenging activity ($R^2 = 0.8984$ in red *S. samarangense jambu* fruit, $R^2 = 0.9975$ in pink *S. samarangense jambu* fruit and R2 = 0.9241 in deep red *S. samarangense*), was found very strong especially in pink *jambu air* and DPPH scavenging activity.

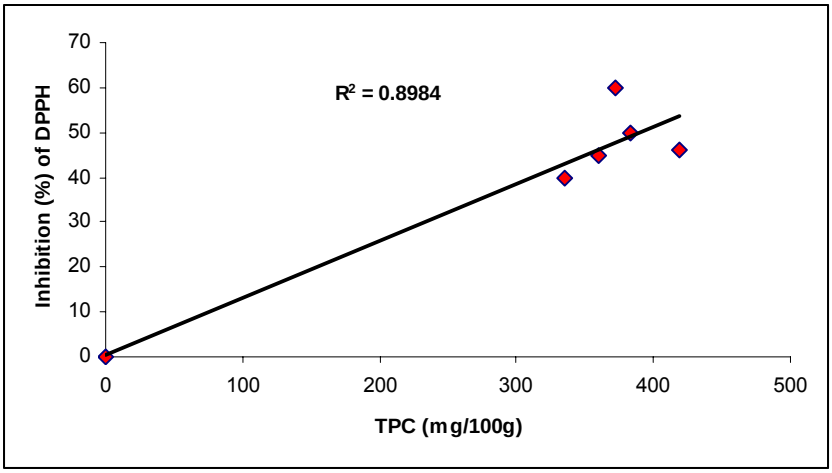

Figure 3.9 Correlation between the DPPH radical scavenging activity and total phenolic content in red *S. samarangense jambu* fruit.

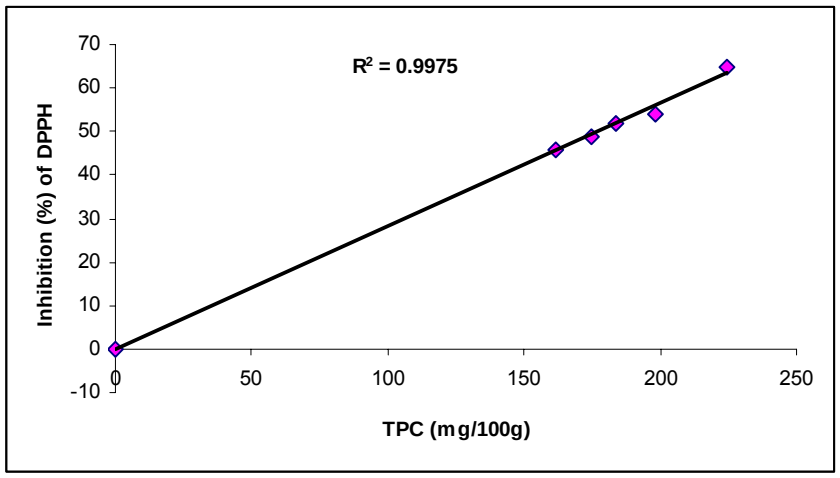

Figure 3.10 Correlation between the DPPH radical scavenging activity and total phenolic content in pink *S. samarangense jambu* fruit.

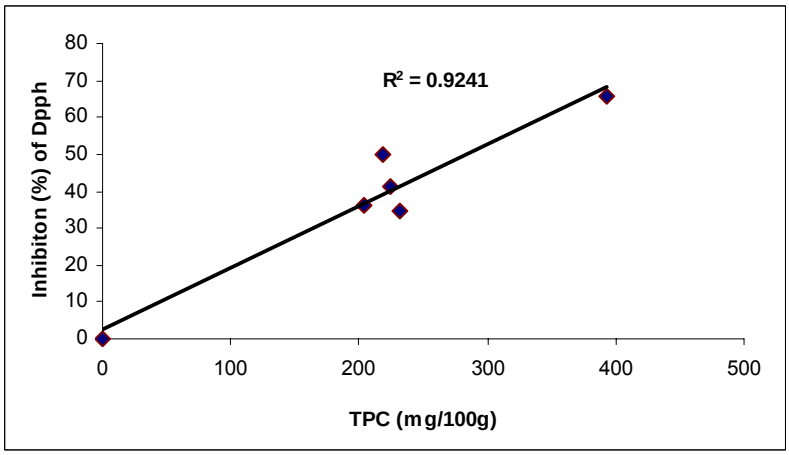

Figure 3.11 Correlation between the DPPH radical scavenging activity and total phenolic content in deep red *S. samarangense jambu* fruit.

