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ENHANCEMENT OF PINEAPPLE BASED JUICE WITH STINGLESS BEE HONEY AND HERBS

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ENHANCEMENT OF PINEAPPLE BASED JUICE WITH STINGLESS BEE HONEY AND HERBS

By

MARTIN KEHENG AJANG

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ABSTRACT

The study was conducted to optimize the use of locally available plant materials such as pineapple, ginger, cinnamon, and stingless bee honey. Pineapple (*Ananas comosus*) is plants that are being actively cultivated by Sarawakians either in the highlands or in the lowlands. Pineapple mixed with ginger and cinnamon would be a health drink with an enhanced nutritional value. The quality of the botanical drink fermented would be analysed in this study. The mixture of the botanical drink was in percentage of 1000 ml of the botanical drink with T1 (comprises of 85% pineapple, 5% ginger, 5% cinnamon, 5% honey) , T2 (comprises 70% pineapple, 10% ginger, 10% cinnamon, 10% honey), T3 (comprises 70% pineapple, 5% ginger, 15% cinnamon, and 10% honey) and T4 (comprises of 65% pineapple, 15% ginger 15% cinnamon and 15% honey). Parameters such as Total Soluble Solid (TSS), acidity (pH), and, Total Flavonoid Content (TFC) were analysed. All samples were also pasteurised at different temperatures of, 63°C, 90°C and unpasteurized. Result revealed that addition of stingless bee honey, cinnamon, and ginger differs the Total Flavonoid content as well as the pasteurization point affected the Total Flavonoid content. Based on the result formulation T2 (unpasteurized) showed the highest contents of antioxidant activities.

ABSTRAK

Kajian telah dibuat menggunakan bahan-bahan tempatan yang mudah di dapati sepserti, nanas, madu kelulut, halia dan kayu manis. Nanas (*Ananas comosus*) merupakan sejenis tumbuhan yang di tanam oleh penduduk Sarawak di kawasan tinggi dan juga rendah. Campuran jus nanas, halia, kayu manis dan madu lebah kelulut akan menjadikan minuman yang lebih berkhasiat. Kualiti jus botanical ditapai ini akan di analisis di dalam kajian ini. Campuran jus botanikal ini akan dalam bentuk peratusan 1 litter atau 1000 ml dari kesuluruhan kuantiti jus dengan T1 (mempunyai 85% nanas, 5% halia, 5% kayu manis, 5% madu), T2 (mempunyai 70% nanas, 10% halia, 10% kayu manis, 10% madu) T3 (mempunyai 70% nanas, 5% halia, 15% kayu manis, and 10% madu) dan T4 (mempunyai 65% nanas, 15% halia 15% kayu manis dan 15% madu). Kualiti pemakanan seperti Pepeja terlarut (TSS), keasidan (pH), komponen antioksidan, dan Jumlah Kandungan Flavonoid (TFC) telah dianalisis. Sample juga diamasak pada kadar yang berlainan iatu 63°C, 90°C dan tidak dimasak. Rumusan menunjukkan penambahan madu kelulut, halia dan kayu manis mengubah Jumlah Flavonoid (TFC) dan juga pempasturan. Berdasarkan rumusan formulasi T2 (Tidak dipasteur) mempunyai Jumlah Flavonoid yang tertinggi.

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APPROVAL SHEET

I certify that this research project report entitled "Enhancement of Pineapple based Juice with Stingless bee Honey and Herbs" has been examined and approved as partial fulfillment 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 ABBREVIATION

UPMKB	University Putra Malaysia Kampus Bintulu
ROS	Reactive Oxygen Species
PCA	Protocatechuic acid
pH	Potential hydrogen
EC	Electrical Conductivity
TSS	Total Soluble Solid
TFC	Total Flavonoid Content
µS	Microseimens
%	Percentage
$NaNO_2$	Sodium Nitrate
$AlCl_3$	Aluminium Chloride
NaOH	Sodium Hydroxide
ANOVA	Analysis of Variance

CHAPTER 1

INTRODUCTION

1.1 Background

Botanical drink or also known as 'organic drinks' are considered to be a popular type of beverage worldwide. This is because of its potential as a source of additional nutrients and other beneficial substances (fiber, antioxidants, etc.) causes a mass production of botanical drinks in western countries. This trend of mixology of different types of agricultural produce has grown popular especially towards groups of people concerned towards their health and well-being. Increase numbers of guru's, health instructors and fitness coach have taken to natural drinks because of its potent and rich nutrients and benefits. As botanical drinks increase in variety and diversity in its line of production, the trend of mixology slowly builds popularity in eastern countries, especially in Asia. One example of this is a popular and well-known Malaysian entrepreneur's take on the growing trend. Products from popular brands such as 'Qu Puteh' produce the type of drinks which utilized agricultural commodities such as tomato, comfrey root, and Goji berry and so on to produce a product that could lighten the skin colour (Products 2012).

