The usage of banana (*Musa acuminata*)
For novel food and beverage production

BY

NKERBU RODOLPHE NWANG

ID: 5329625

Thesis

Master's thesis submitted to the faculty of biotechnology,

Assumption University in partial satisfaction of the requirement

for the degree of Master of Science in Biotechnology

(Joint program with University of California at Davis)

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Title:	**The usage of banana (*Musa acuminata*) for novel food and Beverage production.**
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(Dr. Churdchai Cheowtirakul)

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Dedication

To my parents for their moral support and unconditional love

Acknowledgement

I greatly appreciate the following people for their valued contribution towards the success of this project.

- I would like to express my gratitude to my advisor and Dean of the faculty of Biotechnology Dr. Churdchai Cheowtirakul, for his continuous support in the master program, guidance and encouragement. His supervision enabled me to better apprehend concepts behind the developments of new products. I enjoined doing research under his direction.

- To all my brothers and sister for their moral support and words of encouragement.

- To all my friends for mutual inspiration. Sharing ideas and personal experiences kept me focus on the goal.

- Finally I would like to thank my Family in general for just being there for me as shoulder on which I can lean on at anytime.

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ABSTRACT

Alcoholic beverage derived from ripe banana

The quality properties of alcoholic beverages made from ripe banana were examined. Two banana types of the genus *musa spp* were used that is long hand and short hand respectively, wine yeast (*Saccharomyces cerevisiae* var. ellipsoideus*)* and lactic acid bacteria isolated from yoghurt containing live and active cells of *Lactobacillus bulgaricus and Streptococcus thermophilus* were used as starter cultures. Distilled and mineral water were used respectively as extraction solvents. The extracted juices contained 15 ^{0}Brix, pH in the range of 4.2 to 4.6, TA ranged between 0.21- 0.3g/100ml. Part of The juices was inoculated with wine yeast and some other part with both wine yeast and LAB. Fermentation took about 7 to 10 days to be completed. pH decreased while titratable acidity (TA) increased with increasing fermentation time of the juice. Results revealed that the pH of long hand banana extract fermented with yeast was slightly higher than that of the short hand banana fermented with yeast that is 4.04 and 3.94 respectively. Similar results were observed for long and short hand banana fermented with both yeast and lactic acid bacteria 4.02 and 3.84 respectively. The TA of the long hand banana juices fermented with yeast and both yeast/LAB respectively were lower than that of the short hand banana juice fermented yeast and both yeast/LAB respectively. Fermentation took about 7 to 10 days to be complete and ^{0}brix reduced from 15 ^{o}Brix to 6 ^{o}Brix in all samples. The alcohol content of the various samples varied between 5.5%v/v and 6.5% v/v. Long hand banana had a much fruitier and better aroma than the short hand banana. The colors of the long hand banana extracts (light yellow) were more attractive compared to those of short hand banana (brownish yellow) at the end of the fermentation. Also, short hand banana fermented extracts looked more turbid. The aroma of the long hand banana fermented extracts where fruitier and sweeter compared to those of short hand banana fermented extracts. The sweet aromatic characteristic and light yellow color of the LHB fermented extracts, made us further our study using long hand banana. In the process of furthering this study, the various formulations were used **A: LHB/Distilled water/Yeast, B: LHB/mineral water/yeast, C: LHB/distilled water/yeast and LAB, D: LHB/mineral water/yeast and LAB.** Results revealed pH and specific gravity decreased while titratable acidity (TA) increased with increasing length of fermentation of the juice. A preference test revealed D is most preferred while sample C is least preferred. There was no significant difference between sample B and the rest $P > 0.05$, D was significantly different to A and C $P < 0.05$ also A and C were none significantly different $P > 0.05$. The pH of the distilled water extracts fermented with yeast (3.9 - 4) were slightly higher than those of the mineral water extracts fermented with both yeast and LAB (3.68 - 3.7). TA of the extracts fermented with both yeast and Lab where slightly higher than those of the extracts fermented with only yeast. Fermentation was complete in about 7- 10 days and alcohol contents were: A: 6.4%v/v, B :5.9%v/v, C :6.3%v/v, D: 5.8%v/v the slightly low alcohol content of B and D might be due to the combined effect of both yeast and LAB. Overall the preference test ranked sample D: LHB/mineral water/yeast and LAB as most preferred of all ($2.9 \pm 1.12495^{b)}$ .There was no difference in pH, TA and ^{0}Brix of all the fermented extracts. An alcoholic beverage could be produced from banana using the recipe A to D. Overall there is no significant difference between the various products.

Abstract

Banana puree mixed yoghurt (Banana purero)

The quality properties of a food product made from ripe banana puree were examined. A mixture of banana puree and yoghurt , added with 20 % distilled water and heated at 90^0 c was prepared and fermented by adding yeast and yeast/LAB respectively and stored for a week. A control was set were no microorganism was added. Physicochemical properties of yoghurt samples included pH, TA, texture, flavor, mount feel. Sensory qualities were also determined. Results showed that acidity increases with time while pH decreased. Samples fermented with both yeast and LAB scored the highest TA 1.2g/100ml and a lowest pH 4.15. There was a significant difference in flavor and texture between mixtures fermented with microorganisms and the control $p<0.05$. There is a significant difference in mouth feel between yeast fermented samples and the others $P<0.05$. Sensory evaluation results showed that the sample fermented with both yeast and LAB was the most liked. Improvement of the most liked sample above by adding 40% of distilled water, mineral water and milk respectively. Results revealed no significant difference in color, flavor, texture and mouth feel of the various samples $p>0.05$ and a preference test revealed samples added with mineral water to be the most preferred. Another improvement of the sample added with mineral water above by adding various concentration of sugar that is 0,2,4 and 6 % respectively revealed no significant difference in texture color and mouth feel $p>0.05$ but a significant difference in sweetness and flavor between 6% and 0% sugars $p<0.05$. The pH ranged between 4.04 and 4.12. The lowest pH 4.04 was for the sample added with 6% sugar and the highest pH 4.12 was for the sample in which no sugar was added. TA ranged between 1.03 and 1.5. The highest pH was for the sample in which no sugar was added and the lowest was for sample added with 6% sugar. The results showed that the higher the sugar concentration the lower the pH and TA. Banana puree mixed

with yoghurt, added with 40% mineral water and 6% sugar scored the highest overall acceptability. Comparing colors of the first product in which heat was applied to the improved ones revealed a dark brown color to a white brown color indicating that heating induces browning. The results of this current study demonstrate that banana puree can be used in combination of yoghurt, mineral water , sugar yeast and LAB to produce new food product and also that the addition of sugar and mineral water enhances flavor and overall acceptance of products

Chapter 1: Introduction

A banana is an edible fruit produced by several kinds of large herbaceous flowering plants of the genus *Musa*. Musa ranges from Africa through Asia to the Pacifics **(Nakasone and Paull, 1998).** The banana fruit is variable in size, color and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be yellow, purple or red when ripe. The fruits grow in clusters hanging from the top of the plant. Almost all modern edible parthenocarpic (seedless) bananas come from two wild species – *Musa acuminata* and *Musa balbisiana*. The scientific names of most cultivated bananas are *Musa acuminata*, *Musa balbisiana*, and *Musa* × *paradisiaca* for the hybrid *Musa acuminata* × *M. balbisiana*, depending on their genomic constitution. The old scientific name *Musa sapientum* is no longer used.

Banana was first domesticated on the Kuk valley of New Guinea around 8,000 BCE (Before Common Era). Additionally, though this is the first known location of banana domestication, other spontaneous domestication projects may have occurred throughout the Southeast Asia about 7000 years ago **(D'hont et al, 2012).** Some scientists believe they may have been the world's first fruit. It is also fourth important food crop in terms of gross value after paddy, wheat and milk products and forms an important crop for subsistence farmers. Though banana is mainly known for its fruit as a culinary ingredient, Banana is classified as a functional food because it contains a food component that affects one or a limited number of function(s) in the body in a targeted way so as to have positive effects **(Mohammad et al, 2009).**Owing to its high nutritive value, banana is suitable for people of all age-groups. Banana is the only fruit that has been reported as a source of inulin and oligofructose which can have a positive effect on the host health **(Mohammad et al, 2009).** Since banana is mostly consumed as fresh because of its abundance and easy availability, there has been no serious effort made to convert it to in any other value-added product such as banana juice, banana powder, banana purees, etc. Following the maturity and harvest, there is a rapid rate of deterioration of ripe banana. Though consumed to a considerable extend, large quantities are usually wasted because of inadequate storage facilities and poor handling. Therefore, methods to extend shelf life of banana are useful **(Akubor et al, 2003).**

Many fermented products could be made from bananas such as banana beer, banana wine etc. In the course of this study we decided to focus on the production of an alcoholic beverage from two varieties of ripe banana and various microorganisms like yeast and Lactic acid bacteria isolated from yoghurt and various types of waters such as distilled and mineral. The objective here was to find out if there exist any difference between the quality of the short and long hand banana, the type of liquid used and the types of microorganisms used in the process.

On another hand, we also tried to manufacture a fermented product from banana puree (which we named banana purero). This product involved the usage of banana puree mixed with yoghurt and our objective was to get the best formulation for the product and eventually a better product.

Chapter 2: Banana History

The first bananas are thought to have grown in the region that includes the Malaya Peninsula, Indonesia, the Philippines and New Guinea. From here, traders and travelers took them to India, Africa and Polynesia. There were references to bananas from 600 BC when Buddhist scriptures, known as the Pali Canon, noted Indian traders travelling through the Malaysian region had tasted the fruit and brought plants back with them. In 327 BC, when Alexander the Great and his army invaded India, he discovered banana crop in the Indian Valleys. After tasting this unusual fruit for the first time, he introduced this new discovery to the Western world. By 200 AD bananas had spread to China. According to the Chinese historian Yang Fu, bananas only ever grew in the southern region of China. They were never really popular until the 20th Century as they were considered to be a strange and exotic alien fruit. The bananas we enjoy today are far better than the original wild fruit which contained many large, hard seeds and not much tasty pulp. Bananas as we know them began to be developed in Africa about 650 AD. There was a cross breeding of two varieties of wild bananas, *the Musa Acuminata* and the *Musa Baalbisiana*. From this process, some bananas became seedless and more like the bananas we eat today **http://www.australianbananas.com.au/banana-facts/world-history.**

2.1. Production and consumption of banana

Bananas are the most important traded fruit in the world and the most important fruit in the diet in most Western countries. Almost 60m tones are produced annually, and the general trend of production is still upwards **(International banana trade, 1998).** Bananas and other tropical fruits have grown to be essential commodities in the international market. Developing countries value these fruits both for nutritional content and commercial viability, as they are relatively cheap yet excellent sources of vitamins and minerals. Banana is one of the 5 most commonly eaten fruit in the world. Including Cavendish, the most commonly traded variety, and banana is also the fourth most important food crop in agriculture after rice, wheat, and maize. Most banana exports involve a small number of countries and are mostly controlled by 5 multinational companies. Meanwhile, countries produced tropical fruits solely for domestic consumption until the 1970s. Trade volumes have expanded dramatically as developing countries recognize the benefits of diversifying products, since traditional export crops have been experiencing a downward trend in prices. However, only about 20% of the products are traded internationally. World production of bananas was estimated at more than 102.1 million tons in 2010, about 6% more than 2009. Asia accounted for nearly 61% of world production. Next were the Americas with 27%, Africa with 10%, Oceania with 1.7%, and Europe with 0.4%.The EU banana trade is dominated by Latin American suppliers, with 78.8% of EU imports in 2011. ACP suppliers accounted for the remaining 21.2%. EU banana producers now account for between 11.7% and 12.7% of total EU banana consumption; down from 16% in 2004, although following reforms in 2007, EU production began to grow again, so that by 2010 it was 18.9% higher than in 2007. **(http://agritrade.cta.int/Agriculture/Commodities/Bananas/Executive-Brief-Update-2012-Banana-sector)**

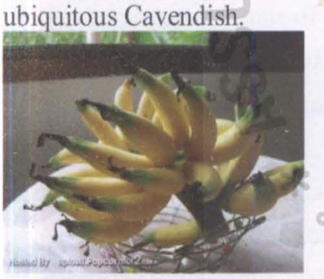
Bananas are grown in all tropical regions and play a key role in the economies of many developing countries. In terms of gross value of production, bananas are the world's fourth most important food crop after rice, wheat and maize. They are a staple food and an export commodity **(FAO, 2002)**

2.2. Types of bananas

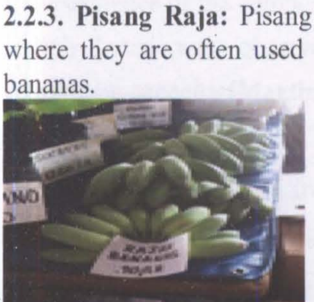
There are over 1,000 types of bananas in the world, many of them differing slightly in appearance, size and taste **(Rodriguez, 2013).** They are often grouped into two categories which are those that people can immediately eat, sometimes called dessert bananas, and those that need to be cooked prior to eating and are higher in starch, called plantains. Again, the number and types that fall into these two groups are striking. There are a few types that are easily distinguishable in a basic grocery store. **http://www.wisegeek.org/what-are-some-different-types-of-bananas.htm**

2.2.1 Cavendish Bananas: Cavendish bananas are the most common variety. They are the long yellow, slightly sweet bananas at supermarkets around the world.

2.2.2. Lady Finger Bananas: Lady Finger bananas are smaller and sweeter than the ubiquitous Cavendish.



2.2.3. Pisang Raja: Pisang Raja bananas (here labeled "rajah") are popular in Indonesia, where they are often used to make banana fritters. They are also known as Musa Belle bananas.



2.2.4. Cooking Bananas: Cooking bananas, like plantains, are better thought of as potatoes than as bananas. They can be roasted, steamed, fried into chips, and otherwise used like any starchy vegetable.

