EFFECTS OF PEELING AND CALAMANSI (*Citrofortunella microcarpa*) JUICE ADDITION ON THE QUALITY PARAMETER OF FRESH AND PASTEURIZED SUGARCANE *(Saccharum officinarum)* JUICE

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by

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Noor Afiqah Md Noor

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
BAM	Bacteriological Analytical Manual
CFU	Colony Forming Unit
DPPH	2,2-diphenyl-1-picrylhydrazyl
FC	Faecal Coliform
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
HPP	High Pressure Processing
HTST	High Temperature Short Time
LTLT	Low Temperature Long Time
MPN	Most Probable Number
PCA	Plate Count Agar
POD	Peroxidase
PPM	Part per million
PPO	Polyphenol oxidase
QE	Quercetin Equivalent
SPSS	Statistical Package Social Science
TA	Titratable Acidity
TC	Total Coliform
TFC	Total Flavonoid Content
TPC	Total Plate Count
TPhC	Total Phenolic Content
TPTZ	2,4,6-tripyridyl-s-triazine
TSS	Total Soluble Solids
UHT	Ultra-high Temperature

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KESAN PENGUPASAN KULIT DAN PENAMBAHAN JUS LIMAU KASTURI *(citrofortunella microcarpa)* TERHADAP PARAMETER KUALITI AIR TEBU *(saccharum officinarum)* SEGAR DAN DIPASTEUR

ABSTRAK

Jus tebu sememangnya terkenal di Malaysia. Namun, jus tebu mudah terdedah kepada perubahan kimia dan pencemaran mikroorganisma akibat pengoksidaan dan pencemaran. Pemasaran jus tebu adalah terhad kerana warnanya yang gelap dan kurang menarik hasil perubahan kimia. Kajian ini dijalankan untuk menentukan kesan pengupasan kulit dan penambahan jus limau kasturi terhadap parameter kualiti air tebu segar dan dipasteur. Bahagian pertama kajian ini dijalankan untuk menyiasat kiraan mikroorganisma dalam jus tebu segar yang diperoleh daripada beberapa penjual jus tebu yang terletak di Pulau Pinang. Jumlah Kiraan Plat (TPC) jus tebu yang didapati dari penjual adalah di antara 4.89-7.40 Log CFU/mL, manakala jumlah koliform (TC) adalah di antara 38-1100 MPN/mL dan koliform najis (FC) di antara 3-75 MPN/mL. Hasil kajian menunjukkan TPC, TC, dan FC jus tebu yang dikupas sebelum diperah adalah lebih rendah berbanding jus tebu yang tidak dikupas. Bahagian kedua dan ketiga dijalankan untuk menentukan kesan pengupasan dan penambahan jus limau kasturi (1.0%, 1.5% dan 3.0%) terhadap sifat-sifat fizikokimia, antioksidan dan mikroorganisma jus tebu segar dan yang dipasteurkan (70ºC selama 10 minit). Kulit tebu mengandungi sebatian polifenolik seperti antosianin yang menyumbang kepada warna dan kandungan antioksidan dalam jus. Oleh itu, jus tebu segar dan dipasteur yang diperah tanpa kulit menghasilkan nilai kecerahan (L*) yang lebih tinggi ($P<0.05$) tetapi mempunyai sifat antioksidan yang lebih rendah. Jus tebu segar yang diperah tanpa kulit menunjukkan kiraan mikroorganisma yang lebih rendah ($P<0.05$)

berbanding jus tebu yang diperah dengan kulit. Penambahan jus limau kasturi menyebabkan peningkatan ketara ($P<0.05$) kepada aktiviti antioksidan (DPPH dan nilai FRAP) jus tebu segar dan dipasteur. TPC jus tebu segar menurun dengan ketara ($P<0.05$) selepas penambahan jus limau kasturi. Penentuan kesan jus tebu (tanpa kulit) selepas rawatan pempasteuran dan penambahan jus limau kasturi (1.5%) pada suhu penyimpanan (4 ºC dan 28 ºC) dan masa (30 hari) yang berbeza dijalankan di bahagian keempat. Semasa penyimpanan, pH dan jumlah pepejal terlarut menurun, manakala TPC meningkat dengan ketara ($P<0.05$) dalam jus tebu pada kedua-dua suhu. Pada suhu 4ºC aktiviti mikroorganisma berkurang dan secara langsung meningkatkan hayat simpanannya dengan ketara ($P<0.05$). Kesimpulannya, pengupasan kulit tebu meningkatkan nilai L* dengan ketara, mengurangkan aktiviti antioksidan dan kiraan mikroorganisma dalam jus yang diperoleh. Sementara itu, penambahan jus limau kasturi pada kadar kepekatan berbeza terbukti dapat menghalang pertumbuhan mikroorganisma. Oleh itu, gabungan pengupasan dan penambahan jus limau kasturi pada 1.5% diikuti dengan pempasteuran mampu meningkatkan kualiti dan keselamatan jus tebu.

EFFECTS OF PEELING AND CALAMANSI (*citrofortunella microcarpa*) JUICE ADDITION ON THE QUALITY PARAMETER OF FRESH AND PASTEURIZED SUGARCANE (*saccharum officinarum*) JUICE

ABSTRACT

Fresh sugarcane juice is popular in Malaysia. However, it is susceptible to chemical changes and microbial spoilage that occur due to oxidation and contamination. Marketability of sugarcane juice is limited due to the unattractive dark colour caused by chemical changes. This study was conducted to determine the effects of peeling and calamansi juice addition on the quality parameters of fresh and pasteurized sugarcane juice. First part of the study was conducted to investigate the microbiological count of fresh sugarcane juice obtained from vendors located in Penang. The total plate count (TPC) of sugarcane juice obtained from vendors ranged from 4.89-7.40 Log CFU/mL, total coliforms (TC) of 38-1100 MPN/mL and faecal coliform (FC) of 3-75 MPN/mL. Result showed that TPC, TC and FC of sugarcane juice that was peeled before pressing was lower than unpeeled sugarcane juice. The second and third part were carried out to determine the effects of peeling and calamansi juice addition (1.0%, 1.5% and 3.0%) on the physicochemical, antioxidant, and microbiological properties of fresh and pasteurized (70^{o}C for 10 minutes) sugarcane juice respectively. Sugarcane peel consists of polyphenolic compounds such as anthocyanin that contribute to colour and antioxidant of juice. Therefore, fresh and pasteurized sugarcane juice pressed without its peel produced significantly ($P<0.05$) higher Lightness (L*) value but lower antioxidant properties. Fresh sugarcane juice pressed without its peel showed significantly ($P<0.05$) lower microbial count compared to sugarcane juice pressed with its peel. The addition of