This is possible because of the agricultural produce properties and nutrients which when extracted and processed could produce cosmetic valuable properties. This is effective in marketing especially for women as they thrive more towards beauty. And because of this, the growing demand for a natural or organic drink is in its incline and high in market value in our country.

The rising popularity of the stingless bee honey and its propolis is mainly due to its highly beneficial nutritional value. Not only being health beneficial, the honey and propolis also produce unique and flavourful taste with hints of sourness, sweetness, and bitterness, which diversify the taste and makes the product liked by many, especially in Sarawak. Though the taste is mainly sweet and sour, it is according to the habitat of the stingless bee being reared. (Yaacob *et al* 2017).

1.2 Problem Statement

Production of pineapple in Malaysia increases rapidly since the past few years. In Sarawak, alone of a total of 1342 hectares had been planted. As of to date, very little high-value products had been derived from pineapple. Only juice, sauce and paste had been producing as a cottage industry and no high-value medicine products had been producing, by the contrary, the ingredients increase the nutritional value or health benefit of the product. Have in realizing this, the objective of the study us to optimize pineapple by combining with Stingless bee honey and selected herbs such as ginger and cinnamon

1.3 Objectives

The specific objectives of this study were:

1. To formulate a preferable botanical drink using locally available raw materials
2. To investigate the physiochemical properties of the formulated botanical drink.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Drink

The act of consuming botanical beverages or organic drinks has shown to have an impact on human society in both the mind and body. Not only these type of beverages are considered versatile, but it is also customizable and is up to the consumer on how to produce it (Products 2012). In the case of this thesis, the customizability gives more room for improvements and better the product. The desire of the consumers to maintain a diet which promotes better health has increased the demand for juices that preserve their natural nutritive value. Therefore, alternative processing methods which potentially increase nutritive properties are necessary (Mphahlele *et al*. 2016).

2.2 Juicing

Juicing is considered to be a healthier alternative for other consuming methods of mainly fresh produce. The juice is defined as unfermented but fermentable juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, and preserved exclusively by physical means(Perez-Cacho and Rouseff 2008). The addition of sugars or acids can be permitted but must be endorsed in the individual standard It extracts the very essence of fruit for example. Extracting the liquids that are concentrated with the benefits and values of the 'said' fruit. It also makes digesting and absorbing the nutrients better. Juices and liquid-based produce, in general, is easier to consume especially for health impaired individuals, children, and people in their

golden age. Thus, increasing the popularity of the use of juicing as a method of consuming, for example, fresh fruits (Aneja *et al.* 2014).

2.3 Pasteurization

Pasteurization of liquid-based product ensures safety for human consumption by reducing the number of viable pathogenic bacteria. This pasteurization temperature is effective against *Escherichia coli* and *Salmonella* but is not effective against ascospores of heat resistant fungi and heat resistant bacteria (Aneja *et al.* 2014). This treatment is used for two distinct reasons which are to make the botanical drink and other liquid based products safe for human consumption by reducing the number of viable bacteria that may be harmful to health and to improve the shelf life by reducing the numbers of spoilage bacteria (Donald *et al.* 2011)

2.4 Benefits of Pineapple

Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour, and immense health benefits. Pineapple contains a considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water, and different minerals that are good for the digestive system and helps in maintaining an ideal weight and balanced nutrition (Farid Hossain 2015), as shown from Table 2.1.

Table 2.1 Nutrients in 100 grams (g) pineapple (Farid Hossain 2015).

Nutrients	Amount
Energy	52 calories
Dietary fiber	1.40 g
Carbohydrate	13.7 g
Protein	0.54 g
Iron	0.28 mg
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Phosphorus	11 mg
Zinc	0.10 mg
Vitamin A	130 mg
Vitamin B 1	0.079 mg
Vitamin B 2	0.031 mg
Vitamin B 3	0.489 mg
Vitamin B 6	0.110 mg
Vitamin C	24 mg

2.4.1 Good source of dietary fiber

Dietary fiber as a general found in beans, oats, flaxseed and oat bran and most fruits can help lower total blood cholesterol levels by lowering low-density lipoprotein, or "bad," cholesterol levels (Farid Hossain 2015). Studies also have shown that high-fiber foods may have other heart-health benefits, such as reducing blood pressure and inflammation. People with diabetes, fiber particular, soluble fiber can slow the absorption of sugar and help improve blood sugar levels. A healthy diet that includes insoluble fiber may also reduce the risk of developing type 2 diabetes (Farid Hossain 2015).

2.4.2 Excellent source of vitamins and minerals

Pineapple fruits exhibit high moisture, high sugars, soluble solid content ascorbic acid, and low crude fiber. Thus pineapple can be used as supplementary nutritional fruit for good health (Farid Hossain 2015). One healthy ripe pineapple fruit can supply about 16.2% of the daily requirement for vitamin C. Vitamin C is the body's primary water-soluble antioxidant, against free radicals that attack and damage normal cells. A powerful antioxidant, vitamin C supports the formation of collagen in bones, blood vessels, cartilage, and muscle, as well as the absorption of iron (Gazdik *et al.* 2008).

