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Determination of harvesting indices and harvesting stage of *Annona muricata* L. (soursop) based on fruit growth

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Abstract

Harvesting time plays a decisive role in ripening, storage and table quality of soursop (*Annona muricata* L.) fruits. This study was conducted with the objective of developing a harvesting index based on the change of selected physical and biochemical parameters at different growth stages of fruits. Growth curves for 3 accessions of *A. muricata* were developed by using weekly changes of fruit length and circumference. Physical, physiological, biochemical and chronological parameters of the fruit were tested at 5 identified stages. Growth curve was identified as double sigmoid with long and highly variable quiescent phase. As harvesting indices fruit skin color (L*= 42.45, b*=25.72, and h*=100.4, Yellow green group -YG 144A of Royal horticulture color chart) and number of spurs per 2.5 cm^2 (1 sq. inch) (3-4) were identified at stage 6 with high total soluble content (17.4 $Brix^0$) with longer shelf life (5.2 days) compared to stage 7 (1.7 days).

Key words: Growth curve, Maturity indices, Physicochemical parameters, Soursop

Introduction

Annona muricata L. (Soursop) of the family Annonaceae produces an exquisite fruit for table and processed food. Recently, soursop is gaining popularity both in local and export markets owing to its nutritional and medicinal properties. It is considered as "the fruit for the health" due to presence of many beneficial phytochemicals of medicinal uses. As cited by Badrie and Schauss (2010) acetogenins is one of such compounds demonstrating anti-cancer properties unique to the family Annonaceae.

All annonas are characterized as climacteric fruits in which full ripening occurs after the climacteric peak and harvested when they reach physiological maturity (Coronel, 1994; Nakasone and Paull, 1998). Fruits left on trees after maturity, result in quick ripening and loos market quality. Fruit pulp taste bitter when they were harvested pre-mutually (Sunil *et al*., 2011). Knowledge of the right maturity indices for the markets are important for all categories in supply chain while assuring the best quality and there by minimizing postharvest losses along the value chain. Selection of suitable maturity indices for harvesting this fruit is very important in achieving extended shelf-life and fruit quality. Physical indices such as fruit skin color, firmness, physiological, indices such as ethylene evolution rate, biochemical indices such as Total Soluble Solids (TSS), Titratable Acidity (TA), pH (Madigu, 2010) and chronological indices (age of the fruit calculated from full bloom or fruit set) are used to establish the maturity indices of many fruits (Kosivacninda *et al*., 1984). However in soursop greater separation between spurs, loss of spur firmness and fruit firmness, division between the loculi protruded, turning of seeds in to shining dark brown color, the change of peal color from dark green to light green are the main physical changes associated with maturation (Lima and Alves, 2011).

The extent of cultivation are increasing continuously due to high demand for this fruit therefore it is important to study the consistent changes of selected physical, physiological, biochemical and chronological indices through each phenological stage to identify an index for harvesting. Therefore this study was undertaken to develop a model for fruit growth and identify maturity variables that behave uniformly over growing period and determine optimum harvesting stage of soursop fruit.

Materials and methods

Three accessions (M1, M2, and M3) from Soursop gene bank of, Fruit Research and Development Institute (FRDI), Horana were selected randomly for the study in year 2015 and data were collected for two consecutive seasons. The study was designed to a RCBD. Form each accession three budded trees of similar vigor and age (8 years) were selected as replicates. During the study period daily weather parameters such as temperature, rainfall and relative humidity (RH%) were recorded and means were calculated. All the trees were managed under standard conditions as recommended. In each replicate, within one season, 10 flowers were selected on all four sides of each tree canopy. Selected flowers were tagged and hand pollinated using pollen collected from the same tree as described by Thanthirige (2001). In weekly intervals, from the day of pollination, until spur development initiated, ovary length (mm) and circumference (mm) was measured and after spur development fruit length (mm) and maximum circumference (mm) was measured until fruits ripened using digital vernier caliper (INSIZE). From the stage which, spurs on skin (individual constituent carpals persist on skin) were separated and skin color became visible, till softening of fruits, change of skin color was noted in all three accessions using Royal Horticulture Color Chart (6^{th} edition) in weekly intervals. When different shades of green color occurred with the growth of fruits, differences in green color were measured with the color reader (Minolta Chroma meter -Model CR 20) which was setup with D65 illuminant and 100 observe angle. The color values recorded were L*, a*, b* and h*. At every color group 03 readings were taken from middle ½ portions of the fruit where color was uniform and mean values were calculated. Growth curves for 3 accessions were drawn using weekly means of fruit length and circumference.

