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Identification of Physical, Chemical Properties and Flavor Profile of *Spondias dulcis* in Three Maturity Stages

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***Abstract*—** *Ambarella (Spondias dulcis) is an underrated tropical tree bearing fruits. Scientific research prove that the fruit contain significant health benefits and nutrients. In the present study, the evolution of some physical and chemical properties has been investigated in three different maturity stages and the distinctive flavor profile of the fruit. The pH, titratable acidity, total soluble solids and fructose content in the fruit increased with maturity while vitamin c and total polyphenolic content expressed a decline with maturation. The vitamin c content in the fruit ranged between 21.31 ppm to 23.79 ppm. Total polyphenolic content was at the lowest value in the second stage of maturity. The highest total polyphenolic content was observed as 81.25 mg/GAE/100g in the unripen fruit. The highest fructose content was observed in the fully ripen fruit expressed as 527.89 ppm. The most common flavor compounds identified in the fruit were limonene, α-pinene, beta pinene and hexanal while many other minor constituents such as alcohols and esters were identified in maturity stages.*

Keywords- *Flavor profile, Fructose content, Maturity stages, Spondias dulcis, Total Phenolic content, Vitamin C.*

I. INTRODUCTION

Spondias dulcis, commonly known as Ambarella or June plum belongs to the family of Anacardiaceae and is a tropical tree which is native from Melanesia through Polynesia and has been widely spread in Sri Lanka, India, Indonesia, Malaysia and many other tropical and equatorial countries [1].

The ambarella tree is a fast growing tree which grows up to 18 m at the homeland and 9-12 m at other areas. Small, white color flowers are borne in the tree and they are assorted in male and female. The tree contain an edible fruit with a fibrous pit which contain a considerable amount of sugars, vitamins and polyphenols. The fruits are oval and 5-10 cm long, containing a fibrous flesh [2]. The color of the fruit flesh changes from white to yellow, become softer and flavor become musky with ripening. Fruit peel changes from green color to golden yellow color upon ripening [3]. The flesh of the ambarella fruit is juicy, crisp and mildly acidic and has a flavor and aroma closely related to pineapple. Ambarella has been used for indigenous medicine in countries like Sri Lanka, India, Vietnam and Malaysia. The fruit is used to treat anemia, regulate blood glucose levels, and to treat digestive problems as ambarella contain high amount of dietary fiber [1]. The *Spondias dulcis* fruit is famous for the distinctive flavor profile of the fruit. A previous study [4] has been carried out to identify some of the main compounds responsible for the distinctive odor of another species of the *Spondias* family yet there are no previous studies on the flavor profile of *Spondias dulcis* and scientific evidence on the physical and chemical properties of the fruit is limited. Thus the objective of the study was to identify some physical and chemical properties of the fruit *Spondias dulcis* in three different maturity stages.

II. MATERIALS AND METHODOLOGY

A. Sample Collection

Ambarella *(Sponidas dulcis)* fruits were collected from Maharagama, Western province, Sri Lanka and the research was carried out at the laboratory of food analysis in the Department of Food Science and Technology, University of Sri Jayewardenepura. The fruits were classified into three different maturity stages based on the subjective evaluation on the peel color.

Unripen- Green color
Semi ripen- Green/ yellow color
Fully ripen- Golden yellow color

Stage 1-Unripen Stage 2-Semi ripen Stage 3-Fully ripen

Fig. 1. Maturity stages of the fruit

B. Identification of the Physical Characteristics

Size of the fruit

The size of the fruit was determined using a Vernier caliper. Ten fruits from each maturity stage was used and the mean value of three replicates from each fruit was used to measure the size of the fruit [3].

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Peel color of the fruit

The Hunter Lab values of surface color of the fruits in different maturity stages were determined using a reflectance chroma-meter (Model CR-400, Konica Minolta Camera Co. Ltd, Osaka, Japan) based on the L* (lightness or brightness), a* (redness/greenness), b* (yellowness/blueness) values. The reflectance chroma-Meter was standardized using a white plate. Three measurements from each fruit was obtained and ten fruits for each maturity stage was considered for the findings.

C. Identification of the Chemical Characteristics

pH

The pH value was determined using a calibrated, Digital pH meter (Intelli CAL pH, pHc 101).

Titratable acidity

The titratable acidity was measured following the AOAC 942.15. About 2 ml of the sample was diluted up to 50 ml and was titrated with standardized 0.1 M NaOH (Analytical reagent) using 0.3 ml of phenolphthalein as the indicator.