calamansi juice had significantly ($P<0.05$) increase antioxidant activities (DPPH and FRAP assay) of both, fresh and pasteurized sugarcane juice. The TPC of fresh sugarcane juice significantly ($P<0.05$) decreased with addition of calamansi juice. The determination on the effect of sugarcane juice (without its peel) after pasteurization treatment and calamansi juice addition (1.5%) at different storage temperatures (4 ºC and 28 ºC) and time (30 days) was conducted in part four. During storage, the pH and total soluble solid decreased whereas, TPC increased significantly ($P<0.05$) in sugarcane juice at both temperatures. At 4ºC the microbial activity reduced, which significantly ($P<0.05$) increase its shelf life. In conclusion, peeling sugarcane significantly increase L* value, reduce antioxidant activity and microbial load of juice obtained. Meanwhile, addition of calamansi juice at different concentrations proved to inhibit microbial growth. Therefore, combination of peeling and addition of calamansi juice at 1.5% followed by pasteurization could improve the quality and safety of sugarcane juice.

CHAPTER 1: INTRODUCTION

1.1 Research Background

Saccharum officinarum or commonly known as sugarcane is a species of perennial plant which is a member of the grass family (Sharpe, 2012). Sugarcane is usually planted in warm temperate regions and is widely used for sugar plantation. According to the Vegetables and Cash Crops Statistics, Malaysia 2017, Malaysia produces 29, 990 Metric tonnes of sugarcane and is mainly planted in Johor, Selangor and Perak. Although primarily planted for sugar production, sugarcane also produces molasses, rum, bagasse, ethanol, and juices (Dotaniya et al., 2016). Sugarcane juice can be found easily especially in countries where sugarcane is cultivated such as Brazil, China, South Asia, and South-East Asia.

Sugarcane juice is a refreshing drink that is usually consumed fresh and is known for several health benefits. Sugarcane juice is rich with minerals (calcium, iron, and phosphorus), vitamins, soluble fibre as well as antioxidant compounds (McCaffrey, 2011). Due to the benefits of sugarcane juice, sugarcane juice drink is easily obtained in cans and even tetra packs. However, although available in these forms, consumers still prefer freshly pressed sugarcane juice that is usually obtained at night markets or street vendors.

Due to the popularity of fresh sugarcane juice, Ministry of Health Malaysia had introduced a program titled ‘The success story of air tebu program’ which was conducted to enhance the compliance status of the fresh sugarcane stall and to educate the food handlers on the requirement of Food Hygiene Regulation 2009 (Ministry of Health Malaysia, 2014). A guide was developed for the food handlers of sugarcane

juice participating in the program to produce safe and high quality juice. The program started with five states namely Kedah, Kuala Lumpur, Johor, Pahang and Sabah in 2014. In 2015 the program was carried out in all states. In Penang, 16 sellers of sugarcane juice voluntarily took part in the program with continuous observation from Penang State Health Department (Khong, 2015). With completion of the program, sugarcane juice vendors received the *"Bersih, Selamat, Sihat"* (BeSS) certificate as an indicator that their sugarcane juice is clean and safe.

According to Bates et al. (2001), sugarcanes used for juicing purposes must be cleaned and peeled well to avoid dark coloured juice. Sugarcane peel should also be removed as a study conducted by Mahale et al. (2008) reported that fruit surfaces contain bacterial counts of 5 Log CFU/cm^2. According to the Australian standard guideline on food contact surfaces, the total viable count above the maximum limit (>1 Log CFU/cm^2) is considered unacceptable (NSW Government Food Authority, 2013). Therefore, the peel of sugarcane should be removed to avoid high microbial count. However, in Malaysia, sugarcane juice is usually pressed on-site through an extractor without peeling and the juice is collected and consumed fresh without pasteurization.

The quality of sugarcane juice is hard to preserve once it is extracted as the juice turns dark brown and sedimentation appears (Sangeeta et al., 2013) due to heat and enzyme. Furthermore, sugarcane juice is low in acidity and has high sugar content which makes it susceptible to microbial spoilage (Yusof et al., 2000). Sugarcane juice has a pH value in the range of 5.28 – 5.54 and a total soluble solid value in the range of 18.0 – 19.5˚Brix (Chauhan et al., 2002). The contamination of fresh juice is due to several factors such as raw materials, equipment used, improper handling of raw material, cross contamination, food handlers and the cleanliness of the environment

where the juice is prepared. These factors contribute substantially to the entry of pathogenic bacteria which can cause spoilage to the juice (Oliveira et al., 2006; Nicolas et al., 2007; Mahale et al., 2008). *Leuconostoc* spp., *Saccharomyces* (yeast) and *Aspergillus* (mould) are some examples of the microorganisms that infest the cane stalks. All these microorganisms which are found in and on the stalk end up in the juice once the sugarcane is pressed. Moreover, according to Ali et al. (2015) coliform bacteria which are indicators of water contaminations are also found in unpasteurized sugarcane juice.

There have been many researches done to maintain the safety and quality of sugarcane juice. Non thermal methods such as high hydrostatic pressure used by Huang et al. (2015) had positive effect on the antioxidant properties, meanwhile Nguyen et al. (2018) and Kayalvizhi et al. (2016) using the pulsed electric fields method reported a microbial inactivation in sugarcane juice. These novelty techniques proved to show great benefits on sugarcane juice, however, due to its high cost and maintenance of the equipment, it is unsuitable and not practical to be used for roadside vendors and small manufacturers.

Besides, there are methods that can be conducted by vendors and small manufacturers themselves to help prolong the shelf life of the sugarcane juice. Combined methods of preservation such as thermal treatment and acid addition to sugarcane juice can easily be conducted and improve the quality of juice. Previous studies reported that, adding moringa seed extract and lemon (Ramachandran et al., 2017), anola juice (Sangeeta et al., 2013), lemon juice and ginger (Khare et al., 2012), ascorbic acid (Mao et al., 2007), and citric acid (Chauhan et al., 2002) into sugarcane juice with thermal treatment had proven to improve the quality and safety of sugarcane juice.

