THE POTENTIAL OF SUBSTITUTION OF BITTER LEAF FOR BITTER HOP IN BEER MAKING

NATTAWEE RUANGCHAWALEE

ID: 6029512

A Thesis submitted in partially fulfillment of the requirement for the degree of Master of Science in Food Biotechnology, Department of Food Biotechnology, Faculty of Biotechnology, Assumption University

2018

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Title	**: The Potential of Substitution of Bitter Leaf for Bitter Hop in Beer Making**
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Nattawee Ruangchawalee

CONTENTS

THE POTENTIAL OF SUBSTITUTION OF BITTER LEAF FOR BITTER HOP IN BEER MAKING

ABSTRACT

The objective of this research was to study the possibility of making good quality craft beer by using Bitter Leaf (*Vernonia amygdalina)* as a supplement ingredient in the brewing. Since the attributes from *V. amygdalina* to human health in medicinal properties could provide health benefits to consumer. Due to health concerned, people trend to live their life healthier by focusing on the consumption of healthy food. Thus, to study the possibility of this Bitter leaf beer to be claimed as health beer by using Sphygmomanometer to measure blood pressure before and after 30 minutes of product testing. Also, to study the substitute bitter hops. The beer formulation using *V. amygdalina* (Bitter Leaf) as a supplement ingredient was successfully developed. From the results obtained from sensory tests of beer brewed by using 1g. of smashed Bitter Leaf has been chosen as the best formula with the best aroma and taste. The results of attributes obtained by 10 trained panelists for sensory evaluation were included foam stability (5.2 ± 1.93), color (6.5 ± 0.97), clarity (5.5±1.58), aroma (5.7 ± 1.95), bitterness (6.2 ± 1.39), alcohol content (5.5 ± 1.43), complexity (6.2 ± 1.39), after taste (6.5 ± 1.08), overall liking (7.0 ± 1.05). The mean score obtained was 7.0 which was greater than other formulations. The uses of *V. amygdalina* could be used for hops substitution since the properties and characteristic which are similar to hops. In addition, the Bitter Leaf Beer has possibility to decrease blood pressure since about 60% of the blood pressure measurement (DIA) has decreased. Therefore, the Bitter leaf beer could be claimed as health beer since it has the availability to decrease blood pressure. However, for a proper health claimed, further medical research is needed to confirm the effectiveness of the developed Bitter Leaf Beer.

Keywords: *Vernonia amygdalina*, Bitter Leaf, Craft beer, Health beer.

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INTRODUCTION

In this era of globalization, the trend of studying herbs are popular since various types of herbs are providing different benefits such as medicinal and supplemental purposes. Medical benefits that are provided from herbs to human health could prevent and treat various diseases. Due to health concerned, people trend to live their life healthier by focusing on the consumption of healthy food such as vegetables, fruits or other nutritional food especially herbs which are promoting good health to them. Therefore, the world came up with the discoveries and studies of herbs that are able to offer lots of surprising health benefits of human. According to the studies, the most famous one is *Vernonia amygdalina*, or known as Bitter Leaf, and "Nan Chao Woei" in Thai language which was adapted from Chinese language. (Asst. Prof. Dr. Srisomphon P) (U. Udochukwu, F.I. Omeje, I. S. Uloma, F. D. Oseiwe).

V. amygdalina was known as Bitter Leaf since its bitterness, and under the specification of Vernonia in short. *V. amygdalina* is a kind of herbs under the Kingdom of Platae, order of Asterales, under the family of Asteraceae, tribe of Vernonia, and genus of Vernonia. *V. amygdalina* is originated from African countries (Ifeoluwa T. Oyeyemia Akinbiyi., A. AkinlabibAderiike., AdewumibAbimbola., O. Aleshinloyeb and Oyetunde T. Oyeyemi) (M. L. K. Bonsi, P. O. Osuji., A. K. Tuah and N. N. Umnna). Previous researches reported that *V. amygdalina* is well known as a medical plant with several uses, including in treating diabetes and fever reduction, etc (Ifeoluwa T. Oyeyemia Akinbiyi., A. AkinlabibAderiike., AdewumibAbimbola., O. Aleshinloyeb and Oyetunde T. Oyeyemi) (Ebenzer O. Farombi and Olantunde Owoeye).

The attributes from *V. amygdalina* to human health in medicinal properties could provide health benefits to consumer (Ifeoluwa T. Oyeyemia Akinbiyi., A. AkinlabibAderiike., AdewumibAbimbola., O. Aleshinloyeb and Oyetunde T. Oyeyemi) (U.Udochukwu, F.I. Omeje, I. S. Uloma, F. D. Oseiwe). Moreover, its bitter taste could go well with the taste of beer. Its characteristics are similar to bitter hops, and it will be used as a supplement ingredient in the brewing. Due to the hops could not grow in Thailand so, we still need to import them. Therefore, the price would be high and also it take long time for the transportation. This study, we try to explore the possibility of

using *V. amygdalina* to substitute bitter hops. This could reduce the cost of beer production and provide more convenience of the raw material availability. In expectation, this beverage after formulated could be explore as a kind of herbs beer which providing health benefits to consumer.

OBJECTIVES

1. To formulate a craft Bitter Leaf beer with appropriate aroma and taste.
2. To study the consumer preference of the Bitter Leaf beer by sensory evaluation.
3. To study the possibility of Bitter Leaf to substitute bitter hops and reduce the cost of the beer production.
4. To study the possibility of beer made by Bitter Leaf to be claimed as health beer.

LITERATURE REVIEW

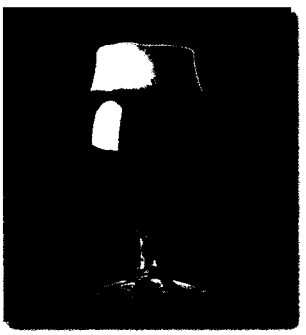


Figure 1. Beer

Beer is the world's most widely consumed and probably the oldest alcoholic beverage, it is the third most popular drink overall, after water and tea. The production of beer is called brewing.

Ingredients of beer making:

There are four main ingredients for making beer. Which are:

1. Malt
2. Water
3. Hops
4. Yeast

1. Malt

Malt is being produced from grain and mostly barley. First of all, the barley from the fields is being thoroughly cleansed. The barley is ready for germination in warm and humid air lasting until the malt sprouts reach about the same length as the grain itself. During this process, valuable enzymes and malt sugar are being generated. For long durability, the grain is being dried over hot air on the called 'kiln'. The higher the kiln temperature the more malt sugar is converted into caramel. The more sugar is converted into caramel, the darker the malt and the brewed beer made out of it. The alcoholic content of beer only depends on the blend ratio of malt and water not on the

color light or dark of the beer. Malted cereal grains are the meat and potatoes of beer. They provide the sugars that are fermented by the yeast to create alcohol and CO^2. They are the primary source of beer color and contribute significantly to flavor and mouth feel. The most common of the malted grains is barley malt. Others include wheat, rye, and oats. In addition to the malted grains, some unmalted cereal grains are used in brewing including corn, rice, wheat, rye, oats, and sorghum. Malting is a process of controlled sprouting and kilning of the grains. The sprouting activates enzymes within the grain that begin to break down the hard, starchy insides into simpler carbohydrates, making them accessible to the brewer. Kilning gives the grains differing degrees of color and flavor. There are four categories of brewing malt. Base malts receive the least kilning. They are the lightest malts and make up the bulk of any beer recipe. Crystal or caramel malts are made by allowing enzymes in the grain to convert complex carbohydrates into simple sugars before kilning. Kilning then caramelizes the sugars in the grain. Crystal malts range in color from light to dark with correspondingly intense flavors. Toasted or kilned malts are dry-kilned to a range of colors and flavors. Roasted Malts are kilned at the highest temperatures until they are very dark brown or even black. (Christopher B. McIlroy, Aaron C. Bandremer, & Ken Takeda, 1999)

2. Water

For the beer production it is of main importance that the brewing water is clean and free of impurities. In contrary to former times, the content of minerals (hardness) is not of crucial importance anymore since it is possible to balance its effects through natural composition alternatives of the malting and brewing process. More important is that the produced beer type is being attuned to the brewing water. Therefore, the brewing recipes are being defined for micro-brewery only after detailed and thorough water analysis. Their compliance is subject to permanent control. (Christopher B. McIlroy, Aaron C. Bandremer, & Ken Takeda, 1999)

3. Hops

Besides the convenient flavor, the hops perform further important tasks during beer production. Due to its natural content of essential oils (as to be found in remedy

herbs like chamomile and eucalyptus), the hops protect the beer against deterioration. Basically, one differentiates between aroma hops and bitter hops, the latter mainly being used due to its considerably lower price even though aroma hops is of higher quality. Hops are the spice of beer. They provide bitterness to balance the sweetness of the malt, as well as flavors and aromas ranging from citrus and pine to earthy and spicy. Hops are the cone-like flower of a rapidly growing vine (a bine actually) in the cannabis family. Waxy yellow Lupulin glands hidden within the leaves of the flower contain the acids and essential oils that give hops their character. Bitterness comes from alpha acids that must be chemically altered through boiling in order to be isomerized. Hop flavors and aromas come from essential oils that are easily dissolved into hot wort but are also highly volatile. Flavor and aroma hops must be added late in the boil or these properties will be lost with the steam. Hops more than any other brewing ingredient are subject to the phenomenon of terrier, as different growing regions produce hops with different flavor and aroma characteristics. The chief hop growing regions are the Northwestern US, Southern England, Germany, Czech Republic, and China. (Christopher B. McIlroy, Aaron C. Bandremer, & Ken Takeda ,1999.)

