

PROCESSING OF *ARENGA PINNATA* (PALM) SUGAR

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ABSTRACT

Arenga pinnata sugar has been consumed by the local people of Indonesia for decades. In Tomohon, Indonesia, the sugar has been processed following the indigenous knowledge which consists of minimal process parameters. The sugar is considered to be potentially better for your health for which sufficient information is not yet available. This study has focused on developing knowledge on the process parameters involved in producing *Arenga pinnata* sugar from the sap, which is needed to improve existing process techniques to enhance the quality of produced sugar, and on the characterization of the sugar which will help the farmers to gain a better market positioning for their sugar.

The first study investigated the changes in *Arenga pinnata* sap, as a raw material, following the harvesting identified by pH, invert sugar and colour changes. As the time increased, the pH of the sap decreased and the invert sugar increased. Colour measurements following CIELAB (L*a*b* colour space) indicated that the change in pH of the sap is more associated with L* and b* values. The results confirmed the hypothesis that these parameters can be used as indicators of deterioration of the sap.

Processing the sap into sugar, by the removal of water through boiling, was carried out in the second study. Physical and chemical transformations involved during the heating process of *Arenga pinnata* sap into sugar were observed by their changes in temperature, pH and Total Soluble Solids (TSS). The pH increased in the beginning of the heating process until it reached a peak at the boiling point, followed by a continuous decrease to the end of the heating process. The final temperature of the concentrated sap was about 127°C corresponding to a TSS of more than 93 (% w/w) and pH values close to the initial pH of the fresh sap.

The colour changed significantly during the process steps involved in sugar processing, i.e., from the heating of the sap until it became a thick syrup, to the production of solid sugar, and the stirring process of the thickened syrup to form granulated sugar. CIELAB ($L^*a^*b^*$ colour space), and several colour determinations, namely total colour difference (ΔE^*), hue angle (h), chroma (C^*) and a^*/b^* ratio were determined in the study. As a function of time, the sap became darker (L^* decreased), more red (a^* increased), and more yellow (b^* increased) during the heating process. During the stirring process the sugar became lighter (L^* increased), more red (a^* increased), and more yellow (b^* increased). The colour of the solid sugar was significantly different than the colour of the granulated sugar (by ΔE^*), nonetheless both sugars can be categorized as having red-orange to yellow-orange colour (by h) with more yellow (by a^*/b^* ratio) and colourful appearance (by C^*).

The final study examined the physico-chemical attributes of granulated *Arenga pinnata* sugar. The sugar showed unique characteristics with an average pH of 6.285, 93.4% total sugar content, 2.98% invert sugar, yellow in colour, and nutritional properties with a high total phenolic compounds content (2432 μ g/g of GAE), and antioxidant potential identified by free radical scavenging activity (IC_{50}) of 0.6 mg/ml. The Fourier transform infrared spectroscopy (FTIR) spectra of the sugar confirmed that sucrose was the main sugar in *Arenga pinnata* sugar, and the tendency of the granules to clump together was identified using scanning electron microscopy (SEM) images.

This study contributes to improving the existing practices applied by indigenous people through better understanding of the important quality parameters involved during processing of the fresh sap into sugar. The findings from this work not only will serve as a

base for future studies on *Arenga pinnata* sugar production, but also help the farmers to develop a better market for their sugar.

RÉSUMÉ

Le sucre issu de la sève de l'*Arenga pinnata* est consommé par la population Indonésienne depuis des décennies. À Tomohon en Indonésie, le sucre est produit par méthode traditionnelle suivant les connaissances locales utilisant peu de moyens. Ce sucre est considéré avoir un potentiel meilleur pour la santé, cependant de l'information justifiant ce constat n'est pas encore disponible. Cette étude s'est intéressée à développer des connaissances sur les paramètres du procédé transformant la sève d'*Arenga pinnata* en sucre, et ce afin d'améliorer les pratiques existantes et améliorer la qualité du sucre ainsi produit, et effectuer la caractérisation du sucre pour aider les producteurs à forger une place de choix pour leur sucre sur les marchés.

La première étude s'est concentrée sur les changements de propriétés de la sève d'*Arenga pinnata*, suivant sa récolte, en particulier les changements de pH, de sucre inverti et de couleur. Avec une augmentation du temps, le pH de la sève diminuait et le sucre inverti augmentait. Les mesures de couleurs basées sur CIELAB (espace couleur L*a*b*) ont indiqué que le changement de pH de la sève s'est enligné avec des changements des valeurs L* et b*. Les résultats ont confirmé l'hypothèse que ces trois caractéristiques de la sève peuvent être utilisées comme indicateurs de la détérioration de la sève.

La transformation de la sève en sucre, par évaporation de l'eau, a été le sujet de la seconde étude. Des transformations des caractéristiques physico-chimiques lors du procédé thermique ont été observées avec l'évolution de la température, du pH et des solides solubles totaux (TSS). Le pH a augmenté au début du procédé thermique pour atteindre son maximum au point d'ébullition, pour ensuite diminuer jusqu'à la fin du procédé. La température finale

de la sève concentrée était d'environ 127°C correspondant à des TSS de plus de 93% (%w/w) et des valeurs de pH proches du pH initial de la sève fraîche.

La couleur a changé de façon significative durant le procédé de transformation pour la production de sucre, de la sève, à sa transformation en un sirop épais, jusqu'à la production d'un sucre solide ou le procédé de brassage menant à la formation de sucre granulé. Les paramètres CIELAB (espace couleur L*a*b*) et les différents qualificatifs couleurs qui en découlent, nommément la différence de couleur (ΔE^*), l'angle hue (h), la chromacité (C*) et le ratio a*/b* ont été déterminés. En fonction du temps, la sève chauffée est devenue plus foncée (diminution de L*), plus rouge (augmentation de a*), et plus jaune (augmentation de b*). Pendant le brassage du sirop, le sucre est devenu plus pâle (augmentation de L*), plus rouge (augmentation de a*), et plus jaune (augmentation de b*). La couleur du sucre solide était significativement différente de la couleur du sucre granulé (ΔE^*), alors que les deux sures peuvent être qualifiés être de couleur rouge-orange à jaune-orange (h) avec plus de jaune (selon le ratio a*/b*) et d'apparence colorée (C*).

La dernière étude a examiné les caractéristiques physico-chimiques du sucre granulé d'*Arenga pinnata*. Le sucre possède des caractéristiques uniques avec un pH moyen de 6.285, un contenu en TSS de 93.4%, de 2.98% en sucre inverti, de couleur jaune (de par son h et son ratio a*/b*) et une propriété nutritionnelle de par son contenu élevé en composés phénoliques totaux (2432 µg/g GAE), et un potentiel antioxydant de 0.6 mg/ml (IC₅₀) déterminé par son activité phagocyte de radicaux libres. L'analyse FTIR (spectrométrie infrarouge par transformée de Fourier) du sucre d'*Arenga pinnata* a confirmé que le sucrose était le sucre principalement présent, alors que la tendance des granules à s'agglomérer a été déterminée par imagerie par microscopie à balayage électronique.

Cette étude contribuera à améliorer les pratiques existantes et utilisées par les populations locales en comprenant mieux les paramètres de qualité qui régissent la transformation de la sève fraîche en sucre. Les résultats de cette étude serviront à paver l'avenir de la recherche sur la production de sucre issu de la sève d'*Arenga pinnata* tout en aidant les producteurs à développer de meilleures ouvertures sur les marchés pour leur sucre.

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4. **Changes in temperature, total soluble solids (TSS), and pH during the heating process of *Arenga pinnata* sap.**
5. **Colour changes during the processing of *Arenga pinnata* sap into sugar.**
6. **Characterization of *Arenga pinnata* (palm) sugar.**

Selected results from the chapters were also used to prepare conference presentations which are specified in the connecting text to each chapter.

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CHAPTER 1

INTRODUCTION

1.1 Background

Arenga pinnata Merr is a type of palm tree growing in tropical forests and is native to the Indo-Malayan archipelago, easily found in South and Southeast Asia (Mogea et al., 1991). Among the 3000 palm species of the tropics and subtropics categorized as multipurpose trees, the *A. pinnata* tree is considered as one of the most diverse multipurpose tree species under culture, and the only one attracting widespread economic interest (Mogea et al., 1991). Having around 150 local names such as arenga palm, black fibre palm, gomuti palm, aren, irok, bagot, kaong, enau, kabung, nira, saguer, and sugar palm illustrates its multipurpose interest (Mogea et al., 1991; Sahari et al., 2012). *A. pinnata* is the prime source of sugar, fermented drinks, syrup, palm wine, vinegar, alcohol, and bioethanol (Sahari et al., 2012). Young fruits of *A. pinnata* are used for cocktails or desserts (Mogea et al., 1991; Sahari et al., 2012), and ijuk (*A. pinnata* fibre) is used in the manufacture of ropes, filters, broom, and roofing (Mogea et al., 1991; Sahari et al., 2012). Starch, the inner part of the tree, is the raw material for the production of noodles, and for local desserts and delicacies (Sahari et al., 2012), while young leaves can be consumed as salads or cooked for soups (Sahari et al., 2012). Wood, the outer part of the stem, can be processed for flooring or furniture (Sahari et al., 2012), and root is traditionally known to be useful as a medicine as it is believed to break stones within the bladder when it is boiled with water (Sahari et al., 2012). Besides utilization of all parts of the plant into a variety of consumable products as described previously, the tree is also

appreciated in landscaping to stabilize the stony slopes, and it contributes to soil stabilization without consuming considerable land areas (Devi et al., 2014; Mogea et al., 1991).

Among those uses, the sweet sap, which has been recognized as the strongest sugar solution of any known trees in tropical areas (Nearing & Nearing, 1950), is claimed to be the most important product (Mogea et al., 1991) and is usually consumed by the local people as a sweetener. Further, local people can generate income from producing sugar processed from the sap as it happens widely for example in Indonesia (Marsigit, 2005; Mogea et al., 1991) and Malaysia (Ho et al., 2008; Sahari et al., 2012).

A. pinnata has not been cultivated significantly on a commercial scale, and most farmers either utilize this plant directly from the forest or from the trees which are grown in their backyard as reported by Devi et al. (2014). However, in Indonesia, a national program of planting palm for industrial purposes was declared by the President in 2007 according to the website of the Ministry of Agriculture of the Republic of Indonesia (Departemen Pertanian). It is estimated that the total area for *A. pinnata* cultivation in Indonesia is approximately 60,482 hectares with a potential of sap production estimated at 303.76 million liters a year resulting annually in 30,376 tonnes of sugar. In North Sulawesi, Indonesia, 3,000 tonnes per year of *A. pinnata* sugar can be produced from a total harvested area of 6,000 ha (Effendi, 2010). Estimated data for the area occupied by *A. pinnata* tree in Indonesia cited by Effendi (2010) is provided in Table 1.1. However this type of data should not be used alone in estimating production from *A. pinnata* planted area, since farmers in each region do not plant the tree in the same density per hectare (Effendi, 2010).

Indonesia has been a net importer of sugar since the sixties. Total sugar consumption increased from 1.8 million tonnes to 2.75 million tonnes in between 1976 and 1996 (FAO,

1997). Nevertheless, between 2006 and 2010, per capita consumption of sugar in Indonesia (16 kg) was below world average per capita consumption (21 kg) (Koo & Taylor, 2011). In 2014, the total sugar consumption was estimated to be 5.7 million tonnes (Perkebunan, 2013). In line with Indonesian population growth and rising incomes, it is expected that per capita consumption of sugar in Indonesia may increase substantially, and therefore increase the total sugar consumption (FAO, 1997; Koo & Taylor, 2011).

Table 1.1 Estimation area of *A. pinnata* in Indonesia

Province	Estimate of total area (ha)
Nanggro Aceh	4,081
Darussalam	
Sumatera Utara	4,357
Sumatera Barat	1,830
Bengkulu	1,748
Jawa Barat	13,135
Banten	1,448
Jawa Tengah	3,078
Kalimantan Selatan	1,442
Sulawesi Utara	6,000
Sulawesi Selatan	7,293
Sulawesi Tenggara	3,070
Maluku	1,000
Maluku Utara	2,000
Papua	10,000
Total	60,482

Source : (Effendi, 2010), p.38

Furthermore in Indonesia, *A. pinnata* sugar also has potential as a product for export market in other countries, especially developed countries where consumers are increasingly looking for natural sweetening products such as palm sugar. These natural sweeteners are perceived “more authentic” food ingredients which have a reputation of being healthier and

safer than more processed ingredients (Ministry of Foreign Affairs). It has been reported for Germany that “*Palm sugar exporters from developing countries (DCs) have an opportunity on the German market by positioning their product as a premium and healthier sugar than conventional white sugar. By using the popularity of organic and fair trade products in Germany, palm sugar producers can market their products in premium market segments. As a healthier ingredient than conventional white sugar, palm sugar has particularly good potential in dietetic foods.*” (Ministry of Foreign Affairs).

Despite its market potential, there are several issues that need to be addressed by *A. pinnata* producers in order to penetrate the sugar market such as product specification i.e., quality, freshness of harvested sap, control of the level of invert sugar, best management and food safety practices through the process steps, and assessment of its improved nutrition.

1.2 Indigenous knowledge of *A. pinnata* sugar processing and its associated challenges

In Tomohon, Indonesia, normally the sap (Figure 1.1) is collected twice a day, once in the morning, when the sap is tapped during the night, and once in the afternoon when the tapping begins in the morning. The sap is tapped from the inflorescence which must be stimulated by beating, usually using a wooden mallet for several minutes each day for about two or three weeks until the stalk itself becomes swollen. Afterwards, the stalk is cut at the swollen section to allow the flow of the sap. The sap is collected in a container, often a bamboo pipe or a jerry can, which is fixed beneath the cut stalk. *A. pinnata* fibre, locally called *ijuk*, is used to cover the mouth of the bamboo pipe and the stalk in order to prevent contamination by outside debris. Following collection of the sap, it is boiled in a round iron pan over a wood-fire. When crystallization has started, the thickened sugar is poured into a

mould which consists of a coconut shell, normally used to form the solid sugar (Figure 1.2). This type of sugar is known locally as “stone sugar” or “red sugar” (Figure 1.3). Besides the produced solid sugar, granulated sugar, locally named as “ant sugar” can also be produced by stirring the thickened syrup until it is dried and forms granules. Considering these two types of sugars, it is the granulated sugar which has the better price and market development potential rather than the solid sugar. In spite of its economic benefits, most farmers refrain from processing granulated sugar as they lack the appropriate knowledge for quality processing, whether the sap can be processed into granulated sugar or not since the quality of the granulation depends of the freshness of the sap at the time of processing. There is a thought and belief among the farmers that only farmers who have lots of experience can successfully produce good quality granulated sugar. This condition is not improving for local farmers since there is a shortage of information with only very few studies that have been reported on the processing of *A. pinnata* sap into sugar, although the sap is traditionally collected and has been consumed for decades as a sweetener.

1.2.1 Problems related to *A. pinnata* sap

Recently, minimal sap characterization, based on its pH, has been used by local people in Indonesia to determine the quality of the sap, whether it can be used or not in the production of granulated sugar. Sap with pH values of 6-7 is considered to be of good quality for use in the production of high quality sugar (Barlina et al., 2006; Marsigit, 2005). On the other hand, fresh sap which has a nearly neutral pH is easily spoiled as identified by a decrease of pH of the sap with time, thus the sap must be processed as soon as possible following harvest (Barlina et al., 2006; Marsigit, 2005). Microorganisms from the environment present during the collecting and transporting processes can easily contaminate

the sap. Marsigit (2005) cited that the microorganism *Saccharomyces cerevisiae* is commonly found in the sap resulting in a rapid fermentation and conversion reaction of the sugars to acids. Further, there is sometimes a time lag that the farmers must wait until the collected amount of sap is substantial enough to be processed into sugar. This brings the challenge of maintaining the quality of the sap at a pH high enough to allow subsequent processing into sugar.



Figure 1.1 *Arenga pinnata* sap

1.2.2 Problems related to the processing of *A. pinnata* sap into granulated sugar

Very few publications on *A. pinnata* sap sugar are available, such as those by Ho et al., 2007; Ho et al., 2008; Ho et al., 2006; Imanda, 2007; Mogea et al., 1991; Rumajar et al., 2008; Sahari et al., 2012. Among those studies, only Ho, et al. (2008) attempted to determine the changes in the physicochemical properties of *A. pinnata* sap during the production of sugar according to the traditional boiling method. Nevertheless, the data they provided did not support the whole production process since it missed information on sap characterization from

the beginning of the process. Other studies by Ho (Ho et al., 2007; Ho et al., 2008; Ho et al., 2006) have also focused on compounds contained in the sap as well as compounds and volatile compounds remaining in the sugar during the sugar processing. Those studies, so far, have not discussed about the control of the processing parameters of the sap into solid or granulated sugars.



Figure 1.2 Processing *A. pinnata* sugar by local farmer in Tomohon, Indonesia

1.2.3 Problems related to the characterization of *A. pinnata* sugar

Given the impact of globalization of sources, the presence of regulatory agencies and consumer groups and the overall demand for safer foods are all driving the food industry to increase its focus on the analysis, authentication and characterization of the raw materials and ingredients that are used in foods, and analyzing the quality of the final food product (Rein, 2012). Information about the characterisation including phytonutrients of *A. pinnata* sugar is very limited, although the indigenous people believe that this sugar might have a good

potential as a healthier sugar compared with refined sugars, but for which sufficient information is not already known.



Figure 1.3 *A. pinnata* solid "stone" sugar produced by local farmer in Tomohon, Indonesia

1.3 Problem Statement

Palm sugar produced from the sap is the main product of *A. pinnata* (Adawiyah et al., 2013; Mogea et al., 1991). Local people can generate income from producing sugar processed from the sap (Adawiyah et al., 2013; Ishak et al., 2013; Marsigit, 2005; Mogea et al., 1991; Sahari et al., 2012), and there are thousands of farmers in Indonesia and Malaysia who earn a living by tapping the sugar palm tree for its sap (Ishak et al., 2013). Although sugar palm has magnificent properties, the work on sugar palm has been restricted to the activity of tapping the palm sap for the production of traditional sugar blocks (Ishak et al., 2013).

There is limited knowledge on how the underlying chemical and physical properties of *A. pinnata* sugar are affected during processing from its sap into the final product. *A. pinnata* sap carries the potential for enhanced food quality and its changes during the process need to

be quantified and described including the measurement of physical, chemical and nutritional properties. Knowledge of process parameters is needed to improve existing techniques applied by indigenous people to enhance quality or minimize loss of product quality, and maintain product identity from the farm to the customer.

1.4 Objectives

The main objective of the study is to screen the importance of the process parameters during the processing of *A. pinnata* sap into granulated sugar, and to characterize the physicochemical nature of *A. pinnata* sugar.

In order to achieve the main objective, several specific objectives are set :

1. To provide accurate information on changes in pH, invert sugar and colour of *A. pinnata* sap as a function of time following the sap collection as part of best management practices in sap postharvest handling;
2. To determine the changes in temperature, Total Soluble Solids, and pH of *A. pinnata* sap during the heating process to concentrate its sugars;
3. To attest that heating can preserve the sap from deterioration by maintaining its pH during storage;
4. To determine colour and quality changes using CIELAB (L*a*b* colour space) with its several derivative quantitative attributes of colourfulness during the processing of *Arenga pinnata* sap into solid and granulated sugars;
5. To characterize *A. pinnata* sugar through an examination of its physico-chemical attributes.

1.5 Outline of the chapters

The overall structure of the study takes the form of seven chapters, including this introductory chapter. Chapter 2 begins by laying out the literature review of the *A. pinnata* tree in general, research on *A. pinnata* sugar, and some references with sugar processing from sap tapped from other type of trees. Chapter 3 presents the findings of the research on the quality changes of *A. pinnata* sap following the harvesting and sap collection. Chapter 4 provides the results of the quality investigation during the heating process of *A. pinnata* sap into sugar. Chapter 5 discusses the determination of colour changes during the processing of *A. pinnata* sap into solid and granulated sugars. Chapter 6 concentrates on the characterization of *A. pinnata* sugar through an examination of its physico-chemical attributes. Chapter 7 is the final chapter which draws upon the entire thesis to sum up process recommendations for the industry and overall implications of the findings on future research into this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Geographical distribution and botany of *Arenga pinnata* (Wumb.) Merr.

Arenga pinnata, naturally a forest species, belongs to the Arecaceae family, the subfamily Arecoideae and tribe Caryoteae of Palmae (Dransfield & Mogea, 1984; Mogea et al., 1991; Sahari et al., 2012). Dransfield & Mogea (Dransfield & Mogea, 1984) based on their 12 years observation on the phenology classified Arenga as follows : *Arenga australasica*, *Arenga borneensis* (Becc.), *Arenga brevipes* Becc., *Arenga caudata* (Mart.), *Arenga engleri* Becc., *Arenga hastata* (Becc.), *Arenga hookeriana* (Becc.), *Arenga listeri* Becc., *Arenga microcarpa* Becc., *Arenga nana* (Griff.), *Arenga obtusifolia* Mart., *Arenga pinnata* (Wumb.) Merr., *Arenga porphyrocarpa* (Mart.), *Arenga retroflorescens*, *Arenga tremula* (Blanco) Becc., *Arenga undulatifolia* Becc., *Arenga westerhoutii* Griff., *Arenga wightii* Griff., *Arenga* sp. (*Didymosperma gracilis* Hook.fil.), *Arenga* sp. (aff. *A. retroflorescens*).

A. pinnata (Figure 2.1) was earlier given several taxonomic names such as *Saguerus rumphii* and *Arenga saccharifera* Labill. However, in 1971, Merill, through the International Congress of Botany in Vienna, officially renamed it *Arenga pinnata* (Mogea et al., 1991; Sahari et al., 2012). In Indonesia alone, *A. pinnata* has around 150 local names such as black fibre palm, *gomuti* palm, *aren*, *irok*, *bagot*, and *kaong* (Mogea et al., 1991; Sahari et al., 2012), while in Malaysia, it is known as either *enau* or *kabung* (Sahari et al., 2012).



Figure 2.1 *Arenga pinnata* (Wumb.) Merr.

Native to the Indo-Malayan archipelago, *A. pinnata* grows in all of the tropical South and Southeast Asia, from 75°E in India and Sri Lanka, to 145°E in Guam and Papua New Guinea, and from 25°N in Myanmar to 10°S in the Nusa Tenggara Timur province of Indonesia (Mogea et al., 1991). Further, those countries where *A. pinnata* can be found are Philippines, Indonesia, Papua New Guinea, India, Thailand, Myanmar, Vietnam, North Australia and Malaysia (Ishak et al., 2013; Sahari et al., 2012).

There are few different reports regarding the altitude at which the tree can grow. Mogea et al. (1991) reported that *A. pinnata* can be found from the lowlands up to an altitude of about 1400 m. Sahari et al. (2012) cited both 1400 m and 1200-1500 m above sea level and areas where the rainfall was 500-1200 cm³ for best condition for the tree to grow.

The bole of the tree is solitary, unbranched and grows a height of 15-20 m, with a diameter of about 30-40 cm. The *A. pinnata* leaves are 6-12 m long and 1.5 m broad. The leaflets are dark green above and whitish beneath. The trunk remaining is covered by leaf sheaths, their margins are fibrous with black hairs, commonly known as *ijuk* or *injuk* (Mogea et al., 1991).

The flowering of *A. pinnata* (Figure 2.2) is reported in Mogea et al. (1991) and Dransfield and Mogea (1984). The palm's first flowering appears at an age of 10 to 12 years, but sometimes occurs as early as 5 to 6 years. Usually, 6-8 inflorescences at the top of the stem are female, the others at the lower nodes are male. Maturity is indicated by simultaneous appearance of two short leaves at the top of the stem. The first inflorescence arises from a node near the top meristem, while subsequent inflorescences arise from the lower located nodes in a descending order from the first flowering node downwards. Each node bears only one inflorescence, since they never multiply. The tree usually dies after a lifetime of 12 to 20 years.



Figure 2.2 Flowering of *A. pinnata* with ladder for tapping the sap

A detailed report of tapping *A. pinnata* sap can only be found in the work of Moga et al. (1991). The sap is tapped from the inflorescences. A farmer cleans an inflorescence stalk from its bracts as soon as the flowers are nearly open. Then, the clean stalk is beaten for several minutes each day for about two or three weeks at a distance of about 45-60 cm from the stalk base, until the stalk itself becomes swollen. Usually a wooden mallet or wooden pestle is used for this purpose. Afterwards, the stalk is cut at the swollen section to allow the sap to flow. One tapping operation can take about 15-20 minutes. The sap is collected in a container, often a bamboo pipe of 1.5 m length and a diameter of about 7 cm, which is fixed beneath the cut stalk (Moga et al., 1991). Nowadays, however, most farmers in Tomohon are using plastics containers of 5 litres size to replace the bamboo pipe. A piece of *A. pinnata* fibre, locally called *ijuk*, is used to cover the mouth of the bamboo pipe and the stalk in order to prevent contamination by outside debris (Moga et al., 1991). Figure 2.3 presents tapping of the sap using bamboo as a container practiced by farmers in Tomohon.



Figure 2.3 Tapping sap from flower stalk with bamboo container

There are male and female inflorescences, but tapping from male inflorescences is preferred rather than female inflorescences, because female inflorescences are believed to produce sap of inferior quality, besides the fibrous stalk of the female inflorescence requires more effort during preparation (Mogea et al., 1991). Nevertheless, there is no formal study that has investigated and confirmed that the male inflorescences produce better quality sap than female inflorescences.

By collecting the sap every morning and afternoon, about 5 litres of sap can be tapped from one inflorescence every day. In a tree, 2-4 inflorescences may be tapped, and one inflorescence can be tapped for a period of 1-2 months (Mogea et al., 1991). These numbers represent the average sap production of *A. pinnata* in Java which might be different with sap production in other regions. In Tomohon, for example, the average yield of *A. pinnata* sap has been reported to be 12-15 liters each day with sugar content of 12-15% and tapping periods of 6-12 months (Mogea et al., 1991). They also pointed that significant differences can occur in the methods practiced by different tribes. However, there is no information provided on what the differences are. Table 2.1 provides sap production of one exceptionally good tree which had a height of 19 m and a diameter of 40 cm. During the time of data collection, they observed that inflorescence 5 was still in production, and a new inflorescence was already appearing. Further, it is interesting to note that the yield was decreasing with the newer inflorescence as a result of shorter tapping period for newer inflorescence. This superior tree did not produce any female inflorescences, as a result it was reported to have a significantly higher yield. From this tree the amount of sap tapped was about 30-33 liters/day, more than 20,000 liters within a period of less than three years or about 6,700 liters/year which is notably much higher than the sap production of 900-1,600 liters/year reported by Effendi (2010).

The harvesting method presented here is similar with the method practiced by *A. pinnata* farmers in Tomohon, North Sulawesi, Indonesia. Although there is no report on how much sap on average the farmers can collect in a day; some farmers inform that they can tap around 10-15 litres of sap each day.

While there is no a prime month or season for collecting sap from *A. pinnata* trees, the farmers can collect the sap through the year, usually twice a day in the morning and in the afternoon. However, there is no report regarding the difference in sap properties as a function of harvesting or collecting time of the sap.

Table 2.1 Yield of a superior *A. pinnata* in Minahasa, Indonesia

Inflorescence	Tapping period (months)	Lifetime yield (liters)
1	6	6,039
2	5	4,506
3	4	3,660
4	4	3,660
5	3*	2,730

* in production

Source : (Mogea et al., 1991), p.125

2.2 Utilization of *Arenga pinnata*

A. pinnata is reported as the most diverse multipurpose tree among the palm species, because it has the widest range of uses involving virtually all parts of the plant as shown in Table 2.2 (Mogea et al., 1991; Sahari et al., 2012). It is considered as the only one attracting widespread economic interest among its genus (Mogea et al., 1991). Besides utilization of all parts of the plant into a variety of consumable products such as sugar sap, starch, *kolang*

kaling (immature fruit), Devi et al. (Devi et al., 2014) also pointed the usage of the plant in terms of agronomic benefits such as soil conservation that helps to prevent erosion, improvement of soil macro conditions, soil porosity, and trapping of rainwater.

Table 2.2 Uses of *A. pinnata* as a function of its different parts

Part of the tree	Uses
Root	Tea to cure bladder stones; insect repellent Erosion control. Post for pepper culture, boards, tool handles, water pipes, musical instruments like drums
Stem core	Sago, fibers
Pith of leaf's rachis	Drinking cup
Fiber from the leaf sheaths margin	Ropes, filters, road construction, basement of sport courts, brooms, brushes, roof material
Hairs of the base of the leaf sheaths	Tinder for igniting fire
Young leaves	Cigarette paper, salads
Leaflet midrib	Brooms, baskets, meat skewers
Mature leaflets	Wrapping material, fruit baskets, decoration
Terminal bud (cabbage)	Salads and cooked vegetable
Male inflorescences	Sap tapped for fresh drink, wine, vinegar and production of palm sugar Fermented sap to produce alcoholic beverages, and bioethanol*
Endosperms of unripe fruits	<i>Kolang kaling</i> (sweetmeat, candied fruit)
Flowers	Source of bee honey
Wood	Flooring, furniture, hand grips of tools*

Source : (Mogea et al., 1991), p.115 , *(Sahari et al., 2012), p. 65, 73

Sap

Among all of *A. pinnata* uses, the sweet sap is claimed to be the most important product from the tree. Sahari et al. (Sahari et al., 2012) reported that *A. pinnata* was the prime source of sugar, fermented drinks, syrup, palm wine, vinegar, alcohol, and bioethanol. One of the most important products of *A. pinnata* sap is sugar which is sweet and brown in colour and normally used as a food sweetener in traditional foods. Sap utilization as the raw material for sugar production is practiced in many areas in Indonesia and was also reported by Moga et al. (1991).

Fruits

The fruits, taken from its fruit bunch, are white in colour which is usually called *kolang kaling*. *Kolang kaling* is made from the endosperm which has a size of 2-2.5 cm by 1-1.5cm. The fruits are boiled for 2-4 hours, and then cut lengthwise until the white, jelly-like elliptic endosperm appears (Moga et al., 1991). *Kolang kaling* is used for the preparation of a cocktail served as a local refreshment (Moga et al., 1991), for preservation in heavy syrup which can also be canned, or can be cooked with sugary syrup for desserts (Sahari et al., 2012). One infructescence yields about 4500 *kolang kaling* (Moga et al., 1991).

Ijuk (A.pinnata fibre)

A. pinnata fibre, known locally as *ijuk* or *injuk*, is wrapped along the *A. pinnata* tree trunk. It is black in colour and up to 0.50 cm in diameter, and up to 1.19 m in length. The tree begins to produce *ijuk* after about 5 years, and each tree can yield about 15 kg. *Ijuk* can be obtained from the trees directly without secondary processes which is unlike other natural fibres such as kenaf and jute which require to be subjected to water retting before the fibres can actually be harvested/separated (Leman et al., 2010). Traditionally, *ijuk* has been used in

the manufacture of ropes, filters, broom, roofing, and in handicraft applications such as for making *kopiah* for Muslim (Sahari et al., 2012). Besides, *ijuk* is also used as traditional roof material for Javanese, Toraja, Minangkabau houses, and for Balinese temples (Mogea et al., 1991). The main advantages of *ijuk* are its durability and good resistance to sea water. It is also unaffected by heat as it has heat resistance up to 150°C (Sahari et al., 2012).

Starch

The inner part of the tree contains starch. The starch flour can be obtained when the first inflorescence is initiated, usually when the tree is about 10-12 years old. One tree can produce 50-75 kg of starch. The starch is used as the raw material for the production of yellow noodles, soun (white noodles), local desserts and delicacies such as *cendol*, *dodol*, etc. Typically, farmers are not tapping the palm if it is to be harvested for its starch, since the farmers believe the tapping may reduce the quantity of starch in the tree trunk (Mogea et al., 1991), or rather, starch is processed when the tree is unproductive in terms of sugar and fruits (Adawiyah et al., 2013).

Young leaves

Young leaves can be eaten fresh or cooked. The young leaves can be consumed as salads or cooked for soups, and they are also used for making cigarette wrappings, making weaving into baskets, for band matting, and for roofing. However, the harvesting of young leaves will shorten the trees' lifetime, therefore they are taken only when really needed (Sahari et al., 2012).

Wood

The outer part of the stem consists of wood, which is extremely hard and durable. It can be processed for flooring, furniture, and as the hand grip of tools (Sahari et al., 2012).

Root

The root is useful for medicinal purposes. It is believed to be capable of breaking down stones within the bladder when consumed as a concoction boiled with water (Sahari et al., 2012). The planted tree, with its expansive root zone, is also appreciated to stabilize stony slopes, and it contributes to soil stabilization without consuming considerable land areas (Mogea et al., 1991).

2.3 Characterization of *Arenga pinnata* sap, syrup and sugar

2.3.1 Sap composition

Table 2.3 presents the chemical composition of *A. pinnata* sap as cited by Rumajar et al. (2008) and Barlina et al. (2006).