2.3. NUTRITIONAL VALUE OF BANANA

Fruits are frequently looked on primarily from the standpoint of acessory substances they contain,but it is well to bear in mind in the case of banana that its caloric value is very high, in fact higher than that of any other common fruit in its natural state.It is also important to note that banana could be obtained at anytime of the year, and at a cost per calory relatively lower than other fruits.The banana peel offers it almost complete protection knowing the condition in which most fruits are sold in our cities is certainly far from sanitary

(Myers and Rose, 1917)

The nutritional value of banana can be reviewed in it unripe and ripe stage. Ripe banana is one of our most valuable fruit. Banana may be used to advantage in dietaries of nephritic patients showing nitrogen retention, for the reason that ripe banana contains small amount of protein and large amounts of readily assimilable carbohydrates. Banana is a starchy food that contains a high proportion of indigestible compounds, such as resistant starch and non-starch polysaccharides. Unripe banana flour has been used as a food ingredient to make pasta (spaghetti) of high quality, on the basis of low-carbohydrate digestibility, and increased resistant starch and antioxidant phenolics contents. Nutritional composition, in vitro kinetics of starch digestion and antioxidant capacity were determined. The addition of banana flour increased the indigestible fraction and the content of phenolic compounds in the spaghetti. As a consequence of the compositional changes, a slow, low rate for the enzymatic hydrolysis of carbohydrates was observed. Moreover, banana flour spaghetti possessed increased antioxidant capacity **(Martinez et al, 2008)**

The nutritional value of a fruit as of any food depends on a wide range of variables, such as its ripeness, climatic condition under which it is grown, the quality of the soil and of particular importance for the banana, the quality of the cultivar **(Gowen, 1995).** The ripe fruit is a rich source of carbohydrates and a fair source of minerals and vitamins, particularly B-complex. The main principal sugars present in ripe fruit are sucrose, glucose, fructose and maltose http://www.health-galaxy.com/Banana-Nutritional-Value.html. Hundred grams of ripe Banana provides approximately 116 Kcal energy that makes it a supplementary staple food **(Sampath et al, 2012)**.An apercu of the nutritional value of banana per serving (100g) is provided below.

2.4. Nutrients in banana per serving (100g)

Nutrients can be extablished according to the state of the banana, that is ripe or unripe.

2.4.1. Banana nutritional value (Unripe banana):

Nutrients in banana	Amount per serving
Moisture:	60-72%
Protein:	1.0- 1.8%
Reducing sugar:	0.1- 0.2%
Non-reducing sugar:	0.5%
Fat:	24 .5%
Ash:	0.9- 1.36%
Calories:	Nil
Starch:	20.5%
Sodium:	67 per 100 gm
Potassium:	382 mg
Calcium:	10 mg
Iron:	0.6 mg
Phosphorus:	30 mg

http://www.health-galaxy.com/Banana-Nutritional-Value.html

2.4.2. Banana nutritional value (Ripe banana):

Nutrients in ripe banana	Amount per serving
Moisture:	60-79%
Protein:	0.4- 1.7%
Reducing sugar:	3.6- 24.6%
Non-reducing sugar:	14.6%
Fat:	16.4%
Ash:	0.7- 1.6%
Calories:	116 cal per 100 gm
Starch:	Nil
Sodium:	34.8 per 100gm
Potassium:	401 mg
Calcium:	9 mg
Magnesium:	28 mg
Manganese:	0.8 mg
Copper:	0.2 mg
Iron:	0.6 mg

Phosphorus:	31 mg
Sulfur:	10.0 mg
Chlorine:	125 mg
Silica:	23.8 mg
Iodine:	Minute quantity
Aluminum:	Minute quantity
Zinc:	Minute quantity
Cobalt:	Minute quantity
Arsenic:	Minute quantity

http://www.health-galaxy.com/Banana-Nutritional-Value.html

2.5. Health benefit of banana

Bananas health benefits are numerous and sustains the body in several ways

. **Healthy bowels**: Banana is Rich in pectin, bananas aid digestion and gently chelate toxins and heavy metals from the body Bananas act as a prebiotic, stimulating the growth of friendly bacteria in the bowel.

· **Cardiovascular health:** Banana is a good source of potassium. Potassium maintains normal blood pressure and heart function. One serving of banana contains 467 mg of potassium; this makes people to believe that one banana a day may help to prevent hypertension or even atherosclerosis.

· **Protection from strokes**: According to research in The New England Journal of Medicine, eating bananas as part of a regular diet can cut the risk of death by strokes by as much as 40%.

.**Protection from ulcers:** Banana has been used as an antacid to protect stomach against ulcers and ulcer damage. It is believed that banana allows a thicker mucus barrier against stomach acids and contains protease inhibitors

. **Improve blood pressure**: This unique tropical fruit is extremely high in potassium yet low in salt, making it perfect to beat blood pressure.

. **May boost mood**: Eating banana reduce PMS symptoms, which regulates blood sugar and produces stress-relieving relaxation due to its content in vitamin B6

http://foodmatters.tv/articles-1/25-powerful-reasons-to-eat-bananas

. **Boost energy:** They are full of nutrients for increased energy and vitality. Bananas contain three natural sugars - sucrose, fructose and glucose combined with fiber. Potassium is also essential for helping muscles to contract properly during exercise and reduces cramping up. A banana gives an instant, sustained and substantial boost of energy

· **Help reduce water Retention** as the potassium content in it will help eliminate fluid retention.

2.6. Fermented banana products

Fermentation is one of the most important technologies used in pre-industrial societies to transform agricultural and wild products into highly edible and nutritional products. The fermentation process is associated with a variety of attributes, some of the most important being the following: Preservation, Decreases spoilage, Decrease harmful attributes, including, Decrease harmful bacteria. There exists a great variety of fermented banana product that vary from beer to wine to bread in Africa (**http://dianabuja.wordpress.com/2012/06/16/banana-beer-and-other-fermented-drinks-in-africa/**).The development of the banana export industry at the end of the 19^{th} century has resulted in a surplus of fruit in producer countries because of the high standards impose on the appearance by the importing countries. **(S. Gowen, 1995).**This fruits could be processed in other products. A wide range of fermented products could be derived from banana for example:

2.6.1. Banana wine

Wine is an alcoholic beverage made by fermentation of fruit juice of ripe grapes using *Saccharomyces cerevisae;* other sugar rich fruits can also be used **(Awe et al., 2013)**

Banana wine is made by adding to banana pulp 100 ppm of SO2 in the form of potassium metabitesulfite (KMS) to suppress the yeast activity. Pectinase enzyme is added at the rate of 0.5% and after holding the same for 8-12h, the pulp is inoculated with *Saccharomyces cerevisiae var. ellipsoideus*. The fermentation is carried out at 22 ^{0}c for 7-10 days. After the completion of fermentation, clear liquid is filtered through a clean muslin cloth and stored in sterile bottles at 21-24 ^{0}C for a month when the suspended matter settles leaving clear wine on the top. The wine is further treated with bentonite and stored in bottles with SO2 as KMS. Bananas make a really versatile wine that can be consumed as is or blended with other wines to add body and a bit of flavor.

- **Dry banana wine**

The ingredient required here are peeled bananas, banana skins, white grape concentrate finely granulated sugar, citric acid, grape tannin, water, yeast nutrient white wine yeast . Banana is Mash and the skins are finely chopped, placing both in primary. Meanwhile, water is brought to boil and in it sugar is dissolved completely. Water is poured over fruit and skins and primary is covered. When cool, all remaining ingredients are added except yeast and stirred well to dissolve. Activated yeast is and primary is recovered. Fermentation is vigorously done for two days and strain through muslin into secondary. An airlock is attached and fermented to dryness. The top is racked and the air lock is refitted. 90 days is allowed for wine to clear. If it does not clear on its own, amylase is added according to its instructions. When clear, it is racked again, toped up and airlock refit. Aged 2 months, stabilized, and refit refitted after final 30 days, racked into bottles and allowed for 3 months rest before drinking **(Keller, 2006)**

2.6.2. Banana beer

It is made from bananas, mixed with cereal flour (often sorghum flour) and fermented to an orange, alcoholic beverage. It is sweet and slightly hazy with a shelf life of several days under correct storage conditions **(Ali, 2008).** There are many variations in how the beer is made. For instance, Urwaga banana beer in Kenya is made from bananas and sorghum or millet and Lubisi is made from bananas and sorghum. Ripe bananas (Musa spp.) are selected. In the rainy season unripe bananas can be left to finish ripening laid on a hurdle over the fire where the cooking is done. During the dry season bananas can be ripened by making a pit in the ground, covering the sides of the pit with green banana leaves, packing the bananas in to the pit and then covering them with banana leaves and earth. On one side of the pit a little ditch should be dug for a fire so that warmth and smoke can enter the pit. This takes about six days.The extraction of a high yield of banana juice without excessive browning or contamination by spoilage micro-organisms and proper filtration to produce a clear product is of great importance. Grass can be used to squeeze the banana so that only a clear juice is obtained. The residue will remain in the grass. One volume of water should be added to every three volumes of banana juice. This makes the total soluble solids low enough for the yeast to act. Cereals are ground and roasted and added to improve the color and flavor of the final product. The mixture is placed in a container, which is covered in polythene to ferment for 18 to 24 hours. The raw materials are not sterilized by boiling and therefore provide an excellent substrate for microbial growth. It is essential that proper hygienic procedures are followed and that all equipment is thoroughly sterilized to prevent contaminating bacteria from competing with the yeast and producing acid instead of alcohol. This can be done by cleaning with boiling water or with chlorine solution. Care is necessary to wash the equipment free of residual chlorine, as this would interfere with the actions of the yeast. Strict personal hygiene is also essential. The main micro-organism is *Saccharomyces cerevisiae var ellipsoideus* which is the same organism that is involved in the production of grape wine. After fermentation the product is filtered through cotton cloth.Packaging is usually only required to keep the product for its relatively short shelf life. Clean glass or plastic bottles should be used **(Borromeo, 2005).**

2.6.3. Fermented Banana Bread

Let bananas ripen to the point that the outer skins are completely black and the bananas are squishy. This is just before the point that they start to get mold. Flour, sugar, baking powder, baking soda, salt, fermented bananas, either shortening or butter or margarine milk, eggs are the ingredients. Mix together flour, sugar, powder, soda and salt. Add bananas, margarine and milk. Beat with electric mixer on low till blended, then on high for 2 minutes. Add eggs and remaining flour and beat till blended. Pour into greased 8x4x2 loaf pan. Bake 55-60 minutes or until tooth pick inserted in center comes out clean. Cool 10 minutes in pan, then Remove from pan and cool thoroughly

http://www.cs.cmu.edu/~mjw/recipes/bread/fermented-banana-bread.html

2.6.4. Banana yoghurt

Banana yoghurt is a product derived from the combination of yoghurt and banana puree added with some microorganisms and allowed to ferment for a given period of time. Addition of different fruit in yogurt manufacture has been attempted increasingly. The use of fruit in yogurt makes its more delicious. Banana yoghurt contains both the refreshing flavor of fruit and beneficial effect of yogurt as reported by **Yousef et al., 2013**. It could also be produced by the combination of banana puree and milk added with some lactic acid bacteria and allowed to ferment under known condition for a given time. There exist various formulations for example. **Cakmakci et al, 2010** produced probiotic yogurt made with banana marmalade (BM). They produced Yogurt from cow milk inoculated with yogurt cultures and probiotic cultures (*Lactobacillus acidophilus*, *Bifi dobacterium bifi dum*, and an equal mixture of the 2 strains), and then added 15% BM yogurt sample.

Chapter 3: Banana usage in alcoholic beverage making

3.1. Materials and methods

Long hand Cavendish banana (*Musa acuminate*), common name in Thailand kluai hom thong and short hand Latundan banana *(Musa acuminata × balbisiana)* common name in Thailand kluai namwa almost at the same stage of ripeness were purchased in a local market in Bangkok, Thailand. The bananas were washed using tap water in the laboratory and allowed to air dry. The two banana types were peeled off and 2000g of each banana pulp was weighed on a balance (zepper Es-3000H, 3000g x 0.1g). The pulps were chopped with a knife and ground in blender. Distilled water was added to the 2000g of pulp in a ratio of 1:2. Each type of banana paste/ distilled water mixture was put in a container and 0.005ml of pectinase (16000 PECTU/ml) was added. **Tapre and Jain, 2012** reported that the addition of pectinase enzymes in the preparation of wines served to reduce viscosity, facilitated pressing of fermented solids and in so doing increased the yield. The containers were covered and allowed to stand overnight at room temperature (25^0c).

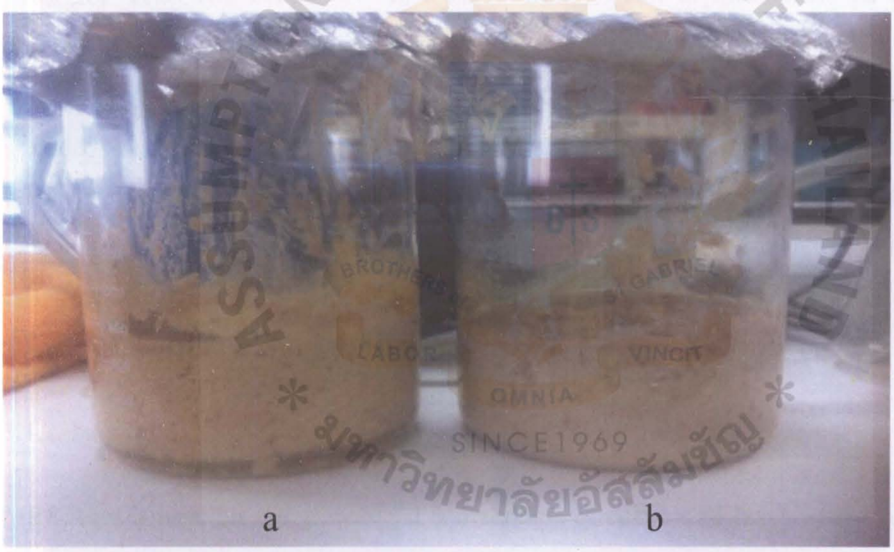


Figure1: Ground Banana in distilled water mixed with pectinase.

a) Long hand banana b) Short hand banana

The next day, we extracted the juice from the mixture by squeezing through a cheese cloth. Various parameters of the juice were measured prior to fermentation that is pH, TA, ^{0}Brix , aroma, color and the results are given in the table below.