2.4.3 Contains beneficial enzymes (Bromelain)

Bromelain is an enzyme mixture present in the pineapple. People tend to use bromelain as a supplement for various health benefits, including relieving sinus problems, and reducing inflammation, Bromelain is also used to remove dead skin from burns, and orally, to reduce inflammation and swelling particularly of the nasal passages (Farid

Hossain 2015). Bromelain is also used as a digestive aid for osteoarthritis and to reduce soreness in aching muscles (Wijeratnam 2015).

2.5 Benefits of Stingless Bee Honey

Antioxidants are agents that save cells from the harmful effects of reactive oxygen species (ROS). Antioxidants act as a saviour to the structure of cells by neutralizing ROS and thus terminating the damaging chain reaction in the body (Dai and Mumper 2010). Both honey and propolis of the stingless bee possess therapeutic action in improving the wound healing process which comes through its antioxidant activity since it can prevent the detrimental effects on the wounded site caused by oxidative stress. Stingless bee honey can also be applied to the treatment of wounds since its antioxidant content is higher or similar to that of other types of honey (Jalil *et al.* 2017). In general, a stingless bee contains a higher level of flavonoids than the honey produced by *A. mellifera*. The total antioxidant activity in the *Tetragonula carbonaria* (stingless bee) honey was proven to be higher than that of the European floral honey. In Malaysia, the researchers from MARDI have revealed that the major free phenolic acid in stingless bee honey consists of protocatechuic acid (PCA) and 4-hydroxyphenylacetic acid (Yaacob *et al.* 2017). PCA is a strong antioxidant that can improve cell proliferation in the wound healing process (Choudhari *et al.* 2013).

2.6 Benefits of Ginger

Ginger is often promoted for the treatment of nausea and vomiting. However, although ginger powder appears to ameliorate nausea of diverse causes, the strength of evidence depends on the context in which it is used for treatment, such as for symptoms following pregnancy, surgery, cancer therapy, or motion sickness (Singletary 2010). In an experimental and clinical motion sickness studies, ginger demonstrated an improvement in symptoms of motion discomfort, nausea, or vomiting, compared with controls or with antiemetic drugs, although statistical significance was not always reached (Singletary, Ali, Hawa, and Asmah 2010).

Ginger extract has demonstrated the capacity in numerous cancer cell culture systems to suppress cell proliferation and induce cell death (Singletary 2010; Shukla and Singh 2007). Ginger and some specific constituents have demonstrated antioxidant effects in several cell culture systems. Furthermore, there are animal studies showing that ginger extracts and individual ginger constituents such as-gingerol can protect several tissues and organs against damage due to a variety of oxidation-inducing stressors (Singletary 2010).

2.7 Benefits of Cinnamon

Cinnamon can act as an antidiabetic agent. Preclinical animal studies provide evidence that constituents of cinnamon may decrease blood glucose and insulin levels both in rats genetically predisposed to diabetes and in rats given high doses of sugars (Singletary 2008 ; Akilen *et al.* 2013 ; Qin *et al.* 2010) Human trials of cinnamon extract do not consistently demonstrate a benefit in lowering diabetes-associated blood cholesterol levels or improving blood lipid profiles. Antioxidant

components of cinnamon have been identified. There are several reports using cell culture models and an experiment in rats demonstrating that cinnamon and cinnamaldehyde have antioxidant activity and scavenge free radicals (Singletary 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

All the materials and methodology used in this research is stated and explained in this section. This includes how to prepare the liquid extract, mixing, pasteurization, preference test, and storage as the first part of the project. A second part will include analysing important parameters such as total flavonoid content, antioxidant determination, total soluble solids, pH value, and total phenolic content.

3.2 Sample Source

All ingredients are obtained through buying it fresh from the market, in Bandar Lama Bintulu, Sarawak. This includes ingredients such as pineapple, cinnamon, and ginger, whereas stingless bee honey is obtained through buying from a stingless bee farmer. Four formulations are produced with different amounts and percentage of ingredients into the individual botanical drink.

3.3 Juice Formulation

For the purpose of the experiment, four juice formulation had been developed. All the juices constitute of pineapple juice and ginger juice as the main formulation. The mixture contents of a total of 1000 ml of juice which includes for T1 [85 % pineapple juice (850 ml), 5% ginger juice (50 ml), 5% amount of stingless honey (50 ml), and 5% cinnamon emulsion 50ml)], T2 [70% pineapple (700 ml), 10% ginger (100 ml), 10% stingless bee honey (100 ml), and 10% cinnamon emulsion (100 ml)],.

