

## Fruit Quality Changes of Salak “Pondoh” Fruits (*Salacca zalacca* (Gaertn.) Voss) during Maturation and Ripening

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### Abstract

Salak is a very important fruit product in Indonesia that has been cultivated throughout Indonesia and has been exported to several countries. The study to determine biochemical and physical as well as physiological changes during fruit maturation and ripening was applied to two superior salak cultivars, “pondoh hitam” and “pondoh super” originally from Sleman, Yogyakarta of Indonesia. Fresh salak fruits of the cultivars “pondoh hitam” and “pondoh super” at three different ripening stages were used, i.e. stage 4, stage 5 and stage 5.5 (4, 5 and 5.5 months after pollination, respectively) for “pondoh hitam” and stage 4, stage 5 and stage 6 (4, 5 and 6 months after pollination, respectively) for “pondoh super”. Immediately after air transport from Indonesia to Germany, determination of fruit colour and texture as well as biochemical analyses were carried out in Berlin. Freeze-dried sample material was used for the determination of minerals, mono- and disaccharides, pectic substances and dietary fibre. Results of the study showed that increase in fruit size and weight as well as changes in peel and pulp colour occurred during maturation and ripening of salak fruits. Different patterns of peel and pulp colour changes were found in “pondoh super” and “pondoh hitam” during ripening. Physiological processes in “pondoh super” occurred to at a later stage but then accelerated faster than “pondoh hitam” in term of changes of mono- and disaccharides, resulting in a poorer marketability and shorter shelf life. In respect to the changes of sugar/acid ratio, there was a faster ripening process in “pondoh super” than in “pondoh hitam”. “Pondoh super” possessed higher content of polysaccharides and lignin, however, the ripening process accelerated earlier in comparison to “pondoh hitam”. Alterations in cell wall and middle lamella structure did not correlated with the physical non destructive texture measurement during ripening of salak.

**Keywords:** *Salacca zalacca*, “pondoh” cultivar, fruit quality, maturation, ripening

### 1. Introduction

Since fresh or processed fruit form an important part of the human diet, there is an increasing demand for both improved quality and extended variety of available fruits (Seymour et al., 1993). Commercially, trade is dominated by a relatively small number of fruit species such as grape, banana, citrus and apple. However, western consumers are becoming more aware of exotic fruits and the trade volume of these commodities is increasing rapidly (Seymour et al., 1993). Among exotic fruits, the salak fruit (*S. zalacca* (Gaertn.) Voss) has a high potential as an export crop.

In Indonesia, salak has been cultivated throughout the islands and the fruit is widely used as fresh fruit. According to the Ministry of Agriculture of Indonesia in 2011, salak production in Indonesia has increased from 423.5 t in 2000 to 862.5 t in 2009. Fresh fruits of this cultivar have also been exported to Singapore, United Kingdom, Malaysia, Thailand, Hongkong and Saudi Arabia (Djaafar, 1998). There are 30 cultivars of salak, which distribute across the Indonesian islands (Sudaryono et al., 1993; Kusumo, 1995). The availability of superior quality fruit of selected cultivars will promote the marketing of salak, such as cultivars “pondoh” from Yogyakarta and “gula pasir” from Bali provinces. Among the various cultivars, “pondoh” is known to reveal superior quality, especially

in respect to its sweeter taste without bitter or sour components in comparison with other cultivars, even at early ripening stages.

Salak belongs to a group of palms, which are 1.5 - 5 m high, extremely spiny and sprout their leaves from the ground level. Salak palms grow as under-storey plants in the low lands of tropical rain forests in Indonesia and other Southeast Asian countries. Fruits of salak are located in tight, globose bunches, round, 2.5 - 10 cm x 5 - 8 cm across. They are covered with regularly arranged scales developing from the peel of the fruit (pericarp) giving it the appearance of a snake or reptiles' skin. The aromatic fruits enclose a soft, translucent pulp with a taste comparable to a combination of apple, pineapple and banana (Schuiling & Moge, 1992). Fruits contain 1 to 3 blackish kernels, which are about 1 cm in diameter (Ochse, 1931).

During fruit development, significant changes e.g. in texture, content of sugars, acids and minerals will occur (Bollard, 1970; Tucker, 1993). Texture modifications which cause softening usually also occur during postharvest ripening of fruits (Reid, 2002). It has been well established that textural changes in fruits are consequence of modifications undergone by polysaccharides that, in turn, give rise to disassembly of primary cell wall and middle lamella structures (Jackman & Stanley, 1995).

In the process of ripening, salak fruits experience an increase of weight, size, percentage of edible portion, sugar/acid ratio, vitamin C, ashes, starch, and content of glucose, fructose, sucrose and total sugar (Sosrodihardjo, 1986; Suhardi, 1997; Tranggono, 1998; Supriyadi et al., 2002). However, a decline in the percentage of peel to fruit ratio, water content, tannins and acids will occur during ripening (Sosrodihardjo, 1986; Suhardi, 1995). The pulp firmness of "pondoh" increase until 174 N until stage 5.5 but then declined to the end of ripening period (stage 6) to 130 N (Supriyadi et al., 2002).

Recommendations are required for determining the optimal developmental stage of salak fruits at harvest in respect to nutritional and sensory attributes as well as to postharvest and storage concerns. Increasing the knowledge about the fruit is prerequisite for optimising the consumer-oriented fruit quality and for extending the marketing period, especially for the export market. Therefore, the aim of the study was to determine biochemical, physical and physiological changes during fruit maturation and ripening of two salak cultivars, "pondoh hitam" and "pondoh super".

## 2. Materials and Methods

### 2.1 Plant Material

For this study, fresh salak fruits of the cultivars "pondoh hitam" and "pondoh super" at three different ripening stages were used, i.e. stage 4, stage 5 and stage 5.5 (4, 5 and 5.5 months after pollination, respectively) for "pondoh hitam" and stage 4, stage 5 and stage 6 (4, 5 and 6 months after pollination, respectively) for "pondoh super". The salak fruits used for the study were grown in the Sleman district, Yogyakarta (Indonesia) and were harvested in September 2003. Immediately after air transport from Indonesia to Germany, the physical measurements and chemical analyses were carried out at the laboratory of the Humboldt University in Berlin. Freeze-dried sample material was used for the determination of minerals, mono- and disaccharides, pectic substances and dietary fibre. The results are expressed on a dry matter (DM) basis.