Table 1: Parameters of Long and short hand banana extracts before fermentation

parameter	LHB extract	SHB extract
pH	4.58	4.28
TA g/100ml (Malic acid)	0.23	0.27
^{0}BRIX	15.1	15.1
aroma	++++	++
color	light yellow	dark yellow

+ indicates the intensity of the aroma

3.1.1 Wine yeast cultivation

Wine yeast was grown on YM agar which is a selective growth medium with low pH useful for cultivating yeasts, molds, or other acid-tolerant or acidophilic organisms, while determining growth of most bacteria and other acid intolerant organisms. It is Malt extract medium modified by the addition of Yeast extract and peptone. The YM of the name stands either for Yeast and Mold, or Yeast extract-Malt extract depending on the source. We prepared a 0.1% peptone solution. To 20ml of this peptone solution we added 0.5g of dry wine yeast. We prepare further decimal dilution to 10^{-3} and allowed to stand overnight. The next day we agitated the tube containing the mixture by means of a vortex and we plated 0.1ml on the YM agar which had previously been autoclaved and put in plates and allowed to solidify. The plates were then incubated at 37^{o}c for 48 hours. After determining the yeast cell growth in the medium, the cells were re-suspended in 10ml of distilled water. The banana pulp was inoculated at 2×10^{6} cells per ml.

3.1.2. Isolation and growth of lactic acid bacteria from yoghurt

MRS Agar is used for the growth and enumeration of cultures of *Lactobacillus* in dairy and other food products. The medium can be used to culture slowly-growing lactobacilli such as *Lactobacillus brevis* and *Lactobacillus fermentum*. Acidified to low pH, it can be used to enumerate *Lactobacillus bulgaricus* in yogurts. Selective media inhibit the growth of certain microbes by eliminating 'suspects'. This kind of media provides general information regarding the bacteria that are able to grow on this specialized type of agar **(Tami, 2012).** We purchased natural yoghurt from Food land (Yalida) containing probiotics i.e. life bacteria cultures of *lactobacillus bulgaricus* and *staphylococcus thermophilus*. We suspended 1g of yoghurt in MRS broth and we incubated at 37 ^{0}C for 24h. We prepare further decimal dilution to 10^{-3}. The next day we plated 0.1ml of the content of the broth on MRS agar plates which had previously been prepared and allowed to solidify and we incubated the plates again at 37^0 c for 48 hours until growth appeared. After determining the lactic acid bacteria cell growth in the medium, the cells were re-suspended in 10 ml of distilled water. The banana pulp was inoculated at 1.6 x 10^6 cells per ml.

3.1.3. Pitching and fermentation of the juice extracted

From each banana extract that is long and short hand respectively, we prepared 2 parts of volume 350 ml. To one part we added 0.5ml of yeast to the other we added 0.5ml yeast and LAB respectively. We monitored the evolution of various parameters during the fermentation. Fermentation end point was determined when the ^{0}Brix remained constant. It should be noted that this was done in duplicate.

Figure 2: Banana extracts

NB: SHB extract on the left and LHB extract on the right

Fermentation took about 8 to 10 days to be complete and the various results were obtained

3.2. Results and discussions

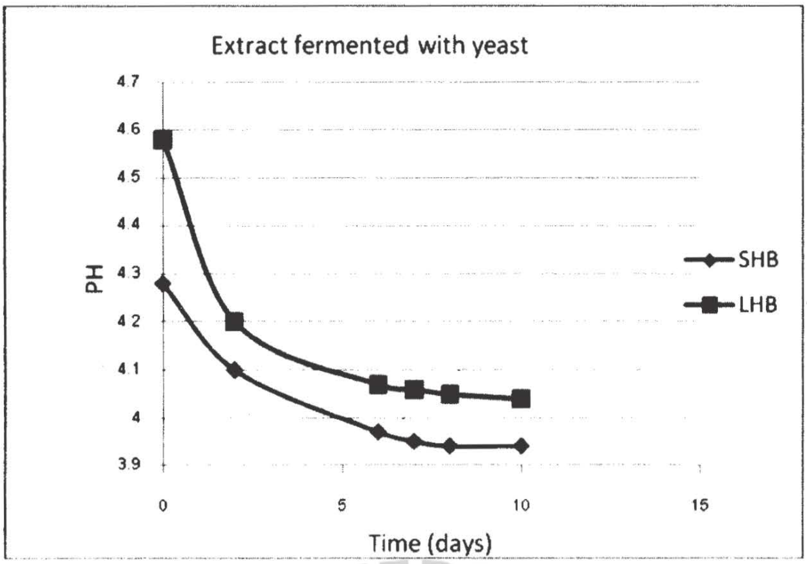


Figure 3: The change of pH with time of banana extract inoculated with yeast

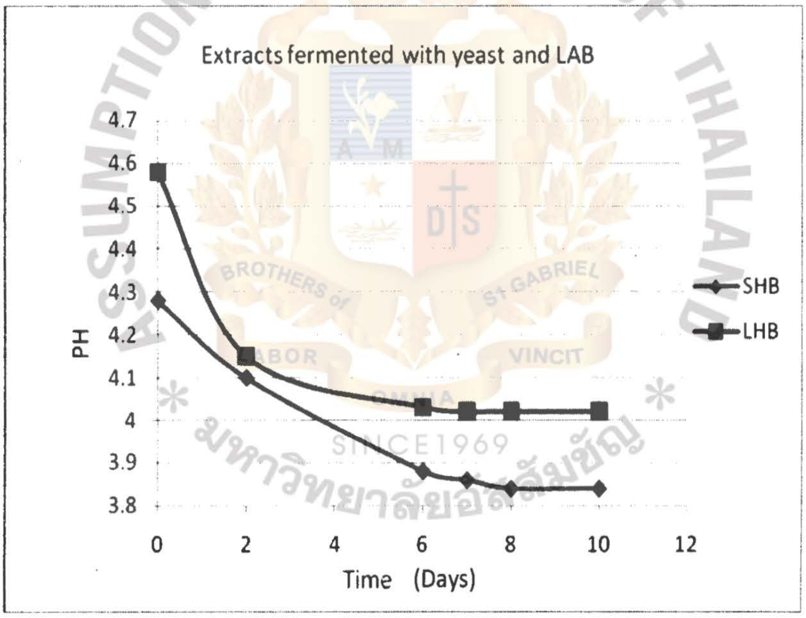


Figure 4: **The change of pH with time banana extract inoculated with yeast and LAB**

The graphs above show us that there is a decrease in pH of the fermented extracts with time. The rate at which pH decreases is faster in LHB extracts fermented by adding yeast and LAB than in SHB extracts fermented by adding yeast (4.04 and 3.94 respectively). Similar results were obtained for extracts fermented by adding both yeast and LAB (4.02 and 3.84).

When comparing both types of banana extracts, we saw that the pH of LHB banana extract in both fermentation were higher than those of the SHB. From a generally perspective, the pH decreased with fermentation time. The pH decrease is assumed to be correlated to the nitrogen consumption. pH is currently used in oenology as an indicator of different aspects, contamination risks, efficiency of sulphating, sensorial properties. Contaminations of grape musts or wines by bacteria or yeasts like *Brettanomyces* sp. are easier at high pH values and the prevention of contaminations by SO2 addition is inefficient when the pH value is too high. Acidity also plays an important role in sensorial analysis for wines and even if pH and acidity taste are not totally correlated, pH can give information on this organoleptic property **(Akin et al, 2008)**.

During alcoholic fermentation of musts, the conversion of substrates (sugars, organic acids, nitrogen) into metabolites such as ethanol and organic acids by yeasts *Saccharomyces cerevisiae* modifies the thermodynamic equilibrium in the medium and consequently the pH **(Akin et al, 2008)**.

Figure 5: The change of TA with time of banana extract inoculated with yeast

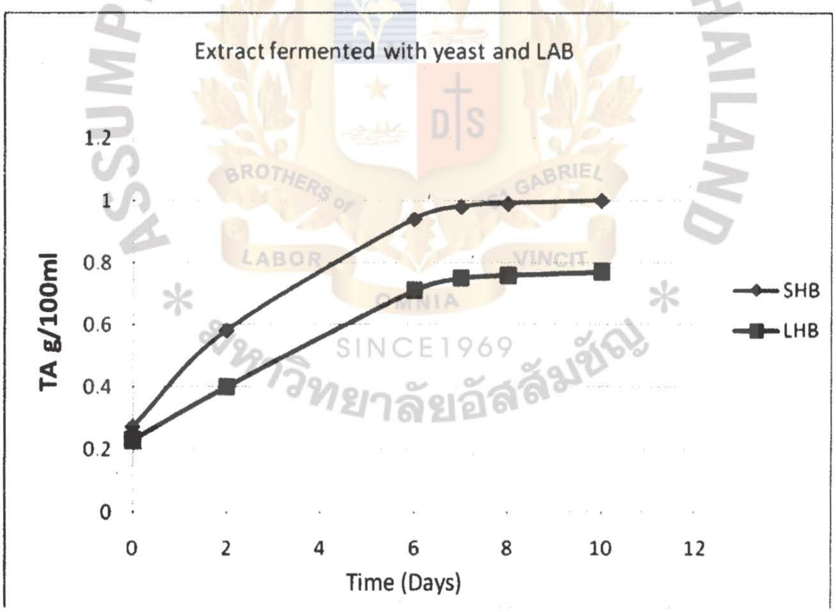


Figure 6: the change of TA with time of banana extract inoculated with yeast and LAB

TA increases with time as shown on the figure above. The increase in titratable acidity at early stage of fermentation was due to production of acid which accompanied the fermentation of sugar. The Constance or decrease towards the end of the fermentation is as a result of the acid being used up by the fermenting yeast to bring about desirable flavor in the wine **(omojasola *et al*, 2003).** TA of LHB extracts fermented by adding either yeast or both yeast and LAB were lower than those of SHB extracts. This might be due to the banana type used and its chemical composition. The drop in pH and corresponding increase in titratable acidity of the juice is attributable to yeast metabolism.

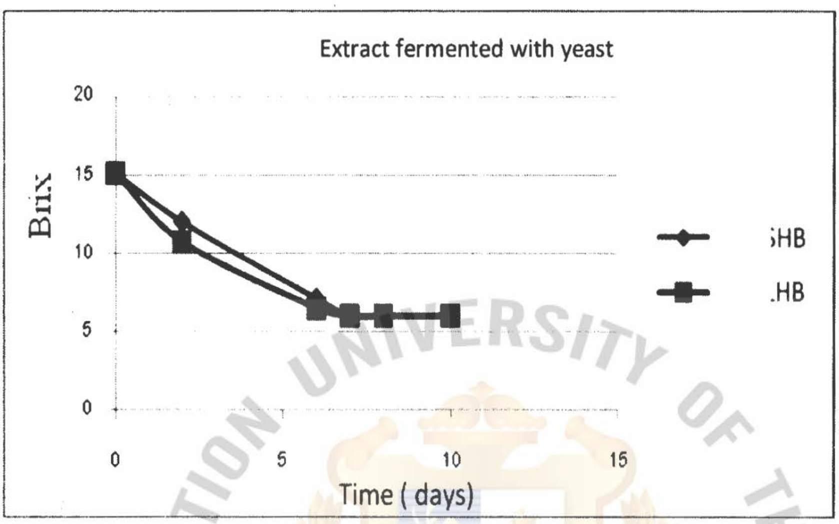


Figure 7: **The change of ^{0}Brix with time of banana extract inoculated with yeast**

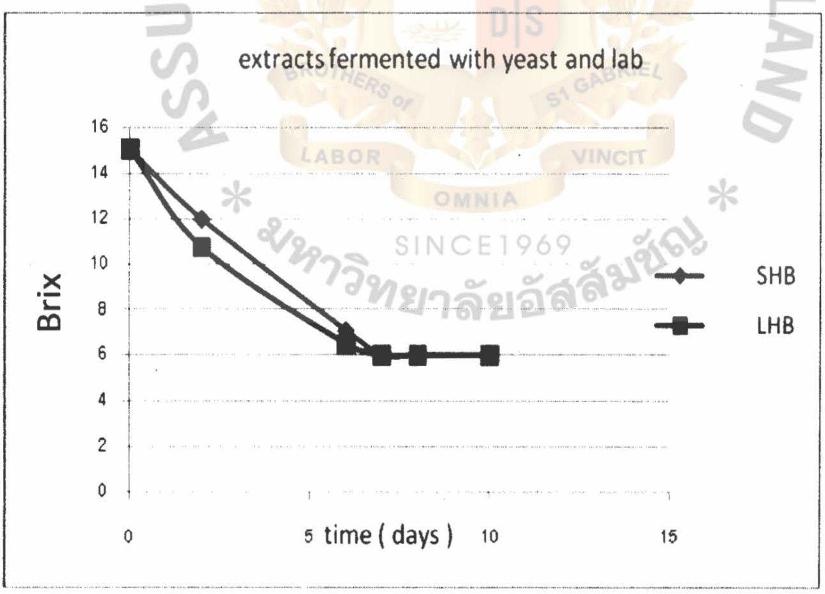


Figure 8: **change of ^{0}Brix with time of banana extract inoculated with yeast and LAB**

^{0}Brix decreases with time in all samples. This is simply due to the consumption of sugar by the microorganisms to produce alcohol and other sub products. The rate at which ^{0}Brix decreases was slightly faster in LHB fermented extracts than in SHB fermented extracts even though at the end, all fermented extracts had the same ^{0}Brix. It took about 7 to 10 days for the fermentation to be complete. This was determined by the constance in ^{0}Brix.