According to the differences in morphology and changes in fruit skin color growth curve was divided in to different stages. Length and circumference of ovary measured prior to pollination was considered as stage 0 and afterwards, stage which the ovary remained quiescent was considered as stage 1. After stage 1 skin color became visible with separation of spurs on the skin and this point was considered as stage 2. Other consecutive different green color groups were identified as stages 3, 4, 5 and 6.

After skin color became visible, till ripening same number of skin color groups were indentified in three replicates of three accessions and in two consecutive seasons. Since change of skin color with growth was same in all accessions, one accession (M1) with tree replicates was selected randomly for the second experiment. At every different color stage five hand pollinated fruits were harvested from each replicate and analyzed their physicochemical parameters. The parameters included fresh weight (g), volume (cm^3), total soluble solids (TSS) (^{0}Brix), percentage titratable acidity (% TA), (equivalent to malic acid %), pH of fruit juice, dry matter percentage (DM %) and number of spurs per 2.3 cm^2 of fruit skin in the middle 1/2 portion of the fruit. Titratable acidity was measured using 0.1 M NaOH as the titer and phenolphthalein as the indicator. Percentage of malic acid was calculated using the formula, malic acid = Titer x acid factor for malic acid (0.0067) x 100 / 10 mL Using the methodology described by Kushman *et al*. (1966) dry matter percentage was determined. Fruit weight was measured using digital top loading balance (KERN) and volume was taken as volume of water which was displaced by the fruit. The total soluble solids of annona pulp were determined by using a hand held refractometer (ATAGO) and pH of fruit juice was measured using digital pH meter (GOnDO electronics).

Using fruits of M1 accession the third experiment was carried out to determine stages of horticulture and physiological maturities and maximum storage life of the fruit under ambient condition. This trial as designed to a CRD. Out of six stages of the growth curve, from 4^{th} , 5^{th} and 6^{th} stages , 15 hand pollinated fruits from each stage were harvested and kept till fully ripened. Before storage fruit fresh weight (g) was recorded. Room temperature (^{0}C) and relative humidity (%) were recorded during the storage period. Number of days from harvesting to ripening of fruits was recorded. Physicochemical parameters such as fruit weight (g) , % TA, pH and TSS % were analyzed after ripening of fruits.

Data were subjected to statistical analysis using SAS 9.1 statistical package. Analysis of variance (ANOVA) and least significant difference (LSD) was used to differentiate between variables. Using Repeated Measures Analysis, growth of fruit was compared between different color stages, accessions and two seasons. Relationships between variables were tested using correlation analysis.

Results and discussion

Six color groups were identified according to Royal Horticulture Color Chart classification (RHCC) from date of hand pollination to commencement of fruit ripening (Table 1) in three replicates and of three accessions and in two consecutive seasons.

Table 1. Classified color groups according to RHCC

Stage / Color	3	4	5	6	7
Color code	Green group 143A	Green group 141B	Green group 141C	Yellow- green group 144A	Yellow- green group 144 C
Name of the color	Strong yellow green A	Deep yellowish green B	Strong yellow green C	Strong yellow green A	Strong yellow green C

Color meter reading of stage 2 was ignored since difficulty of gauging color due to high spur density. When consider the color meter readings (Table 2) of three accessions in two seasons, within each color group variation was non significant in all color variables determined. This proved that variable skin color is not affected with differences among accessions. Since a* chromaticity values remained negative and b* chromaticity values remained positive throughout the growth, skin color of the fruit represented different intensities of yellow and green colors.