Table 2.3 Chemical composition of *A. pinnata* sap

Component	Content (%)
Water content	87.20
Sucrose	11.28
Protein	0.20
Fat	0.02 ; 0.20*
Mineral	0.24
Ash*	0.24*

Source : (Rumajar et al., 2008), p.4, *(Barlina et al., 2006), p.167

A. pinnata sap was reported to have 13.12% total sugar, 4.80% reducing sugar and 0.05% total N in the experiment conducted by Apriyantono et al. (2002). A study by Kismurtono (2012) employed *A. pinnata* sap for a fermentation process and the sap was reported to have contents of sucrose, glucose and total sugar of the sap before fermentation ranging from 29.12 - 30.12%, 26.19 - 26.30%, and 56.90 - 58.00%, respectively. Those

figures reveal that the content of sugar is almost 60% with almost equal proportions of sucrose and glucose. It seems that Kismurtono failed to provide accurate data of *A. pinnata* sap's composition as the author also pointed out that the analysis of sugar content of *A. pinnata* sap before fermentation was around 14.4%. Another consideration is the content of glucose in the sap. While Kismurtono (2012) identifies high content of glucose in the sap, both Rumajar et al. (2008) and Barlina et al. (2006) presented no isolated data for the glucose content (Table 2.3).

Some nutrients have been identified in *A. pinnata* fresh sap by Rumajar et al. (2008) and they are presented in Table 2.4.

Table 2.4 Nutritional composition of *A. pinnata* sap

Element	Content
P	775.69 mg/kg
K	8,296.22 mg/kg
Ca	53.45 mg/kg
Mg	732.73 mg/kg
Na	619.07 mg/kg
Fe	10.05 mg/kg
Cu	5.51 mg/kg
Ash	1.50 %

Source : (Rumajar et al., 2008), p. 12

Further, Barlina et al. (2006) cited the presence of the following acid compounds and their concentration in *A. pinnata* sap with malic acid content being the highest (17.0 ppm), followed by succinic acid (5.1 ppm), lactic acid (4.0 ppm), and pyroglutamic acid (3.9 ppm). Acid compounds which were less than 1 ppm also found in *A. pinnata* sap were citric acid (0.9 ppm), tartaric acid (0.6 ppm), and fumaric acid (0.1 ppm). There were also plentiful sucrose

and polar side chain amino acids particularly asparagine and glutamine contained in *A. pinnata* sap (Ho et al., 2007).

2.3.2 Composition of *A. pinnata* syrup

So far, the only record that can be found on the composition of *A. pinnata* syrup is for the inverted sugar syrup provided by Imanda (2007). The inverted sugar syrup contained water at 32.1%, ash at 2.39%, Total Soluble Solids (TSS) at 69.23%, water-insoluble at 0.52%. The physical properties studied were viscosity of 291.49 cP, density of 1.3467 g/ml, and pH value of 5.63 (Imanda, 2007).

2.3.3 Composition during boiling of *A. pinnata* sap into sugar

Information on the volatile compounds released during the heating process involved in producing sugar from *A. pinnata* sap was reported by Ho et al., 2007; and Ho et al., 2006. Using headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS), thirty six volatile compounds were identified in *A. pinnata* sugar (Ho et al., 2006). The thirty six compounds comprised of fourteen pyrazines, six aromatics, five furans, four acids, two furanones, two aldehydes, two ketones and one pyran. Further information on the volatile compounds found in *A. pinnata* sugar processing and their concentration was presented in another work by Ho et al. (2007). Thirty volatile compounds were identified as seventeen pyrazine compounds (N-Heterocyclic), seven furan derivative compounds (O-Heterocyclic), four aldehydes, and two ketones. Of the four compound categories identified, N-Heterocyclic was the highest contributor to the aroma profile of *A. pinnata* sugar (83.69%). Among the thirty volatile compounds, the highest value was given by 2,5(6)-Dimethyl pyrazine (2323 ppb), followed by 2-Ethyl-5-methyl pyrazine (1386 ppb), 2-Methyl pyrazine (1190 ppb), and others were less than 685 ppb for each compound. In total

for N-Heterocyclic, O-Heterocyclic, aldehydes, and ketones concentrations were 7523 ppb, 1299 ppb, 140 ppb, and 27 ppb, respectively. The pyrazines were considered to be giving a significant contribution to the typical sensory attributes of *A. pinnata* sugar such as its roasted nut aroma (Ho et al., 2007).

2.3.4 Composition of *A. pinnata* sugar

The values of *A. pinnata* sugar composition and the information written on the label of a package of *A. pinnata* sugar manufactured by Masarang factory in Tomohon, Indonesia are given in Table 2.5. From the two articles reporting on *A. pinnata* sugar composition, one is the original measurement by Rumajar et al. (2008), and another article presents cited data (Sihombing, 1995).

Rumajar et al. (2008) identified the characteristics of *A. pinnata* crystal sugar which was manufactured with the addition of sucrose seed crystals sized < 60 mesh. In a comparative study between cane sugar and *A. pinnata* sugar, Sihombing (1995) cited the composition of *A. pinnata* sugar, however the units of measurement given were not complete except for sucrose. Furthermore, the content of sucrose in Sihombing's (1995) paper is much lower compared to the other two sources. Imanda (2007) characterized *A. pinnata* solid sugar and reported that it contained 75.8% sucrose, 11.8% reducing sugars, 10.3% water content, 2.8% ash, 1.7% fat, 1.5% protein, 0.3% water-insoluble and 83.8° Brix of total dissolved solids. Although Parjoko et al. (1996) did not state a specific type of palm sugar used in their experiment, the composition and the concentration of the palm sugar was reported in their experiment and should be compared and reported in the study of *A. pinnata* sugar composition. The composition of the palm sugar reported in that study was for sucrose (769g/kg), glucose (60g/kg), fructose (47g/kg), xylose (39g/kg), ash (16g/kg) and water

(105g/kg) (Parjoko et al., 1996). This data of unspecified palm sugar is in closer agreement with the values reported by Sihombing (1995).

Table 2.5 Composition of *A. pinnata* sugar

[1]	[2]	[3]			
Sucrose	70%	Sucrose	97.58%	Sucrose	96.8%
Calorie	372 cal	Reducing sugar	1.81%	Invert sugar	2.64%
Water content	7 g	Water content	0.57%	Other minor components	0.5%
Mineral	1 g	Ash	0.03%		
Protein	0				
Fat	0				
Potassium	344.10 mg				
Calcium	85 mg				
Sodium	44.10 mg				
Phosphor	35 mg				
Iron	3 mg				
Niacin	0.2 mg				
Riboflavin	0.07 mg				
Thiamin	0.02 mg				
Ascorbic acid	0 mg				

Source : [1] (Sihombing, 1995); [2] (Rumajar et al., 2008); [3] (Masarang Factory)

Despite the little information available on the composition of *A. pinnata* sap, syrup and sugar, *A. pinnata* sugar is a type of palm sugar believed to be an alternative sweetener which is healthier and a more nutritious sugar than the one obtained from sugarcane (Tamunaïdu et al., 2013). Compared to conventional sugar, which mostly consists of sucrose, palm sugar has additional components such as protein, minerals, calcium, phosphor, iron and copper, in addition to having a lower glycemic index (GI of 30-35) when compared to regular refined sugars (GI of 60) (Ministry of Foreign Affairs).

2.4 *Arenga pinnata* sap, syrup and sugar processing

A. pinnata sugar is produced by heating the sap on the same day following its collection to prevent quality loss of the sap. A study on *A. pinnata* sap reporting on the characteristics of the raw sap material *A. pinnata* for sugar production was conducted by Barlina et al. (2006). In the study, coconut coir extracted from a husk of coconut was used, as a preservation additive in the tapping process of *A. pinnata* sap. Coconut coir contained about 3.12% of tannin which might be the compound attributed to the improved sap preservation. Coconut coir with three different weights were used i.e., 50, 100 and 150 g. The coconut coir was inserted in each container used in tapping the sap. It was concluded that 50 g of coconut coir can retain the quality of *A. pinnata* sap for 3 hours after harvesting. However, using coconut coir in preserving the sap imparted a darker colour to the sap which is not preferable in producing quality *A. pinnata* sugar. Besides, in this exploratory investigation Barlina et al. (2006) fail to provide the size of the container or the volume of the sap, so it requires clarification and further studies before farmers can safely employ this method.

Apriyantono et al. (2002) conducted a research to analyse the rate of browning reaction during preparation of *A. pinnata* and coconut sugars. *A. pinnata* sap was obtained from a rural area of Bogor, Indonesia. The experiments were divided into three parts, and the first and the third parts are related with *A. pinnata* sap sugar processing, while the second part is related to coconut sugar. The composition of *A. pinnata* sugar namely total sugar, organic acids, and volatiles was analysed in the first experiment. The third experiment was done in order to study the rate of brown colour formation during heating of 10 ml of *A. pinnata* sap with the pH adjusted to 8.0 in a closed system at 105, 115 and 121°C for 300 minutes. This process seems far from the method being applied by local people who do not apply a pH

adjustment of *A. pinnata* sap and do the heating process in an open system. Also, the initial pH of *A. pinnata* sap used in the experiment was not provided. On the other hand, the preparation of coconut sugar from the sap in this work is similar with the processing method of producing *A. pinnata* sugar from its sap: heating 2500 ml of coconut sap which had initial pH of 6.4 in a wok pan at a temperature of 110°C for about 90 minutes.

An experiment undertaken by Ho et al. (2007) processed *A. pinnata* sap obtained from a local traditional manufacturer in Kuala Pilah, Malaysia. The boiling sap was sampled at every 30 minutes intervals for 4 hours of heating to determine volatile compounds and relative concentrations. Several flavour compounds formed during the heating process, including compounds from the Maillard reaction and the Strecker degradation, as well as lipid oxidation products. Ho et al. (2007) argue that the heating process of the palm sap is important to obtain the characteristic aroma of *A. pinnata* sugar, and suggest the necessity of applying a temperature higher than 110°C to gain the typical roasted, nutty and sweet caramel-like aroma notes.

In another study, Ho et al. (2008) attempted to determine the changes in the physicochemical properties of *A. pinnata* sap during the production of sugar. Approximately 50 L of *A. pinnata* sap were heated in a big wok in an open process. Temperature employed to heat the sap was up to 150°C, and about 200 ml of heated sap was sampled at intervals of 30 minutes for the 4 hours heating process. Analysis of colour, soluble solid, pH, temperature, sugar and amino acid concentration of the samples were determined for each sample. Besides the hydrolysis of the carbohydrates to generate reducing sugars and degradation of amino acids, many physicochemical changes were identified during the heating process. The results further support the idea that high temperature is necessary to create an environment which was

rich in essential precursors for subsequent reactions such as the Maillard reaction. It also showed that other quality characteristics of *A. pinnata* sugar based on colour and soluble solids ($^{\circ}$ Brix) shared an increase as a function of time. Comparing with other studies on the processing of *A. pinnata* sap into sugar, this study gives more information especially about the quality characteristics of *A. pinnata* sap sugar such as moisture content, TSS, pH, colour, and amino acids. However, the data provided does not support the whole process because the quality characteristics assessed were determined only after 30 minutes of heating, and therefore the information to date is still missing the characterization of the initial sap and the initial stage of heating.

2.5 Other palm sap, syrup and sugar processing

The works on other palms sap, syrup, and sugar processing can be useful in studying *A. pinnata* sap, syrup, and sugar processing, since very little research has been found focusing on the processing of *A. pinnata* sap into syrup or sugar.

A study to produce syrup from Palmyra palm (*Borassus flabellifer* Linn.) was carried out by Naknean et al. (2013). The sap was filtered using cloth sheet after tapping and harvesting in a 12 hours period. Then, it was boiled using an open pan and a vacuum evaporator at 70°C and 80°C . The TSS of the syrups was 70.23° Brix and 70.07° Brix for syrups produced by an open pan and by vacuum evaporator, respectively. This study monitored the changes in the properties of palm sugar syrup produced by both open pan and a vacuum evaporator during storage at 4°C and 30°C for 12 months monitored at monthly intervals. During storage, the Maillard reaction took place in the samples while the reaction for samples stored at 4°C was lower than those stored at 30°C . This study provided information on the processing of palm sugar syrup and the storage conditions that could

influence the properties of palm sugar syrup. Palm sugar syrup produced by a vacuum evaporator retained desired quality attributes in syrup better than palm sugar syrup produced by heating with an open pan. Moreover, the loss of quality of the palm sugar syrup due to a non-enzymatic browning reaction increased with increases in storage temperature and time. The results obtained from the different treatments suggest that non-enzymatic browning of palm sugar syrup during storage could be reduced. This could be done by using a vacuum evaporator for the production of palm sugar syrup and storing it at low temperature (Naknean et al., 2013). This article provides some evidence in syrup processing using open pan and vacuum evaporator, nevertheless it would have been more useful if the authors had included many of the important process parameters which are required during the heating process in order to produce good quality syrup.

Concentrating sap into syrup by evaporation was also studied by Ben Thabet et al. (2009). The sap was collected from date palm, *Phoenix dactylifera* L. in Tozeur, Tunisia. The initial total soluble solids content of the juice was 11% (w/w). Before boiling it in an open stainless steel pan over a hotplate at 100°C, the sap was filtered through a fine cloth. During the heating process, a continuous agitation was applied. As they compared ° Brix of maple syrup which ranged from 62-74 (a mean of 67° Brix) with the ° Brix of date syrup which was 75° Brix, they chose to stop the concentration of the sap when the total soluble solids content of syrup reached 64, 68, and 74° Brix. In this study, determination of selected physicochemical characteristics of the syrup produced included water activity, protein, ash, colour (L*a*b*), total phenolic content, radical scavenging activity, rheology, differential scanning calorimetry, and sensory evaluation.

Another study concerning the heating process was conducted by Rao et al. (2009). The sap was tapped from two different types of palm trees namely Palmyra palm (*Borassus flabellifer* L.) and date palm (*Phoenix sylvestris* L.), and the sap was processed to manufacture jaggery sugar. The initial TSS content of Palmyra palm sap was 16 (% w/w), whereas it was 12.9 (% w/w) for date palm sap. The sap was filtered through a fine muslin cloth and boiling was carried out in an open shallow aluminum pan. During boiling, a mild bleaching agent at the rate of 5 g/50 L juice was added intermittently to clarify the Palmyra palm juice. However, no clarifying agent was added during boiling of the date palm sap. Both Palmyra palm and date palm juices were boiling for more than 2 hours before they attained a TSS of 81 (% w/w) corresponding with a temperature of about 120°C. Two parts were considered in this study. The first part was monitoring at the site of the preparation of *jaggery* from the sap. Measurements of temperature of the juice and TSS were done periodically while the evaporation was in progress. The second part of the study was measuring other properties i.e., density, viscosity, thermal conductivity, thermal diffusivity, specific heat and colour of the juice or syrup, collected at different stages of boiling. There were three phases of boiling of the sap during the process monitored in the study. Phase 1 was a rise in temperature to boiling, phase 2 was a slow rise in both boiling temperature and TSS, and phase 3 was a rapid rise in boiling temperature with concomitant increase in viscosity and TSS. Among these phases, phase 3 was found to be very critical and the heating must be carefully regulated in this phase to retain the quality of the *jaggery*. The temperature of 104-120°C was argued to be a critical temperature range (Rao et al., 2009). This detailed article provides valuable information on palm sugar processing as it does not only present data of changes in density, thermal

conductivity, and specific heat of *jaggery*, but also discusses the time-temperature, time-TSS, colour-TSS profiles of different juices during *jaggery* making process.

Palm syrups produced and reported previously show that there is no specific characteristics of palm syrup especially $^{\circ}$ Brix or TSS of the syrup; Naknean et al. (2009) produced syrup of about 70° Brix, while Ben Thabet et al. (2009) made syrup of 64, 68, and 74° Brix. Rao et al. (2009) on the other hand concentrated their research on manufacturing solid palm sugar during their study.

2.6 Maple sap, syrup and sugar processing

Among the syrups and sugars produced from sap tapped from a tree, maple syrup and maple sugar processing are considered as the most developed. In North America, maple sap is tapped in late winter and early spring during the months of February, March and April when night time temperatures are below freezing, followed by days when there is a rapid warming above freezing which is ideally to about 4°C (Chapeskie et al., 2006; Wilmot, 2008).

Comprehensive information on maple sap, syrup and sugar processing including information from the maple tree resource to the marketing of maple products are provided in some books such as the North American Maple Syrup Producers Manual (Heiligmann et al., 2006; Koelling et al., 1996) and Sugar Maple Research: Sap Production, Processing and Marketing of Maple Syrup (Anonymous, 1982).

In processing maple syrup by heating/evaporation of the sap, proper equipment and techniques are critical to economically produce quality syrup. Maple syrup evaporators have undergone a great evolution in design. While early evaporators were heated exclusively by wood fires, modern evaporators are available which use a variety of heat sources including

wood, fuel oil, gas, and steam. More information on the development of evaporators used in maple syrup production which use sap preheaters to be more economical is reported by (Garret, 1982; Garret et al., 1982; Huyler, 1982).

Since the processing of maple syrup and maple sugar from maple sap has been well developed, most current papers on maple sap, syrup and sugar have emphasized on the chemical properties of the sap, syrup and sugar, and most of the time the studies of the product samples are conducted in collaborations with the maple syrup and maple sugar processing industries such as reported by (Clement et al., 2010; González-Sarriás et al., 2012; Ngadi & Yu, 2004; Rees, 1982; Stuckel & Low, 1996; Theriault et al., 2006). Only few studies have reported on the processing parameters of the sap into syrup or sugar during their experiment such as Jones & Alli (Jones & Alli, 1987) who processed maple sap to syrup on a batch basis by boiling on a kitchen range, while Akochi-K, et al. (1997) reported the processing of maple sap to maple syrup at the laboratory scale by heating the sap in glass beakers of 1 L capacity placed on a sand bed within a stainless steel pan. The period of heating, pH and water content of the sap were considered in determining the pyrazines formed during the conversion of maple sap to syrup. The use of microwave energy to concentrate maple sap to syrup has also been studied (Favreau et al., 1997; Favreau et al., 2001).

Changes in some quality parameters observed during the evaporation process of maple sap are well described in the work of Akochi-K, et al. (1997). The sap was boiled at the temperature of 105°C with an estimated rate of evaporation of 6 ml per minute on average. The pH and total soluble solid contents were monitored during 120 minutes of the boiling process, and changes in pH were observed both for the open boiling process and under reflux boiling. The pH values of sap heated in open boiling process showed an increase from 7.2 to

9.2 after 30 minutes boiling, before it decreased to 7.3. A similar pattern of pH changes was observed for the sap heated under refluxing conditions i.e., pH of 7.2 increased to about 8.6, before decreasing to around 7.6 at the end. Total soluble solids increased gradually from 3% to 5% in the first 40 minutes of heating before it showed a rapid increase to 63% in the subsequent 20 minutes, and reached 65% in the next 30 minutes, where it remained till the end of the 120 minutes heating process (Akochi-K et al., 1997). The findings of this study would have been more useful if the author had provided enough information on the initial characteristics and amount of the sap, and had offered explanations for the distinction between processing the sap by using open boiling and under reflux boiling. Further, there are some questions that need to be asked, for example why was a constant total soluble solids of the syrup experienced during the last 30 minutes of boiling of the total 120 minutes of heating time (i.e., why not shorten the boiling time if no further concentration is occurring).

Changes for both pH and total soluble solids were also reported by Heiligmann (1996). The sap had a pH of 7 when the evaporation began, then it reached a pH of 8 to 9 before it decreased until the end of the heating period to about 7; whereas the degree Brix increased slowly at the beginning then increased more rapidly as the evaporation progressed. It was reported that the rate of color formation increased dramatically when the syrup reached about 25° Brix (Heiligmann, 1996).

The production of maple syrup in both Canada and the United States must follow federal grading standards and guidelines. Maple syrup must have a density of 66° Brix at 68°F (20°C), and shall not have any off flavors or other undesirable characteristics. Maple syrup grade designations are based on colour in relation to light transmittance, and in Canada, for example, the grades of Canada No.1 Extra Light (AA), Canada No. 1 Light (A), Canada No.1

Medium (B), Canada No.2 Amber (B), Canada No. 3 Dark (C), have light transmissions of \geq 75.0%, 60.5-74.9%, 44.0-60.4%, 43.9-27.0%, and $< 27.0\%$, respectively (Marckres et al., 2006). A detailed all inclusive grading system for maple syrup is provided in the North American Maple Syrup Producers Manual (Heiligmann et al., 2006; Koelling et al., 1996).

These are several activities which must be performed to produce quality syrup once the evaporator is fired (Koelling et al., 1996), these are as follows :

- Monitor and maintain the depth of sap within the evaporator;
- Keep a constant heat to maintain the desired density gradient and evaporate the sap as quickly as possible;
- Keep the foaming under control, and when it happens a defoaming agent such as cream, butter and vegetable oil should be used to reduce the foaming mass;
- Skim the scum off the top of foam as it appears;
- Watch for any accumulation of solids in the bottom of the syrup pan;
- Monitor the density of the liquid near the takeoff valve and be ready to draw off finished syrup or concentrated sap.

Maple syrup also serves as the raw material for other maple products including maple sugar which is available in numerous different forms such as soft sugar, hard sugar, block sugar, molded sugar, or granulated (stirred) sugar. Making maple sugar is a difficult process and it even requires much more experience and care than for the making of maple syrup (Nearing & Nearing, 1950). Several factors influence the quality results in maple confections processing such as the syrup's boiling temperature, barometric pressure, air temperature, humidity, the amount of invert sugar in the syrup, and the elevation above sea level (Koelling

et al., 1996). A summary of maple confections, their names, boiling points and characteristics is presented in Table 2.6.

Table 2.6 Maple confections, boiling points and characteristics

Product name	Cook to boiling point of water plus an additional :	Process	Characteristics
Rock candy	8-10°F (4-6°C)	Boil, allow to set for weeks	Hard, clear sucrose crystals
Maple cream (maple spread, maple butter)	22-24°F (12-14°C)	Boil, cool, and stir	Creamy smooth, paste-like
Sugar-on-snow	22-27°F (12-15°C)	Boil, pour over packed snow	Chewy to brittle, depending on boiling temperature, no crystals
Taffy	23-26°F (13-14°C)	Freeze immediately	Thick and chewy, no crystals
Fondant type candy (maple cream)	27-29°F (15-16°C)	Boil, cool, stir, and mold	Smooth, putty-like
Molded sugar (soft sugar, maple candy)	32-34°F (18-19°C)	Boil, stir hot, pour into molds	Firm, but not rock solid, very small crystals
Hard sugar (block sugar)	34-38°F (19-21°C)	Boil, stir hot, pour into molds	Harder than molded sugar candies, in block form
Granulated sugar (Indian sugar, stirred sugar)	45-50°F (25-28°C)	Boil, stir until granulated, sift	Loose granulated, like brown (cane) sugar

Source : (Heiligmann et al., 2006), p.198

In producing maple sugar, however, not all grades of maple syrup can be used. For example, maple syrup classified as grade D which has a high inverted sugar content cannot be used to produce granulated sugar using the traditional boiling method due to the presence of a high level of glucose which makes the crystallization process of sugar impossible by applying the traditional stirring method (Aider et al., 2007a). Nevertheless, maple syrup, with high inverted sugar, can successfully produce granulated maple sugar by adapted process parameters using an evaporator-crystallizer and by arranging the vacuum values in the evaporator, controlling mixing speeds of a blade stirring system used with a motor, and setting the duration of crystal growth after adding sugar crystals with dimensions less than 10 μm in the syrup to start crystallization (Aider et al., 2007a; Aider et al., 2007b).

2.7 Consideration factors in maple syrup/sugar processing

2.7.1 Boiling point elevation

A primary method to monitor the boiling sap and to determine when it has reached the correct density is by measuring the elevation in the boiling point with a thermometer. Boiling temperature elevation refers to the elevation of boiling temperature above the boiling temperature of pure water at the time and place where syrup is being made. Pure water boils at 212°F (100°C) only when the barometric pressure is 29.92 inches (760 mm) of mercury. Therefore, it is necessary to determine the temperature of pure boiling water at the time and place where syrup is being made, since the barometric pressure might not be 29.92 inches when the syrup is being made. The boiling temperature increases as the sugar concentration in the solution, such as maple sap or syrup, increases. Therefore, the boiling point can be used as a measure of maple sap or syrup density (Heiligmann et al., 2006; Koelling et al., 1996).

2.7.2 Refractometer use and the Brix scale

Using a refractometer to determine the density of syrup is an accurate, simple method. A refractometer works by measuring the refractive index of a sugar solution which is directly related to the amount of dissolved solids (sugar) present in the solution in $^{\circ}$ Brix unit (Heiligmann et al., 2006; Koelling et al., 1996). The Brix value indicates what the percentage of sugar would be if the density of the solution were due only to dissolved sugar. Even if it does not express the true percentage of sugar in a solution containing sugar plus other dissolved solids, for practical purposes the Brix value equals the percentage of sugar in the syrup (Heiligmann et al., 2006; Koelling et al., 1996).

2.7.3 Invert sugar

When it comes from the tree, sucrose is the predominant sugar in sap. However, microbial contamination during processing and storage of sap can convert some of the sucrose into invert sugar (degraded sucrose into glucose and fructose). Generally, all grades of maple syrup contain some concentration of invert sugar, and the amount varies among different grades. Lighter syrup has the least invert sugar, whereas very dark syrup has the most invert sugar. In producing maple sugar and maple confections, a small amount of invert sugar is desirable in the maple syrup. However, too little invert sugar in the syrup will cause the maple products to be grainy, while too much may prevent formation of small crystals which are required for example in making maple cream (Heiligmann et al., 2006; Koelling et al., 1996).

2.8 Transformations during evaporation by heating

2.8.1 Density changes

According to Perkins & van den Berg (2009), the change in density, especially sugar content, is the first and most obvious transformation that occurs during evaporation of maple sap. Sap enters the process at an average of around 2° Brix, and by the time 90 percent of the water has been removed the density has increased to only 19° Brix. A concomitant increase in boiling temperature is found as the density gradient within the evaporator develops. It is important to monitor the boiling syrup as it approaches the desired concentration, for example for maple syrup it is at 66.5° Brix. The slower it moves or the longer it is exposed to heat, the darker the syrup becomes (Heiligmann et al., 2006; Koelling et al., 1996; Perkins & van den Berg, 2009).

2.8.2 Changes in colour

The second most obvious transformation during heating of maple sap is a change in colour (Perkins & van den Berg, 2009). Colour develops as a result of chemical reactions occurring during the process. There are several points of consideration. First, the longer the sap is boiled, the darker it becomes. Therefore, anything that slows the evaporation process will produce darker and stronger flavored syrup. Second, for a given length of boiling, little colour is relatively produced at low sugar concentrations, whereas at higher sugar concentrations more colour develops for the same length of boiling time. The length of time when the Brix value is low until up to about 25° Brix is not really important in the formation of colour as the rate of colour formation increases dramatically at around 25° Brix and above. Therefore, final processing at this stage should not be prolonged and should be carefully monitored (Heiligmann et al., 2006; Koelling et al., 1996).

2.8.3 Changes in pH

Sap pH increases rapidly as the solution becomes more concentrated during the heating process, usually from neutral or slightly acidic to slightly to moderately alkaline (Perkins & van den Berg, 2009), before it decreases in alkalinity until the end of the period when the syrup is produced, where it is then about neutral as shown in works by (Akochi-K et al., 1997; Koelling et al., 1996).

2.9 Conclusion

In recent years, there has been a slowly increasing amount of literature on *A. pinnata*. However, the evidence presented in this chapter suggests that only few writers have worked on the monitored processing of *A. pinnata* sap into syrup or sugar. Moreover, those researchers have not treated specifically the processing itself and its operating details. The composition of *A. pinnata* sap and sugar has been reported to some extent following different parameters among the researchers, and in general the information provided is not complete or is unclear. Studies from other types of palms have provided more information on the processing of sap/syrup/solid sugar, however there are still some uncertainties, for example there is no general agreement on which density the syrup should be brought to. Literature on maple sap/syrup/sugar production provides more complete and detailed information on the syrup/sugar processing, and it should be considered as a vital guideline in exploring the processing of *A. pinnata* sap into syrup/sugar. Moreover, the many differences between *A. pinnata* sap and maple sap as the raw material for syrup or sugar production together with different environmental and process conditions should be taken into account before adapting

techniques or methods applied in maple sap/syrup/sugar processing to *A. pinnata* sap sugar processing.

CONNECTING TEXT

The potential to produce sugar from *A. pinnata* sap has been discussed in **Chapter 2**, and it can be seen that there is no comprehensive report available on the quality of the sap as the raw material. Knowing the characteristics of the sap is important as the quality of the sap determines the quality of the final product (sugar). While there is no complete information on the quality of the sap and its changes following the harvesting and sap collection, it is generally known that freshness is important to produce sugar from the concentrated sap. The first experiment was setup to study the characteristics of the sap particularly through some specific parameters namely pH, invert sugar and colour (**Chapter 3**). It was hypothesized that these parameters can be used as indicators to identify the freshness of the sap.

Some of the results presented in **Chapter 3** have been disseminated through a conference presentation.

- Inneke Victor and Valérie Orsat. 2014. **Changes in pH, invert sugar and colour of *Arenga pinnata* sap.** Northeast Agricultural and Biological Engineering Conference. Oral presentation. Ontario, Canada. July, 27-30, 2014.

CHAPTER 3

STUDY OF pH, INVERT SUGAR AND COLOUR CHANGES OF *ARENGA PINNATA SAP*

3.1 Abstract

This study examined the changes in pH, invert sugar and colour parameters, L*a*b* colour space, taking place in *Arenga pinnata* (sugar palm) sap as a function of time following sap harvest. The pH decreased significantly ($p < 0.01$) from 6.538 to 5.201 during the first 240 minutes of the experiment. In another set of samples there was an increase of the invert sugar over a period of 270 minutes from 1.01 mg/ml to 1.86 mg/ml, while the pH decreased from 6.973 to 5.492. The L* values decreased significantly ($p < 0.01$) from 53.0 to 44.5, b* values decreased significantly ($p < 0.01$) from 9.8 to 6.5, and there was no significant effect of time on a* values for the 12 hours of observation of colour change of the sap. Colour quantifications i.e., ΔE^* , hue angle h , chroma C^* and a*/b* ratio were determined for the 12 hours of observation. The colour determinations revealed that the colour of the sap was yellow (by hue angle h and a*/b* ratio), but it was not obviously changed in the first 3 hours (by ΔE^*), while the sap was rich in colour in the beginning and became dull over the study time (by chroma C^*). The findings imply that the pH, invert sugar, and both L* and b* values can be used as indicators to identify deterioration of the sap's freshness.

Keywords : *Arenga pinnata* Merr sap, pH, invert sugar, CIELAB (L*a*b* colour space), colour parameters (ΔE , hue angle h , chroma C^* , a*/b* ratio)

3.2 Introduction

Arenga pinnata Merr is a type of palm tree growing in tropical forests and is native to the Indo-Malayan archipelago, easily found in South and Southeast Asia (Mogea et al., 1991; Sahari et al., 2012). Among the 3000 palm species of the tropics and subtropics categorized as multipurpose trees, the *A. pinnata* tree is considered as one of the most diverse multipurpose tree species under culture (Sahari et al., 2012), and the only one attracting widespread and growing economic interest (Mogea et al., 1991). Having around 150 local names such as arenga palm, black fibre palm, gomuti palm, aren, irok, bagot, kaong, enau, kabung, nira, saguer, and sugar palm illustrates its multipurpose interest with the local use of the sap, fruits, fibres, young leaves, steamed core, wood and roots of the tree (Mogea et al., 1991; Sahari et al., 2012).

Among those uses, the sweet sap, which has been recognized as the strongest sugar solution of any known trees in tropical areas (Nearing & Nearing, 1950), is claimed to be the most important product (Mogea et al., 1991) and is usually consumed by the local people as a sweetener. Further, local people can generate income from producing sugar processed from the sap as it happens widely for example in Indonesia (Marsigit, 2005; Mogea et al., 1991) and Malaysia (Ho et al., 2008; Sahari et al., 2012).

Recently, minimal sap characterization, based on its pH, has been used by local people in Indonesia to determine the quality of the sap, whether it can be used or not in the production of sugar. Sap with pH values of 6-7 is considered to be of good quality for use in the production of high quality sugar (Barlina et al., 2006; Marsigit, 2005). However, the sap is easily spoiled and therefore it must be processed as soon as possible following tapping to prevent the sap/sugar loss as indicated by the measured reducing pH caused by fermentation

(Marsigit, 2005). Several preventive methods were reported for the collection of the sap to prevent it from fermentation, for instance using additives such as coconut husk (Barlina et al., 2006), or candle nut and coconut oil (Marsigit, 2005) which are added to the sap as preservative agents.

