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Changes in physico-chemical properties during fruit development of Japanese pumpkin *(Cucurbita maxima)*

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Abstract

Changes in physico-chemical properties such as specific gravity (SG), color development, fruit firmness, moisture content (MC), dry matter (DM), total carotenoids, total chlorophyll, crude fiber, starch content, sugar content and total soluble solids in Japanese pumpkin fruit *'Cucurbita maxima'* were investigated during fruit development. The results revealed that the fruits gradually accumulated dry matter, total soluble solids, total sugars, total carotenoids, total chlorophylls, starch content and crude fiber along fruit development. The values of the aforementioned parameters were 6.93-16.00%, 4.5-12.0%, 16.0-23.55 mg/g, 1.86-2.83 mg/100g, 12.56-49.42 mg/100g and 1.56-2.54%, respectively. Based on the changes of these parameters during fruit development, harvesting at 50 days after fruit set was considerably recommended due to the fruits had optimum maturity and the quality also agreed with market and consumer acceptability.

Introduction

Kabocha or Japanese pumpkin *(Cucurbita maxima)* belongs to the family Cucurbitaceae, genus *Cucurbita*. It is considered a health promoting vegetable due to its high contents of multivitamins and antioxidative compounds such as carotenoids and phenolic compounds (Hurst *et al*., 1995). Both internal and external qualities are the most important indices of Japanese pumpkin fruit maturity. Some internal quality parameters of agricultural products are related to fruit development and maturity such as changing of physical properties (flesh color, dry matter and fruit firmness), chemical properties (total soluble solids, tritratable acidity, sugar content, pectin substance, carotenoid and chlorophyll contents) and physiological properties (respiration rate and ethylene production). In the case of Japanese pumpkin, fruit maturity is difficult to define. Most of the popular edible Japanese pumpkin fruit have relatively small fruit size and somewhat the same size as those at immaturity stage. Whereas the change in rind color from light green to dark green can be a beneficial characteristic of Japanese pumpkin to determine harvest time, but it is not truly related to fruit maturity. Harvest time should be based on internal quality and fruit maturity. In most varieties, high starch content is strongly associated with good eating quality and about two months after fruit set is recommended as an appropriate harvest time (Loy and Noseworthy, 2008). Normally, producers evaluate maturity of Japanese pumpkin fruit by using day counting after planting and observing stem dryness before harvesting. Although these methods can assess maturity of Japanese pumpkin fruit and provide reliable results, some losses arising from the mix of immature fruit are unavoidably observed. Thus, the objective of the present study was to study the changes of physico-chemical properties during fruit development, and to identify a maturity index that meets market and consumer satisfactory for Japanese pumpkin.

Materials and Methods

Raw materials

One-hundred-eighty samples of Japanese pumpkin fruit were used in this study. All fruit

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were obtained from "Mont Ngo" Station of the Royal Project Foundation Development Center, Chiang Mai, Thailand (The highland area and low temperature). Thirty fruit were harvested at every ten days after fruit set (DAFS) for 6 times (10, 20, 30, 40, 50 and 60 DAFS, totaling 180 fruit). Then, they were transported to a Postharvest Technology Research Center Laboratory, Chiang Mai University, Chiang Mai, Thailand by refrigerated truck under controlled temperature at 25°C. All fruit were subsequently assessed for physico-chemical properties.

Determination of physical characterization

Each fruit was individually measured for fruit weight by a digital balance (2 positions, Mettler Toledo, Switzerland). Fruit height and diameter were recorded by digital vernier caliper (Mituyo, Japan) prior to further quality determination.

Determination of physico-chemical properties during fruit development

Color determination

Rind and pulp color were determined using color meter (CR-400, Minolta, Osaka, Japan). Three readings L^*, a^* and b^* values were taken and transformed to hue angle (H^*) and chroma (C^*). The measurements were made on 4 positions for each fruit sample.

Specific gravity (SG)

Each intact pumpkin fruit was collected its SG by measuring fruit weight (g) in air and in water. Afterward the SG was calculated by using Equation (1) (Haq and Rab, 2012).

$$SG = \left(\frac{weight\ in\ air}{(weight\ in\ air - weight\ in\ water)}\right) \quad (1)$$

Fruit firmness determination

Fruit firmness was measured at two positions on fruit equatorial line by texture analyzer (TA.XT plus, Texture Analyzer, England). Stainless steel cylinder probe with 6.0 mm diameter was used for punching into fruit rind and pulp at 10.0 mm of depth. The speed of probe was 1.0 mm/sec. Firmness was measured as the maximum force (gram) required for the probe to penetrate into the fruit.