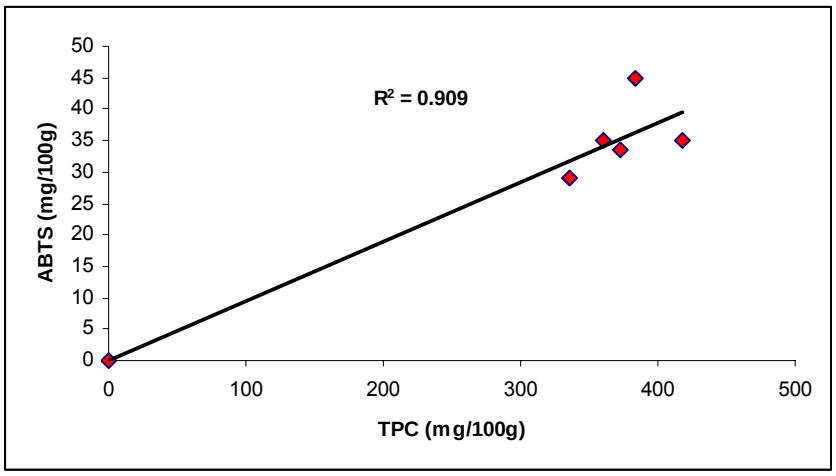

Figure 3.12 Correlation between the ABTS method and total phenolic content in red *S. samarangense jambu* fruit.

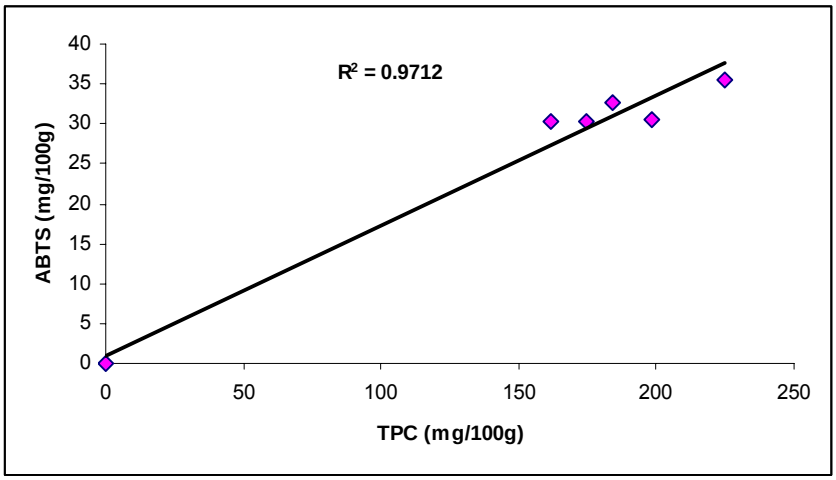

Figure 3.13 Correlation between the ABTS method and total phenolic content in pink *S. samarangense jambu* fruit.

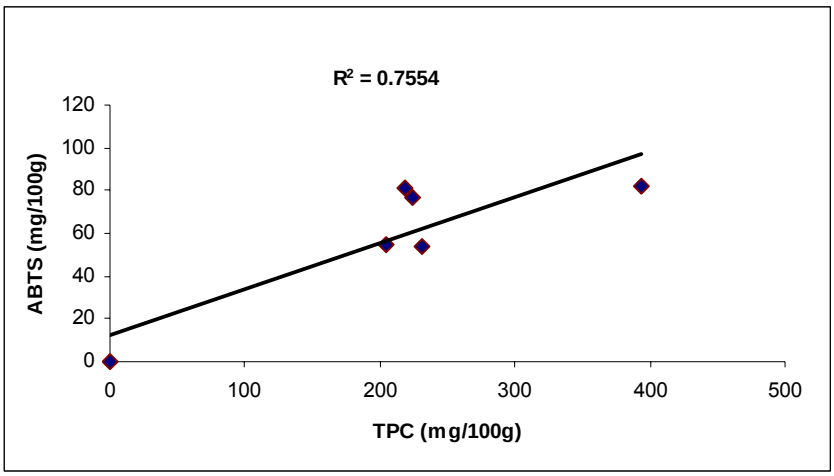

Figure 3.14 Correlation between the ABTS method and total phenolic content in deep red *S. samarangense jambu* fruit.

In the Figures 3.15 to 3.17, it is shown that increase of TPC has caused increase of DPPH radical scavenging.