### 2.2 Fruit Properties

The fruit ( $n = 7$ ) of each ripening stage was measured (length and diameter) using callipers. Fresh fruits were weighed and the edible portion was determined. Dry weight of each ripening stage was determined after drying the sample at 103°C for 24 hours until constant weight (Maier, 1990).

### 2.3 Colour

Measurements of the fruit peel and pulp colour were conducted using a Minolta Colorimeter (CR-321, Minolta, Japan) with a standardised light type D65. The equipment was calibrated with a white standard plate. Colour measurement were expressed in the  $L^* a^* b^*$  scale, where  $L^*$  indicates the luminescence on a 0 to 100 scale from black to white. The colour coordinates of  $a^*$  and  $b^*$  locate on a rectangular-coordinate grid perpendicular to the  $L^*$  axis with 0 to 60 scale. The colour at the grid origin ( $a^* = 0, b^* = 0$ ) is achromatic (gray). On the horizontal axis, positive  $a^*$  indicates red and negative  $a^*$  green colour. On the vertical axis, positive  $b^*$  indicates yellow and negative  $b^*$  blue colour. Chroma represents the hypotenuse of a right triangle created by joining points (0, 0), ( $a^*, b^*$ ) and ( $a^*, 0$ ). As chroma increase, colour becomes more intense. Hue angle is the angle between the hypotenuse and 0° on the  $a^*$  (green/red) axis. Hue angle of 0° indices red, 90° yellow, 180° green and 270° blue. Chroma was calculated as follows:

$$\text{chroma} = \sqrt{(a^*)^2 + (b^*)^2}$$

whereas hue angle was computed as follows:

$$\text{hue angle} = \tan^{-1} (b^*/a^*) \text{ (McGuire, 1992).}$$

Colour was recorded on each of the fruits per cultivar. Peel colour was recorded on the equator region of each fruit with eight measurements. Peel colour was computed on the tip and base regions of each fruit with four measurements.

#### 2.4 Firmness

The pulp firmness was measured non-destructively using a Shore A instrument (HHP-2001, Bareiss, Germany) equipped with a 2.5 mm diameter probe and a standardized metal disc. The measurement for each fruit was carried out on 3 equidistant records of the equatorial region. The average value per treatment was computed on 7 fruits per cultivar and was expressed as Shore a units.

#### 2.5 Soluble Solid Content and Titratable Acidity

Measurements of soluble solid content (SSC) were performed on extracted juice of 8 fruits for each ripening stage with two replicates. SSC was measured using a digital refractometer (model PR 101, Kuebler, Germany). A calibration with distilled water was conducted before each measurement. Refractometric reading was expressed as index of refraction (°Brix) at 20°C (AOAC, 1990). Measurement of the titratable acidity was carried out by titration of juice samples with 0.1 N NaOH up to pH 8.1 (AOAC, 1990) and expressed as malic acid, the dominant organic acid in salak fruit. The sugar/acid ratio was calculated as SSC/titratable acidity.

#### 2.6 Minerals

Freeze-dried fruit samples (500 mg), triplicates per each ripening stage, were used for the determination of K, Ca, Mg and P. Fruit mineral contents were analysed from dried and ground samples (Mikro-Feinmühle-Cullati DCFH 48, Janke & Kunkel, Germany) and were analysed according to a modification method as described by Evenhuis and de Waard (1980). The samples were dry-ashed at 490 °C for 4 h. Ashes were dissolved in 25% HCl and were evaporated in a sand bath for 20 min until the complete disappearance of solid residues. P was determined photometrically (Eppendorf 1101 M, Germany). The other minerals were analysed using atomic absorption spectrophotometry (AAS) (905 A, GBC, Australia). The data of the minerals were expressed as mg/g DM.

#### 2.7 Mono- and Disaccharides

Mono- and disaccharides (glucose, fructose and sucrose) were determined by High Performance Liquid Chromatography (HPLC) (Ulrichs, 1999). Analyses were performed in triplicates per cultivar and the data were expressed in mg/g DM. Freeze-dried samples (100 mg) were added with 80% ethanol, and placed in a stirring water bath at 70°C for 20 minutes. Thereafter, the samples were centrifuged at 3000 rpm for 15 min. The supernatant was collected in a volumetric flask, whereas pellets were stirred with 80% ethanol. Extraction with ethanol was conducted three times, thereafter HPLC water was added to the samples. The supernatants were evaporated in a rotavapor (RE 120, Buechi, Switzerland). A defined volume of HPLC water and saturated lead acetate solution were added to the dried samples, which were centrifuged thereafter at 3000 rpm for 20 min. The supernatant was transferred to an Eppendorf-funnel with ion exchanger V (Merck) and shaken for 30 min. Finally, the samples were purified in extract clean columns (C18, Alltec, Germany) and kept at -30°C until further analysis (HPLC).

A HPLC instrument (Model 25, Fa. Bischoff, Germany), equipped with an autosampler (Alcott 708) and a RI-detector (8110, Fa. Bischoff, Germany) was used. A waters-spherishorb amino (250 mm x 3.0 mm) column was used with a 3 µm-packing material. The mobile phase was acetonitrile/water (85:15). HPLC was operated at an ambient temperature with a flow rate of 1 ml/min. 10 µl sample was injected into the autosampler. Quantification was accomplished by determination of the area under the chromatographic peak and calculation of the level of each component on the basis of standard curves generated with pure compounds. Standard sugar solution (Fa. Seroa and Boehringer, Germany) contained 10 µg/10 µl of both for fructose and glucose, and 25 µg/10 µl of sucrose.

#### 2.8 Pectic Substances

Freeze-dried fruit samples (1.5 g) were used for the determination of pectin. Cell walls were prepared following the method described by McComb and McCready (1952), Blumenkrantz and Asboe-Hansen (1973) and modified by Huyskens (1991). Triplicates of 1.5 g freeze-dried per ripening stage fruit samples were mixed with 100 ml

99.9 % acetone and boiled for 30 min. Thereafter, the suspension was vacuum filtered. The residue on the filter paper (Schleicher and Schuell No 589/3) was resuspended in 99.9% acetone, 70% ethanol and again in 99.9% acetone to remove mono- and disaccharides. The final white residue on the filter paper was dried overnight at 70°C. The alcohol insoluble solids (AIS) fraction was weighed and stored in a vacuum desiccator.

