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DEVELOPMENT OF PINEAPPLE-BASED HEALTH DRINK ACCORDING TO THEIR ANTIOXIDANTS AND SENSORY EVALUATION

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By

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ABSTRACT

The study was conducted to optimize the use of two major crops, pineapple, and tamarillo. Pineapple (*Ananas comosus*) and tamarillo (*Cyphomandra betacea*) are plants that are being actively cultivated by Sarawakians either in highlands or in lowlands. Mixed pineapple and tamarillo juice health drink is premixed with either highland salt, grounded cinnamon and stingless bee honey will enhance their nutritional value. On the other hand, consumer preference will determined the best formulation. The mixture of the health drinks were 1500 mL pineapple juice + 200 mL tamarillo juice (F1), 1500 mL pineapple juice + 200 mL tamarillo + 20 mL stingless bee honey (F2), 1500 mL pineapple juice + 200 mL tamarillo juice + 20 mL stingless bee honey + 1 g highlands salts (F3), 1500 mL pineapple juice + 200 mL tamarillo juice + 25 mL cinnamon (F4) and 1500 mL tamarillo juice + 40 mL cinnamon + 1 g highland salt (F5). Nutrition quality such as Total Soluble Solid (TSS), acidity (pH), antioxidant components, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were analysed. Result revealed that adding of stingless bee honey and cinnamon contribute to higher TPC (F3) and TFC (F5). Based on the result formulation F3 showed the highest contents of antioxidant activities. The sensory evaluation study of the formulations found that F2 was more favourable followed by F3. Both formulation F2 and F3 showed highest acceptance score and also nutritional score. Therefore, to producing these nutritious beverages not only nutritional quality should be taken into account, but also the level of consumer acceptance in taste.

ABSTRAK

Kajian ini telah dijalankan untuk mengoptimumkan penggunaan dua utama tanaman, nanas, dan tamarillo. Nanas (*Ananas comosus*) dan Tamarillo (*Cyphomandra betacea*) adalah tumbuhan yang sedang giat diusahakan oleh rakyat Sarawak sama ada di kawasan tanah tinggi atau di kawasan tanah rendah. Jus nanas dan tamarillo adalah minuman kesihatan pracampuran dengan sama ada garam tanah tinggi, kayu manis dan madu kelulut akan meningkatkan nilai pemakanan mereka. Sebaliknya, keutamaan pengguna akan ditentukan formulasi terbaik. Campuran jus minuman kesihatan adalah 1500 mL jus nanas + 200 mL jus tamarillo (F1), 1500 mL jus nanas + 200 mL jus tamarillo + 20 mL madu kelulut (F2), 1500 mL jus nanas + 200 mL jus tamarillo + 20 mL madu kelulut + 1 g garam Bario (F3), 1500 mL jus nanas + 200 mL jus tamarillo + 25 mL kayu manis (F4) dan 1500 mL jus tamarillo + 40 mL kayu manis + 1 g garam Bario (F5). Kualiti pemakanan seperti JumLah Larut Solid (TSS), keasidan (pH), komponen antioksidan, JumLah Kandungan Fenolik (TPC) dan JumLah Kandungan Flavonoid (TFC) telah dianalisis. Keputusan menunjukkan bahawa penambahan madu kelulut dan kayu manis menyumbang kepada TPC lebih tinggi (F3) dan TFC (F5). Berdasarkan rumusan keputusan F3 menunjukkan kandungan tertinggi aktiviti antioksidan. Kajian penilaian deria formulasi mendapati F2 adalah lebih baik diikuti oleh F3. Kedua-dua formulasi F2 dan F3 menunjukkan skor penerimaan tertinggi dan juga skor pemakanan. Oleh itu, untuk mengeluarkan produk-minuman berkhasiat bukan sahaja kualiti pemakanan perlu diambil kira, tetapi juga tahap penerimaan pengguna dalam rasa.

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APPROVAL SHEET

I certify that this research project report entitled "Development of Pineapple - Based Health Drink according To Their Antioxidants and Sensory Evaluation" has been examined and approved as partial fulfilment of the requirement for the degree of Bachelor Bioindustrial Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TSS	Total Soluble Solid
$NaNO_2$	Sodium nitrite
$AlCl_3$	Aluminium chloride
NaOH	Sodium hydroxide

CHAPTER 1

INTRODUCTION

Fresh juices from fruits are good sources of valuable nutrients and completed our diet with essential vitamins and minerals, dietary fibers, small quantities of lipids and proteins, being in the same time good sources of carbohydrates. Fruits are not only valued for the taste, texture and color they add to a diet, but for their contribution to health. Consuming of fruit juice had a rich source of vitamins and minerals. In the tropics, a great variety of fruits are produced all year round. The food market has stimulated the development of new products that present good sensory acceptance and of high nutritional value (Ijah *et al.* 2015). Development of new products where two or more kinds of fruit juices are blended to obtain a product that combines the nutritional value of both fruits with the benefit of a pleasant taste, has been encouraged by the food industry and has been well accepted by consumers (Ameh *et al.* 2015).