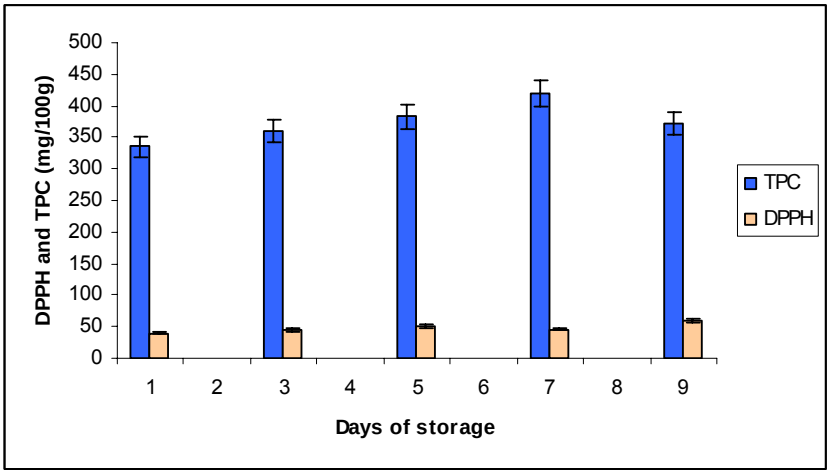

Figure 3.15 Correlation between IC50 DPPH values and total phenolic content in red *S. samarangense jambu* fruit.

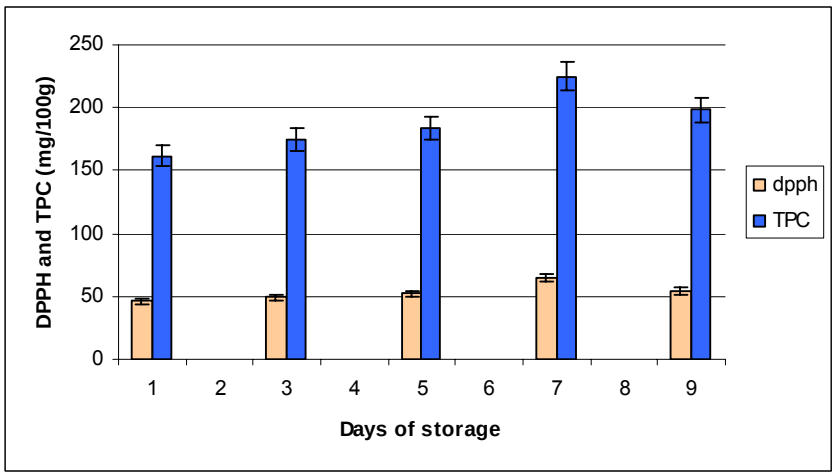

Figure 3.16 Correlation between IC50 DPPH values and total phenolic content in pink *S. samarangense jambu* fruit.

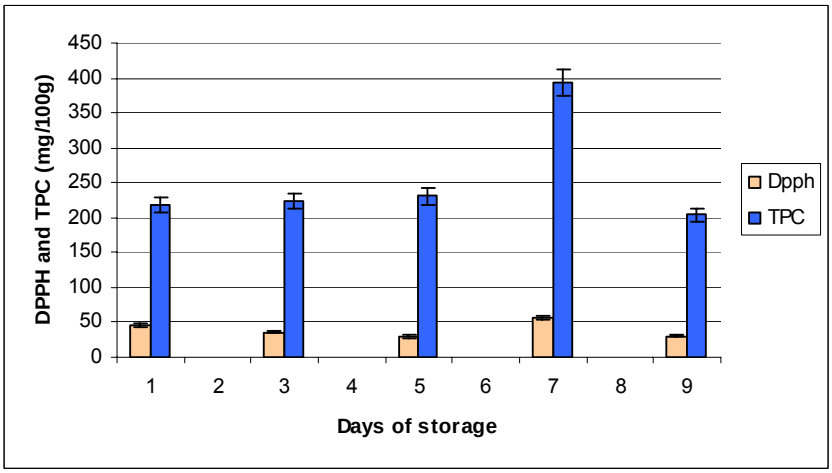

Figure 3.17 Correlation between IC50 DPPH values and total phenolic content in deep red *S. samarangense jambu* fruit.

3.3.6 Total Protein (TP)

The results showed that the total protein content of the three types of *jambu air* fruits studied increased significantly till day 5 but subsequently after that decreased till the end of period of storage (day 9) . The average TP content was 6.94 ± 2.3 mg / 100g in the red *S. samarangense*, 6.88 ± 1.69 mg / 100g in the pink *S. samarangense* and

7.99 ± 2.01 mg / 100g in the deep red *S. samarangense* fresh fruit. The highest total protein content recorded was 10.67 mg/100 g, 9.31 mg/100 g and 10.68 mg / 100g in 5 day-old red and pink *S. samarangense* and deep red *S. samarangense jambu* fruits respectively (Fig. 3.18).