Moisture content (MC) and dry matter (DM) determination

A hot-air oven (Air oven, Venticell 111, MMM Medcenter Einrichtungen GmbH, Germany) was used to determine MC by setting temperature at 105ºC for 48 hours (AOAC, 2000). Moisture content was reported as percent wet basis (%wb) calculated from Equation (2). Dry matter was also determined by the aforementioned apparatus, however setting temperature at 70ºC for 48 hours (AOAC, 2000). The DM was reported as percent dry matter (%DM) obtained from Equation (3).

$$MC(\%wb) = \left(\frac{(fresh\ weight - dry\ weight)}{fresh\ weight}\right) \times 100 \quad (2)$$

$$DM\ (\%) = \left(\frac{dry\ weight}{fresh\ weight}\right) \times 100 \quad (3)$$

Carotenoids and chlorophyll concentrations determination

Carotenoids and chlorophyll concentrations were determined by methods of Joomwong (2003) with some modifications. Ten grams of pumpkin (pulp and rind) were blended by a high-speed blender prior to adding 20 ml 95% alcohol. The sample was kept in a refrigerator for 24 hours, then it was filtered through filter paper Whatman No.1. Filtered solution was measured its optical density (O.D.) at 420, 447, 645 and 663 nm by spectrophotometer UV-VIS (Specord 40, Analytik jena, Germany). Carotenoids and chlorophyll concentrations were calculated by Equation (4) and (5), respectively.

$$\text{carotenoids concentration (mg/100g)} = A \times \frac{454}{196} \times L \times W \quad (4)$$

Where

A = Value of abs. optical density at 420, 447 and 474 nm

L = Cell length in centimeter.

W = gram product/ final dilution Convent by Cx0.22

chlorophyll concentration (mg/100g) (5)

$$total\ chlorophyll = (20.2D_{645} - 8.02D_{663}) \times V/1000W$$

$$chlorophyll\ a = (12.7D_{663} - 2.69D_{645}) \times V/1000W$$

$$chlorophyll\ a = (22.9D_{645} - 4.68D_{663}) \times V/1000W$$

Where

D=value of abs. optical density at 645 and 663 nm

V=volume of pigment solution (ml)

W=fresh weight of sample (g)

Crude fiber determination

Crude fiber of Japanese pumpkin was done by modified method of Joomwong (2003). One hundred

grams of samples were blended in 100 ml boiled water for 10 minutes. Subsequently, 12.5 ml of 50% NaOH solution was added and mixed thoroughly for 15 minutes in order to obtain fiber extract. The fresh fiber was washed and drained through 30 meshes net prior to drying in hot air oven at 105ºC for 2 hours. Dried residue was weighed and calculated for crude fiber by Equation (6).

$$crude\ fiber\ (\%) = \left(\frac{dry\ weight\ of\ fiber}{fresh\ weight}\right) \times 100 \qquad (6)$$

Starch, sugar and total soluble solids contents determination

Starch content was measured by a total starch assay kit (Megazyme, Catalog No. K-TSTA, Wicklow, Ireland). Dried sample was screened through a 0.5 mm screened mill "Cyclotec 1093" (Foss Tecator AB, Hoganas, Sweden) then added the flour sample (100 mg) into 5.0 ml of aqueous ethanol (80% v/v), and incubated at 80ºC for 5 minutes. Mixed the content and added 5.0 ml of 80% aqueous ethanol. Then centrifuged at 3,000 rpm for 10 minutes and discard supernatant. Immediately added 3.0 ml of thermostable α-amylase in MOPS buffer (50 mM, pH 7.0) and incubated in a boiling water bath for 6 minutes. Placed the tube in a water bath at 50ºC, added sodium acetate buffer (4 ml, 200 mM, pH 4.5), followed by amyloglucosidase (0.1 ml, 20U) and mixed thoroughly for 30 minutes. Transferred the solution to 100 ml volumetric flask and adjusted the volume to 100 ml with distilled water. Mixed and centrifuged the solution at 3,000 rpm for 10 minutes. Transferred sample solution to test tube then added 3.0 ml of GOPOD reagent, and incubated the tube at 50ºC for 15 minutes. Then, the absorbance at 510 nm for sample and glucose control was read against the reagent blank by spectrophotometer (UV/VIS model specord 40 Analytik, Jena, Germany). The amount of starch contents was calculated as 90% of measured glucose (McCleary *et al.* 1997) as explained in Equation (7).

