

APPENDIX A

Table 2.1(a) Changes in the dimension (length and width) of the *Jambu* fruits during growth.

Week	1 th	2 th	3 th	4 th	5 th	6 th	7 th	8 th	9 th
Length	1.6	1.8	2	2.1	2.4	3.5	4.5	5.2	5.8
Width	0.8	0.9	0.95	1	1	1.4	2.9	3	3.6

Table 2.2 (a) Color changes and % weight loss for Pink *S. samarangense*, Red *S. samarangense* and Deep red *S. samarangense* fruits. Mean values are significantly different ($P \leq 0.05$).

Days of storage		D1	D2	D3	D4	D5	D6	D7	D8	D9
Pink <i>S. samarangense</i>	Pulp colour (L*a*/b*)	53.04	51.83	50.25	49.53	48.58	44.50	46.18	44.23	43.87
	% Weight loss	0.00	0.45	1.00	1.88	2.80	3.52	4.15	5.1	5.93
Red <i>S. samarangense</i>	Pulp colour (L*a*/b*)	73.88	73.98	70.85	70.28	71.73	69.23	68.06	67.86	66.04
	% Weight loss	0.00	0.19	0.44	1.44	3.72	4.85	6.98	8.5	9.15
Deep red <i>S. samarangense</i>	Pulp colour (L*a*/b*)	46.94	45.28	46.05	51.67	46.07	42.93	43.23	44.98	41.18
	% Weight loss	0.00	0.62	1.09	1.55	1.91	2.37	3.55	3.84	4.38

Table 2.3 (a) TSS, pulp firmness, pH, total sugar and starch content changes in pink *Sizygium samarangense jambu* fruit. Data are means of five replicates \pm SD. Mean values are significantly different ($P \leq 0.05$).

Days of storage	D1	D3	D5	D7	D9
Firmness (kg ^o f)	0.72	0.64	0.55	0.47	0.37
TSS(°Brix)	7.36	7.2	7.8	8.3	9.5
pH	4.31	4.18	3.94	4	3.9
Titration acidity (%)	0.179	0.144	0.145	0.110	0.105
Total sugar (g/100g)	3.56	3.72	4.38	4.73	5.15
Starch content(g/100g)	1.55	1.29	1.07	0.96	0.83

Table 2.4 (a) TSS, pulp firmness, pH, total sugar and starch content changes in Red *Sizygium samarangense jambu* fruit. Data are means of five replicates \pm SD. Mean values are significantly different ($P \leq 0.05$).

Days of storage	D1	D3	D5	D7	D9
Firmness (kg ^o f)	0.63	0.51	0.45	0.42	0.41
TSS(°Brix)	8.1	8.4	8.2	8.43	9.15
pH	4.9	4.84	4.77	4.76	4.62
Titration acidity (%)	0.179	0.153	0.127	0.102	0.089
Total sugar (g/100g)	3.64	3.98	4.01	4.02	4.11
Starch content(g/100g)	2.42	1.9	1.29	0.85	0.65

Table 2.5 (a) TSS, pulp firmness, pH, total sugar and starch content changes in Deep red *Sizygium samarangense jambu* fruit. Data are means of five replicates \pm SD. Mean values are significantly different ($P \leq 0.05$).

Days of storage	D1	D3	D5	D7	D9
Firmness (kg [°] f)	0.8	0.73	0.67	0.62	0.56
TSS(°Brix)	5.63	5.89	7.19	8.38	9.84
pH	4.80	4.75	4.70	4.60	4.50
Titration acidity (%)	0.19	0.175	0.140	0.125	0.117
Total sugar (g/100g)	4.52	4.86	5.05	5.19	5.37
Starch content(g/100g)	2.44	2.24	1.82	1.73	1.51

Table 3.1 (a) Changes in AEAC (by ABTS and DPPH methods), total phenolic and flavonoid contents during ripening in the red *S. samarangense* fruits. Values are means of five replications \pm S.D (n = 5). Mean values are significantly different ($P \leq 0.05$).