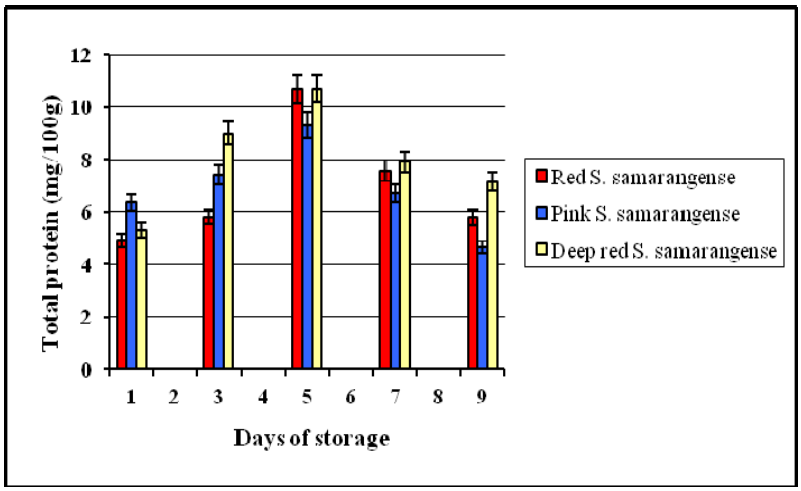

Figure 3.18 Total protein content (TP) in *jambu* fruits during storage (■ red *S. samarangense*, ■ pink *S. samarangense* and ■ deep red *S. samarangense*).Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different (P≤0.05, t-test).

3.3.7 PAL Enzyme Activity

The results showed in Fig. 3.19 exhibits the PAL enzyme activity in the red *S. samarangense* and deep red *S. samarangense jambu* fruits. It increased significantly up to day 5 (70 days after bloom) and then decreased till the end of period of storage (day 9). In the pink *jambu air* fruits, PAL activity increased slightly till day 5, but decreased subsequently until day 9. As can be seen, the PAL activity are almost similar in all the *jambu* varieties whereby it showed an initial increasing trend followed by a decrease after day 5 till day 9. The average activity was 0.138 ± 0.07 units per mg protein per hour in the red *S. samarangense*, 0.178 ± 0.04 units in the pink *S. samarangense* and 0.134 ± 0.04 units per mg protein per hour in the deep red *S. samarangense* fresh fruit.

The highest level of PAL activity (0.248 ± 0.02 units per mg protein per hour) was observed in the red *S. samarangense jambu* fruits.

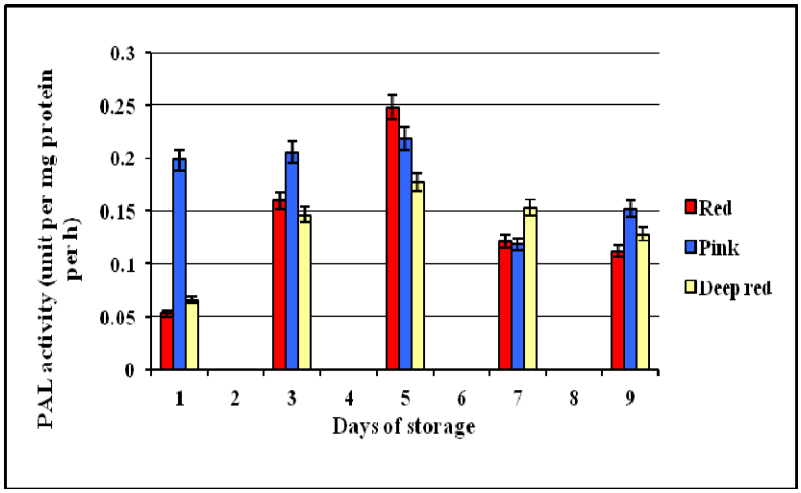

Figure 3.19 PAL activity in *jambu* fruits during storage (■ red *S. samarangense*, ■pink *S. samarangense* and ■ deep red *S. samarangense*). Values are means of five replications ±S.D (n = 5). Mean values at the same day, are significantly different ($P \leq 0.05$, t-test).

3.4 Discussion

3.4.1 Total Phenolic Content (TPC) and Total Flavonoids Content (TFC)

Phenolic compounds are a large group of secondary metabolites widespread in the plant kingdom. They are particularly important in fruits and vegetables, to which they contribute color and flavor. Phenolic compounds are normally synthesized in the cell cytoplasm and stored in the cell vacuole and possess various important biological activities, including antioxidant activity, as mentioned earlier in the Literature Review chapter. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on their stability in different systems, as well as the number and location of hydroxyl groups. In many *in*

vitro studies, phenolic compounds have demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Podsedek, 2007).