Studies on *A. pinnata* have been reported for their multiple industrial uses highlighting the thermo-mechanical behaviors of arenga's thermoplastic starch (Sahari et al., 2013), the composition of *A. pinnata* starch (Adawiyah et al., 2013), the effect of aging in *A. pinnata* fiber-reinforced epoxy composites (Ali et al., 2010), the toughness characteristics of concrete containing *Arenga pinnata* fibre (Razak & Ferdiansyah, 2005), and identifying the compounds contained in the sap as well as the volatile compounds and the changes in the physico-chemical properties of the sap during the production of *A. pinnata* sugar (Barlina et al., 2006; Ho et al., 2007; Ho et al., 2008; Ho et al., 2006; Sihombing, 1995). However, no comprehensive report could be found on how the pH of the sap decreases following its harvest, whilst without any preservation processes, time becomes the main essential issue since pH of the sap will decrease rapidly over time from fermentative activity.

Among the few available publications on *A. pinnata* sap and sugar, this work aims to provide accurate information on changes in pH, invert sugar and colour of *A. pinnata* sap as a function of time following the sap's collection. pH, invert sugar and colour of the sap were considered since they have been used as indicators of the freshness of a variety of raw materials and food products. For example, pH was reported to be a factor affecting freshness of beef (Page et al., 2001), and changes in pH were suggested to be used as a measure of deterioration of sweet sorghum (Lingle et al., 2013). Invert sugar, which is the separation of sucrose to glucose and fructose (which are not conducive to crystallization), is considered as

an important indicator of post harvest quality changes during storage and it was mostly reported in studies on post harvest deterioration in sugarcane (Bhatia et al., 2009; Kenter & Hoffmann, 2009; Rakkiyappan et al., 2009; Saxena et al., 2010; Singh et al., 2009; Uppal et al., 2008), whereas using colour changes as a freshness indicator of food has mostly been reported for fruits (Gonçalves et al., 2007; Lysiak, 2012), fish (Yagiz et al., 2009), beef (Page et al., 2001), and other fresh and processed food products (Pathare et al., 2013).

The knowledge of these characteristics can help *A. pinnata* farmers and *A. pinnata*-related industries to identify sap's deterioration with time, to have a better understanding on how to appropriately handle the sap, and in taking preventive actions to prolong the freshness of the sap in order to produce a good quality sugar.

3.3 Materials and Methods

The sap used in all experiments was tapped from *A. pinnata* trees and was obtained from farmers in two locations in Tomohon, Indonesia. The sap was tapped from different trees with one inflorescence tapped from each tree.

Three characterization experiments were conducted for the study. The first experiment focused on the effect of time on the pH changes of fresh sap. Sap samples of 50 ml were taken and kept at room temperature. The measurement of pH was done every 30 minutes for 240 minutes. The second experiment focused on the progress of both the pH and invert sugar in the sap with time. Fifty ml of sap was sampled every 30 minutes for 270 minutes at room temperature and was analyzed for both pH and invert sugar. The last analysis concentrated on measuring the pH and colour changes of the sap as a function of time. Samples were measured every hour for 12 hours for pH and colour.

3.3.1 Measurements

pH

The measurement of pH was done using a pH meter (WTW Sentix 81, pH 3110, Weilheim 2008, Germany). Calibration was accomplished employing pH 7.0 and 4.0 buffer solutions.

Invert sugar

The invert sugar percentage was determined using a glucometer (GlucoDr Biosensor Type GM 2100, Korea) following the procedure described by Childs & Chabot (Childs & Chabot, 2007).

Colour

Colour was measured using a Konica Minolta Colour Reader CR 10 (Konica Minolta Optics, Inc., Japan). Sap samples were placed inside the plastic cell CM-A131, the white calibration plate CR-A43 was attached to one side of the plastic cell and the measurement was taken. The colour measurement was expressed in CIELAB ($L^*a^*b^*$ colour space); with L^* representing the lightness of colours (0=black, 100=white), a^* positive is red while negative a^* is green, and b^* positive is yellow whereas negative b^* is blue (Loughrey, 2000; Minolta Co., 2007).

Total colour difference (ΔE^*) is expressed by the following equation:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (\text{Mercali et al., 2014; Minolta Co., 2007}).$$

Hue angle is calculated by:

$$h = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (\text{Minolta Co., 2007})$$

Chroma C^* is computed by :

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (\text{Minolta Co., 2007})$$

and the red/yellow colour ratio is simply a^*/b^* ratio (Garcia & Calixto, 2000)

Where L_0^* , a_0^* and b_0^* represented the reading at time zero (the initial sap), and L^*, a^*, b^* represented the individual reading over the duration of observation.

3.3.2 Statistical Analysis

A one-factor repeated-measures Anova with univariate test was used for the experimental design and statistical analysis. All the statistical analysis was done using JMP 10 software program (SAS Institute Inc.). A repeated measure design was chosen since it offers an advantage as a type of design in which it is possible to obtain information concerning a pattern of changes over time by measuring the same sample repeatedly at each specific time point (Davis, 2002; Turner, 2001). A following post-hoc test Tukey's HSD was conducted when there was a significant effect indicated from the univariate repeated-measure Anova test.

3.4 Results

3.4.1 Effect of time on the pH of *A. pinnata* sap

The pH of twelve sap samples from different batches were measured every 30 minutes for 240 minutes and summary statistics of the results are presented in Table 3.1. Since the samples ($n=12$) were taken from several different batches of *A. pinnata* sap, standard error of the mean was preferable to use in the report rather than standard deviation of the samples, as we were interested in showing an estimation of the population mean of *A. pinnata* sap utilized by the farmers in producing *A. pinnata* sugar. It also means that the standard error obtained from this study should decrease if the sample size increases, thus the estimate of the population would improve.

Table 3.1 pH of *A. pinnata* sap during 240 minutes of observation

Time (minute)	Level	N	Min	Max	Mean	Std Err
0	1	12	6.100	6.986	6.538	0.131
30	2	12	5.728	6.887	6.341	0.156
60	3	12	5.413	6.772	6.155	0.174
90	4	12	5.199	6.679	5.979	0.192
120	5	12	5.038	6.574	5.817	0.198
150	6	12	4.920	6.387	5.649	0.190
180	7	12	4.815	6.207	5.505	0.179
210	8	12	4.727	6.044	5.359	0.165
240	9	12	4.659	5.851	5.201	0.143

Nine levels (one level for every 30 minutes measurement) resulted from the one-way repeated-measure Anova design and the data was analysed first for the sphericity condition. Mauchly's test indicated that the assumption of sphericity had not been met, hence degrees of freedom had to be corrected. Univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) corrections were considered and they had the values of the estimation of sphericity (ϵ) of 0.217 for G-G and 0.253 for H-F. Since these ϵ values were less than 0.75, G-G correction was chosen to be used. The G-G test showed that there was a significant effect of time on the pH of the sap ($p < 0.01$) with the average pH decreasing significantly every 30 minutes for 240 minutes. Figure 3.1 shows the sample means on a scatter plot for the sap's pH during the 240 minutes of observation. It presents that the 1st level (0 minutes) had the highest value of pH (6.538) and it decreased gradually until the last level (240 minutes) which showed the

lowest pH value (5.201). Following this result a post-hoc test Tukey's HSD ($\alpha = 0.05$) was conducted and it indicated that all 9 levels of the experiments were different from each other implying that every 30 minutes, the pH of the sap had changed. From the figure we also can see a line of best fit which has an R^2 of 0.997.

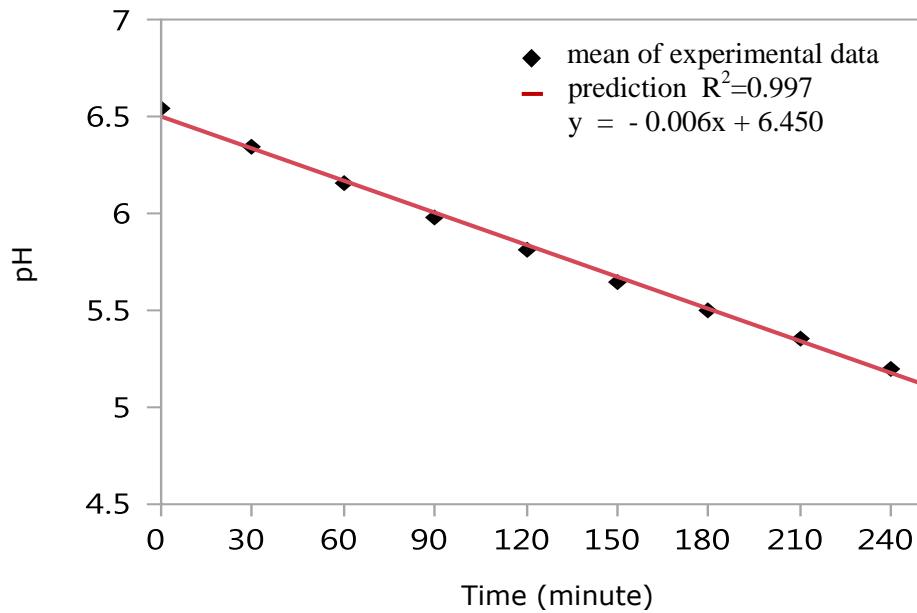


Figure 3.1 pH changes of *A. pinnata* sap as a function of time (240 minutes)

3.4.2 Effect of time on the pH and invert sugar of *A. pinnata* sap

Table 3.2 presents the experimental data of the pH and invert sugar measurements taken from three samples of the sap every 30 minutes for a total period of 4 hours 30 minutes. Ten experimental levels resulted from the one-way repeated-measure Anova design and were used to analyse the significance of time on pH and invert sugar of the sap. Sphericity tests for both pH and invert sugar were unavailable indicating that the assumption of sphericity had not been met. Consequently univariate G-G and H-F corrections were considered. Sphericity estimations (ε) calculated for pH were 0.156 (G-G) and 0.413 (H-F), and for invert sugar were

0.19 (G-G) and 1 (H-F). G-G correction was selected for pH, but both G-G and H-F were considered to be used for invert sugar, since the values of ϵ were < 0.75 for G-G and > 0.75 for H-F. The G-G test showed that there was a significant effect of time on the pH of the sap ($p < 0.01$), and both of the G-G and H-F tests indicated that time had a significant effect on invert sugar ($p < 0.01$). A post-hoc Tukey's HSD test ($\alpha = 0.05$) was done for both pH and invert sugar. It showed that all 10 levels of the experiment were different from each other for the pH of the sap indicating that the pH of the sap changed every 30 minutes. However, among the 10 levels used for this repeated-measure design, 6 different groups were identified suggesting that the invert sugar of the sap also changed during the 270 minutes period studied, but a significant change was not necessarily occurring at every 30 minutes.

Table 3.2 pH and invert sugar of *A. pinnata* sap over a period of 270 minutes

Time (minute)	Level	pH	Invert sugar (mg/ml)
0	1	$6.973^a \pm 0.012^b$	$1.01^a \pm 0.05^b$
30	2	6.861 ± 0.017	1.03 ± 0.04
60	3	6.756 ± 0.015	1.11 ± 0.03
90	4	6.654 ± 0.037	1.15 ± 0.04
120	5	6.496 ± 0.027	1.30 ± 0.08
150	6	6.360 ± 0.042	1.38 ± 0.02
180	7	6.186 ± 0.028	1.46 ± 0.02
210	8	6.019 ± 0.041	1.59 ± 0.03
240	9	5.813 ± 0.055	1.70 ± 0.08
270	10	5.492 ± 0.044	1.86 ± 0.03

^amean ^bstandard deviation

Further, as shown in Figure 3.2 as the time increased, the pH of the sap, on the average, decreased gradually from 6.973 to 5.492 over the 270 minutes. The invert sugar, on the other hand, starting from 1.01 mg/ml initially increased continuously to 1.86 mg/ml at the end of the observation period. The invert sugar of the sap had a slow increase in the beginning as it remained constant around 1.01-1.03 mg/ml in the first 30 minutes, before it had a little increase around 1.10-1.15 mg/ml during the first 90 to 120 minutes, then it rose from 1.30 mg/ml to 1.86 mg/ml at the end of the 270 minutes test period.

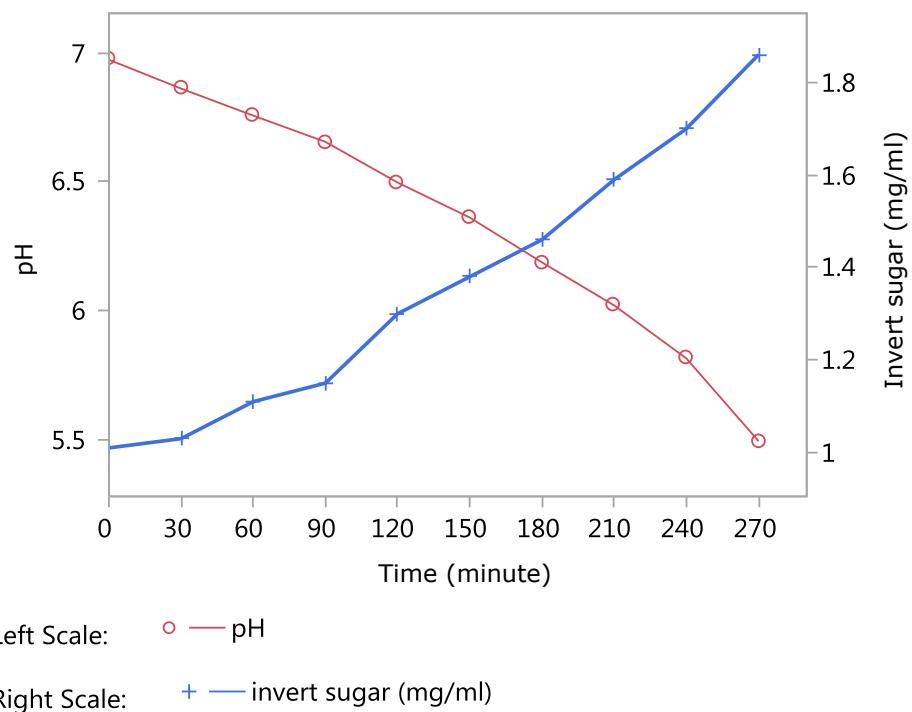


Figure 3.2 Effect of time on *A. pinnata* sap's pH and invert sugar

Correlation analysis which measures the strength of association between pH and invert sugar was conducted. The data was tested for its distribution and it showed that both variables (pH and invert sugar) did not follow normal distribution assumption. Hence, non-parametric Spearman rank correlation test, which does not make any assumptions about the distribution

of the data, was chosen. The data also met a second assumption of employing Spearman's test since the data indicated a monotonic relationship between pH and invert sugar. The analysis showed Spearman's correlation coefficient (ρ) of - 0.9923, indicating a very strong negative association between pH and invert sugar (Figure 3.3). Rapid changes occurred in both pH and invert sugar of the sap which implies that the quality of the sap was easily and rapidly spoiling.

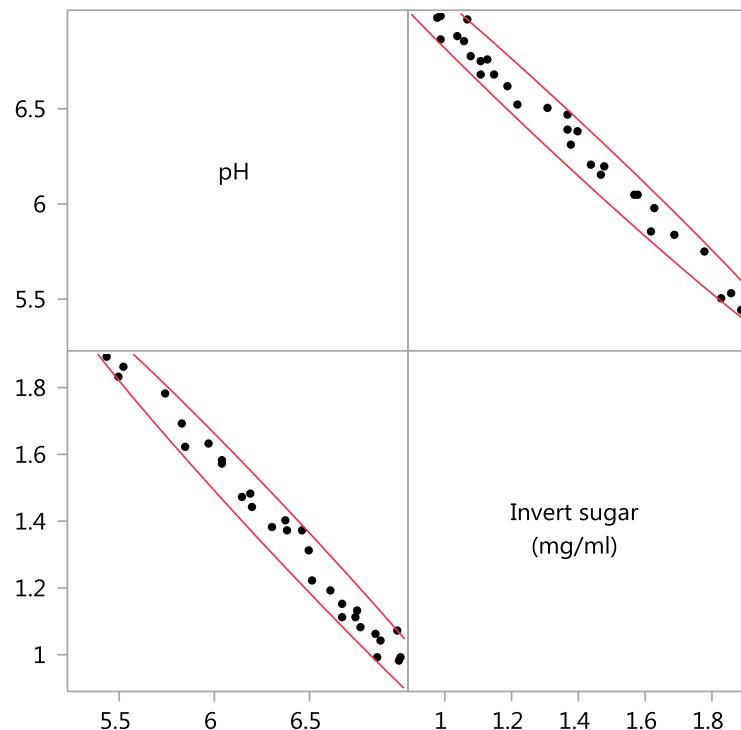


Figure 3.3 Correlation between invert sugar and pH of *A. pinnata* sap

3.4.3 Effect of time on the pH and colour of the *A. pinnata* sap

The results obtained from the measurements of pH and L*a*b* colour values of the sap during 12 hours are shown in Table 3.3. A one-way repeated-measure Anova was employed to test the effect of time on pH and colour parameters L*a*b*.

Table 3.3 pH and L*a*b* colour values of *A. pinnata* sap during 12 hours of observation

Time (hour)	Level	pH	L*	a*	b*
0	1	6.387 ^a ± 0.006 ^b	53.0 ^a ± 0.4 ^b	1.5 ^a ± 0.3 ^b	9.8 ^a ± 0.1 ^b
1	2	6.310 ± 0.033	54.8 ± 0.7	1.6 ± 0.1	9.5 ± 0.1
2	3	6.052 ± 0.009	52.0 ± 0.1	1.4 ± 0.1	9.3 ± 0.1
3	4	5.814 ± 0.051	51.4 ± 0.3	1.4 ± 0.1	9.2 ± 0.1
4	5	5.573 ± 0.011	49.5 ± 0.8	1.4 ± 0.1	8.9 ± 0.1
5	6	5.335 ± 0.009	49.5 ± 0.5	1.4 ± 0.2	8.5 ± 0.2
6	7	5.240 ± 0.008	48.1 ± 0.5	1.6 ± 0.1	7.7 ± 0.3
7	8	5.126 ± 0.006	48.5 ± 0.4	1.4 ± 0.1	8.0 ± 0.2
8	9	5.057 ± 0.004	48.0 ± 0.4	1.5 ± 0.2	7.5 ± 0.4
9	10	5.027 ± 0.006	47.5 ± 1.3	1.3 ± 0.2	7.5 ± 0.2
10	11	4.964 ± 0.003	46.6 ± 0.4	1.3 ± 0.1	7.3 ± 0.2
11	12	4.897 ± 0.006	46.1 ± 0.2	1.2 ± 0.0	7.0 ± 0.1
12	13	4.883 ± 0.003	44.5 ± 0.5	1.2 ± 0.1	6.5 ± 0.1

^amean ^bstandard deviation

The G-G and H-F corrections were considered since the Mauchly's test was too small for pH measurements and was unavailable for the L*a*b* values. The univariate G-G estimates of sphericity (ϵ) for pH, L*, b*, and a* values were 0.147, 0.118, 0.129, and 0.134 respectively, whereas they were 1 (pH), 0.317 (L*), 0.491 (a*), and 0.602 (b*) for the H-F correction. The G-G correction values were chosen since those values are less than 0.75. The results showed that for 12 hours there was a significant decrease ($p < 0.01$) of pH from 6.387 to 4.883, of L* values from 53.0 to 44.5, and of b* values from 9.8 to 6.5. However, there was no significant effect of time on a* values ($p > 0.01$). Following the results of a Tukey's

HSD ($\alpha = 0.05$), a post-hoc test was done for the parameters showing a significant difference in the pH, L* and b* values. The test showed that the pH values of the sap were different from each other for the first 7 hours, but there were no differences between 8 and 9 hours and between 11 and 12 hours. During the experiment, seven different groups were identified in a Tukey's HSD test for L* values, whereas eight different groups were detected for b* values. It can also be seen from Figure 3.4, Figure 3.5, Figure 3.6, and Figure 3.7 that R^2 values of a line of best fit for pH, colour L*, colour a*, and colour b* were 0.912, 0.925, 0.508, and 0.969, respectively.

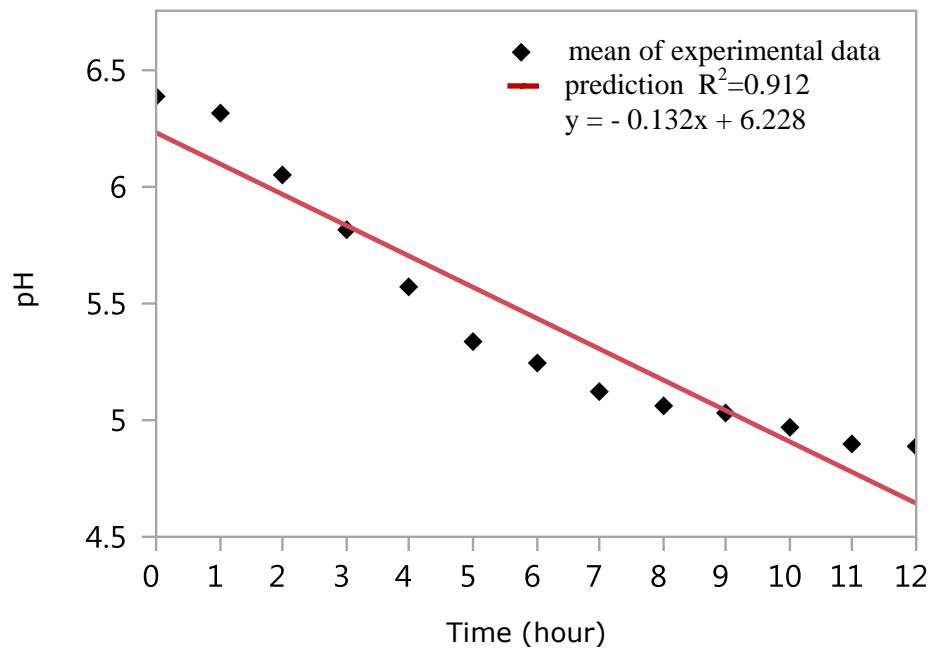


Figure 3.4 pH changes as a function of time (12 hours)

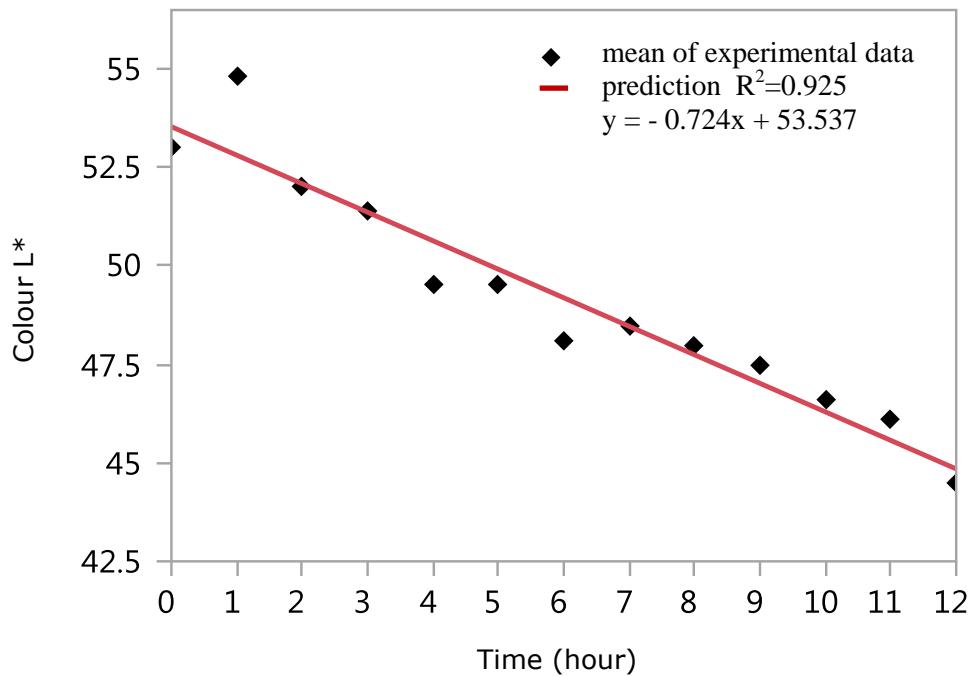


Figure 3.5 Colour L* changes as a function of time

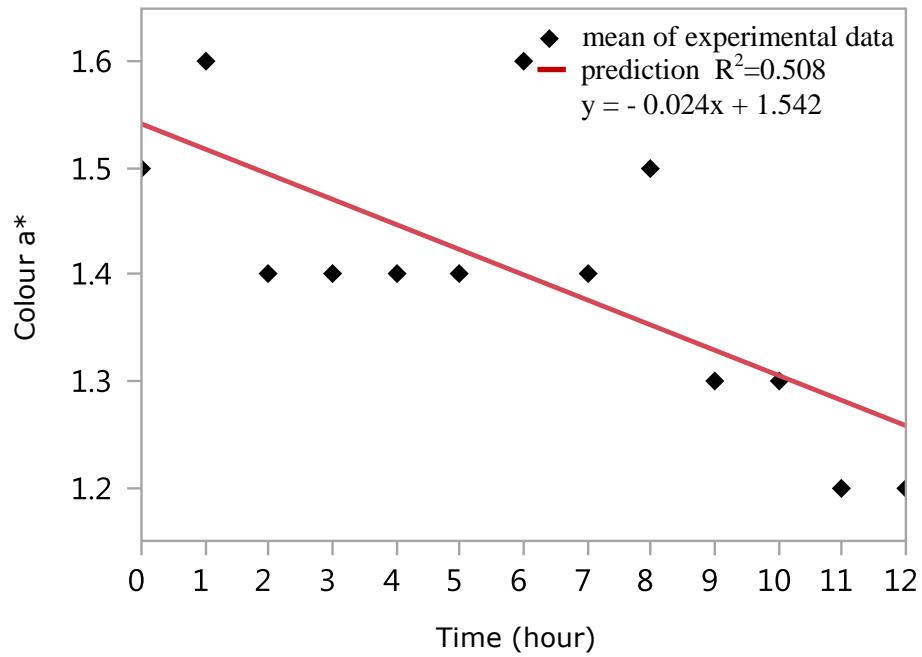


Figure 3.6 Colour a* changes as a function of time

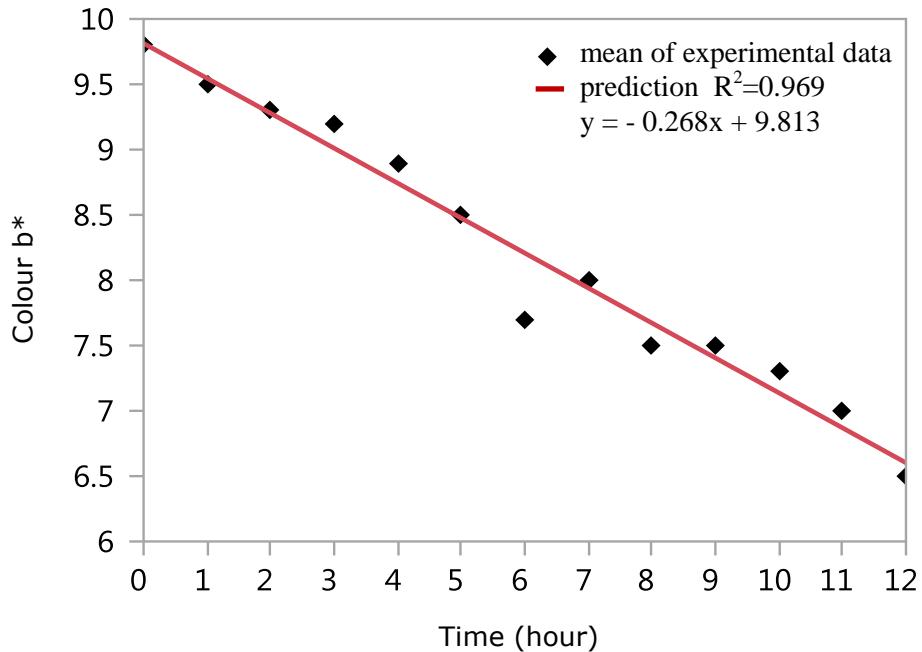


Figure 3.7 Colour b* changes as a function of time

3.4.4 Colour quantification of *A. pinnata* sap

To expose the distinction of colour in *A. pinnata* sap following 12 hours from harvesting, colour quantification namely ΔE^* , hue angle h , chroma C^* , and a/b ratio were derived from $L^*a^*b^*$ values and the results are presented in Table 3.4. It is apparent from this table that ΔE^* tended to increase as the time increased, hue angle h was in the range from 78.3° to 81.4° , chroma C^* decreased continuously from 9.9 to 6.6, and the a^*/b^* ratio was constant at 0.2.

3.5 Discussion

This study reports for the first time the changes in pH, invert sugar and colour of *A. pinnata* sap as a function of time following harvest.

Table 3.4 Colour quantification of *A. pinnata* sap during 12 hours of observation

Time (hour)	Colour quantification			
	ΔE^*	h	C^*	a^*/b^*
0	0.0	81.3°	9.9	0.2
1	1.8	80.4°	9.6	0.2
2	1.1	81.4°	9.4	0.2
3	1.7	81.3°	9.3	0.2
4	3.6	81.1°	9.0	0.2
5	3.7	80.6°	8.6	0.2
6	5.3	78.3°	7.9	0.2
7	4.8	80.1°	8.1	0.2
8	5.5	78.7°	7.6	0.2
9	6.0	80.2°	7.6	0.2
10	6.9	79.9°	7.4	0.2
11	7.5	80.3°	7.1	0.2
12	9.1	79.5°	6.6	0.2

3.5.1 The changes of pH

While there is little information on the initial pH of sap reported in previously published studies for *A. pinnata*, this study clearly presented the initial pH of fresh *A. pinnata* sap and how pH changed continuously over time following harvest. Saps with different initial pH varied from 6.103 to 6.973, monitored over different times of observation (4 hours, 4hours 30 minutes, and 12 hours), were used and all the results confirmed that the pH of the sap decreased as a function of the time from harvest (Figure 3.1, Figure 3.2, Figure 3.4). The initial pH of the sap samples used were similar with the initial pH of *A. pinnata* sap reported

by others to range between 6.34 - 6.50 (Barlina et al., 2006), and 6 - 7.4 (Marsigit, 2005), and of other sap such as coconut sap at pH 7 reported by Vidanapathirana et al. (1983), and 6.40 by Apriyantono et al. (2002), and 6.8 for Tunisian date palm sap (Manel et al., 2011). The measurements showed a continuous decrease of pH of approximately 1.337 in 4 hours, 1.481 after 4 hours 30 minutes and 1.503 after 12 hours (Figure 3.1, Figure 3.2, Figure 3.4). Though there were similarities of the tendencies of the pH to decrease as shown in this study with other reported palm sap studies, the pH results from this experiment were somehow lower than the reported findings from previous studies which showed a pH drop for coconut sap of about 3 (from 7 to 4) in 15-20 hours (Vidanapathirana et al., 1983), and of Tunisian date palm of about 2.5 (from 6.8 to 4.3) after 9 hours due to fermentation (Manel et al., 2011).

The present findings are consistent with other research which found pH changes with time, indicative of sap deterioration, such as untreated cane juice which had the largest pH change in the first 14 hours following harvest (Eggleston, 2002), sweet sorghum juice from pH 5.16 to 4.34 in 2 days of storage (Lingle et al., 2013), sugarcane juice from several varieties with pH changes from 5.30 to 4.50 in 96 hours (Saxena et al., 2010). The monitoring of pH changes, as an indicator of the deterioration of raw products, was also reported for Norway lobsters, but in that case there was a reported increase in pH from 7.03 to 8.08 during 7 days of iced storage (Albalat et al., 2011).

The changes of pH in collected tree sap is likely related to the microorganism activity which mostly comes from the inflorescences and spathes of the tree. The rapid growth of microorganisms reduces the pH by producing organic acids, thus indicating varying levels of deterioration. When acids increase in the sap, the pH decreases since the acids release hydrogen ions in the sap. Bacteria spoilage is inevitable because food products provide a

suitable nutrient environment for vegetative cells to grow, and yeasts tend to grow well in mild acid conditions where the sugar concentration is high as is the case in *A. pinnata* sap. Similarly, in sugarcane juice deterioration, acids, mainly D-lactic acid, are produced from the microbial degradation of sugars, which lowers the pH (Eggleston et al., 2004). The decrease of pH by lactic acid bacteria was also reported for palm sap (*P. dactylifera*) (Manel et al., 2011). In a study of coconut sap, a phase, indicated by a rapid drop of pH from 7 to 4 was dominated by bacteria, and a phase, characterized by a constant pH of 4, was dominated by yeasts (Vidanapathirana et al., 1983). A study which investigated autochthonous yeasts and their functions during the fermentation of pineapple juice found that the pH of the juice decreased slightly from an initial pH of 3.9 down to 3.6 after six days of fermentation (Chanprasartsuk et al., 2010).

Temperature is another environmental factor which interacts with pH. In a reported study, sap which was kept untreated at room temperature (~24°C), was spoiled in relation with its decreasing pH, as supported by the fact that any non-sterile food is liable to spoil with time if it is held between the temperatures of -5°C and 70°C (Jay, 2006).