$$starch = \Delta E \times F \times \left(\frac{1}{W}\right) \times 90 \qquad (7)$$

Where:

ΔE = Absorbance (reaction) read against the reagent blank

F = 100 (µg of glucose) / abs. of 100 µg of glucose

W = The weight in milligrams of the flour being analyzed

Sugar content was determined by means of glucose, fructose and sucrose with modified method of Saranwong (2003). The sugars were extracted by homogenizing the sample with 5.0 ml of distilled water. The sample was centrifuged at 1,000 rpm for 5 minutes, and subsequently collected the supernatant. The final volume was adjusted to 10.0 ml using distilled water. Suspended solids were filtered through 0.45 µm model Watchman No. "507". Thereafter, 20 microlitres of aliquot was injected to measure each sugar concentration. The HPLC (Hewlett Packard Model 1100 Series, Agilent Technologies, Inc., Waldbrom, Germany) was operated under the following conditions; column: shincarbon ST, mobile phase: water:acetonitrile (75:25), flow rate: 1.0 ml/sec, column temperature: 25 ºC and detection: RI detector. Total soluble solids content (TSS) was determined from pumpkin juice by a refractometer PAL-1 (ATAGO, Japan).

Sensory evaluation

The evaluation was done by 10 trained panels consisting of five females and five males (age 25-35 years). All panelists had experience and were trained in evaluating cooked Japanese pumpkin quality from former experiments. Japanese pumpkin fruit were rated for cooked quality attributes. Overall color using score of 10 to 1, in which 10 indicated like extremely and 1 was dislike extremely. Furthermore, stickiness was scored from 1 to 10, in which 1 was considered the lowest stickiness level and 10 indicated the highest stickiness level. Sweetness was scored from 1 to 10, in which 1 was considered the lowest sweetness level and 10 showed the highest level. Softness was scored from 1 to 10, in which 1 was considered the lowest softness level and 10 indicated the highest level. Then overall acceptable in term of consumer acceptability was also tested to determine the acceptability of cooked Japanese pumpkin in each stage using a scored in a scaling test by using 10-point scale (1 = dislike extremely; 5 = neither like nor dislike; and 10 = like extremely). The overall acceptability of cooked Japanese pumpkin was classified as unacceptable when the score was below 6.

Statistical analysis

Statistical analysis was performed using SPSS v.16.0 software (SPSS Inc., IL, USA). All the experiments were conducted in triplicate and the mean ± standard deviation of three values are reported. Data were initially evaluated using analysis of variance (ANOVA) and Duncan's multiple range test was used to determine significance ($P < 0.05$) between samples.

Table 1. Physical characterization of Japanese pumpkin during fruit development

Fruit age	Fruit weight (g)			Fruit diameter (cm)			Fruit height (cm)		
	Min	Max	Average	Min	Max	Average	Min	Max	Average
10 DAFS	21.32	30.61	25.87±3.68	3	3.5	3.27±0.17	3.2	3.7	3.53±0.14
20 DAFS	612.5	867.5	771.60±113.14	10.9	12.9	9.80±0.76	9	11.1	9.86±0.85
30 DAFS	1881	2231	2028.40±152.34	16.5	18.8	17.72±1.01	9.1	10.3	9.96±0.49
40 DAFS	1850	2570	2252.22±244.12	18	20.9	19.88±1.04	10.2	12.2	11.41±0.84
50 DAFS	2107	2548	2377.20±177.95	18.5	21.2	19.36±1.16	9	11.8	10.72±1.04
60 DAFS	2223	2957	2539.40±408.21	18.6	24.2	20.51±2.16	9.2	11.1	10.26±0.77

:DAFS=Day after fruit set; Min= Minimum value; Max=Maximum value
:The values are the means of thirty determinations ± standard deviation