Table 2: Average values of parameters of long and short hand banana beverage (Mean± SD)

parameters	SHB/yeast	SHB/yeast/LAB	LHB/yeast	LHB/yeast/LAB
pH	3.94±0.057^a	3.85± 0.028^a	4.02±0.042^a	4.04±0.014^a
TA g/100ml	0.94 ±0.014142^a	0.95 ±0.014142^a	0.8±0.028284^a	0.77±0.113137^a
^{0}BRIX	6	6	6	6
ALCOHOL	6.45±0.071^a	6.±0.282^a	6.3±0.707^a	5.5±0.424^a
Color	Dark yellow	Dark yellow	Light yellow	Light yellow
Aroma	+	+	++	++

Same letters in same row or column means no significant difference

The Alcohol contents of the various fermented extracts given in the table 2 above show that yeast fermented extracts register higher alcohol content than yeast and LAB fermented extracts. This is attributed to the competition between yeast and LAB to consume the sugar molecules. Also, we found out that the SHB fermented extracts had a higher alcohol content than the LHB fermented extracts. The color of the LHB extract (light yellow) was more attractive at the end of the fermentation than that of SHB (dark yellow). This can be seen on **figure** above. The aromas of the LHB fermented extracts were fruitier and sweeter compared to those of the SHB fermented extracts. Mainly because of the sweeter Aroma of LHB fermented extracts, lower TA and higher pH, and its more attractive color we decided to further the study using the long hand banana.

3.3. Banana alcoholic beverage improvement

3.3.1 Preparation of banana juice

Long hand banana (*Musa acuminata*) commonly referred to as Cavendish group bananas was purchased from a local market in Thailand and washed. The banana was peeled off and by means of a balance (zepper Es-3000H, 3000g x 0.1g) we weighed 2000g. The banana pulp was then chopped by means of a knife and ground in a blender while adding mineral water (aura mineral water) to a total volume of 1L which corresponds to 50% 0f the weighed banana pulp. The mixture was then put in a container and 0.005ml of pectinase was added. One of the main hindrances in the production of highly desirable beverages is the pectinaceous nature of the banana fruit, which makes juice extraction and clarification very difficult. Commercial enzyme applications seem to be the major way forward in solving processing problems in order to improve banana juice and wine quality **(Byarugaba and William, 2008)**. We covered the container and allowed the mixture to stand for 24 hours at room temperature. The juice extraction was done by squeezing the mixture above through a cheese cloth. The same procedure was carried out as mentioned above but this time using distilled water as the solvent of extraction.

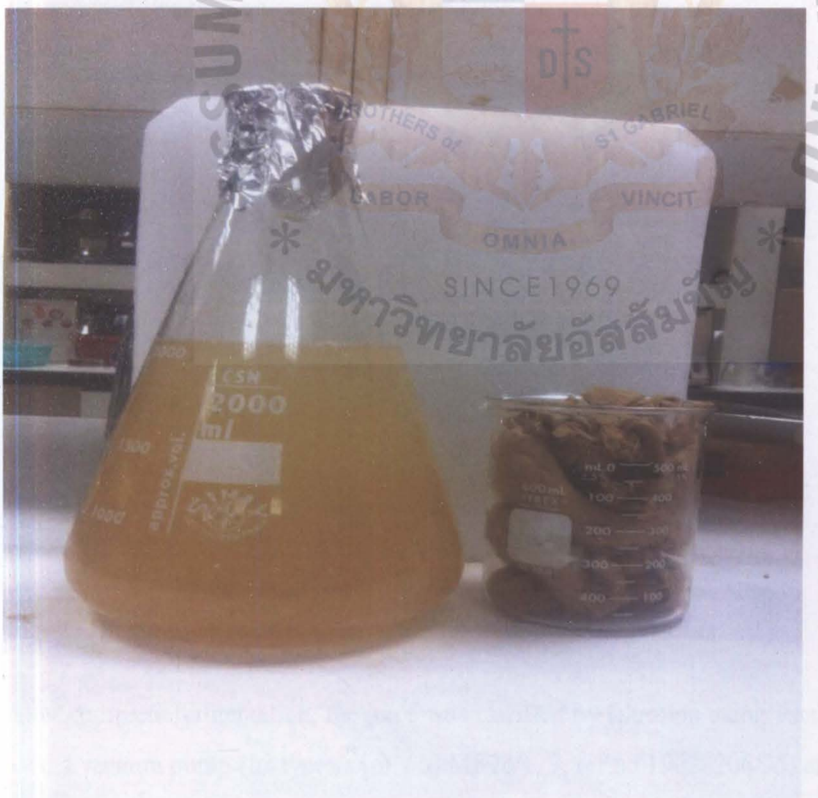


Figure 9: Banana juice extract and puree

Prior to fermentation of the must, some physicochemical properties were analyzed that is pH, soluble solid (^{0}Brix), TA, color, and aroma the results are shown in table below.

Table 3: physicochemical properties of the various recipes before fermentation of improved banana beverage extracts

samples	pH	^{0}BRIX	TA g/100ml	color
Banana/mineral water extract	4.54	15	0.22	Yellowish brown
Banana/distilled water extract	4.52	15	0.23	Yellowish brown

3.3.2. Pitching and fermentation of extracted juice

The experiment was carried out in duplicate. To 350 ml of the banana/mineral water extract, we added 0.5ml wine yeast and to another 350 ml of banana/ mineral water extract we added 0.5ml of wine yeast and lactic acid bacteria respectively. Similar inoculations were done with banana /distilled water extract. We let the juice ferment while controlling various parameters.

Figure 10: Banana extract after pitching with microorganisms

After complete fermentation, the juice was clarified by filtration using vacuum aspirator. We used a vacuum pump (Its types/s [m^3 / h] ME2c/1, 7, ser no 19028206/95) and what man filter paper 4 of pore diameter 110mn □ to get rid the suspended particles and consequently obtain a clearer solution.

Figure 11: Banana extracts after fermentation and clarification

3.3.3. Physicochemical analysis

I. pH: pH was measured by Electronic digital type Hana, meter No. H 8416 according to method **No. 981.12 AOAC (1990)**.

Table 4: pH of the various fermented banana extracts

Samples	**pH mean**
A	3.9
B	3.68
C	4
D	3.7

A: LHB/Distilled water/Yeast B: LHB/ mineral water/yeast

C: LHB/distilled water/yeast and LAB D: LHB/mineral water/yeast and LAB

II. Total Titratable Acidity: Acidity was measured by AOAC **Physico-chemical analysis** Method No.947.05 (1990).

III. Brix (Soluble solid): was measured with Brix refractometer with scale range of 0-32^0 Brix unit)

IV. Alcohol content: Alcohol percentage was determined by means of an Ebulliometry as outlined by Iland et al., 2000.

3.3.4. Consumer sensory testing for product development

A test was carried out to determine which product was the most liked.

3.3.5. Selection of the most likely preferred fermented banana beverage

The four types of fermented banana extract were used in other to find out which is the most preferred and eventually predict why.

Table 5: List of tasted samples formulations.

Set	sample	water	Microorganism
LHB	1	Mineral water	yeast
	2	Mineral water	Yeast/LAB
LHB	1	Distilled water	Yeast
	2	Distilled water	Yeast/LAB

Each sample was assigned with a 3 digit code and served in 3 oz. k-resin plastic cup at room temperature (25 ± 5^{0})

3.3.6. Panel test

Panel test was conducted in the sensory evaluation laboratory of Assumption university.30 panelist for each sample was asked to participate in the study. A 4- point hedonic scale questionnaire was used to score the liking of each sample. All the samples were served and placed in front of each panelist at the same time. Alongside we placed a cup of mineral water which we advised the panelist to rinse their mouth with before starting the test as well as in between two different samples to be tested. A form containing the instructions to follow was given to each subject. We asked the panelist to begin with the sample on their left and also that they could restart the tasting as often and they needed to. After they had finished tasting, they could start ranking the samples from most preferred (4) to least preferred (1).

3.3.7. Statistical analysis.

A preference test conduct on the banana beverage tells us which of the samples is mostly preferred and which was the least preferred.

3.4. Results and discussion

The changes in pH of the banana wines during fermentation are presented in the figure 12 and 13 below.

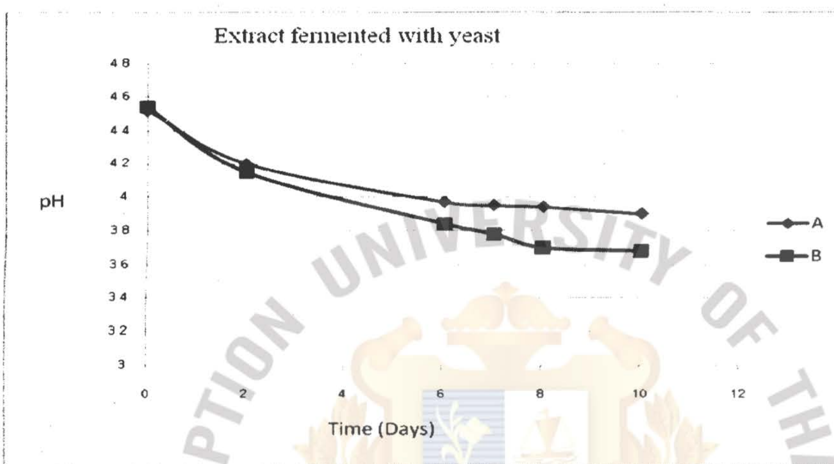


Figure 12: The change of pH in banana extract fermented by adding with yeast

A: banana/distilled water inoculated with yeast

B: banana/ mineral water inoculated with yeast

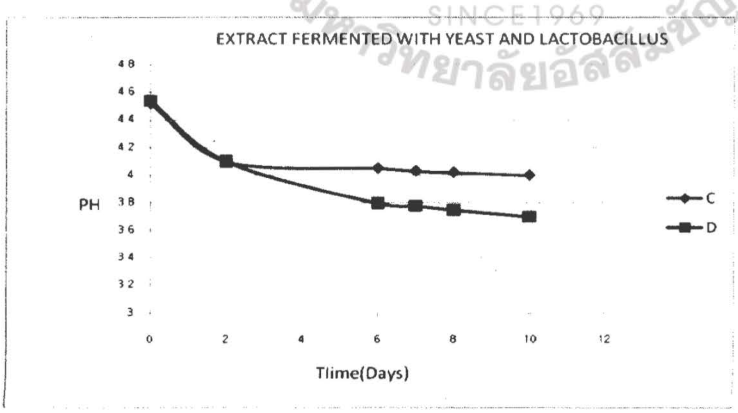


Figure 13: The change of pH of banana extract fermented by adding yeast and LAB

C: banana/distilled water extract inoculated with both yeast and LAB

D: banana/ mineral water extract inoculated with both yeast and LAB

It was observed that the pH values reduced with fermentation time apparently due to the production of acids from the fermentation. This result agrees with reports of **(Emmanuel and Ikenna, 2011)**.The initial pH was 4.52 for the banana/ distilled water extract and 4.54 for banana/ mineral water extract. At the end of the fermentation, the samples containing mineral water scored lower pH than those fermented with distilled water even though overall there was no significant difference in pH of the various products P> 0.05

The changes in TA of the banana wines during fermentation are presented in the figure 14 and 15 below.

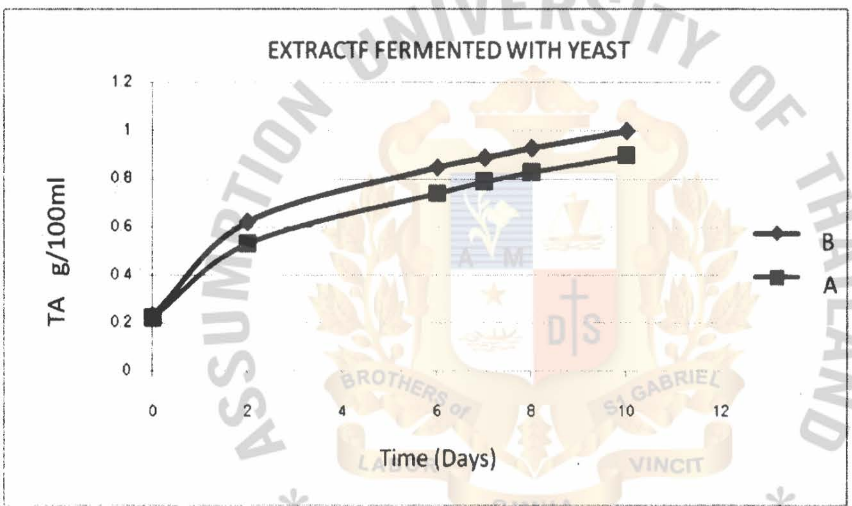


Figure 14: The change of TA with time of extract fermented by adding yeast

A: banana/distilled water inoculated with yeast

B: banana/ mineral water inoculated with yeast

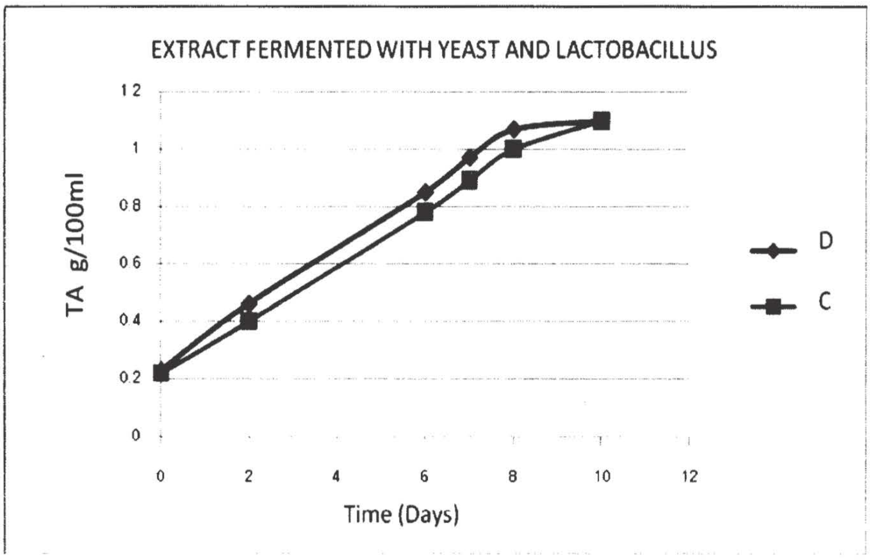


Figure 15: The change of TA with time of extract fermented by adding yeast and LAB

C: banana/distilled water extract inoculated with both yeast and LAB

D: banana/ mineral water extract inoculated with both yeast and LAB

It was observed that titratable acidity increased with period of fermentation. It was also observed that the samples fermented with both yeast and lactic acid bacteria had acidities higher than those fermented with yeast only. This could be due to the presence of a lactic acid fermentation arising from succession of the yeast cells by lactic acid bacteria during fermentation. There was no significant difference between the acidity of the various samples. A wine undergoing malo-lactic conversion will be cloudy due to the presence of bacteria and this can be observed on figure 9 above. It is a desirable phenomenon in wine production due to the attendant buttery flavor. Inoculating musts with yeast and lactic acid bacteria (LAB) concurrently in order to induce simultaneous alcoholic fermentation (AF) and lactic acid fermentation (MLF) can be an efficient alternative to overcome potential inhibition of LAB in wines because of high ethanol concentrations and reduced nutrient content (**Jussier et al., 2008).**

The changes in soluble sugar of the banana beverages during fermentation are presented in the figure 16 and 17 below.