Included in the same group of compounds are the flavonoids which occur widely in the plant kingdom and are a major source of the colors of flowers, leaves, stalks, etc. From ancient times, flavonoids have been utilized as natural colors and as active constituents of galenicals/ crude drugs and herbal medicines. Many flavonoids have a hydroxyl group in their structure and are called "polyphenols". Recently, polyphenols are in the spotlight for their various anti-oxidative properties and their ability to reduce blood cholesterol levels. Polyphenol consumption as flavonoids has been shown to decrease the risk of heart disease and cancer in an epidemiological study (Arts and Hollman, 2005).

The color changes during ripening of fruits result largely from the loss of chlorophyll, the synthesis of carotenoids and the synthesis of pigmental phenolic compounds such as anthocyanins. In any one fruit, typical color change during ripening may result from only one or from any combination of these processes (Pedisic *et al.*, 2007). The phenolic content is responsible for the formation of various colored pigments, anthocyanins, which are a very diverse range of pigments localized within the vacuole of plant cell The water soluble anthocyanins which are responsible for the various shades of red and blue of many fruits are one of the major flavonoid classes (Pedisic *et al.*, 2007; Ezhilarasi and Tamilmani, 2009).

The Folin–Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic content but it is known that different phenolic compounds give different responses to the Folin–Ciocalteu method (Kahkonen *et al.*, 1999). Furthermore, the Folin-Ciocalteu colorimetric method is not very specific method for total phenolics. Many others compounds like sugar, chinonnes also react with Folin-Ciocalteu reagent and they can give apparently high results. Phenolics are very complex compounds

whose biosynthesis and degradation occur during whole plant life and large number of groups exists (Peacock *et al.*, 1986).

Changes in TPC during fruit ripening have been associated with pigment development of anthocyanins in fruit tissue such as in blackberry where it has been reported to decrease while in raspberries it increases (Wang and Lin, 2000). Wang (Wang and Lin, 2000) reported that raspberry fruits have the highest TPC in green and ripe berries with the lowest concentrations in the pink fruit. TPC changes, have also been associated with PAL activity in apple (Lister *et al.*, 1996) and loquat fruit (Ding *et al.*, 2001), which behave similarly to raspberry. They have their highest concentrations of phenolic compounds and PAL activity in unripe and almost ripe stages, with the lowest levels in between. They have not given any reason for the decrease in phenolic compounds during growth between 4 and 2 weeks prior to harvest of the loquat fruit but they suggested that the increase of chlorogenic acid contributed to the increase of total phenolics during fruit ripening. Others have reported that phenolic biosynthesis is dependent on the environmental temperature during fruit development (Tang and Tigerstedt, 2001). The phenolic content has been reported to increase at higher temperatures of growth and decrease at lower temperatures of the growing season (Wang and Zheng, 2001).

The level and composition of phenolic compounds vary greatly with cultivar, season, location, stage of maturity and postharvest storage conditions (Ding *et al.*, 2001). It is important to know the concentration of individual phenolic compounds in fruit and their changes during maturation and in different cultivars. Ding (Ding *et al.*, 2001) reported that the increase of chlorogenic acid contributed to the increase of total phenolics during loquat fruit ripening.

The result for TPC reported in this study showed that TPC, in all the three types of *jambu* fruits, increased and then decreased and a similar trend was observed for TFC.

The increase of the total phenolic compounds have been reported to be a response to oxidative stress (Ayala-Zavala *et al.*, 2004) and probably also due to an increase in fruit color (Zhang *et al.*, 2008). Zhang (Zhang *et al.*, 2008) reported that with the increase in fruit color across the cultivars, the contents of total phenolics, flavonoids, and anthocyanins all increased. Fruit phenolic compounds include mainly flavonoids (e.g. flavonols, flavones, isoflavone, anthocyanins, flavanones, chalcones), phenolic acids, quinones, and tannins. Increase in fruit color across the cultivars is usually associated with an increase in these compounds (Zhang *et al.*, 2008). Chang (Chang *et al.*, 2003) also reported that the concentrations of TSS, soluble protein and total phenolic compounds in the fresh wax apple fruit discs decreased rapidly from anthesis until the middle stage. This could be due to either consumption or dilution during rapid growth. However, most of these components accumulated again during fruit maturation. However, this study showed no correlation between skin color (Fig. 2.11), which did not increase throughout the storage period and TPC and TFC content. However, the increase of TPC observed in this study is in agreement with reports from previous studies on strawberry (Ayala-Zavala *et al.*, 2004), raspberry (Wang and Lin, 2000) and sour cherry (Pedisic *et al.*, 2007). Ayala-Zavala (Ayala-Zavala *et al.*, 2004) reported that an increase of phenolic compounds was observed for all the treatments during storage period, but their levels decreased at the end. Pedisic (Pedisic *et al.*, 2007) reported that the content of phenolic compounds increased during ripening as well as the content of anthocyanins, while antioxidant activity decreased with ripening. Wang (Wang and Lin, 2000) also reported that total phenolic content and total anthocyanin content increased with maturity for all three species of fruits that included blackberry, raspberry and strawberry.