Results and Discussion

Physical characterization

Physical properties of the Japanese pumpkin are shown in Table 1. Shama and Ramana Rao (2013) reported that the developmental process is characterized by irreversible increase in volume as consequences of cell division and cell elongation. In the present study, the fruit of Japanese pumpkin were about 3.53±0.14 cm in height, 3.27±0.17 cm in diameter, and weighed 25.87±3.68 g at 10 DAFS. The fruit dimensions increased gradually along an increase of DAFS, in which, the fruit reached up to their maximum values at 60 DAFS with 10.26±0.77 cm in height, 20.51±2.16 cm in diameter and weighed 2539.40±408.21 g. The results indicated that fresh weight gain of Japanese pumpkin fruit is mainly achieved by an increase in its diameter rather than the height. Fruit development and maturation of Cucurbita can be divided into three phases: (i) fruit expansion, (ii) dry matter and starch accumulation in pericarp tissue, and (iii) seed maturation (Loy 2004).

Physico-chemical properties during fruit development

Color development

On the basis of color evaluated in CIE system, lightness *(L^*)* of zero denotes black and one hundred indicates diffused white. Chroma value *(C^*)* represents the purity or intensity of a color. The hue angle *(H^*)* represents the actual color of sample surface. Figure 1a and 1b illustrate the changes of rind and pulp color, respectively. At early stage, the color was notably turned rather than the once observed in the later period. Rind color started with bright green at 10 DAFS and successively turned into dark green after fruit development at 60 DAFS. The average of color parameter L^*, Chroma *(C^*)* and Hue angle *(H^*)* in the rind of the Japanese pumpkin fruit at 10 DAFS were 54.65, 28.57 and 120.2°, respectively, while the highest hue angle value of rind color (141.41°) was recorded at 40 DAFS. The rind color parameter L^*, C^* and H^* at 60 DAFS were 32.29, 11.15 and 80.2°, respectively (Figure 1a).

Interestingly, pulp color development changed from bright green to dark yellow during fruit growth. Pulp color appeared in bright green at 10 DAFS (L^*=80.47, C^*=43.88, H^*=104.35°), then turned into dark yellow after fruit development at 60 DAFS, reaching L^*, C^* and H^* at 66.63, 70.57 and 80.2°, respectively. Pulp color development showed a rapid increase within 60 DAFS.

Specific gravity (SG)

Typically, the specific gravity of fruit is generally correlated with chemical compositions such as starch content, dry matter, cell size and intercellular spaces and has been used as maturity and/or quality index in several fresh horticultural commodities, such as apricots, strawberries, tomato, mango and peach (Zaltzman *et al*., 1987; McGlone *et al*., 2007). Figure 1c shows a gradual decrease of SG during fruit development. The SG reached its minimum value at 50 DAFS before arising during the last 10 days. This scenario supported that maturity stage was correlated with the size of internal cavity of the pumpkin fruit during fruit development (Harvey *et al*. 1997).

Fruit firmness

Fruit firmness is one of the main factors limiting quality and the postharvest quality in Japanese pumpkin fruit. Figure 1d illustrates the changes in fruit firmness during fruit development. The result showed that rind firmness increased gradually and reached the highest value at 50 DAFS (raised from 4846.74 g at 10 DAFS to 16884.60 g at 50 DAFS). However it continued to decline and arrive to 15831.93 g at 60 DAFS. Likewise rind firmness,