4. Yeast

Yeast is the most important ingredient in beer brewing. It is a single-celled organism; a fungus (phylum Ascomycetes; class Hemiascomycetes; including 10 different families). Yeast is a living creature, metabolizing, reproducing, and living off the ingredients in the beer. It is responsible for the converting of sugar to alcohol and carbon dioxide in the fermentation stage. Yeast is also the final component that determines the flavor of the beer. There are thousands of varieties and strains of yeast. Even in the air, wild yeast is floating around ready to contaminate. Only cultivated strains of yeast should be utilized in the brewing of beer. If other yeast contaminates the beer, the results can be over carbonation, strange flavors, and all kinds of fermentation peculiarities. Therefore, picking the right yeast for the desired beer is an absolutely critical. The two main varieties of yeast used for beer brewing are top-fermenting yeast (*Saccharomyces cerevisiae*) and bottom-fermenting yeast (*Saccharomyces uvarum*). The names of both are descriptive of where the fermentation takes place. The top-fermenting yeast is similar to the yeast for baking bread. It is applied for making ales

and stouts. The bottom-fermenting yeast is utilized for production of lagers and steam beer. Top fermenting yeast is named as such because most strains exhibit the tendency to flocculate (gather) at the surface of the beer during the first few days of fermentation. After which the yeast settles to the bottom of the fermenter while a large percentage stay in dispersion. Top Fermenting yeast, 'Ale yeast', finds optimum performance in the temperature range of 55-75 deg F. Lower temperatures tend to inhibit fermentation, causing the yeast to become dormant. Bottom fermenting yeast, 'Lager yeast', is best suited for the temperature range 55-32 deg F. The process of fermentation takes substantially longer when using Lager yeast, this time is often referred to as 'Laagering'. Bottom fermenting yeast, as is expected, flocculates at the bottom of the vessel and spends most of its life-cycle in the sedimentary state. The main difference between the beers produced from either yeast variety is that top fermented beers bear a flowery and fruity taste. (Christopher B. McIlroy, Aaron C. Bandremer, & Ken Takeda , 1999)

- **Process of beer making**

The beer production for the commercial consists of 9 steps

1. Grinding
2. Mashing
3. Lautering
4. Wort boiling
5. Cooling
6. Fermentation
7. Storage
8. Filtration
9. Filling

1. Grinding

The grinding is a coarse milling, even better a crushing of the relatively mellow malt grain. In doing so, it must be observed that the outer shell of the malt grain, the so-called husk, remains nearly intact. In the lautering step, these husks serve as a natural filter layer.

2. Mashing

The word mashing originally derives from mixing. The beer production starts with mixing the grist of first step with warm brewing water. The water being applied for mashing-in is called main mash water in contrary to the so called second wort during lautering step. This grist-water mixture is gradually being heated in the brew vessel. According to individual recipe, the temperature must be hold correspondingly long at each temperature step. During this so-called rest, the starch contained in the malt grain is being converted into malt sugar and valuable amino acids develop from indigestible proteins.

3. Lautering

Lautering means the separation of the hazy mash particles from the clear liquid. This process can be compared to filtering coffee where the coffee grounds are being restrained and a clear fluid containing the dissolved coffee particles runs through the filter. The more water is being poured over the coffee grounds, the more exhaustive the diffusion where by the running off coffee becomes more and more watery. The lautering starts with the transfer of the entire mash into the lautertun. The lautertun is the second copper vessel in the brew house equipped with a false bottom with thin slits approximately 1.5 cm above the original bottom. Since the husks are heavier than the other mash particles, they depose at the false bottom thus forming a natural filter layer. The false bottom only serves as a support of this husk filter. The brewer calls the thereby almost clear running-off sugar water wort. As soon as the wort ran-off entirely, the solid mash particles remain within the lautertun, called "spent grains". In the beginning, these spent grains still contain a fair amount of malt sugar being rinsed out with hot water. The water being applied for this purpose is called second wort and is being poured over the spent grains without destroying their layering. The lautering must be done very carefully because if the wort would run-off freely, the developing suction would contract the husk layer to almost impermeable extent.

4. Wort boiling

The entire run-off wort is being re-collected in the brew vessel and boiled together with the hops for at least one hour. The wort must be boiled until the desired sugar concentration is reached due to evaporating water. During boiling, also the composition of the wort changes whereby insolvable components like for example hops oils are being dissolved, others simply drop out or evaporate in form of solid components, the so-called break. The sugar concentration of the wort after boiling is the well-known original extract. Since the original extract is converted into alcohol during fermentation, the later alcoholic content of the beer directly depends on the original extract. By the time enough water has evaporated, the wort with the whole hops will again be transferred to the Lautertun. The decocted whole hops and the break remain on the false bottom whereas the wort runs-off at the bottom now bearing a distinctive taste of hops.

5. Cooling

The boiling hot wort must be cooled down to the starting temperature of the yeast.

From this point of time, an extremely neat and clean operation is required because otherwise lactic acid bacteria instead of the yeast might start the fermentation of the wort. These lactic acid bacteria convert the sugar into lactic acid and not into alcohol like the yeast does. Thereby the beer turns sour and therefore becomes denaturized. Lactic acid bacteria are not harmful to humans in any way. For top fermentation, the wort is being cooled down to approximately at 15°C, for bottom fermentation to approximately at 5°C. These temperatures lie about 5°C below the optimum temperature of the respective yeast because fermentation ought to start slowly. For cooling down the wort to approximately 20°C it is possible to use cold tap water. During this process, the tap water heats up to approximately at 85°C and is available as hot water. In order to further cool down the wort, artificially cooled "ice water" of approx. 1°C is required.

6. Fermentation

The yeast converts the sugar of the wort into alcohol, CO_2 and heat. The wort turns into green beer. The fermentation performed slowly because otherwise big quantities of undesirable fermentation by-products accumulate besides higher alcohol is the main cause for headaches. In order to produce high-quality beer, appropriate cooling ensures a fermentation period of approx. 8-10 days. Since at this point sugar is converted into alcohol, the measured content of original extract continuously drops during fermentation. The accumulated CO_2 can freely escape from the open fermenter. Because CO_2 bubble escapes through the green beer, undesirable fermentation by-products negatively influencing the taste and digestibility of the beer are being washed out. Through fermentation, white foam builds up at the beer surface collapsing again at the end of the main fermentation.

7. Storage

The residual sugar ferments to alcohol and CO2. For this purpose, the beer is being transferred from the open fermenter to the closed storage tank. The storage tank is being closed with a bunging apparatus. By the use of this bunging apparatus, the desired CO2-content of the beer can be adjusted. Therefore, the now accumulating CO2 remains in the beer. In order for the sugar to further ferment, the temperature ought to remain at approx. 5°C for a while. Afterwards, the beer is ready for slowly being cooled down to maturity temperature of 1°C. Under these temperatures, the green beer matures for several weeks. During this time, the beer almost completely purifies and finally reaches full maturity.

8. Filtration

The residual yeast is being filtered from the beer. For this purpose, the beer from the storage tank is being pressed through a Kieselguhr-filter layer under pressure. The hereby secreted yeast remains in the filter together with the Kieselguhr and can be disposed after termination of the filtration process. After filtration, the beer is being collected in the pressure tank and from there arrives at the filling facilities.

9. Filling

The filtered beer is being filled into bottles. In general, bottle filling is returnable bottles with swing stopper, so called Rick layer's bottles, are being used. First of all, the bottles must be manually cleaned with the aid of a bottle washing machine comparable to an industrial dishwasher. The bottles are being cleansed of yeast deposits and afterwards sterilized. Next, the bottles are manually being removed from the machine and placed on a table for filling. For filling purpose, the brew master applies hand operated Isobarometric filler. Each bottle is separately being removed from the table. The brew master ensures a careful filling of the beer without the development of foam and tight closure of the bottles with a ceramic seal. Filled beer can be stored up to 6 – 12 weeks until consumption.

- **Bitter Leaf** (*Vernonia amygdalina*)

Figure 2. Bitter Leaf

Vernonia amygdalina Del (Asteraceae) is a small shrub with dark green leaves and rough barks growing predominantly in tropical Africa but has been domesticated in many parts of West Africa (Igile et al., 1994). It is a perennial plant with height between 1m and 6m (Nwosu et al., 2013). *V. amygdalina Del* is soft wooded and is a multipurpose and rapid regenerating shrub. Its bitter taste has made it to be fondly called ''Bitter leaf" and it is also referred to by several local names in different languages of different regions. Anti-nutritional phytochemicals within the plants are responsible for its bitter taste (Bonsi et al., 1995). The leaves are consumed as green leafy vegetable. Its richness in minerals and vitamins has made it an important human diet (Sobukola et al.,2007). Some of the identified bioactive compounds of *V. amygdalina* responsible for its ethnobotanical uses include alkaloids, saponins, terpenes, lignans, flavonoids, phenolic acids, steroids, anthraquinone, coumarins, sesquiterpenes, xanthones and edotides (Izevbigie,2003; Cimanga et al., 2004; Muraina et al., 2010).

Figure 3. Bitter Leaf Tree

V. amygdalina is found in nature closed to rivers and lakes, in forests margins, woodland and grassland up to 2800 m altitude, in areas with mean annual rainfall 750–2000 mm. The plant can tolerate drought although humid environment is more suitable for its growth (Ndaeyo, 2007). It can thrive on all types of soil but grows better on humus-rich soils, these probably underscores its ability to thrive on a range of ecological zones.