Days of storage	AEAC by ABTS method	AEAC by DPPH method	Total phenolic content	Total flavonoids content
D1	29.02	20.47	335.48	23.14
D3	34.90	24.08	360.41	29.66
D5	44.94	26.60	382.98	34.47
D7	35.03	24.71	418.35	38.08
D9	34.42	33.06	372.37	37.47

Table 3.2 (a) Changes in AEAC (by ABTS and DPPH method), total phenolic and flavonoid contents during ripening in the pink *S. samarangense* fruits. Values are means of five replications \pm S.D (n = 5). Mean values are significantly different ($P \leq 0.05$).

Days of storage	AEAC by ABTS method	AEAC by DPPH method	Total phenolic content	Total flavonoids content
D1	30.24	12.61	161.67	16.55
D3	30.20	14.02	174.39	16.94
D5	32.74	15.39	183.91	17.51
D7	35.56	21.66	224.73	19.71
D9	30.56	16.54	198.28	15.71

Table 3.3 (a) Changes in AEAC (by ABTS and DPPH method), total phenolic and flavonoid contents during ripening in the deep red *S. samarangense* fruits. Values are means of five replications \pm S.D (n = 5). Mean values are significantly different ($P \leq 0.05$).

Days of storage	AEAC by ABTS method	AEAC by DPPH method	Total phenolic content	Total flavonoids content
D1	80.84	29.6	218	10.65
D3	85.18	43.61	224	11.02
D5	99.31	53.42	231	11.65
D7	79.62	43.71	393	25.69
D9	70.06	30.93	204	17.18

Table 3.4 (a) Inhibition (%) of DPPH radical scavenging activity by methanolic extract of sample fruits. Values are means of five replications \pm S.D (n = 5). Mean values are significantly different ($P \leq 0.05$).

Day	Red <i>S. samarangense</i>	Pink <i>S. samarangense</i>	Deep red <i>S. samarangense</i>
1	40	46	50
3	45	49	41
5	50	52	34
7	46	65	65
9	60	54	36

Table 3.5 (a) Effect of different concentrations of red *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

Conc.(g/L)	% In (1 st D)	%In (3 rd D)	% In (5 th D)	% In (7 th D)	% In (9 th D)
0.1	12.01	15.58	9.15	14.55	8.97
0.2	25.3	32.39	20.23	30.15	19.01
0.3	38.04	47.81	25.46	48.32	27.33
0.4	50.02	64.38	34.47	65.77	36.01
0.8	60.87	83.04	61.8	92.98	63.95
1.2	91.75	98.05	78.47	98.53	82.77
1.6	97.08	99.51	95.48	99.45	92.49
1.8	98.01	99.61	96.2	99.6	93.15
2	98.18	99.75	97.05	99.73	93.86

Table3.6 (a) Effect of different concentrations of pink *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

Conc.(g/L)	%In (1 st D)	%In (3 rd D)	%In (5 th D)	%In (7 th D)	%In (9 th D)
0.1	18.51	13.98	11.39	12.23	15.05
0.2	34.93	27.53	25.09	25.72	29.15
0.3	51.47	43.21	37.93	38.58	48.61
0.4	69.67	49.06	50.16	51.05	64.32
0.8	97.01	79.34	77.08	78.31	93.07
1.2	97.98	82.07	79.86	84.92	98.49
1.6	99.09	93.5	91.73	97.56	99.05
1.8	99.14	94.38	92.56	98.67	99.63
2	99.23	95.01	93.13	99.53	99.76

Table3.7 Effect of different concentrations of deep red *S. samarangense jambu* extract on 1,1-diphenyl –2-picrylhydrazyl (DPPH) free radicals.

Conc.(g/L)	% In (1 st D)	% In (3 rd D)	% In (5 th D)	% In (7 th D)	% In (9 th D)
0.1	12.01	10.91	9.15	14.55	8.97
0.2	25.3	22.15	20.23	30.15	19.01
0.3	38.04	33.58	25.46	48.32	27.33
0.4	50.02	41.3	34.47	65.77	36.01
0.8	60.87	73.69	61.8	92.98	63.95
1.2	91.75	90.06	78.47	98.53	82.77
1.6	97.08	96.51	95.48	99.45	92.49
1.8	98.01	96.99	96.2	99.6	93.15
2	98.18	97.49	97.05	99.73	93.86

Table 3.8 (a) Changes in total protein (TP) and PAL enzyme activity during ripening in the three varieties of *jambu* fruits. Values are means of five replications \pm S.D (n = 5). Mean values are significantly different ($P \leq 0.05$).