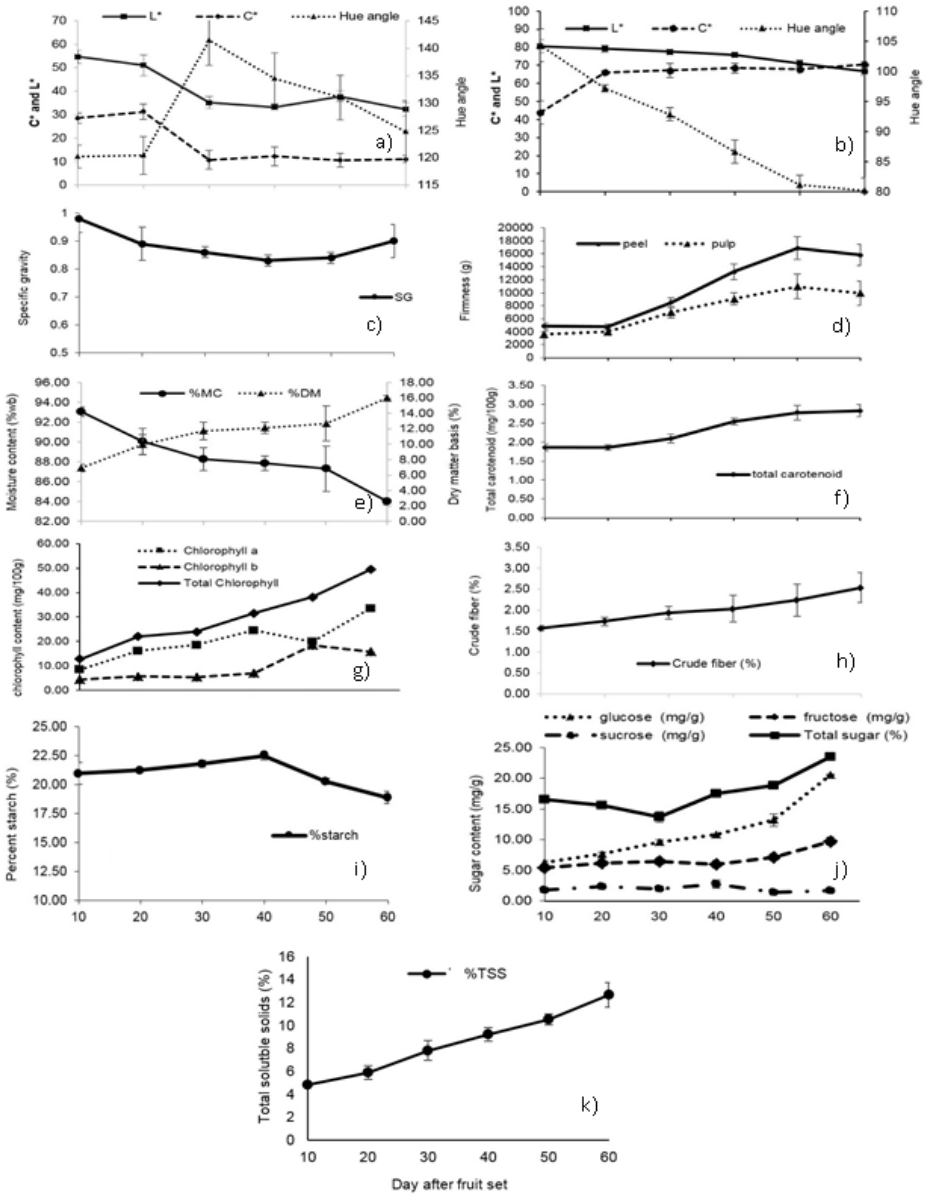

Figure 1. Change of physico-chemical properties of Japanese pumpkin fruits during fruit development

pulp firmness determined in this study apparently increased from 3598.21 g at early stage (10 DAFS) to 10970.55 g in 50 DAFS. Then it slightly decreased to 9925.91 g afterward. The texture properties of fruit and vegetables are mainly due to the cell wall and polysaccharide components. The firmness declined during fruit maturation and fruit firmness began to lose and soften after maturation (Joomwong, 2004). The softening during fruit development is associated with fruit ripening. During fruit ripening, the physiological changes of pumpkin fruit literally related with characterized action of changing in cell wall, middle lamella structure and the action of enzyme such as polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase and cellulase (Huber, 1983; Tucker and Grierson, 1987; Sharma and Ramana Rao, 2013).

Moisture content and dry matter

Dry matter is one of the most important properties that directly related to quality of squash fruit (Ferriol and Pico, 2008). In our study, the results showed that dry matter of pumpkin fruit increased during fruit growth. On the other hand, the result of moisture content at early stage (10 DAFS) was 93.07%wb which was greater than at later stage (60 DAFS), 84%wb. This result corresponded to the studies of Saeleaw and Schleining (2011), who reported that the composition of fresh pumpkin fruit at maturity stage exhibited high level of dry matter and carbohydrate, on the other hand, low content of fat (0.15%), protein (0.98%), ash (0.76%), crude fiber (0.58%) and moisture content (70%). Figure 1e clearly illustrates the decrease tendency of moisture content along fruit development. Percent dry matter of Japanese pumpkin fruit rapidly increased from