Table 2. Mean values of color parameters at different stages in three accessions

Acc.	L*					(-)a*				
	3	4	5	6	7	3	4	5	6	7
M1	31.8^{a}	24^{a}	34.3^{a}	43.6^{a}	49.9^{a}	4.7^{a}	5.2^{a}	5.1^{a}	4.4^{a}	4.1^{a}
M2	31^{b}	23.6^{a}	33.8^{a}	42.8^{ab}	49.6^{a}	4.4^{a}	5.2^{a}	5a	4.2^{a}	4.1^{a}
M3	29.3^{b}	23.1^{a}	33.6^{a}	42.4^{ab}	49.5^{a}	4.3^{a}	4.8^{ab}	4.6^{ab}	4.1^{a}	4^{a}
Mean	**30.4**	**23.5**	**33.9**	**42.9**	**49.6**	**4.4**	**5.0**	**4.9**	**4.2**	**4.0**

Table 2. Cont.

Acc.	b*					h*				
	3	4	5	6	7	3	4	5	6	7
M1	20.7[a]	11.8[a]	18.3[a]	26.7[a]	31.7[a]	103.6[a]	110.6[a]	107.6[a]	101.1[a]	97.4[a]
M2	19.4[a]	11.8[a]	17.8[a]	26[a]	31.5[a]	103.4[a]	109.9[a]	107[a]	100.9[ab]	97.1[a]
M3	19.2[ab]	11.5[a]	17.2[a]	25.6[a]	31.3[a]	102.9[a]	109.7[a]	106.5[a]	100.3[ab]	97.1[a]
Mean	**19.7**	**11.7**	**17.7**	**26.1**	**31.5**	**103.3**	**110.0**	**107.0**	**100.7**	**97.2**

Note : Same letters indicate no significance difference at the 0.05 probability. L: lightness, a (-)*: green chromaticity, b*: yellow chromaticity, h*:hue angle.*

In soursop, chlorophyll a and b is developed on fruit skin at early stages of growth and declined at latter stages (Worrell et *al*., 1994). (Table 3)

Table 3. Mean values of color parameters at different growth stages

Growth Stages	Color Parameters			
	L*	(-)a*	b*	h*
3	30.65 [d]	4.48 [bc]	19.82 [c]	103.11 [b]
4	23.01 [e]	5.02 [a]	11.50 [e]	109.96 [a]
5	33.81 [c]	4.89 [a]	17.82 [d]	107.09 [a]
6	42.30 [b]	4.23 [dc]	25.46 [b]	100.22 [b]
7	49.49 [a]	4.11 [d]	31.54 [a]	97.20 [c]

Note : Same letters indicate no significance difference at the 0.05 probability. L: lightness, a(-)*: green chromaticity, b*: yellow chromaticity, h*:hue angle*

Variable (-) a* of the color meter readings, explains the intensity of green color in an object (Gomez *et al*., 2002). In the growth curve, stages 4 and 5 acquired significantly higher values for variable a* (-5.02 and -4.89 respectively) compared to other stages. According to RHCC color classification stage 4 was identified as most dark stage out of all stages and stage 5 less dark than stage 4. Therefore these two stages could be considered as maximum chlorophyll deposited points. Variable b* represents the yellow chromaticity (Hunter Laboratories, 2012), it reads accumulated carotenoids (yellow color) in vacuoles of epidermal cells (Fernandez-Lopez *et al*., 1992; Ikoma *et al*.,2001). From stage 3 to stage 4 a significant reduction of variable b* was recorded (from 19.82 to 11.5) while from