Days of storage		D1	D2	D3	D4	D5
Pink <i>S. samarangense</i>	Total protein (TP)	6.36	7.41	9.31	6.7	4.63
	PAL enzyme activity	0.198	0.205	0.218	0.118	0.152
Red <i>S. samarangense</i>	Total protein (TP)	4.90	5.79	10.67	7.56	5.78
	PAL enzyme activity	0.053	0.160	0.248	0.121	0.112
Deep red <i>S. samarangense</i>	Total protein (TP)	5.28	8.98	10.68	7.89	7.15
	PAL enzyme activity	0.066	0.146	0.177	0.153	0.128

APPENDIX B
STANDARD CURVES

B.1 Glucose standard curve

The standard solutions of glucose were prepared in the rang of 5-100 μ g / ml. 2ml of glucose solution was added to 1ml of phenol. Then, 5ml of concentrated H₂SO₄ was added to the mixture. Mixture was left at room temperature for 10 minutes. Then, the mixture was shaken (mixed thoroughly). Absorbance was read at 485 nm.

Table B.1 Concentrations of glucose standard solution and absorbance at 485 nm

Glucose (100 μ g /ml) stock solution (ml)	0.00	5	10	15	20	25	50	75	100
DdH ₂ O(ml)	100	95	90	85	80	75	50	25	0
Concentration (μ g / ml)	0.00	5	10	15	20	25	50	75	100
Absorbance	0.00	0.113	0.150	0.201	0.295	0.364	0.524	0.721	0.951

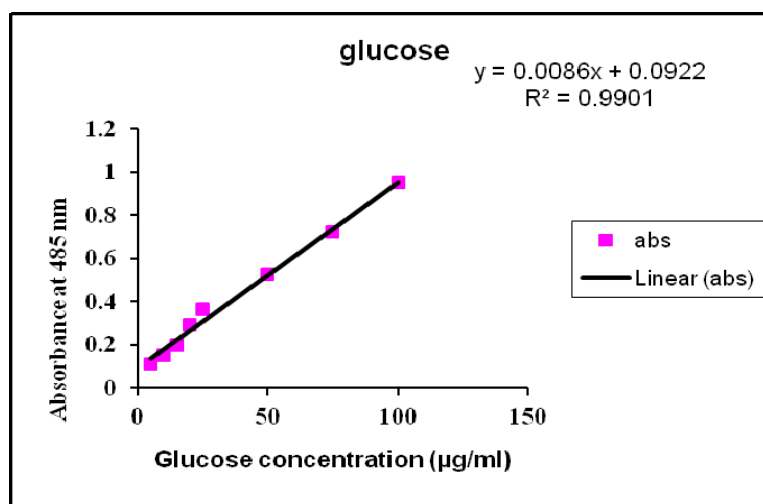


Figure B.1 Glucose standard curve

B.2 Gallic acid standard curve

A range of gallic acid concentrations from 0.5 – 0.005 mg / ml was used to prepare the calibration curves (Table B.2 and Fig B.2).

Table B.2 Different concentrations of gallic acid standard solution and their absorbance at 765 nm

Gallic acid 5mg/ml stock solution (ml)	0	0.1	0.5	1	2	5	10
DdH ₂ O(ml)	100	99.9	99.5	99	98	95	90
Concentration (mg / ml)	0	0.005	0.025	0.05	0.1	0.25	0.5
Absorbance	0.00	0.033	0.076	0.154	0.282	0.657	1.246