AIS were fractionated into water soluble pectin (WSP), ethylene diamine tetra acetic acid-soluble pectin (EDTA-SP) and insoluble pectin (ISP) according to the method of Blumenkrantz and Asboe-Hansen (1973). For the determination of WSP, AIS was mixed with 20 ml distilled water using a magnetic stirrer (IKA-Labortechnik, Germany) at room temperature for 1 h. The solution was brought to pH 4.5 by adding 1:1 diluted acetic acid. Then 0.1 ml pectinase (ca. 20 µg) (Pectinex Ultra SP-L, Novo Nordisk Ferment Ltd, Dittingen, Switzerland) was added to the solution, which was stirred for 1 h. The solution was placed in 100 ml centrifugal tubes and centrifuged (Biofuge 22R, Heraeus Sepatech, Germany) at 4°C at 11.000 rpm for 10 min. The supernatant and the pellets were filtered through Miracloth in a 50 ml volumetric flask. The flask was filled with 0.5% EDTA-solution (pH 4.5) up to 50 ml. The supernatant was refiltered through a filter paper (Schleicher and Schuell No 589/3). The remaining pellet was kept in deep freezes for further extraction of the EDTA-SP fraction. For this extraction, a similar procedure as for water soluble pectin fraction was applied. Instead of mixing the AIS samples with 20 ml distilled water, the pellet of the WSP fraction was mixed with 20 ml 0.5% EDTA (pH 6.0). The remaining pellet at the end of procedure was kept for further extraction of ISP. For this extraction, the pellet of the EDTA-SP fraction was mixed with 20 ml 0.5% EDTA (pH 11.5) and stirred for 30 min. After 10 min, the pH was adjusted to 11.5.

The pectin fractions WSP, EDTA-SP and ISP were determined as described by McComb and McCready (1952). One aliquot of 0.7 ml, 0.5 ml and 0.2 ml filtrate of each fraction sample (WSP, EDTA-SP and ISP, respectively) was filled up to 1 ml by adding 0.5% EDTA-solution (pH 6). Then 6 ml of ice-cold concentrated sulphuric acid were added to the samples to solubilise the polysaccharides. The samples were boiled in a water bath at 100°C for 10 min to hydrolyse the polysaccharides to monomeric sugars and then cooled-down to room temperature. MHDP-solution 0.1 ml 0.15% was added to the sample. The pectin content was determined spectrophotometrically at a wavelength of 520 nm (UV/VIS-8730, Phillips, UK). Standard solutions of D-galacturonic acid were used for the calibration. Data were expressed as mg galacturonic acid/g DM.

### 2.9. Cellulose, Hemicellulose and Lignin

One g of freeze-dried fruit sample was used for the determination of cellulose, hemicellulose and lignin. Cellulose, hemicellulose and lignin were analysed according to the method of Goering and Soest (1972) and AOAC (1984). 1 g freeze-dried sample material was extracted with 100 ml Neutral Detergent Fibre (NDF) reagent or Acid Detergent Fibre (ADF) reagent using the hot extractor unit (Fibertec System M 1020, Tecator, Sweden) to get content of NDF or ADF respectively. NDF reagent comprised of 18.61 mol/l EDTA, 6.81 mol/l natriumtetraborat, 30 mol/l laurylsulphate, 10 ml/l ethyleneglycolmonoethylether and 4.56 mol/l dinatrium-hydrogenphosphate. ADF reagent comprised of 20 g cetyltrimethylammoniumbromide, which was adjusted to 11 by adding 1mol/l H<sub>2</sub>SO<sub>4</sub>. After extraction, the solution was vacuum filtered, washed with boiled water until acid removal and again washed with 90% acetone. NDF and ADF residues were dried at 105°C for 24 h, weighed, ash-dried at 500°C for 24 h and weighed again to calculate NDF and ADF contents.

ADF residue served as material for further lignin determination. The residues were again submerged with 72% H<sub>2</sub>SO<sub>4</sub> for 3 h at room temperature (20 to 23°C), washed with hot water until acid removal and washed again with 90 % acetone. Subsequently, the material was dried at 105°C for 24 h, continued by ash-drying at 500°C for 24 h.

NDF, ADF and lignin were calculated from the ratio before and after the samples have been ash-dried and the value was expressed in % DM. The content of hemicellulose was calculated by the difference between NDF and ADF, whereas the content of cellulose was calculated by the difference between ADF and lignin content.

### 2.10 Statistical Analyses

All data except those of soluble solid content, titratable acidity, cellulose, hemicelluloses and lignin were subjected to the standard analysis of variance (ANOVA), with significant differences between means determined ( $p \leq 0.05$ ) (Steel et al., 1997) and then further analysed with Duncan test using the statistic program SPSS 11.5 for Windows (SPSS Inc., Chicago, USA, 2000). The data of soluble solid content, titratable acidity, cellulose, hemicelluloses and lignin was taken duplicates.

## 3. Results and Discussion

### 3.1 Fruit Properties

Physical changes of salak “pondoh hitam” and “pondoh super” during ripening are summarised in Table 1. The fruit size of both salak cultivars increased significantly during the ripening process. This result is in agreement to

Sosrodihardjo (1986). Fresh weight (FW) of the fruits increased significantly during ripening from stage 4 to stage 5.5 for “pondoh hitam” by 170.8% and from stage 4 to stage 6 for “pondoh super” by 266.9%. The edible portion of both cultivars increased until stage 5 and remained constant thereafter. These findings are consistent with findings of Djaafar and Mudjisihono (1998), who reported that fruit weight and edible portion of salak “pondoh hitam” and “pondoh super” cultivars increased until stage 5 and then remained constant until stage 6. Supriyadi et al. (2002) reported that fruit weight and edible portion of salak pondoh increased during maturation. Dry weight of salak fruits increased during ripening by 11% from stage 4 to stage 5.5 for “pondoh hitam” and by 5% from stage 4 to stage 6 for “pondoh super”. This indicated that the fruit growth in terms of volume increase continuously during maturation and ripening period.