Citrus fruits, has low pH and contains a variety of vitamins, minerals, and phytochemicals that are full of health benefits (Turner & Burri, 2003). It is sometimes used to preserve food and drinks due to its antimicrobial potentials. Calamansi is an example of citrus fruit that is commonly found in South East Asia. Calamansi juice is used for cooking and added into beverages due to its refreshing taste. The juice is acidic and contains antioxidant compounds (Cheong et al., 2012b) that can be beneficial for consumers' health. Therefore, in this research, calamansi juice will be used to reduce the pH of the sugarcane juice, which would greatly impact the survival and growth of microorganisms.

1.2 Problem Statement

i. There is insufficient information regarding the microbiological quality of sugarcane juice obtained from street and night market vendors.
ii. The presence of soil and dirt on the external surface, high sugar content and the low acid of the sugarcane may reduce the physicochemical, antioxidant and microbiological properties of fresh sugarcane juice.
iii. Unless sugarcane juice has been pasteurized or otherwise treated, the sugarcane juice could be contaminated.
iv. Changes in physicochemical properties and the presence of microorganisms will shorten the quality and shelf life of sugarcane juice.

1.3 Objectives

General objective:

To study the effects of peeling and calamansi juice addition on the physicochemical, antioxidant and microbiological properties of fresh and pasteurized sugarcane juice.

Specific objectives:

i. To investigate the microbiological quality of sugarcane juice obtained from street and night market vendors.
ii. To determine the effects of removing the sugarcane peel and calamansi juice addition on the physicochemical, antioxidant and microbiological properties of fresh sugarcane juice.
iii. To determine the effect of peeling and calamansi juice addition on the physicochemical, antioxidant, and microbiological properties of pasteurized sugarcane juice.
iv. To study the quality of sugarcane juice after pasteurization treatment and calamansi juice addition at different storage temperatures and time.

CHAPTER 2: LITERATURE REVIEW

2.1 Sugarcane

Saccharum officinarum or commonly known as sugarcane belongs to the kingdom *Plantae*, in the tribe of *Andropogonaeae*, family of *Gramineae* and is a species of the genus *Saccharum* (Sharpe, 2012). Sugarcane is a perennial plant which is the member of the grass family. There are five species of sugarcane that are cultivated in different parts of the world such as *S. officinarum, S. robustum, S. barberi, S. spontanuem* and *S. sinese.*

Saccharum officinarum also known as 'Noble Cane' is rich in sucrose, but the stem contains less fibre. Its stem is thick, juicy and hardy. In the *S. officinarum* species, there are many varieties, and around the world, these varieties are known in different names. Blackburn (1984) noted that the four most important varieties were the Creole, the Otaheite (Bourbon), the Cheribon (Transparent) and the Tanna (Caledonia). *Saccharum robustum* stems are hard and contain less juice. They grow in the wild and the stem contains higher fibre and lower sugar content compared to *S. officinarium*; it is known as the robust cane and is normally found in New Guinea. *Saccharum barberi* originates from northern India and the species are known based on its short robust stem. *Saccharum spontanuem* is also known as wild sugarcane and is native to the Indian Subcontinent. This variety of sugarcane have very low sucrose content but contains high fibre (James, 2004). *S. spontanuem* resembles to grass more than sugarcane, and this vast species is said to be an invasive species due to its ability to quickly colonize disturbed soil and take over croplands and pasturelands. *Saccharum sinese* is also known as the Chinese cane. The stems have high fibre content and

intermediate sucrose content (James, 2004). Their stems are usually red in colour and hardier than other species.

The basic structure of the sugarcane plant is closely related to other members of the *Gramineae*. The cylindrical-shaped stem of sugarcane can grow up until six to seven meters tall and 2.5- 5.0 cm in diameter (James, 2004). Sugarcane usually grows in a clump which consists of several strong unbranched stems. Each stem has a hard, wax-covered rind surrounding a mass of softer tissue where sugar is accumulated. The wax layer helps protect the stem from water loss due to evaporation, and the fibrous rind provides the plant with strength and rigidity (James, 2004). Figure 2.1 shows the black stem sugarcane; the stem composes a series of nodes and internodes and the stems hardness is dependent on the variety of sugarcane (Singh et al., 2013). The lengths of the nodes and internodes or joints vary in length, between 5.0 - 25.0 cm, however, the range will become shorter at the top of the stalk where elongation or growing is taking place (James, 2004). The internodes of sugarcane come in many shapes; these shapes include cylindrical, conoidal, barrel or bobbin, and circular or oval in cross-section. James (2004) also classified nodes into four different shapes, tall root band, constricted root band, conoidal root band and obconoidal root band.

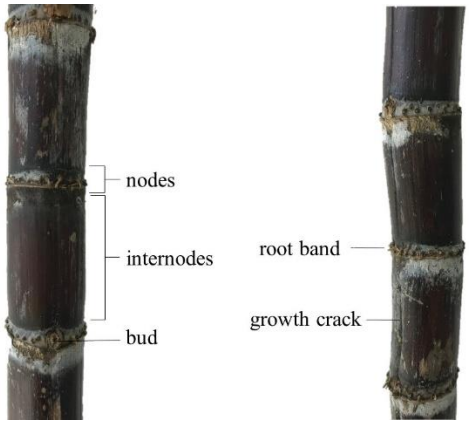


Figure 2.1 Black stem sugarcane

Due to many varieties of sugarcane within a species, sugarcane can be found in many colours. The stems of sugarcane may vary in a range of colour from pale green to green, red to purple and dark purple to almost black (Sandhu et al., 2016). The skin colour of sugarcanes is derived from two basic pigments: the red color of anthocyanin and the green colour of chlorophyll (Panda, 2011). The red and blue anthocyanins is usually found in the epidermal cell meanwhile the green of the chlorophyll is usually found in deeper tissue of the stem. The ratio of concentration of these pigments results in the various colours of stem (Sandhu et al., 2016). It is also reported that when both anthocyanin and chlorophyll pigments are absent in the stem, the cane will be yellow in colour (James, 2004).