The findings from the present study, therefore, provide important insights for considerations during the sap tapping processes and for taking measures to prevent sap deterioration. A need for reducing microbial contamination in fresh sap is necessary to produce sugar of good quality. Sterilization of the container especially bamboo container with the smoke of a wood fire is commonly practiced by *A. pinnata* farmers in Tomohon. While this method can affect in flavor of the sugar, several approaches presented below which are generated from techniques employed in maple syrup and sugar industries (Heiligmann et al., 2006) should be considered to kill or prevent the growth of microorganisms in *A. pinnata* sap.

- Knives to cut inflorescence stalk and ijuk (*A. pinnata* fibers) to cover the cut stalk from outside debris should be kept as clean as possible to minimize microbial contamination.
- Use of a cover (besides the ijuk) to keep out rain since rain dilutes sap, thus more sap, time and fuel is required to produce the sugar.
- Container must be kept as clean as possible, and should be used only for collecting *A. pinnata* sap. The container must be washed in detergent or bleach solution, thoroughly rinsed, and dried before being used to collect the sap. Utilization of alcohol might also be considered.
- After collecting the sap, the containers must be cleaned and should be stored in a clean and dry location. This post harvesting cleaning should not be postponed, because the thin layer of dried sap on the surface of the container will support the growth of microorganisms. Further, it will be more difficult to remove sap and debris accumulations if washing of the container is delayed.
- When container is not used for a while for example there is a time lag between tapping one inflorescence to another inflorescence, it is a good idea to wash the container with hot water, rinsed with a bleach solution, and thoroughly rinsed to make sure all possible sources of contamination and all traces of the bleach solutions are removed.

As a whole, a major requirement for producing good quality sugar is to gather and process the sap as soon as possible, and to practice cleanliness during the tapping and collecting of the sap to prevent microbial contamination in the sap.

3.5.2 Changes and interaction of pH and invert sugar

To the best of our knowledge, there is no available report on invert sugar changes and the relationships between pH and invert sugar changes of *A. pinnata* sap following harvest. In order to observe the changes of invert sugar and how they relate to the pH of the sap, this experiment was conducted over a fixed period of time of 4 hours 30 minutes. Figure 3.2 illustrates that there was a negative correlation found between pH and invert sugar concentration wherein, as the time increased, the pH of the sap decreased gradually and the invert sugar content increased.

A glucometer was used to measure the invert sugar of *A. pinnata* sap following the procedure commonly used in maple sugar industries (Childs & Chabot, 2007). The value of the invert sugar shown was thus specifically for glucose content and not for other invert sugars. As shown in Table 3.5, the initial invert sugar (glucose) and the corresponding pH of *A. pinnata* sap were similar with those of *Phoenix dactylifera* L. sap, while pH and the corresponding invert sugar of *Borassus flabellifer* Linn sap were lower than those of *A. pinnata* sap.

The relationship of the changes of invert sugar and pH identified in the current study is consistent with a report on pH and invert sugar changes during coconut sap fermentation. Whilst the invert sugar increased from the beginning and reached the maximum in about 30 hours, the pH of the coconut sap decreased and reached a constant value of 3.8 – 4.0 in a day (Vidanapathirana et al., 1983). A similar pH and invert sugar correlation was also revealed for sugarcane juice (Saxena et al., 2010).

Table 3.5 The initial pH and invert sugar of fresh sap from several palm trees

Type of palm	pH	Invert sugar (glucose)	Source
<i>Borassus flabellifer</i> Linn.	4.19 - 5.23	0.50% - 1.85%	(Naknean et al., 2010)
<i>Phoenix dactylifera</i> L.	6.86 ± 0.05	2.51%	(Ben Thabet et al., 2009a)
<i>Arenga pinnata</i> Merr.	6.973 - 5.492	2.02% - 3.72%	Present study

There is a possible explanation for this result. Invert sugars are produced by the breakdown (hydrolysis) of sucrose into glucose and fructose, commonly by the action of microorganisms (Heiligmann et al., 2006). In *A. pinnata* sap, sucrose is the predominant sugar when it comes from the tree, and accounts for between 11.28% - 30.12% (Kismurtono, 2012; Rumajar et al., 2008). In the current study, following harvesting, some of the sucrose was converted to invert sugar mostly as a result of microbial contamination and it continued to the end of the observation time (4 hours 30 minutes) causing a significant increase of the invert sugar. In the degradation process, microorganisms produce organic acids which lead to a lowering of the sap's pH.

The correlations between pH and invert sugar identified in this study, while preliminary, suggest that invert sugar can also be used as an indicator of sap degradation, besides pH, to determine the quality of *A. pinnata* sap following its harvest as a raw material prior to sugar processing.

3.5.3 The changes of pH and colour L*a*b* values

This experiment aimed to determine the pH and colour (L*a*b* values) changes that take place during the hours following harvest of *A. pinnata* sap. The results showed that the average pH decreased significantly ($p < 0.01$) from 6.387 to 4.883 over 12 hours, while L* values decreased significantly ($p < 0.01$) from 53.0 to 44.5, b* values decreased significantly ($p < 0.01$) from 9.8 to 6.5, and there was no significant effect of time on a* values ($p > 0.01$). Moreover, the pH decreased rapidly from 6.387 to 5.126 in the first 7 hours, then it decreased slowly to 4.883 at the end of the period of observation. These results suggested that as the pH decreased, the colour (L* and b* values) of the sap changed over time.

Table 3.6 compares the L*a*b* values obtained from the current and published studies using different palm saps. Vidanapathirana et al. (1983) also reported a yellowish brown clear liquid as the colour of the coconut sap with a pH of 7. Manel et al. (2011) reported the colour changes of *P. dactylifera* sap to a milky white during the sap fermentation with lactic acid bacteria considered to change the consistency and the colour from transparent to whitish through their production of gums such as dextran. However, there is no information found in the literature on the changes of L*a*b* values with time for palm saps including *A. pinnata* sap.

A range of L*, lightness (55) to darkness (45) of colour was reported after 12 hours and it was significantly different ($p < 0.01$) with time. Following the first hour, the sap was increasingly lighter (Figure 3.5) compared with the colour of the original sap. The b* parameter showed a similar tendency with L* as its values decreased over time from 9.8 to 6.5 (Figure 3.7), indicating that the sap was more yellow in the beginning. The a* did not show a

significant difference ($p > 0.01$) over time since a^* values ranged from +1.6 to +1.2 (Figure 3.6), which indicated a reddish color.

Table 3.6 The pH and L*a*b* values of several palm saps

Type of palm	pH	Colour space			Source	Instrument
		L*	a*	b*		
<i>Borassus flabellifer</i> Linn.	4.19 – 5.23	61.49 - 87.53	1.46 - 3.52	12.41 - 19.31	(Naknean et al., 2010)	Hunter Lab Colourflex
<i>Phoenix dactylifera</i> L.	6.86 ± 0.05	72.01 ± 0.07	0.64 ± 0.02	15.64 ± 0.02	(Ben Thabet et al., 2009a)	Lovibond Tintometer PFX 195
<i>Arenga pinnata</i> Merr.	4.883 – 6.387	44.5 - 54.8	1.2 - 1.6	6.5 - 9.8	Present study	Minolta Reader

In the present study, L* and b* values were positively correlated to the pH of the sap, showing that as pH of the sap decreased, L* and b* values decreased (Figure 3.5, Figure 3.7). The connection between pH and colour changes identified in the study seems to be consistent with other findings with food such as fruit colour (Gonçalves et al., 2007), and beef colour (Page et al., 2001). The pH of the sap was associated with L* and b* values, indicating that pH was more a function of lightness/darkness and yellow/blue rather than the red/green hues in *A. pinnata* sap. Referring to the specific colour values, as identified in this experiment, it was somewhat similar with other reports such as that for beef muscle where pH is most highly associated with a* and b* than for values of L* (Page et al., 2001), while the a* value was a better ripening indicator of Sampion and Royal Gala apples rather than L* and b* values (Łysiak, 2012).

In addition, based on our subjective observations, higher pH caused *A. pinnata* sap to appear more transparent (clearer), whereas lower pH caused the sap to appear white. This experiment supports the hypothesis that colour is also a good indicator of quality change which can be correlated to pH changes and can be used to predict the corresponding quality deterioration resulting from post-harvest sap handling and storage.

3.5.4 Colour quantification ΔE^* , hue angle h , chroma C^* , and a^*/b^* ratio

It has been suggested that the total color difference (ΔE^*) indicates the colour difference but not in what way the colours are different (Minolta Co., 2007). The total colour difference (ΔE^*) can be classified as small difference ($\Delta E^* < 1.5$), distinct ($1.5 < \Delta E^* < 3$), and very distinct ($\Delta E^* > 3$) (Adekunle et al., 2010). Using the initial sap as a control sample in calculating ΔE^* , it is apparent that there was a minor difference on the colour of *A. pinnata* sap in the beginning (the initial sap) and the sap after 3 hours of harvesting, however after 4 hours the total colour difference was very clear as indicated by a ΔE^* of 3.6 to 9.1 (Table 3.4).

The hue angle (h) is a basic unit of colour and is considered as the qualitative attribute of colour. Hue angle h is the most obvious characteristic parameter to define differences in colour and it traditionally identifies colour as reddish or greenish, etc. Hue angle h is expressed in degrees ; 0° would be red, 90° would be yellow, 180° would be green, and 270° would be blue (Pathare et al., 2013). The results of the present study, as shown in Table 3.4, indicate that the colour of *A. pinnata* sap was yellow, and it cannot be obviously differentiated between the initial sap and after 12 hours of observation (78.3° to 81.4°).

Chroma C^* is the saturation or vividness of colour (Itle & Kabelka, 2009), and is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness (Minolta Co., 2007). A colour becomes more intense as C^* increases, and it becomes

more dull as C^* decreases (Itle & Kabelka, 2009; Minolta Co., 2007). As shown in Table 3.4, the colour of *A. pinnata* sap looked rich and full in the beginning before it became dull indicated by a decrease of C^* values over the observation period.

A measurement of a^*/b^* (red/yellow) ratio can be classified as yellow ($a^*/b^* < 1$) or red ($a^*/b^* > 1$) (Garcia & Calixto, 2000). Data in Table 3.4 indicates that the saps were yellow in colour because the a^*/b^* ratio of the saps were less than 1. Further, the distinction among the saps was difficult as the same value of a^*/b^* ratio was estimated (0.2). This result is comparable to the result for the hue angle h .

3.6 Conclusion

This study was designed to determine the effect of time on pH, invert sugar and colour $L^*a^*b^*$ changes of fresh *A. pinnata* sap. Colour quantifications i.e., ΔE^* , hue angle h , chroma C^* and a^*/b^* ratio were also estimated. The findings suggested that pH, invert sugar and colour $L^*a^*b^*$ of the sap changed over the duration of time tested, and there was a correlation between each; while pH decreased, the invert sugar increased, and the colour L^* and b^* values decreased. Colour quantification measurements indicated that *A. pinnata* sap was yellow (h and a^*/b^* ratio), and that the sap was very distinct after 4 hours (ΔE^*) and it became dull over time (C^*). Taken together, these results indicate that the freshness of the sap can be estimated using pH, invert sugar or L^* and b^* values. The results of this study will be useful to boost the awareness of the need of clean conditions of tapping and collecting the sap to insure a high quality sap for high quality sugar production.

3.7 Acknowledgment

The authors would like to acknowledge the financial support of the Higher Education of Ministry of Education and Culture of Indonesia for the principal author, and thank you to Mr. Nico Tatontos for helping to obtain the samples, and farmers in Tomohon for providing the sap.

CONNECTING TEXT

The effect of time on pH, invert sugar and colour changes of fresh *A. pinnata* sap was studied in **Chapter 3**, and the findings showed that these parameters can be used to assess the freshness of the sap. Sap deteriorates quickly as indicated by the rapid changes in pH, invert sugar and colour, as a result the sap must be processed immediately following the harvesting to maintain the quality of the sap which then has an effect on the quality of the final product (sugar). The following study (**Chapter 4**) focuses on processing the sap into sugar by the removal of water, primarily by evaporation through boiling. Controlled boiling was chosen for the processing of *A. pinnata* sugar as it is the most practical and useable method for the local community. During the heating process many complex physical and chemical transformations are involved including temperature, pH, and Total Soluble Solids (TSS).

Some of the results presented in **Chapter 4** have been disseminated through a conference presentation.

- Inneke Victor and Valérie Orsat. 2014. **Exploring the processing of *Arenga pinnata* sugar.** American Society of Agricultural and Biological Engineers. Food and Process Engineering Division Poster Session. Montreal, Canada. July, 13-16, 2014.

CHAPTER 4

CHANGES IN TEMPERATURE, TOTAL SOLUBLE SOLIDS (TSS), AND pH DURING THE HEATING PROCESS OF *ARENGA PINNATA* SAP

4.1 Abstract

This study aims to examine the changes in temperature, Total Soluble Solids (TSS), and pH during the heating process of *Arenga pinnata* (sugar palm) sap with different initial pH without adjustment i.e., 7.020, 6.388, 6.092 and 5.713 and to assess the evolution of pH changes between untreated sap (the sap which was kept at room temperature), and pre-boiled sap (the sap heated at its boiling point). Preboiling of the sap was chosen as it was the most applicable preservation method for the local farmers. A trend of behaviour of the *Arenga pinnata* sap during the heating process was identified in a first set of experiments: an increase of pH until it reached a peak at the boiling point (98-98.7°C), before it had a continuous decrease to the end of the heating process. The final temperature of the concentrated sap was about 127°C corresponding to a TSS > 93 (% w/w) with pH values close to the initial pH of the fresh sap. The initial sap should be maintained at pH of 6 or higher in order for granulated sugar processing to occur. Changes of pH in untreated sap and pre-boiled sap were tested at 0 and 18 hours in the second set of experiments. In contrast to untreated sap, which showed a strong decrease in pH after 18 hours (from 6.388 to 3.760), the pH of pre-boiled sap remained stable after 18 hours (from 8.532 to 8.465). Consequently, after 18 hours, untreated sap could not be used as a raw material for processing into sugar, while pre-boiled sap could be successfully processed into sugar. This work contributes to improved knowledge on the heating process of *Arenga pinnata* sap in addition to proposing to help the farmers to preserve

their sap from deterioration by heating the sap to its boiling point as soon as possible following harvest.

Keywords : *Arenga pinnata* Merr sap heating process, temperature, Total soluble solids (TSS), pH.

4.2 Introduction

A. pinnata Merr, a type of palm tree growing in tropical forests of Asia, is considered as one of the most diverse multipurpose tree species under culture and currently attracting widespread economic interest (Mogea et al., 1991; Sahari et al., 2012). Among its multipurpose uses, with local preference for the sap, fruits, fibres, young leaves, steamed core, wood and roots of the tree, the sweet sap is claimed to be the most important product and is usually widely consumed by the local population in Indonesia, Thailand and Malaysia as a sweetener (Mogea et al., 1991; Panyakul, 2001; Sahari et al., 2012). The sugar is usually produced through a simple process where the sap is collected, then it is transferred into a big iron pan and heated on a wood fired stove for a few hours until it becomes sufficiently thickened. Afterwards, the thickened liquid is poured into a mold (Ho et al., 2007; Ho et al., 2008; Mogea et al., 1991). A solid sugar is thus produced through this type of process known as palm sugar or neera sugar in Malaysia (Sahari et al., 2012), brown sugar in Indonesia and Thailand (Mogea et al., 1991; Panyakul, 2001), and jaggery in India (Rao et al., 2009). In Tomohon Indonesia, granulated sugar, locally named as 'ant sugar' is also produced by stirring the thickened syrup to form a granulation.

Though local people can generate income from producing sugar processed from the sap (Ho et al., 2008; Sahari et al., 2012), far too little attention has been paid to the

evaporation process of the sap, a method of producing the sugar simply applied by the local people. Previous studies (Ho et al., 2007; Ho et al., 2008; Ho et al., 2006) have focused on compounds contained in the sap as well as compounds and volatile compounds during the sugar processing. Those studies, so far, have not discussed about processing of the sap with different initial pH, a parameter used to determine whether the sap can be used or not in the production of granulated sugar. On the other hand, fresh sap which has a nearly neutral pH is easily spoiled as identified by a decrease of pH of the sap with time, thus the sap must be processed as soon as possible (Barlina et al., 2006; Marsigit, 2005). A method commonly applied by the farmers in Tomohon to prevent the sap deterioration during harvesting is using mangosteen rind or a piece of mangosteen tree bark which is inserted into the container installed at the tapped inflorescences. Rind and tree bark of mangosteen contain xanthones which have remarkable biological activities such as antibacterial and antifungal (Dweck; Palakawong et al., 2010), and therefore utilizing mangosteen rind and tree bark helps in the preservation of *A. pinnata* sap. Another method proposed by Rindengan et al. (2006) is employing coconut coir, a natural fibre extracted from the husk of coconuts, which can be added as a preservative agent, during the sap tapping as a natural means of preserving the quality of the sap. According to Rindengan et al. (2006) an amount of coconut coir was placed in the container used in collecting the sap tapped in the afternoon (17:00 to 18:00 hours) which was sampled in the morning (06:00 hours). It was reported that using coconut coir preserved the quality of the sap up to 3 hours following the harvesting (sampling) as indicated by pH values ranging from 6.34 to 6.50. Tannins, which were estimated at about 3.12% in coconut coir, could be the factor causing longer sap storage times by helping to reduce the amount of living microorganisms. Consequently, the sap can be used as a raw material in *A.*

pinnata sugar processing since the manufacture of the sugar requires a pH of 6 or higher (Barlina et al., 2006; Marsigit, 2005).

Sometimes, however, there is a time lag that the farmers must wait until the collected amount of the sap is substantial enough to be processed into sugar. In several cases, the amount of the sap tapped in the afternoon and collected in the morning is not enough, and therefore the farmers need to collect another batch of the sap which usually requires more than 3 hours, usually by the end of day. It can also happen that when the amount of the sap tapped in morning, along with that collected in the afternoon is not enough, the farmers have to collect another batch of sap which requires another whole night of tapping. This brings the challenge of maintaining the quality of the sap at a pH high enough (close to 7) to allow processing into sugar.

The quality of *A. pinnata* sap can be maintained by preventing the growth of bacteria, fungi or other micro organisms. Several preservation methods have been reported using in postharvest technology such as UV radiation which is employed to maintain the quality of fruits such as strawberries, apples, watermelon, pomegranates, grapes, and fresh vegetables such as carrot and spinach (Ribeiro et al., 2012). Another food preservation method is freezing which is a noticeable preservation method to the appropriate climates. Any areas that have freezing temperatures for even part of a year might use this climate to preserve foods. An example of freezing temperature that affected sugar industry in Louisiana has been reported by Eggleston et al. (2004). The sugarcane harvesting occurred when the minimum temperature in the area were reported from -3.5°C to -5.1°C (Eggleston et al., 2004). The freezing method is also available by utilization of energy generated from electricity or other energy sources, thereby requiring more consideration on the initial capital costs and maintenance costs.

Among the few available publications on *A. pinnata* sap and sugar, this work aims: 1) to determine the changes in temperature, Total Soluble Solids (TSS), and pH of *A. pinnata* sap during the heating process of saps with different initial pH and ; 2) to attest that pre-heating can preserve the sap from deterioration by maintaining its pH during storage. *A. pinnata* sap, with several different initial pH, was used in the current study and pH is one of the most important parameters governing the quality of the subsequently produced sugar; whereas heating the sap was chosen among other preventive methods in postharvest technology such as UV radiation or freezing, since the heating method is likely a more practical and useable method for the local people.

This work contributes to the very scarce existing knowledge of the physico-chemical changes which occur during the heating process of *A. pinnata* sap and it will help the farmers and related industries to maintain the sap from deteriorating due to fermentation during sap harvesting, handling and storage.

4.3 Materials and Methods

The sap used in all experiments was tapped from *A. pinnata* trees and was obtained from farmers in Tomohon, Indonesia, who tapped the sap from different trees with one inflorescence tapped from each tree. Tomohon has an altitude ranging between 700-1000 meters above sea level. Total soluble solid (TSS) of the sap ranged from 12 to 14.9%. There was no chemical adjustment of the initial pH of the saps, and the process of heating the saps began two to five hours after the saps were harvested.

Two characterization experiments were conducted for the study. The first experiment focused on the temperature, TSS, and pH changes of harvested saps. The saps, with four different initial pH i.e., pH of 7.020, 6.388, 6.092 and 5.713 were used in the first set of

experiments. The boiling process was established following the procedure performed in making maple syrup and maple sugar confections (Koelling et al., 1996) with some modifications. Four liters of the filtered sap (filtered through a 100 microns filter fabric) at each pH were boiled in an open stainless steel pan over a gas fire with a constant supply of heat. The foam produced during the heating process was controlled using palm oil as a defoaming agent. The scum was skimmed off as it appeared. The sap was heated to 25-28°C above the boiling point of water. The syrup was cooled to about 93°C and stirred manually until granulation was achieved. Ten ml of the sample was collected at each specified time during the heating process. The intervals of sampling were 30 minutes from 0-60 minutes, 20 minutes from 60-150 minutes, and 5 minutes from 150-155 minutes, and 2 minutes at the end of the heating process. The changing time intervals were considered from a preliminary experiment showing that the TSS change in the beginning of the heating process was not noticeable but changing very fast at the end of the process.

The second set of experiments focused on pH changes in both untreated sap and pre-boiled sap. One liter of fresh filtered sap (filtered through a 100 microns filter fabric) was boiled in an open stainless steel pan over a gas fire until it reached its boiling point (about 10-15 minutes boiling). One hundred ml of sample was taken and kept until 18 hours. The pH of the pre-boiled sample was measured at 0 and 18 hours. Some amount of the fresh sap from the same batch was kept untreated until 18 hours, and the initial pH of the untreated samples was measured as well as the pH after 18 hours.

4.3.1 Measurements

Temperature

The temperature was measured using a digital stainless steel probe thermometer (BG 363, Blue Gizmo, Acez).

Total soluble solids (TSS)

TSS (% w/w) was measured using an Atago Pocket Refractometer (PAL-3, Atago, Japan) (0-93% Brix).

pH

The sample was diluted ten-fold, and the measurement of pH was done using a pH meter (WTW Sentix 81, pH 3110, Weilheim 2008, Germany). Calibration was accomplished employing pH 7.0 and 4.0 buffers.

4.3.2 Statistical Analysis

All samples were studied in triplicates, and all the statistical analysis was done using JMP 10 software program (SAS Institute Inc.).

4.4 Results and discussion

4.4.1 Temperature, TSS and pH changes during heating process of *A. pinnata* sap with different initial pH

Starting the heating process of the saps at room temperature (~ 24°C), the experiments were terminated in 157 minutes when the temperature reached about 122-127°C with the corresponding TSS of approximately 93 (% w/w). Figures 4.1 - 4.4 show the time-temperature profile and corresponding change of TSS for *A. pinnata* sap with different initial pH.

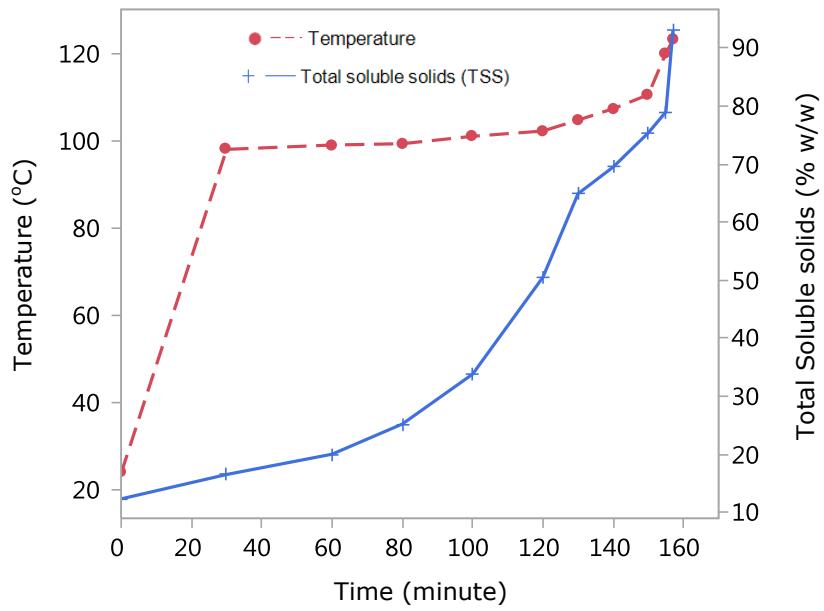


Figure 4.1 Changes in temperature and TSS values in *A. pinnata* sap with initial pH of 7.020

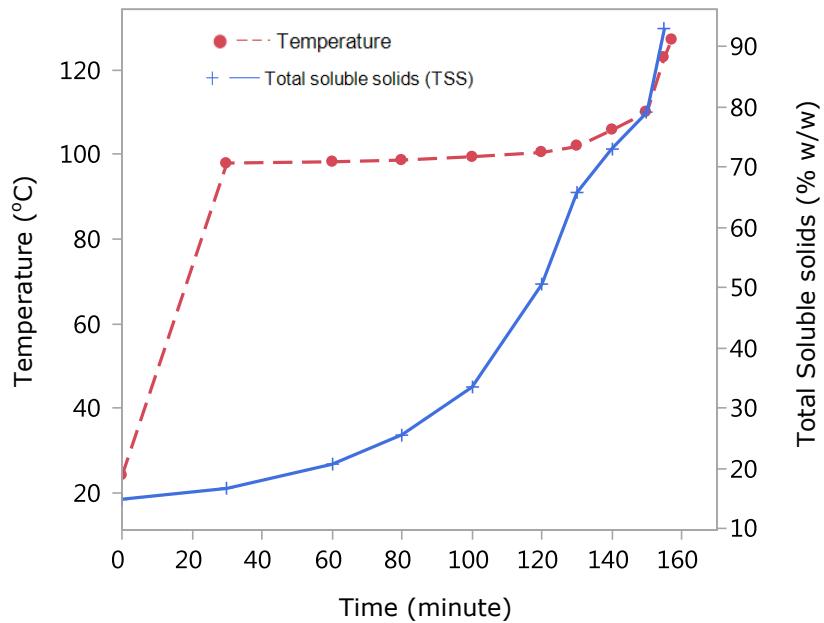


Figure 4.2 Changes in temperature and TSS values in *A. pinnata* sap with initial pH of 6.388

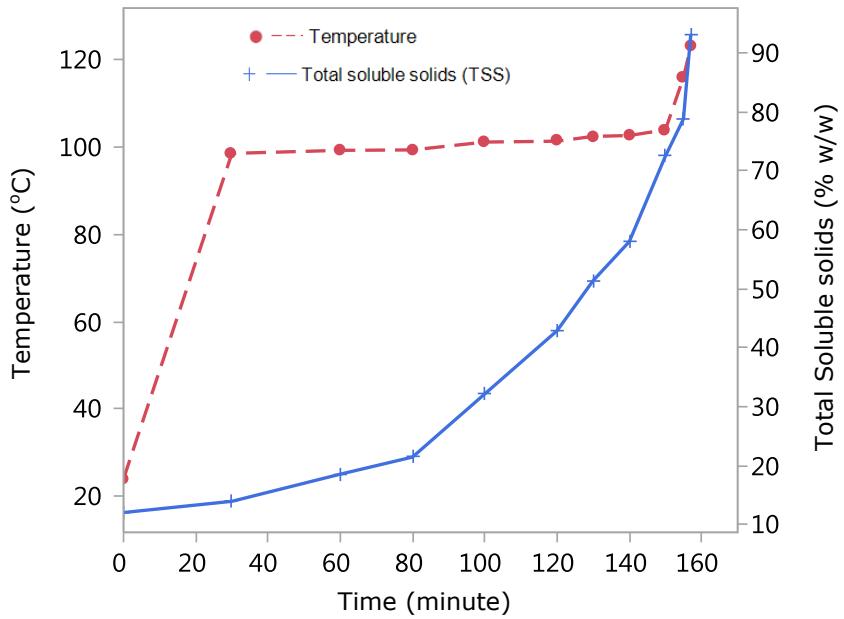


Figure 4.3 Changes in temperature and TSS values in *A. pinnata* sap with initial pH of 6.092

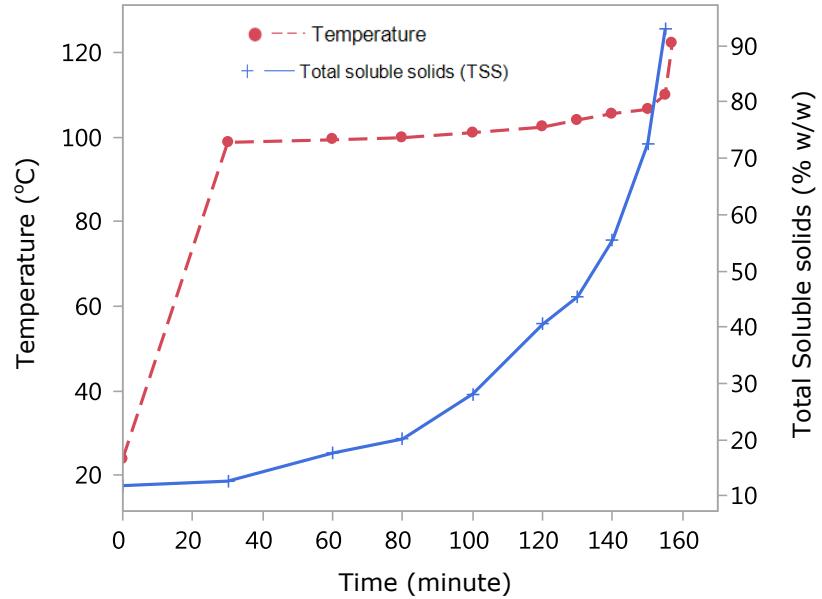


Figure 4.4 Changes in temperature and TSS values in *A. pinnata* sap with initial pH of 5.713

In general, the temperature of the sap increased rapidly in the first 30 minutes from room temperature to the boiling point determined within the range of 98-98.7°C. During this period, TSS of the sap did not change significantly, with approximately a 2% average increase in TSS within the first 30 minutes of heating. Then, it showed a slow increase of temperature from the boiling point to a temperature range from 104-110.5°C in the next 150 minutes, associated with the increase of TSS by about 60%, followed by a sharp rise in both temperature, ranging between 122-127°C and a corresponding increase of around 18% TSS in 7 minutes.

Based on our observations, the length of time required for boiling the sap depends on numerous parameters such as volume, initial TSS of the sap and type and amount of energy used during the boiling process. Therefore, it was not possible to attain the same temperature and TSS at the specific time under the experimental conditions. The boiling temperature observed during the study ranged from 98-98.7°C. It was surprising because the sap contained initial TSS of 12-14.9% which should actually increase the boiling point. The observed boiling point was lower compared to the reported boiling temperature of some juices which had initial TSS of 12.9-17.2% (100-104°C) (Rao et al., 2009). A possible explanation for this might be because the place of the study has an altitude of 700-1000 meters above sea level which causes a lower boiling point (around 2.3°C).

The four runs of the samples showed that the final temperature reached in the study (122-127°C) was quite lower than the temperature (147.6-150°C) reported by Ho et al. (Ho et al., 2007; Ho et al., 2008). It is difficult to explain this difference as the values come from different studies, but it might be related to the altitude, the equipment, instrument and method used to measure the temperature during the experiments. However, the final temperature

values measured in this study were similar with those described by other researchers (Favreau et al., 2001; Heiligmann et al., 2006; Koelling et al., 1996; Rao et al., 2009) who reported the final temperatures of processing sweet sap used in their studies to be less than 128°C. It was noticed during the current study that the period near the end of the process, where the temperature was about 127°C, was crucial as the concentrated sap was easily burnt by the finishing heating process.

The linkages of time-temperature and corresponding TSS changes, which occur during the evaporation process of *A. pinnata* sap, might be divided into three main phases as described by (Rao et al., 2009). The first phase (0-30 minutes) can be considered as the initial increase of *A. pinnata* sap temperature to the boiling point; the second phase (30-150 minutes) corresponds to the removal of the water present to concentrate the sugar content; and the final phase (150-157 minutes) indicates a sharp rise in both temperature and TSS. Consequently, the increase of TSS is very slow in the first phase, moderate in the second phase and mostly sharp in the last phase. Further, no obvious difference can be identified relating the increase in temperature and TSS in the samples with different initial pH.

The evolution of pH and temperature during the boiling process of *A. pinnata* sap with several different initial pH is presented in Figure 4.5. Starting with a specific initial pH at 24°C, an increase in the pH values was observed in each run, reaching a peak at 98-98.7°C, followed by a continuous decrease till the end of the heating process which corresponded to temperatures of 122-127°C. For example, during the heating of the sap with an initial pH of 7.020, the pH increased after 30 minutes boiling and reached a peak of pH 9.265 at 98°C, before it decreased to pH 6.630 at the end of the process. A similar pattern was found for the

three other sap samples (Figure 4.5), and their initial, maximum and final pH values are presented in Table 4.1 and compared to pH values reported from other studies.