V. amygdalina has been widely used for the traditional treatment and/or management of various diseases in humans and animals in Africa. The leaves are effective against fevers and are common substitute for quinine in many African countries including Nigeria (Masaba, 2000). The plant is a potent malaria regimen in different regions of Africa (Madureira et al.,2002; Njan et al., 2008; Ene and Atawodi, 2012; Tugume et al.,2016). The leaves are used traditionally to induce fertility in women (Adedapo et al., 2014), as laxatives/purgative and enema (Kupcham, 1971). It is used widely to cure several parasitic ailments such as amoebic dysentery and schistosomiasis (Huffmanet al., 1996), helminthosis (Nabukenya et al., 2014), hiccups, typhoid fever (Fadimu et al., 2014), yellow fever, (Ene andAtawodi, 2012), stomach-ache, convulsions (Tugume et al., 2016),measles (Sonibare et al., 2009), boils, burns, diabetes (Ajibesinet al., 2008), jaundice (Simbo, 2010), inflammatory diseases(Ogbole et al., 2010), candidiasis (Mustapha et al., 2013), pile (Nwauzoma and Dappa, 2013), cancer, viral diseases (Koubéet al., 2016), bacterial infection, gastrointestinal (GIT) disorders, liver diseases, kidney problems, nausea (Atangwho et al., 2012).Others include diarrhea, hepatitis (Olorunfemi et al., 2012),eczema, anemia (Akindahunsi and Salawu, 2005), hypertension, cough (Amira and Okubadejo, 2007), febrile convulsion (Moshiet al., 2010), urinary tract inflammation, wound dressing, menstrual pain, and other sexually transmitted diseases (Farombi, 2003; Fasuyi, 2006). In Southern Ghana, the

young fresh leaves are used in treating diabetes, fever, constipation, high blood pressure and as laxative (Asante et al., 2016). The leaves, root and twig of the plant are used for treating wounds, venereal diseases and hepatitis (Nwanjo, 2005; Erasto et al., 2006). The leaves are also used for breast milk enhancement in nursing mothers (Kankara et al., 2015), treatment of fever in poultry (Nalubegaet al., 2012), helminthous is in livestock (Nabukenya et al., 2014), mastitis in cattle (Moshi et al., 2010) and cattle de-ticking (Regassa, 2000). Moreover, the aqueous extract of the leaves is commonly recommended for the treatment of diabetes, induce dabrosia nausea, emesis, loss of appetite, dysentery and other gastrointestinal tract problems (Adedapo et al., 2014), scabies, headache, stomach-ache, joint pain associated with AIDS, gingivitis and toothache due to its antimicrobial activity (Akah and Okafor,1992; Alabi et al., 2005; De Boer et al., 2005; Fasuyi, 2006;Innocent and Deogracious, 2006) by herbalist and naturopathic doctors for their patients.

There are more than thirty compounds belonging to several classes of compounds with differing bioactivities have been isolated and characterized from *V. amagdalina*. These include sesquiterpene lactones (Kupchan et al., 1969; Erasto et al.,2006; Owoeye et al., 2010; Luo et al., 2011), steroidal saponins (vernoniosides) (Jisaka et al., 1992), steroid glycosides and flavonoids (Igile et al., 1995). The sesquiterpene lactones are peculiar to *V. amygdalina* (Abay et al., 2015) and have been shown to be the main active compounds responsible for most of the plant's activities (Luo et al., 2011). The isolated compounds have shown activities ranging from antifeedant (Ganjian et al., 1983), antischis-tosomal (Koshimizu et al., 1994), antiplasmodial (Abay et al.,2015), antioxidant (Erasto et al., 2007a; Sinisi et al., 2016), anti-inflammatory (Sinisi et al., 2016) and anticancer activities (Koshimizu et al., 1994; Owoeye et al., 2010; Luo et al., 2011).

MATERIALS

Materials

1. Raw-materials

- Fresh bitter leave (*Vernonia amygdalina)*
- Water
- Malt Extract
- Malt barley (Pale Ale Malt)
- Bitter hop (Cascade)
- Aroma hop (Cascade)
- Dry yeast (*Saccharomyces Cerevisiae*); Pasteur Red yeast

2. Lab analysis equipment

- pH meter
- Thermometer
- Balance
- Refractometer (0-32o Brix)
- Ebulliometer
- Filtration equipment
- Sphygmomanometer

3. Chemical agent

- Distilled water
- Liquid Carbon-dioxide
- 70% ethanol

Materials

V. amygdalina or Bitter Leaf was purchased from a Chinese Pharmacy in Bangkok Province, Thailand. The type of malt barley grain used was Pale Ale Malt from Weyermann®, yeast (Saccharomyces cerevisiae) from Red Star® and hops (Cascade) with 8.1% of alpha acid from Brew-By-Me.

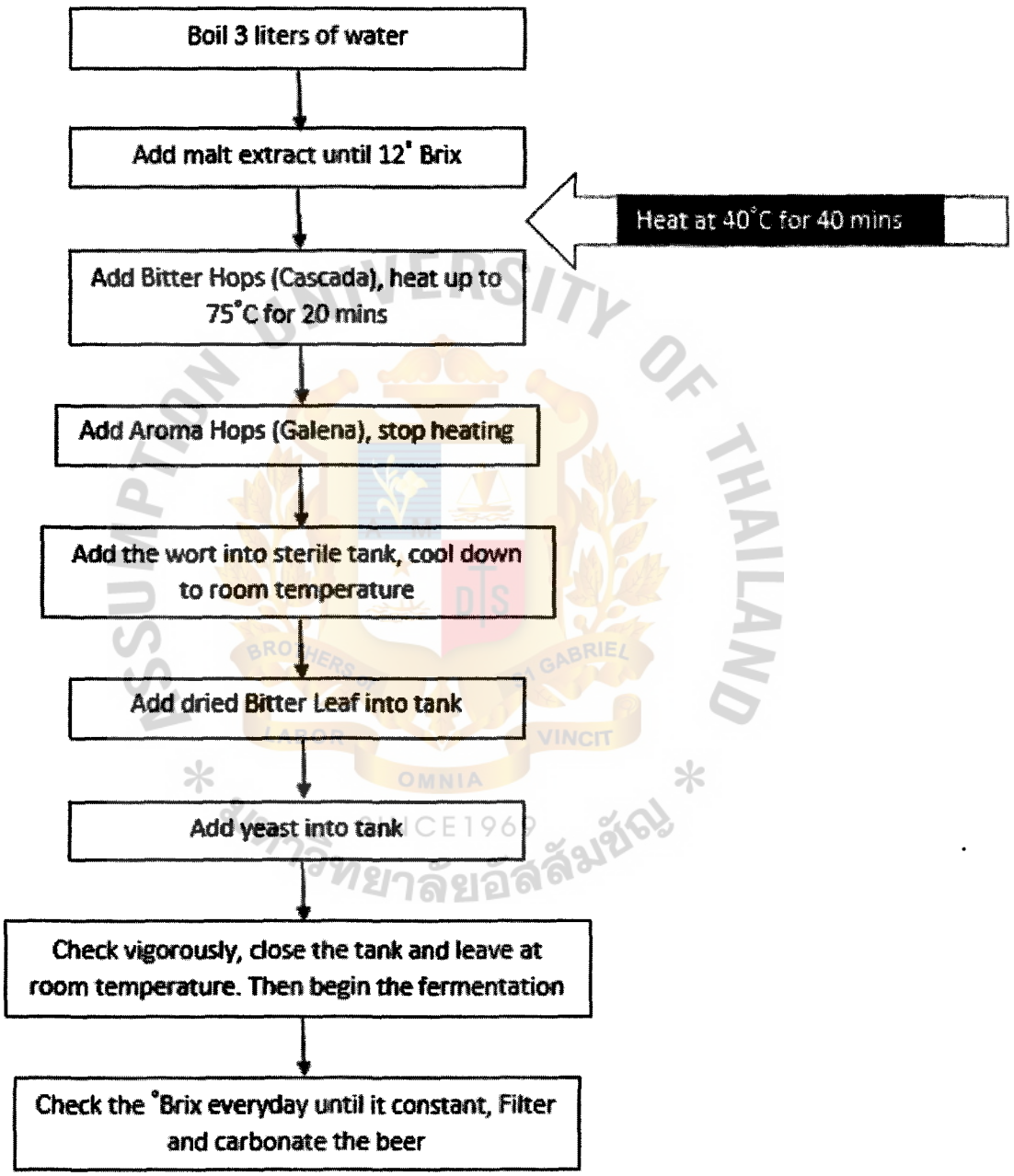
METHODOLOGY

- Brewing Process
- Beer formulation
- Sensory Evaluation
- Experimental Design (Surface Response)
- Statistical Analysis "SAS Program" (SAS Version 9.4 (32))
- Blood Pressure Measurement
- Lab Analysis
  - pH
  - *Brix
  - % Alcohol

Methods

Brewing process for all treatments

Figure 4. The flow chart of Bitter Leaf Beer brewing process.



Brewing processes

3 liters of water were boiled, milled malts or malt extracts were added to the boiled solution and adjusts the °Brix until 12 °Brix. Then, malting process was started by heating the solution at 40 °C for 40 minutes. Bitter hops (Cascade) was added and the solution was heated up to 75 °C for 20 minutes. After boiling for an hour, stop heating and aroma hops (Cascade) was added. Whirlpool was done to let the residual of hops formed a cone in the middle of wort. The wort was transferred into a sterile tank and cooled down to room temperature. Weighed amount of Bitter Leaves were added then followed by activated yeast. In this period, the fermentation was started. The value of °Brix was checked every day until it was constant. The filtration was done, and filtered beer has been carbonated and stored in cold room.

Method for Bitter Leaf beer formulation

The experiment has been separated into three formulations by varying the amount of Bitter Leaf, bitter hops and aroma hops to improve the formulation.