The decrease in phenolic content observed toward the end of storage may be due to the breakdown of the cellular structure, the vacuoles in fruit cells form the main

compartment in which phenolic compounds accumulate (Toor and Savage, 2006). The loss of phenolic compounds in fruit stored was correlated with an increased susceptibility to fungal attack and also could be related to a decline in tissue resistance to infection (Lattanzio *et al.*, 2001; Nguyen *et al.*, 2003).

The total phenolic concentration (TPC) of fruits studied so far are; 330 mg gallic acid equivalents GAE/100 g in strawberry (*Fragrariax ananassa*), 126 mg GAE/100 g in orange, 60 mg GAE/100 g in pear (*Pyrus communis*), 48 mg GAE/100 g in apple, 38 mg GAE/100 g in peach, 38 mg GAE/100 g in banana and 30 mg GAE/100 g in tomato (Proteggente *et al.*, 2002). The TPC concentration of three types of *jambu* fruits was less than strawberry, but more than orange, pear, apple, peach, banana and tomato. The TFC in the red variety of *S. samarangense* was higher than yellow apple (peeled), green apple (peeled), peach, sweet cherry and fig (Marinova *et al.*, 2005). However, TFC in plants are strongly influenced by extrinsic factors such as variations in plant type and growth, season, climate, degree of ripeness, food preparation and processing (Aherne and O'Brien, 2002).

From this study it can be seen that the red *jambu air* variety seemed rich in TPC and also TFC and this is probably due to the rich abundance of anthocyanidines in combination with the other flavonoids in these fruits (Marinova *et al.*, 2005).

3.4.2 Total Antioxidant Capacity of *Jambu* Fruits

The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals (Namiki, 1990). Since antioxidants can be classified according to their protective properties at different stages of the oxidation process and since they act by different mechanisms, they are generally arranged into two categories: primary and secondary antioxidants. Primary antioxidants can inhibit or retard oxidation by

scavenging free radicals by donation of hydrogen atoms or electrons, which converts them to more stable products. Examples of primary antioxidants include tocopherols, which constitute the principal antioxidants in vegetable oils, and butylated hydroxyanisole (BHA), a synthetic antioxidant often used in the food industry to retard lipid oxidation in foods.

Secondary antioxidants function by many other mechanisms, including binding of metal ions scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen (Gordon, 1990; Maisuthisakul *et al.*, 2007). Ascorbic acid functions as an oxygen scavenger and asserts its antioxidant action when oxidised to form dehydroascorbic acid.

The DPPH radical is a stable free radical and its radical-scavenging activity can be determined by observing the decrease in absorbance at 517 nm due to its reduction by an antioxidant (Gordon, 1990). The DPPH solution which is deep purple in color will turn yellow on reduction and its absorbance will drop. The decrease in absorbance is proportional to the number of DPPH molecules being scavenged and the change in absorbance produced in the reaction is assayed to evaluate the antioxidant potential of extracts. This then means that the more, rapidly the absorbance decrease, the more potent the antioxidant activity of the extract. The greater the bleaching action, the higher the antioxidant activity (AEAC value), and this is reflected in a lower IC50 value. Primary antioxidant properties are generally measured by the DPPH assay, expressed as AEAC and IC50.

The ABTS method measures the relative antioxidant ability of a solution to scavenge the $ABTS^{\bullet+}$ radical in the aqueous phase, as compared with a standard amount of ascorbic acid. The $ABTS^{\bullet+}$, generated by potassium persulfate, is used as a tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavengers of lipid peroxyl

radicals). Rice-Evans (Rice-Evans *et al.*, 1995; Rice-Evans *et al.*, 1996) and Roberta (Roberta *et al.*, 1999) have demonstrated that the ABTS assay can be used to measure the antioxidant activity of a broad diversity of substances. It is widely used to evaluate antioxidant activity in foods and biological systems and a high value of AEAC value would indicate a high level of antioxidant activity.

The present study has shown that the antioxidant capacity (AC) of red and pink *S. samarangense* varieties increased and then decreased during storage, when both assay methods (DPPH and ABTS) were used. The antioxidant capacity (AC) of the deep red *S. samarangense* variety was significantly different from the red and pink *S. samarangense* varieties. AC in the deep red *S. samarangense* variety increased in the first five days of storage period and later decreased. Similar increases in antioxidant capacity after post harvest storage have also been reported in raspberries and strawberries (Kalt *et al.*, 1999) and blueberry(Connor *et al.*, 2002).