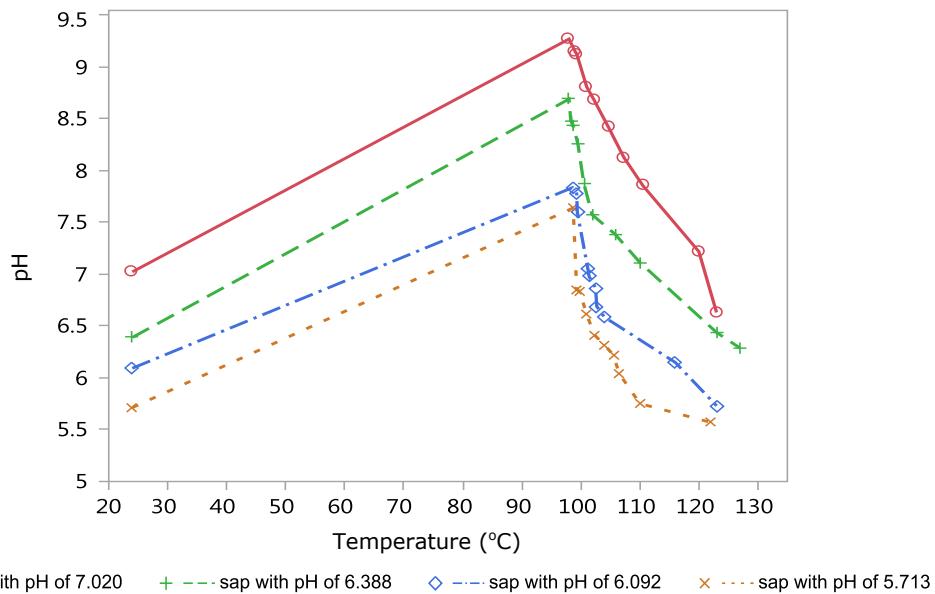


Figure 4.5 Changes in pH values in *A. pinnata* sap with different initial pH during the heating process

It is interesting to note that in all runs of this experiment the final pH, after it increased and decreased during the heating process, went back close to its initial pH value. A higher or lower value of final pH compared with the corresponding initial pH shown in Table 4.1 was related to the different concentrations of the samples reached at the final stage (93 and 66% w/w). Although the result of the current study was consistent with some studies (Table 4.1), it was not in accordance with observations from Apriyantono et al. (2002) who demonstrated a pH decrease through the whole heating process for coconut sap. Our observation indicated that an increase of pH in the beginning of the heating process occurred in the first 30 minutes of a total of 157 minutes of heating of four liters of sap, before the pH decreased continuously till the end of the boiling process. There is a possibility that the trend of pH changes during

heating of *A. pinnata* sap is similar with that of coconut sap. If it is true, then the interval time set in the work of Apriyontono et al. (2002) probably could not detect the increase of pH occurring in the beginning of the heating process. Based on our results, we suggest that the interval time during the observation should be short enough in order to detect the pH changes (an increase) in the beginning of heating process since it occurs over a short period.

Table 4.1 Initial, maximum and final pH values of *A. pinnata* sap and maple sap during the heating process

Samples	Initial pH	Peak pH	Final pH	TSS (% w/w)	Source
<i>A. pinnata</i> sap	7.020 ± 0.008	9.265 ± 0.016	6.630 ± 0.016	> 93	Present study
<i>A. pinnata</i> sap	6.388 ± 0.014	8.686 ± 0.008	6.285 ± 0.007	> 93	Present study
<i>A. pinnata</i> sap	6.092 ± 0.006	7.833 ± 0.016	5.730 ± 0.009	> 93	Present study
<i>A. pinnata</i> sap	5.713 ± 0.014	7.637 ± 0.011	5.570 ± 0.019	> 93	Present study
<i>A. pinnata</i> sap	n.a	~ 8	5.80	~ 87.89	(Ho et al., 2008)
Maple sap	7.2	9.2	7.3	65	(Akochi-K et al., 1997)
Maple sap	7.46	n.a	7.96	66	(Clement et al., 2010)
Maple sap	7.40	n.a	7.42	66	(Clement et al., 2010)

n.a : not available

The initial and final pH values of the samples in the current study indicate that *A. pinnata* sap and sugar are slightly acidic, implying the presence of some compounds such as minerals and organic acids rather than simply a pure solution of sugars. According to Akochi-K, et al. (1997) the formation of Amadori rearrangement, which are more basic than amino

acids, may cause the increase of pH at the beginning of the boiling process before the boiling sap reached a peak. Moreover, the Strecker degradation of amino acids might contribute to the increase in pH by losing CO₂ from the acid moiety. The following decrease of pH occurred after it reached a peak, indicating the loss of the basic amino groups. It might be due to the decomposition of Amadori products and other products with various acidities such as carboxylic acid which reduced the pH and slowed the reaction (Akochi-K et al., 1997; Ho et al., 2008).

The results of the current study suggest that a link may exist between the initial and the final pH of the sap. The reason for this link is not clear yet, thus a more in-depth study on this topic needs to be undertaken before the association between the initial and final pH values of *A. pinnata* sap-sugar is more clearly understood. It is possible, however, based on the current findings to utilize the pH of *A. pinnata* sugar, as the final product, to determine its initial pH (the sap), and vice versa.

The findings of the study may help us to have a better understanding of the changes in temperature, TSS and pH during the heating process of *A. pinnata* sap. It also provides important information to *A. pinnata* sugar farmers/industries to highlight the fact that heating must be regulated more carefully in the last period of the boiling process as the heated and concentrated sap is more sensitive to the heat and can easily get burnt.

4.4.2 pH change of untreated and pre-boiled saps

The second set of data from this experiment focused on the evolution of pH in untreated sap and pre-boiled sap between 0 hour and 18 hours following harvest. The initial pH of the fresh sap was measured and some amount of the sap was kept untreated at room

temperature until 18 hours. One liter of the sap was heated until it reached the boiling point of 98-98.7°C. The pH results of this experiment are presented in Table 4.2.

A decrease of pH between measurements made at 0 and 18 hours for both untreated and pre-boiled saps was identified (Table 4.2). It is important to bear in mind that the pH of untreated sap and pre-boiled sap did not have the same starting point at 0 hour since pH of sap increased during the heating process, as described in the previous section, and there was no pH adjustment taken during the experiment. The observations on pH changes between untreated and pre-boiled sap observed in the current study are in accordance with those of non-boiled and boiled sweet potato (Panda et al., 2009) and of untreated and heated cane juices (Eggleston, 2002).

Table 4.2 pH of untreated and pre-boiled *A. pinnata* sap at 0 and 18 hours

Treatment	0 h	18 h
Untreated sap	$6.388^a \pm 0.014^b$	$3.760^a \pm 0.013^b$
Pre-boiled sap (98°C)	8.686 ± 0.008	8.620 ± 0.019
Pre-boiled sap (98.3°C)	8.477 ± 0.015	8.427 ± 0.007
Pre-boiled sap (98.7°C)	8.433 ± 0.010	8.348 ± 0.014

^a mean

^b standard deviation

The lowering of pH which occurred in untreated and pre-boiled saps at 18 hours was probably due to the accumulation of organic acids such as lactic acid, acetic acid and butyric acid produced by the activity of microorganisms (Eggleston, 2002; Eggleston et al., 2004; Saxena et al., 2010). Furthermore, the very little decrease of pH in pre-boiled saps indicated

some level of inactivation of the enzymes and microbes which are responsible for the change (Eggleston, 2002). The heating process might have denatured the enzymes and destroyed or reduced the number of microbes, and therefore the decrease of pH of pre-boiled saps occurred only very slightly in 18 hours when comparing with a sharp decrease of pH in the untreated sap.

The results from the experiments demonstrated that the pH of untreated sap was 3.760 ± 0.013 at 18 hours, thus this sap could not be used as a raw material for sugar processing since the making of sugar requires the pH of the sap to be greater than 6 (Barlina et al., 2006; Marsigit, 2005). However, as the pH of all pre-boiled saps remained same after 18 hours (from 8.532 ± 0.135 to 8.465 ± 0.140 on average), pre-boiled saps can still be processed successfully into quality sugar. It can thus be suggested that heating the sap to its boiling point can maintain the pH, and therefore it is possible to preserve *A. pinnata* sap for at least 18 hours prior to its processing into sugar.

4.5 Conclusion

The present study was designed to investigate the changes of temperature, TSS and pH which occurred during the heating process of *A. pinnata* sap with different initial pH and to determine the pH changes between untreated sap and pre-boiled sap.

This work contributes to the processing knowledge of *A. pinnata* sap during the boiling process to produce sugar. First, the study has shown that there is no obvious difference in temperature and TSS changes of sap with different initial pH; second, the trend of pH changes is increasing in the beginning, reaching a peak at its boiling point, then decreasing till the end of the process to reach a final pH which has a close value with the initial sap's pH.

The results from this study also provide an important suggestion to be made to farmers to help them preserve the quality of their sap for at least 18 hours by heating it to its boiling temperature prior to storage.

4.6 Acknowledgment

The authors thank Ms. Praycillia Karundeng for her keen help during the preliminary experiments, and the farmers for providing the sap. The financial support of the Higher Education of Ministry of Education and Culture of Indonesia for the first author is acknowledged.

CONNECTING TEXT

Chapter 4 studied the changes of temperature, TSS and pH which occurred during the boiling process of *A. pinnata* sap into sugar. Subsequently in **Chapter 5** the quality of sugar was determined through colour changes since colour is one of the most important considerations for the customer to make decision for buying the product. Solid sugar and granulated sugar were produced from the boiling process of the sap. We hypothesized that colour changes would take place during the heating process and the colour of solid sugar would be different from the colour of granulated sugar. CIELAB measurements ($L^*a^*b^*$ color space), several derivative quantitative attributes of colourfulness i.e., total colour difference (ΔE^*), hue angle (h), chroma (C^*) and red/yellow ratio (a^*/b^* ratio) and their correlation with time, temperature, Total Soluble Solids (TSS), and pH during the processing of *A. pinnata* sap into sugar were analyzed in the following study.

Some of the results presented in **Chapter 5** have been disseminated through a conference presentation.

- Inneke Victor and Valérie Orsat. 2014. **Colour changes during the processing of *Arenga pinnata* (palm) sap into sugar.** International Union of Food Science and Technology. Poster presentation. Montreal, Canada. August, 17-21, 2014.

CHAPTER 5

COLOUR CHANGES DURING THE PROCESSING OF

ARENGA PINNATA SAP INTO SUGAR

5.1 Abstract

This is the first study reporting on the quality of granulated sugar, produced from *Arenga pinnata* sap. The purpose of the current study was to determine the colour changes using CIELAB measurements ($L^*a^*b^*$ color space) and their correlation with time, temperature, Total Soluble Solids (TSS), and pH during the processing of *Arenga pinnata* sap into sugar. Several colour indicators, namely total colour difference (ΔE^*), hue angle (h), chroma (C^*) and red/yellow ratio (a^*/b^* ratio) were monitored. Three quality assessment experiments were conducted for the study. The first and second experiments focused on the colour changes during the boiling process and during the granulation process respectively, whereas the last set of experiments emphasized on the study of colour differences between solid and granulated sugars. Descriptive statistics, regression analysis and a paired t-test were used. The colour space $L^*a^*b^*$ values changed noticeably during boiling of the sap. As a function of time, the sap became darker, more red, and more yellow indicating a continuous decrease of L^* values, and an increase in both a^* and b^* values. However, a^* and b^* were not significantly affected by temperature in the beginning of processing. A constant decrease of L^* and an increase of a^* and b^* were particularly observed when TSS of the sap was more than 33.5%. During the process, regression analysis showed that a linear pattern was indicated for ΔE^* with time, whereas a non linear relationship was shown for ΔE^* with TSS, temperature and pH. This implies that during the heating of *Arenga pinnata* sap, ΔE^*

increased as time increased, while relationships of ΔE^* with TSS, temperature and pH were not easily established. Stirring the syrup changed the $L^*a^*b^*$ values while ΔE^* showed a decrease throughout the process. The results indicate that the sugar became lighter (L^* increased), more red (a^* increased), and more yellow (b^* increased) during the stirring process. The type of sugar produced, solid or granulated sugar influenced the $L^*a^*b^*$ colour space of *Arenga pinnata* sugar (paired t-test, $p < 0.01$). The colour determination confirmed that the colour of solid sugar was very distinct from that of granulated sugar (by ΔE^*), the colour of both solid and granulated sugar was red-orange to yellow-orange (by h), granulated sugar was more colourful than the solid sugar (by C^*), and granulated sugar was more yellow than solid sugar (by a^*/b^* ratio). This research will serve as a base for future developments for the manufacture and quality grading of *Arenga pinnata* sugar.

Keywords : *Arenga pinnata* sap sugar, CIELAB ($L^*a^*b^*$ colour space), time, temperature, TSS, pH, colour determination (ΔE^* , h , C^* , a^*/b^* ratio).

5.2 Introduction

Colour is widely present throughout nature in fresh foods as well as in processed foods. Colour of agri-food products is derived from natural pigments which vary in their physical and chemical properties (Maskan et al., 2002; Pathare et al., 2013). Many of those are sensitive to cultivar, oxidation, pH change, light, and temperature and time especially during processing. Changes in the structure of foods, including color modification, are produced during their manufacturing process (Maskan et al., 2002). In the food industry, colour change during the manufacture of a processed food is of vital interest since colour is among the

primary considerations of consumers to make a decision of purchasing (Arabhosseini et al., 2011; Maskan et al., 2002). Hence colour, among other parameters such as appearance, taste and odour, is employed to monitor a food quality's assessment throughout the processing and distribution chain (Maskan et al., 2002).

Palm sugar, produced from *A. pinnata* sap, has been known as a local delicacy commonly consumed in Asia in cakes and desserts (Lasekan & Abbas, 2010). In Indonesia, for example, adding palm sugar in certain Indonesian foods is still a preference since palm sugar has a specific flavour profile that cannot be replaced by white sugar or other types of sweeteners (Sihombing, 1995).

Processing *A. pinnata* sugar from the sap is achieved by thermal processing through boiling and it usually does not involve any purification process, or use any chemical additive in order to bleach the colour. Thermal processing is one of the most important methods of food preservation to inactivate enzymes, deteriorative microorganisms and reduce water activity by concentration and dehydration. However, although necessary, this preservation method can change the color and final appearance of the product and therefore may affect the commercial quality of the processed food (Maskan et al., 2002).

The effect of thermal processing on the colour of food materials has been studied by many such as for Palmyra-palm and date-palm sugars (Rao et al., 2009), to assess colour change of red pepper (Rhim & Hong, 2011), and colour change of double concentrated tomato paste (Barreiro et al., 1997). Arabhosseini et al. (2011) studied the effect of drying on the colour of tarragon leaves, while Krokida et al. (1998) reported the effect of temperature and air humidity on colour changes of apple, banana, carrot and potato. Thermal degradation kinetics of pigments and visual colour in watermelon juice was investigated by Sharma et al.

(2008), whereas Maskan et al. (2002) demonstrated the effect of concentration and drying on colour change of grape juice and fruit leather. Colour changes were also found in concentrated fruit pulp (Lozano & Ibarz, 1997), and in pear puree during thermal treatments (Ibarz et al., 1999). Whilst many studies have reported on the colour change in fruits, vegetables and their derivatives, only a few studies have been conducted on *A. pinnata* sap/sugar such as studied by Ho and colleagues (Ho et al., 2007; Ho et al., 2008; Ho et al., 2006). Little work was carried or focused on heating *A. pinnata* sap/sugar processing, and there are no reports on a relationship between colour changes and process and quality parameters such as temperature, Total soluble solids (TSS), pH development from the time of sap harvest through the process of concentrating the *A. pinnata* sap. Therefore, this study was aimed at determining the colour changes using CIELAB ($L^*a^*b^*$ colour space) with its several derivative quantitative attributes of colourfulness (total colour difference ΔE^* , hue angle h , chroma C^* , and red/yellow a^*/b^* ratio) during the processing of *A. pinnata* sap into solid and granulated sugars. Colour change evaluation in food products using CIELAB colour space has become very popular among food researchers since this technique mimics well the human perception of this quality attribute (Quintas et al., 2007), and it is simple and faster than chemical analysis (Itle & Kabelka, 2009; Rattanathanalerk et al., 2005). The results of this work provide some information about the mechanisms of colour change during processing of *A. pinnata* sap into sugar which is important for the quality optimization of the manufactured sugar. This present study is also believed to be the first paper reporting on quality parameters of granulated sugar produced from sap tapped from *A. pinnata* palm trees although the sap is traditionally collected and has been consumed for decades as a sweetener.

5.3 Materials and Methods

The sap used in all experiments was tapped from *A. pinnata* trees and was obtained from farmers in Tomohon, Indonesia, where the altitude ranges between 700-1000 meters above sea level. The farmers tapped the sap from different trees while one inflorescence was tapped from each tree.

Three characterization experiments were conducted for the study. The first experiment focused on the colour changes during the heating process, the second experiment emphasized on the colour changes during the granulation process, while colour assessment between solid and granulated sugars was a focal point in the last experiment.

In the first trial, four liters of the sap were boiled in an open stainless steel pan placed over a gas fire. For monitoring purposes, ten ml of the sample was collected at each specified time interval throughout the heating process. The time intervals of sampling were every 30 minutes from 0 to 60 minutes, every 20 minutes from 60 to 150 minutes, every 5 minutes from 150 to 155 minutes, and every 2 minutes at the end of the heating process. The changing interval time sampling was considered and required as a result of the preliminary experiments which showed that colour changes in the beginning of the heating process were not noticeable whereas colour changes were very fast at the end of the process. In the second part of the experiments, the thickened processed sap, from the first experiment, was stirred continuously until it formed granulated sugar. The heating of the sap was terminated at a temperature of 127°C, and the thickened syrup was cooled to around 93°C before stirring it to form granulated sugar. Samples were taken at three phases of the stirring process: before the liquid was stirred, during the stirring process, and at the end when granulated sugar was finally produced. Four liters of two different batches of *A. pinnata* sap with the initial pH of 6.388

and 6.758 for the first batch and the second batch respectively, were boiled and processed into both solid and granulated sugar in the last set of experiments. The samples for analysis were taken from the produced solid and granulated sugars.

5.3.1 Measurements

Temperature

The temperature was measured using a digital stainless steel probe thermometer (BG 363, Blue Gizmo, Acez) which has a temperature range of -10 to 200°C with an accuracy of \pm 1°C.

Total soluble solids (TSS)

TSS (% w/w) was measured using an Atago Pocket Refractometer (PAL-3, Atago, Japan) (0-93% Brix).

pH

The sample was diluted ten fold, and the measurement of pH was done using a pH meter (WTW Sentix 81, pH 3110, Weilheim 2008, Germany). Calibration was accomplished employing pH 7.0 and 4.0 buffer solutions.

Colour

Colour was measured using a Konica Minolta Color Reader CR 10 (Konica Minolta Optics, Inc., Japan). Samples were placed into a clear glass petri dish, the white calibration plate CR-A43 was attached to one side of the dish and the measurement was taken. The colour changes measurement during the processing of *A. pinnata* sap sugar was recorded in three CIELAB parameters (L*a*b* colour space) with L* representing the lightness of colour (0=black, 100=white), +a* is red while -a* is green, and +b* is yellow whereas -b* is blue. Total colour difference (ΔE^*) is expressed using the following equation:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (\text{Mercali et al., 2014; Minolta Co., 2007}).$$

Hue angle is calculated by:

$$h = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (\text{Minolta Co., 2007})$$

Chroma is computed using:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (\text{Minolta Co., 2007})$$

While the red/yellow colour ratio is simply a^*/b^* ratio (Garcia & Calixto, 2000).

L_0^* , a_0^* and b_0^* represented the reading at time zero, and L^* , a^* , b^* represented the individual reading during thermal treatment.

5.3.2 Statistical Analysis

All samples used in the measurements of pH, TSS and colour space ($L^*a^*b^*$) were taken in triplicate and the average values were displayed in the figures provided in the results and discussion section. Further analyses, namely colour quantifications (total colour difference ΔE^* , hue angle h , chroma C^* , and red/yellow a^*/b^* ratio), used the average values of colour space $L^*a^*b^*$ in their calculation. Descriptive statistic was mainly used in the experiments, while regression analysis was incorporated in the first set of experiments and a paired samples t-test was conducted in the third experiment. All the statistical analysis was done using JMP 10 software program (SAS Institute Inc.).

5.4 Results and discussion

Marsigit (2005) and Barlina et al. (2006) reported on the pH values of *A. pinnata* sap that are recommended in order to produce sugar, however from these publications it was not clear whether solid sugar or granulated sugar was produced from the sap. As a consequence of

this uncertainty of information, some preliminary experiments were conducted to clarify the pH parameter and other essential conditions in producing *A. pinnata* granulated sugar. It was determined from the preliminary testing that the pH of the sap must be higher than 6, the total soluble solids (TSS) of the syrup must be greater than 93 (% w/w), and final temperature during boiling of the sap should be around 127°C (as an indicator of ° Brix).

5.4.1 Colour changes during the heating process of *Arenga pinnata* sap

The changes of some parameters i.e., time, temperature, TSS, pH, and L*a*b* colour space values were recorded during the evaporation of *A. pinnata* sap into sugar and the results are presented in Figure 5.1. The temperature of the sap increased rapidly in the first 30 minutes from room temperature (~24°C) to 98°C which was observed to be the sap's boiling point. Then, the temperature rose slowly to 105.8°C at 140 minutes heating time, followed by a sharp increase to the final temperature of 127°C at 157 minutes. TSS of the sap, on the other hand, showed a very small rise of less than 2% in the first 30 minutes, before it increased continuously and reached about 93% during the following 155 minutes of heating. The heating was continued for the next 2 minutes until the temperature reached 127°C, but a precise TSS value of the boiled sap was not recorded as the experiment had reached the limitation of the refractometer. Figure 5.1 illustrates the increase of pH from the initial pH of 6.388, reaching a peak of 8.686 in the first 30 minutes, before it decreased continuously to 6.285 at the end of the 157 minutes boiling process. All L*a*b* values investigated changed noticeably as presented in Figure 5.1. A gradual decrease of L* and an increase of both a* and b* were identified from the beginning of the heating process. However the L*a*b* trends showed dramatic changes near the final process stage from 155 to 157 minutes of boiling

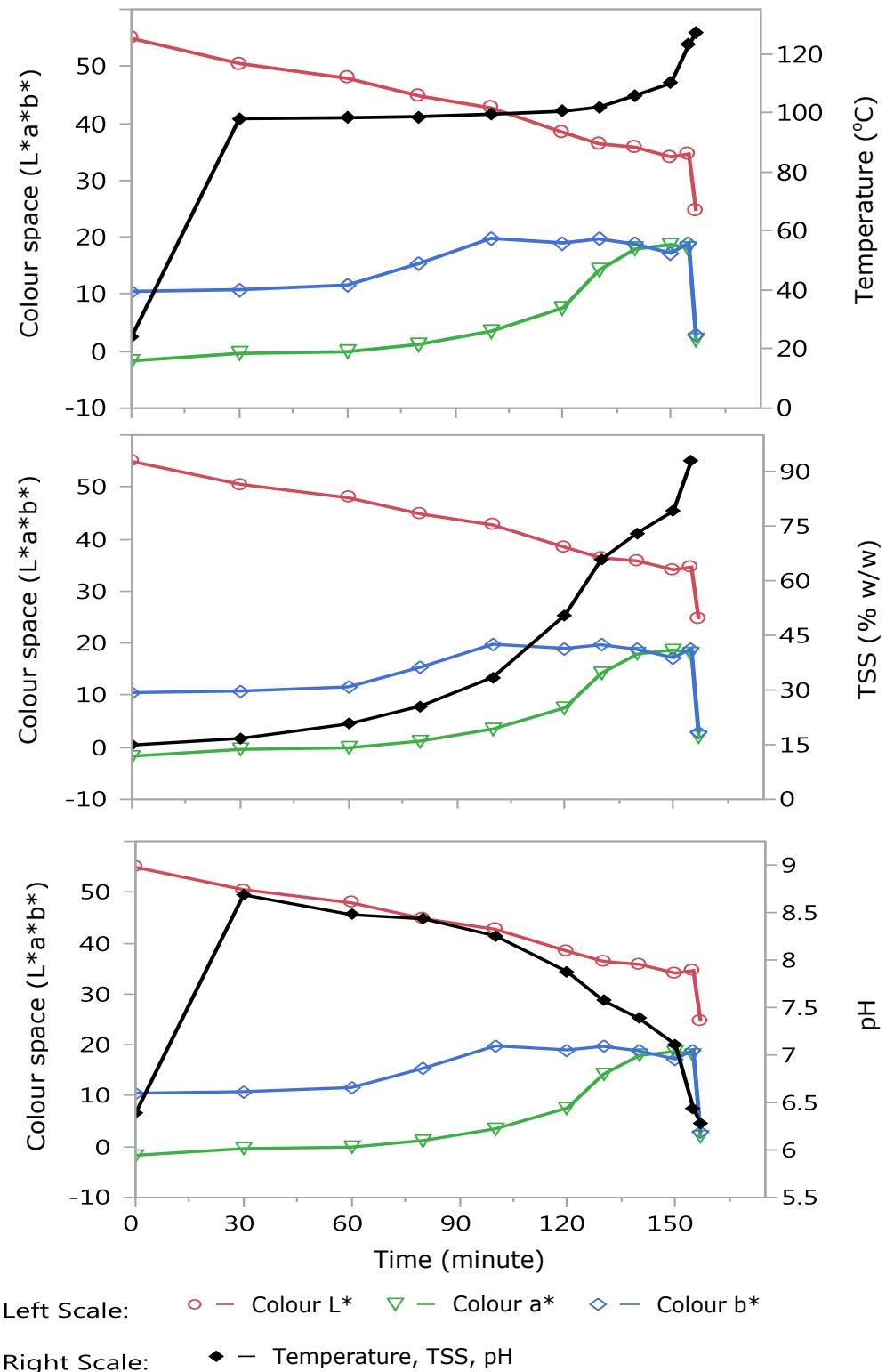


Figure 5.1 Changes in L*a*b* colour space, temperature, TSS, and pH during the sap evaporation process

when the corresponding temperature, pH, and TSS were from 123 to 127°C, from 6.437 to 6.285, and greater than 93 (% w/w), respectively.

In contrast to earlier findings, these results have not been previously described in any palm juice processing studies as published by Ho et al. (2008) for *A. pinnata*, by Rao et al. (2009) for Palmyra-palm and date-palm, and by Ben Thabet et al. (2009) for date palm. The differences may be explained by the fact that previous studies reported on the heating process of sap into solid sugar with TSS of 88% (Ho et al., 2008), and 82% (Rao et al., 2009), whereas the present study described changes which occurred during the heating process resulting in both solid and granulated sugar with TSS greater than 93%.

5.4.1.1 Colour-time

As a function of time, the sap became darker, more red, and more yellow from beginning up to 155 minutes indicated by a continuous decrease of L* values, and an increase both in a* and b* values. Starting with a value of 54.9, L* decreased continuously to 24.4 in 157 minutes of boiling process. A slow increase of a* was indicated from -1.7 to -0.1 in the first 60 minutes, then it increased up to 8.7 at 150 minutes, before it decreased dramatically to 1.9 which was likely due to the caramelization of sugars. Values of b* rose slowly starting from 10.5 at the beginning, and a gradual increase to 19.7 at 130 minutes, then it showed a huge decline to reach 2.8 at the end of the heating process. Trends of decreasing L* and both increasing a* and b* observed in 155 minutes of boiling in the current study are consistent with those of Rao et al. (2009) for boiling sugarcane, palmyra-palm and date-palm juices, of Maskan et al. (2002) for grape juice concentration, and Ibarz et al. (1999) for heating pear puree. The onset of non-enzymatic browning, especially because of the ascorbic acid

degradation might favour the decrease of L^* as reported by Mercali et al. (2014) for acerola pulp. A strong colour change, particularly the L^* decrease as a function of time, is often reported for food items, as reported by Marquex & Anon (1986) for frying potatoes.

5.4.1.2 Colour-temperature

When the temperature rose to reach boiling, from 24°C to 98°C, the L^* values showed a slight decrease from 54.9 to 50.5, whereas a very small increase was experienced by both a^* from -1.7 to -0.4 and b^* from 10.5 to 10.8. L^* decreased gradually to 34.1 at 110°C, followed by a considerable decline to 24.6 at 127°C. A drastic decrease was experienced by a^* from 18.7 at 110°C to 1.9 at 127°C, after it had demonstrated a gradual increase from the beginning. Values of b^* increased consistently from 10.8 at 98°C to 19.7 at 101.9°C, before showing an inconsistent trend until the temperature of 123°C, followed by a steep decrease to 2.8 at 127°C.

From the graph it can be seen that in the beginning, when the temperature rose drastically, there was no clear change on the colour parameters especially a^* and b^* , and only a slight decrease of L^* . This implied that a^* and b^* of the boiling sap were not affected by temperature in the beginning of the process, whereas there was a considerable effect on the $L^*a^*b^*$ colour space values at 127°C. L^* decreased and both a^* and b^* increased as temperature increased from 24°C to 123°C, in agreement with Rao's (Rao et al., 2009) and Ibarz's (Ibarz et al., 1999) findings. However these results differed from Quintas et al. (2007) who reported that processing temperature did not affect the lightness of the final product during the caramelization of sucrose, though it influenced the final colour difference, and from Krokida et al. (1998) who described that the lightness of samples used was not affected by temperature. A possible explanation for this might be the difference in the composition and

purity of the raw material used in these different studies. The present study utilized *A. pinnata* sap which contained sucrose and other phyto-compounds, whereas a solution mixture of sucrose of 83% (w/w) and distilled water was used in Quintas's study while dehydrated food materials were used in Krokida's study (Krokida et al., 1998; Quintas et al., 2007). Nevertheless, both a^* and b^* values increased during Krokida's study (Krokida et al., 1998), indicating that both a^* and b^* were affected by temperatures of 50-90°C used in drying of some fruits and vegetables (apple, banana, carrot and potato). An increase of a^* as affected by a process temperature of 55-75°C was demonstrated in Maskan's drying of pestil (grape leather) (Maskan et al., 2002), whereas b^* was reported to be sensitive to the change of temperature between 70-90°C during heating of pineapple puree (Chutintrasri & Noomhorm, 2007).

5.4.1.3 Colour-TSS

The initial total soluble solids (TSS) of the sap used in the present study was 14.9 (% w/w) with associated $L^*a^*b^*$ values of 54.9, -1.7 and 10.5, respectively. L^* changed gradually to 34 with a TSS of 79.1%, followed by a considerable decrease of L^* to 24.6 when the TSS reached greater than 93%. Both a^* and b^* did not change significantly in the beginning until the sap was about 20.7% in TSS. Then, a continuous increase was shown by a^* to 18.7 and by b^* to 17.2 associated with TSS of 79.1%, before demonstrating a sharp decline to 1.9 for a^* and 2.8 for b^* when the boiled and concentrated sap was more than 93% TSS.

A trend of decreasing L^* and increasing a^* and b^* from the beginning of the heating process until the boiled sap reached a TSS of about 93 (% w/w) as determined in this study corroborates the earlier finding by Rao et al. (2009). Indeed in that study, a constant change in

L^* and an obvious increase of a^* and b^* particularly when TSS of the sap was more than 33.5% indicated the onset of caramelization, the process which became more obvious when the juice reached around 60 (% w/w) (Rao et al., 2009).

5.4.1.4 Colour-pH

In the beginning of the heating process, the $L^*a^*b^*$ colour space values of the sap, with a pH of 6.388, were 54.9, -1.7, 10.5, respectively. Afterwards, the pH increased drastically to 8.686 associated with the decrease in L^* to 50.5, and the increased values of both a^* to -0.4 and b^* to 10.8. Then, the pH values decreased continuously throughout the heating process and reached a pH of 6.285. After the sap reached a peak pH, L^* and pH values decreased gradually to 34.6 and 6.437, respectively, whereas both a^* and b^* increased to 18.1 and 19.0. Then, a^* and b^* further decreased to 1.9 and 2.8, respectively, associated with a continuous decrease of the pH down to 6.285.

No obvious changes of a^* and b^* , and only a slight increase of L^* was observed during the increasing of pH from the beginning until it reached a peak of 8.686. This suggests that a^* and b^* were not affected by pH changes in the beginning. Further, the decrease of L^* and increase of a^* and b^* identified in the present study was consistent with Maskan's finding during the concentration process for grape juice which had a pH value of 7.6 (Maskan et al., 2002). A correlation between pH and colour was also reported for ground starch-glucose-lysine extrudates (Ames, 1998) for which, as the pH increased, the L^* value decreased and the a^* value increased, whereas the effect on b^* values was less clear. A decrease of pH from 7.8 to 7.3 associated with a decrease of L^* and b^* and an increase of a^* was also identified in syrup from date palm (Ben Thabet et al., 2009b). The changes of pH detected in the present work and previous studies might explain that there was a destruction of anthocyanins and an

occurrence of Maillard reactions during the heating process (Ben Thabet et al., 2009b; Maskan et al., 2002). During thermal processing, anthocyanin pigments degrade and change the colour and may affect nutritional properties of the product (Patras et al., 2010). Bakhshayeshi et al. (2006) in their work to evaluate the stability of anthocyanin in *Malus* demonstrated that increases in pH and temperature or exposure to light were leading to damage of the anthocyanin pigments. The intensity and stability of the anthocyanin pigments were also reported to be affected during various steps of processing and storage which include pH, temperature, light, oxygen, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide, etc. (Bakhshayeshi et al., 2006; Patras et al., 2010).