Apart from the physical aspects, the nutritional composition of the sugarcane juice depends on the cane variety, soil and quality of fertilisers used, climatic conditions during growth, harvesting, irrigation, disease and pest infestation, state of maturity and time duration between harvesting and crushing (Meyer and Wood, 2001). Generally, sugarcane can grow in various type of soils from heavy clay, organic, to sandy soils. It can grow on both acidic and alkaline soils but grows best in soil with a pH of 6.5.

Sugarcane is native to the warm temperate to tropical regions of South Asia and is widely used for sugar production. It is a highly versatile plant and can be grown successfully under a wide range of condition. Although sugarcane can be cultivated in temperate zones, productivity is much higher in tropical climates. When sugarcane is cultivated in the tropics and subtropics areas, generous supply of water will be provided for a continuous period of more than six to seven months each year through natural rainfall or irrigation. Irrigation is important as sugarcane is a crop that highly

depend on the water intake. Less water supply will retard the growth which would produce short and thin internodes of sugarcane stem (James, 2004).

Age of harvest is an important factor that could affect sugarcane productivity, as harvesting sugarcane under-aged or over-aged may lead to a loss in cane yield, sugar recovery, poor juice quality and would cause problems in milling (Yesuf et al., 2016). The maturation state of sugarcane is dependent on the optimum content of sucrose and variety used as different varieties meet maturation state at different time frame. Some sugarcane varieties have high sucrose content in early seasons at 10-12 months, which makes them already suitable to be harvested. These sugarcanes are defined as early maturing, while sugarcane that mature at 14-16 months are defined as late maturing (Calderon et al., 1996). Sugarcane quality is also affected by the time duration between harvesting and juice crushing. A study by Yusof et al. (2000) reported that upon delayed crushing of the sugarcane, the sucrose content of the juice decreases due to its conversion to reducing sugar. Furthermore, the yield of juice also decreased with an increase in time of storage.

The Food and Agriculture Organization of the United Nations reported that in 2017, Brazil produced 758, 548, 292 metric tonnes of sugarcanes making it the largest producer of sugarcane, followed by India which produced 206,069,000 metric tonnes. Based on the ranking of sugarcane production in the year of 2017, including 98 countries, Malaysia had the 82nd spot (FAO, 2017). In Malaysia, sugarcane has been grown since the 19th century (Tan, 1989). According to vegetables and cash crops statistics obtained from the Department of Agriculture, Malaysia produced 29,990 metric tonnes of sugarcane in 2017. Johor was the biggest producers in Malaysia in the year 2017 producing 10,836.75 metric tonnes.

2.2 Sugarcane Juice

In Malaysia, sugarcane is usually sold by street and market vendors. The juice is usually prepared fresh on-site and not only available at the street stalls but also in food courts in malls and shopping centres. The colour of freshly extracted sugarcane juice is usually pale green to olive green depending on the variety of sugarcane (Singh et al., 2014). The presence of various types of pigments such as chlorophyll, xanthophyll, carotene and anthocyanins gives effect to the colour of sugarcane juice (Rupa and Asokan, 2008). The skin colour of the sugarcane stem contributes to the colour of the sugarcane juice. A darker peel colour of sugarcane will produce a darker colour of sugarcane juice (Rupa and Asokan, 2008).

There are two types of equipment's that are normally used for sugarcane juice extraction such as a traditional cranked machine or a powered machine. Figure 2.2 shows a sugarcane juice extractor. The sugarcane juice extractor machine usually consists of rollers with immense interlocking and corrugated teeth on the circumference of each roll. These rolls are set close to each other and sugarcane passing through it is broken into short pieces and matted to an even layer. The surface of the rollers is grooved to increase their grip power and facilitate juice drainage. The juice will be then squeezed out and is run through a collector and stored in a large tank. The yield of juice obtained from sugarcane depends mostly on the quality of the cane as well as the efficiency of the juice extraction (Panda, 2011) through the way sugarcane stem is cut or peeled, or how many times the sugarcane stem is passed through the extractors.

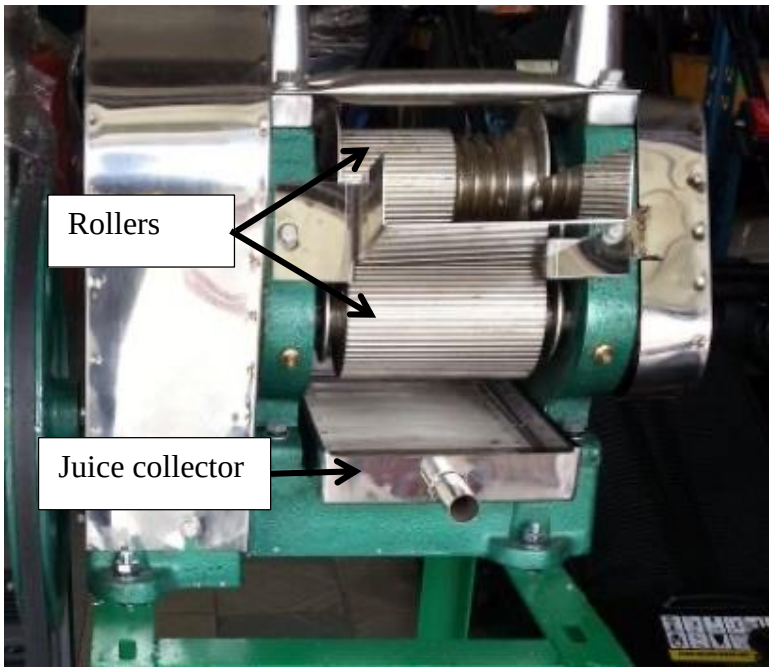


Figure 2.2 Sugarcane Juice Extractor

Generally, sugarcane juice is extracted from *S. officinarum* species because the stem is thick and have more juice as well as containing high sugar and low fibre content. Black, red, green and yellow stems are the common varieties of colour found in *S. officinarum.* Although there are many varieties of sugarcane in the same species, the yellow sugarcane variety or locally known as "tebu kuning" is the most popularly selected sugarcane cultivated for its juice (Salunkhe and Desai, 1988). The selection of yellow stem sugarcane might be due to the distinct flavour and the abundance of juice produces as it has a softer and less fibrous stem (Siti and Baharuddin, 1994).