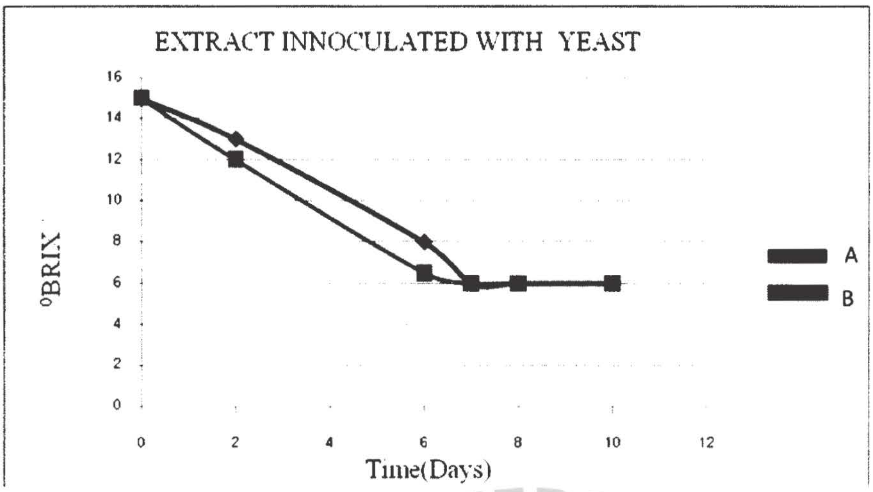


Figure 16: The change of °Brix in extracts inoculated with yeast

A: banana/distilled water inoculated with yeast

B: banana/ mineral water inoculated with yeast

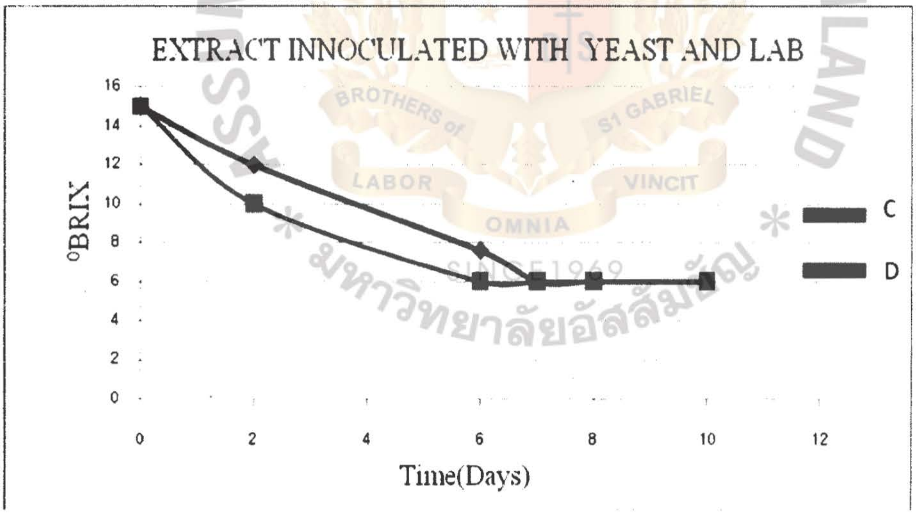


Figure 17: The change of °Brix in extract inoculated with yeast and LAB

C: banana/distilled water extract inoculated with both yeast and LAB

D: banana/ mineral water extract inoculated with both yeast and LAB

The above figures show us that ^{0}Brix decreases with time and the rate at which the ^{0}Brix (sugar content) decreases with time is greater for the sample C and D than for sample A and B. This may be due to both starter culture competing for nutrients thus rapid consumption of sugar molecules such as glucose unlike in the samples A and B which only contained yeast as the starter culture. There was also a slight reduction in fermentation time observed in A and C compared to sample B and D i.e. it took about 6 to 10 days to reach a constant sugar level unlike 8 to 10 days in the samples B and D this could be accounted for by the type of water (mineral water) that most have provided some kind of optimum condition for the cultures to grow and consume the sugar molecules (**http://www.eckraus.com/wine-making-stuck-9**). Minerals increase the yeast's ability to metabolize or consume sugar. Without a supply of minerals you have yeast that consume sugar at a slower pace. Using distilled water can cause big problems for the unsuspecting winemaker. There are two reasons for this. The first being distilled water has had all of the excess or free oxygen removed from it. The second reason is that distilled water has no minerals either. Both of these conditions are direct results of the distilling process and both conditions have inhibiting effects on fermentation (**http://www.eckraus.com/wine-making-stuck-9/).**

The alcohol percentages (%v/v) of the banana beverage after fermentation are presented in the Figure below.

Table 6: Alcohol content of various banana beverages

SAMPLES	ALCOHOL CONTENT (%v/v)
A (LHB/Distilled water/Yeast)	6.4%
B (LHB/ mineral water/yeast)	5.9%
C (LHB/distilled water/yeast and lab)	6.3%
D (LHB/mineral water/yeast and lab)	5.8%

It was observed that the sample inoculated with both yeast and LAB scored a little less alcohol content compared to those inoculated with only yeast (A 6.4%, B 5.9%, C 6.3%, D 5.8%) the slightly low alcohol content might be due to the combine effect of both yeast and LAB. lactic acid fermentation can occur spontaneously as a result of LAB that occurs naturally, or it can be induced with commercial starter cultures. Yeasts produce compounds i.e. ethanol, SO2 and medium chain fatty acids that inhibit the growth of LAB. Both yeasts and LAB compete with each other for nutrients thus leading to low alcohol content in samples inoculated with both starter cultures since the LAB consumes some of the sugar to produce lactic acid which of course would have been used by yeast to produce ethanol thus leading to a lower alcohol content. This agrees with **Heinrich and Neil, 2000**. But from an overall perspective there was no significant difference between the alcohol contents of the various samples $p \geq 0.05$.

We also observed a decrease in turbidity of the fermented liquids with times due to the sedimentation of the suspended particles which fall due to gravity on another hand, we realized that the color left from deep brown color to a more yellowish color as a result of the settlement of the suspended particles that created some turbidity thus blocking light from passing through. There was also a decrease in aroma of the various wines (sweetness) we could simply attribute this to the volatility of the sweet banana aromatic compounds. The aroma responsible for the flavor of fruits is highly susceptible to temperatures in storage. **(Facundo et al, 2012)**

Table 7: Average values of parameters of various improved banana beverages (Mean ± SD)

parameters	A	B	C	D
pH	3.45±0.212[a]	3.68±0.113[a]	3.7±0.283[a]	4±0.282[a]
TA g/100ml	1±0.141[b]	0.9±0.141[b]	1.1±0.354[b]	1.1±0.212[b]
BRIX	6	6	6	6
ALCOHOL%	6.4±0.566	5.9±0.141	6.3±0.283	5.8±0.141

Statistical analysis.

Overall liking score of each sample were analyzed by using spss trial version

Table 8: Mean of overall liking of each sample

samples	Mean ± S.D
A	2.33 ± 1.09334[a]
B	2.7 ± 1.02217[ab]
C	2.066 ± 1.11211[a]
D	2.9 ± 1.12495[b]

A: banana/distilled water extract fermented by adding yeast **B:** banana/ mineral water extract fermented by adding yeast **C:** banana/mineral water extract fermented by adding both yeast and LAB **D:** banana/ distilled water extract fermented by adding both yeast and LAB. Sample D was the most liked while sample C was the least liked. Results also revealed that there was no significant difference between A, B and C $p \geq 0.05$ meanwhile D was significantly different to A and C $p \leq 0.05$ but non significantly different to B $p \geq 0.05$

3.5. Conclusion

Banana is a fruit that can eventually be used to produce an alcoholic beverage without any adjustment of the ^{0}Brix and addition of preservatives. It took about 7-10 days for fermentation to be complete. . The combination of both yeast and LAB lead to a most appreciated beverage than that fermented by adding only yeast. The combination of both cultures in fermentation plays a great role in the improvement of the organoleptic properties of the banana beverage. Beverages produced recorded very high level Of TA ranges of 0.9-1.1 g/100ml.This high TAs might influence the taste of the beverage by increasing its sourness. The type of water used might also have a great impact on the quality of the drink. In the study above, we found out that all the samples fermented with mineral water where highly preferred compared to those fermented with distilled water. Also the combination of water type (Mineral water) and both starter cultures (yeast/LAB) lead to best result as liking the product is concerned. Banana/mineral water extract fermented by adding both yeast and lactic acid bacteria was mostly preferred compared to the others followed by banana/ mineral water extract fermented by adding yeast only. Statistical analysis showed some difference in the overall liking of the various fermented extracts $P < 0.05$ that is, the banana/mineral water extracts fermented by adding yeast and LAB was significantly different to the banana /distilled water extracts fermented by adding yeast or both yeast and LAB. The alcoholic beverages produced showed no appreciable differences in the tested parameters such as pH, Acidity, and total soluble solid left after fermentation and alcohol percentage $P > 0.05$.The beverages produces are very close to dry wine based on their physicochemical properties

Chap 4: Banana puree mixed yoghurt (Banana purero)

4.1 Materials and methods.

4.1.1. Preparation of puree.

Long hand ripe banana (*Musa acuminata*) was purchased in a local market in Thailand. The Bananas were peeled off and 2000g of the pulp was weighed. To the weighed pulp, 50% distilled water was added and the mixture was blended in a blender. After blending, 0.005ml of pectinase was added. The homogenized mixture was put in a container and allowed to stand for a day at room temperature. After 24 hours, the mixture was filtered using a cheese cloth to squeeze out the liquid in order to obtain the puree.

Figure 1: Banana puree

4.1.2. Preparation of starter cultures

Wine yeast cultivation as well as isolation and cultivation of LAB from yogurt were done as previously mentioned in section 3.1.1 and 3.1.2.

4.1.3. Preparation banana puree/ yoghurt mixtures

400 g of banana puree was weighed in a container and to it 400g of Bio Dutchie yoghurt natural flavor with live active lactic acid bacteria culture was added and homogenized with a stirring rod. To the 800g mixture of puree and yoghurt, we added 20% distilled water and heated at 90^0 c for 15 minutes on a hot plate while stirring continuously with glass rod. We then prepared 3 portions of weight 240 g each. To a portion, we added a 0.5ml of wine yeast *(Saccharomyces cerevisiae)*, to another 0.5ml of wine yeast *Saccharomyces cerevisiae* and LAB (isolated from yoghurt) respectively and to the third portion no microorganisms were added. Prior to fermentation, pH and TA of the mixtures were measured and recorded. We then allowed the mixtures to ferment at room temperature while observing the aroma until a presumed best aroma was attained. After 24 hours of fermentation. We then recorded values of pH and TA of the samples.

Figure 2: Banana puree/yoghurt texture

Figure 3: Banana puree/ yoghurt added with yeast.

4.2. Physicochemical Analysis

I. pH: pH was measured by Electronic digital type Hana, meter No. H 8416 according to method No. 981.12 AOAC (1990)

II. Total Titratable Acidity: Acidity was measured by AOAC Physico-chemical analysis Method No.947.05 (1990).

Table 1: Mean of pH and Titratable acidity of samples before fermentation

SAMPLE	pH	TA g/100ml (Malic acid)
Yoghurt (Yolida)	4.3	0.9
Puree (banana)	4.5	0.3
Puree/yoghurt	4.4	0.8
Puree/yoghurt/yeast	4.35	0.8
Puree/yoghurt/ yeast/LAB	4.35	0.82

4.3. Organoleptic evaluation

Analysis was carried out to rank the three products from most preferred to least preferred using 30 panelists randomly selected around the Assumption university campus. 1for most preferred, 2 for intermediate preference; 3 for least preferred. Also, flavor, texture and mouth feel was evaluated on a 7 point scale with 7 points for excellent quality & 1 point for bad quality.