5.4.2 Total colour difference (ΔE^*)

A single numerical value can be determined for describing colour change and it is expressed as total colour difference (ΔE^*). It has become one of the best parameter since it combines all colour parameters $L^*a^*b^*$ (Ibarz et al., 1999). Previous studies have reported using ΔE^* for example to express the colour degradation of pineapple puree (Chutintrasri & Noomhorm, 2007), to present colour differences during heating of peach, apple and plum (Lozano & Ibarz, 1997), and to describe the total colour change of pineapple juice (Rattanathanalerk et al., 2005). The ΔE^* values presented here are derived from $L^*a^*b^*$ values recorded during the evaporation process of *A. pinnata* sap. To obtain ΔE^* values, the initial sample's colour value was used as a reference colour (reading at time zero), hence ΔE^* of the first sample was zero. An empirical relationship between ΔE^* and the process parameters specifically time, temperature, TSS and pH was incorporated.

A pattern of data between ΔE^* and each parameter viz. time, temperature, TSS and pH was examined. It can be seen that ΔE^* and time showed a linear pattern (Figure 5.2).

Therefore, a linear correlation between ΔE^* and time was determined, and it showed R^2 value for correlation between ΔE^* and time of 0.9717 (Table 5.1).

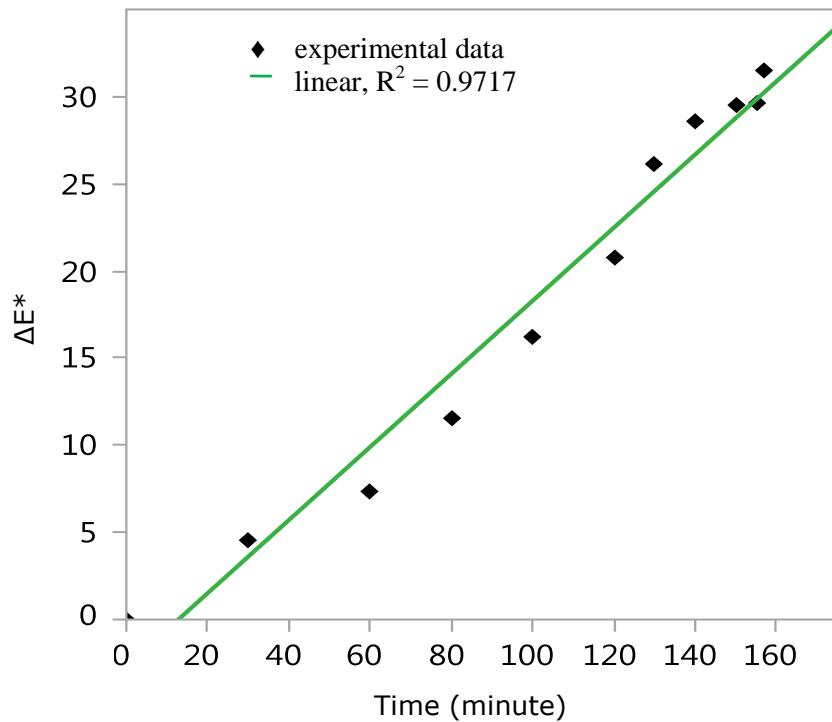


Figure 5.2 Total colour difference (ΔE^*) changes as a function of time

Table 5.1. Linear model identified for relationship between total colour difference (ΔE^*) and time

Model	Prediction model	R^2
Linear	$y = a + bx$ a = intercept = - 2.6845 b = slope = 0.2097	0.9717

Since the scatter plot of ΔE^* and TSS did not obviously show linear or non linear patterns (Figure 5.3), both linear model and non linear model i.e., three parameters exponential were considered in examining the correlation between ΔE^* and TSS (Table 5.2). The results showed the exponential model provided a better statistical fit than did the linear

model as shown by R^2 values for fit linear and exponential of 0.9236 and 0.9899, respectively.

Figure 5.3 presents the experimental data and the exponential model as the best fit model.

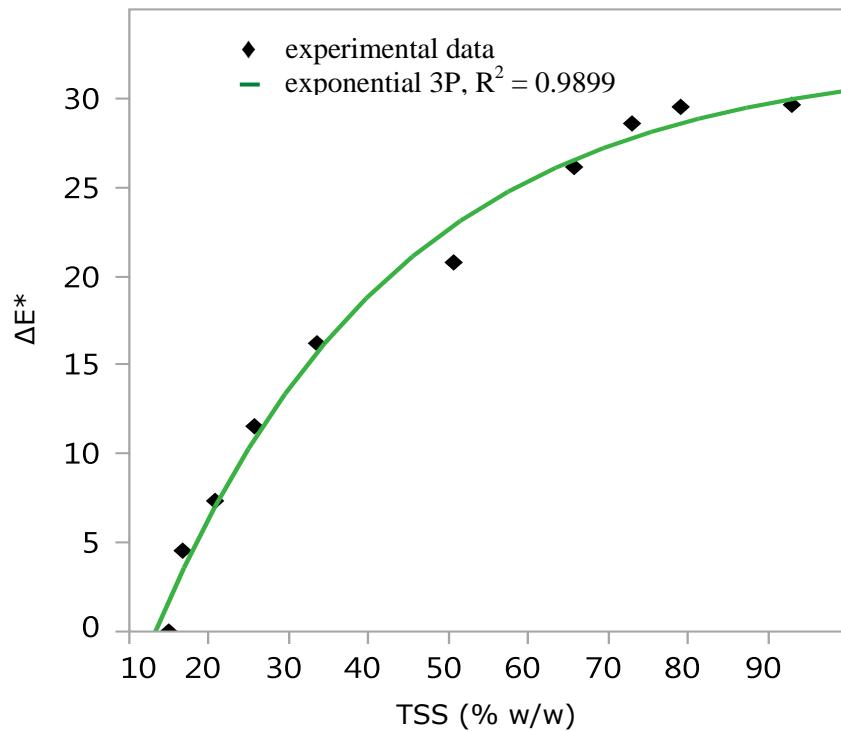


Figure 5.3 Total colour difference (ΔE^*) changes as a function of TSS

Table 5.2 Linear and exponential models identified for relationship between total colour difference (ΔE^*) and TSS

Model	Prediction model	R^2
Exponential 3P	$y = a + b * \text{Exp}(c * x)$ a = asymptote = 32.2902 b = scale = - 49.9692 c = growth rate = - 0.0329	0.9899
Linear	$y = a + bx$ a = intercept = - 0.1012 b = slope = 0.3706	0.9236

On the other hand, the scatter plot of ΔE^* and temperature showed an obvious non linear pattern Figure 5.4, as a result only non linear model specifically three parameters Gompertz was investigated (Table 5.3). The result showed a strong correlation between ΔE^* and temperature with R^2 of 0.9946.

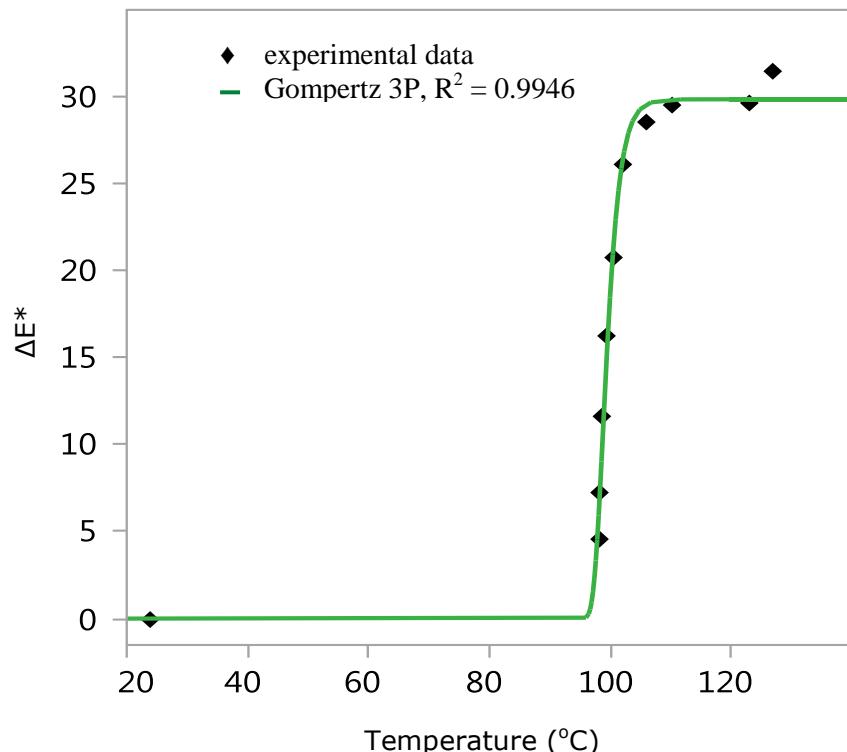


Figure 5.4 Total colour difference (ΔE^*) changes as a function of temperature

Table 5.3 Gompertz model identified for relationship between total colour difference (ΔE^*) and temperature

Model	Prediction model	R^2
Gompertz 3P	$y = a\text{Exp}(-\text{Exp}(-b(x-c)))$ a = asymptote = 29.8591 b = growth rate = 0.6466 c = inflection point = 98.7915	0.9946

An obscure pattern of the scatter plot data between ΔE^* and pH was identified during the heating process of *A. pinnata* sap as a result of a single data at pH of 6.388 occurred before the boiling point of the sap/syrup. Consequently, the data was excluded from further analysis. Both linear and non linear patterns could be employed on the scatter plot data of ΔE^* and pH past the boiling point of the syrup (Figure 5.5), and therefore linear and non linear models namely three parameters Gompertz were considered (Table 5.4). Gompertz model revealed the best prediction model with R^2 of 0.9862 as shown in Figure 5.5. Further, Table 5.5 provides the best models obtained by the regression analysis of ΔE^* and each parameter viz. time, TSS, temperature and pH.

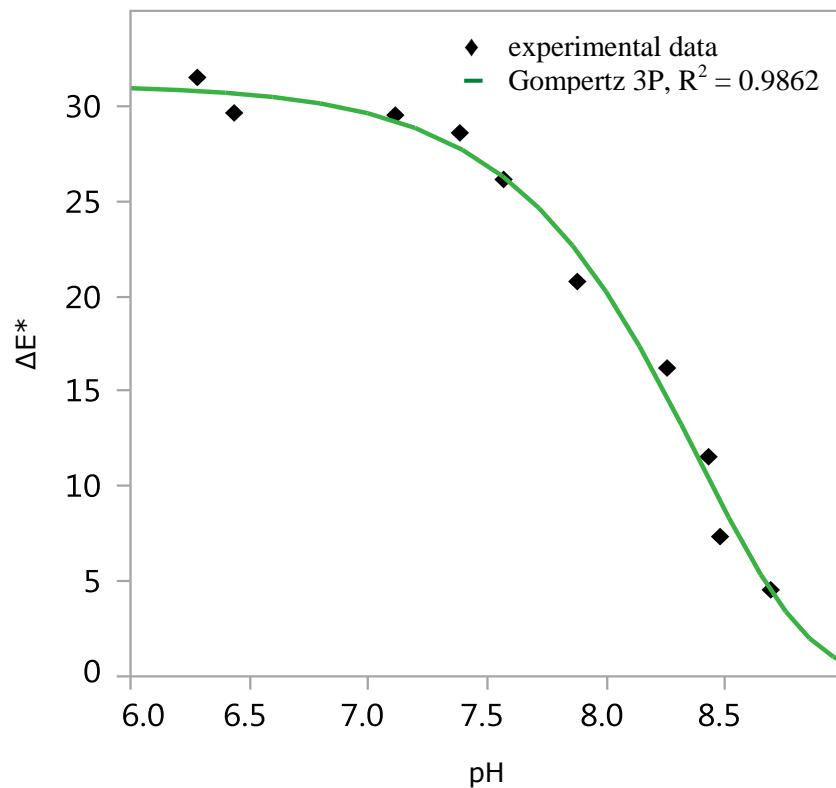


Figure 5.5 Total colour difference (ΔE^*) changes as a function of pH during heating past boiling point of the syrup

Table 5.4 Linear and Gompertz models identified for relationship between total colour difference (ΔE^*) and pH during heating past the boiling point of the syrup

Model	Prediction model	R^2
Gompertz 3P	$y = aExp(-Exp(-b(x-c)))$ a = asymptote = 31.1094 b = growth rate = - 2.1672 c = inflection point = 8.3875	0.9862
Linear	$y = a + bx$ a = intercept = 103.5840 b = slope = - 10.8481	0.8422

Table 5.5 Regression models identified for relationship between total colour difference (ΔE^*) and time, TSS, temperature and pH

Parameter	Prediction model	R^2
ΔE^* - time	Linear : $y = -2.6845 + 0.2097x$	0.9717
ΔE^* - TSS	Exponential : $y = 32.2902 - 49.9692Exp(-0.0329x)$	0.9899
ΔE^* - temperature	Gompertz : $y = 29.8591Exp(-Exp(-0.6466(x-98.7915)))$	0.9946
ΔE^* - pH (past boiling point)	Gompertz : $y = 31.1094Exp(-Exp(2.1672(x-8.3875)))$	0.9862

5.4.2.1 Time

A strong positive correlation between ΔE^* and time implies that during the heating of *A. pinnata* sap, the ΔE^* increased as time increased shown by high value of $R^2 = 0.9717$. These results are consistent with those of other studies reporting significant change in color during heating of peach, apple and plum (Lozano & Ibarz, 1997), colour degradation of

pineapple puree (Chutintrasri & Noomhorm, 2007), heating pear puree (Ibarz et al., 1999), during double concentration of tomato paste (Barreiro et al., 1997), and for heating pineapple juices (Rattanathanalerk et al., 2005).

5.4.2.2 TSS

The correlation between ΔE^* and TSS determined was an exponential decay model in increasing form or limited growth as it showed the negative growth rate ($c = -0.0329$) with an asymptote of 32.2902. The model indicated a slow growth at first, followed by a period of moderate growth, and then leveled off to become asymptotic to the upper limit of ΔE^* . This exponential model can be rewritten as a logarithmic expression since logarithm is an exponent, therefore exponential and logarithm models can define the same function, and as such can be used interchangeably. The result obtained through the regression analysis is in agreement with colour changes observed during the evaporation of maple sap (Koelling et al., 1996). Using a colour index as an indication of colour changes in their study showed that little colour change was produced in the beginning, but it increased further as the concentration of the sap approached syrup consistency with TSS of 30% and above which associated with the value of colour index from 0 to about 1.5 (Koelling et al., 1996). Study by Ho et al. (2008) also showed that the absorbance increased slowly from 0 to about 0.1 when the sap had TSS of 45%, and then it went up to 1.0 for sap with 88% in TSS.

5.4.2.3 Temperature

The regression analysis shows a non linear Gompertz model for correlation between ΔE^* and temperature with R^2 of 0.9946. The Gompertz model indicates that an increase of ΔE^* was slow in the beginning of the heating process shown by a positive growth rate ($b = 0.6466$) until it reached the boiling point (around 98°C) as revealed by the inflection point

($c = 98.7915$), then followed by a period of rapid growth. This result further supports the idea of the significant effect of temperature on ΔE^* when using high temperatures from 80-98°C as reported for the thermal treatment on pear puree (Ibarz et al., 1999), by heating treatment at 70-110°C for pineapple puree (Chutintrasri & Noomhorm, 2007), by thermal treatment at 70-100°C for double concentrated tomato paste (Barreiro et al., 1997), and by heating of pineapple juice at 55-95°C (Rattanathanalerk et al., 2005). The increase of ΔE^* with development of colour was also reported to result in a significant darkening of a heated sucrose solution (Quintas et al., 2007). ΔE^* was stated to be the most sensitive parameter for the measurement of colour degradation in pineapple puree in response to temperature treatment (Chutintrasri & Noomhorm, 2007). During observation of colour change of pineapple juice, Rattanathanalerk et al. (2005) proposed that higher temperature accelerated the chemical reactions and most of the colour change occurred early on during the heating period. It was also argued that colour changes may be a result of more than one reaction and these reactions may not occur simultaneously at one temperature. Therefore, temperature was an important driving force behind the changes in the colour of heated samples. The results from their study suggested that both non-enzymatic browning and pigment destruction influenced the change in ΔE^* , and the Maillard reaction was found to be dominant rather than pigment destruction (Rattanathanalerk et al., 2005). Another possible explanation for this is that when temperature increases the sucrose is easily hydrolyzed resulting in the liberation of more glucose and fructose which are the substrates leading to increasing Maillard reactions, the non-enzymatic browning reactions (Ibarz et al., 1999). Ames (2003) reported that Maillard reactions are affected by metals, oxygen, components in the food which act as reaction precursors, temperature and time of heating, pH and water activity, the presence of inhibitory

agents, e.g., sulfite, and this reaction can be minimized by removing one of the required reaction partners, i.e., the reducing sugar. It is possible, therefore, to assume that besides temperature, the Maillard reaction occurred during heating of *A. pinnata* sap as it might also be affected by its reducing sugar content, the time of heating, pH, TSS of the syrup, and compounds contained in the sap.

5.4.2.4 pH

The Gompertz model resulted by the regression analysis implies a decrease in the correlation between between ΔE^* and pH after the boiling point showed by a negative growth rate ($b = -2.1672$) and had a turning point at pH of 8.388 (by the inflection point, c). A correlation between ΔE^* and pH both Gompertz and linear models could only be expressed after the pH of the sap reached a peak at boiling temperature when the pH was around 8.686. The results suggest that the correlation between ΔE^* and pH could only be identified during that particular period of boiling process. This result complies with earlier observations made during maple syrup processing (Koelling et al., 1996) showing a continuous decrease of pH after it reached a peak at pH of 9 to 7 associated with an increase on colour formation. Similarly, the sugar processing of *A. pinnata* by others (Ho et al., 2008) revealed a decrease in pH values from 7.8 to 5.8 which was also associated with an increase of colour absorbance.

Overall, the regression analysis shows that the correlation between ΔE^* and some processing parameters namely time, temperature, TSS and pH was mostly identified as having non linear relationship rather than linear relationship.

5.4.3 Colour changes during the formation of granulated sugar

Granulated sugar was produced by boiling the sap up to 127°C with a TSS greater than 93%, and the thickened syrup was cooled to about 93°C followed by stirring it continuously

until it was dry and the granulation was formed (Figures 5.8-5.10). Table 5.6 provides the colour changes during the processing of granulated sugar through stirring of the thickened syrup recorded as $L^*a^*b^*$ colour space values together with ΔE^* . L^* values had a slight increase at the beginning from 24.6 to 29.0, before increasing sharply to 42.6 as the granulated sugar was produced. Likewise, both values of a^* and b^* also showed an increase in the beginning to the second phase of the stirring process from 1.9 to 6.7 and 2.8 to 7.3 for a^* and b^* , respectively. Then, b^* increased significantly to 13.5, while a^* indicated a minor increase to 7.4 at the end of the stirring.



Figure 5.6 Phase 1 of processing thickened syrup to granulated sugar



Figure 5.7 Phase 2 of processing thickened syrup to granulated sugar



Figure 5.8 Phase 3 of processing thickened syrup to granulated sugar

Table 5.6 Colour changes during the formation of *A. pinnata* granulated sugar

Phase	L*	a*	b*	ΔE*
1	24.6 ^a ± 0.5 ^b	1.9 ^a ± 0.5 ^b	2.8 ^a ± 0.3 ^b	31.3
2	29.0 ± 0.9	6.7 ± 0.4	7.3 ± 0.6	27.4
3	42.6 ± 0.4	7.4 ± 0.7	13.5 ± 0.6	15.6

^amean^bstandard deviation

It is apparent from Table 5.6 that a* had less significant change than b*, and L* changed the most among these colour space values. On the question of the overall colour value, ΔE* showed a decrease from the beginning to the end of the stirring process. These results suggest that stirring the syrup changed all the colour space values L*a*b*. Specifically, the result of the current study suggests that an improvement in colour was observed, as it became lighter (L* increased), more red (a* increased), and more yellow (b* increased) throughout the stirring process, yielding a pleasantly coloured granulated sugar. There are similarities between the colour changes expressed by a* and b* in this study and those described by Aider et al. (2007) where b* was found to give more adequate information on the colour change of maple sugar. The colour of the sugar produced in their study varied from yellow to brown, and b* parameter provided information on the colour in the yellow spectral zone (Aider et al., 2007b). Similarly, *A. pinnata* sugar produced in the current study varied from red to yellow in colour, confirming that this colour parameter, b* value, might be considered as a stronger indicator of colour changes in the production of *A. pinnata* sugar when comparing with L* and a* values.

5.4.4 Colour quantification of solid sugar and granulated sugar

As colour is usually considered the most important attribute of any foods' appearance (Pathare et al., 2013), the colour difference between solid and granulated sugar produced from the same syrup were analyzed, and a paired t-test was used to assess the difference between them (Table 5.7). There was a statistical evidence ($p < 0.01$) that the type of sugar (solid and granulated sugar) influenced the colour parameters $L^*a^*b^*$ of *A. pinnata* sugar. Determination of several quantitative attributes defining colourfulness i.e., total colour difference ΔE^* , hue angle h , chroma C, and red/yellow a^*/b^* ratio were considered to corroborate the difference between solid and granulated sugar as identified by the paired t-test (Table 5.8).

Table 5.7 $L^*a^*b^*$ colour space and paired t-test of *A. pinnata* solid and granulated sugar

Colour space	Solid sugar (N=6)	Granulated sugar (N=6)	t ratio	Prob > t
L^*	25.8	45.0	27.79	< 0.01
a^*	3.4	7.9	9.02	< 0.01
b^*	5.0	16.0	40.37	< 0.01

Table 5.8 Colour quantifications of *A. pinnata* solid and granulated sugar

Colour quantification	Solid sugar	Granulated sugar
Total colour difference (ΔE^*)	0.0	20.7 - 25.0
Hue angle (h)	$47.3^\circ - 65.7^\circ$	$60.6^\circ - 67.5^\circ$
Chroma (C*)	3.2 - 9.7	14.7 - 21.6
Red/yellow ratio (a^*/b^* ratio)	0.5 - 0.9	0.4 - 0.6

Total colour difference (ΔE^*) indicates the magnitude of the colour difference but not in what way the colours are different (Minolta Co., 2007). In terms of ΔE^* , differences in perceivable colour can be classified as very distinct ($\Delta E^* > 3$), distinct ($1.5 < \Delta E^* < 3$), and small difference ($1.5 < \Delta E^*$) (Adekunte et al., 2010). Using solid sugar as a control sample in calculating ΔE^* , it is apparent that the solid sugar was very distinct from the granulated sugar as the ΔE^* value was much higher than 3 (Table 5.8).

Hue angle (h), which traditionally identifies colour as reddish or greenish, etc., is considered as the qualitative attribute of colour and is the most obvious characteristic parameter to define differences in colour. Hue angle h is defined as starting at the $+a^*$ axis and is expressed in degrees ; 0° would be $+a^*$ (red), 90° would be $+b^*$ (yellow), 180° would be $-a^*$ (green), and 270° would be $-b^*$ (blue) (Pathare et al., 2013). The results, as shown in Table 5.8, indicate that the colour of both solid and granulated sugar was red-orange to yellow-orange where the solid sugar (47.3° to 65.7°) was more red/less yellow than granulated sugar (60.6° - 67.5°).

Chroma (C^*) is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness. The higher the chroma values, the higher is the colour intensity of the samples perceived by the human eye. In summary, chroma tells how pure a hue is. It means there is no white, black, or gray present in a colour that has high chroma; the colour of a product looks rich and full if it has high chroma, but it looks dull and grayish if it has low chroma (Minolta Co., 2007). From Table 5.8, the solid sugar (3.2-9.7) was showed to have lower C^* values than the granulated sugar (14.7-21.6) which implied that granulated sugar was more colourful than the solid sugar, thus giving a pleasing impression for the granulated sugar.

A measurement of $a^*/(red)/b^*(yellow)$ ratio can be classified as yellow ($a^*/b^* < 1$) or red ($a^*/b^* > 1$) (Garcia & Calixto, 2000). Data in Table 5.8 demonstrates that both solid and granulated sugars were in the yellow colour category because the a^*/b^* ratios of both solid and granulated sugars were less than 1. However, the granulated sugar was more yellow than the solid sugar since the average of a^*/b^* ratio of granulated sugar ($a^*/b^* = 0.5$) was lower than that of solid sugar ($a^*/b^* = 0.7$). Colour interpretation of solid and granulated sugar resulting from a^*/b^* ratio was comparable to that of hue angle.

In the market, although the colours of both solid sugar and granulated sugar look different, ranging from light to dark, there is currently no colour grading system established for sugars.

5.5 Conclusion

The present study was designed to examine the colour changes occurring during boiling of *A. pinnata* sap and during its processing into solid and granulated sugars. This study has shown that $L^*a^*b^*$ colour space values changed during the evaporation of the sap associated with the evolution of the process as a function of time, temperature, TSS and pH. During the heating process, time showed a linear correlation with ΔE^* , TSS revealed an exponential (limited growth) correlation with ΔE^* , correlation between temperature and ΔE^* was indicated as Gompertz model, whereas pH demonstrated a strong correlation with ΔE^* for particular ranges in Gompertz model. It was also shown that $L^*a^*b^*$ changed during the formation of granulated sugar when stirring was applied on the thickened syrup. Several colour quantifications conducted on solid and granulated sugar proved that the colour of both solid and granulated sugar was red-orange to yellow-orange, but they were very distinct from

each other, with granulated sugar being more yellow and colourful than solid sugar. This research will serve as a base for future studies on *A. pinnata* sap sugar production especially granulated sugar since this is the first study reporting on the production of granulated sugar from boiling *A. pinnata* sap. The current findings enhance our understanding about the colour change mechanism which took place in processing *A. pinnata* sap into sugar. Furthermore, this information can be used to develop targeted interventions on process and colour development aimed at producing both *A. pinnata* solid and granulated sugars of high market quality.

5.6 Acknowledgment

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CONNECTING TEXT

The colour changes which occurred during the processing *A. pinnata* sap into both solid and granulated sugar were presented in **Chapter 5**. *A. pinnata* sugar is believed to have potential health benefits according to the indigenous Indonesian community, but there is no comprehensive study on the nutritional analysis of this sugar. The fact that *A. pinnata* sugar is traditionally produced without a purification process leads us to the hypothesis that the sugar might contain interesting phyto-compounds such as phenolic compounds with potential antioxidant activity. The characterization of *A. pinnata* sugar through examination of some of its physicochemical attributes namely pH, colour, invert sugar, total sugar content, total phenolic content, antioxidant activity, FTIR spectra and structure by SEM imaging is, therefore, a focus in the following study (**Chapter 6**).

CHAPTER 6

CHARACTERIZATION OF ARENGA PINNATA (PALM) SUGAR

6.1 Abstract

Information on the quality parameters of *A. pinnata* (palm) sugar and its composition is very limited, although the sugar is believed to be a more nutritious sugar than other types of purified sugars. This study aimed at the characterization of *A. pinnata* sugar through an examination of its physicochemical attributes i.e., pH, colour space ($L^*a^*b^*$), invert sugar and total sugar contents, total phenolic content, antioxidant activity, FTIR spectra and its crystallization via SEM imaging. The results indicate average values for pH, invert sugar, total sugar content, total phenolic content, and free radical scavenging activity (IC_{50}) of 6.285 ± 0.007 , $2.98 \pm 0.08 \%$, $93.4 \pm 1.3 \%$, $2432 \pm 32 \mu\text{g/g}$ of GAE, and 0.6 mg/ml , respectively. Colour parameters obtained were 42.6 ± 0.4 (L^*), 7.4 ± 0.6 (a^*), and 13.5 ± 0.6 (b^*). The spectrum of *A. pinnata* sugar displayed absorbance bands at 3386.87, 3331.91, 2360.93, 2158.44, 2034.53 cm^{-1} in the functional group region, and at 1343.18, 1115.62, 1067.89, 1052.46, 1004.25, 989.79, 908.79 cm^{-1} in the fingerprint region. The SEM images confirmed that granulated *A. pinnata* sugar tended to clump together, and was different in sizes and shapes when compared with white cane sugar. The results of this investigation showed that *A. pinnata* sugar has unique characteristics and interesting nutritional properties. The findings from the present study can help the *A. pinnata* farmers and sugar processing industries to gain better market for their products.

Keywords : *A. pinnata* (palm) sugar, characterization, nutritional values, FTIR, SEM images.

6.2 Introduction

There are various types of sugar derived from different sources including sugar produced from concentrated sap tapped from trees such as palm trees growing generally in tropical countries. This type of sugar or sweetener has been reported to be produced into sugar from sap tapped from *Arenga pinnata* merr in Malaysia, and Indonesia (Marsigit, 2005; Mogea et al., 1991; Sahari et al., 2012), from *Borassus flabellifer* tree in Thailand (Panyakul, 2001), sap from the coconut tree (Apriyantono et al., 2002), and from the *Palmyra* palm and date palm (Rao et al., 2009) in some other countries. The sugar is known as palm sugar or neera sugar in Malaysia (Sahari et al., 2012), brown sugar in Indonesia and Thailand (Mogea et al., 1991; Panyakul, 2001), and jaggery in India (Rao et al., 2009).

Information on the characteristics and properties of *A. pinnata* sugar is very limited, even though the sugar is believed to be an alternative sweetener and a more nutritious sugar than from sugarcane (Tamuñaidu et al., 2013). Among available studies on *A. pinnata*, only a few have examined the properties of the sap, and the changes that occur during the sugar production or syrup produced from the *A. pinnata* sugar as reported by Apriyantono et al. (2002), Ho et al. (2007), Ho et al. (2008) and Imanda (2007). The fact that *A. pinnata* sugar usually does not undergo any purification process, or use any synthetic chemical in order to bleach the colour may result in a sugar with potential nutritional benefits since the raw sugar may contain interesting phyto-compounds such as polyphenols (Ben Thabet et al., 2009b; Nayaka et al., 2009). In addition, characterization of the raw materials and ingredients used in foods is increasing recently and is driven by regulatory agencies and consumer groups demanding for safer and healthier foods, especially given the impact of the globalization of sources (Rein, 2012). Therefore, the aim of this study is to characterize *A. pinnata* sugar

through an examination of some of its physico-chemical attributes namely pH, colour, invert sugar, total sugar content, total phenolic content, antioxidant activity, FTIR spectra and its sugar particles via SEM imaging.

Sugar from palm sweeteners is mostly in the form of sucrose in addition to varying amounts of invert sugar present (Naknean et al., 2013). Sucrose is reported as the major sugar in *A. pinnata* sap (Apriyantono et al., 2002; Barlina et al., 2006; Rumajar et al., 2008), and other palm saps such as Palmyra-palm (*Borassus flabellifer* L.), coconut palm (*Cocos nucifera* L.), wild date-palm (*Phoenix sylvestris* L.), and sago palm (*Caryota urens* L.) (Rao et al., 2009). Similarly in maple sap, sucrose is the only sugar when it comes from the tree. Because of microbial activity, some of the sucrose in the sap is converted to invert sugar during handling and processing (Childs & Chabot, 2007; Clement et al., 2010). Invert sugar is also known as reducing sugar while the term “invert” refers to the way these sugars bend polarized light, whereas “reducing sugar” refers to their chemical reactivity (Childs & Chabot, 2007). These sugar compounds have been found to play a role in governing the final products’ organoleptic characteristics such as in palm syrup (Rao et al., 2009) and in maple syrup (Clement et al., 2010).

Invert sugar is the mixture of equal amounts of glucose and fructose (Yu et al., 2011). Determination of invert sugar content is crucial based on the fact that in the maple sugar industry the presence of invert sugars is one of the most important factors influencing the quality results in the manufacture of maple confections (Heiligmann et al., 2006; Koelling et al., 1996), particularly in producing granulated maple sugar (Aider et al., 2007a; Aider et al., 2007b; Childs & Chabot, 2007).

One of the most essential parameters used during the processing of sap into sweeteners is pH as reported for *A. pinnata* sugar (Barlina et al., 2006; Marsigit, 2005), coconut sugar (Apriyantono et al., 2002), Palmyra-palm syrup (Naknean et al., 2013), date-palm syrup (Ben Thabet et al., 2009b), and maple syrup (Clement et al., 2010; Stuckel & Low, 1996; Takano, 2005).

Colour is an important quality attribute in food products that can determine their overall quality and consequently consumer's decision on whether or not to buy the products (Krokida et al., 1998; Maskan et al., 2002; Quintas et al., 2007; Rhim & Hong, 2011). Using colour parameters $L^*a^*b^*$ as a method to measure a product's colour is valuable in providing useful information of the quality appearance of the products, and in addition this method is simpler and faster than using chemical analysis (Maskan et al., 2002).