In Malaysia, it has been observed that there has been an increase in sugarcane juice vendors who has been using black stem sugarcane as a substitute for the usual yellow stem. A study by Chong (2017) reported that yellow stem sugarcane juice had a total soluble solid of 11.4˚Brix while black stem sugarcane juice had a total soluble solid of 18.8˚Brix. Hence, the increase in the use of black stem sugarcane could be due to the black stem sugarcane producing sweeter juice. A study conducted by

Chauhan et al. (2002) discovered that the physicochemical characteristics such as juice yield, moisture, pH, total soluble solids, and acidity of sugarcane juice obtained from different varieties differed significantly. However, to date, there has been very little published information regarding the physicochemical and sensory characteristic of black stem sugarcanes. Therefore, this study aims to gather information on the properties of black stem sugarcane juice to fill the gap in the literature in the field.

2.2.1 Nutritional Composition of Sugarcane Juice

Sugarcane juice is usually consumed as a refreshing drink. The juice has a rich source of carbohydrates which provides 40 Kcal of energy, 10 mg of iron and 6 μg of carotene for every 100 mL of sugarcane juice consumed (Parvathy, 1983). Sugarcane juice is slightly acidic in nature with a pH value of 5.28-5.54 and an acidity of 0.24-0.39%. A number of organic acids contributes to its acidic pH value which includes aconitic acids (83%), malic acids (14%) and citric acid (<1%) (Gutierrez et al., 1989). However, juice composition may vary with the variety of sugarcane, geographical location and stage of maturity (Yasmin et al., 2010). Generally, sugarcane juice contains 80.00-81.70% of moisture, 0.39-0.60% of protein and 0.14-0.19% of fat. The highest mineral found in sugarcane is calcium with the value of 125.68-180.72 mg/100 g, followed by iron and phosphorus, which is 36.25-71.29 mg/100 g and 25.33-29.73 mg/100 g respectively (Chauhan et al., 2002).

The sugar content in sugarcane is highly dependent on the state of maturity of the cane during harvesting. Mostly sugar found in sugarcane juice are sucrose (18-19.5%) and only a small amount of reducing sugars (0.20-0.65%) such as glucose and fructose (Chauhan et al., 2002). As the sugarcane matures the sucrose contents increases and the glucose and fructose content decreases (Qudsieh et al., 2002).

Besides sugarcane juice also contains vitamins, soluble fibre, and numerous other health-supportive compounds (McCaffrey, 2011).

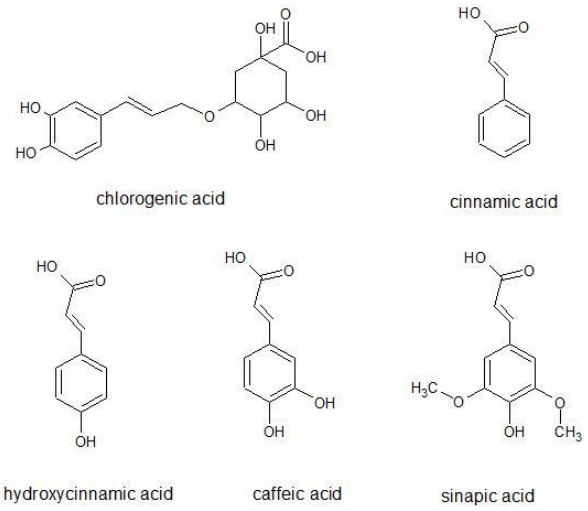
2.2.2 Health Benefits of Sugarcane Juice

Sugarcane juice has a low glycemic index which keeps the body healthy and makes sugarcane juice suitable for diabetic patients; it also hydrates the body quickly when exposed to prolonged heat and physical activity (Sangeeta et al., 2013; Yusof et al., 2000). The hydrating and thirst-quenching ability of sugarcane juice is one of the reasons the juice is famous in Malaysia where the weather is hot and humid. One of the other benefits of sugarcane juice is that it is packed with phenolic acids and flavonoids which contribute to the antioxidant properties (Singh et al., 2015). It is widely known that antioxidants have a very strong and positive effect on the body. According to Kadam et al. (2008), the phenolic compounds in sugarcane juice such as hydroxycinnamic acid, sinapic acid, and caffeic acid can scavenge free radicals and also reduce the production of iron complex and inhibit lipid peroxidation.

Flavonoid compound such as apigenin, luteolin and tricin derivatives present in sugarcane juices falls into the classification of flavones within polyphenolics (Duarte-Almeida et al., 2007). Flavonoid plays an important role in human health by acting as an anticancer, anti-inflammatory, antiviral and anti-allergic substance (Kadam et al., 2008). Previous studies also showed that polyphenols exhibit the role of preventing chronic diseases involving oxidative stress (Packer and Cadenas, 2002). Studies also show that apigenin, induces tumours growth inhibition effects and has potential and may be developed as a promising chemotherapeutic agent against the development of chemical carcinogenesis (Jeyabal et al., 2005).

Figure 2.3 shows the structures of the phenolic compounds found in sugarcane juice. Phenolic compounds found in sugarcane juice consists of phenolic acid (chlorogenic acid, cinnamic acid, hydroxycinnamic acid, sinapic acid and caffeic acid) and flavones (apigenin, luteolin and tricin) (Singh et al., 2015).

a) Phenolic acids



b) Flavones

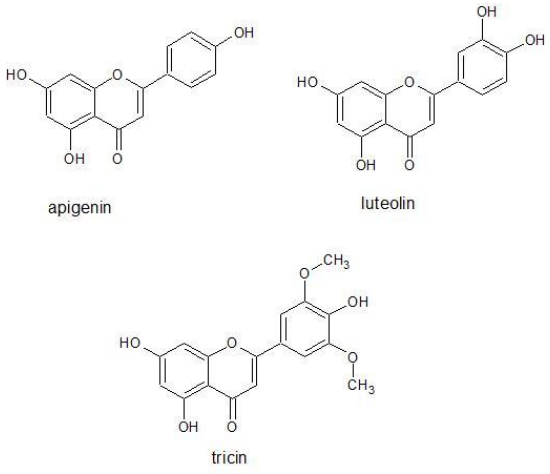


Figure 2.3 Phenolic and flavonoids compounds identified in sugarcane juice (Singh et al., 2015)

Sugarcane juice is known to help our bilirubin levels normal and was traditionally used to treat jaundice. Bilirubin, an orange-yellow pigment that occurs normally when part of your red blood cells breaks down. The liver takes the bilirubin from your blood and changes it so most of it is found in bile. Bilirubin can build up in your body due to liver problems. Jaundice is usually caused by poor functioning of liver and clotting of the bile duct (Rawat and Pokhriyal, 2014). Consumption of sugarcane juice also helps protect the liver from damages and acts as a palliative to a damaged liver (Koge et al., 2005). In addition, sugarcane juice is a great preventive and healing source for sore throat, cold and flu (Sangeeta et al., 2013; Khare et al., 2012).