Table 1. First batch beer formulation used for primary consumer's sensory evaluation.

Formula	Ingredients				
	Water (L)	Bitter Leaf (g)	Bitter Hops (g)	Aroma Hops (g)	Yeast (g)
Control	3	-	5	5	3
1	3	1	5	5	3
2	3	2	5	5	3
3	3	3	5	5	3
4	3	4	5	5	3

Remarks: Adjusting the wort °Brix with malt extracts until 12 °Brix; - : none of Bitter Leaf added.

From the consumer's sensory evaluation, the results were used to improve the formulation in second batch of formulation. The bitterness provided from bitter hops were reduced from 5 g to 4 g. The amount of Bitter Leaf used in this formulation was varied into 1, 2, and 3g.

Table 2. Second batch of beer formulation improved from primary consumer's sensory evaluation.

Formula	Ingredients				
	Water (L)	Bitter Leaf (g)	Bitter Hops (g)	Aroma Hops (g)	Yeast (g)
Control	3	-	4	5	3
1	3	1	4	5	3
2	3	2	4	5	3
3	3	3	4	5	3

Remarks: Adjusting the wort °Brix with malt extracts until 12 °Brix; - : none of Bitter Leaf added.

The amount of bitter hops used in third formulation was reduced from 4 g to 3 g. The variation of Bitter Leaves was 3 g and 4 g and aroma hops were reduced from 5 g to 4 g. In this batch, formulation was varied into two different batches which brewed by using malt extracts and milled malt grains.

Table 3. Beer formulation brewed by malt extracts.

Formula	Ingredients				
	Water (L)	Bitter Leaf (g)	Bitter Hops (g)	Aroma Hops (g)	Yeast (g)
Control	3	-	3	4	3
1	3	1	3	4	3
2	3	2	3	4	3

Remarks: Adjusting the wort °Brix with malt extracts until 12 °Brix; - : none of Bitter Leaf added.

Table 4. Beer formulation brewed by using milled malt.

Formula	Ingredients				
	Water (L)	**Bitter Leaf (g)**	**Bitter Hops (g)**	**Aroma Hops (g)**	**Yeast (g)**
Control	3	-	3	4	3
1	3	1	3	4	3
2	3	2	3	4	3

Remarks: Adjusting the wort °Brix with malt extracts until 12 °Brix; - : none of Bitter Leaf added.

The fourth formulation was varied by cut and smashed Bitter Leaf and brewed by using milled malt.

Table 5. Beer formulation for the formulation using cut Bitter Leaf.

Formula	Ingredients				
	Water (L)	**Bitter Leaf (g)**	**Bitter Hops (g)**	**Aroma Hops (g)**	**Yeast (g)**
1	3	1	3	4	3

Table 6. Beer formulation for the formulation using smashed Bitter Leaf.

Formula	Ingredients				
	Water (L)	**Bitter Leaf (g)**	**Bitter Hops (g)**	**Aroma Hops (g)**	**Yeast (g)**
1	3	1	3	4	3

Sensory analysis

Sensory tests were done to obtain the final formulation of the beer. Samples used for sensory tests were the formulation 1 from the third batch which brewed by milled malt and formulation using smashed Bitter Leaf brewed by milled malt from fourth formulation. 9-point Hedonic Scale sensory evaluation was used and the attributes evaluated were foam stability, color, clarity, aroma, bitterness, alcohol content, complexity, after taste and overall impression. The sensory analysis was done by 10 trained panelists.

Blood Pressure Measurement

Measure blood pressure before beer testing and after 30 minutes of beer testing in Systolic Blood Pressure (SYS), Diastatic Blood Pressure (DIA) and Pulse Pressure (PUL) by using Sphygmomanometer.

RESULTS AND DISCUSSIONS

The study of beer formulation of Bitter Leaf Beer

According to table 1., 2., 3., 4., 5., 6., there were 4 batches of formulation showed. The improvement of formulations was adjusted according to the results obtained from consumer's sensory evaluation. The improvement was adjusting by varying amount of Bitter Leaf, bitter hops and aroma hops used. First and second batches were preliminary test only. The formulation from third and fourth batches were used for sensory test. The best sensory test samples have been repeated and tested by using 9-Point Hedonic Scale.

Table 7. The results of sensory evaluation for beer brewed by malt extract.

Attributes	Average scores ± SD		
	Control	1	2
Foam stability	5.4 ± 0.55^{b}	5.4 ± 0.55^{a}	5.2 ± 0.84^{b}
Color	5.6 ± 0.55^{b}	5.6 ± 1.14^{a}	5.0 ± 1.22^{c}
Clarity	5.6 ± 1.14^{bc}	5.6 ± 0.55^{a}	4.8 ± 0.84^{c}
Aroma	5.4 ± 0.55^{cd}	4.4 ± 2.30^{a}	5.2 ± 0.45^{cd}
Complexity	4.4 ± 0.55^{b}	3.8 ± 2.59^{a}	4.8 ± 0.84^{b}
Bitterness	4.2 ± 1.64^{b}	5.2 ± 1.48^{a}	5.2 ± 0.84^{b}
After Taste	3.4 ± 1.52^{b}	4.4 ± 2.51^{a}	4.4 ± 1.14^{b}
Alcohol content	5.4 ± 1.14^{bc}	5.2 ± 1.64^{a}	5.0 ± 0.71^{c}
Overall liking	5.2 ± 1.30^{c}	4.8 ± 0.84^{ab}	4.6 ± 0.55^{cb}

Remark: mean ± SD, Means with the same letter are not significantly different ($p \leq 0.05$)

Table 8. The results of sensory evaluation for beer brewed by milled malt.

Attributes	Average scores ± SD		
	Control	1	2
Foam stability	6.4 ± 0.55^{a}	6.4 ± 0.55^{b}	6.6 ± 0.89^{a}
Color	7.0 ± 0.71^{a}	7.0 ± 0.00^{bc}	6.6 ± 0.89^{ab}
Clarity	6.8 ± 0.84^{a}	7.2 ± 0.45^{bc}	6.4 ± 1.14^{ab}
Aroma	8.4 ± 0.55^{ab}	7.6 ± 0.89^{d}	6.6 ± 1.14^{bc}
Complexity	7.6 ± 1.14^{a}	7.4 ± 0.55^{b}	7.6 ± 0.89^{a}
Bitterness	7.8 ± 0.84^{a}	7.2 ± 1.09^{b}	7.4 ± 0.89^{a}
After Taste	6.8 ± 0.89^{a}	7.4 ± 0.89^{b}	7.8 ± 0.45^{a}
Alcohol content	7.6 ± 0.89^{a}	7.6 ± 0.55^{c}	6.2 ± 0.84^{b}
Overall liking	7.0 ± 0.00^{a}	7.6 ± 0.55^{c}	6.2 ± 0.84^{b}

Remark: mean ± SD, Means with the same letter are not significantly different ($p \leq 0.05$)

Table 9. The results of sensory evaluation of beer formulated in fourth batch.

Attributes	Average scores ± SD	
	1	2
Foam stability ^{ns}	6.0 ± 1.15	5.2 ± 1.93
Color ^{ns}	6.4 ± 1.35	6.5 ± 0.97
Clarity ^{ns}	5.3 ± 2.11	5.5 ± 1.58
Aroma ^{ns}	5.1 ± 2.68	5.7 ± 1.95
Complexity ^{ns}	5.8 ± 1.87	6.2 ± 1.39
Bitterness ^{ns}	4.0 ± 2.40	6.2 ± 1.39
After Taste*	4.8 ± 1.87	6.5 ± 1.08
Alcohol content ^{ns}	4.9 ± 2.18	5.5 ± 1.43
Overall liking*	6.0 ± 1.05	7.0 ± 0.82

Remark: 1: Formulation brewed by using cut Bitter Leaf; 2: Formulation brewed by using smashed Bitter Leaf; attributes with * means the two mean are significantly different ($p \leq 0.05$), ns means the two mean are not significant different ($p \geq 0.05$).

Results of sensory evaluation of Bitter Leaf beer formulation

There were two batches of formulation which classified by beer brewed using cut Bitter Leaf and smashed Bitter Leaf. According to the results, the formulation of using cut Bitter Leaf would contain less aroma compared to smashed Bitter Leaf. Therefore, this experiment was studied to find out the appropriate formulation for making Bitter Leaf beer before studying the possibility of bitter hops substitution.

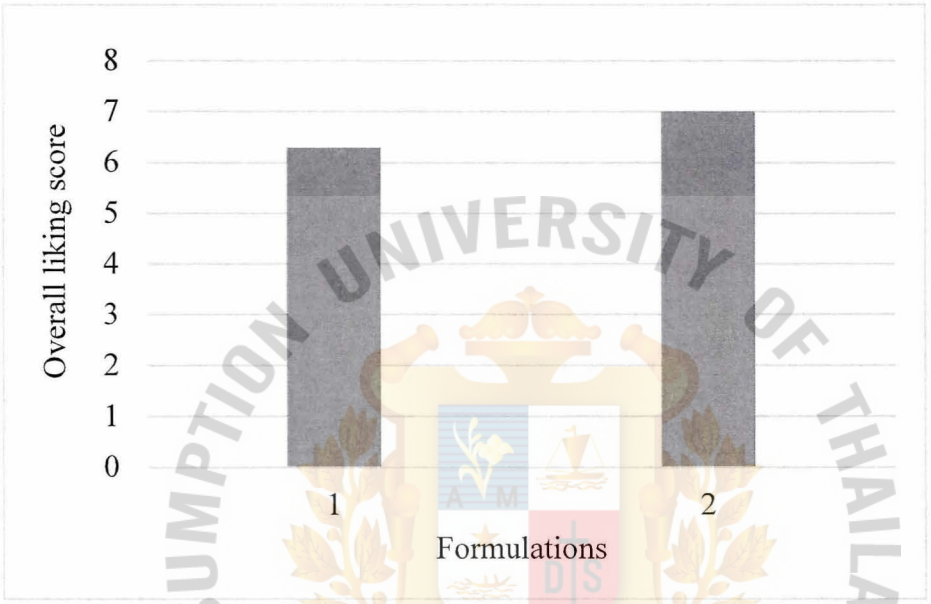


Figure 5. The sensory test chart for overall impression of two formulations.