T3 [contains 70% pineapple juice (700 ml), 5% ginger (50 ml), and 15% cinnamon emulsion (150 ml) and a 10 % stingless bee honey (100 ml)], and T4 [consists of 65% pineapple (650 ml), 15% ginger (150 ml), 15% cinnamon emulsion (150 ml) and a 15% stingless bee honey (150 ml)] as shows in table 3.1 below.

Table 3.1 Formulation of botanical drink

Ingredients	**Pineapple**	**Ginger**	**Cinnamon**	**Stingless Bee Honey**
Formulation	**(ml)**	**(ml)**	**(ml)**	**(ml)**
T1	85%	5%	5%	5%
T2	70%	10%	10%	10%
T3	70%	5%	15%	10%
T4	65%	15%	15%	15%

3.4 Juice Preparation

3.4.1 Pineapple and Ginger Juice Extraction

Pineapple and ginger were peeled using a disinfected knife and cut into a cube before doing juice extraction using juice extractor (Juicer model MJ-DJ01SSL Panasonic). The skin of the ginger and pineapple eyes were removed. The cube was then put into the juicer where both pulp and juice were separated automatically. The juice was then kept in a clean and pasteurized beaker prior to mixing.

3.4.2 Cinnamon Emulsion Preparation

Cinnamon barks were grounded and sieved using 0.02 mm. The powder was sieved and then immersed into hot water at the rate of 1 (w):10 (v) in a 1000 ml beaker and kept for 24 hours. After 24 hours, the emulsion was then sieved to separate the cinnamon bark powder from the concentrated emulsion and keep in pasteurized 500 ml beaker.

3.5 Pasteurization

The formulated juice was bottled and sealed before undergoing pasteurization. Pasteurization method applied was by soaking the bottle in 90°C circulating hot bath for 30 minutes. After 30 minutes, the bottle was then kept cool under room temperature before being stored into a chiller (11.5°C) and another replicate stored at room temperature (24°C) for 1 month prior to compositional analysis. This is also repeated at 63°C pasteurization point, unpasteurized, and stored in both chilled and room temperature

3.6 Parameter Determination

The formulated juice underwent further analyse. The parameters analyses were Total Soluble Solid (TSS), Refractive Index, Acidity (pH), and Total Flavonoid contents. These results determined the composition of the formulated botanical drink.

3.6.1 Total Soluble Solid

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

3.6.2 Juice Acidity (pH)

The pH of the juice was measured using a digital pH meter (Ino Lab 720, Germany) followed the Horwitz and Latimer (2006) method. Fifty milliliters (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.

3.7 Antioxidant Determination

Juice from the different formulation which is T1, T2, T3, and T4 were sampled. Sample of 10 ml was taken from each sample prior to further process. The juice was then extracted with 70% methanol at 100 rpm for 2 hours at ambient temperature and stirred 17 conical flasks using an orbital shaker. The juice extracts were filtered using

Whatman qualitative filter paper No. 2 and filled into falcon tube (50 ml) then labeled according to the formulation.

3.8 Determination of Total Flavonoid Content

Total Flavonoid Content (TFC) was determined by using modified Aluminum trichloride coulometric assay following 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. The final volume was made up with distilled water to 10 ml. The mixture absorbance was measured at 510 nm.

3.9 Statistical Analysis

The data obtained were analysed and interpreted by analysis of variance (ANOVA) and Least Significant Difference (LSD) means comparison test at a level of 5% of significance, using SAS version 9.4. Values were presented as mean ± standard deviations of 3 observations.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physicochemical and Phytochemical Analysis of Botanical Drink

After a month of observation with data collected per week for 1 months, mean for each pasteurization points (unpasteurized, 63°C and 90°C) was collected and presented by the tables 4.1, 4.2 and 4.3 for storage under room temperature (25°C) and 4.4, 4.5 and 4.6 are tables representing data collected under chilled conditions (11.5°C) with each representing juice acidity (pH), electrical conductivity, total soluble solids, and its reflective index, under each respective pasteurization points.

It is also noted that at a certain point, some of the samples, that is stored in room temperature were considered compromised in a way that there was the growth of fungus floating on the sample and most of this samples which were indicated as the data NA. Most of the 63°C treatments were considered to be compromised and this was considered invalid and was discarded, as the sample was considered to be contaminated. The factor in which the growth of fungus under room temperature condition was apparent because the growth of fungus correlates towards the storage condition (Packter 1994). At lower temperature, fungus growth decreases and halts at a certain point, and is either non-existent or dormant. It is also noted that, the pasteurization point in which the samples were cooked also influence the growth of fungus. There is also the factor of raw ingredients contracting spores during harvesting, storage, and preparation (Packter 1994). Even with proper washing or cleaning of equipment and the raw material, fungus spores are considered to be resistant and

without proper procedures such as SOPs of food manufacturing, there is a chance of the samples contracting fungus spores, bacteria and as such (Onyekwelu 2017)