Fruit juicing is one of the method applied in postharvest handling of fruit. Fruit juice is commonly a healthy beverage by itself. Simple processing of fruit juice ensure the conservation of their nutritional benefits. With the development of fruit production industry in Sarawak, fresh juice will be a big industry. According to Malaysian Pineapple Board (2016), total area planted with pineapple is 1805 ha and concentrated in Kuching, Samarahan and Sibu area of Sarawak. Moris, Gandol (N19), Sarawak, MD2, Josapine and Maspine are varieties of pineapple

available in Malaysia (Taufiq *et al.* 2015). Pineapple commonly consumed fresh or processed into other product such as nectar and juice due to its pleasant aroma and flavor (Laorko *et al.* 2013). Pineapple juice generally drinkable in the form of single-strength or concentrated, and blend for development of new flavors for beverages and other new products due to its strong acid flavor (De Carvalho *et al.* 2007; Jan *et al.* 2012; Shamsudin *et al.* 2014). Producing of juices can reduces the suffering of post-harvest losses due to inadequate processing technologies. It has a part of others alternative to reduce post-harvest losses by exploring the affordable and easily adoptable food processing and preservation methods that can convert the abundant fruits into shelf stable products like juices, jams and jellies which are easy, cheap and economically.

Cyphomandra betacea is known as "Buah Cinta," "Moginiwang," or "Tamarillo" among local Malaysia. *C. betacea* usually grow naturally in the higher-humidity and low-temperature area. In Malaysia *C. betacea* is cultivated in Cameron Highland (Peninsular Malaysia), and Kundasang (Sabah). Tamarillo had been planted in other highland such as Bario due to its taste and health benefit. Tamarillo had been used for producing juice or botanical drink. The ripe fruit of *C. betacea* is usually eaten raw by local community (Ali Hassan and Abu Bakar 2013). The fruits is an egg-shaped with a bright red fruit with yellow-orange flesh and black seeds are edible which is surrounded by purple gelatin. There are two varieties of the fruits which are yellow and red. But the red varieties are more popular and more common (Lister *et al.* 2005).

Cinnamon bark is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Approximately, there have 250 species have been identified among the cinnamon genus, with trees being scattered all over the world (Sagal 2011; Vangalapati *et al.* 2012). Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (Huang *et al.* 2007). The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde (*Cin*), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (Yeh *et al.* 2013). In Bario, the species has been found grow naturally and collected by the local community as their source of income.

Stingless bees is a small bee from the species of *Trigona* or *Meliponine* and also known as stingless bee (Kelulut). Stingless bee produce honey that has a high medicinal beneficial than other bee species which had alleged by the traditional medical practitioner (Biswa *et al.* 2017). It can be found mostly in tropical and subtropical area. The color of the honey is usually clearer, liquid and has a sweet and sour taste (Roowi *et al.* 2012). The recent studies showed that the stingless bee honey has the potential to treat colorectal cancer (Yazan *et al.* 2016), anti-inflammatory (Borsato *et al.* 2014), antimicrobial (Zainol *et al.* 2013; Nobre da Cruz *et al.* 2014; Massaro *et al.* 2014; Nishio *et al.* 2016; Medeiros *et al.* 2016; De Sousa *et al.* 2016) and has an antioxidant property (Almeida Da Silva *et al.* 2013; Duarte *et al.* 2012).

Salt is an important seasoning ingredient added during cooking to increase the flavor of dishes. It is also commonly added in processed foods as a preservative, stabilizer, texture enhancer, color enhancer, and more. About 90% of salt is largely added in food processing, in restaurant foods, in sauces and cooking (Choong *et al.* 2012). Highland salt produced high mineral contain (natural iron, potassium, and magnesium) that helps to ensure good health, thus making it a highly desirable product from the highlands.

Mixing pineapple, tamarillo, stingless bee honey, cinnamon and salt could result in a juice product with more vitamins and minerals as well as different and more acceptable sensory characteristics when compared to the raw materials. In addition, production of mixed fruit juices from pineapple and tamarillo will create variety in the fruit juice market and reduce post-harvest losses.

The objective of this study were to determine the phytochemical and investigate the consumer acceptability of a mixed fruit juice produced by mixing of pineapple and tamarillo juice and some other natural flavor enhancer.

CHAPTER 2

LITERATURE REVIEW

2.1 Fruit Juice and Their Health Benefit

The manufacture of juices from fruits and vegetables is as old (or older) than agriculture. In simple words, juice is the extractable fluid contents of cells or tissues. It is defined as fermentable but unfermented juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, preserved exclusively by physical means. The juice may be turbid or clear. The addition of sugars or acids can be permitted but must be endorsed in the individual standard (Bates *et al.* 2001; ICMSF 2005; Bevilacqua *et al.* 2011).