According to the result obtained from figure 5., the formulation 2 which brewed by using smashed Bitter Leaf with milled malt obtained the highest score of overall impression which was 7.0. The formulation was brewed by using 1 g of Bitter Leaf. From sensory evaluation, small amount of Bitter Leaf used in brewing provided a better taste of aroma of beer compared to the formulation which used more than 1 g of Bitter Leaf.

Figure 6. Bitter Leaf beer sample No.1 and sample No.2

In addition, the sample 1 and 2 have been used to analyzing for their pH, Alcohol content and Brix value. And the results have shown in figure.

	**pH** 4.44	**% Alcohol** 5.75%	***Brix** 5.6
	**pH** 4.5	**% Alcohol** 5.7%	***Brix** 5.8

Figure 7. Lab analysis pH, % alcohol and *Brix of 2 formulations

Table 10. Mean scores of each attributes and overall impression done by 10 trained panelists.

Attributes	Mean Score
Foam stability	5.2
Color	6.5
Clarity	5.5
Aroma	5.7
Complexity	6.2
Bitterness	6.2
After taste	6.5
Alcohol content	5.5
Overall impression	7.0

From the results showed in table 10., trained panelists were preferred the formulation of beer brewed by using smashed Bitter Leaf. Thus, the formulation brewed by smashed Bitter Leaf was used as final beer formulation. The overall impression of consumers toward the develop beer product obtained a mean of 7.0 which was moderately good score.

Bitter hops substitution by using Bitter Leaf

Since the cost of the bitter hops use in beer brewing are high and also in Thailand has to import only. Thus, the hops could not grow in Thailand. In an economic point view, if the bitter leave could use to substitute bitter hops, there would be a lot of benefits to reduce the production cost of bitter hops. According to previous researches, *V. amygdalina* contained bioactive compounds such as tannins, flavonoids, alkaloids, and phenolic compound (U.Udochukwu, F.I. Omeje, I. S. Uloma, F. D. Oseiwe, 2013)[1]. Due to its characteristic and bitter taste, its flavor is suitable for substituting hops. Bitter taste from *V. amygdalina* could be used for hops substitution but the amount of leaves added need to be in an appropriate amount to prevent the over-bitterness and off-flavor of beer. From the research, alpha acid, beta acid, essential hop oils and polyphenols are the main components of hops, contribute to bitterness (Yarong H., Johannes T., and Thomas B., 2013). Also, the hop chemistry typical analysis of dried cone hop included 4% of Tannin (polyphenol) which is the same compound as in bitter leave that gives bitterness flavor (T.R. Roberts, in Brewing Materials and Processes, 2016). In this research, the beer was brewed by using both *V. amygdalina* and hops. According to the formulation, the bitterness provided from the addition of Bitter Leaf could reduce the amount of hops used. The anti-microbial properties of *V. amygdalina* could improve the shelf life of beer as hops did. Additionally, the previous amount of bitter bops used in this study was 5g. then, the last batch which is the most preference formula was used only 3g. It could mean that, there is 60% of hops reduction in the brewing by using bitter leaf as a supplement ingredient for hops substitution. Therefore, to substitute the hops by using *V. amygdalina* is possible but the flavor might be different, and the sterilization steps need to be done very well since hops is an important ingredient which conducted to the shelf life and stability of the beer. The foam stability of the beer still needs to obtain from hops. From this research, it could give the idea that there is a possibility to use bitter leave to substitute bitter. Which could reduce the cost of hops use in brewing industry.

The study about possibility of Bitter Leaf beer to be claimed as a health beer on reducing blood pressure.

Table 11. Data of blood pressure measured before of beer tasting done by trained 10 panelists.

No.	Blood Pressure Measurement		
	SYS	DIA	PUL
1	121	72	95
2	106	56	90
3	137	75	61
4	132	88	98
5	116	69	83
6	138	72	82
7	171	89	98
8	166	85	70
9	102	62	82
10	104	72	88

Table 12. Data of blood pressure measured after 30 minutes of beer tasting done by trained 10 panelists.

No.	Blood Pressure Measurement		
	SYS	DIA	PUL
1	123	78	84
2	101	62	81
3	115	71	64
4	153	94	84
5	112	69	83
6	113	80	86
7	143	87	98
8	137	81	62
9	100	60	81
10	101	69	85

According to the table11 and 12, the results showed that value of blood pressure measurement before and after 30 minutes in Systolic Blood Pressure (SYS), Diastatic Blood Pressure (DIA) and Pulse Pressure (PUL). In this case, the result would measure from the DIA value since most of the panelists are at the age about 20-40. For example, the measurement of panelist no.2 was 106/56 mmHg before consuming beer then after 30 minutes of beer consumed, the value became 101/62 mmHg. From the result, the SYS number has decreased but the DIA number has increased. It showed that the blood pressure has decreased but for the DIA value that increased may refer to the increasing of oxygen amount in blood vessel when the blood vessel was released. The result showed that the Bitter Leaf Beer has possibility to decrease blood pressure since about 60% of the blood pressure measurement (DIA) has decreased. However, this experiment used only 10 panelists for the measurement, therefore the result might not be confirmed 100% effective. According to the conditions and results obtained from 10 panelists, the Bitter Leaf Beer could be claimed as health beer since it has the availability to decrease blood pressure. Thus, this study is just a preliminary test to give out some idea that there is a possibility for blood pressure reduction. However, for a proper health claimed, further medical research is needed to confirm the effectiveness of the developed Bitter leaf beer.

Further study suggestion

For the further study suggestion, according to the references, bitter leaf has lots of medicinal benefits. The use of bitter leaf could give the health benefits for this beer product. However, the clinical study to prove the health benefits to ensure the efficacy of using this product as claimed is needed.

CONCLUSION

In conclusion, the beer formulation using *V. amygdalina* (Bitter Leaf) as a supplement ingredient was successfully developed. From the results obtained from sensory evaluation of beer brewed by using 1g. of smashed Bitter Leaf has been chosen as the most consumer preference formula with the best aroma and taste. The scores evaluated by using 9-Point Hedonic Scale was ran by SAS Program and the results showed the significant different than others. The results of attributes obtained by 10 trained panelists for sensory evaluation were included foam stability (5.2 ± 1.93), color (6.5 ± 0.97), clarity (5.5±1.58), aroma (5.7 ± 1.95), bitterness (6.2 ± 1.39), alcohol content (5.5 ± 1.43), complexity (6.2 ± 1.39), after taste (6.5 ± 1.08), overall liking (7.0 ± 1.05). The mean score obtained was 7.0 which was greater than other formulations. The uses of *V. amygdalina* could be used for hops substitution since some of the chemical compound in its are the same with bitter hops. Also, the properties and characteristic which are similar to hops. It may benefit to the reduction of hops cost in brewing industry. By the way of contrast, the flavor of beer might be different from commercial beers which brewed by using bitter hops. To guarantee the beer's shelf life, a modern preservation is needed. In addition, the Bitter Leaf Beer has possibility to decrease blood pressure since about 60% of the blood pressure measurement (DIA) has decreased. Therefore, the Bitter leaf beer could be claimed as health beer since it has the availability to decrease blood pressure. However, for a proper health claimed, further medical research is needed to confirm the effectiveness of the developed Bitter Leaf Beer. This study only a preliminary test for give the idea of blood pressure reduction by using bitter leaf.

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APPENDIX A

Analysis Methods

1. Alcohol determination by using Ebulliometer

An Ebulliometer is an instrument used for determination of the alcohol content of water-alcohol solutions by determining the difference in boiling points between pure water and the solution. Based on the comparison, the percentage alcohol (v/v) can be determined by referring to tables or using the calculating dial.

Procedure

Determine the boiling point of water

1. Fill the lamp with 95% reagent alcohol
2. Rinse the boiler and pour through the opening "A" 20 ml pure water measured with the sample vial to the mark "Eau"
3. Place the thermometer in position by inserting into the opening "A"
4. Light the alcohol burner and place it under "B"
5. When the thermometer reading becomes stable, read the temperature
6. This is the temperature reading for water to be used for further calculation

.

Using the Ebulliometer

1. Opening the stopcock, "F", empty the boiler, rinse it with some beer to be tested pour out again and blow through upper tube "C" to clear away the condensed steam.
2. Pour into boiler 50 mL of sample, using the sample measure, and filling up to the mark "VIN"
3. Place the thermometer in "A", fill the condenser "D-E" with cold water, and heat s preciously discussed.
4. The mercury will rise and stabilize; wait until the mercury is motionless to take the reading.

Determination alcohol concentration

1. On the calculation dial, setting the zero on boiling point of water, find the corresponding % alcohol for the boiling point of the sample.