2.2.3 Microbiological Guidelines for Sugarcane Juice

In Malaysia, food is likely to be sold anywhere with little attention being paid to the food hygiene (New et al., 2017). However, to strengthen and ensure the food safety in Malaysia, the Ministry of Health (MOH) has introduced a Food Safety and Quality division (FSQD) since 2010 (Ministry of Health Malaysia, 2011). The FSQD is responsible for ensuring food safety along the food supply chain in order to protect the public against health hazards and fraud in the preparation, sale and use of food (Ministry of Health Malaysia, 2011). According to the Malaysia Food Regulations 1985, it is stated that there should be no pathogenic microorganisms present in a drink. In the Malaysian Standard (MS 601:1994) specification for ready-to-drink beverages which includes fruit drinks, it is stated that the beverage should contain a total colony count of < 3.70 Log CFU/mL, viable yeasts and moulds count of <10/mL, and presumptive coliform organisms should not be found in the drink (Department of Standard Malaysia, 1994). It also stated in the standard that all drinks prepared should be under strict hygienic conditions in accordance with Good Manufacturing Practices

and relevant public health requirements currently enforced (Department of Standard Malaysia, 1994).

The MOH microbiological guidelines for ready-to-eat foods and prepared beverages which includes fruit juices, stated that the total plate count of beverage should be $<10^5$ cfu/g meanwhile, *E. coli* count should be $<10^2$ MPN/g. To monitor and control food safety, these guidelines are used to provide assistance to law enforcers or officers to interpret the microbiological information and prove appropriate action and solution (Centre for Food Safety, 2014).

Different country has different standards, food acts and microbiological guidelines for ready to eat foods and prepared beverages developed by government agencies and industry. Standards, food acts and guidelines of neighbouring countries are compiled in Table 2.1. Similar to Malaysia, although from the same country the standards, food acts and guidelines might differ to one another.

Table 2.1 Microbiological standards and regulations for fruit drink or juice in Malaysia and neighbouring countries

Country	Standard/Regulations	Scope/Including	Specification
Malaysia	MS 601:1994 Carbonated water-based beverages	- Ready to drink beverages including fruit drinks and flavoured drinks	- Total colony count: <50 CFU/mL - Yeast and moulds: <10 CFU/mL - Presumptive coliform organisms: Negative
	Food Regulation 1985 Carbonated water-based beverages	- Soft drink composed of potable water and permitted flavouring substances with or without sugar glucose, high fructose glucose syrup or edible portions of extract of fruit or other plant substance. May contain carbon dioxide.	- No pathogenic microorganisms - Aflatoxin or any other mycotoxins: <5 µg/kg
Singapore	SS 62:1997 Carbonated and non-carbonated beverages	- Beverages containing natural extracts - Fruit-flavoured carbonated beverages - Flavoured carbonated beverages - Non-flavoured and unsweetened carbonated beverages	- Total bacteria count: <10 CFU/mL - Coliform count: negative/mL - Yeast and mould count: negative/mL
	Food Regulations 2006 Soft drinks	- Any flavoured drink ready for consumption without dilution - Soda water, Ginger beer and any beverage made from any harmless herbal or botanical substance; Fruit drink or fruit crush.	- *Escherichia coli*: < 20 CFU/mL - Total colony Count at 37°C for 48 hours: <100,000 CFU/mL
Thailand	Notification of Ministry of Public Health No.214 B.E. 2543 (2000)	- Beverage, which is containing or made from fruits, plants or vegetables, and may also contain dissolved carbon dioxide or oxygen gas.	- Coliform count: >2.2/100mL - *Escherichia coli*: negative - Yeast and mould: negative
Vietnam	QCVN 6-2:2010/BYT -	- Soft drink produces which includes fruit beverages, nectar beverages, and ready to drink without alcohol.	- Total plate count: 100 CFU/mL - Coliforms: 10 CFU/mL - Yeast and Moulds: 10 CFU/mL

	National technical regulation for soft drinks		- *Escherichia coli*: negative - *Staphylococcus aureus* : negative
Hong Kong	Microbiological Guidelines for Food (2014)	- For ready to eat food in general and specific food items including fruit juice.	- Total viable count: $\geq 10^7$ CFU/g - *Escherichia coli*: <100 CFU/mL - *Staphylococcus aurus :* < 100 CFU/mL

(Extracted from: International Life Sciences Institute, 2012)

2.3 Factors Affecting the Quality of Sugarcane Juice

There are a few factors that influence the organoleptic quality of sugarcane juice. Sugarcane juice, which has low acidity, high water activity and sugar content promotes fermentation process immediately after extraction. Which makes it deteriorate rapidly even though stored at low temperature (Qudsieh et al., 2002; Yusof et al., 2000).

The colour of freshly extracted sugarcane juice is varied from pale green to dark green which depends on the variety of sugarcane. Unfortunately, extracted juice obtained from sugarcane usually turn dark brown and sedimentation appears during storage (Yasmin et al., 2010; Chauhan et al., 2002). This is a problem to sugarcane juice vendors as the browning reaction brings a negative impact towards the acceptability of sugarcane juice (Mathew et al., 2016; Kunitake et al., 2014).

The colour change in the sugarcane juice from greenish to yellowish and browning is due to oxygen, light and internal enzymes that may convert the chlorophyll present in the sugarcane to a more stable form (Yusof et al., 2000). The degradation of chlorophyll by the loss of the Mg^{+} ion or by the enzyme chlorophyllase produces products such as pheophytin and peoporbide which are olive green and brown coloured respectively. The darkening of sugarcane juice occurs through the formation of brown pigments by enzymatic and non-enzymatic reactions (Kunitake et al., 2014).