4.3.1 Sensory evaluation for banana puree/yoghurt.

a) Aim

To determine whether significant difference exists between product samples.

b) Scope

Three types of banana puree/yoghurt were used, that is banana puree added only with yoghurt, banana puree added with yoghurt and yeast and banana puree added with yoghurt, yeast and LAB.

c) Methods

All products were self manufactured. Tasting took place in the sensory facilities of Assumption University, Huamak Campus. All assessors were randomly selected and made aware of what kind of products before taking part. Products were assigned random three-digit codes in order to eliminate bias. All products were stored in the refrigerator. The product samples were prepared immediately before assessment by putting a couple of spoons into serving cups. The served portions were all of a similar quantity.

Figure 4: presentation banana puree products serving for sensory evaluation

Participants were presented with a questionnaire, water to cleanse the palate and a tray with the three product samples, which were labeled with their three-digit codes. The order in which the participants were asked to taste the samples were altered so that each combination was used an equal number of times. Participants were asked to assess the 3 product samples and rank them according to overall preference, flavor, texture and mouth feel.

4.4. Results and discussion.

pH and titratable acidity of various samples were measured after complete fermentation.

Table 2: pH and Titratable acidity mean, color and texture of various samples after fermentation

SAMPLES	pH	TA g/100ml	color	Texture
Puree/yoghurt	4.26	0.8	Dark brown	Rough
Puree/yoghurt/yeast	4.18	1.1	Dark brown	Rough
Puree/yoghurt/yeast/LAB	4.15	1.2	Dark brown	Rough

We observed a decrease in pH with time. The initial pH values of the puree/ yogurt, puree/yoghurt/yeast, puree/yoghurt/yeast/LAB were 4.4, 4.35, and 4.35 respectively. After fermentation, these values were 4.26, 4.18, 4.15 respectively this values agree with **Cackmacki et al., 2010**.These various values fall within yoghurts pH range 4.1 to 4.9 as reported by **Jamie, 2010**. The pH of samples added with yeast and LAB were lower than those added with yeast only which were is return lower than those in which no microorganisms where added at all. This rate can only be attributed to the presence and growth of microorganisms. Inversely, we observed an increase in titratable acidity of puree/yoghurt/yeast/LAB and puree/yoghurt/yeast with fermentation time mean while that of banana puree/yoghurt stayed relatively constant. The decreased in pH may be attributed to the increasing percentage of lactic acid and yeast during fermentation. Acidity changes could be evaluated as an indirect Characteristic of the growth of lactic acid bacteria **Shima et al., 2012.** Yeast and LAB are responsible for the post acidification of yogurt. Titratable acidity of the mixtures were respectively 0.8, 1.1 and 1.2 as shown in table 2 above this acidities fall within the acidity range of pure yoghurt. In yoghurt production, According to the prevailing Physical, standards of yoghurt, final acidity vary between 0.7% (IDF, 1992) to 0.9% LA (FDA, 1996). FSSA (2006), India requirement is to have 0.85% to 1.2% LA during the shelf life of yoghurt. Acidity influences the quality attributes of yoghurt such as flavor, texture, whey syneresis, shelf life etc **(Narayana and Gupta, 2013)**. PH range for yogurt is pH 4.25 to 4.5. We see that our products quite respect those boundaries of pH and acidity. Banana puree/ yoghurt mixtures added with starter cultures scored higher TA than the ones in which no microorganisms was added. This indicated that the microorganisms play a crucial role in acid production by using the substrates in the puree/yogurt mixture to produce more acids. The presence of LAB produces even more acids.

Table 3: Participant ranking of the 3 products

	Banana puree/yoghurt	Banana puree/yoghurt/yeast	Banana puree/yoghurt/yeast/LAB
Rank sum	85	71	59
Mean ±SD	2.833 ± 1.262^{b}	2.366 ± 1.129^{ab}	1.9667 ± 0.128^{a}
Overall ranking	3rd	2nd	1st

Table 4: Statistically significant results

		Banana Puree /yoghurt	Banana Puree/yoghurt/ yeast	Banana Puree/yoghurt/yeast/LAB
	Rank sum	85	71	59
Puree/yoghurt	85	X	NSD	SD
Puree/yoghurt/yeast	71	NSD	X	NSD
Puree/yoghurt/yeast/ LAB	59	SD	NSD	X

SD = Significant Difference NSD = No significant Difference

The results above show us that there is a preference for the banana puree/ yoghurt added with both yeast and LAB followed by banana puree/ yoghurt added with only yeast and finally banana puree Added with yoghurt only. Banana puree/yoghurt added with no microorganism was the least preferred. This could be attributed to the presence of the microorganisms that might have developed some particular flavor, texture or mouth feel. These starter culture can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Examples are lactic acid bacteria that produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, or useful enzymes **(Lero and Vuyst, 2004).** Lactic acid bacteria (LAB), with trophic and non-trophic relationships, produce important flavor components. Flavor compounds are key elements for consumer acceptance and product identification **(Rehman et al., 2006).** Even though banana puree/yoghurt added with both yeast and lactic LAB was the most preferred in terms of ranking, yet statistical analysis revealed no significant difference with banana puree/yoghurt added with yeast only $P > 0.05$ but there is a significant difference with banana puree/yoghurt added no microorganisms $p<0.05$. This difference could be attributed to the presence of microorganisms that most have had some beneficial influence in the development of some aromatic compounds as well as textural composition of the mixture. There was no significant difference between banana puree/yoghurt added with yeast and banana puree/ yoghurt in which no microorganism was added $p>0.05$. Because of the presence of Malic acid in banana LAB might contribute in the flavor complexity of the product by inducing a malo-lactic fermentation **(Plessis and Jolly, 2000).** This might confirm the fact that the combination of both microorganisms in banana Puree/ yoghurt/ yeast/LAB leads to its preference.

Table 5: Average values of flavor, texture and mouth feel of various banana yoghurt recipes.

	Mean ± SD		
Banana Samples	Flavor	texture	Mouth feel
puree/yoghurt	1.86 ± 0.99^{a}	2.00 ± 0.53^{a}	1.75 ± 1.04^{a}
Puree/yoghurt/yeast	3.00 ± 1.28^{ab}	2.75 ± 1.28^{ab}	2.86 ± 0.83^{b}
Puree/yoghurt/yeast/LAB	3.25 ± 1.39^{b}	3.13 ± 0.99^{b}	1.75 ± 0.71^{a}

Same letters in the same row/column means no significant difference at $p>0.05$

From the above table we see that there is no significant difference between flavor of banana/yoghurt/yeast and banana/yoghurt/yeast/LAB $p> 0.05$ but there is a significant difference between flavor of banana/yoghurt and banana/yoghurt/yeast/LAB $P<0.05$. When we get to comparing means of flavors, we see that, that of banana/yoghurt/yeast/LAB was the highest indicating that it was the most preferred flavor followed by banana/yoghurt/yeast. This could only be accounted for by the combination of both yeast and LAB. There exist a significant difference between the texture of banana/yoghurt/yeast/LAB and that of banana/yoghurt $P<0.05$. When comparing mean, the texture of banana/yoghurt/yeast/LAB was preferred to the other two products even though statistically speaking, there is no textural difference between banana/yogurt/yeast and banana/yoghurt/yeast/LAB. Banana puree/yoghurt showed no significant difference to banana/yoghurt/yeast/LAB $P>0.05$. We can only attribute this preference to both types of microorganisms used. In general, the associations of yeasts and LAB are of key importance for a broad range of fermented foods. However, surprisingly, little information is available about molecular-interaction mechanisms **(Sieuwerts et al., 2008)**. There was a significant difference between the mouth feel of banana/yoghurt/yeast and the other banana puree/yoghurt products $p<0.05$.The mouth feel of the banana/yoghurt/yeast was highly preferred to the other two products. The sole presence of yeast might be at the origin of the most preferred mouth feel. Maybe when both Yeast and LAB are present there exist competitions between the two microbes which prevent the yeast from fully expressing its self in the development of mouth feel. Yeast autolysis also releases substances that enhance the mouth feel, color and flavor of food products **(Dharmadhikari, 1995)**. The reason for this difference still needs to be explored.

3.5. Conclusion:

There was an order of preference amongst participants and that order was 1st: Banana puree/yoghurt/yeast/LAB, 2nd: Banana puree/yoghurt/yeast/, 3rd: Banana puree/yoghurt. The results showed us that there is a significant difference between banana puree/yeast/LAB and banana puree/yoghurt $p<0.05$.There is a significant difference between flavor and texture of Banana puree/yoghurt/yeast/LAB and Banana puree/yoghurt $p < 0.05$. We could presume that Banana puree/yoghurt/yeast/LAB was mostly preferred because of the combination of both yeast and LAB that might have contributed in the establishment of a particular flavor and texture. Mouth feel of all the products compared to texture and flavor were low. This can be seen by the mean values recorded in table 5 above. The mouth feel were low maybe due to astringency which might have been developed during heating of the banana puree as mentioned in the method. If it's the case then it would agree with **Mendoza et al, 1992.**

4.6. Banana puree/ yoghurt added with yeast and LAB first improvement

From the above results we found out that banana puree mixed with yoghurt and added with yeast and LAB was highly preferred to the others so we decided to improve this sample.

4.6.1. Materials and methods

After obtaining the puree as previously mentioned, we weighed 400 g of the puree in which we added 400g of bio Dutchie yoghurt (1:1) making a total weight of 800g. The mixture was homogenized by means of a blender.

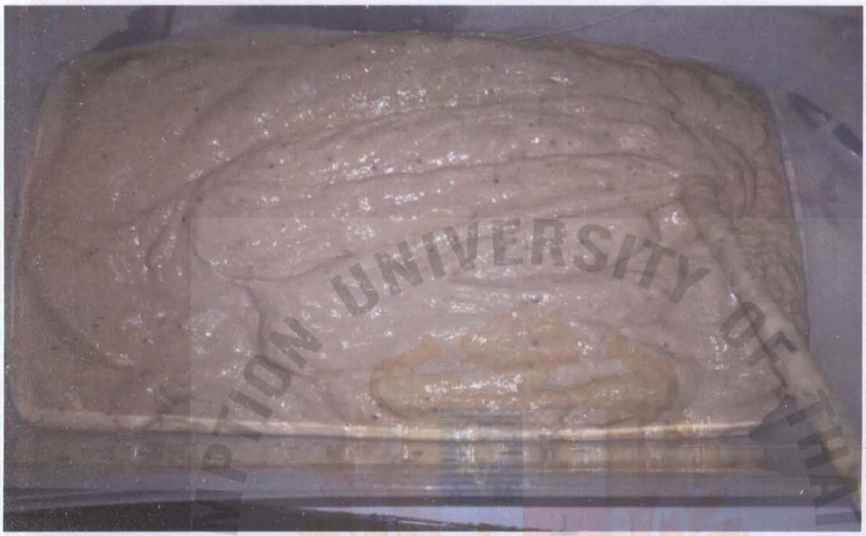


Figure 5: Banana puree mixed with yoghurt and homogenized in blender

We then prepared three equal portions of weight 240g.To one portion we added 40 % mineral water to another 40% distilled water and to the last portion, 40% milk. To every sample we added a 0.5ml of yeast and LAB respectively cultured on YM and MRS agar respectively. We should note here that the LAB was isolated from yoghurt containing live active bacteria.

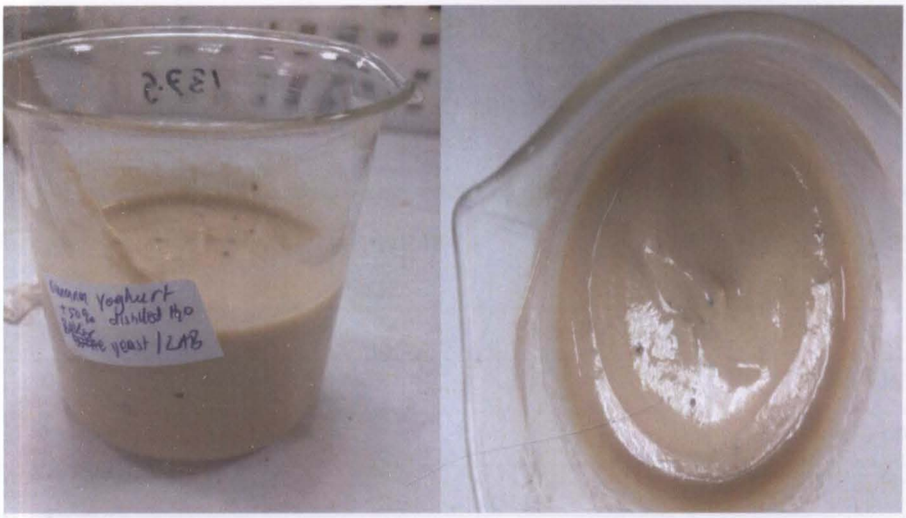


Figure 6: Banana puree mixed with yoghurt and added with water, yeast and LAB

The various samples were allowed to stand at room temperature for 24 hours, after which we carried a consumer test to pull out the best product.

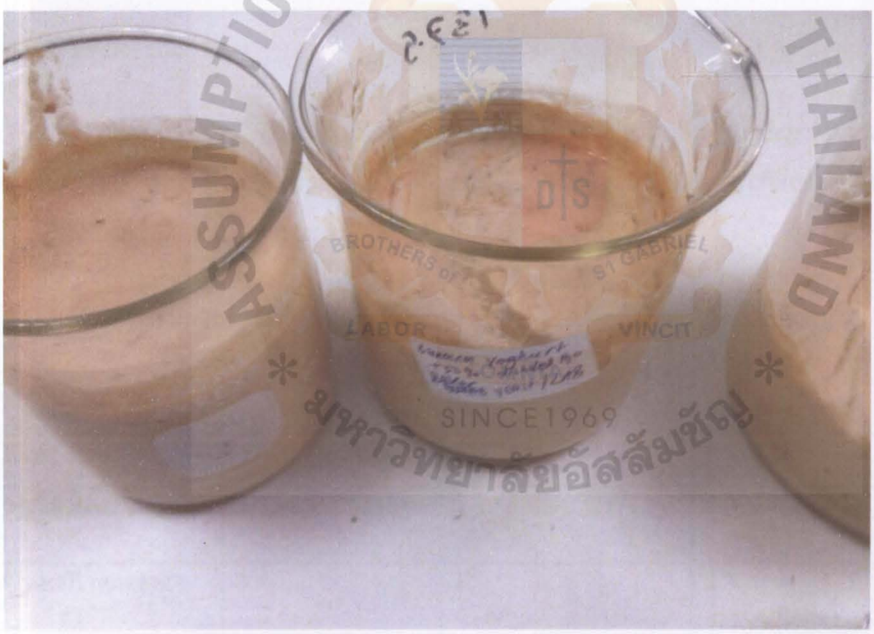


Figure 7: Appearance of products after 24 hours fermentation at room tempera

4.6.2. Physicochemical properties

pH, TA and color were measured. Results are given in the table below.