4.1.1 Room Condition

Table 4.1 Unpasteurized and 90°C pasteurization point

Treatment	Week	Unpasteurized			90°C		
		EC	pH	TSS	EC	pH	TSS
T1	0	1086.88^{a} ± 1.55	4.89^{a} ± 0.05	10.79^{c} ± 0.02	1369.25^{b} ± 0.45	4.90^{a} ± 0.01	13.80^{a} ± 0.01
	1	1120.00^{b} ± 1.54	4.91^{b} ± 0.04	10.79^{c} ± 0.01	1220.64^{a} ± 0.55	5.24^{b} ± 0.02	15.51^{b} ± 0.01
	2	1619.67^{c} ± 1.56	4.99^{c} ± 0.01	8.41^{b} ± 0.02	1501.77^{c} ± 0.52	5.34^{c} ± 0.01	18.36^{c} ± 0.02
	3	3559.33^{d} ± 1.52	5.19d ± 0.02	7.86^{a} ± 0.01	2796.03^{d} ± 0.45	5.44^{d} ± 0.01	20.93^{d} ± 0.01
T2	0	1129.33^{a} ± 0.95	4.99^{b} ± 0.02	18.00^{c} ± 0.01	1173.07^{b} ± 0.55	4.98^{a} ± 0.02	13.44^{a} ± 0.02
	1	1128.07^{a} ± 0.85	4.99^{b} ± 0.02	11.21^{a} ± 0.02	1129.13^{a} ± 0.45	5.11^{b} ± 0.01	19.10^{d} ± 0.01
	2	1457.11^{b} ± 0.81	4.07^{a} ± 0.02	11.19^{a} ± 0.01	1225.63^{c} ± 0.45	5.19^{c} ± 0.02	18.31^{c} ± 0.02
	3	2757.49^{c} ± 0.86	5.19^{c} ± 0.01	17.71^{b} ± 0.02	2137.17^{d} ± 0.56	5.34^{d} ± 0.02	17.82^{b} ± 0.02
T3	0	1128.47^{b} ± 1.21	4.76^{a} ± 0.02	18.01^{c} ± 0.02	1232.67^{b} ± 0.56	5.20^{b} ± 0.01	15.26^{a} ± 0.01
	1	1111.88^{a} ± 1.23	4.91^{b} ± 0.01	12.30^{a} ± 0.01	1128.97^{a} ± 0.55	3.59^{a} ± 0.01	17.20^{b} ± 0.01
	2	NA	NA	NA	1224.70^{b} ± 1.54	5.08^{d} ± 0.01	17.61^{c} ± 0.02
	3	1533.88^{c} ± 1.23	5.00^{c} ± 0.01	17.60^{b} ± 0.02	1773.70^{c} ± 1.55	4.76^{c} ± 0.01	20.09^{d} ± 0.01
T4	0	1159.52^{a} ± 1.11	4.96^{b} ± 0.02	15.66^{b} ± 0.02	1231.02^{c} ± 1.23	4.98^{a} ± 0.02	13.39^{a} ± 0.02
	1	1156.01^{a} ± 1.10	4.96^{b} ± 0.02	12.70^{a} ± 0.01	1120.17^{a} ± 1.44	4.98^{a} ± 0.01	13.39^{a} ± 0.01
	2	1241.33^{b} ± 1.56	3.83^{a} ± 0.01	17.40^{c} ± 0.02	1209.40^{b} ± 1.21	5.19^{c} ± 0.02	18.50^{b} ± 0.02
	3	2684.00^{c} ± 1.55	5.39^{c} ± 0.02	19.41^{d} ± 0.02	1954.00^{d} ± 1.43	5.02^{b} ± 0.02	19.39^{c} ± 0.01

All values are given as mean ± standard deviation and value in parenthesis at the range a>b>c>d>e. The different alphabets in the same column indicate significant difference at $p \leq 0.05$ (ANOVA, LSD).

4.2 Juice Acidity

The pH of each sample provided from the tables shows a significant change as samples become less acidic along the observation weeks. This is because the reaction between the bacteria within the samples causes the production of alcohol from the carbohydrate contents within the sample. The most fermentation process that revolves around the usage of the anaerobic process that reacts certain bacteria that would cause the production of alcohol. For instance, the production of rice wine and such (Krebs 1940).

In this case, the thriving of bacteria that are not killed from the pasteurization process would cause the fermentation. Alcohol at its purest form would have no pH value, but as for alcohol production from natural processes would have a more alkaline nature, and thus causing the samples to become less acidic over time(Leahu et al. 2007). As can be seen from most of the treatment, there is an incline pH value upon elongated storage time. For example, treatment 1 (unpasteurized) shows a change from 4.89 to 4.91 to 4.99 to 5.19 at the end of the observation period. It is not only noted from the pH, but there is also the presence of alcohol from the smell and the pressurized gas that emits after opening the sample bottle.