### 3.2 Colour

The peel colour values ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma and hue angle) of both salak cultivars increased significantly during ripening (Table 1), indicating lighter peel with more red and yellow components. Colour values of “pondoh super” were higher at all developmental stages in comparison to those of “pondoh hitam”. These reflected a darker peel with less red and yellow colour components of “pondoh hitam” in comparison to “pondoh super”. Similar changes in the peel colour of salak during ripening were reported by Djaafar and Mudjisihono (1998), who found that peel colour changed from blackish brown to yellowish brown for “pondoh super” and from blackish brown to black for “pondoh hitam”.

$L^*$  and  $a^*$  values of the pulp of “pondoh hitam” declined during ripening, whereas  $b^*$  and chroma remained constant and hue angle increased. This indicated an acceleration in the development of green compounds (chlorophyll), but no changes in yellow pulp colour occurred during ripening. On the other hand,  $L^*$  of “pondoh super” pulp remained constant and  $a^*$  declined during ripening, whereas  $b^*$ , chroma and hue angle values increased significantly. This indicated a faster acceleration of green and yellow pulp colour compounds during ripening in “pondoh super” as compared to those in “pondoh hitam”. Pulp colour changes of both cultivars showed that there was no degradation of chlorophylls. According to Tucker (1993) the degradation of chlorophylls could in turn unmask previously present pigments, particularly  $\beta$ -carotene, which reflected a colour range from yellow to orange. Eventhough, as reported by Setiawan et al. (2001), salak fruit was found to be an excellent source of provitamin A, i.e. containing 1130  $\mu\text{g}$  lycopene/100 g FW and 2997  $\mu\text{g}$   $\beta$ -carotene/100 g FW. Most of the colour values of “pondoh super” were higher at all ripening stages in comparison to those of “pondoh hitam”, assumingly due to a progressed ripening pattern of “pondoh super”. Similar changes in the pulp colour of salak during ripening were reported by Djaafar and Mudjisihono (1998), who found that the pulp colour of “pondoh super” was more yellow in comparison to “pondoh hitam” with a whitish pulp.

Colour change of the fruit peel and pulp is associated with ripening and represents a key attribute for the determination of quality (Seymour et al., 1993). Plant pigments, which are responsible for fruit peel and pulp colouration, include chlorophylls (green), carotenoids (yellow and orange) and anthocyanins (red, blue and purple) (Kader & Barret, 1996).

### 3.3 Firmness

There were no significant differences in fruit pulp firmness of “pondoh hitam” and “pondoh super” during ripening, varying from 51.6 to 55.3 Shore A units for “pondoh hitam” and from 46.3 to 53.3 for “pondoh super”, respectively (Table 1). These values reflected a very firm fruit pulp during the entire maturation process. This result was in contrast with the finding of Supriyadi et al. (2002) that the firmness in pulp of “pondoh” fruits increased until stage 5.5, but declined thereafter to the end of ripening period. These contradictive results were possibly due to the different firmness measurement method applied. Supriyadi et al. (2002) measured the texture of a piece of salak pulp 1 x 1  $\text{cm}^2$  in size. On the other hand, the non destructive method applied in this study that involves compression and tension might not be sensitive enough for texture measurement of salak fruit pulp, which contained 1 to 3 hard kernels. At the last ripening stage 6, “pondoh super” was significantly softer than “pondoh hitam”. This might indicate a faster break down of pectic substances in the middle lamella which could lead to more higher limitation in shelf life of “pondoh super” as compared to that of “pondoh hitam”.

Table 1. Quality attributes of salak fruits at different ripening stages

Quality attribute	Salak "pondoh hitam"			Salak "pondoh super"		
	stage 4	stage 5	stage 5.5	stage 4	stage 5	stage 6
Fruit length (mm)	47.05 ± 1.26 a	53.50 ± 1.27 b	53.80 ± 1.21 b	40.84 ± 1.66 a	60.47 ± 2.76 b	70.50 ± 1.26 c
Fruit diameter (mm)	30.14 ± 0.56 a	39.00 ± 0.34 b	45.43 ± 0.73 c	32.21 ± 0.76 a	42.31 ± 1.15 b	52.84 ± 0.70 c
Fresh weight (g)	17.87 ± 0.60 a	34.59 ± 0.92 b	48.40 ± 1.04 c	20.17 ± 0.68 a	39.94 ± 1.79 b	74.00 ± 3.47 c
Edible portion (mg/g)	517.10 ± 19.9 a	592.00 ± 23.9 b	570.20 ± 11.4 ab	365.80 ± 49.4 a	665.90 ± 58.0 b	674.20 ± 18.7 b
Dry weight (mg/g)	165.55 ± 0.80 a	183.40 ± 9.00 b	186.20 ± 1.40 b	177.90 ± 12.4 a	195.90 ± 2.50 a	186.20 ± 9.50 a
Peel L*-value	29.15 ± 0.25 a	29.87 ± 0.16 a	30.96 ± 0.13 b	30.34 ± 0.37 a	33.16 ± 0.59 b	33.59 ± 0.48 b
Peel a*-value	4.87 ± 0.25 a	5.24 ± 0.17 a	7.51 ± 0.21 b	7.66 ± 0.33 a	10.19 ± 0.43 b	10.14 ± 0.37 b
Peel b*-value	3.02 ± 0.24 a	3.50 ± 0.16 a	5.21 ± 0.24 b	6.50 ± 0.36 a	10.34 ± 0.93 b	10.50 ± 0.78 b
Peel chroma	5.73 ± 0.33 a	6.30 ± 0.23 a	9.14 ± 0.30 b	10.05 ± 0.46 a	14.55 ± 0.94 b	14.61 ± 0.80 b
Peel hue angle	31.56 ± 2.54 b	33.66 ± 0.42 ab	34.67 ± 0.79 a	40.22 ± 0.98 b	44.80 ± 1.75 a	45.69 ± 1.19 a
Pulp L*-value	76.61 ± 0.41 b	77.30 ± 0.54 b	74.80 ± 0.66 a	78.86 ± 0.87 a	78.15 ± 0.43 a	78.93 ± 0.46 a
Pulp a*-value	0.60 ± 0.23 b	-0.43 ± 0.16 a	-0.25 ± 0.29 a	0.47 ± 0.19 b	-1.08 ± 0.07 a	-1.29 ± 0.13 a
Pulp b*-value	16.86 ± 1.12 b	19.69 ± 0.44 a	17.04 ± 1.27 b	15.52 ± 0.69 a	24.55 ± 0.37 b	26.77 ± 0.35 c
Pulp chroma	16.89 ± 1.13 a	19.70 ± 0.44 a	17.05 ± 1.27 a	15.52 ± 0.69 a	24.57 ± 0.37 b	26.80 ± 0.35 c
Pulp hue angle	87.95 ± 0.75 b	91.28 ± 0.43 a	90.87 ± 0.30 a	88.40 ± 0.65 b	92.69 ± 0.21 a	92.77 ± 0.28 a
Pulp firmness	51.59 ± 1.77 a	55.31 ± 1.24 a	54.10 ± 1.13 a	50.25 ± 4.13 a	53.29 ± 1.14 a	46.30 ± 2.22 a