Figure 8. Ebulliometer

2. °Brix measurement

Using Refractometer sugar °brix 0-32%

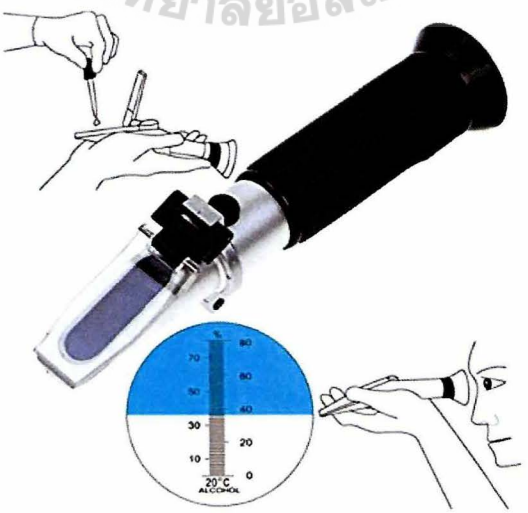


Figure 9. Refractometer

3. pH Determination by using a pH meter

1. Switch on the power supply at the plug point
2. Switch on the meter (press the "power" key)
3. Rinse the electrode with distilled water
4. Select using the mode button the desired measurement mode
5. Calibrate the meter using at least 2 buffers and record value when the meter indicates "ready"
6. Carry out the measurement by dipping the electrode in the sample
7. Rinse the electrode with distilled water and store in electrode storage solution after use
8. Switch off the meter by pressing the "power"

Figure 10. pH meter

4. Acid determination by titration method

1. Put the sample into a conical flask
2. Add 2-3 drops of Phenolphthalein indicator
3. Fill the burette with 0.1N NaOH
4. Titrate until the color turn into pink

Formula for finding percent Total acidity

$$M_{acid} \times V_{acid} = M_{base} \times V_{base}$$

M_{acid} = Molarity of the acid M_{base} = Molarity of the base

V_{acid} = Volume of the acid V_{base} = Volume of the base

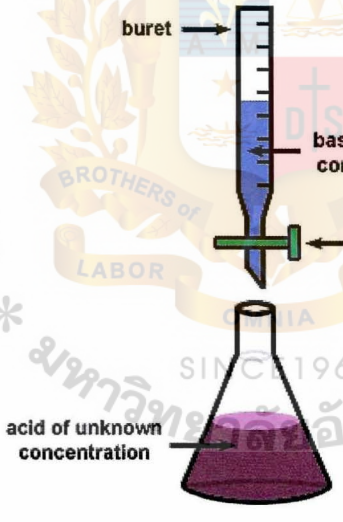


Figure 11. Titration method

APPENDIX B

Questionnaire: Sensory evaluation

Study of Bitter Leaf Beer Formulation

Panelist name: Date:

Introduction:

Please rate the samples how much you like or dislike the sample using the sample using the scale below. Please test from left to right rinse your mouth with water and preference test.

1=Dislike extremely
2=Dislike very much
3=Dislike moderately
4=Dislike slightly
5= Neither like nor dislike
6=Like slightly
7= Like moderately
8= Like very much
9=Like extremely

Attributes \ Sample No.	____	____	____
Foam stability ฟอง			
Color สี			
Clarity ความใส			
Aroma กลิ่น			
Complexity ความกลมกล่อม			
Bitterness รสขม			
After taste รสสัมผัสหลังชิม			
Alcohol content ความเข้มข้นของแอลกอฮอล			
ความชอบโดยรวม Overall Liking			

.....THANK YOU FOR YOUR TIME.....

Figure 12. Sensory evaluation questionnaire.

APPENDIX C

SAS Output

SAS Output for beer brewed by malt extract.

Randomized Complete Block

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
Block	5	1 2 3 4 5
Treatment	3	A B C

Number of Observations Read	15
Number of Observations Used	15

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Foam stability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.80000000	0.13333333	0.24	0.9527
Error	8	4.53333333	0.56666667		
Corrected Total	14	5.33333333			

R-Square	Coeff Var	Root MSE	Foam stability Mean
0.150000	14.11449	0.752773	5.333333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	0.66666667	0.16666667	0.29	0.8739
Treatment	2	0.13333333	0.06666667	0.12	0.8905

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Color

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	8.13333333	1.35555556	1.98	0.1819
Error	8	5.46666667	0.68333333		
Corrected Total	14	13.60000000			

R-Square	Coeff Var	Root MSE	Color Mean
0.598039	15.30814	0.826640	5.400000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	6.93333333	1.73333333	2.54	0.1223
Treatment	2	1.20000000	0.60000000	0.88	0.4521

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Clarity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	4.13333333	0.68888889	0.77	0.6172
Error	8	7.20000000	0.90000000		
Corrected Total	14	11.33333333			

R-Square	Coeff Var	Root MSE	Clarity Mean
0.364706	17.78781	0.948683	5.333333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	2.00000000	0.50000000	0.56	0.7013
Treatment	2	2.13333333	1.06666667	1.19	0.3541

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Aroma

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	9.46666667	1.57777778	0.76	0.6184
Error	8	16.53333333	2.06666667		
Corrected Total	14	26.00000000			

R-Square	Coeff Var	Root MSE	Aroma Mean
0.364103	28.75181	1.437591	5.000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	6.66666667	1.66666667	0.81	0.5544
Treatment	2	2.80000000	1.40000000	0.68	0.5348

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Complexity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	19.20000000	3.20000000	1.81	0.2140
Error	8	14.13333333	1.76666667		
Corrected Total	14	33.33333333			

R-Square	Coeff Var	Root MSE	Complexity Mean
0.576000	30.67293	1.329160	4.333333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	16.66666667	4.16666667	2.36	0.1403
Treatment	2	2.53333333	1.26666667	0.72	0.5171

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Bitterness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	15.06666667	2.51111111	1.88	0.1999
Error	8	10.66666667	1.33333333		
Corrected Total	14	25.73333333			

R-Square	Coeff Var	Root MSE	Bitterness Mean
0.585492	23.72672	1.154701	4.866667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	11.73333333	2.93333333	2.20	0.1592
Treatment	2	3.33333333	1.66666667	1.25	0.3370

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Aftertaste

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	26.93333333	4.48888889	2.24	0.1436
Error	8	16.00000000	2.00000000		
Corrected Total	14	42.93333333			

R-Square	Coeff Var	Root MSE	Aftertaste Mean
0.627329	34.77574	1.414214	4.066667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	23.60000000	5.90000000	2.95	0.0902
Treatment	2	3.33333333	1.66666667	0.83	0.4691

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Alcohol content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	11.46666667	1.91111111	2.21	0.1487
Error	8	6.93333333	0.86666667		
Corrected Total	14	18.40000000			

R-Square	Coeff Var	Root MSE	Alcohol content Mean
0.623188	17.90287	0.930949	5.200000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	11.06666667	2.76666667	3.19	0.0761
Treatment	2	0.40000000	0.20000000	0.23	0.7990

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Overall liking

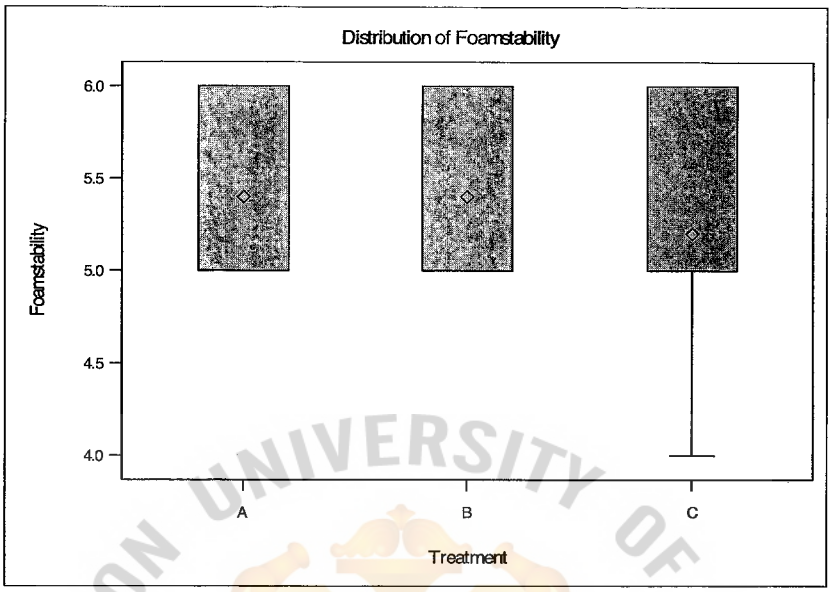
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	6.66666667	1.11111111	1.75	0.2260
Error	8	5.06666667	0.63333333		
Corrected Total	14	11.73333333			

R-Square	Coeff Var	Root MSE	Overall liking Mean
0.568182	16.35252	0.795822	4.866667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	5.73333333	1.43333333	2.26	0.1513
Treatment	2	0.93333333	0.46666667	0.74	0.5085

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Foam stability

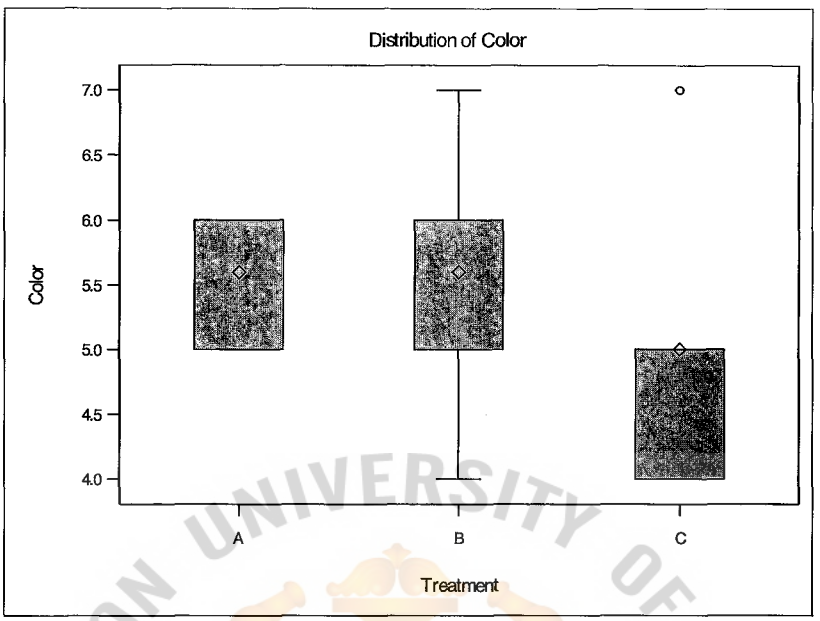
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.566667
Critical Value of t	2.30600
Least Significant Difference	1.0979