4.3 Electrical Conductivity

The electrical conductivity defines as the measure of electrical current a material can carry or its ability to carry a current. This corresponds and correlates EC towards salinity of a sample, soil or water. The current capability is affected by the amount of salt within a sample (Schön 2015). This was seen on most of the sample having higher EC over time. This is because the sample is become diluted, having less salt causing

more conductivity. The main would because of dilution is because of sedimentation, most of the pulp of the ingredients would be present within the sample. In addition, as time passes, larger particles, pulp, and ingredients within the sample would cause sedimentation on the bottom of the falcon tube. This causes the water to be on top of the sample which when kept in longer periods of time, would be more dilute because of bacterial activity that consumes the pulp and nutrients (Schön 2015).

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4.4 Total Soluble Solids

Sugar acts as an important constituent of fruits, as it is considered to a natural preservative for fruits. As shown on the table, an example of pasteurization of 90°C of T1 at week 0 had 13.80% which increases 15.51% to 48.36% over the course of 3 weeks, this indicates the total soluble solids with a sample would have an increase over time. This is because hydrolysis of polysaccharides like starch, cellulose or pectin is converted into simpler sugar (glucose, fructose) which increases its quantity (Leahu et al. 2007). As for certain samples with an incline of the TSS percentage for example unpasteurized sample T1 that had 10.79% decreases to 7.86% is because of the bacteria.

4.5 Storage Condition

4.5.1 Chiller Condition

Table 4.2 Data of unpasteurized, 63°C, and 90°C

	Week	Unpasteurized			63°C			90°C		
		EC	pH	TSS	EC	pH	TSS	EC	pH	TSS
T1	0	1251.00^{b} ± 1.44	5.01^{b} ± 0.01	11.60^{a} ± 0.02	1321.22^{a} ± 1.43	5.53^{c} ± 0.01	11.29^{a} ± 0.02	1144.11^{a} ± 1.10	5.54^{c} ± 0.01	19.71^{d} ± 0.02
	1	1404.18^{d} ± 1.23	5.02^{b} ± 0.02	15.81^{c} ± 0.01	1321.00^{a} ± 1.23	5.51^{c} ± 0.02	11.29^{a} ± 0.01	1343.33^{b} ± 0.55	5.51^{c} ± 0.02	16.30^{c} ± 0.02
	2	1310.56^{c} ± 1.33	4.99^{a} ± 0.02	13.13^{b} ± 0.02	1481.33^{b} ± 1.23	5.02^{a} ± 0.02	16.81^{c} ± 0.02	1424.67^{c} ± 0.54	5.02^{a} ± 0.02	15.71^{a} ± 0.01
	3	770.75^{a} ± 1.45	5.09^{c} ± 0.01	19.03^{d} ± 0.01	1534.55^{c} ± 1.55	5.33^{b} ± 0.01	14.90^{b} ± 0.02	2873.78^{d} ± 0.89	5.33^{b} ± 0.02	15.73^{b} ± 0.02
T2	0	771.79^{a} ± 1.56	5.06^{d} ± 0.01	12.29^{a} ± 0.01	1244.56^{b} ± 1.87	5.51^{d} ± 0.01	19.28^{b} ± 0.02	1105.89^{a} ± 0.76	5.51^{d} ± 0.02	19.60^{c} ± 0.01
	1	1193.70^{b} ± 1.12	4.03^{a} ± 0.02	20.00^{b} ± 0.02	1141.33^{a} ± 1.43	5.12^{a} ± 0.02	14.31^{a} ± 0.02	1251.00^{c} ± 0.55	5.12^{a} ± 0.01	14.32^{a} ± 0.02
	2	2530.14^{d} ± 1.43	4.67^{b} ± 0.01	20.88^{c} ± 0.01	1243.00^{b} ± 1.33	5.29^{c} ± 0.03	19.61^{c} ± 0.01	1213.67^{b} ± 0.54	5.29^{c} ± 0.01	16.61^{b} ± 0.01
	3	1222.42^{c} ± 1.34	4.89^{c} ± 0.02	20.88^{c} ± 0.02	2224.11^{c} ± 1.23	5.20^{b} ± 0.01	20.11^{d} ± 0.02	1253.33^{c} ± 0.98	5.20^{b} ± 0.01	20.11^{d} ± 0.01
T3	0	1220.26^{b} ± 1.45	4.93^{c} ± 0.02	18.61^{c} ± 0.01	1223.00^{b} ± 2.12	5.20^{b} ± 0.01	17.99^{a} ± 0.02	1257.44^{b} ± 0.55	5.20^{c} ± 0.02	18.09^{b} ± 0.02
	1	1224.70^{c} ± 2.34	4.08^{a} ± 0.01	14.61^{a} ± 0.01	1222.44^{b} ± 2.11	5.05^{a} ± 0.01	17.98^{a} ± 0.02	1222.22^{a} ± 1.45	5.05^{b} ± 0.02	16.63^{a} ± 0.02
	2	2530.11^{d} ± 1.11	4.83^{b} ± 0.02	17.56^{b} ± 0.01	1215.67^{a} ± 1.23	5.01^{a} ± 0.02	19.50^{c} ± 0.02	1297.67^{c} ± 1.32	5.01^{a} ± 0.02	18.21^{d} ± 0.01