Table 6: pH and TA mean and color of the first improved banana puree/yoghurt products after overnight fermentation

sample	pH	TA (g/100ml)	color
A	4.22	1.5	Whitish Brown
B	4.1	1.3	Whitish brown
C	4.09	1.4	Whitish Brown

A: Banana puree/yoghurt/ milk/yeast/LAB

B: Banana puree/yoghurt/mineral water/yeast/LAB

C: Banana puree/yoghurt/distilled water/yeast/LAB

4.6.3. Preference test:

We carried out a preference test as previously mentioned above using 30 judge panelists. Participants where as to rank the product according to their preference that is 1=most preferred and 3= least preferred

Table 7: Participant ranking of the first improved banana purees products

	A	B	C
Rank sum	67	52	61
Mean ±SDV	2.23 ± 0.85836	1.70 ± 0.70221	2.07± 0.82768
Overall ranking	3^{rd}	1^{st}	2^{nd}

A: Banana puree/yoghurt/ milk/yeast/LAB

B: Banana puree/yoghurt/mineral water/yeast/LAB

C: Banana puree/yoghurt/distilled water /yeast/LAB

Table 8: Statistically significant results of first improved banana puree/yoghurt products

Samples		A	B	C
Samples	Rank sum	52 least liked	67 Most liked	61
A	67 least liked	X	SD	NSD
B	52 Most liked	SD	X	NSD
C	61	NSD	NSD	X

SD = Significant Difference NSD = No significant Difference

A: Banana puree/yoghurt/Dutch milk/yeast/LAB **B:** Banana puree/yoghurt/mineral water/yeast/LAB **C:** Banana puree/yoghurt/distilled water/yeast/LAB

Table 9: Average values of flavor, texture and mouth feel of the first improved banana puree yoghurt products.

	Mean ± SDV				
SAMPLES	COLOR	FLAVOR	TEXTURE	MOUTHFELL	OVERALL
A	4.33 ± 1.10^{a}	3.87 ± 0.97^{b}	4.13 ± 1.04^{c}	4.00 ± 1.17^{d}	3.93 ± 0.87^{e}
B	4.53 ± 1.07^{a}	3.97 ± 0.99^{b}	4.37 ± 0.89^{c}	4.10 ± 0.82^{d}	4.33 ± 0.76^{e}
C	4.43 ± 0.99^{a}	3.90 ± 1.06^{b}	3.83 ± 1.09^{c}	4.03 ± 0.99^{d}	4.13 ± 0.86^{e}

Same letters in same column means no significant difference $p>0.05$

A: Banana puree/yoghurt/Dutch milk/yeast/LAB,

B: Banana puree/yoghurt/mineral water/yeast/LAB,

C: Banana puree/yoghurt/distilled water/yeast/LAB.

From the above results product B was the most preferred in all aspects that is overall linking, color, flavor, texture and mouth feel. We decided to further the study using sample B **(Banana puree/yoghurt/mineral water/yeast/LAB)**.

4.7. Banana puree/ yoghurt added with yeast LAB second improvement.

From the above results we realized that sample B (Banana puree/yoghurt/mineral water/yeast/LAB) was the most preferred of the entire samples, so we decided to improve its quality by using a different formulation. We weighed 400g of banana puree which we added to 400g of yoghurt and we blended. To the mixture we added 40% mineral water (320ml of mineral water).We then prepared 4 equal portions of this mixture (240g per portion). To one portion we added 0% sugar to another 2% (4g) sugar, 4% (8g) and 6% (12g) sugar respectively. To each portion we added a 0.5ml of yeast and LAB respectively and allow the whole mixture stand overnight at room temperature. The next day we measured certain parameters such as pH and TA and next we proceeded to a consumer test to assess the overall likeness, color, flavor texture, mouth feel and sweetness of the products using a 7 point hedonic scale.

3.7.1. Results and discussions.

Table 10: pH and TA mean values and color of the Second improved banana puree/yoghurt products

samples	pH	TA (g/100ml)	color
D	4.15	1.5	Whitish Brown
E	4.12	1.3	Whitish Brown
F	4.08	1.08	Whitish Brown
G	4.04	1.03	Whitish Brown

D: Banana puree/yoghurt/mineral water/microorganism/0%sugar

E: Banana puree/yoghurt/mineral water/microorganism/2% Sugar

F: Banana puree/yoghurt/mineral water/microorganism/4% sugar

G: Banana puree/yoghurt/mineral water/microorganism/6% sugar

The pH ranged between 4.04 and 4.12. The lowest pH 4.04 was for the sample added with 6% sugar and the highest pH 4.12 was for the sample in which no sugar was added. TA ranged between 1.03 and 1.5.The highest pH was for the sample in which no sugar was added and the lowest was for sample added with 6% sugar. The pH and TA range agree with those of **Yousef et al., 2013**.The higher the sugar concentration the lower the pH and TA.

Tablel 11: Average values of flavor, texture and mouth feel of the second improved banana puree/ yoghurts products.

SAMPLES	COLOR	FLAVOR	TEXTURE	MOUTH FEEL	SWEETNESS	OVERALL
D	3.77±1.43^{a}	3.23±1.22^{a}	3.50±1.50^{a}	3.53±1.28^{a}	2.90±1.125^{a}	3.23±1.22^{a}
E	3.60±1.40^{a}	3.53±1.55^{ab}	3.47±1.36^{a}	3.43±1.41^{a}	3.33±1.45^{ab}	3.43±1.55^{ab}
F	3.73±1.44^{a}	3.43±1.41^{ab}	3.50±1.46^{a}	3.53±1.43^{a}	3.66±1.47^{ab}	3.43±1.40^{ab}
G	3.53±1.41^{a}	3.87±1.63^{b}	3.53±1.59^{a}	3.63±1.45^{a}	4.00±1.68^{b}	3.87±1.63^{b}

Same letters in same column means no significant difference p>0.05

D: Banana puree/yoghurt/mineral water/microorganism/0%sugar

E: Banana puree/yoghurt/mineral water/microorganism/2% Sugar

F: Banana puree/yoghurt/mineral water/microorganism/4% sugar

G: Banana puree/yoghurt/mineral water/microorganism/6% sugar

In this study, we found out that higher concentrations of sugar inhibit TA production. The reason for which sugar inhibits TA production remains to be explored. We also observed that higher sugar concentrations increased the overall likeness of the product.

The consumer test revealed that the sample added with 6% sugar was the most liked followed by those added with 4%, 2% and finally 0% sugar but the statistical analysis revealed no significant difference between the overall ratings of the products P > 0.05. We observed that there was no significant difference in color, texture and mouth feel of the various products p>0.05 but there was a significant difference in flavor and sweetness of the product added with 6% sugar and that added with 0% $p<0.05$.There was no significant difference between 6%, 2% and 4% sugar $p>0.05$. We can presume that sugar enhances flavor.

3.8. Comparison between various improved banana puree/yoghurt products

The most preferred sample at each stage of production were compared

Table 12: Comparison between various improved products

					Mean ± SDV	
samples	pH	TA (g/100ml)	COLOR	TEXTURE	FLAVOR	MOUTH FEEL
1^{ST}	4.15	1.5	Dark brown	rough	3.25 ± 1.39^a	1.75 ± 0.71^a
2^{nd}	4.1	1.3	Light brown	smooth	3.97 ± 0.99^b	4.10 ± 0.82^b
3^{rd}	4.04	1.03	Light brown	smooth	3.87 ± 1.63^b	4.00 ± 1.68^b

1^{ST}: banana puree/yoghurt/yeast and LAB

2^{nd}: Banana puree/yoghurt/ mineral water/yeast and LAB

3^{rd}: Banana puree/yoghurt/ mineral water/yeast and LAB added with 6% sugar

From the above table, we see that the pH value of first sample is higher than that of the second which is in return higher than the third. This result can be attributed to the effect of mineral water in the second and the combined effect of both mineral water and sugar in the third. The titratable acidities of the first and second samples are not quite different that is 1.5 and 1.3 g/100ml respectively. We also observe that the TA of the first and second sample are higher than that of the third sample1.03g/100ml.The low TA Of the third sample could be attributed to the addition of sugar that most have exerted and osmotic stress on the lactic acid bacteria thus reducing their efficiency in converting sugar molecules into Lactic acid and consequently reducing the TA. According to **Guven and karaca, 2002**, “It was concluded that an increase in sugar content in frozen yogurt had slowed down the activation of microorganisms and prevented any increase in acidity”. When we turn to compare the colors of all samples as seen on the figure above, we observe that the color of the first sample is less attractive (dark brown) compared to the others that reveal a light brown color. This dark brown color could be a result of the heating of the banana puree. Heating might have induced caramelization of the sugar thus causing a dark brown color unlike in the other samples were heat was not applied. Heating puree to 90^0C inactivated PPO and reduced browning in puree and juice, especially in puree from more mature bananas which had greater PPO activity **(Sims et al., 2007).** But in our study, we found out that heating of the first sample increased browning. We can only attribute browning to the effect of heat on the sugars. Caramelization occurs during dry heating and roasting of foods with a high concentration of carbohydrates (sugars). We observe a lack in color uniformity of the first sample compared to the improved ones. This could be attributed to the poor homogenization done by stirring using a rod unlike in the improved samples whereby homogenization was done by means of a blender.

Figure 8: Comparing texture of banana puree/yogurt before and after improvement

Looking at the textures of the various samples as shown on the pictures above, we realize that the first sample shows a rough and non uniform structure compared to the other samples that show a smooth and more uniform structure. The roughness and non uniform structure could be attributed to the lack of homogenization of the mixture in a blender. The fact that not enough liquid was added to the first sample that is 20% compared to 40 % in improved samples might have also contributed to a much harder and rough texture. There is a significant difference in texture $p > 0.05$.When we compare the mean values of flavor of all three samples, we see that the flavor of the first sample was the lowest while those of the second and third are higher and almost similar (the results are shown in the table above). We could attribute this to the type of water used in the preparation. In the first sample we used distilled water and in the others we used mineral water. We could assume that minerals present in the water contributed to the development of a much better flavor in the second and third sample unlike the first in which distilled water (water in which most of its impurities has been removed by distillation) was used. Finally the mouth feel reveals a significant difference. The first sample is highly disliked compared to the others which are almost similar. Again we could only attribute this to the roughness and hardness of the first sample in which heat was applied and less liquid was used or astringency caused by heating.

4.9. General Conclusion

Banana puree can successfully be used to develop a new product if mixed with yoghurt and fermented by adding both yeast and lactic acid bacteria. When grading the products in term of liking, there is a significant difference between the products fermented by adding yeast only compared to the combination of both yeast and LAB which may imply there is a synergy between both microbes that contribute to a better product quality.

When banana puree is mixed with yoghurt homogenization by means of a blender is necessary so as to ensure a smoother and uniform texture unlike homogenizing by means of a stirrer (spoon or piston). Heating of the banana puree to soften its texture before adding in yoghurt could not be a good solution because we saw that the heat might influence the color and texture in a negative way since it might lead to a darker and less appreciated color and also a non uniform texture of the product. In the future, it would be interesting to find the optimum heating temperature in case we would like to use heat. The dark color might be due to caramelization of sugar and the poor texture might be due to the formation of clumps by agglomeration. Addition of a liquid (water) in a reasonable proportion ensures a better texture and appearance of the product. It should be noted here that the type of water used plays an important role in the product. When comparing the flavors of the products in which mineral or distilled water was used, we realize that those in which mineral water was used always had a higher score.

In the first step of our improvement we found out that overall, there was no significant difference between **B:** Banana puree/yoghurt/mineral water/yeast/LAB **and C:** Banana puree/yoghurt/distilled water/yeast/LAB $p>0.05$ but when we compared overall consumer liking, we decided to proceed the improvement using samples added with 40% mineral water because it scored the highest mean overall.

Addition of sugar also contributes to a better flavor of the product .Consumers were sensitive to the sweet taste. In the second part of our improvement when using banana puree mixed with yoghurt added with 40% mineral water and 0,2,4 and 6% sugar respectively, we found out that there was a significant difference between 0% sugar 6 % sugar $p<0.05$.

We can say that the best ingredients so far in the development of our new product “banana purero” are Banana puree, yoghurt, mineral water, yeast, lactic acid bacteria and sugar we successfully developed a new product from banana puree that could possibly attract a reasonable group of consumers.

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APPENDIX A

MEDIA FORMLATION

LACTOBACILLI MRS AGAR

Formula / Liter

Enzymatic Digest of Animal Tissue....................................10 g

Beef Extract ....10 g

Yeast Extract ....5 g

Dextrose ....20 g

Sodium Acetate .... 5 g

Polysorbate 80 .... 1 g

Potassium Phosphate ....2 g

Ammonium Citrate .... 2 g

Magnesium Sulfate .... 0.1 g

Manganese Sulfate .... 0.05 g

Agar ....15 g

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Directions

Suspend 70 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121 ^{0}C for 15 minutes

Source: De Man. J.C et al., 1960 Lactobacillus Broth acc, Appl. Bact. 23. 130-135

Yeast Mold agar (YM)

Formula / Liter

Yeast Extract... 3.0 g

Malt Extract... 3.0 g

Peptone.. 5.0 g

Dextrose.. ..10.0 g

Agar.. 20.0 g

Direction

. Suspend the powder in 1 L of purified water:. Heat the agar medium with frequent agitation and boil for

1 minute to completely dissolve the powder. Autoclave the agar or broth media at 121 ^{0}C for 15 minutes.