The increase in AC might be attributed to an increase in the concentration of anthocyanin pigments or the accumulation of other phenolics (Zheng *et al.*, 2003; Kulkarni *et al.*, 2007). Toor (Toor and Savage, 2006), working with tomatoes, reported that during storage there was a slight increase in the levels of total phenolics and ascorbic acid and their possible synergistic interactions may have been responsible for the observed increase in the soluble antioxidant activity in the tissue. An increase in total AC in strawberry was observed, associated with the accumulation of phenolics (Ayala-Zavala *et al.*, 2004). The strawberries were stored at high oxygen atmospheres for 14 days at 5 °C.

The total antioxidant activity is normally attributed to the levels of phenolic acids, ascorbic acid as well as anthocyanin and this is why the seasonal changes in total antioxidant activity are different to those seen for the total phenolic content (Ayala-Zavala *et al.*, 2004; Kulkarni and Aradhya, 2005). Kulkarni (Kulkarni and Aradhya,

2005) reported that the phenolic compounds in pomegranate juice are used up in the biosynthesis of the flavylium ring during anthocyanin pigment formation, leading to a reduction in their content but not a reduction in the level of total antioxidant activity.

The decreased AC at the end of storage in the three types of *jambu* fruits could be due to a reduced concentration of total phenolics and ascorbic acid (Kulkarni and Aradhya, 2005). However, preharvest factors, such as genetic background and cultural practices (Hodges *et al.*, 2004), environmental conditions (temperature, light, water and nutrient availability), production techniques used (plant growth regulators, date of harvest, etc.) and postharvest storage conditions (Leonardi *et al.*, 2000; Dumas *et al.*, 2003) have been reported to influence antioxidant capacity in crops. Tian (Tian and Yang, 2004) and Lata (Lata *et al.*, 2005) have reported that antioxidant activity of fruits and vegetables may vary by variety, time of harvest, and post-storage handling factors. The activity of the fruit extract to scavenge free radicals is classified into four categories (Leong and Shui, 2002). The fruit with AEAC using $ABTS^{\bullet+}$ of over 600 mg AAeq/100 g is classified as containing very high antioxidant capacity. On the other hand, fruits with AEAC from 200 to 600 mg AAeq/100 g, 70 to 200 AAeq/100 g and less than 70 mg AAeq/100 g are classified as containing high, medium and low antioxidant capacities respectively. It was reported (Leong and Shui, 2002) that the highest antioxidant capacity, followed by *ciku* fruit, strawberry, plum, star fruit, guava, seedless grape, *salak* fruit, mangosteen, avocado, orange, solo papaya, mango, kiwi fruit, *cempedak* fruit, pomelo, lemon, pineapple, apple, foot long papaya, rambutan, rambutan king, banana, coconut pulp, tomato, rockmelon, honeydew, watermelon and coconut water. The total antioxidant capacity of the *jambu* fruit was found to be low (compared with the other fruits examined by Leong (Leong and Shui, 2002)). The results in this study showed that the AEAC value for the *jambu* fruit was more than that of rockmelon (26.2 ± 3.5 mg AAeq/100 g) but less than tomato (38.0 ± 1.7 mg AAeq/100 g).

The results showed that the two methods are compatible when used to assess free radical-scavenging activity, but values from the DPPH assay were lower than those for the ABTS assay. However, Wang (Wang *et al.*, 1998) reported that some compounds which have ABTS scavenging activity may not show DPPH scavenging activity. The red *jambu* fruits tested with a high AEAC in the ABTS assay also showed a high AEAC in the DPPH assay. This high correlation may partly result from a similar mechanism and, also that both the antioxidants are soluble in aqueous and ethanol systems (Leong and Shui, 2002). However, Tabart (Tabart *et al.*, 2009) working on a different fruit and vegetable juices reported that the ABTS and DPPH methods provided widely different results.

3.4.3 Correlation between TPC and Antioxidant Activity

As demonstrated in Figures 3.9, to 3.14, the high correlation values between total phenols and the antioxidative activity suggest that the major antioxidant compounds in this study are probably phenolics. Previous studies have shown that most of the antioxidant capacities in fruits are associated with their phenolics, particularly the flavonoids. In bayberry, cranberry, apple and grape, a linear correlation was observed between phenolic content and total antioxidant activity (Proteggente *et al.*, 2002; Orak, 2006; Zhang *et al.*, 2008). Gil (Gil *et al.*, 2002) also found high correlation ($r > 0.9$) between antioxidant activity, as determined by DPPH assays and the TPC in nectarines, peaches and plums.