Fruits and vegetables form a versatile and complex substance group category of foods. The relevant substance groups are carbohydrates, acids, minerals, polyphenols (tannins) including the colorful anthocyanins, water-soluble vitamins, amino acids, aroma compounds, carotenoids, fibers and other bioactive substances. During processing, they are essentially transferred into the pressed juice or into the puree (Bates *et al.* 2001).

The health benefits of fruits and fruit juices have been reviewed by Landon (2007). The high potassium and low sodium characteristic of most juices help maintain a healthy blood pressure, furthermore the lack or near absence of fat in fruit juices is beneficial for the cardiovascular system. (Delichatsios and Welty

2005). The fortification of juices with calcium (Andon 1996) and phytosterol (Devaraj *et al.* 2004) provide some supplemental bone and cardiovascular benefits. Recently, several reviews have summarized the health benefits of fruit polyphenols (Spencer 2010; Chong *et al.* 2010; Gonzalez- Gallego *et al.* 2010; Hardcastle *et al.* 2010).

Vitamins have a special role since they are essential for life and most are not produced by the body. Vitamin C (ascorbic acid), naturally present or added to most juices, is necessary for the body to form collagen, cartilage, muscle, and blood vessels, and aids in the absorption of iron. The enzymatic and non-enzymatic functions of vitamin C were reviewed by Levine *et al* (1993). Its role as an antioxidant has been extensively examined, however many vitamin C effects appear to be due to its role as a coenzyme in many biochemical reactions (Levine *et al.* 1993). More recently, the influence of vitamin C in gene modulation and biochemical pathways modifications has been shown, particularly in blood vessel endothelium (Wu *et al.* 2007) and atherosclerosis (Schmidt and Lykkesfeldt 2009). Among the vitamins found in fruit juices, folate from citrus and pineapple is essential for the prevention of spina bifida (Bell and Oakley 2009) and premature birth (Bukowsky *et al.* 2009). It also helps in maintaining a low level of the amino acid homocysteine, a marker of inflammation that has been associated with a higher risk for heart disease, stroke, and heart failure (Sánchez-Moreno *et al.* 2009).

The health benefits of minerals, vitamins, and micronutrients have been well characterized but many beneficial properties of juices have been shown to come from phytochemicals, mainly polyphenols, carotenoids and limonoids. It should be pointed out that data on the health benefits of fruit juices are still fragmentary and that many studies have been done on cell cultures and animal models. Efforts are to be made to perform extended clinical studies with doses of juices similar to those ingested in normal life. If health claims are to be accepted, it will be necessary to determine the bioavailability of the main fruit juice phytochemicals and to define some type of RDI values (Williamson and Holst 2008).

Most of the diseases that seem to be targeted by phytochemicals are those induced by chronic inflammation. This process, which had been called the silent killer, is an attempt by the organism to remove injurious stimuli and to initiate healing. The diseases that may be initiated by chronic inflammation include aging diseases such as Alzheimer's disease (Granic *et al.* 2009; Kanapuru 2009), diabetes, insulin resistance (King 2008; Blüher 2008) and cardiovascular disease, particularly atherosclerosis (Bucova *et al.* 2008). In addition, bone diseases such as osteoporosis and arthritis (Hardy and Cooper 2009), cognitive functions and brain diseases (Wärnberg *et al.* 2009) and some forms of cancer (Gonda *et al.* 2009) may be induced by chronic inflammation.

Christian Mertz *et al.* (2009) reported that tamarillo has higher carotenoid content. According to Bobbio *et al.* (1983); Wrolstad and Heatherbell, (1974) anthocyanins were detected in the tree tomato. Rodriguez Amaya *et al.* (1983),

observed the presence of Carotenoids in tree tomato. Olson (1996) revealed that, except for the well-known provitamin activity of some carotenoids, they could be involved in protective effects against degenerative or cardiovascular diseases and are known for having antioxidant capacity. From the results of the study conducted by Hassan *et al.* (2013), it can be concluded that *C. betacea* has a significant amount of phenolic, flavonoids, anthocyanin, and carotenoid which contribute to the antioxidant activity of the fruit extracts. The acceptable amount of phytochemicals in the fruits showed that *C. betacea* is one of the richest sources of antioxidant phytonutrients and has anti-cholinesterase properties that can enhance human health.

Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It contains 10-25 mg of vitamin (Rasid; Kader and Hosain 1987). Pineapple composition has been investigated mainly in the edible portion. Pineapple contains 81.2 to 86.2% moisture, and 13-19% total solids, of which sucrose, glucose and fructose are the main components. Carbohydrates represent up to 85% of total solids whereas fiber makes up for 2-3% of the organic acids, citric acid is the most abundant in it. The pulp has very low ash content, nitrogenous compounds and lipids (0.1%). From 25-30% of nitrogenous compounds are true proteins. Out of this proportion, Ca. 80% has proteolytic activity due to a protease known as Bromelain. Fresh pineapple

contains minerals as calcium, chlorine, potassium, phosphorus and sodium (Dull 1971).