Continued Table 4.2

	3	1175.00^{a} ± 1.23	5.06^{d} ± 0,02	18.67^{c} ± 0.02	2145.56^{c} ± 1.32	5.64^{c} ± 0.02	18.31^{b} ± 0.01	2445.67^{d} ± 1.12	5.64^{d} ± 0.01	18.14^{c} ± 0.01
T4	0	1175.00^{a} ± 1.34	5.06^{d} ± 0.01	16.58^{b} ± 0.01	1191.44^{a} ± 1.21	5.25^{c} ± 0.02	19.28^{c} ± 0.01	1162.11^{a} ± 0.78	5.25^{c} ± 0.01	19.73^{c} ± 0.02
	1	1176.42^{a} ± 1.54	5.03^{c} ± 0.02	14.58^{a} ± 0.01	1211.67^{b} ± 1.33	5.12^{b} ± 0.02	18.41^{b} ± 0.02	1175.67^{b} ± 0.98	5.12^{b} ± 0.01	15.63^{a} ± 0.02
	2	1275.80^{b} ± 1.43	4.28^{a} ± 0.02	19.95^{c} ± 0.01	1231.33^{c} ± 1.45	4.91^{a} ± 0.01	18.06^{a} ± 0.02	1221.11^{c} ± 0.87	4.91^{a} ± 0.02	19.60^{b} ± 0.01
	3	2619.62^{c} ± 2.11	4.41^{b} ± 0.01	20.10^{d} ± 0.02	2555.00^{d} ± 1.54	5.35^{d} ± 0.02	20.50^{d} ± 0.02	2448.00^{d} ± 0.58	5.35^{d} ± 0.01	20.41^{d} ± 0.01

All values are given as mean ± standard deviation and value in parenthesis at the range a>b>c>d>e. The different alphabets in the same column indicate significant difference at $p \leq 0.05$ (ANOVA, LSD).

4.6 Effects of Treatment on Parameters

There is also an apparent difference in parameter data (EC, pH, and TSS) when compared against treatments (T1, T2, T3, T4) at both storage conditions. This because of the treatments were formulated differently, which cause different effects on the parameters, as shown in table 4.3.

Table 4.3 Effects of treatments on parameters

	pH	EC	TSS	TFC
T1	$4.89^{b} \pm 0.02$	$1086.00^{a} \pm 1.54$	$10.79^{a} \pm 0.01$	$5.93^{a} \pm 0.01$
T2	$4.99^{d} \pm 0.01$	$1129.33^{c} \pm 1.44$	$18.00^{c} \pm 0.02$	$7.79^{c} \pm 0.01$
T3	$4.76^{a} \pm 0.02$	$1128.47^{b} \pm 1.98$	$18.01^{c} \pm 0.02$	$7.46^{b} \pm 0.01$
T4	$4.96^{c} \pm 0.01$	$1159.52^{d} \pm 1.72$	$15.66^{b} \pm 0.01$	$6.35^{d} \pm 0.01$

All values are given as mean ± standard deviation and value in parenthesis at the range a>b>c>d>e. The different alphabets in the same column indicate significant difference at $p \leq 0.05$ (ANOVA, LSD)

In general, the EC of T1 should show the highest amount because of its composition consisting mainly of pineapple, where its pulp was mainly filtered during the extraction. This causes less sedimentation floating within the samples and fewer amounts of sedimentation after a while causing more conductivity (Method 2000; Schön 2015) in the samples of T1, whereas it should show lowest at T4 because of the viscosity of the sample having highest amounts of stingless bee honey which had affected the EC value. Followed by T2 and T3 where the cinnamon emulsion (which is higher than T2) is thicker than the ginger extract having more sediments within the juice.

The pH of the samples should also vary but not much as the source of the acidity should come from the pineapple extract which is present in all of the samples. The slight difference is mostly caused by the amount of extracted pineapple composition in the samples and the total soluble solid show is highest at T4 because of the amount of stingless bee honey which should have the highest complex sugar of all the ingredients. The more the complex sugar, the more the reaction of bacteria which breaks down the polysaccharides into simpler sugar such as glucose (Bartolomé, Rupérez, and Fúster 1995) causing more value in TSS.

4.7 Total Flavonoid Content

In this experiment, the use of only unpasteurized samples and 90°C samples are used as the samples from 63°C are not preferable as can be seen on storage time, and would not be a productive and efficient use of materials and chemicals. Both respective pasteurization points have a more suitable outcome and thus, undergoes the next phase of analysis. The experiment shows the contents of anti-oxidant in TPC (Total Phenolic Content) of all the composition comprised of T1, T2, T3, and T4 and the difference in pasteurization points when compared with its similar composition such as T1 (90°C) and T1 (Unpasteurized).