3.4.4 Total Protein Content (TP)

Protein is one of the important components contributing to the nutritional value of food. Chang (Chang *et al.*, 2003) reported that soluble protein concentration in wax apple (*Syzygium samarangense*) decreased rapidly from 20 mg g^{-1} (2000 mg /100g) at

petal fall to about 2.5 mg g^{-1} (250 mg/100g) at week 5 and increased to about 5 mg g^{-1} (500 mg/100g) before the harvesting began. In this study, we observed an increasing trend during storage for all the 3 varieties of *S. samarangense* fruits up to 5 days during storage but subsequently the total protein content decreased. The initial increase could be probably due to an increased synthesis of enzymes involved in ripening and senescence of the fruit (Aydin and Kadioglu, 2001). Generally, the decrease in total protein content after 5 days in the 3 types of *jambu* fruits, during storage, could be attributed to a breakdown of proteins which is normally observed during the senescence of fruits (Kulkarni and Aradhya, 2005). However, it has also been reported that decreased protein content could be attributed to an increased protein degradation or decreased protein synthesis or both (Ezhilarasi and Tamilmani, 2009). A similar increase in total protein content up to the full-ripe stage and a sudden decrease has been reported in white and pink guava types (Abu-Goukh and Bashir, 2003), pomegranate arils (Kulkarni and Aradhya, 2005) and in medlar (Aydin and Kadioglu, 2001).

3.4.5 PAL Enzyme Activity

PAL is the key enzyme linking the all important pathways, the shikimate pathway to phenylpropanoid metabolism in higher plants and plays an important role in the biosynthesis of phenylpropanoids, flavonoids, and phenolic compounds (Dixon and Paiva, 1995). It also plays an important role in plant defense mechanisms as well (El-Shora, 2002). PAL has been shown to be induced during development and ripening in some climacteric (Diallinas and Kanellis, 1994; Assis *et al.*, 2001) and non-climacteric fruits (Given *et al.*, 1988). Shu (Shu *et al.*, 1998) reported that total phenolic compounds, anthocyanin and PAL activity increased in the wax apple (*Syzygium samarangense*) during development of the skin color. It has also been reported that in wax apples that, anthocyanin accumulation and maximal PAL activity increased from

the immature to the initial ripe stage and then decreased at the full ripe stage. Other studies have also shown that the PAL enzyme plays an important role in the development of skin color in *jambu* air fruits where it has been reported that the change in PAL activity was associated with a change in anthocyanin accumulation (Wang *et al.*, 2000; Wang and Lin, 2000). Working with loquat fruits, Ding (Ding *et al.*, 2001) reported that the changes of PAL enzyme activity seemed to be associated with variations in chlorogenic acid concentration during development, maturation, and ripening. They observed the biosynthesis of chlorogenic acid (as a phenolic compound) during loquat ripening and the increase in enzyme activity leads to an accumulation of this phenolic compound. The decrease in PAL activity after 5 days in the three types of *jambu* fruits might be attributed to breakdown of proteins which is normally observed during senescence of fruits (Kulkarni and Aradhya, 2005).

The results in this study show a similar trend to that for total phenolic content and total flavonoids content especially in pink *S. samarangense* and deep red *S. samarangense jambu* fruits (Figures 3.6 and 3.7).

CHAPTER 4

Conclusion

It can be concluded that all of the three *Syzygium samarangense* varieties studied reached minimum maturity index, in terms of fruit color, titratable acidity and other fruit maturity indices. It is a non- climacteric fruit and harvesting at the ripe stage do not lead to any significant change after harvest. The fruits mature in 64 days from the time of full opening of the flowers till the fully ripe stage and harvest date.

From this study it can be concluded that weight loss, total soluble solids (TSS) and pH of the three types of *jambu* fruits increased with time whilst pulp firmness, titratable acidity (TA) and the color index of the fruits decreased. Total sugar increased slightly during post harvest storage.

Analysis of the antioxidant activity using the DPPH and ABTS methods showed that the antioxidant activity generally increased gradually during the post harvest period and correlated well with the increases in total phenolic and flavonoid contents. The amount of total phenolic and flavonoid contents also showed high values comparable with that reported in many studies on other common and popular fruits. It was also observed that the PAL enzyme activity, an enzyme important in phenolic and flavonoid biosynthesis, correlated well with the accumulation of total flavonoid and total phenolic content in the fruits during storage.

These results present new postharvest data on the *Syzygium samarangense* fruits, an increasingly popular fruit in the Asian region. This study has also demonstrated that *jambu* fruits are a rich source of antioxidant compounds. Jambu fruits are regularly grown and consumed in tropical part of the world, and this report suggests that these fruits have potential for development as a functional food.