Pineapple juice contains ascorbic acid and is a good source of Vitamin C Ascorbic acid or vitamin C fights bacterial and viral infections which is an effective antioxidant and helps the body absorb iron. According to Jillian Michael website, half a cup of pineapple juice provides 50 percent of an adult's daily recommended amount of vitamin C. Several essential minerals exist in pineapples, including manganese, a trace mineral instrumental to the formation of bone, as well as the creation and activation of certain enzymes. Pineapples also include copper, another trace mineral. It assists in the absorption of iron and regulates blood pressure and heart rate (Debnath et *al.* 2012).

Food preservation is defined as to control the growth of spoilage and pathogenic organisms (Aneja *et al.* 2008). Preservation of fruit juice depends on the low pH, pasteurization, refrigeration and on the addition of preservatives. Pasteurization of fruit juices is often done by applying temperature of 85-95° C for 2 minutes. However, some problems associated with this technique, as pasteurization temperature is only effective against pathogens such as *E. coli* and *Salmonella* but are not effective against aeciospores of heat resistant fungi and heat resistant bacteria. In addition the thermal treatment also affects the sensory and nutritional quality of fruit juices (Salomao *et al.* 2007; Kutama *et al.* 2010; Smit *et al.* 2011; Steyn *et al.* 2011; Mosqueda-Melgar *et al.* 2012).

As far as the use of natural antimicrobials contains great diversity of the compounds, however their use in foods is concerned; the lack of reproducibility of their activity is one of the major restrictions. There is variation in qualitative and quantitative analysis of bioactive phytochemicals in plant extracts result in their variable effectiveness. Further, the extrapolation of results obtained from in-vitro experiments with laboratory media to food products is not straightforward as foods are complex, multicomponent systems consisting of different interconnecting microenvironments. Herbs, spices and essential oils are used by the food industry as natural agents for extending the shelf life of juices. There are more than 1340 plants with defined antimicrobial compounds and over 30,000 components have been isolated from phenol group containing plant oil compounds and used in food industry (Tiwari *et al.* 2009; Tajkarimi *et al.* 2010; Negi 2012; Lucera *et al.* 2012). The essential oil of cinnamon also has antimicrobial properties, which can aid in the preservation of certain foods when added to food, it inhibits bacterial growth and food spoilage, making it a natural food preservative.

2.2 Cinnamon and It's Health Benefits

Cinnamon is widely believed to be high in anti-oxidants. Regular drinking of cinnamon tea could be beneficial to oxidative stress related illness in humans. Cinnamon tea offers helpful relaxation for the stomach upset by the tension and strain of modern living. Numerous studies show that cinnamon regulates blood sugar, making it a great choice for diabetics and hypoglycemic alike. That's also

great news for anyone who wants stable energy levels and moods. It reduces LDL cholesterol levels. LDL is also known as the harmful cholesterol. Reducing it may help reduce the risk of cardiovascular disease. It has natural anti-infectious compounds. Cinnamon has been shown in studies at the Department of Internal Medicine, Kangnam Korean Hospital, to reduce cytokines linked to arthritic pain. According to research at the University of Texas, published in the journal Nutrition and Cancer, shows that cinnamon may reduce the proliferation of cancer cells, holding promise for cancer prevention and sufferers of the disease. It's a natural food preservative. It's been proven effective for menstrual pain and infertility.

Cinnamon contains a natural chemical called cinnamaldehyde, which studies show increases the hormone progesterone and decreases testosterone production in women, helping to balance hormones. Cinnamon holds promise for various neurodegenerative diseases, including: Alzheimer's disease, Parkinson's disease, multiple sclerosis, brain tumor, and meningitis, according to research at the Cytokine Research Laboratory, Department of Experimental Therapeutics, and University of Texas. Their research shows that cinnamon reduces chronic inflammation linked with these neurological disorders. Cinnamon powder is used as traditional medicine against herpes virus had an effect on HIV-1 and a significant effect on controlling type-2 diabetes by controlling the blood glucose levels (Premnathan *et al.* 2000)

2.3 Stingless Bee Honey and It's Health Benefits

Previous study showed that stingless bee honey can act as anti-inflammatory (Borsato *et al.* 2014), anti-cancer (Kustiawan *et al.* 2014; Yazan *et al.* 2016), anti-microbial (Miorin *et al.* 2003; Demera *et al.* 2004; Garedew *et al.* 2004; Temaru *et al.* 2007; Kimoto-Nira and Amano 2008; Chanchao *et al.* 2009; Boorn *et al.* 2010; Rodríguez *et al.* 2012; Ilechie *et al.* 2012; Andualem 2013; Ewnetu *et al.* 2013; Mercês *et al.* 2013; Queiroz *et al.* 2013; Zainol *et al.* 2013; Nobre da Cruz *et al.* 2014; Massaro *et al.* 2014; Zamora *et al.* 2014; Nishio *et al.* 2016; Medeiros *et al.* 2016; De Sousa *et al.* 2016) and possessed antioxidant properties (Duarte *et al.* 2012; Almeida da Silva *et al.* 2013; De Sousa *et al.* 2016). However, the beneficial of stingless bee honey has been abandoned in modern medicine due to the paucity of systematic scientific studies for supporting its medical properties (Pe´rez *et al.* 2006).