Total phenolic content

The total polyphenol content in different maturity stages of ambarella was determined following the Folin-Ciocalteu's reagent [5] with slight modifications [6]. An aliquot of 0.2 ml of the sample was transferred into a tube containing 1.0 ml of Folin-Ciocalteu's reagent diluted in water in 1/10 ratio. The mixture was incubated at room temperature for 10 minutes followed by the addition of 0.8 ml of a 7.5% sodium carbonate solution. The tubes were again allowed to stand at room temperature for 30 minutes and absorbance was measured at 743 nm. The standard gallic acid curve was constructed using the same methodology and a dilution series was prepared between 10 mg/GAE/100g to 100 mg/GAE/100g. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 ml in the sample.

Fructose content

The fructose content was determined by the method for reducing sugars [7]. 10 ml of the sample was digested with 50 ml of 2M HCl in a 100 ^{o}C water bath for 2 hours followed by the neutralization from 50 ml of 2M NaOH before the test procedure. D (-) Fructose was used as the fructose standard and to identify the exact wavelength for the identification of fructose content, a series of wave lengths were used to plot a graph for the same sample and the wave length which provides the highest absorbance was selected as the most suitable wave length. The absorbance correspond to the concentration of the sample was determined at 523 nm.

Vitamin C content

The ascorbic acid content was determined in different maturity stages [8]. An aliquot of 4 ml from the centrifuged sample was mixed with 0.23 ml 3% bromine water followed by the addition of 1.3 ml of 10% thiourea which was added to remove surplus bromine water and osazone was formed by an addition of 1 ml of 2, 4 - Dinitro phenyl hydrazine solution. After that, the samples were incubated in a 37 ^{0}C water bath and were cooled in an ice bath for 30 minutes. The samples were treated with 5 ml of chilled 85% sulphuric acid with constant stirring and the absorbance was measured at 521 nm from UV visible Spectophotometer – UV Mini – 1240.

D. Identification of the Flavor Profile of Sponidas dulcis Fruit

The flavor profile of the *Sponidas dulcis* extract prepared from maturity stage two was determined by GC-MS technique coupled with SPME method. The fruit extract samples were prepared by taking 10 ml from the sample and immersing the sample in a closed container at 37 ^{o}C for 20 minutes while the SPME fiber is inserted [9]. The SPME fiber, 50/30µm divinylbenzene/ carboxen on polymethyl silioxone on a 1 cm stable flex fiber was used to identify the flavor compounds. Gas chromatography (GC) (Agilent 7890A) combined with a mass spectrophotometer (MS) (5975C) was used to headspace flavor analysis. The DB-225MS (Agilent) capillary column (30 m x 250 µm x 0.25 µm) was used in GC/MS to separate the flavor compounds. The GC-MS column type was DB-WAX fused silica capillary column (60×0.32 nm 1d, 0.25 µm thickness). Helium was used as carrier gas at a constant flow rate of 1 ml/min. The initial column temperature of the program was started with the oven temperature at 40 ^{o}C, then 2 ^{o}C/min to 70 ^{o}C, hold at 70 ^{o}C for 5 min, then heated up to 170 ^{o}C by 5 ^{o}C / min, then 50 ^{o}C / min up to 220 ^{o}C and finally maintained at 220 ^{o}C for 5 min.

E. Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) 21.0 version statistical software. Differences were considered statistically significant when $p<0.05$.

III. Results And Discussion

Table 1 depicts the luminosity of the fruit peel which was increasing with maturity indicating the increment of lightness of the fruit peel. This is a sign of the pigment transformation of the fruit peel with maturity and the complex biochemical reactions which occur with maturity[10]. In Ambarella fruit, the green color of the fruit peel was transforming into golden yellow color with maturity and this can be used as an indices to identify the correct maturity stage to harvest the fruit. The color change in the peel occur due to the degradation of chlorophyll and synthesis of carotenoids or anthocyanin [2].

TABLE 1. The fruit peel color of Ambarella in three different maturity status.

Maturity Stage	L*	a*	b*
Unripen	38.43±0.64^{c}	-6.69±0.79^{b}	15.49±1.28^{c}
Semi-Ripen	54.39±0.48^{b}	-2.53±1.94^{c}	29.81±0.67^{b}
Fully Ripen	65.21±1.08^{a}	4.89±1.12^{a}	42.41±0.55^{a}

Average values (±S.D.) are presented. Different letters on rows mean statistically significant differences ($P<0.05$) between the measured parameters at different maturity stages.