Source: Difco™ & BBL™ Manual, 2nd Edition

Appendix B

Plate count

Spread plate

CFU/ml= CFU/plate x dilution factor x1/0.1

Ym agar

Dilution	Count 1	Count 2
1/10	TNTC	TNTC
1/100	TNTC	TNTC
1/1000	205	190

Plate count per ml = (205 + 190) x 10^3 x 1/0.1
= 197.5 x 10^4
= 2 x 10^6 CFU/ml

MRS agar

Dilution	Count 1	Count 2
1/10	TNTC	TNTC
1/100	TNTC	TNTC
1/1000	200	120

Plates count per ml = (200+120) x 10^3 x 1/0.1
= 160 x 10^4
= 1.6 x 10^6 CFU/ml

Source: krasaekoopt. W, 2008, Food microbiology practical manual 1st edition, p: 114-118

APPENDIX C

Table of data

➢ **Preference test Data for various produced banana beverages**

consumer	A	B	C	D
1	3	4	1	2
2	2	3	4	1
3	1	4	2	3
4	2	4	3	1
5	1	2	3	4
6	3	4	1	2
7	3	2	1	4
8	4	1	3	2
9	3	4	2	1
10	4	3	2	1
11	1	4	3	2
12	2	4	3	1
13	1	3	4	2
14	3	4	2	1
15	2	1	4	3
16	2	1	4	3
17	2	3	1	4
18	2	4	1	3
19	2	4	3	1
20	4	3	2	1
21	4	3	1	2
22	4	2	1	3
23	2	4	1	3
24	4	1	2	3
25	3	1	2	4
26	3	2	4	1
27	4	3	2	1
28	3	2	4	1
29	4	3	2	1
30	3	4	2	1

A: banana/distilled water inoculated with yeast

B: banana/ mineral water inoculated with yeast

C: banana/Distilled water extract inoculated with both yeast and LAB

D: banana/ mineral water extract inoculated with both yeast and LAB

➢ **Preference test data for produced banana puree/ yogurt before improvements**

consumers	A	B	C
1	3	2	1
2	2	1	3
3	1	3	2
4	1	1	2
5	3	3	2
6	1	2	3
7	2	3	1
8	2	3	1
9	2	1	3
10	3	2	1
11	1	3	1
12	3	2	1
13	3	1	2
14	2	3	1
15	3	2	1
16	5	3	1
17	4	2	3
18	3	2	1
19	5	4	3
20	4	1	2
21	5	2	3
22	3	4	2
23	2	1	2
24	4	5	1
25	3	1	2
26	4	3	1
27	5	2	3
28	2	5	3
29	1	2	3
30	3	2	4
	85	71	59

A:Puree/yoghurt

B:Puree/yoghurt/yeast

C: Puree/yoghurt/yeast/LAB

APPENDIX D

TA Measurement

Method using a colored indicator

TA Measurement Materials

Titratable acid can be measured by a simple titration procedure using a calibrated sodium hydroxide solution. Phenolphthalein solution is used as an indicator to show the titration end point. The following materials are needed for this measurement.

- 10-ml pipettes (two pipettes are convenient)
- 250-ml Erlenmeyer flask or other clear glass container
- 0.1 normal sodium hydroxide
- 1% phenolphthalein solution
- Distilled water

TA Measurement Procedure

The following procedure is satisfactory for measuring the TA of juices and white or blush wines.

1. Draw 5 milliliters (ml) of juice or wine into a pipette and transfer the sample into the flask.
2. Add about 50 ml of distilled water and three or four drops of phenolphthalein solution.
3. Fill a 10-ml pipette with 0.1 N sodium hydroxide solution.
4. Titrate with the sodium hydroxide while mixing the wine sample by rocking the flask.
5. Stop titration when the sample turns a faint pink.
6. Read the pipette scale and record the quantity of sodium hydroxide solution used.
7. Rinse the flask and pipettes with clean water and allow them to dry.

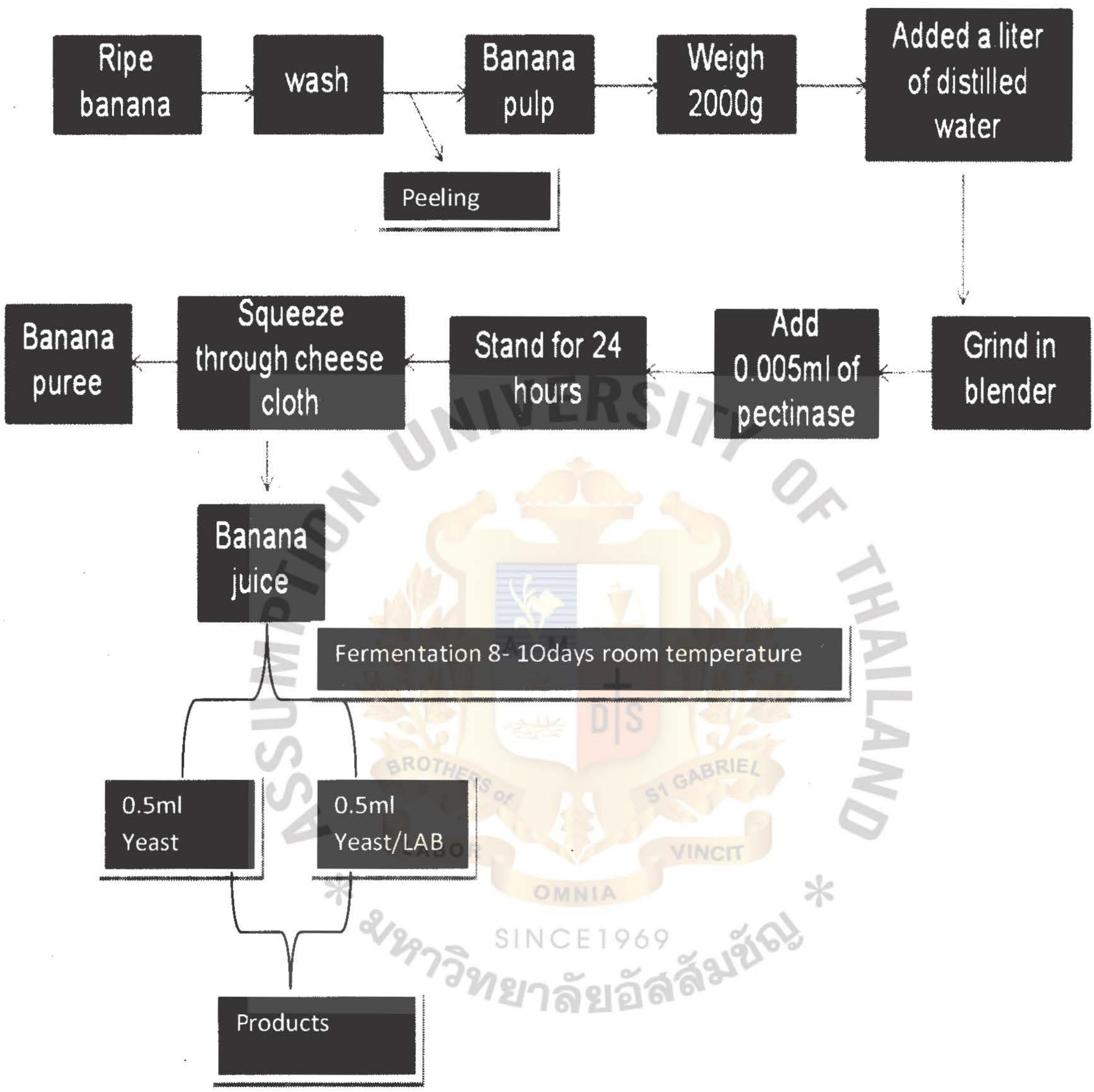
Results expressed as acid in gram/litre:

g/l acid = (Titre x acid factor x 100 x 10) ÷ 10 (ml juice) **Malic Acid factor = 0.064**

Source:http://www.extension.iastate.edu/wine/titratable-acidity, accessed 20/09/2014

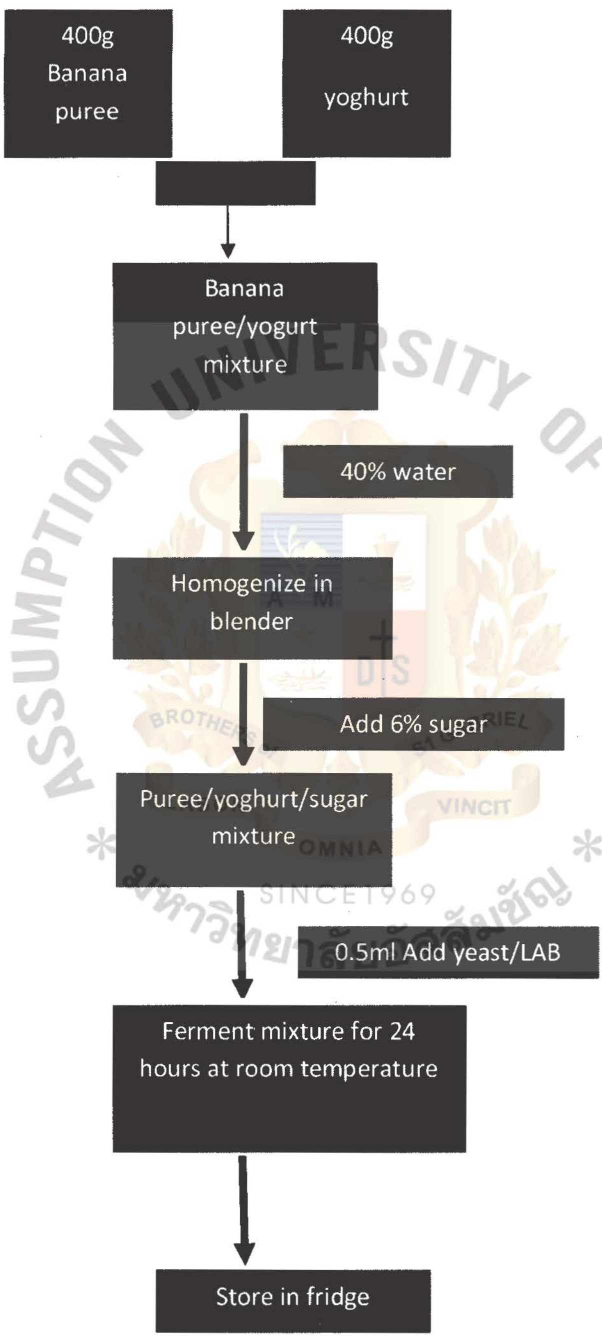
APPENDIX E

PROCESS Chart of banana alcoholic beverage making



APPENDIX F

Process chart of Banana puree/ yoghurt making



APPENDIX G

Preference Test- Ranking form

Subject No________ Session code ______ Date_________ ____

Please rinse your mouth with water before starting, after each sample and anytime you want to In front of you are four samples. Beginning with the sample on your left taste each one After you taste each sample, you may restart as often as you need to.

Rank the samples from the most preferred (=1) to least preferred (=4).

Sample	Rank (1 to 4) Ties are not allowed
643	______
296	______
781	______
528	______

Thank you

APPENDIX H

Consumer sensory testing for product development

Panelist No____________ sample No______________ Date ________________

Please consume your entire sample in order to evaluate all attributes. Please remember one page of score sheet is for one sample. Please answer the following questions by completely filling in the square that best reflects your feeling about this sample.

1. Over all how would you rate this sample?

Dislike extremely	Dislike very much	Dislike slightly	Neither like nor dislike	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐

2. How much would you rate the color of this sample?

Dislike extremely	Dislike very much	Dislike slightly	Neither like nor dislike	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐

3. How would you rate the flavor of this sample?

Dislike extremely	Dislike very much	Dislike slightly	Neither like nor dislike	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐

4. How would you rate the texture of this sample?

Dislike extremely	Dislike very much	Dislike slightly	Neither like nor dislike	Like moderately	Like very much	like extremely
☐	☐	☐	☐	☐	☐	☐

5. How would you rate the mouth feel of this sample?

Dislike extremely	Dislike very much	Dislike slightly	Neither like nor dislike	Like moderately	Like very much	Like extremely
☐	☐	☐	☐	☐	☐	☐

Thank you very much for your time

APPENDIX I

Paw statistic viewer Spss results for color, flavor, texture and mouth feel for second improved banana puree/ yogurt/mineral water/yeast/Lab

Y

	TR	N	Subset
			1
Duncan[a,b]	3.00	30	4.3333
	1.00	30	4.4333
	2.00	30	4.5333
	Sig.		.495

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.121.

a. Uses Harmonic Mean Sample Size = 30.000.

b. Alpha = .05.

Y

	TRT	N	Subset
			1
Duncan[a,b]	1.00	30	3.8667
	3.00	30	3.9000
	2.00	30	3.9667
	Sig.		.722

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.025.

a. Uses Harmonic Mean Sample Size = 30.000.

b. Alpha = .05.

Y

	TRT	N	Subset
			1
Duncan[a,b]	3.00	30	3.8333
	1.00	30	4.1333
	2.00	30	4.3667
	Sig.		.055

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.018.

a. Uses Harmonic Mean Sample Size = 30.000.

b. Alpha = .05.

Y

	TRT	N	Subset
			1
Duncan[a,b]	1.00	30	4.0000
	3.00	30	4.0333
	2.00	30	4.1333
	Sig.		.633

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1.016.

a. Uses Harmonic Mean Sample Size = 30.000.

b. Alpha = .05.