The composition of stingless bee honey differ from other species according to some physicochemical parameters (Özbalci *et al.* 2013) and other studies prove that honey from stingless bees are more valuable and it has been used for a long time to treat various diseases(Souza *et al.* 2006).

2.4 Bario Highland Salt and It's Health Benefits

Bario salts is rich in high mineral content (natural iron, potassium and magnesium). According to Zaleha *et al.* (2003), it was found that salt produced from one of the wells in Bario contained high quantity of iodine. Bario salt is

natural and not over processed hence it's naturally brownish color. Not only that, it is also less than other commercial salts which easily found in supermarkets.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Source

Convenience sampling was used to obtain the sample for this study. Freshly harvested Tamarillo (*Cyphomandra betacea*) were purchased from local stall in Cameron Highland and also from Bario, pineapple (*Ananas comosus*) were purchased from local stall in Bario and Bintulu. Pineapple cultivars selected are *Nanas Sawit* and *Moris*. Others sample such as cinnamon (*Cinnamomum* sp.), stingless bee honeys and highland salt were purchased from local stall in Bario.

3.2 Sample Preparation

3.2.1 Tamarillo Puree Preparation

Tamarillo fruits was cleaned using 10% diluted detergent and thorough washed using tap water prior to peeling and coring. After coring both pulp and seeds then blend using fruit blender without adding any water. The puree further sieved to remove the seeds and only pure puree will be used for further study. The ready puree then kept in cleaned jar prior to mixing with pineapple juice.

3.2.2 Pineapple Juice Preparation

Pineapple fruits were peeled using disinfected knife and cut into cube before doing juice extraction using juice extractor (Juicer model MJ-DJ01SSL

Panasonic). Skin and the pineapple eyes were removed. Cube then put into the juicer where both pulp and juice were separated automatically. Juice then keep in clean and pasteurized beaker prior to mixing. The fruit pulp then add 10% (w/v) to the fruit juice and this make the concentrated juice.

3.2.3 Cinnamon Emulsion Preparation

Cinnamon bark were chop into pieces and ground into fine mesh and sieved using 0.02 mm. The powdered then immersed into hot water at the rate of 1 (w):10 (v) in a 1000 mL beaker and keep for 24 hours. After 24 hours, the emulsion then sieved to separate the cinnamon bark powder from the concentrated emulsion and keep in pasteurized 500 mL beaker.

3.2.4 Stingless Bee Honey

Stingless bee honey used was obtained from the meliponi farm situated in remote area. For this reason, the honey was obtained from Bario and from the species of *Heterotrigona itama.*

3.2.5 Highland Salt

The dehydrated salt was obtained from the local trader in Bario. The salt was used according to the intended formulation as stated in section 3.3 below.

3.3 Juice Formulation

For the purpose of experiment, five juice formulation had been developed. All the juices were constitute of pineapple juice and tamarillo juice as the main formulation. The mixture contents of 1500 mL of pineapple juice and 200 mL of tamarillo juice that made the F1 formulation. Then the based juice formulation (F1) then mixed with 20 mL stingless bee honey to make F2 formulation. To make formulation F3, the formulated F2 then mixed with 1g of highland salt and shake well till the salt dissolved. Somehow, formulation F4 was the mixture of F1 with 25 mL of cinnamon emulsion and final formulation was F5 that's contained tamarillo juice (1500 mL) mixed with 40 mL of cinnamon and 1 g of salt (Table 3.3).

Table 3.3: Formulation for mixed juice

Formulation	Pineapple (mL)	Tamarillo (mL)	Stingless Bee Honey (mL)	Cinnamon (mL)	Highland Salt (g)
F1	1500	200	0	0	0
F2	1500	200	20	0	0
F3	1500	200	20	0	1
F4	1500	200	0	25	0
F5	0	1500	0	40	1

Upon mixing, all the formulated juice then poured into pasteurized 200 mL glass bottle and each formulation had 5 replicates.

3.4 Pasteurization of Bottled Juice

The formulated juice were bottled and sealed before undergone pasteurization. Pasteurization method applied was by soaking the bottle in 90°C circulating hot bath for 20 minutes. After 20 minutes, the bottled then keep to cool under room temperature before keeping into chiller (5°C) for 1 month prior to compositional analysis.

3.5 Parameter Determination

After 1 month of keeping refrigerated, the formulated juice then gone further analyse. The parameters analyses are Total Soluble Solid (TSS), Refractive Index, acidity (pH), total flavonoid and total phenolic contents. These result will determine the composition of the formulated juice apart from the standard contents of the fruits (tamarillo and pineapple), cinnamon, stingless bee honey and salt.