Values represent means ± SE. Different letters in the same line indicate significant differences by Duncan test ( $P < 0.05$ ).

### 3.4 Soluble Solid Content and Titratable Acidity

Fruits differ in their relative contents of sugar and acids (Ulrich, 1970; Whiting, 1970). Changes in the chemical composition of salak fruits during ripening are shown in Table 2. Soluble solid contents (SSC) of "pondoh hitam" remained constant during the ripening period (varying from 18.5 to 18.9 °Brix). In contrast, SSC of "pondoh super" at stage 4 was relatively low (11.8 °Brix), but markedly increased (55%) until stage 5 and declined thereafter by only 8%. The increase of sugar during ripening was possibly due either sugar import from the leaf or to the break-down of starch reserves of the fruit (Seymour et al., 1993). Titratable acidity (TA) of "pondoh hitam" declined gradually (29%) from stage 4 to stage 5.5, whereas that of "pondoh super" showed a strong decline of 81 % from stage 4 to stage 6. Similar results were reported by Djaafar and Mudjisihono (1998), who found that acidity of both salak cultivars declined during ripening. The decline of acids during ripening, presumably due to their utilisation as respiratory substrate, has been explained by Ulrich (1970). The sugar/acid ratio of "pondoh super" strongly increased from 13.1 (stage 4) to 99.4 (stage 6), while this ratio in "pondoh hitam" increased only slightly, i.e. from 48.7 (stage 4) to 69.3 (stage 5.5). These results showed that respiratory processes of "pondoh super" were on a higher level than that of "pondoh hitam" during ripening, which might indicate a different climacteric ripening patterns of "pondoh super" and "pondoh hitam".

Table 2. Soluble solid content (SSC) and organic acids of salak fruits at different ripening stages

Index	Salak "pondoh hitam"			Salak "pondoh super"		
	stage 4	stage 5	stage 5.5	stage 4	stage 5	stage 6
SSC (°Brix)	18.5	18.9	18.7	11.8	18.3	16.9
Titrateable acidity (malic acid) (%)	0.38	0.34	0.27	0.90	0.43	0.17
Sugar/acid ratio	48.68	55.59	69.26	13.11	42.56	99.41

### 3.5 Minerals

According to Bollard (1970) developing fruits may receive nutrients “directly” from the environment or nutrients enter the developing fruit “indirectly”. Direct uptake means that nutrients are obtained either from the roots via the xylem stream and from soluble carbohydrates of the leaves via the phloem. In contrast to that, nutrients supplied to the leaves via the xylem stream and then recirculated to developing fruits through the phloem are regarded as absorbed “indirectly”. In salak “pondoh hitam”, K, Ca and Mg declined during ripening, whereas P content remained relatively constant (Table 3).

These results are consistent with Imad et al. (1995), who reported a decline of K, Ca and Mg during ripening of date fruit. This may be caused by the active competition between the developing fruits and the growing leaves and shoots as it has been reported for grapes (Coombe, 1962), Japanese pear (Buwalda & Meekings, 1990) and sapodilla (Sulladmath, 1983).

Table 3. Mineral content of salak fruits at different ripening stages

Element	Salak “pondoh hitam“			Salak “pondoh super“		
	Mineral content (mg/g DM)					
	stage 4	stage 5	stage 5.5	stage 4	stage 5	stage 6
K	18.29±0.26 b	12.66±0.37 a	12.59±0.56 a	20.28±0.33 c	10.65±0.04 b	12.52±0.37 a
Ca	1.17±0.00 c	0.85±0.01 b	0.55±0.03 a	1.00±0.02 c	0.42±0.02 b	0.26±0.00 a
Mg	0.79±0.00 c	0.60±0.01 b	0.54±0.02 a	0.86±0.01 c	0.61±0.01 b	0.58±0.01 a
P	0.75±0.00 a	0.74±0.01 a	0.79±0.03 a	1.15±0.01 b	1.05±0.04 a	0.90±0.03 a

Values represent means ± SE. Different letters in the same line indicate significant differences by Duncan test ( $P < 0.05$ ).

### 3.6 Mono- and Disaccharides

During ripening, the contents of glucose and fructose in “pondoh hitam” increased significantly by 67 % from stage 4 to stage 5.5, whereas the content of sucrose remained constant until stage 5, and declined by 5.7 % thereafter (Figure 1). On the other hand, the content of glucose, fructose and sucrose in “pondoh super” increased from stage 4 to stage 5 by 50 %, 41 % and 33 %, respectively. The content of glucose and sucrose decreased by 24 % and 6.5 % thereafter, whereas the fructose content remained constant (Figure 2). The decrease of sucrose content in “podoh hitam” and “pondoh super” fruits after stage 5 indicated the occurrence of the hydrolysis of sucrose by sucrase to yield glucose and fructose, which reflected accelerated ripening processes in the fruit. This result was in agreement with Supriyadi et al. (2002) and Lestari et al. (2004). The increase of glucose and fructose content in “pondoh hitam” especially after stage 5 might also due to sucrose hydrolysis process. On the other hand, the decrease of glucose content and the constant content of fructose in “pondoh super” after stage 5 might cause by the usage of these monosaccharides, mainly glucose as primary respiration substrates. This possibly indicated a later but faster ripening process and less shelf life in “pondoh super” than in “pondoh hitam” fruits.