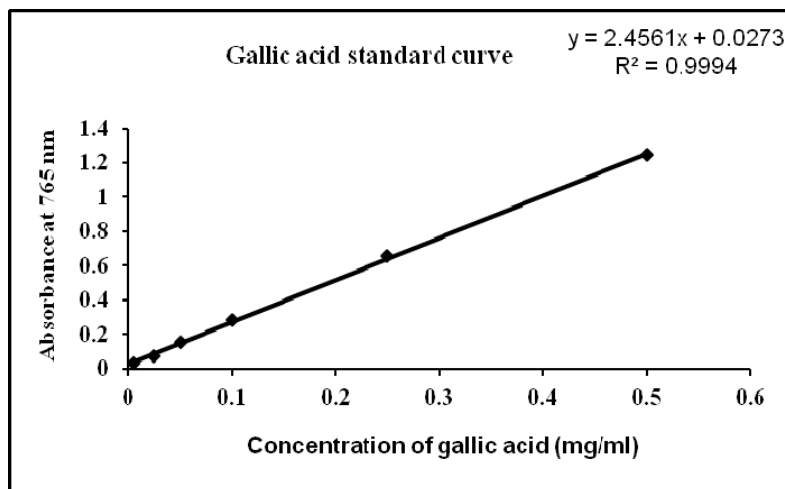


Figure B.2 Gallic acid standard curve

B.3 Catechin standard curve

Determination of flavonoids was performed according to the colorimetric assay of Kim et al. (2003). The standard solutions of catechin were prepared in the range of 10-100 μg / ml (Table B.3 and Fig B.3).

Table B.3 Concentrations of catechin standard solution and absorbance at 510 nm

Catechin (100 μg /ml) stock solution (ml)	0	1	2	3	4	5	6	7	8	9	10
DdH ₂ O(ml)	10	9	8	7	6	5	4	3	2	1	0
Concentration (μg / ml)	0	10	20	30	40	50	60	70	80	90	100
Absorbance	0.00	0.050	0.090	0.261	0.378	0.471	0.575	0.661	0.796	0.805	0.859

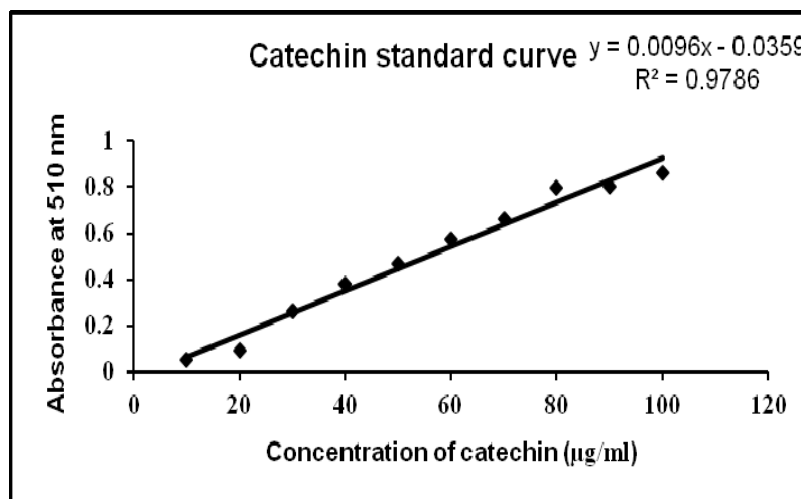


Figure B.3 Catechin standard curve

B.4 DPPH standard curve

A methanolic stock solution of the ascorbic acid (AA) (concentrations of stock solutions were 2, 4, 6, 8, 10 $\mu\text{g} / \text{ml}$) was placed in a cuvette, and 2 ml of 4×10^{-4} M methanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined by spectrophotometer after 30 min for all samples. Methanol was used to zero the spectrophotometer (Table B.4, and Fig B.4).