3.5.1 Total Soluble Solid

Total soluble solids (TSS) content of a solution is determined by the index of refraction. This is measured using a digital refractometer (AR-2008, Krus Germany), and is referred to as the degrees Brix. A drop of the juice was placed on the prism and press calculate to read the data given (AOAC, 2005).

3.5.2 Juice Acidity (pH)

The pH of the juice was measured using digital pH meter (Ino Lab 720, Germany) followed AOAC (2006) method. Fifty milliliter (50 mL) of the juice sample was transferred into a beaker and pH was determined after the meter was calibrated using standard buffer solutions of pH 4.0 and 7.0.

3.6 Antioxidant Determination

Juice from the different formulation which are F1, F2, F3, F4 and F5 were sampled. Sample of 10 mL was took from each sample prior to further process. The juice then extracted with 70% methanol at 100 rpm for 2 hours at ambient temperature and stirred in conical flask using an orbital shaker. The extraction of the juice were filtered using Whatman qualitative filter paper No. 2 and filled into falcon tube (50 mL) then labelled according to the formulation.

3.6.1 Determination of Total Flavonoid Content

Total Flavonoid Content (TFC) was determined by using modified Aluminum trichloride colometric assay followed Meda *et al.* (2005). To quantify the TFC, quercetin was used as the standard. A standard curve of quercetin concentrations was generated at the ranges 0- 100mg/l. According to Ramaiya *et al.* (2014), 0.1 mL of the sample was extracted into 25 mL falcon tube, then added with 0.3 mL of 5% $NaNO_2$ and left for five minutes. 0.3 mL of 10% $AlCl_3$ were added and left for another five minutes before added 2 mL of 1M NaOH. Final volume was made

up with distilled water to 10 mL. The mixture absorbance was measured at 510 nm.

3.6.2 Determination of Total Phenolic Content

Total phenolic content (TPC) was determined by spectrophotometer using Follin-Ciocaltue's reagent method (Singleton and Rossi, 1965) with some modification. Followed to Ramaiya *et al.* (2014), 1 mL of the juice extraction was added with 0.3 mL of Follin-Ciocaltue and vortex it for one minute to mix the mixture well. The juice was left for six minutes. Then, added with 10 mL of 7% Sodium Carbonate (Na_2CO_3) and final volume was made up with distilled water to 25 mL. After incubation at room temperature in the dark for 90 min, the mixture's absorbance was measured against the Gallic acid standard at 740 nm. Total phenolic content were considered as Gallic acid equivalent (GAE)/100 g sample.

3.7 Sensory Evaluation

An acceptance test was conducted with 30 respondents (15 women and 15 men), aged between 18 and 30 years of age. Attributes evaluated were color, sweetness, sourness, aroma, flavor and overall liking, through hedonic scale. The scale are; like extremely (6 scores), like moderately (5 scores) to like slightly (4 scores), neither like nor dislike (3 scores) and dislike slightly to dislike extremely (0-2 score). Each tester were given 5 mL of juice sample and coded as A, B, C, D and E. The test was carried out in Food Processing Laboratory, UPMKB. After

each sample test, the tester have to rinse the mouth by gargling using plain water. This will be repeated until all the juice sample (A, B, C, D and E) finished.

3.8 Statistical Analysis

The data obtained were analyzed and interpreted by analysis of variance (ANOVA) and Tukey means comparison test at a level of 5% of significance, using SAS version 9.3. Values were presented as mean ± standard deviations of 3 observations.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Physicochemical and Phytochemical Analysis of Mixed Fruit Juice

The physicochemical parameters of mixed fruit juice obtained from the analysis are presented in Table 4.1. From the table, there was significant ($p<0.05$) difference in the TSS and pH value of the mixed fruit juice. The highest TSS content was observed in F2 with 13.37 followed by F3 (12.80), F4 (10.87), F1 (10.37) and F5 (7.80). According to Antune, *et al.* (2008), Malaysian Josapine pineapple had a TSS value of 13.5%. Formulation 2 have the highest value of Brix due to stingless bee honey. By adding cinnamon and salt in the mixed fruit juice, TSS of the juice also decrease due to the ability of the elements to reduce and eliminate sugar (Hertzler 2011).

The pH value of F5 was much higher than the others formulation with 4.23 and followed by F4 (3.99), F1 (3.98), F2 (3.94) and F3 (3.91). According to Kaanane, *et al.* (1988), the minimal change in pH can be explained by relationship between pH and free acid content. The high pH of a food is used as an indicator of bacterial spoilage (i.e. the food with high pH is more susceptible to microbial spoilage). The pH value of 3 to 4 may give juice a good potential inhibiting the growth of pathogenic bacterial (Jay 1984). The spiced fruit drinks were acidic in nature therefore, becoming more resistant to bacterial spoilage (Richard and St-Piere 2006).