10 DAFS (6.93%) to 60 DAFS (16.0%). Generally, Japanese pumpkin contains the highest dry matter when it reaches fully mature stage. Moreover our results revealed a relatively lower than that observed by Nakkanong *et al.* (2012). Likewise, Harvey *et al.*, (1997) reported that percent dry matter of squash mesocarp did not change significantly from the first day after pollination (DAP) to 12 DAP but after it rapidly increased until it reached the maximum value at 60 DAP. Typically, dry matter contents in kabocha and buttercup cultivars *(C. maxima)* were considered to the highest quality fruit containing dry matter which was accounted as about 20-30%.

Carotenoids accumulation

Carotenoids contents were assessed on the basis of mg/100g of β-carotene equivalents. The changes of carotenoids content are shown in Figure 1f. The carotenoids content in Japanese pumpkin fruit showed an increased tendency since initial stage until 60 DAFS. Carotenoids content varied from 1.86 to 2.83 mg/100g. The highest amount was found in 60 DAFS (2.83 mg/100g), followed by 50 and 40 DAFS (2.77 and 2.57 mg/100g, respectively). The accumulation of certain compounds is called carotenogenesis, which is typically occurred during ripening stage of pumpkin fruit (Sharma and Ramana Rao, 2013). Carotenoids dramatically increased by eleven folds in its young and ripened stage (0.67±0.03 to 7.47±0.10 mg/100 g FW, respectively). In addition, Murkovic *et al.* (2002) determined the range of the carotenoids from 0.06 to 7.4 mg/100 g for β -carotene, from 0 to 7.5 mg/100 g for α -carotene and from 0 to 17 mg/100g for lutein in pumpkin fruit. About total carotenoid content, increased during maturation and ripening stage of carotenogenic fruit and vegetables. The biosynthesis and metabolism of carotenoids in vegetables were significantly affected by the difference in fruit development environment, for example, temperature, soil, light intensity and ripening stage as reported by Sharma and Ramana Rao (2013). During pumpkin fruit maturation, carotenoids dramatically increased, responsible for development of color from light yellow at young stage to dark yellow on ripening at 50-60 DAFS. In addition, *C. maxima* and *C. moschata* were found to be enriched in α-carotenoid, β-carotenoid and lutein (Provesi *et al*., 2011).

Chlorophyll accumulation

In Figure 1g, chlorophyll content of pumpkin fruit during 50-60 DAFS accumulated slightly greater than fruit at 10-40 DAFS. In which total chlorophyll content of pumpkin fruit at 10 DAFS was obviously lower than the fruit at full maturation (12.65 and 49.42 mg/100g, respectively). In pumpkin fruit, chlorophyll accumulation typically occurred in two phases; growth and maturation. Our results showed that the compounds were accumulated during maturation (8.92 mg/100g) rather than the growth phase (6.31 mg/100g). Likewise, chlorophyll a and b also increased after fruit development; from 8.33 to 33.60 mg/100g in chlorophyll a and from 4.32 to 15.85 mg/100g in chlorophyll b.

Crude fiber

Japanese pumpkin crude fiber continually increased during fruit development (figure 1h). This result corresponded to that of pumpkin (*Curcubita moschata* D.) reported by Alfawaz (2004) and Valenzuela *et al.* (2011). In addition, the crude fiber also indicated the similar range as determined by Saeleaw and Schleining (2011).

Starch content, sugar content and total soluble solids

In this study, Figure 1i showed that Japanese pumpkin accumulated starch approximately up to 40 DAFS, and thereafter declined towards to 60 DAFS. The result showed an increase in concentration of total starch during fruit development. The initial total starch was recorded in 10 DAFS (20.96%) and the highest amount was found in 40 DAFS (22.47%). Starch content gradually decreased to 18.88% when it reached 60 DAFS. In *Cucurbita* species, Ferriol and Pico (2008) reported that starch is the main component of fruit mesocarp, so-called dry matter, which is responsible for flesh dryness. It is one of the factor that directly related to eating quality of squash fruit. Starch is the main storage carbohydrate in early stage of fruit development, which degrades with the onset of ripening. Sharma and Ramana Rao (2013) reported that the pumpkin fruit accumulated starch approximately by two-fold up to premature stage (138.47±0.7 mg/g FW), and thereafter declined towards the ripening (68.55±0.7 mg/g FW). Starch content was related with the texture of squash fruit and quality. Squash was higher in dry matter and starch content at harvest. Moreover, starch and dry matter contents were correlated well with texture attributes of *Cucurbita maxima* (Hurst *et al*., 1995; Corrigan *et al.*, 2001; Cumarasamy *et al.*, 2002). Nakkanong *et al.* (2012) reported that starch content increased rapidly at the early stage of fruit development and rose to the peak at 48 days after pollination, followed by slight decrease at 60 days after pollination.