Table B.4 % inhibition of ascorbic acid ($A_{C(0)} = 0.719$)

Concentration of AA ($\mu\text{g} / \text{ml}$)	0	2	4	6	8	10
Absorbance After 30 min	0.719	0.403	0.341	0.221	0.113	0.063
% inhibition	0.00	43.95	52.57	69.26	84.28	91.24

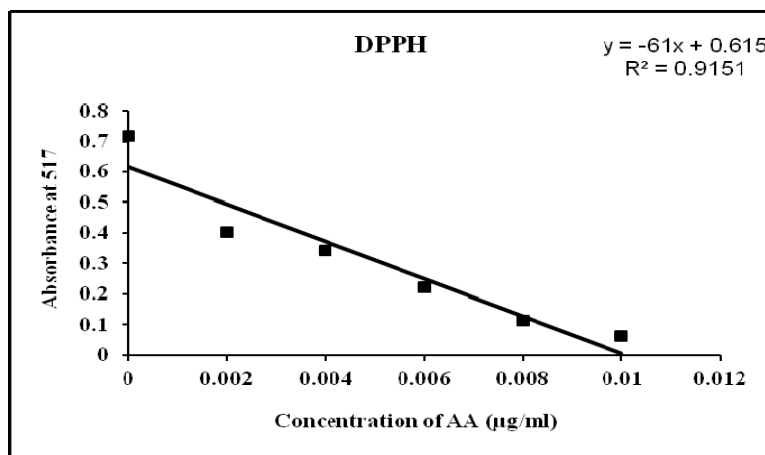


Figure B.4 DPPH standard curve

B.5 ABTS standard curve

A methanolic stock solution of the ascorbic acid (AA) (concentrations of stock solutions were 1, 2, 3, 4, 5, 6 $\mu\text{g} / \text{ml}$) was placed in a cuvette, and 3ml of methanolic solution of ABTS was added. Absorbance measurements commenced immediately. The decrease in absorbance at 734 nm was determined by spectrophotometer after 60 min for all samples. Methanol was used to zero the spectrophotometer (Table B.5, and Fig B.5).

Table B.5 Antioxidant activity of ascorbic acid by ABTS

Concentration of AA ($\mu\text{g} / \text{ml}$)	0	1	2	3	4	5	6
Absorbance After 1 h	0.830	0.471	0.395	0.304	0.297	0.256	0.183

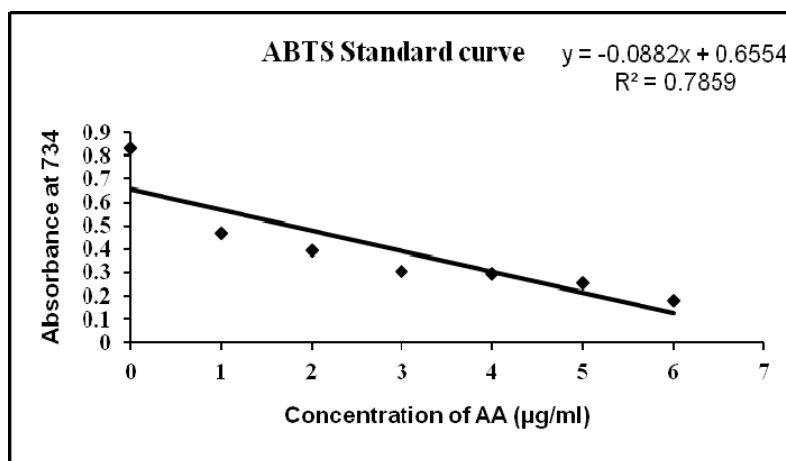


Figure B.5 ABTS standard curve

B.6 Protein standard curve

In any protein assay, the ideal protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, another protein must be selected as a relative standard. The best relative standard to use is one that gives a color yield similar to that of the protein being assayed. Selecting such a protein standard is generally done empirically. Alternatively, if only relative protein values are desired, any purified protein may be selected as a standard. The two most common protein standards used for protein assays are BSA (Bovine Serum Albumin) and gamma-globulin. With the quick start Bradford protein assay; dye color development is significantly greater with BSA than with most other proteins, including gamma-globulin. Therefore, the BSA standard would be an appropriate standard if the sample contains primarily albumin, or if the protein being assayed gives similar response to the dye. For a color response that is typical of many proteins, the gamma-globulin standard is appropriate.

The standard solutions of BSA were prepared in the range of 0.125 – 2 $\mu\text{g} / \text{ml}$. Different volumes of each standard solution into a clean, dry test tube were taken. Then, was added 5.0 ml of diluted dye reagent to each tube and vortex. The mixture was incubated at room temperature for at least 5 minutes. Absorbance was measured at 595 nm.