The pH value is an internal indicator of the maturity of a fruit [11]. The pH value and the titratable acidity serve as an indication of the acidic nature of the fruit with maturity. According to the results, the pH value and the titratable acidity was increasing with maturity while a decline in TA was observed in the fully ripen stage of maturity. Formation of organic acids with maturity indicates the increasing values of

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titratable acidity with maturity stages and the highest value was observed as 1.08 in the fully ripen stage of maturity.

The Vitamin C content was increasing at the first and the second stages of maturity and was declining at the final stage of maturity. The increasing value of pH goes hand in hand with the increasing of titratable acidity and decreasing of ascorbic acid content. In contrary to the findings, similar pattern of the reduction of Vitamin C has been observed for *Spondias dulcis* fruit with maturity[3]. The highest Vitamin C content was observed in the second stage of maturity as 27.72 mg/100g and the average value of ascorbic acid content in three maturity stages was observed as 24.27 mg/100g. Vitamin C and A are the most abundant vitamins in Ambarella fruit pulp[2]. The results suggests that semi ripen fruits contain higher amount of vitamin C when compared to the ripen fruits. Ascorbic acid is readily susceptible to temperature and oxygen. So with ripening the fruit remain in the atmosphere for a prolonged period of time which increase the rate of oxidation reactions, thus the ascorbic acid content decreases with maturity.

The total soluble solid content was highest at the fully ripen stage of maturity being 7.52^0 Brix. The Total soluble solid content vary between different fruit varieties, maturity stages and cultivations yet commonly the TSS content is increasing in many types of fruits such as mango, pomegranate, blueberries, with maturity[12], [13], [14] and similarly our experiment express the same result.

The size of the fruit was increasing with maturity and the increasing rate of the length of the fruit was higher when compared with the width of the fruit indicating a final shape of an oval fruit.

Polyphenols are large structural compounds with the presence of multiple phenolic structures. The polyphenolic compounds present in trees are phytochemicals and they are an indication of the antioxidant compounds in fruits. The Total Poly Phenolic Content in Ambarella was expressed as mg/GAE/100g and the highest TPC content was observed in the unripen fruits as 81.25 mg/100g and the TPC content was decreasing in the semi-ripen stage and again was increased at the final stage of maturity. The declining of TPC can occur due to the oxidation of polyphenolic compounds by polyphenol oxidase in fruits. A declining of total phenolic content has been observed for other fruit types with maturity such as high bush blueberries, red raspberries[15] and also for *Spondias dulcis* species in another study[3].

Monosaccharaides such as fructose, glucose and mannose are the major sugars that can be found in Ambarella[2]. Fructose is the predominant carbohydrate in fruits being called as fruit sugar. Ambarella fruit contain a significant amount of fructose in all three maturity stages and the level of fructose content express a significant increment with maturity ($p<0.05$) and the highest fructose content was observed as 527.89 ppm in the fully ripen stage of maturity. The fructose content increase with maturity as the reducing sugar content increase in fruit with maturation. Similar results have obtained for different fruits such as blueberries[14], mango[16] and pineapple[17].

TABLE 2. Physical and Chemical parameters of Ambarella fruit at different maturity stages.

Maturity Stage	Unripen	Semi-Ripen	Fully Ripen
pH	4.63 ± 0.06^c	4.94 ± 0.07^b	5.47 ± 0.07^a
Titratable Acidity	0.92 ± 0.08^c	1.35 ± 0.05^a	1.08 ± 0.03^b
Total Soluble Solids (Brix)	4.50 ± 0.45^c	6.18 ± 0.27^b	7.52 ± 0.26^a
Length (cm)	5.53 ± 0.33^c	6.55 ± 0.19^b	7.52 ± 0.18^a
Diameter (cm)	4.58 ± 0.15^c	5.16 ± 0.14^b	5.62 ± 0.16^a
Vitamin C	23.79 ± 0.93^c	27.72 ± 0.88^a	21.31 ± 0.97^b
Total Poly Phenolic Content (mg/GAE/100g)	81.25 ± 2.41^a	47.66 ± 1.11^b	56.97 ± 1.49^c
Fructose Content	381.89 ± 7.67^c	440.43 ± 7.76^b	527.89 ± 6.57^a

Average values (±S.D.) are presented. Different letters on rows mean statistically significant differences ($P<0.05$) between the measured parameters at different maturity stages