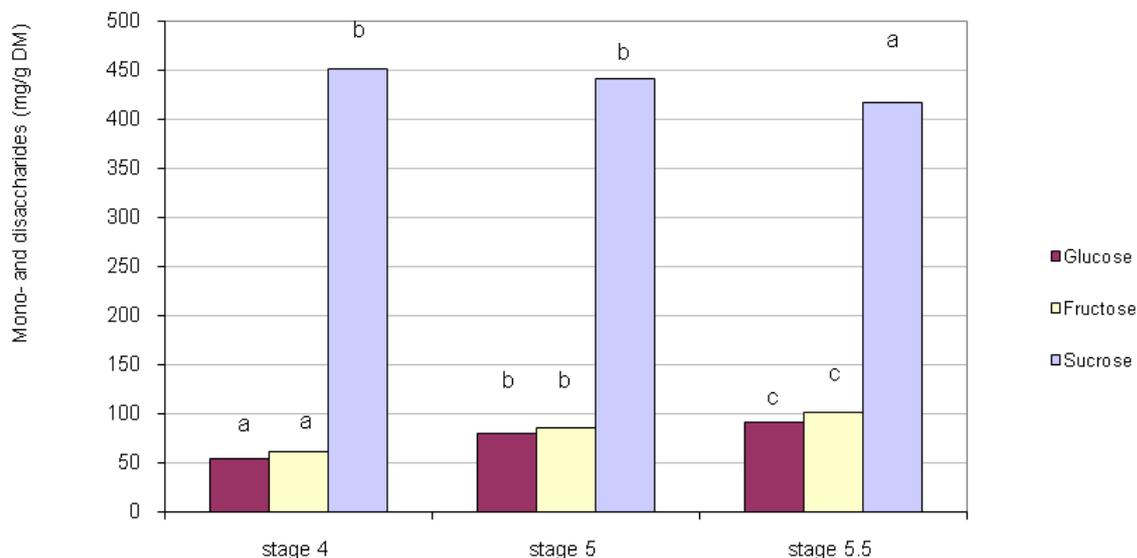


Figure 1. Mono- and disaccharides of salak "pondoh hitam" fruits at different ripening stages. Different letters in the same parameter indicate significant differences by Duncan test ( $P < 0.05$ )

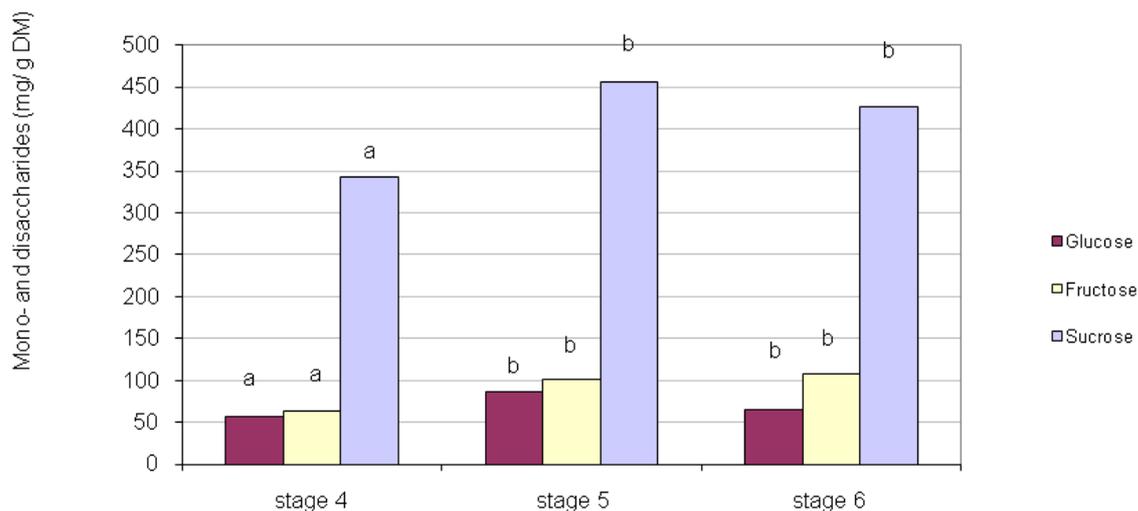


Figure 2. Mono- and disaccharides of salak "pondoh super" fruits at different ripening stages. Different letters in the same parameter indicate significant differences by Duncan test ( $P < 0.05$ )

### 3.7 Polysaccharides and Lignin

#### 3.7.1 Pectic Substances

The breakdown of pectic substances and hemicelluloses in the middle lamella weakens the cell wall and reduces the cohesive binding forces between cells. This is strongly associated with the reduction of the fruit firmness (Wills et al., 1981). During ripening, fruits undergo a softening, which is a major quality attribute that often limits shelf life (Tucker, 1993). As ripening progresses, insoluble pectin in the cell wall is converted into soluble pectin by the action of cell wall hydrolysis, which is indicated by the decrease in the content of insoluble pectin (ISP) and the increase of water soluble pectin (WSP). During ripening of salak "pondoh hitam", the ratio of WSP to ISP remained constant until stage 5 and increased by 12 % thereafter. This was caused by the increase of WSP and ISP contents in "pondoh hitam" until stage 5 (Figure 3).

On the other hand, the ratio of WSP to ISP of "pondoh super" increased more than twice from ripening stage 4 to stage 5 and increased thereafter by 17 % at stage 6. This was due to the increase of WSP in "pondoh super" until stage 5, whereas ISP content in this cultivar decreased during ripening (Figure 4). On the other hand, EDTA

soluble pectin of “pondoh hitam” decreased until stage 5 and remained constant thereafter, whereas that of “pondoh super” decreased continuously from stage 4 to stage 6 (Figure 3 and 4). These results reflected the faster breakdown of pectic substances in “pondoh super” than in “pondoh hitam” during ripening process, assuming the limited shelf life of “pondoh super” than of “pondoh hitam” fruits.