Table 4.1: TSS and pH of the tested formulation

Formulation	TSS (%)	TSS Refractive Index (RI)	pH
F1	10.37 ± 0.033^{d}	1.347	3.98 ± 0.007^{b}
F2	13.37 ± 0.033^{a}	1.348	3.94 ± 0.003^{c}
F3	12.80 ± 0.058^{b}	1.347	3.91 ± 0.003^{d}
F4	10.87 ± 0.033^{c}	1.347	3.99 ± 0.006^{b}
F5	7.80 ± 0.058^{e}	1.345	4.23 ± 0.006^{a}

All values are given as mean ± standard error and value in parenthesis at the range a>b>c>d>e. The different alphabets in same row indicate significant difference at $p \leq 0.05$ (ANOVA, HSD). Key: F1=1500 mL pineapple juice + 200 mL tamarillo juice, F2=1500 mL pineapple juice + 200 mL tamarillo + 20 mL stingless bee honey, F3=1500 mL pineapple juice + 200 mL tamarillo juice + 20 mL stingless bee honey + 1 g highlands salts, F4=1500 mL pineapple juice + 200 mL tamarillo juice + 25 mL cinnamon, F5=1500 mL tamarillo juice + 40 mL cinnamon + 1 g highland salt.

The phytochemical of mixed fruit juice are shown in Table 4.2. Polyphenols are important group of compounds regarding the appearance and the functional in fruits. Polyphenolic compounds have been implicated in antioxidant activity of fruits, vegetables and derived beverages (Trueba 2003). It is also a members of a class of natural compounds, recently considered as high scientific and therapeutic interest. Fruits is not only give as a nutrient but also act as medicine. The result, shows that TPC of the mixed fruit juice was in F3 with 12.20 mg GAE/100 g followed by F4 (11.85 mg GAE/100 g), F5 (10.15 mg GAE/100g), F2 (7.48 mg GAE/100 g) and F1 (4.58 mg GAE/100 g). There were significant difference in

mixed fruit juice in term of TPC value. The value of TPC in F3 was greater due to the addition of honey and salt. Stingless bee honey contain its own phenolic contain and highland salt enhance the phenolic substance of honey. The concentration and type of phenolic substances in fruit juice depend on several factors; differences of varieties, ripeness and also the processing methods. According to Sobhana *et al.* (2015) the development of mixed fruit products is an alternative to add nutritional value since, mixed fruit products unite nutritional characteristics of two or more fruits.

Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cell against all stages of carcinogenesis (Okwu 2004). Flavonoid, in intestinal tract lowers the risk of heart disease (Okwu 2005). TFC of F5 was higher than the others formulation with 8.57 mg QE/100 g and followed by F4 (7.63 mg QE/100 g), F2 (7.22 mg QE/100 g), F1 (6.46 mg QE/100 g) and F3 (5.53 mg QE/100 g). There was significant difference in TFC in the mixed fruit juice. F3 shows the lower value of TFC due to addition of stingless bee honey and highland salts. This was occur when the mixed honey and salts can reduce the flavonoid content. TFC of F5 is higher than the other because it containing high concentration of tamarillo and cinnamon. Cinnamon have high in flavonoid contain that can cover tamarillo juice. Deepshikha (2013) reported that the extracts of cinnamon and cloves exhibited strong antioxidant properties owing to the presence of high phenolic and flavonoid contents in them.

Table 4.2: Antioxidants properties of tested formulation based on TPC and TFC

Formulation	TPC (mg GAE/100 g)	TFC (mg QE/100 g)
F1	4.58 ± 0.002^{c}	6.46 ± 0.194^{d}
F2	7.48 ± 0.073^{d}	7.22 ± 0.002^{c}
F3	12.20 ± 0.018^{a}	5.53 ± 0.004^{e}
F4	11.85 ± 0.145^{b}	7.63 ± 0.009^{b}
F5	10.15 ± 0.001^{c}	8.57 ± 0.004^{a}

All values are given as mean ± standard error and value in parenthesis at the range a>b>c>d>e. The different alphabets in same column indicate significant difference at $p \leq 0.05$ (ANOVA, HSD). Key: F1=1500 mL pineapple juice + 200 mL tamarillo juice, F2=1500 mL pineapple juice + 200 mL tamarillo + 20 mL stingless bee honey, F3=1500 mL pineapple juice + 200 mL tamarillo juice + 20 mL stingless bee honey + 1 g highlands salts, F4=1500 mL pineapple juice + 200 mL tamarillo juice + 25 mL cinnamon, F5=1500 mL tamarillo juice + 40 mL cinnamon + 1 g highland salt.