Ethanol, Acetic acid, ethyl acetate, Hexanal, Ethyl butanoate and Limonene are the major compounds that have been identified in fruit varieties belong to *Spondias* family which contribute to odor[18]. Professional perfumers has described the odor of the hexane extracts of ripen *Spondias* species as cognac-rum like, weak fruity, fresh green nutty while the unripen fruits contain more fresh-green nutty, weak fruity rum like odors[4]. About 4 major compounds which are common in different maturity stages were identified in the study namely as D-Limonene, Alpha-pinene, Beta-pinene, and Nonanal. Most of the ester compounds, alcohols, aldehydes that were identified in the study are responsible for the distinctive flavor of the fruit. Minor alcohols, esters and acids are especially related with the aroma in the fruit[4].

The maturity stage 1 mostly comprise of compounds which provide grassy green odor (3-hexen-1-ol), essential oil compounds (1R-alpha-pinene, Beta-pinene, Caryophyllene**)** and fruity odor compounds (1.3.3-trimethyl Bicyclo(2.2.1)heptan-2-one**)**. Citrus fruit odor compounds (Succinic acid di(3.5-dimehylcyclohexyl)ester**)** were also found in the maturity stage 1. Most of the compounds identified in the stage 1 are secondary plant metabolites which are responsible for the distinctive flavor and odor of the fruit.

The maturity stage 2 mainly possesses a fruity odor. Therefore compounds like 1-penten-3-one, Hexanal, 2-Hexenal which contributes for fruity flavors were present in the fruit extract. The compounds responsible for the grassy green odor were not present in the stage 2 indicating the loss of grassy odor with maturity. Also there were essential oil compounds (1R-alpha-pinene, Tetradecane) and several esters in the stage 2 of maturity.

The maturity stage 3 mainly contained several alcoholic compounds, ethanol, 3-methyl-1-Butanol indicating the fermentation of fruit sugars towards the end of the maturity. Also esters (2-Hexenal2-Butenoic acid, methyl ester (E), Trifluoroacetic acid, 1-cyclohexyl ethyl ester), volatile oil constituents (1S-alpha-pinene, Beta-pinene and 2-methyl-3-Buten-1-ol) and fruity flavor compounds were present in the fruit extracts of maturity stage 3. The weak fruity aroma in the maturity stage 3 of the fruit is associated with 2-Hexenal and other compounds including 2-Butenoic acid methyl ester, Trifluoroacetic acid 1-cyclohexylethyl ester, Napthalen-2-ol.
The results are compatible with the previous studies on the odor of the fruits in the same species. Due to the distinctive

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flavor and aroma of the fruit, it can be used in flavoring other food products such as juices, sweets and tea.

TABLE 3. The major flavor compounds identified for each maturity stage with their relative peak areas in *Spondias dulcis* fruit extract

Maturity Stage 1 (Unripen)	Relative peak area
1-Hexanol	124074454
Ethyl alaninate	46726226
3-butyn-1-ol	6991050
Beta-pinene	6496739
Caryophyllene	4950727
1.3.3-trimethyl Bicyclo(2.2.1)heptan-2-one	3891522
1R-alpha-pinene	3496317
8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)(1s-cis)	2457097
Copaene	1706534
D-Limonene	1447786
1-Heptanal	860350
Succinic acid di(3.5dimehylcyclohexyl)ester	778581
H-lindole	250920

Maturity Stage 2 (Semi ripen)	Relative peak area
2-Hexenal	445962070
Hexanal	331731706
Ethanol, 2-methoxy acetate	225750797
D-Limonene	43175190
1-penten-3-one	8824452
Nonanal	8259034
1R-alpha-pinene	7062746
Tetradecane	5906395
Sulfurous acid, hexyl pentyl ester	1462710
Normorphine bis(trimethyldilyl) ether	210451

Maturity Stage 3 (Fully ripen)	Relative peak area
Ethanol	124509988
2-Hexenal-2-Butenoic acid, methyl ester (E)	55438751
Hexanal	52592119
3-methyl-1-Butanol	12583781
1S-alpha-pinene	6939150
Beta-pinene	1356868
Nonanal	1073737
1-(1H-1,2,4-triazol-3ethylminomethyl) Napthalen-2-ol	248510
2-methyl-Decane	230132
1-cyclohexylethyl ester	166954

IV. Conclusion

The study provides information on physical and chemical properties of the *Spondias dulcis* fruit during three different maturity stages which can be used as an indication to identify the correct maturity stage. The identification of composition of the fruit is important in value addition for the fruit in future.

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