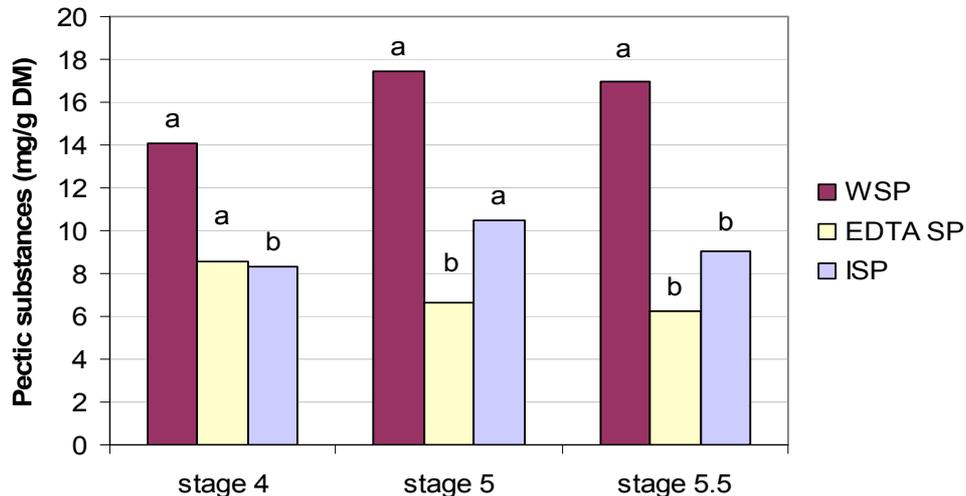


Figure 3. Pectic substances of salak “pondoh hitam” fruits at different ripening stages WSP: Water Soluble Pectin; EDTA SP: EDTA Soluble Pectin; ISP: Insoluble Pectin. Different letters in the same parameter indicate significant differences by Duncan test ( $P < 0.05$ ).

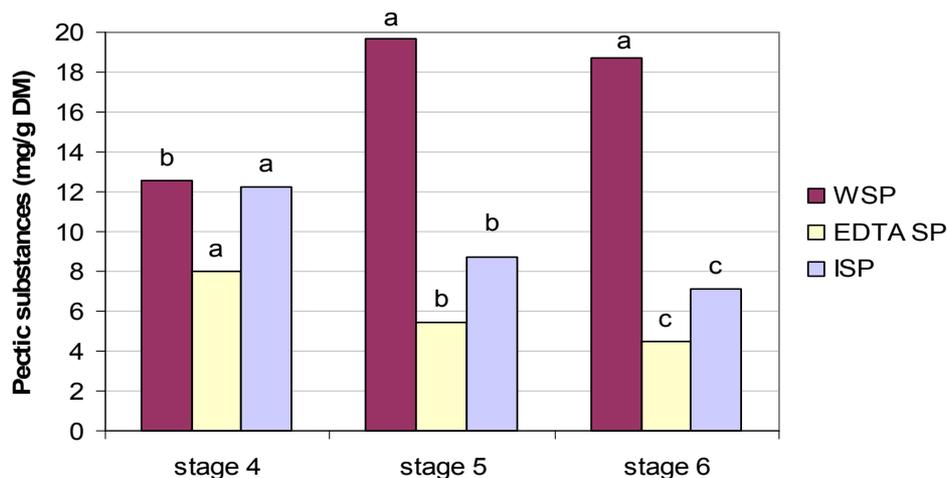


Figure 4. Pectic substances of salak “pondoh super” fruits at different ripening stages

WSP: Water Soluble Pectin; EDTA SP: EDTA Soluble Pectin; ISP: Insoluble Pectin. Different letters in the same parameter indicate significant differences by Duncan test ( $P < 0.05$ ).

Differences in the change patterns of pectic substances might be due to different activities of pectic enzymes between salak cultivars during ripening stages, such as pectinmethylesterase (PME), polygalacturonase (PG) and cellulase. In some studies, PME activity in tomatoes and bananas during ripening can decline or remain constant or increase depending on the cultivar. On the other hand, PG and cellulase activities appear only with the onset of ripening and tend to increase dramatically during ripening (Seymour and Thompson, 1997). The monomeric building block of pectin’s polymer backbone consists of  $\alpha$ -D-galacturonic acid and methyl  $\alpha$ -D-galacturonic acid along with rhamnose units (Cho et al., 1997). The remainder of the structure is made up side chains of the neutral sugars arabinose, galactose, glucose and xylose. PME acts to remove the methyl group from C-6 position of a

galacturonic acid, PG hydrolyses the  $\alpha(1-4)$  link between adjacent demethylated galacturonic acid residues, whereas cellulase hydrolyses the  $\beta(1-4)$  link between adjacent glucose residues (Seymour et al., 1993). In respect to content of pectic substances of salak fruits during ripening, the activities of PME, PG and cellulase in “pondoh super” might be faster in comparison to those in “pondoh hitam” resulting in different softening pattern.

### 3.7.2 Cellulose, Hemicellulose and Lignin

Salak “pondoh hitam” fruits had less cellulose (by 23%), hemicellulose (by 43%) and lignin (by 39%) in comparison to “pondoh super” at ripening stage 4 (Figure 5 and Figure 6). However, a higher reduction of cellulose, hemicellulose and lignin of “pondoh super” occurred at stage 5 (by 45%, 64% and 41%, respectively) in comparison to “pondoh hitam” at stage 5 (by 20%, 27% and 30%, respectively). These structural carbohydrates content in both salak cultivars decreased continuously thereafter (Figure 5 and Figure 6). In a study of Manrique and Lajolo (2002), cellulose residues of papaya fruit exhibited decreasing quantities of galacturonic acid and non-glucose monosaccharides during ripening, indicating that an association between polysaccharides from the matrix and microfibrillar phases may be involved in the softening process. Another study gave evidence for the depolymerisation of hemicellulose in tomato during ripening (Huber, 1983). On the other hand, Gross and Walner (1979) reported that the levels of sugars associated with cellulose and hemicellulose in tomato were found to be constant throughout the ripening process. In pears, no correlation between flesh firmness and cellulose content was found and only a slight difference in cellulose and hemicellulose content occurred between firm and soft fruit (Murayama et al., 2002). Depolymerisation of the hemicellulosic fraction in papaya fruit was not evident during ripening (Manrique & Lajolo, 2002).