Generally, the reduced quality of fresh sugarcane juice is due to non-enzymatic and enzymatic activities which leads to browning as well as microbial spoilage. Non-enzymatic browning occurs in the juice through caramelization or Maillard reaction while enzymatic browning occurs through the formation of the brown pigment in the

juice through oxidation of phenolic compounds forming melanin (Kunitake et al., 2014). At the same time, microbial spoilage in sugarcane juice could also occur due to a few factors such as the plantation environment and contamination via human beings and utensils used.

2.3.1 Non-Enzymatic Browning

Maillard reaction, thermal and alkaline degradation as well as caramelization are some examples of non-enzymatic reactions (Kunitake et al., 2014). These reactions give rise to a brown pigment in sugarcane juice through chemical reactions. Alkaline degradation and condensation of reducing sugar resulted in an uncharged medium to high molecular weight brown pigments in pasteurized sugarcane juices (Chen and Chou, 1993). Banerji et al., (2012) has reported that non-enzymatic browning rate in sugarcane juice increases with increasing pH and temperature. Therefore, decreasing the pH of the sugarcane juice could help reduce the browning caused by the Maillard reaction.

Maillard reaction is not only responsible for the brown colour but also the flavours produced in cooked meat, roasted coffee and toasted bread. Maillard reaction proceeds effectively at temperatures >50°C and the optimum pH is between pH 4-7 (Kroh, 1994). According to Mastrocola et al. (2000) Maillard reaction occurs between the sugar and the amino acid present in the sugarcane juice during the heating process. Figure 2.4 shows the general outline of the Maillard Reaction. The Maillard reaction produces a glycosylamine compounds which is rearranged to develop a ketosamine compound (Misral, 2008). The final stage consists of ketosamine compound reacting in a number of ways to produce several different compounds such as reductones, diacetyl, asprin, pyruvaldehyde and other short-chain hydrolytic fission products

(Ogutu et al., 2017). Melanoidins are one of the potential end products and are responsible for the brown appearance on the juice.

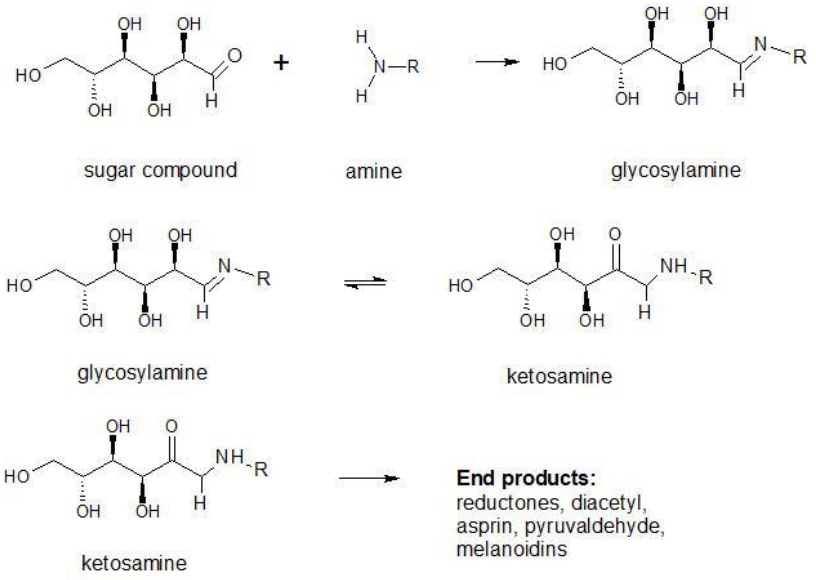


Figure 2.4 General Outline of Maillard Reaction (Ogutu et al., 2017)

Caramelization process is the oxidation of sugar, which is used extensively in cooking to obtain flavour and brown colour. Figure 2.5 shows the general outline of the caramelization process which involves sucrose and heat. This caramelization process is a decomposition of sucrose brought by high temperature (160°C) or pH in a range of 3-9 (Kroh, 1994). This complex process of caramelization produces a lot of intermediate flavour compounds and its product is similar to that of Maillard reaction (Davies and Labuza, 1997). The key intermediates of the thermal caramelization are α-dicarbonyl compound, which leads to the formation of the caramel colour and the important volatile products (such as diacetyl and hydroxymethylfurfural) which are typical of caramel flavour (Kroh, 1994). In sugarcane juice, caramelization usually occurs during processing when sugarcane juice is pasteurized before bottled or canned.

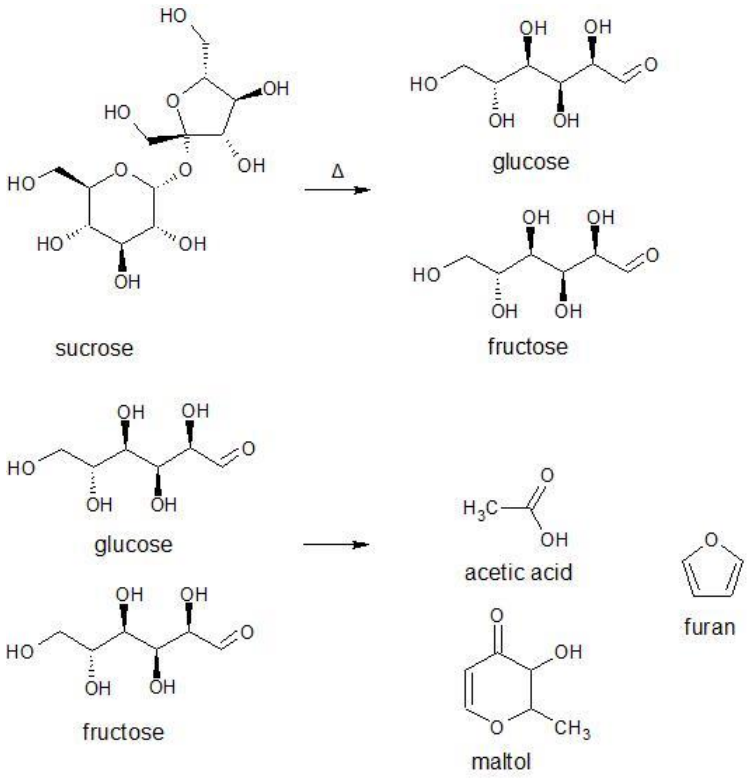


Figure 2.5 General Outline of Caramelization Reaction (Ashish, 2019)

2.3.2 Enzymatic Browning

Enzymatic browning of sugarcane can occur during the post-harvest and processing stage. During the post-harvest, enzymatic browning can occur by roughly handling, storing and transporting the sugarcane. When cracks and bruises emerge on sugarcane stems during the post-harvesting process, the cell integrity of the sugarcane gets damaged which allows enzyme and its substrates to mix. By exposing sugarcane juice to oxygen, enzymatic browning will take place by the activity of peroxidase (POD) and polyphenol oxidase (PPO) enzyme (Qudsieh et al., 2002).