The interest in natural antioxidants has increased in recent years, including in the diet, by increasing awareness to consume foods that contain antioxidants. Despite of this fact, the promotion of the presence of antioxidant compounds in sugar has not preferably been targeted among sugar manufacturers or vendors (Sia et al., 2010). Since most types of refined sugars on the market do not contain any antioxidant (Sia et al., 2010), determining the antioxidant content in *A. pinnata* sugar can benefit the *A. pinnata* sugar industries by positioning their products as a healthier sugar than conventional white sugar. Further, the antioxidant capacity/activity and the total phenolic content were reported to be correlated (Poiana et al., 2012), and they are found mainly in plant products, and play a role in preventing many chronic diseases (Theriault et al., 2006).

FTIR spectroscopy is an effective method at analyzing, characterizing and verifying the composition of raw materials and ingredients used in food, and it can provide clear insight

into the identity of the sugar especially through comparisons with spectral library databases (Rein, 2012). FTIR has been used for rapid authentication and detection of adulteration of foods (Rodriguez-Saona & Allendorf, 2011), adulteration study of maple syrup (Paradkar et al., 2002), detection of inverted beet sugar adulteration of honey (Sivakesava & Irudayaraj, 2001), and to identify selected wheat varieties (Amir et al., 2013). For the purpose of systematic evaluation, the IR spectrum is commonly divided into three regions namely the functional group region (4000 to 1300 cm^{-1}), the fingerprint region (1300 to 910 cm^{-1}), and the aromatic region (910 to 650 cm^{-1}) (Sherman Hsu, 1997). For example, the study by Sivakesava (2001), used a spectral range of 3700-700 cm^{-1} for the identification of pure honey to cover the three regions. So far no previous study has investigated the spectra of *A. pinnata* sugar. In this work, the spectra of *A. pinnata* sugar were obtained by FTIR and verified using the spectral library matching capability of the Nicolet FTIR analyzer, and therefore it provides clear insight into the identity of the sugar. Identification of the structure of the organic compounds present in *A. pinnata* sugar and the sugar's functional group is one of the objectives of the study, thus working over the spectra wavelength from 3700 to 800 cm^{-1} was chosen.

Scanning electron microscopy (SEM) has been used to provide remarkable images of materials across a range of magnifications to give detailed insights on the morphology of a variety of samples (Dhillon & Kant, 2013). In this study, SEM images with several magnifications ranging from 50X to 600X were studied in order to obtain surface information, and size and shapes of the granules of *A. pinnata* sugar. Previous works using SEM have reported on the morphology and functional properties of starches (Barrera et al., 2013; Sujka & Jamroz, 2013; Utrilla-Coello et al., 2013), investigation of foreign substances in food

(Charbonneau, 2001), and also in numerous other fields of application (Boehm et al., 2013; Conner & Williams, 2004; Nakagawa et al., 2000).

The findings of the present study make a major contribution to knowledge on *A. pinnata* sugar by determining its composition and basic quality parameters. Further, it will help the farmers and related processing industries of *A. pinnata* sugar to gain better access to markets for their quality product.

6.3 Materials and Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl, Folin & Ciocalteu's phenol reagent and gallic acid standard were purchased from Sigma-Aldrich, Dextrose (D-glucose) anhydrous, sodium carbonate and sulfuric acid were purchased from Fisher Scientific Canada, while the anhydrous ethyl alcohol was obtained from Commercial Alcohols, Brampton, Ontario, Canada.

Samples preparation

Four liters of the filtered sap with a pH of 6.388 and 14.9° Brix, obtained from farmers in Tomohon, Indonesia, were boiled in an open stainless steel pan placed over a gas fire to evaporate the moisture content. The sap was heated to the final temperature of 127°C at 157 minutes, before it was cooled to about 93°C and stirred manually until it formed granulated sugar. The obtained granulated sugar was used for the analysis, i.e., determination of pH, colour, total sugar content, invert sugar, total phenolic content, antioxidant activity, FTIR and SEM imaging. Besides *A. pinnata* sugar's sample described previously, some other samples namely ground *A. pinnata* sugar, maple sugar, and white sugar were used for the comparative

study of SEM images. Maple sugar was procured from a maple sugar factory (Ferme St-Ours Inc., Quebec, Canada), and white sugar was purchased from a local supermarket (Ste-Anne-de-Bellevue, Quebec, Canada).

6.3.1 Measurements

pH

A sugar sample of 10 g was diluted in 90 ml of distilled water, and the measurement of pH was done using a pH meter (WTW Sentix 81, pH 3110, Weilheim 2008, Germany). Calibration was accomplished employing buffer solutions at pH 7.0 and 4.0.

Colour

Objective colour measurements were made using a Konica Minolta Color Reader CR 10 (Konica Minolta Optics, Inc., Japan). Samples were placed as a uniform layer on a clear glass petri dish, the white calibration plate CR-A43 was attached to one side of the dish and the measurement was taken. The measurements were recorded in three CIELAB parameters ($L^*a^*b^*$ colour space) and further colour quantification namely hue angle h and a^*/b^* ratio were estimated as follows.

Hue angle h :

$$h = \tan^{-1} \left(\frac{b^*}{a^*} \right) \text{ (Minolta Co., 2007)}$$

and the red/yellow colour ratio (a^*/b^* ratio) (Garcia & Calixto, 2000).

Invert sugar

The invert sugar percentage was determined using a glucometer (GlucoDr Biosensor Type GM 2100, Korea) following the procedure described by Childs & Chabot (Childs & Chabot, 2007).

Determination of total sugar content

The total sugar content of *A. pinnata* sugar was verified colorimetrically using Sulfuric Acid-UV method proposed by Albalasmeh et al. (Albalasmeh et al., 2013). The sugar sample of 0.02 g was diluted in 200 ml of distilled water. A one ml aliquot of sample solution was mixed with 3 ml of concentrated sulfuric acid in a test tube and vortexed for about 30 seconds. The solution was cooled in ice for 2 minutes to bring it to room temperature given that the temperature of solution rose rapidly within 10-15 seconds after the addition of sulfuric acid. Absorbance was measured by UV spectrophotometer (Ultraspec 1000, Biochrom) at 315 nm using glucose (0-0.15 g/L) as a spectrophotometric standard. Standard solutions were prepared following the procedure described above, replacing the sugar solution with glucose at 0.005, 0.010, 0.015, 0.020, 0.025, 0.030 g, diluted each with 200 ml of distilled water (Figure 6.1).

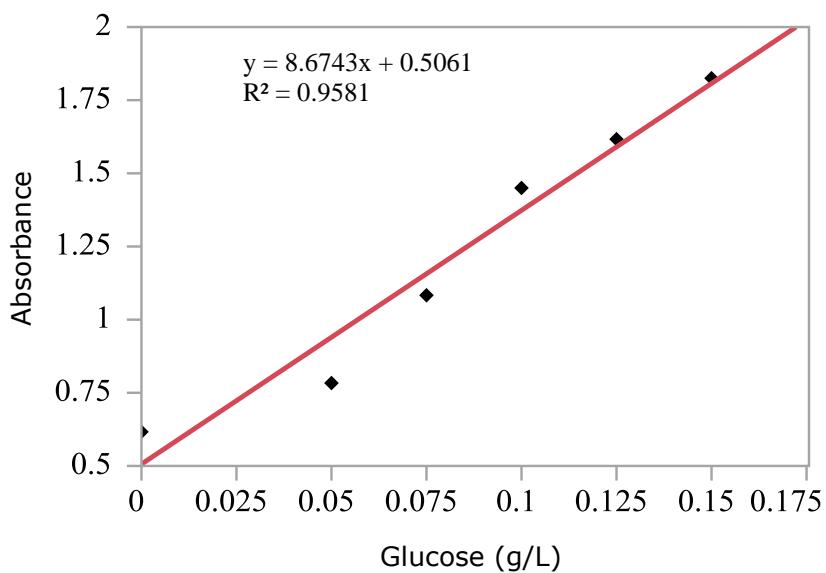


Figure 6.1 Standard curve for determination of total sugar content

Determination of total phenol content

The total phenol content of *A. pinnata* sugar was determined colorimetrically using the Folin-Ciocalteu method as described by Nayaka et al. (Nayaka et al., 2009) with some modifications. A sample aliquot (10 g/L) of 1 ml was added and mixed with 5ml of Folin-Ciocalteu reagent, previously diluted 10 fold with distilled water (0.2 N), and 4 ml of saturated sodium carbonate solution (100 g/L). Samples were left at room temperature for 2 h, and the absorbance was measured at 765 nm using a UV spectrophotometer (Ultraspec 1000, Biochrom). Gallic acid was used as the spectrophotometric standard (0-0.12 g/L) and the total phenolic content was expressed as micrograms of gallic acid equivalent (GAE) per gram of sample. Figure 6.2 presents the standard curve developed in the experiment.

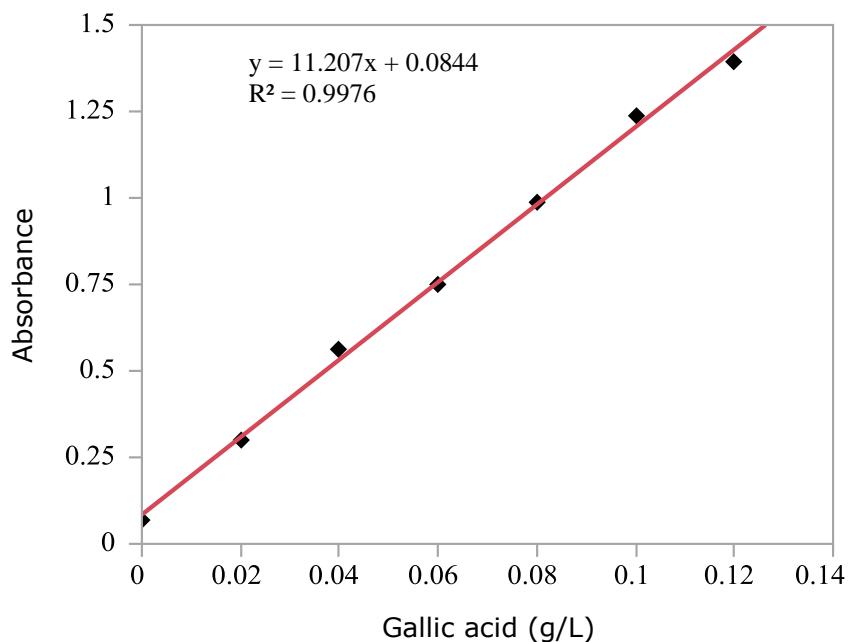


Figure 6.2 Standard curve for determination of total phenolic content

DPPH radical scavenging activity

The free radical scavenging activity of *A. pinnata* sugar was estimated according to the 1-Diphenyl-2-picrylhydrazyl (DPPH) assay (Valli et al., 2012) with minor modifications. Solutions of samples were prepared in ethanol/water 70:30 v/v at different concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10.0 mg/ml), and 100 µL of 0.208 mM DPPH in ethanol/water (70:30 v/v) was added to 100 µL of each solutions. The obtained mixtures were left to stand in the dark for 60 min, and the absorbance at 515 nm was measured with a spectrophotometer (Ultraspec 1000, Biochrom). The radical scavenging activity was calculated by using the following formula : Scavenging effect (%) = [(Abs_{control} – Abs_{sample}) / Abs_{control}] x 100

Fourier Transform Infrared Spectroscopy (FTIR)

A Nicolet iS5 FTIR equipped with ID5 ATR (Thermo Fisher Scientific, USA) was used to analyse the samples. The spectra were obtained in the range 3700-800 cm⁻¹ at 4 cm⁻¹ resolution presenting the average of 32 scans, and the surrounding air spectra was taken and used as the background.

Scanning Electron Microscopy (SEM)

The observations of surface and morphology for size and shape of *A. pinnata* sugar were done with scanning electron microscopy (SEM) (The TM3000 Olympus TableTop Microscope). The dry sugar specimen (*A. pinnata* sugar) was used without further preparation as the sample met the requirement of the SEM. The sugar was mounted onto an adhesive double-sided carbon tape attached to the specimen stub. The observations were made using an annular multi-segmented detector at acceleration potential of 5kV which enhanced the surface structure and optimized imaging of materials. Further analysis of SEM micrographs was done using the program freeware ImageJ (National Institutes Health, Bethesda, MD, USA). Other

samples (maple sugar, white sugar, ground *A. pinnata* sugar) were treated in the same procedure for capturing their SEM images for comparison.

6.3.2 Statistical Analysis

All samples were studied in triplicates for the analysis of pH, colour space ($L^*a^*b^*$), invert sugar and total sugar contents, total phenolic content, and antioxidant activity of *A. pinnata* granulated sugar. The statistical analysis was done using JMP 10 software program (SAS Institute Inc.).

6.4 Results and discussion

Table 6.1 presents the physicochemical properties of *A. pinnata* sugar.

6.4.1 pH

The pH measurements indicated that the *A. pinnata* sugar solution was slightly acidic (6.285 ± 0.007), and it was close to the initial pH of the sap (6.388). The present findings seem to be consistent with previous studies which have demonstrated that some sweeteners such as maple sugar, maple syrup, and date palm syrup have reported pH of 6.30-7.20, 6.35-8.69, and 7.45 to 7.90, respectively (Ben Thabet et al., 2009b; Clement et al., 2010; Heiligmann et al., 2006). All these sugars had similar production processes of thermal evaporation which did not have any purification process and their pH values showed that the sugars solutions were a little acidic, implying a complex composition including minerals and organic acids rather than a pure solution of sugars.

We observed the processing of the sugar sampled in the present study from its sap including the changes of pH during the heating process. From the result of the study, it is

likely that a connection exists between the pH of the solution of the resulting sugars and the initial pH of their parent saps/juices. Even though not many studies have reported the connection between initial pH of the sap/juice and pH of the corresponding sweetener/sugar produced, this hypothesis is supported by the findings of a few previous studies especially during the concentration of saps into syrup or sugar (Apriyantono et al., 2002; Ben Thabet et al., 2009b; Clement et al., 2010; Ho et al., 2007).

Table 6.1 Physico-chemical properties of *A. pinnata* sugar

Composition	Content
pH	6.285 ^a ± 0.007 ^b
Colour space	
L*	42.6 ± 0.4
a*	7.4 ± 0.6
b*	13.5 ± 0.6
Hue angle <i>h</i>	60.6 - 67.5°
Red/yellow a*/b* ratio	0.4 – 0.6.
Total sugar content	93.4 ± 1.3 %
Invert sugar	2.98 ± 0.08 %
Total phenolic content	2432 ± 32 µg/g of GAE
Antioxidant activity	(%)
0.1 mg/ml	45.979 ± 3.325
0.2 mg/ml	46.997 ± 2.645
0.5 mg/ml	48.699 ± 2.608
1.0 mg/ml	52.519 ± 2.081
2.0 mg/ml	59.626 ± 2.967
5.0 mg/ml	71.421 ± 2.504
10.0 mg/ml	77.411 ± 1.378

^a mean

^b standard deviation

6.4.2 Colour

The measurements of CIELAB parameters represent L^* as the lightness of colour (0=black, 100=white), $+a^*$ is red while $-a^*$ is green, and $+b^*$ is yellow and $-b^*$ is blue. Hue angle h is the most obvious characteristic parameter to define differences in colour. Hue angle h is expressed in degrees; 0° would be red, 90° would be yellow, 180° would be green, and 270° would be blue (Pathare et al., 2013). Colour ratio a^*/b^* can be classified as yellow ($a^*/b^* < 1$) or red ($a^*/b^* > 1$) (Garcia & Calixto, 2000).

The colour space ($L^*a^*b^*$) values of the granulated *A. pinnata* sugar are presented in Table 6.1. The values indicate that the sugar had an intermediate lightness ($L^* = 42.6$) (between no lightness i.e., absolute black and maximum lightness i.e., absolute white), with red ($a = +7.4$) and yellow ($b = +13.5$). Further analysis showed that the sugar was definitely yellow as indicated by both the hue angle h (60.6 - 67.5°) and the red/yellow a^*/b^* ratio (0.4 – 0.6). Previous studies have reported a similar colour change of the sap especially becoming darker as a function of time during syrup/sugar processing (Ibarz et al., 1999; Maskan et al., 2002; Rao et al., 2009). The onset of non-enzymatic browning, especially because of the ascorbic acid degradation might favour the decrease of L^* (becoming darker) (Mercali et al., 2014), and its dark colour might be also related to the presence of polyphenol compounds (Garcia & Calixto, 2000).

6.4.3 Total sugar content

The total sugar content of *A. pinnata* sugar was determined colorimetrically using the Sulfuric Acid-UV method. In our preliminary experiment two analysis of total sugar content were tried namely a Phenol-Sulfuric acid method (Ben Thabet et al., 2009b), and the Sulfuric-Acid method which is reported in this study. The present method (Sulfuric-Acid method) was

chosen as it had higher R^2 (0.9581) than the Phenol-Sulfuric acid method (R^2 of 0.9572) (the result does not show here), besides the present method eliminated the hazards posed by usage of phenol since it did not use phenol in its analysis. The sample dilution employed in the present study was a result of our previous work with *A. pinnata* syrup which had a concentration of 66 (% w/w). In the study, 0.1 g of *A. pinnata* syrup was diluted with 1, 1.1, 1.2, 1.3, and 1.4 L of distilled water. Therefore, in the present study, the dilution of *A. pinnata* sugar which had concentration more than 93% (w/w) was tested first with 0.02 – 0.1 g/L before the final concentration (0.1 g/L) was chosen.

The total sugar content of *A. pinnata* sugar determined in the present study was $93.4 \pm 1.3\%$. The sugar content can be compared to the findings of published works. Sihombing (1995) cited that *A. pinnata* sugar contains 70% of sucrose, whereas Rumajar et al. (2008) measured 97.58% sucrose in *A. pinnata* sugar processed in their experiment. Another unspecified palm sugar reported by Parjoko et al. (1996) contained 77% sucrose. This discrepancy may be explained by the fact that there might be a difference in the final concentration of the corresponding juices (higher residual moisture content) which produced the sugars. While there is no report of final concentration of the sugar juices in the reports of Sihombing (1995), Rumajar (2008), and Parjoko (1996), the heating process of the sugar in the present study, for example, was terminated when the concentration of the syrup was above 93 (% w/w).

The *A. pinnata* sugar samples used for the analysis were not subjected to any pre-treatment, consequently it is possible there were interferences in the method analysis due to the presence of phenolic compounds. Nevertheless, the total sugar content detected in this study is still comparable with the concentration of the final syrup recorded during the

experiment (TSS of more than 93%). Therefore, according to these data, we can infer that the level of phenolic compounds identified in the samples (as reported in another section of this chapter) might be in an admissible level which would not interfere in the Sulfuric Acid-UV method analysis. In spite of this, pre-treatment or pre-screening of UV absorbance of the samples should be taken into account in future studies.

6.4.4 Invert sugar

It is apparent from Table 6.1 that the invert sugar of *A. pinnata* sugar produced in the present study (2.98%) was higher than the invert sugar of *A. pinnata* sugar reported (1.81%) by Rumajar et al. (2008). This different result might be related to the characteristics of the initial saps such as pH and sugar content, besides the heating process employed to produce the sugars. The sugar analysed in the present study was produced by heat evaporation of the sap having TSS of 14.9° Brix and pH of 6.388. In published work on date palm syrup, Ben Thabet et al. (2009) reported that the reducing sugar present in the syrup was 10.63-11.37% of total sugars, and suggested that a part of sucrose, the major sugar of date palm sap, was converted to reducing sugars by thermal treatment during the concentration process. In maple sugar industry, the amount of invert sugar in the syrup is one of the most important factors influencing the quality results of maple confections (Koelling et al., 1996). For example, maple syrup with 3% of inverted sugar content cannot be used to produce granulated sugar (Aider et al., 2007a; Aider et al., 2007b), while syrup containing less than 4% invert sugar can be used for making maple cream as syrup with more than 4% invert sugar will not crystallize, or will crystallize only if heated to a higher temperature than the usual temperature (12°C to 13°C above the boiling point) employed in producing maple cream (Heiligmann et al., 2006; Koelling et al., 1996).

6.4.5 Total Phenolic Compounds

A. pinnata sugar contained total phenolic compounds of $2432 \pm 32 \text{ } \mu\text{g/g}$ of GAE as quantified by the Folin-Ciocalteu method (Table 6.1). This result can be compared with the published data presented in Table 6.2 which shows the total phenolic content of some sweeteners. It can be seen that the phenolic content of *A. pinnata* sugar was about 80-90 times higher compared to white and refined sugars, was six times higher compared to brown sugar, and was nearly the same amount as with jaggery sugar. It is possible to hypothesize that a high content of total phenolic compounds is likely present in *A. pinnata* sugar as a result of there being no purification process applied during its manufacture, whereas refined sugar is produced by employing additional steps which remove colour and any other non-sugar components (Phillips et al., 2009).

Polyphenolic compounds are found in plant products (Theriault et al., 2006), and these compounds can be modified and can deteriorate after harvesting due to the storage and food processing conditions (Poiana et al., 2012). The findings of this study can be explained by the fact that the process of concentrating the sap, which initially contained phenolic compounds, into sugar will concentrate the components contained in the sap. This fact agrees with the values of total phenolic compounds of date syrup (*Phoenix dactylifera* L.) reported by Ben Thabet (2009b) where the total phenolic compounds increased as the syrup was more concentrated. Processing temperature and time could also play a significant role in the amount of total phenolic compounds determined in the samples of *A. pinnata* sugar produced from the processing which had a final process temperature of 127°C after 157 minutes of boiling. Naknean & Meenune (2014) demonstrated that Palmyra palm (*Borassus flabellifer* L.) syrup with TSS of 70°Brix produced at temperature of 100°C (using open pan) presented a higher

polyphenol content (1.8 mg/g) than those that were produced at temperature of 60°C (using evaporator) (1.4 mg/g). Different heating methods (open pan vs evaporator) might contribute to changes in polyphenol content in this experiment besides simply the processing temperature (100°C vs 60°C), likely the extended time of the process.

Table 6.2 Reported values of total phenolic contents of some sweeteners found in the literature

Products	Total phenolic compounds (µg/g)	Standard used	Source
Refined sugar (sugarcane)	26.5	Gallic acid	(Nayaka et al., 2009)
White sugar (sugarcane)	31.5	Gallic acid	(Nayaka et al., 2009)
Brown sugar (sugarcane)	372	Gallic acid	(Nayaka et al., 2009)
Brown sugar (sugarcane)	108.1 – 418.1	Gallic acid	(Payet et al., 2005)
Jaggery sugar	3837	Gallic acid	(Nayaka et al., 2009)
Sugar cane molasses	381.62 ± 6.82	-	(Valli et al., 2012)
Sugar beet molasses	62.25 ± 1.72	-	(Valli et al., 2012)
Date palm syrup (64° Brix)	147.6 ± 7.3	Ferulic acid	(Ben Thabet et al., 2009b)
Date palm syrup (68° Brix)	195.1 ± 11.2	Ferulic acid	(Ben Thabet et al., 2009b)
Date palm syrup (74° Brix)	224.5 ± 2.3	Ferulic acid	(Ben Thabet et al., 2009b)

Nevertheless, Ames's work (Ames, 1998) showed that temperature-time was among some various factors influencing the Maillard reaction, a reaction which might occur during the processing of *A. pinnata* sugar. The Maillard reaction increases with temperature, and the

temperature should be kept as low as possible in order to stop or limit the reaction. However, the temperature and heating time will produce a different profile of the product, and product profiles produced at high temperatures will differ significantly from the products processed at a lower temperature for a long time (Ames, 2003). A similar idea was also noted in the previous study by Poiana (2012) that processing method including the number of processing steps and technique, heating temperature, processing period can have a significant impact on the phenolic content, whether positive or negative.

6.4.6 DPPH radical scavenging activity

Antioxidants are compounds with a reductive-oxidative potential, and therefore they can prevent oxidative damage induced by free radicals and other reactive oxygen species (Phillips et al., 2009). Antioxidants are considered important nutraceuticals on account of their potential benefit to human health as shown by several examples that antioxidants reduce risks of developing oxidative stress-related diseases such as cancer and cardiovascular diseases (Abbes et al., 2013; Phillips et al., 2009; Salluca et al., 2008; Sharma & Bhat, 2009). There are a number of different mechanisms such as free radical activity and reducing capacity which might contribute to the antioxidant activity and those need different analytical methods to evaluate the effectiveness of the antioxidants (Abbes et al., 2013). DPPH is one of the methods widely used for evaluation of antioxidant activity of various samples (Abbes et al., 2013; Ben Thabet et al., 2009b; Phillips et al., 2009). DPPH is a stable organic free radical, and the antioxidants reduce the DPPH radical indicated by colour changes from violet colour to yellow colour during the test (Abbes et al., 2013).

Table 6.1 presents the dose-response of DPPH scavenging activities of *A. pinnata* sugar. The results of this study show that *A. pinnata* sugar exhibited a potential free radical

scavenging activity in an amount-dependent manner. The higher the concentration, the higher will be the radical scavenging effect and the lower will be the remaining DPPH. A concentration-dependent activity of the radical scavenging effect demonstrated by *A. pinnata* sugar was in agreement with previous findings on date palm (*Phoenix dactylifera* L.) syrup (Abbes et al., 2013), brown sugar and jaggery sugar (Nayaka et al., 2009). The radical scavenging activity of the samples on the DPPH can also be expressed by IC₅₀ i.e., the concentration of sample needed to reduce the initial DPPH concentration by 50%. Using a linear analysis (Abbes et al., 2013), the IC₅₀ of *A. pinnata* sugar was calculated and the result is presented in Table 6.3 together with the reported IC₅₀/EC₅₀ of some other sweeteners.

Table 6.3 Inhibitors IC₅₀ for some sweeteners

Products	IC ₅₀ (mg/ml)	Source
<i>A. pinnata</i> sugar	0.6	Current study
Jagery sugar	0.00781 *	(Nayaka et al., 2009)
Brown sugar	0.0594 *	(Nayaka et al., 2009)
White sugar	insignificant inhibitory activity *	(Nayaka et al., 2009)
Refined sugar	insignificant inhibitory activity *	(Nayaka et al., 2009)
Sugarcane molasses	1.47	(Valli et al., 2012)
Sugarbeet molasses	7.25	(Valli et al., 2012)
Date palm syrup	10.22-42.62	(Abbes et al., 2013)

* EC₅₀ (effective concentration) was used instead of IC₅₀ (inhibitory concentration). However, in this case the term IC₅₀ and EC₅₀ had the same meaning since they were generated using the same procedure.

The lower value of IC_{50} reflects better protective action, thus as it is apparent from Table 6.3, that *A. pinnata* sugar showed higher antioxidant capacity among sugarcane molasses, sugarbeet molasses and date palm syrup, whereas it was lower compared to the antioxidant capacity of jaggery sugar and brown sugar reported by Nayaka et al. (2009). The antioxidant capacity is usually correlated with the phenolic compounds (Abbes et al., 2013; Ben Thabet et al., 2009b; Payet et al., 2005; Poiana et al., 2012). Abbes et al. (2013) reported a significant linear correlation ($R^2=0.8322$) between DPPH radical scavenging activity and the total phenolic content of date (*Phoenix dactylifera* L.) syrup.

A study by Payet et al. (2005) investigated the polyphenol content and volatile composition, in relation to their free radical scavenging capacity, of cane brown sugars and found that the increased content of polyphenolics increased the antioxidant activity. Those antioxidant properties of the sugars seemed to be related to the polyphenol components native to the plant of origin. In addition, Maillard reaction products formed during the heating process might also contribute to the antioxidant activity (Ben Thabet et al., 2009b; Payet et al., 2005). While antioxidant capacity of some plants such as berries was reported to decrease after jam processing (Poiana et al., 2012), Maillard reaction products were noted among the components contributing to the higher antioxidant activity in the date syrup processed traditionally than that in enzyme-treated date syrup examined in the study of Abbes et al. (2013). The impact of the Maillard reaction on antioxidants can also be seen in the findings of Abbes et al. (2013) and those described by González-Sarriás et al. (2012) where it was indicated that the darker grades of maple syrup were found to be more active than the lighter grades due to the higher concentration of the most active phenolics.

Some of Maillard reaction products are able to retard lipid oxidation, thereby influencing the oxidative stability of foods (Ames, 2003). Wang et al. (2011) in their review of melanoidin properties produced by the Maillard reaction which related to the beneficial health mention that the Maillard reaction occurs during the preparation of food leads to the production of melanoidin. They pointed out that one of main mechanisms for the antioxidant activity of melanoidins is their radical-scavenging activity. Ames (2003) describes that the formation of nondialyzable melanoidins in the Maillard reaction products is enhanced by an increase in pH and the amino compound sugar molar ratio. Nondialyzable melanoidins then scavenge hydroxyl radicals and other active oxygen species. It has been suggested that antioxidant activity increased with formation of melanoidins to a maximum level before it decreases due to further heating. Reducing activity is one of the factors that might account for antioxidant activity, however the structures responsible for antioxidant activity remain uncertain (Ames, 2003).

In general, the antioxidant activity of *A. pinnata* sugar shows a proportional relation to the concentration and the sugar can be a good source of residual antioxidant components from the plant sap since it is not subjected to any purification process prior to processing, in addition to the generation of antioxidant molecules formed during the heating process thanks to the presence of Maillard reaction products.

6.4.7 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 6.3 presents the FTIR spectra of *A. pinnata* sugar manufactured in the study. Using spectrum search tool and spectral library database, the spectra of *A. pinnata* sugar was found best matched at 87.86% with pure sucrose obtained from HR Aldrich Alcohols and Phenols at the range of wavenumbers of 3700 - 800 cm^{-1} as shown in Figure 6.4 and Table

6.4. The peaks locations of *A. pinnata* sugar were further identified by the spectral library database and the results are given in Table 6.5.

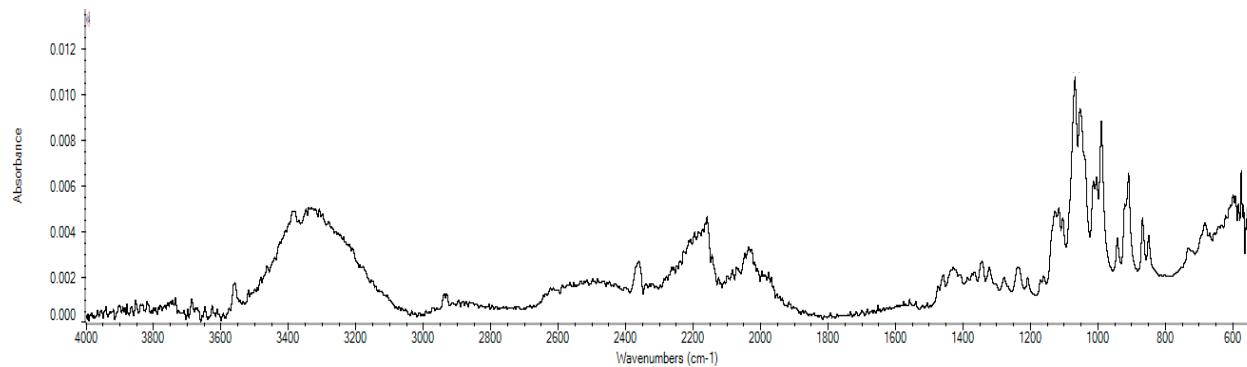


Figure 6.3 FTIR spectra of *A. pinnata* sugar

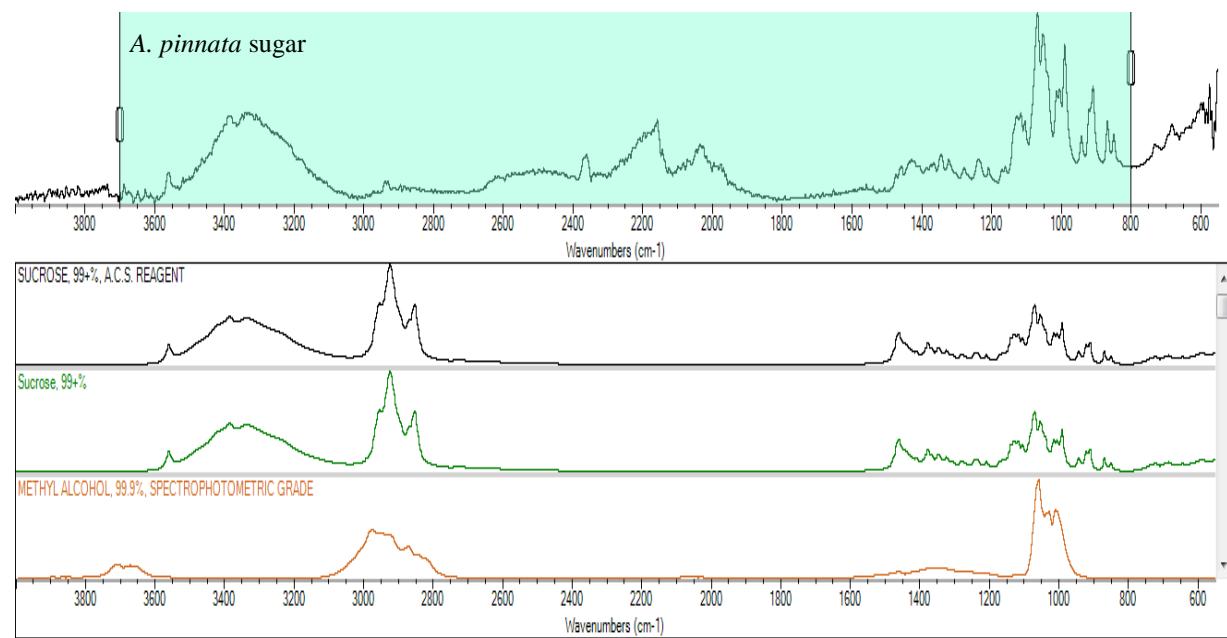


Figure 6.4 FTIR spectra of *A. pinnata* sugar compared with three other substances

Table 6.4 Three substances which showed best matches with the spectra of *A. pinnata* sugar

Match (%)	Component	Library database
87.86	Sucrose, 99+%, A.C.S.Reagent	HR Aldrich Alcohols and Phenols
62.50	Sucrose, 99+%	HR Nicolet Condensed Phase Academic Sample
40.94	Methyl Alcohol, 99.9%, Spectrophotometric grade	Aldrich Vapor Phase Sample Library

The peaks of *A. pinnata* sugar spectrum identified in the range of 3700-800 cm^{-1} were used to identify the structural elucidation namely the functional group and the finger print (Sherman Hsu, 1997). The functional group of *A. pinnata* sugar was examined in five different zones ranging from 3700 to 1450 cm^{-1} as shown in Table 6.6, whereas the finger print was analysed in the range of 1300-800 cm^{-1} . However, the spectral interpretations were not confined to one or two bands and the whole spectrum was examined as suggested by Sherman Hsu (1997).