Cellulose, hemicellulose and lignin are regarded to play a major role in the texture of plant tissue. Instantaneous elasticity of tissue is attributed to the combination of cell turgor and primary cell wall strength, as dictated by cellulose. Viscoelastic properties are related to hemicellulose and pectic components and steady state viscous behaviour to exosmosis and to increased wall fluidity arising from the breakdown of cell wall and/or middle-lamellar polymers (Jackman & Stanley, 1995). The different amount in the declines of structural carbohydrates between the two salak cultivars tested in this study might imply a faster break-down of the fruit cell wall in “pondoh super” in comparison to “pondoh hitam” during ripening. This result might also reflect a limited shelf life of “pondoh super” than of “pondoh hitam” fruits.

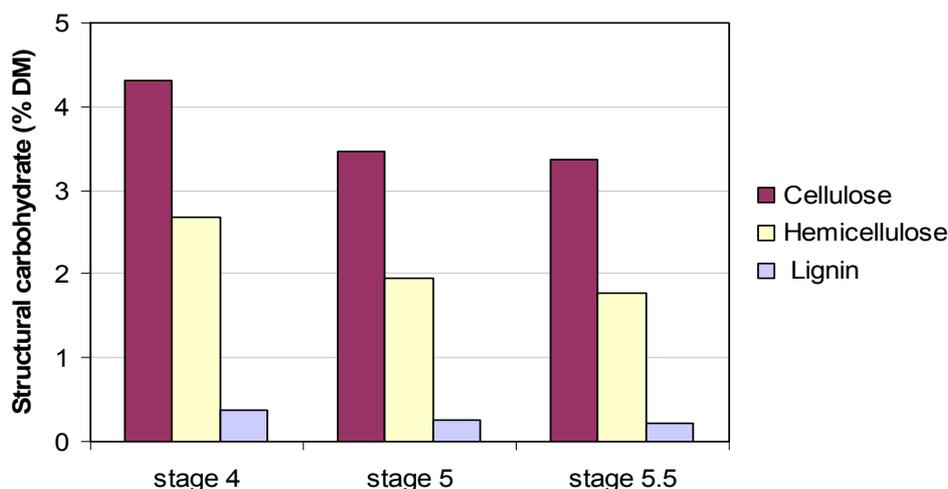


Figure 5. Structural carbohydrate of salak “pondoh hitam” fruits at different ripening stages

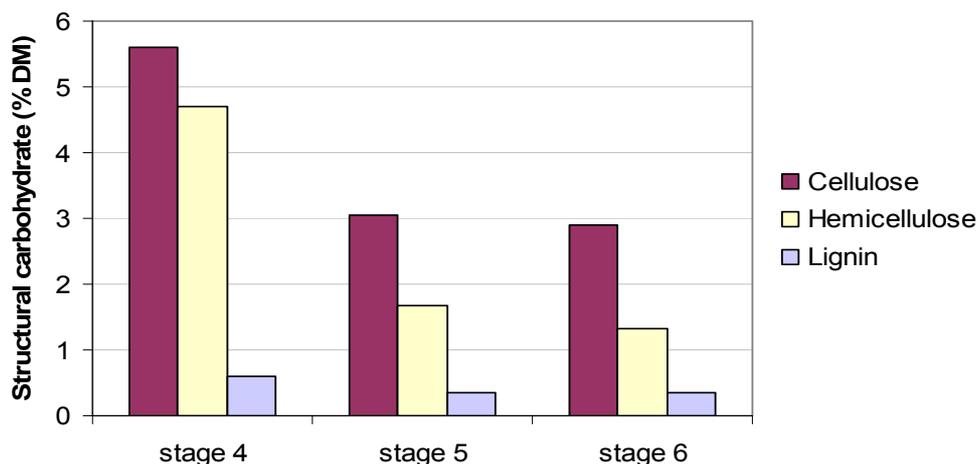


Figure 6. Structural carbohydrate of salak “pondoh super” fruits at different ripening stages

### 3.8 Firmness and Associated Cell Wall Compounds

There was no significant difference in pulp firmness being measured non destructively between “pondoh hitam” and “pondoh super” at all ripening stages. However, there was a marked loss of cell wall compounds during ripening. These results indicated that the method for determining textural properties of salak fruit had not been sensitive enough. Similar findings were also reported for tomato fruit (Hobson & Grierson, 1993). Non-destructive methods that involve compression and tension can result in a misleading texture evaluation, which should be measured objectively in terms of force, distance and time (Jackman & Stanley, 1995). Another possible explanation might relate to the specific enzyme activities in salak fruits. Giovannoni et al. (1989) reported the over-expression of polygalacturonase in non-softening mutants of transgenic *rin* (ripening inhibitor) tomato fruits failed to induce softening, although pectin depolymerisation and solubilisation occurred. Tieman and Handa (1994) noticed a marked loss in tissue integrity during senescence but little modification of transgenic tomato fruit firmness during ripening, which contained an antisense pectinmethylesterase gene, whose expression leads to a 10-fold reduced enzyme activity.

## 4. Conclusions

Increase in fruit size and weight as well as changes in peel and pulp colour occurred during maturation and ripening of salak fruits. Different patterns of peel and pulp colour changes were found in “pondoh super” and “pondoh hitam” during ripening. From the results obtained non-destructive colour measurement might be used to estimate optimum ripening stage at harvest. Physiological processes in “pondoh super” occurred to a later stage of ripening but then accelerated faster than “pondoh hitam” in term of changes of mono- and disaccharides, resulting in a poorer marketability and shorter shelf life. In respect to the change of sugar/acid ratio, there was a faster ripening process in “pondoh super” than in “pondoh hitam”. “Pondoh super” possessed higher content of polysaccharides and lignin, however, the ripening process accelerated earlier in comparison to “pondoh hitam”. Alterations in cell wall and middle lamella structure were not associated with the non destructive texture measurement during ripening of salak, assumingly due to the non sufficient sensitivity for salak fruits.

Fruits of salak “pondoh hitam” and “pondoh super” should be harvested from 4 to 6 months after pollination, depending on the market orientation. Thus, if the target is to achieve optimal sensory attributes and nutritional contents, harvest time could be delayed in order to improve those quality compounds. Fruits at stage 6 reached a maximum amount of flavour component, but had lower mineral content and structural carbohydrates in comparison to fruits of an early ripening stage. On the other hand, if the mineral and structural carbohydrate contents are the predominant criterion for the market, i.e. for processing purposes, salak fruits should be harvested at an earlier ripening stage (stage 4).

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