stage 5 to 6 (from 17.82 to 25.46) a significant increment was observed. Stage 7 recorded the highest value (31.54). Above readings proved that soursop fruit represents one of the three typical patterns of carotenoids accumulation on fruits. It is the pattern which shows minimum carotinoids accumulation in mid development stages and maximum at latter stages (Gross, 1987). Transform of fruit skin color from greenness to yellowness towards maturity was due to accumulation of carotinoid and degradation of chlorophyll at latter stages of the growth curve. When consider the L* variable, it describes the luminosity of a particular color (Hunter Laboratories, 2012). Stage 4 showed a significantly lower value of variable L* (23.5) than other stages, since it was one of the highest accumulation point of chlorophyll and least accumulation point of carotinoids. With degradation of chlorophyll at latter stages and higher accumulation of carotinoids, L* value was increased up to 49.49 at stage 7 . The variable h* represents angle in the color wheel (0^0 – 360^0), where 90^0 is yellow and 180^0 is green (Hunter Laboratories, 2012). Values of h* variable varied between 109.96 to 97.2 from stage 3 to 7 respectively where skin color was more closer to yellowness than greenness and towards maturity yellowness increased significantly. Color variables L* and b* measured using color reader were significantly different at all growth stages and variables (-)a* and h* variable were significantly different at certain growth stages. Further growth stages identified using RHCC were easily distinguishable to naked eye. Therefore skin color could be successfully used as a parameter of determining harvesting indices.

When analyzed the time range required to complete each stage (Table 4), within one accession time taken for stage 1 was highly variable in both seasons. Therefore time factor was not considered as a parameter in formulating harvesting indices. In all accessions, mean percentage time required for the completion of stage 1 was nearly 50% and remaining 50% time period was spent for remaining six stages. This long quiescent period was also reported by Worrell *et al*. (1994).

Table 4. The range of time (minimum and maximum) required for each growth stage of each accession , in two seasons.

Acc	Season	Minimum and maximum time period (wks)						
		S1	S2	S3	S4	S5	S6	S7
1	**1**	8-26	1-2	2-3	1-2	2-4	1-3	1
2	**1**	7-28	1-3	2-4	1-3	2-4	1-3	1-2
3	**1**	8-25	1-2	2-3	2-3	2-4	1-3	1-2
1	**2**	6-18	1-2	2-4	1-3	3-5	1-3	1
2	**2**	6-23	1-3	2-5	1-3	2-4	1-3	1
3	**2**	6-20	1-3	2-5	1-3	3-5	1-2	1

Acc: Accession, S1-S7: Stage 1-Stage 7

Figure 1 illustrates the overall changes of all color attributes gauged from color meter along the fruit growth curve. Each point in the graphs represents the means of three accessions.

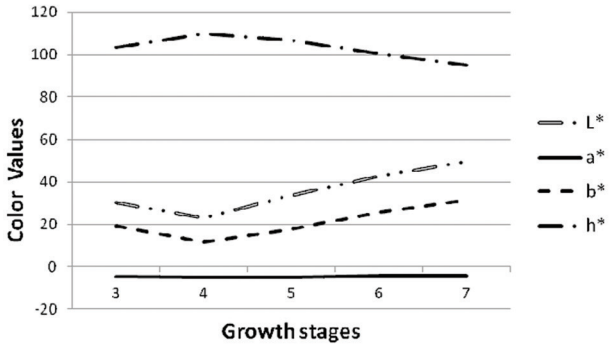

Figure 1. Graphical presentation of behavior of color parameters at different growth stages. L*: lightness, a(-)*: green chromaticity, b*: yellow chromaticity, h*:hue angle.

Fruit growth (length and diameter) of all accessions increased continuously till initiation of ripening. Double sigmoidal curve with 03 distinct phases (phase I, phase II and phase III) were depicted in the growth curve of soursop (Figure 2) as previously reported by Worrell *et al*. in 1994 (Figure 1) with pictures representing each growing stage of the fruit.