Processing operations such as washing, scrubbing, peeling, trimming and cutting can cause stress to the sugarcane stem which would activate the enzymatic activity. If equipment used to peel and cut the plant is not sharp, bruising and damaging to the stem may occur which would increase the ethylene production in the plant, that

causes higher cellular metabolism and higher enzymatic activity (Francis et al., 2012; Rocculi et al., 2009). During the juice extraction process, phenolic compounds in sugarcane juice will oxidize to the chemically more reactive quinone (Bucheli and Robinson, 1994). The oxidation of the phenolic substrates by PPO is considered to be the major cause of the brown colouration that is usually seen in many fruits and vegetables during handling, storage and processing (Queiroz et al., 2008).

Polyphenol oxidase (PPO) is a group of copper-proteins enzymes which catalyses the oxidation of phenolics to quinones (Taranto et al., 2017). Quinones formed which are highly reactive would undergo secondary reactions and polymerize to form the red, black, and brown pigment which is associated with browning. During sugarcane juice extraction, the tissue is damaged and ruptured; PPO that is located in cellular compartment comes in contact with phenolic compounds and causes the browning reaction (Queiroz et al., 2008; Bucheli and Robinson, 1994).

Figure 2.6 shows a simplified schematization of the browning process. The figure shows two-stage reactions which may be catalysed by the PPO enzyme (Taranto et al., 2017). Mishra and Gautam (2016) reported that PPO may exhibit either or both of these reaction, the hydroxylation of *mono*-phenol to *Ortho*-diphenols, and the oxidation of *Ortho*-diphenols to their corresponding quinones. The initial reaction catalysed by PPO produces o-quinones which are highly reactive and so they continue to undergo reaction to produce brown melanin pigments.

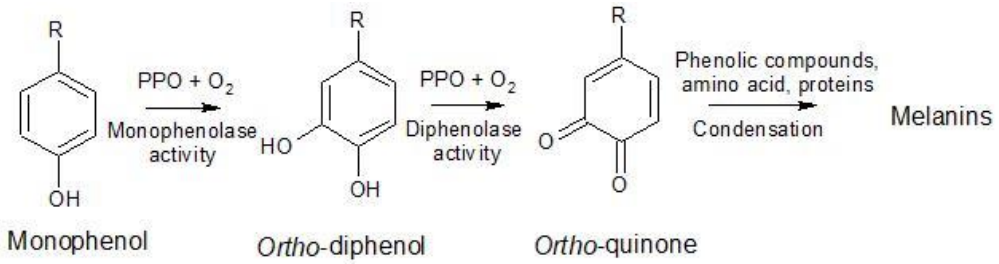


Figure 2.6 Schematization of enzymatic browning by the reaction between polyphenol oxidase and polyphenolic compounds (Taranto et al., 2017)

Generally, PPO activity is optimal at a pH of 4.0-8.0, where different pH affects the binding of the substrates and the catalysis (Taranto et al., 2017). PPO enzyme is not heat stable. Arroyo and Lyng (2017) reports that in most cases this enzyme will inactivate at temperature of 70 – 90°C. This finding was consistent with Kunitake et al. (2014), who reported that PPO activity was inactivated at pasteurization temperature of 85°C for 30 seconds.

Peroxidase (POD) is a member of a large group of enzymes called the oxidoreductase. Plant peroxidases utilize H_2O_2 to oxidase a wide range of phenolic substrates. POD is not an oxygen-dependent reaction, but it could reduce diphenols and promote darkening in sugarcane juice (Bucheli and Robinson, 1994). PPO and POD can be inactivated by heat treatment, but compared to PPO, POD is more heat stable enzyme. Therefore, inactivation of POD which is considered to be among the most heat resistant plant enzyme is widely used to indicate the adequacy of blanching sufficiency fruits and vegetables (Severini et al., 2015). Kunitake et al. (2014) found that a thermal treatment at 95°C for 30 seconds could inactivate POD enzymes in an acidified sugarcane juice beverage. Besides that, POD activity could also be reduced by lowering the pH of the juice to less than 4.0 (Kunitake et al., 2014). Acidification and heat treatment combination could be a preferred option to reduce enzymatic

browning. Pasteurization treatment would, however, cause unwanted changes on the sensory properties, antioxidant activities and micronutrients composition.

2.3.3 Microbial Spoilage

Microbial spoilage in sugarcane juice can occur at the field and through handling. The origin of bacterial contamination may have occurred in the sugarcane field itself. The physiology and nature of the sugarcane plant and how it grows facilitates the contamination of microorganisms. On the sugarcane plant itself, microorganisms detected include *Bacillus, Enterobacteriaceae, Lactobabillus, Erwina, Leuconostoc, Flavobacterium, Xanthomonas* as well as yeast and mould (Sperber and Doyle, 2009).

As sugarcane matures and grows, the surface of the cane sometimes gets damaged by animals or through cuts and splits that occur during growth. These cracks and damages obtained during the growth and harvesting of sugarcane are one of the factors that influence the growth of microorganisms (Mahale et al., 2008). When the sugarcane is cracked or damaged, the stems which are rich in sucrose are exposed. Microorganism found on the surface of the sugarcane such as *Leuconostoc* would then penetrate and colonize in the internal tissue (Misra et al., 2017). *Pseudomonas* which is a Gram negative bacteria which exist in water and soil is also found on the sugarcane plant (Sperber and Doyle, 2009). This contamination may arise from the debris or fine particles that are stuck on the sides or joints of troughs on the plant. It could also be due to the soil adhering to the cane stalk as sugarcane grows upwards from soil.

The environment and geographical in which sugarcanes are planted, harvested and extracted also plays a role in the contamination of microorganisms. A study found high *Leuconostoc* spp. bacteria count and high survival rate in sugarcane when in an