4.3 Sensory Analysis

The result of sensory scores of the mixed fruit juice formulations has been shown in Table 4.3. There are no significant difference ($p<0.05$) in the flavor for formulation F1, F2, F3 and F4 the reference formulations (4.20, 4.47, 3.90 and 4.17), respectively. The score for sample F5 (2.33) was significantly low than the other sample. Sweetness score of the mixed fruit juice were increase from formulation 1 (3.17) to 4 (4.10) and then a decline through to formulation 5 (1.97). Score of sourness of the mixed juice fruits was high, ranging from 4.20 – 4.13 respectively. F1 (4.20), F2 (4.13), F3 (3.83) and F4 (3.87) has no significant difference as compared to F5 (3.17) has significant difference. There was a decline in the scores of aroma from F1 to F5 and also no significant difference between F1 (3.90), F2 (3.60), F3 (3.20) and F4 (3.03). The score of color decrease from F1 (4.69) to F2 (4.33) and increase to F3 (4.37) and decrease again from F4 (4.03) to F5 (3.20).

The flavor of the mixed juice among all that content pineapple juice did not have significant difference even though had been mixed with honey, cinnamon or salt. Tamarillo based juice has the lowest flavor score (significant) and some situation recorded on the juice sweetness. According to the mix reading, all the formulation were significantly different. Thus indicate that the test bud not able to detect slight differences on the sweetness of the juice. Formulation F1 and F2 on the other hand has highest sourness score detected but based on pH reading, there were slightly difference of the pH. For all the formulation F1 – F4, the aroma and color score are the highest and tamarillo, F5 was found to be the lowest. Therefore, all

the F1 – F4 has highest acceptability score and the highest was F2 and lower is F5.

Table 4.3: Sensory evaluation properties of mixed fruit juice.

Formulation	Sensory evaluation					
	Flavor	Sweetness	Sourness	Aroma	Color	Acceptability
F1	4.20 ± 0.269^{a}	3.17 ± 0.318^{a}	4.20 ± 0.232^{a}	3.90 ± 0.182^{a}	4.69 ± 0.170^{a}	4.03 ± 0.222^{a}
F2	4.47 ± 0.171^{a}	3.27 ± 0.172^{a}	4.13 ± 0.133^{a}	3.60 ± 0.201^{a}	4.33 ± 0.221^{a}	4.50 ± 0.184^{a}
F3	3.90 ± 0.188^{a}	3.53 ± 0.229^{a}	3.83 ± 0.220^{ab}	3.20 ± 0.251^{ab}	4.37 ± 0.189^{a}	4.13 ± 0.224^{a}
F4	4.17 ± 0.173^{a}	4.10 ± 0.205^{a}	3.87 ± 0.164^{ab}	3.03 ± 0.242^{ab}	4.03 ± 0.169^{ab}	4.23 ± 0.177^{a}
F5	2.33 ± 0.292^{b}	1.97 ± 0.269^{b}	3.17 ± 0.325^{b}	2.57 ± 0.317^{b}	3.20 ± 0.334^{b}	2.57 ± 0.302^{b}

All values are given as mean ± standard error and value in parenthesis at range a>b. The different alphabets in same row indicate significant difference at $p<0.05$ (ANOVA, HSD) Key: F1=1500 mL pineapple juice + 200 mL tamarillo juice, F2=1500 mL pineapple juice + 200 mL tamarillo + 20 mL stingless bee honey, F3=1500 mL pineapple juice + 200 mL tamarillo juice + 20 mL stingless bee honey + 1 g highlands salts, F4=1500 mL pineapple juice + 200 mL tamarillo juice + 25 mL cinnamon, F5=1500 mL tamarillo juice + 40 mL cinnamon + 1 g highland salt.

4.4 Health Drink Requirements

The result of the preliminary sensory tests to select the mixed fruit juice with good acceptance and the higher pineapple juice concentration indicated the following formulations: F1 (1500 mL pineapple juice + 200 mL tamarillo juice), F2 (1500 mL pineapple juice + 200 mL tamarillo + 20mL stingless bee honey), F3 (1500 mL pineapple juice + 200 mL tamarillo juice + 20 mL stingless bee honey + 1 g highlands salts), F4 (1500 mL pineapple juice + 200 mL tamarillo juice + 25 mL cinnamon) and F5 (1500 mL tamarillo juice + 40 mL cinnamon + 1 g highland salt), particularly in the brix, pH and total phenolic and flavonoid content. Although the potential benefits of fruit juices are enormous, the need to evaluate their nutritional constituents and safety cannot be under estimated so as to provide information that may influence their choice and selection for human consumption.

CHAPTER 5

CONCLUSION

The experiment implies the prospect of the processing of mixed fruit juice as well as investigates the nutritional and commercial aspects of mixed fruit juice. Consumer acceptability of F2 implies mixed fruit juice can be prepared by using a combination of 80% pineapple juice, 20% tamarillo juice and 2% stingless bee honey. Slight variation of TSS, pH, TPC and TFC has been seen but might not affect the eating quality of the products.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Development of Pineapple - Based Health Drink according To Their Antioxidants and Sensory Evaluation" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and publish in the form approved by the faculty.

Angela Cindy Anak Kasim

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