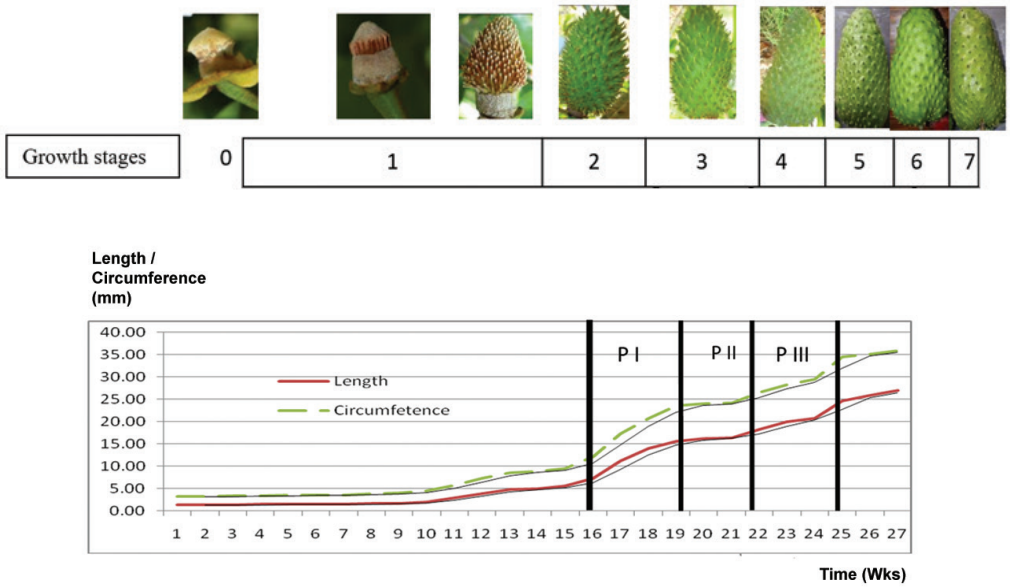

Figure 2. Double sigmoidal growth curve with identified growth stages (0-7) P1- Phase 1, P11- Phase 11, PIII- Phase III.

The rapid growth phase is termed as phases I (PI) in a double sigmoid growth curve. In the growth curve of soursop, out of identified seven color stages stage 3 can be identified as phase I. Phase II (PII), which is a slow growing phase depicted in stage 4 in the growth curve. Stage 5, which represented the second slow growth stage could be recognized as phase III (PIII). This triphasic growth has been reported in blueberry, fig, grape, kiwifruit and pineapple (Coombe, 1976).

Out of three accessions ovary size was significantly higher in M3 (length 1.4 mm and circumference 3.36 mm) (Table 5) but when growth proceeded fruit size was independent of size of the ovary. In repeated measurement analysis effect of growth stages, growing season and difference in accession on fruit growth were tested. Fruit growth in terms of length and circumference were significantly different among identified seven stages at $p< 0.05$ ($P= 0.0001$).The effect of season, accession and replication were not significantly different. It indicated that the demarcation of stages was accurate and stages were common to all accessions. The identified stages are reliable to use in

designing the growth curve and formulating harvesting index. Further overall means of length and circumference were significantly different in two seasons (P=0.001). In both seasons variation of climatic factors were not significantly different. First season was from November 2015 to October 2016 and second was from November 2016 to October 2017. Mean temperature, rainfall and RH%, during first season were 31.4 ^{o}C, 6.5 mm and 83.1% respectively and 31.1 ^{0}C, 7 mm and 82.9% respectively during the second season. Therefore climatic factors were not the cause for the size variation during two seasons. Though in this study fruit size appeared to be a reliable index for harvesting it is highly related to the proportion of carpals fertilized and environment factors prevail during pollination (Heenkenda *et al*., 2011). Therefore size of a fruit can be used to formulating harvesting indices for hand pollinated fruits which is growing in uniform environmental conditions.

Table 5. Mean values of fruit length and fruit circumference of three accession of seven stages in two seasons

Stages	Length (mm)			Circumference (mm)		
	M1	M2	M3	M1	M2	M3
0	1.37^{b}	1.38^{b}	1.40^{a}	3.39^{b}	3.25^{b}	3.36^{a}
1	3.36^{a}	3.24^{a}	3.45^{a}	6.56^{ab}	6.20^{b}	6.79^{a}
2	5.82^{b}	6.10^{b}	6.57^{a}	9.51^{b}	9.47^{b}	10.07^{a}
3	12.30^{b}	13.00^{b}	14.51^{a}	17.54^{b}	18.17^{b}	19.91^{a}
4	17.08^{a}	17.96^{a}	18.57^{a}	23.76^{a}	24.87^{a}	25.77^{a}
5	19.45^{b}	20.40^{ab}	21.05^{a}	27.00^{b}	27.56^{ab}	28.50^{a}
6	25.46^{a}	26.16^{a}	26.68^{a}	34.58^{a}	34.64^{a}	36.29^{a}
7	27.50^{b}	28.07^{ab}	28.52^{a}	36.48^{b}	37.23^{ab}	39.20^{a}