Total sugar concentration illustrated in Figure 1j shows the increased tendency from 16% FW at early stage of fruit development to 23.55% FW at the later

stage. The scenario was found to be coincided with the decreased pattern of starch content. Sucrose content exhibited a different trend to the values observed for glucose and fructose. The lowest glucose (6.31 mg/g) and fructose (5.37 mg/g) were recorded in 10 DAFS, while the lowest sucrose was recorded in 50 DAFS (1.43 mg/g). Cumarasamy *et al.* (2002) reported that glucose and fructose varied from 2.43 to 12.48 mg/g in six uncooked squash *(Cucurbita maxima)* cultivars. On the other hand, they were higher in sucrose and dry matter at commercially-mature stage. Fruit ripening is associated with the pattern of sugar metabolism and catabolic processes, leading to degradation of starch into glucose and fructose, which enters the metabolic partway where they are used as respiratory substrates. (Paliyath and Murr, 2008). The mechanism of starch formation along with the corresponding enzymes and genes responsible for starch biosynthesis was investigated in Cucurbita species.

The result showed that total soluble solids changed during fruit development. The lowest TSS (4.85%) was recorded in 10 DAFS. The results regarding TSS changes were shown in Figure 1k. The highest TSS was found in 60 DAFS (12.68 %). The increases in TSS, total sugar and sugar contents may be attributed to hydrolysis of starch into simple sugars (Biale, 1960; Kulkarni and Aradhya, 2005). Starch is the main carbohydrate in early stage of fruit development of Japanese pumpkin, which degrades along the ripening stage. Changes in the sugar content and TSS of Japanese pumpkin fruit were closely related to the decrease in starch. Total soluble solids, sugar and starch were reported as important maturity indices for Cucurbita maxima (Biale, 1960; Loy, 2004; Ferriol and Pico, 2008).

Sensory evaluation

Figure 2 shows the scores of all parameters used for quality evaluation. The evaluation revealed that cooked Japanese pumpkin fruit at 50 DAFS had the highest acceptability score for color, firmness, softness and overall acceptability. While other stages had significantly lower acceptability (scored less than 6.0) in terms of color, firmness softness and overall satisfaction than the fruit at 50 DAFS. The sweetness score of cooked Japanese pumpkin gradually increased during fruit development, in which, the score of over mature stage showed higher score than other stages.

Conclusions

Changes of physico-chemical properties of Japanese pumpkin fruit during fruit development were studied. Fruit rind color changed from bright green at the early stage to dark green at mature stage, while pulp color changed from bright green to dark yellow along fruit development. During 10 DAFS to 60 DAFS, pumpkin fruit gradually accumulated considerable amounts of dry matter, total soluble solids, total sugars, total carotenoids, total chlorophylls, starch content and crude fiber. Whilst moisture content of fruit pulp slightly declined along the aforementioned periods. The optimum harvesting time of Japanese pumpkin fruit was found to be at 50 DAFS due to the quality was considerably met consumers' acceptability.

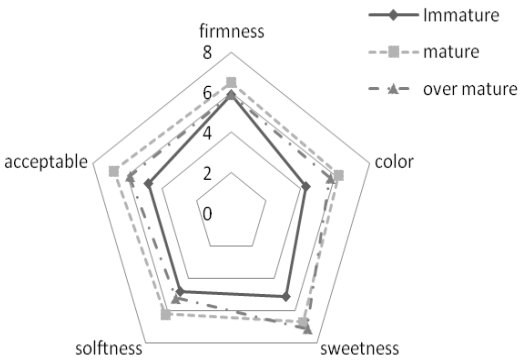

Figure 2. Spider plots of five sensory attributes of cooked Japanese pumpkin fruit at each stage of maturation

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