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.4000	5	A
A			
A	5.4000	5	B
A			
A	5.2000	5	C

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Color

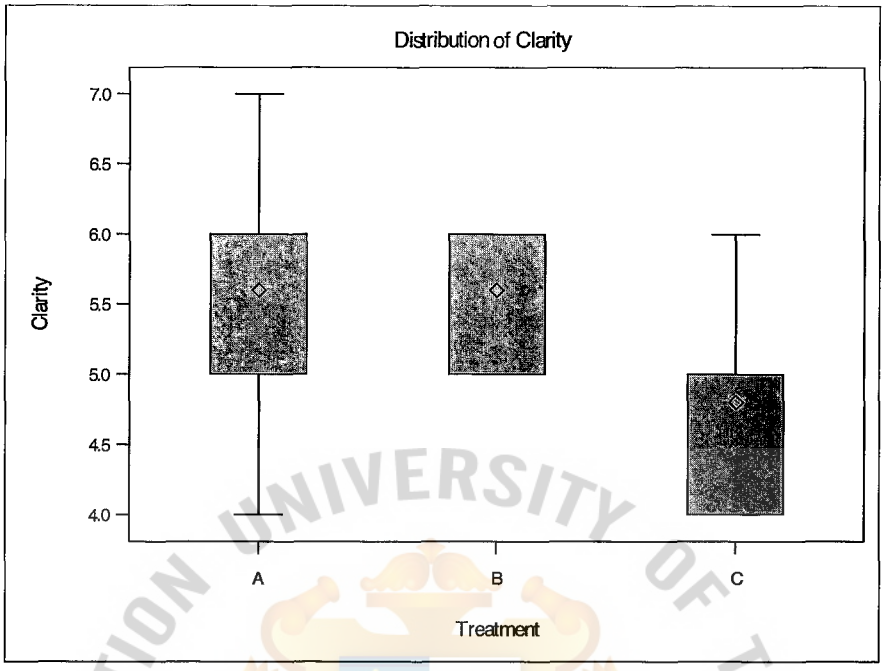
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.683333
Critical Value of t	2.30600
Least Significant Difference	1.2056

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.6000	5	A
A			
A	5.6000	5	B
A			
A	5.0000	5	C

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Clarity

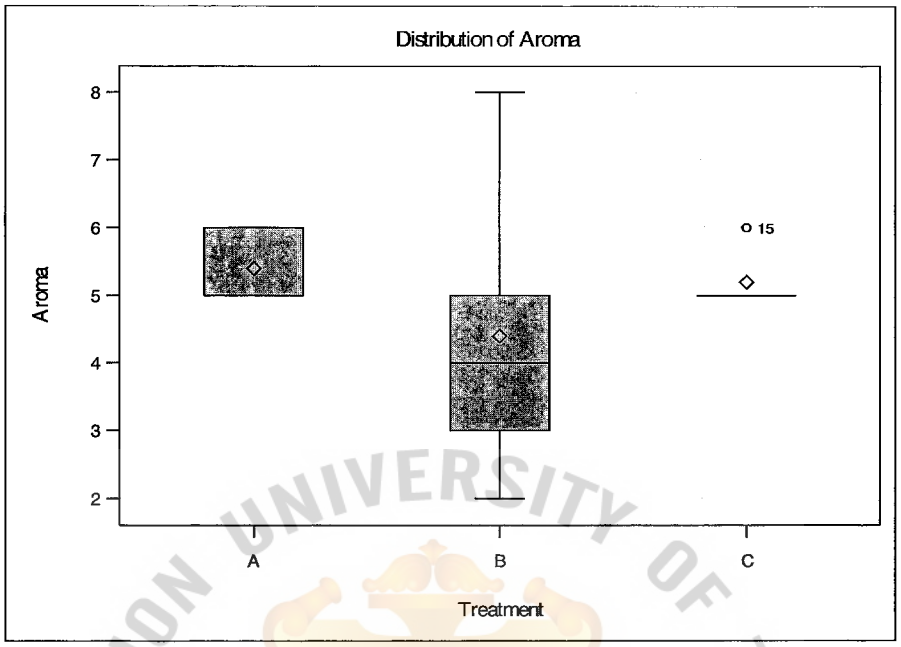
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.9
Critical Value of t	2.30600
Least Significant Difference	1.3836

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.6000	5	A
A			
A	5.6000	5	B
A			
A	4.8000	5	C

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Aroma

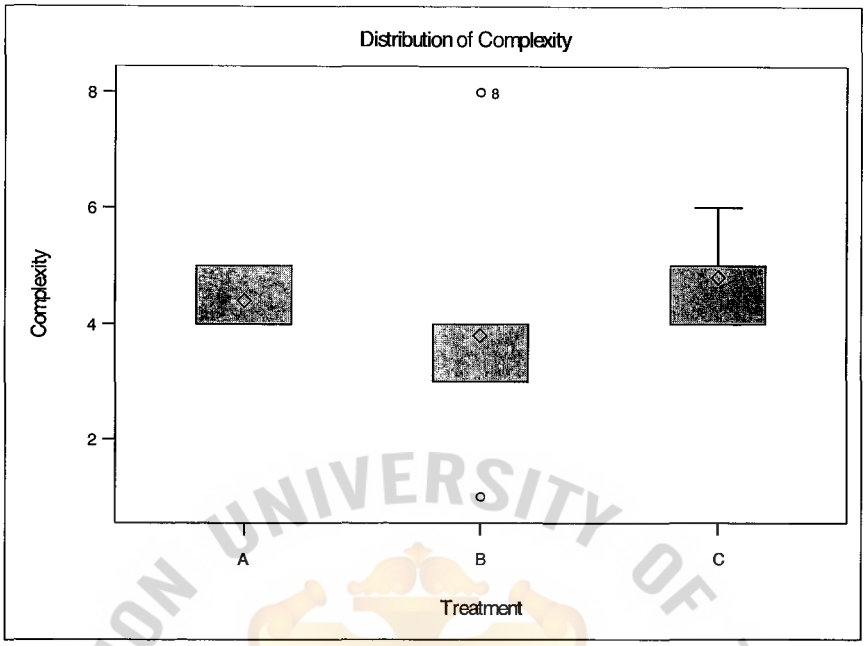
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	2.066667
Critical Value of t	2.30600
Least Significant Difference	2.0966

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.4000	5	A
A			
A	5.2000	5	C
A			
A	4.4000	5	B

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Complexity

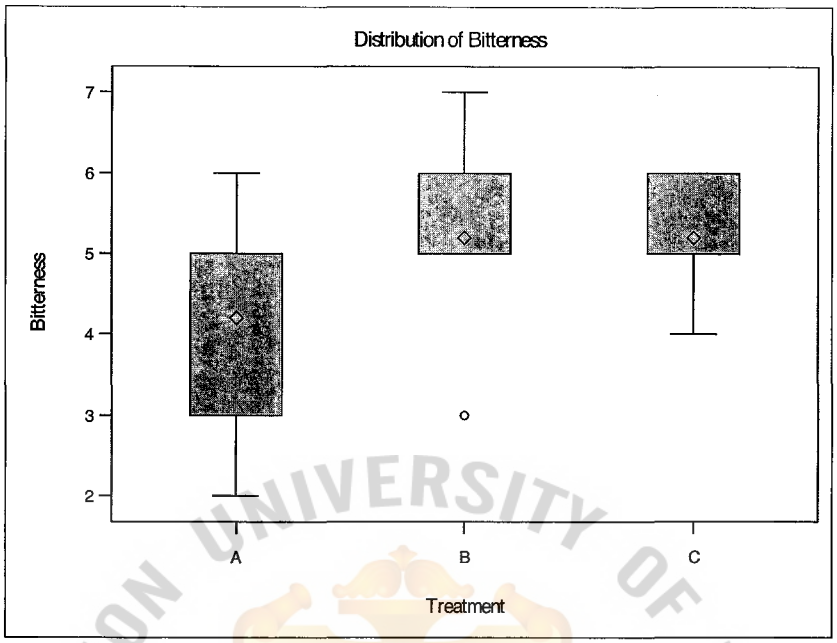
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	1.766667
Critical Value of t	2.30600
Least Significant Difference	1.9385

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	4.8000	5	C
A			
A	4.4000	5	A
A			
A	3.8000	5	B