Note : Same letters indicate no significance difference at 0.05 probability.M1,M2,M3-Accsession 1,2 and 3

In the experiment which physiochemical properties (Table 6) of different stages were analyzed, analysis of variance revealed that all biochemical components except pH and all physical components except number of spurs, increased significantly with

maturation. According to Kuo-Tan Li, (2012), at the initial stages of fruit development accelerated cell division is taking place where fruit size is not significant. Since cell division is and energy demanding process, at stages 2 and 3, DM% (7.87 and 8.6 respectively) were significantly lower than stage 4 (13.83), therefore stages 2 and 3 could be considered as stages of cell division. A drastic increase of % dry matter (%DM) could be observed when the fruit entered to the slow growing stage (stage 4) where rapid internal physiological and morphological changes taking place. Until harvest %DM was increased. The presence of high dry matter content towards maturity indicates that the fruits have attained to its physiological maturity (Saranwong *et al*., 2004). In most of the fruits titratable acidity (TA%) is reduced during maturity (Kays, 1997) but in soursop TA% was increased from 0.11% at stage 2 to 0.27% at stage 7. The increase of TA% towards maturity was also noted by Wills *et al*. (2007) in atemoya. According to Lima and Alves (2003) this significant increase of TA% towards maturity is due to that, transformed sugars in to acids is underutilized in respiration during ripening. Since underused acids were increasing towards maturity, accordingly pH was heading towards acidic nature (6.0 at stage 2 and 5.03 at stage 7). Increased acid level can be used for formulating an index for harvesting. The gradual increment of TSS content towards maturity (from 4.9 to 7.4 ^{0}Brix) was mainly due to hydrolysis of reserve carbohydrates which were accumulated during the growth (Wills *et al*., 2007). Accordingly to Lima and Alves (2003), in soursop fruits TSS content increases gradually during different stages and reaches 7.0^{0}Brix when fruit is physiologically matured. TSS content in fruits has been used as a maturity indicator since it increases with the maturation (Wills *et al*., 2007). With the increase of fruit size (length and circumference) density of spurs per 2.5 cm^2 reduced significantly from one stage to another along the growth curve, starting from 19.63/2.5 cm^2 at stage 2 and 2.63/2.5 cm^2 at stage 7. When fruit size is increased skin got stretched and spur density reduced accordingly. At different stages spur density differed significantly number of spurs per 2.5 cm^2 can be used successfully for developing an indicator for soursop fruit harvesting. Fruit weight (FW) and fruit volume (FV) not significantly different in stages 2 and 3 though stage 3 represented in rapid growth phase (phase I) of the double sigmoid growth curve. According to Carini *et al*. (2001) during cell division phase fruit size and weight not significant but rapid morphological changes occurs. In the current context as morphological changes skin color development and

protrusion of skin spurs occurred. After stage 4 a rapid increment of weight volume was evident due to the reason that the fruit has entered its cell expansion phase (phase III) where size of individual is expanded and intercellular spaces started to increase. When cell enlarges its vacuole increased in size and occupies most of the volume inside and air spaces increased to a maximum (Schwab and Raab, 2004). Fruit weight and volume at stage 7 is (1509.5 g and 1594.44 cm^3 respectively) is approximately 5 folds of weight and volume of stage 2 (263.14 g and 230.33cm^3 respectively). Fruit weight and volume highly depend on level of pollination and the proportion of carpel development. Hand pollination will increase the uniformity of fruit weight and volume (Heenkenda *et al*., 2011). Therefore fruit weight and volume could be taken as reliable parameters in developing an maturity index for hand pollinated fruits.