Between formulations T1-T4, there would be significant differences because of the number of ingredients used. Highest amounts of flavonoid content would be from T2. This is because of the quantity of cinnamon and ginger, which is a balance in the treatment. Even with the amounts of cinnamon to be higher at T4, the amounts of stingless bee honey would also cause a reduction of the flavonoid content of a sample (Dai and Mumper 2010).

As in the factor of pasteurization points, there is also a significant difference from all the samples as the heat would affect flavonoid content. As in all general nutritional degradation, heat plays a vital role in the stability of the nutrients (Xu *et al.* 2007). Some nutrients would degrade or decrease at certain points of temperature, as in the view of the table 4.2.1, the unpasteurized samples have generally slightly higher amounts of flavonoid activity when compared to the 90°C pasteurization point (Xu *et al.* 2007)

Table 4.4 Total Flavonoid Content

Pasteurization point	Treatment	Total Flavonoid Content
Unpasteurized	T1	$5.93^{f} \pm 0.01$
	T2	$7.79^{a} \pm 0.01$
	T3	$7.46^{d} \pm 0.01$
	T4	$6.35^{e} \pm 0.01$
90°C	T1	$5.50^{f} \pm 0.01$
	T2	$7.50^{b} \pm 0.01$
	T3	$7.20^{c} \pm 0.01$
	T4	$6.10^{e} \pm 0.01$

All values are given as mean ± standard deviation and value in parenthesis at the range a>b>c>d>e. The different alphabets in the same column indicate significant difference at $p \leq 0.05$ (ANOVA, LSD)

CHAPTER 5

CONCLUSION

The experiment shows the quality of a botanical drink, which is produced using locally available products within the Bintulu area. All variation in the formulation shows a promising contribution towards well-being or considered to be health benefits in their own respect. The balance of all respected parameters with little difference from its original state (1st reading) and its last (4th reading) at the end of the observation is the most optimum outcome for the sample. This is to ensure a better botanical drink which can last longest with it's nutrient and health beneficial compound does not differ from its fresh composition. In this project, T2 would have the most balance or least change out of all the samples and unpasteurized samples would have a higher amount of Total Flavonoid content as heat affects the degradation of the compound; T2 would also have the highest amount of flavonoid compound.

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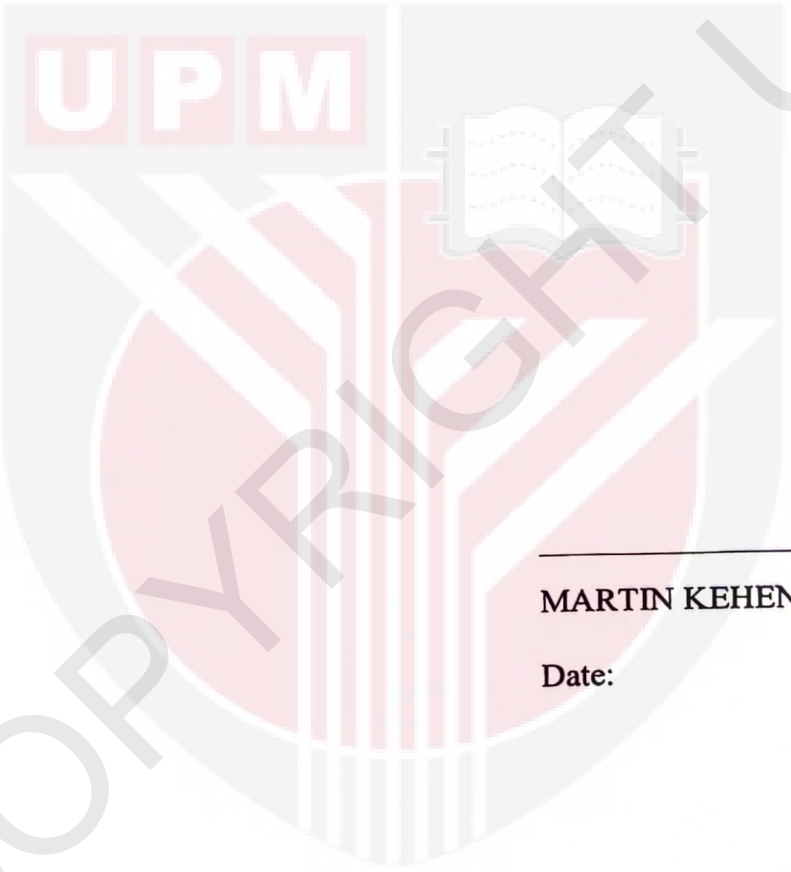
PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Enhancement of Pineapple based juice with Stingless bee Honey and Herbs" by the supervisor in joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.



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