Table B.6 Concentrations of BSA standard solution and absorbance at 595 nm

protein(2µg/ml) stock solution (ml)	0	6.25	12.5	25	37.5	50
DdH ₂ O(ml)	100	93.75	87.5	75	62.5	50
Concentration (µg/ml)	0	0.125	0.25	0.5	0.75	1
Absorbance	0.00	0.136	0.220	0.485	0.574	0.701

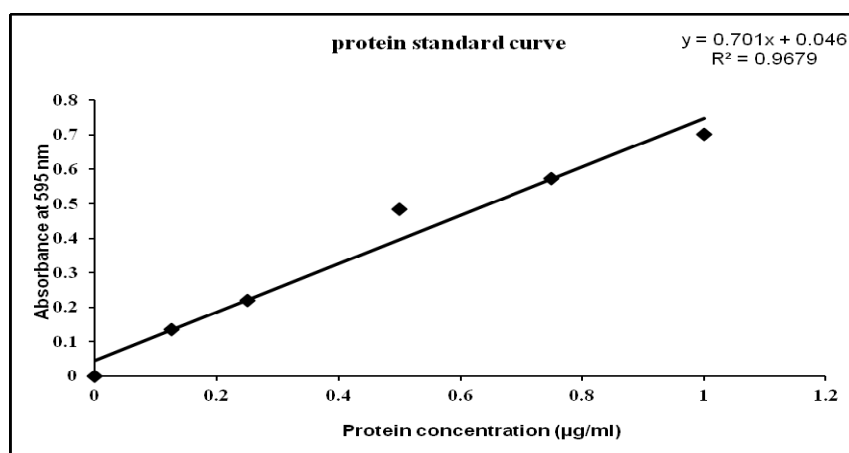


Figure B.6 Protein standard curve

APPENDIX C

C.1 Preparation of extracts

C.1.1 Protein extraction

Proteins were extracted by adding 4g of *Jambu* pulp to 8 ml of a modified kanellis *et al.* (1989) 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, (tissue to volume ratio = 4:8). The mixture was then vortexed thoroughly, left on ice for 10 minutes and then centrifuged at 25,000 g for 30 minutes at 4°C in a refrigerated centrifuge.

C.1.2 Preparation of extract for the determination of total sugar and starch

One gram of *jambu air* tissue was ground in a mortar and pestle in 4 ml of 0.5M NaOH. The homogenate was then transferred to polypropylene centrifuge tubes and centrifuged at 3500g for 20 minutes. The mixture was next filtered through a Whatman No. 1 filter paper and the supernatant neutralized with 4 ml of 0.5M acetic acid. The resulting extract was diluted to 25 ml with distilled water and stored in the refrigerator (4°C) before further analysis for total sugar and starch.

C.2 Preparation of reagents

C.2.1 Preparation of Bradford reagent

Bradford reagent was prepared by dissolving 100 mg coomassie brilliant blue G-250 in 50 ml of 95% ethanol, then, was added 100 ml of 85% (w/v) phosphoric acid. The reagent was diluted to 1 liter when the dye had completely dissolved, and filtered through Whatman #1 paper just before use.

C.2.2 Preparation of glucose reagent for the determination of starch content

Reagent I

1.65g Na_2HPO_4 , 1.09g NaH_2PO_4 , 2.4 mg peroxidase (Podsdek) and 3.6 g glucose oxidase were dissolved in 150 ml of distilled water.

Reagent II

One mg of dianicyanidin hydrochloride was dissolved in 2 ml of distilled water.

The glucose reagent was obtained by adding 50 ml of reagent I to 0.5 ml of reagent II.