Table 6. Mean values of physiochemical parameters of different growth stages of the fruit

Stage	Factor						
	DM%	TA%	pH	TSS ($Brix^0$)	No. Spurs	FW(g)	FV(cm^3)
2	7.87^e	0.11^c	6^a	4.9^d	19.63^a	263.14^d	230.33^d
3	8.6^e	0.12^c	6.01^a	5.41^c	10.76^b	408.3^d	364.8^d
4	13.83^d	0.13^c	6.02^a	6.07^b	7.16^c	598.75^c	573.93^c
5	17.43^c	0.14^b	5.95^a	6.5^b	6.03^d	828.56^b	910.1^b
6	19.26^b	0.2^b	5.71^b	7.84^a	3.73^e	1340.18^a	1446.67^a
7	21.56^a	0.27^a	5.03^c	7.4^a	2.63^f	1509.5^a	1594.44^a

Note :Same letters indicate no significance difference at the 0.05 probability. DM%-dry matter %, TA%-Titratable acidity%, TSS-Total soluble solids. No. Spurs- Number of spurs, FW- Fruit weight, FV- Fruit volume

In the experiment conducted in identifying the optimum harvesting stage, 30 fruits harvested at stage 5 in two seasons did not ripped at all. After 8- 10 days all 30 fruits were decayed due to postharvest diseases. Fruits harvested at stage 6 ripe after 5.2 days and at stage 7 ripe after 1.7 days. The physiochemical parameters analyzed before and after storage of fruit are given in Table 7.

Table 7. Physicochemical parameters of fruits of stages 6 and 7 after and before ripening

Stage	Factor							
	After ripening			Before ripening			Days to ripe	%Weight loss after ripped
	TSS ($Brix^0$)	TA%	pH	TSS($Brix^0$)	TA%	pH		
6	17.47^a	1.01^a	3.57^a	7.84^a	0.2^a	5.71^a	5.2^a	10.5^a
7	15.97^b	0.97^a	3.44^a	7.4^a	0.27^a	5.03^b	1.7^b	2.11^b

Note: Same letters indicate no significance difference at the 0.05 probability. DM%-dry matter %, TA%-Titratabel acidity%, TSS-Total soluble solids.

A drastic increment of TSS (17.47 and 15.97 Brix0 respectively) and decrease in TA% (1.01 and 0.97% respectively) and pH (3.57 and 3.44 respectively) compared to unripe fruits were observed (Table 5). Aziz and Yusof *et al*. (1994) and Lima and Alves (2003) also reported the abrupt drop of pH (3.6-3.7) during ripening. As described by Chandra in 1990 rapid increase of TSS was due to hydrolysis of stored starch and conversion of acids into simple sugars. Biale and Young. (1986) reported a biphasic respiratory climacteric in soursop. According to Kuo-Tan Li (2012) ripening is highly energy demanding process especially in climacteric fruits. Due to high energy demand stored food is depreciated. Therefore harvesting before the onset of climacteric respiratory peak can result high TSS value. Based on the results of this study stage 6 was identified as the optimum maturity stage for harvesting soursop fruits with long shelf life and high TSS value. However, weight loss was highest (10.5%) during the storage in fruits of stage 6 and it could be due to the evaporation loss caused during the long storage compared to stage 7.

A linear correlation was not apparent among variables during correlation analysis. A relationship between physical and biochemical parameters could be establish using some selected variables. As physical parameters, variables L*, b* and h* of skin color readings and number of spurs on skin and as a biochemical parameter TSS could be successfully used for establishing harvesting index for soursop fruits.

Conclusion

Soursop fruit exhibits double sigmoidal growth curve with a long and highly variable initial quiescent period. Of several parameters evaluated, fruit skin color and number of spurs per 2.5cm^2 of fruit skin were identified as promising physical parameters for determining the harvesting index. Growth stage 6 was the optimum maturity stage for harvesting soursop fruits with Long shelf life compared to stage 7 and high total solids content compared to stage 5.

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