Table 6.5 Identified peak locations of sucrose and *A. pinnata* sugar

Sucrose* (cm^{-1})			<i>A. pinnata</i> sugar (cm^{-1})		
3386.39	1322.93	1238.08	3386.87	3331.91	2360.93
1116.58	1105.01	1068.37	2158.44	2034.53	1343.18
1052.94	1004.73	991.23	1115.62	1067.89	1052.46
943.02	908.31	867.81	1004.25	989.79	908.79
848.53					

* sucrose, 99+%, A.C.S. Reagent, HR Aldrich Alcohols and Phenols

In the region of functional group, the spectrum of *A. pinnata* sugar showed absorbance bands at 3386.87, 3331.91, 2360.93, 2158.44, and 2034.53 cm^{-1} . The bands in the 3700-3200 cm^{-1} region were assigned to alcohol O-H, amide/amine N-H, or terminal Alkyne $\equiv\text{C-H}$. *A. pinnata* sugar's spectrum also showed peaks at 2360.93, 2158.44, and 2034.53 cm^{-1} which could be assigned to alkyne C \equiv C and nitrile C \equiv N (zone 3). However, alkynes have peaks at both 3300 and 2250-2100 cm^{-1} , whereas nitriles have peaks specifically in the range of 2240-2260 cm^{-1} . The spectrum of *A. pinnata* sugar did not show absorbance in the range of 2240-2260 cm^{-1} , thus it could be hypothesised that nitrile was not present in the sugar. In contrast, both peaks at around 3300 cm^{-1} (refer to zone 1) and 2250-2100 cm^{-1} (refer to zone 3) were identified in the spectrum of *A. pinnata* sugar, and it can therefore be assumed that the sugar contained alkyne.

Table 6.6 Five zones approach to identify functional groups of *A. pinnata* sugar

Zone	Wavenumber (cm^{-1})	Functional group	Peak of <i>A. pinnata</i> sugar spectra (cm^{-1})
1	3700 – 3200	Alcohol O-H Amide/Amine N-H Terminal Alkyne $\equiv\text{C-H}$	3386.87 3331.91
2	3200 – 2700	Alkyl C-H (peak $< 3000 \text{ cm}^{-1}$) Aryl or vinyl C-H (peak $> 3000 \text{ cm}^{-1}$) Aldehyde C-H Carboxylic Acid O-H	-
3	2300 – 2000	Alkyne C \equiv C Nitrile C \equiv N	2360.93 2158.44 2034.53
4	1850 – 1650	Carbonyl functional groups	-
5	1680 – 1450	Alkene C = C Benzene	-

The second region to analyse in the spectrum of *A. pinnata* sugar was the fingerprint region (1300- 910 cm⁻¹). The spectrum of *A. pinnata* sugar showed absorbance bands at 1343.18, 1115.62, 1067.89, 1052.46, 1004.25, 989.79, and 908.79 cm⁻¹. According to (Paradkar et al., 2002; Sivakesava & Irudayaraj, 2001), 1500-800 cm⁻¹ region corresponds to the absorption zone of the 3 major sugars namely sucrose, fructose and glucose. In *A. pinnata* sugar, sucrose is the main sugar as it was identified to have a 87.86% matching spectrum with sucrose spectrum (by HR Aldrich Alcohols and Phenols, Table 6.4). The bands in the 904 to 1153 cm⁻¹ regions are assigned to C-O and C-C stretching modes, and those around 1199 to 1474 cm⁻¹ are due to the bending modes of O-C-H, C-C-H, and C-O-H angles (Paradkar et al., 2002).

6.4.8 Scanning Electron Microscopy (SEM)

Figures 6.5 - 6.8 display the scanning electron microscopy (SEM) micrographs of different magnifications of granulated *A. pinnata* sugar, maple sugar, white sugar, and ground *A. pinnata* sugar. Since the scanning electron microscopy (SEM) is operated under high vacuum, the samples must be compatible with high vacuum (~ 10⁻⁵ mbar) which means liquids and materials containing water and other volatile components cannot be studied directly (Central Facility for Advanced Microscopy and Microanalysis).

Scanning electron micrographs of granulated *A. pinnata* sugar (Figure 6.5a-c) showed that the sugar particles tended to clump. The reason for this is not clear but there are several possible explanations for this result. First, it might be related to the purity of the sugar as there was no purification process applied in manufacturing *A. pinnata* sugar and a variety of phyto compounds were present in the sap and subsequently in the sugar, perhaps clinging to the outside of the sugar granules. Second, *A. pinnata* sugar might contain more liquid on the sugar

surface than on white sugar which had a hot air drying step during the purification process, resulting in a tendency of *A. pinnata* sugar to clump which was not shown by the white sugar. This hypothesis was supported by comparison with the SEM micrographs of maple sugar (Figure 6.6) and white sugar (Figure 6.7a-b). The tendency to clump as demonstrated by *A. pinnata* sugar was also seen in maple sugar, another sugar which has no purification and/or hot air drying step during the process, but it was not found in white sugar which employs purification and hot air drying process during its manufacturing. Therefore, employing more drying process on *A. pinnata* sugar crystals might eliminate clumping while still retaining a high phyto-chemical content.

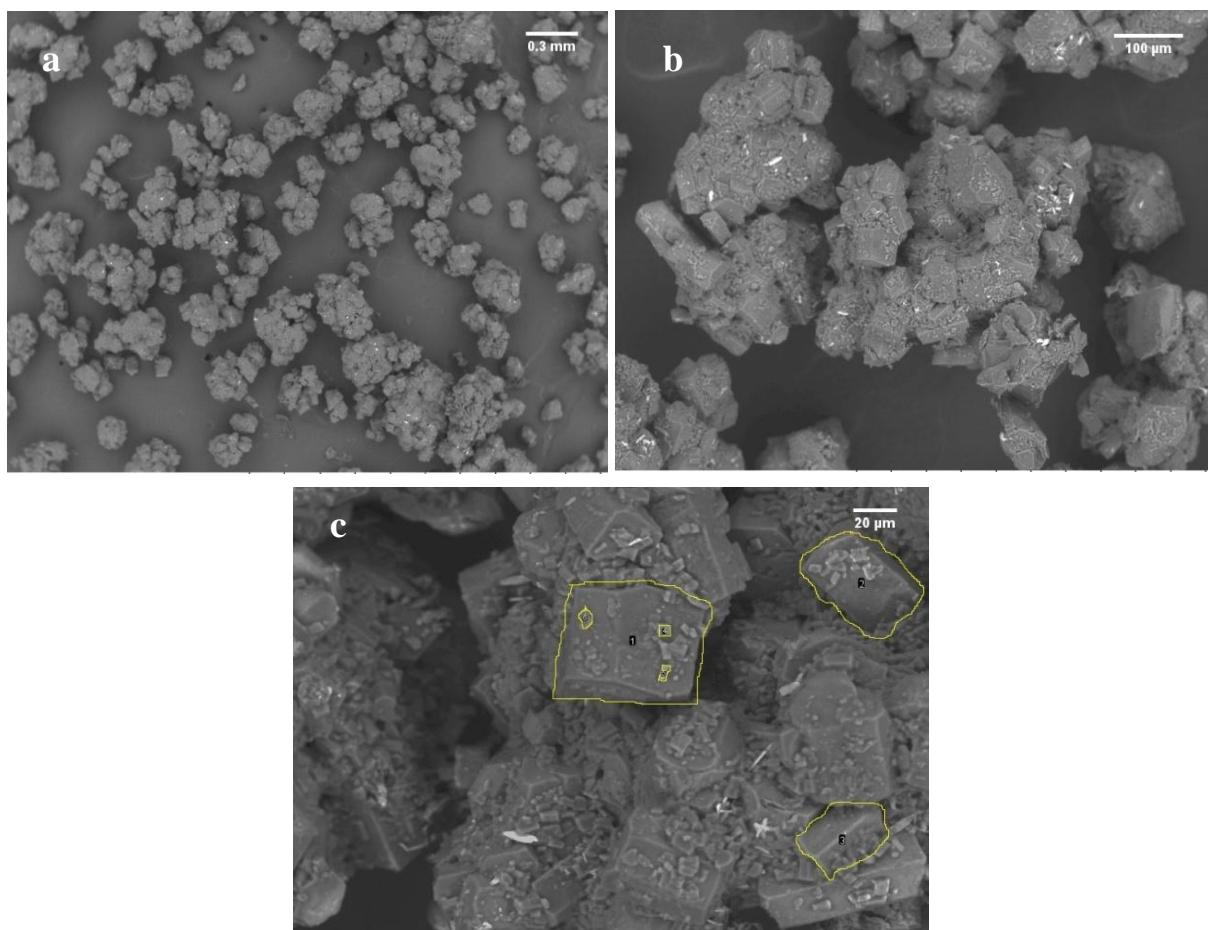


Figure 6.5 Scanning electron micrographs of *A. pinnata* sugar

(a) 50X magnification (b) 200X magnification (c) 600X magnification

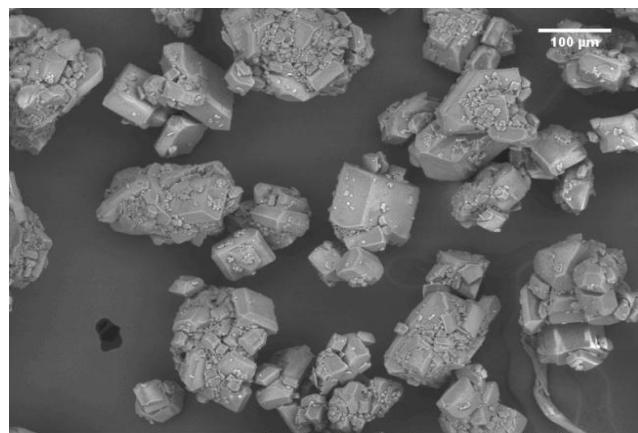


Figure 6.6 Scanning electron micrographs of maple sugar
(200X magnification)

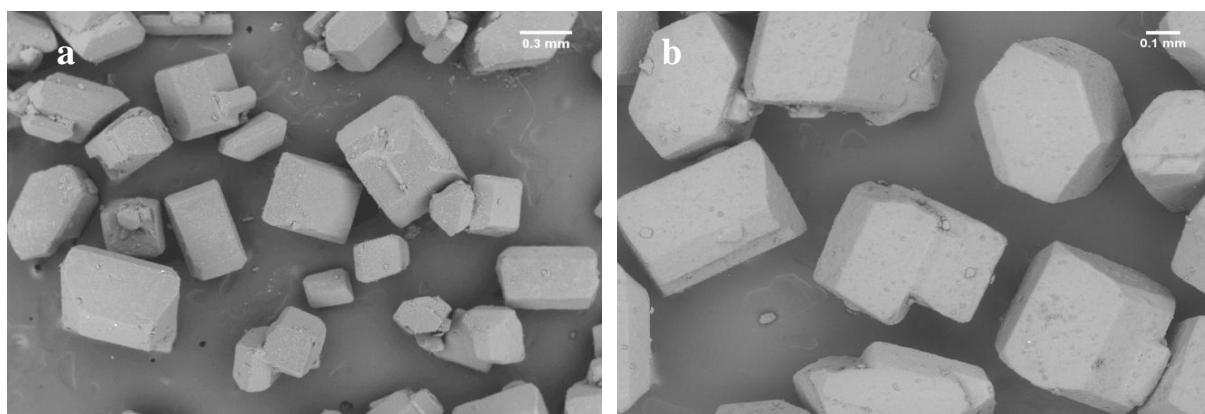


Figure 6.7 Scanning electron micrographs of white sugar
(a) 50X magnification (b) 100X magnification

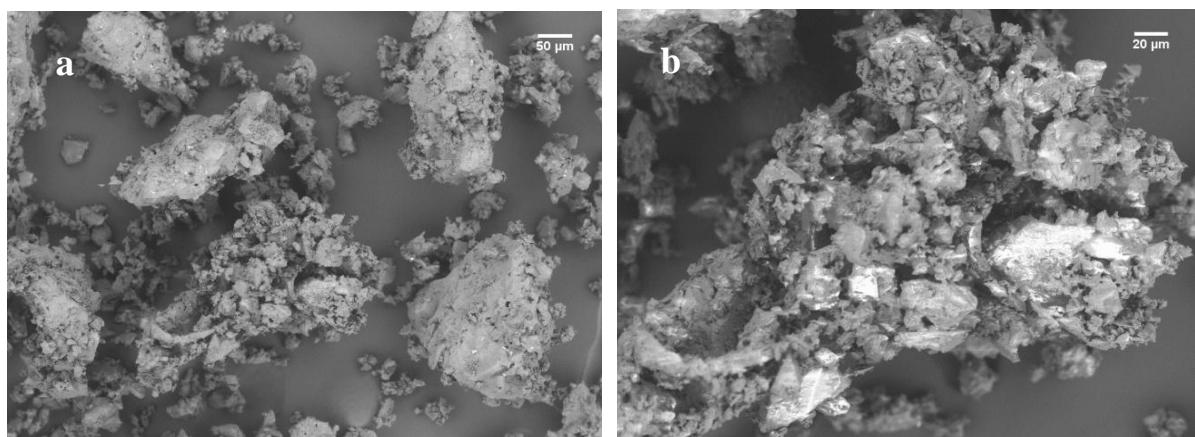


Figure 6.8 Scanning electron micrographs of ground *A. pinnata* sugar
(a) 200X magnification (b) 500X magnification

Variations in size and shapes of *A. pinnata* sugar are clearer at the higher magnification which reveals that the sugar tended to be irregular and angular in shape (Figure 6.5c). It also shows the presence of granules of different sizes with small granules visible on the surface and in between the larger ones.

In addition, no crystals could be observed in SEM micrographs of ground *A. pinnata* sugar (Figure 6.8a-b) as a consequence of particle size reduction of the sugar by crushing the sugar which damaged the crystal sugar. However, the results of the present study do not explain the particle size distribution that affects the quality of the foods in which the sugar is added, and a further study with more focus on the crystal size distribution of *A. pinnata* sugar is therefore recommended for future.

6.5 Conclusion

The main goal of the current study was to characterize *A. pinnata* sugar by determining its pH, colour, invert sugar, total sugar content, total phenolic content, antioxidant activity, FTIR spectra and structure by SEM imaging. The results of this investigation show that *A. pinnata* sugar has unique characteristics with pH of 6.285 ± 0.007 for the sugar solution, $93.4 \pm 1.3\%$ total sugar content, $2.98 \pm 0.08\%$ invert sugar, yellow-red in colour (by hue angle h and a^*/b^* ratio values), and interesting nutritional properties with a high total phenolic compounds content ($2432 \pm 32 \mu\text{g/g}$ of GAE), and antioxidant potential identified by a free radical scavenging activity (IC_{50}) of 0.6 mg/ml . Spectra of *A. pinnata*, examined in the range of $4000\text{-}800 \text{ cm}^{-1}$, showed peaks in the functional group region of $3700\text{-}3200 \text{ cm}^{-1}$ and $2300\text{-}2000 \text{ cm}^{-1}$ which were assigned to alcohol O-H, amide/amine N-H, or terminal Alkyne $\equiv\text{C-H}$, and Alkyne $\text{C}\equiv\text{C}$, and in the fingerprint region ($1400\text{-}910 \text{ cm}^{-1}$) which corresponded to the

absorption zone of sucrose, fructose and glucose. Scanning electron microscopy (SEM) micrographs of *A. pinnata* sugar displayed the variations in size and shape of *A. pinnata* sugar. The granules of the sugar tended to clump, and there were small granules lying on the surface of bigger granules. The empirical findings from this study provide good baseline information on *A. pinnata* sugar especially to extend our knowledge of this particular sugar's composition. The finding of the study will help *A. pinnata* sugar farmers and industries to have a better market for their products as they will be able to promote their products as having antioxidant capacity and an appreciable total phenolic content.

6.6 Acknowledgment

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CHAPTER 7

GENERAL CONCLUSION, CONTRIBUTION AND RECOMMENDATION FOR FUTURE STUDIES

7.1 Introduction

Palm sugar produced from the sap is the main product of *A. pinnata*, one of the most diverse multipurpose palm tree (Adawiyah et al., 2013; Moga et al., 1991), and it has been consumed by the local people in Indonesia as a sweetener for decades. In Tomohon, Indonesia, normally the sap is collected twice a day, in the morning and in the afternoon using a container, then it is boiled in a round iron pan over a wood-fire until it is thickened before pouring it into a mould which consists of a coconut shell to form solid sugar or stirring the thickened juice to form granules.

A. pinnata sap processing into sugar carries the potential for enhanced quality however changes in its physico-chemical characteristics during the process into sugar need to be quantified more precisely including measurements of physical parameters and chemical and nutritional properties. The indigenous knowledge for processing practices by local people in Tomohon and available literature are both incomplete and somewhat inconclusive especially in the context of the optimized processing of *A. pinnata* sugar from its sap. Therefore this study was set up to develop a better understanding of the processes which are needed to improve existing techniques applied by indigenous people to enhance or minimize the loss of product quality, and maintain product identity from the farm to the customer. In order to achieve this goal, the study has sought to determine the important baseline process parameters in order to produce granulated sugar and to characterize the phytochemical content of the

obtained *A. pinnata* sugar. Monitored sap boiling was chosen for the processing of the *A. pinnata* sap into sugar as it is the most practical and useable method for the local communities of Indonesia.

7.2 Synthesis of findings

The desire and need to produce a natural, high quality sugar have been realized as the primary goal of the present study. Consequently, the study followed the guidelines practiced by maple syrup/sugar producers in North America, considering the industrial development and standardization of the maple syrup/sugar production, as described in **Chapter 2** for conducting the experiments for processing *A. pinnata* sugar from its sap.

A. pinnata sap is the raw material from which *A. pinnata* sugar is made. It is a completely natural product obtained by tapping the inflorescences of *A. pinnata* tree. A pattern of changes occurring within the *A. pinnata* sap over time was studied in **Chapter 3**. Without any preservation process, time becomes the main essential issue/parameter to monitor since the sap will deteriorate rapidly over time from fermentative activity. The experiments focused on the effect of time on the changes in pH, invert sugar and colour space ($L^*a^*b^*$) of fresh sap. Since we were interested in understanding the pattern of changes over time, a repeated measure design was chosen for the experiment. Sap with different initial pH varying from 6.103 to 6.974 was observed at different times and all the results confirmed that the pH of the sap decreased as a function of time. The study also identified a negative correlation between pH and invert sugar wherein, as the time increased, the pH of the sap decreased and the invert sugar increased gradually. The measurement of pH and colour ($L^*a^*b^*$ values) changes implied that the pH of the sap was more closely associated with L^* and b^* values, indicating

that change in pH was more a correlated function of lightness/darkness rather than red/green hues in the sap.

Preventing the sap from deterioration following harvest by maintaining the pH of the sap constant for more than 3 hours was studied in **Chapter 4**. The experiment was conducted to observe the pH changes in both untreated and pre-boiled saps over a period of 18 hours. Following 18 hours of storage, the pH of untreated sap decreased from the initial pH of 6.388 to 3.760 at which point the sap cannot be processed into sugar, whereas the pH of pre-boiled sap remained stable (from 8.686 to 8.620) at a pH level where the sap can still be processed into sugar. The result from this experiment suggested that heating the sap to its boiling point can maintain the pH constant by inactivation of microorganisms, and therefore it is possible to preserve the sap for at least 18 hours following harvest, prior to its processing into sugar.

Converting the sap into sugar is accomplished by the concentration of the sugar content in the sap through boiling to evaporate the water as studied in **Chapter 4**. The process involves many complex physical and chemical transformations that together affect the final product, whether it being solid or granulated sugar. The behaviour of *A. pinnata* sap during the heating process was studied through the measurement of several process parameters i.e., temperature, total soluble solids (TSS), and pH changes. In this experiment, four liters of the filtered sap were boiled in an open stainless steel pan over a gas fire. Palm oil was used as a defoaming agent to control the foam generated during the heating process. The sap was boiled to 23-25°C above the boiling point of water. Following the sap concentration, the syrup was cooled to about 93°C and stirred manually to form granulated sugar. This process of producing *A. pinnata* sugar took 157 minutes of boiling the sap before it was terminated at a temperature around 123°C which corresponded to more than 93% TSS. The results showed that the

increase of temperature during heating of the sap had three phases. In the first phase (0-30 minutes), the sap increased rapidly from room temperature (~24°C) to the boiling point determined within the range of 98-98.7°C; the second phase (30-150 minutes) occurred when the temperature indicated a small increase from 98-98.7°C to about 110°C; and the final phase (150-157 minutes) was when the temperature increased another 13°C. Through these three phases the TSS during the heating process was determined. The increase of TSS was very small in the first phase (around 2% TSS in 30 minutes), moderate in the second phase (60% TSS in 120 minutes), and high in the last phase (18% TSS in 7 minutes).

The evolution of pH and temperature during the boiling process of *A. pinnata* sap with several different initial pH was also determined. The observations of initial and final pH of the samples indicated that *A. pinnata* sap and sugar are slightly acidic, implying the presence of a complex mixture including minerals and organic acids rather than simply of a pure solution of sugars. It was also interesting to note that the final pH, although it experienced increases and decreases during the heating process, went back close to its initial pH value once the sap was processed into sugar. This result suggested that a link might exist between the initial pH of the sap and the final pH of the sugar. Therefore, it is possible while preliminary, to utilize the pH of *A. pinnata* sugar, as the final product, as an indicator of the initial pH of the sap, and vice versa. This might be used to monitor the quality of the supply.

The colour changes and their correlations with time, temperature, TSS and pH during the processing of the sap into sugar were examined in **Chapter 5**. This study provides some information about the colour changes that took place during processing of *A. pinnata* sap into sugar which can be a useful and important tool for the quality optimization of the manufactured sugar. During this experiment, the colour space L*a*b* and several colour

indicators i.e., total colour difference (ΔE^*), hue angle (h), chroma (C^*) and red/yellow ratio (a^*/b^* ratio) were monitored. The results showed that as a function of time, the sap became darker, more red, and more yellow from beginning up to 155 min indicated by a continuous decrease of L^* values, and an increase both in a^* and b^* values. Colour space a^* and b^* were not affected by temperature in the beginning of the process, however there was a big effect on the $L^*a^*b^*$ colour space after it reached a temperature of about 125°C. Correlation between colour and TSS was determined. It showed that there was a trend of decreasing L^* and increasing a^* and b^* from the beginning of the heating process until the boiled sap reached a TSS of about 93%. No obvious changes of a^* and b^* , and only a slight increase of L^* was observed during the increase in pH from the beginning until it reached a peak suggesting that a^* and b^* were not affected by pH changes in the beginning of the boiling process.

A correlation between total color difference (ΔE^*) and each parameter (time, temperature, TSS and pH) was considered particularly because it combines all colour parameters $L^*a^*b^*$. The analysis showed a positive correlation between ΔE^* and time, while a relationship between ΔE^* with temperature and time showed a non-linear trend. A non-linear correlation between ΔE^* and pH can only be identified for particular ranges i.e., the period after the sap reached a peak pH (at the boiling temperature) to the end of the heating process.

Producing granulated sugar from *A. pinnata* sap and the study of the processing parameters affecting the granulated sugar was reported here for the first time. As a result, some findings from the present study were in contrast to earlier findings such as studies by Ho et al. (2008) for *A. pinnata*, by Rao et al. (2009) for Palmyra-palm and date-palm, and by Ben Thabet et al. (2009) for date palm. Since those studies reported on the heating process of sap

into solid sugar with TSS of 88% (Ho et al., 2008), and 82% (Rao et al., 2009), the present study described changes which occurred during the heating process with TSS greater than 93% resulting in both solid and granulated sugars. In order to produce granulated sugar, boiling the sap up to 127°C with a TSS greater than 93% must be followed by stirring of the thickened syrup continuously until it produces a dry granulation. The colour space L*a*b* of the thickened syrup changed throughout the stirring process and it showed an improvement as it became lighter (L* increased), more red (a* increased), and more yellow (b* increased), yielding a pleasantly coloured granulated sugar. The observations on solid sugar and granulated sugar by several colour indicators showed that solid sugar was very distinct from granulated sugar (identified by ΔE^* value), the colour of both solid and granulated sugar was red-orange to yellow-orange where the solid sugar was more red/less yellow than granulated sugar (identified by hue angle h), and granulated sugar had more strength in colour than the solid sugar (identified by chroma C*), thus giving delightful impression for the granulated sugar.

The sugar characterization was done through an examination of its physicochemical attributes i.e., pH, colour space (L*a*b*), invert sugar, total sugar content, total phenolic content, antioxidant activity, FTIR spectra and its images by SEM in **Chapter 6**. The total sugar content of *A. pinnata* sugar was determined to be $93.4 \pm 1.3\%$, with an invert sugar content of $2.98 \pm 0.08\%$. Total phenolic compounds in the sugar was $2432 \pm 32 \mu\text{g/g}$ of GAE which was higher compared to total phenolic compounds contained in other reported sugars by Nayaka et al. (2009) and Payet et al. (2005) (26.5, 31.5, and 108-418 $\mu\text{g/g}$ of GAE for refined sugar, white sugar, and brown sugar, respectively).

The DPPH test showed that *A. pinnata* sugar exhibited a potential free radical scavenging activity in a concentration-dependent manner. Further analysis expressed by IC₅₀ (the concentration of sample needed to reduce the initial DPPH concentration by 50%) indicated that the antioxidant capacity contained in *A. pinnata* sugar (0.6 mg/ml) was stronger compared to antioxidant capacity of sugarcane molasses (1.47 mg/ml), sugarbeet molasses (7.25 mg/ml) (Valli et al., 2012), and date palm syrup (10-43 mg/ml) (Abbes et al., 2013).

The mid-infrared spectrum of *A. pinnata* sugar was identified in the range of 3700-800 cm⁻¹ by FTIR. The results showed absorbance bands at 3386.87, 3331.91, 2360.93, 2158.44, 2034.53 cm⁻¹ at the functional group region, and absorbance bands at 1343.18, 1115.62, 1067.89, 1052.46, 1004.25, 989.79, 908.79 cm⁻¹ at the finger print region which corresponds to the absorption zone of sucrose, fructose and glucose.

Scanning electron microscopy (SEM) micrographs of *A. pinnata* sugar displayed variations in size and shape of *A. pinnata* sugar. The granules of the sugar had a tendency to clump together, and small visible granules were over the surface of and between bigger ones. It is possible, therefore, to hypothesise that these conditions might be related to phyto-compounds present in the sap and subsequently in the sugar due to the lack of a purification process employed during the manufacturing of *A. pinnata* sugar, in addition to liquid present on the granules as there was no hot air drying step employed during the sugar processing. In general, the results showed that *A. pinnata* sugar contained higher bioactivity compared with other refined sugars, and a good source of residual antioxidant activity from the palm plant.

7.3 Contribution to knowledge

This thesis is the first study reporting on the comprehensive processing of *A. pinnata* sap into both solid and granulated sugars and on the quality parameters of granulated sugar produced from the process. The outcome of this study has contributed to our knowledge in several ways including a better understanding of the scientific and process application challenges. The following are a few of the several commendable contributions of this research.

1. Knowledge was developed on the underlying chemical and physical properties of *A. pinnata* sugar as affected during processing from the fresh sap into sugar. This study reports for the first time the changes in pH, invert sugar and colour of *A. pinnata* sap as a function of time following harvest. The correlation between pH and invert sugar in *A. pinnata* sap was first identified in this study. Furthermore, it is likely that such connection exists between the initial pH of the sap and the final pH of the sugar, and therefore it is possible to utilize the pH of *A. pinnata* sugar, as the final product, to determine its initial pH (the sap), and vice versa as a quality indicator.
2. A trend of behaviour of the *A. pinnata* sap during the heating process was identified in this study through the examination of changes in temperature, Total soluble solids (TSS), pH and colour ($L^*a^*b^*$ colour space). The empirical findings from this study contribute to the baseline of knowledge available in the very limited literature on the production of *A. pinnata* solid sugar especially to extend our knowledge of the palm syrup/sugar processing provided by Ho et al. (2008) for *A. pinnata*, by Rao et al. (2009) for Palmyra-palm and date-palm, and by Ben Thabet et al. (2009) for date palm which produced only solid sugar from the heating/concentration process.

3. Interesting nutritional properties such as total phenolic compounds and antioxidant potential were identified in the study and represent a major contribution to the knowledge on *A. pinnata* sugar's composition. An important practical implication from the finding of the study is that it will help *A. pinnata* sugar farmers and industries to develop a better market for their products.
4. This work contributes to the very scarce existing knowledge of the physico-chemical changes which occur during the heating process of *A. pinnata* sap into sugar. The results of the study will help the farmers and related industries to maintain the quality of the sap from deterioration, to develop targeted interventions on colour aimed at producing both solid and granulated *A. pinnata* sugar, and will serve as a base for future studies on *A. pinnata* sap sugar production.

7.4 Recommendations for further research

This study contributed to improving the existing practices applied by indigenous people, especially to have better understanding of the principal chemical and physical properties of *A. pinnata* sugar which are affected during its processing from the fresh sap into sugar. Nevertheless, this study has unravelled many questions in need of further investigation in addition to specific concerns on the economic benefits of producing quality sugar. Therefore, the following are some of the recommendations for future research.

1. The findings from the present study provide the following insights for future research on pH and invert sugar mechanism in the sap and during the heating process. The precise mechanisms governing the change of pH and invert sugar in *A. pinnata* sap as well as during the heating process remain to be elucidated. Further research needs to examine

more closely the links between the initial pH (sap) and the final pH (sugar) identified in the study.

2. Further studies are needed to investigate more specifically the physico-chemical properties of *A. pinnata* sap and sugar. The mixture and interaction of compounds contained in *A. pinnata* sap, which change during the heating process affect the quality of *A. pinnata* sugar and need to be further explored. Further research regarding *A. pinnata* sugar's nutritional properties and their potential for human health benefits would be worthwhile.
3. Further research would be of great help in promoting food safety standards during processing of *A. pinnata* sugar. Safety during harvesting and handling of the sap has not been a concern to local farmers yet. The challenge of future works is to minimize microbial growth, and knowing the risk of loss of quality and production due to microorganism sap contamination needs to be more clearly understood by farmers and processors. *A. pinnata* sugar is a food product, thereby a large number of steps involved during the processing will require care to maintain a high level of quality, and to insure there is no contamination with any potentially harmful substances.
4. The process of converting *A. pinnata* sap to sugar is essentially one of boiling the sap for a period of time to evaporate water and concentrate the sugar in the sap. While the evaporation process is simple in concept, proper evaporation equipment and technique are critical to economically produce quality *A. pinnata* sugar. The current study was limited by local unavailability, in Indonesia, of a wide variety of equipment and practices during the process of producing *A. pinnata* sugar. Therefore, it is recommended that further research might explore utilization of an evaporator for *A. pinnata* sugar processing.

Evaporators used in maple syrup production should be the point of reference; however some modifications should be taken into account. Findings from the present study should be used as a guideline to justify the size of the evaporator. Nevertheless, backyard models of maple syrup evaporators which commonly have a size of 2x4 ft or 2x6 ft for the boiling pan is proposed for future designing of an *A. pinnata* evaporator.

5. A future study investigating the cost of producing *A. pinnata* sugar would be very helpful. So far, economics of *A. pinnata* sugar production has not yet been a consideration, since the local people produce the sap as a sweetener for household use or sell it in traditional local markets. There has been to date no analysis for costs of production or economic evaluation of *A. pinnata* sugar production which is important in business development to ensure profit, especially if new equipment will be purchased for larger scale production.

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