Postharvest physico-chemical and mechanical changes in *jambu air* (*Syzygium aqueum* Alston) fruits

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Abstract

Little data is available in the scientific literature on postharvest changes taking place in *Syzygium aqueum* fruits, an increasingly popular fruit in the Asian region. In this study the postharvest physico-chemical and mechanical properties, namely, fruit color, weight loss, pulp firmness, total soluble solids, pH and titratable acidity were determined during the postharvest storage period under ambient conditions of *Syzygium aqueum* fruits. It was observed that weight loss, total soluble solids (TSS) and pH of the *jambu air* fruits increased with time whilst pulp firmness and the color index of the fruits decreased. Analysis of the antioxidant activity, determined in the methanol extracts of the *jambu* fruit extracts over a period of 18 days after harvesting, using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, showed that the antioxidant activity increased gradually during postharvest ripening. The total phenol content determined by the Folin-Ciocalteu method revealed a high concentration of phenol content in the *jambu air* fruits, with values around 344.25 ± 107.68 mg gallic acid equivalent (GAE) / 100g fresh fruit. Similarly, the flavonoid content measured spectrophotometrically, using the aluminium chloride colorimetric assay, also showed an increasing trend over the same period. The results were expressed as mg of ascorbic acid equivalent (AAE), gallic acid equivalent (GAE) and catechin equivalent (CE) per 100g of sample. These results represent new data on postharvest changes occurring in *Syzygium aqueum* fruits and show that this increasingly popular fruit has great potential for future development in the agriculture sector.

Keywords: *Syzygium aqueum*, postharvest physico-chemical changes, antioxidant, flavonoid and phenolic content**Abbreviations:** AAE_ ascorbic acid equivalent; CE_ catechin equivalent; DPPH_ 2,2-diphenyl-1-picrylhydrazyl; F&C_ Folin-Ciocalteu; GAE_ gallic acid equivalent; TA_ titratable acidity; TFC_ total flavonoid content; TPC_ total phenolic content; TSS_ total soluble solids;**Introduction**

The *jambu air*, literally translated as water guava or water apple, fruit is a tropical fruit that is fairly widely cultivated and grown throughout Malaysia, on a small scale, where the climate is very suitable for its production all year round. It belongs to the genus *Syzygium* in the family Myrtaceae. There are four types of wax *jambu air* fruits, namely, pink, red, pale green and green. The fruits are pear shaped, often juicy, with a subtle sweet taste and an aromatic flavor. There is great scope to develop the potential wax *jambu air* fruits which can fulfill the local as well as foreign demands for it. Currently in Malaysia it is cultivated mainly as smallholdings ranging from 1 to 5 ha with its hectare age estimated at 1,500 ha in 2005. Fruit harvesting is an important aspect in the fruit growing production industry. This due to the fact that when fruits are removed from the trees and plant it starts to age and ripen and is also naturally susceptible to damage, when it is plucked from the branches and also when it comes into contact with other fruits. This is also true for fruits that fall to the ground. These injuries to the fruits are usually in the form of bruises, punctures and splits. Furthermore additional damage can occur when fruits are raked, collected, loaded and shipped to distant destinations. As transportation of the fruits can take several days, it will bring about changes in several physico-chemical and mechanical properties of fruits as it ripens. Fruit growth and development involves many changes to its morphology, anatomy and physiology and

biochemistry (El-Otmani et al., 1987). When a fruit matures the changes associated with it includes changes in rind texture, juice composition and taste (Chahidi et al., 2008). For the fruit grower it is important to have information on the differences in fruit quality among the selections available and the changes occurring in fruit quality parameters over time. These changes and their rate of occurrence with time will inform the fruit growers and sellers, on the best possible strategies and logistics to employ to ensure its successful production and marketing. Thus the post harvest physico-chemical characteristics of the fruits are important in determining the adoption and design of various handling, packaging, storage and transportation systems. Several parameters in fruits have been evaluated and studied to determine the maturity in fruits and one such index is fruit firmness. In most fruits there is a change in firmness during ripening and the softening process which begins whilst the fruits are on the tree continues during harvesting, handling and storage (Joseph and Aworh 1991; Mizrach, 2000; Singh and Reddy, 2006; Chahidi et al., 2008). Another important external factor and parameter that is used to determine fruit quality is its color. To prospective consumers the appearance of the fruit can have a great influence and is an important determining factor. The relationship between color and degree of maturation in many fruits have been widely studied, such as in tomatoes (Choi et al., 1995), peaches, and nectarines (Luchsinger and Walsh, 1993) and is well documented. Water content is another parameter that affects fruit quality and during ripening and storage of fruits water