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Bitterness

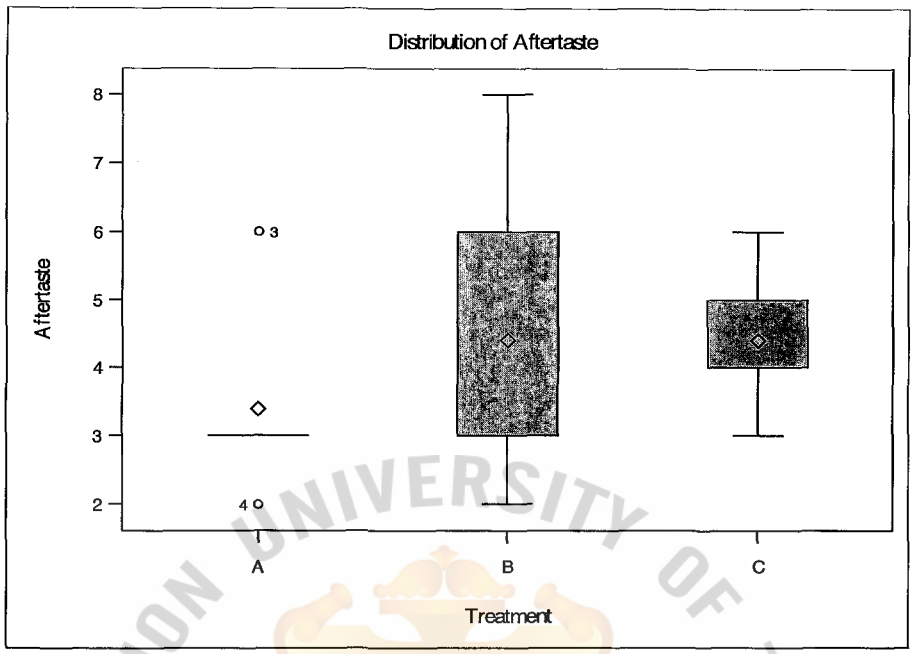
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	1.333333
Critical Value of t	2.30600
Least Significant Difference	1.6841

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.2000	5	C
A			
A	5.2000	5	B
A			
A	4.2000	5	A

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Aftertaste

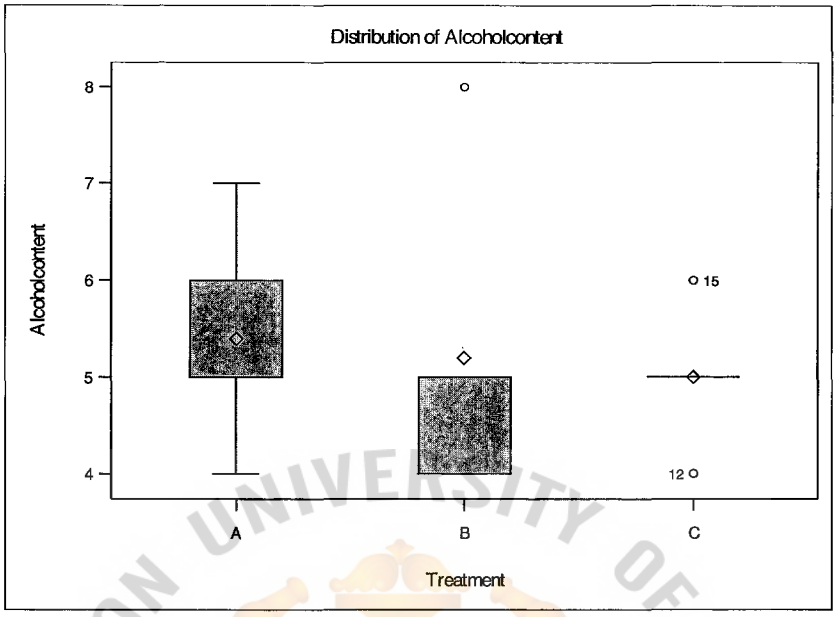
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	2
Critical Value of t	2.30600
Least Significant Difference	2.0626

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	4.4000	5	C
A			
A	4.4000	5	B
A			
A	3.4000	5	A

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Alcohol content

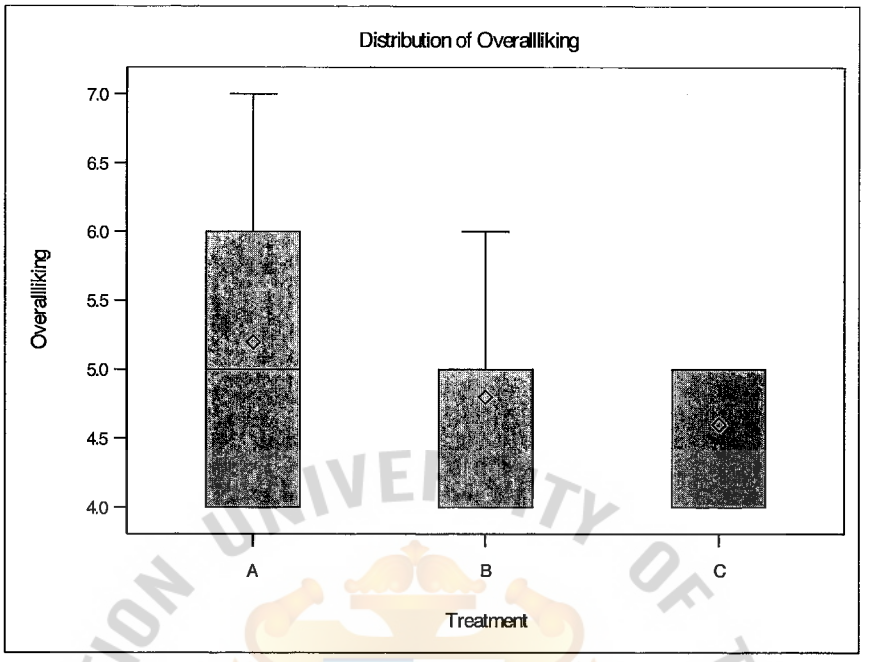
Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.866667
Critical Value of t	2.30600
Least Significant Difference	1.3577

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.4000	5	A
A			
A	5.2000	5	B
A			
A	5.0000	5	C

Randomized Complete Block

The ANOVA Procedure



t Tests (LSD) for Overall liking

Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.633333
Critical Value of t	2.30600
Least Significant Difference	1.1607

Means with the same letter are not significantly different.			
t Grouping	Mean	N	Treatment
A	5.2000	5	A
A			
A	4.8000	5	B
A			
A	4.6000	5	C

SAS Output for beer brewed by milled malt.

Randomized Complete Block

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
Block	5	1 2 3 4 5
Treatment	3	A B C

Number of Observations Read	15
Number of Observations Used	15

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Foam stability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2.53333333	0.42222222	1.06	0.4580
Error	8	3.20000000	0.40000000		
Corrected Total	14	5.73333333			

R-Square	Coeff Var	Root MSE	Foam stability Mean
0.441860	9.780240	0.632456	6.466667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	2.40000000	0.60000000	1.50	0.2894
Treatment	2	0.13333333	0.06666667	0.17	0.8493

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Color

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.93333333	0.15555556	0.26	0.9414
Error	8	4.80000000	0.60000000		
Corrected Total	14	5.73333333			

R-Square	Coeff Var	Root MSE	Color Mean
0.162791	11.28053	0.774597	6.866667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	0.40000000	0.10000000	0.17	0.9494
Treatment	2	0.53333333	0.26666667	0.44	0.6561

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Clarity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2.66666667	0.44444444	0.46	0.8203
Error	8	7.73333333	0.96666667		
Corrected Total	14	10.40000000			

R-Square	Coeff Var	Root MSE	Clarity Mean
0.256410	14.45871	0.983192	6.800000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	1.06666667	0.26666667	0.28	0.8856
Treatment	2	1.60000000	0.80000000	0.83	0.4713

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Aroma

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	13.86666667	2.31111111	4.78	0.0232
Error	8	3.86666667	0.48333333		
Corrected Total	14	17.73333333			

R-Square	Coeff Var	Root MSE	Aroma Mean
0.781955	9.228608	0.695222	7.533333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	5.73333333	1.43333333	2.97	0.0892
Treatment	2	8.13333333	4.06666667	8.41	0.0108

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Complexity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3.20000000	0.53333333	0.65	0.6894
Error	8	6.53333333	0.81666667		
Corrected Total	14	9.73333333			

R-Square	Coeff Var	Root MSE	Complexity Mean
0.328767	11.99597	0.903696	7.533333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	3.06666667	0.76666667	0.94	0.4886
Treatment	2	0.13333333	0.06666667	0.08	0.9224

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Bitterness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	6.66666667	1.11111111	1.75	0.2260
Error	8	5.06666667	0.63333333		
Corrected Total	14	11.73333333			

R-Square	Coeff Var	Root MSE	Bitterness Mean
0.568182	10.65834	0.795822	7.466667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	5.73333333	1.43333333	2.26	0.1513
Treatment	2	0.93333333	0.46666667	0.74	0.5085

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Aftertaste

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2.53333333	0.42222222	0.50	0.7953
Error	8	6.80000000	0.85000000		
Corrected Total	14	9.33333333			

R-Square	Coeff Var	Root MSE	Aftertaste Mean
0.271429	12.57211	0.921954	7.333333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	0.00000000	0.00000000	0.00	1.0000
Treatment	2	2.53333333	1.26666667	1.49	0.2818

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Alcohol content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	12.26666667	2.04444444	11.15	0.0016
Error	8	1.46666667	0.18333333		
Corrected Total	14	13.73333333			

R-Square	Coeff Var	Root MSE	Alcohol content Mean
0.893204	6.002445	0.428174	7.133333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	5.73333333	1.43333333	7.82	0.0072
Treatment	2	6.53333333	3.26666667	17.82	0.0011

Randomized Complete Block

The ANOVA Procedure

Dependent Variable: Overall liking

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	7.20000000	1.20000000	5.54	0.0152
Error	8	1.73333333	0.21666667		
Corrected Total	14	8.93333333			

R-Square	Coeff Var	Root MSE	Overall liking Mean
0.805970	6.713577	0.465475	6.933333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	4	2.26666667	0.56666667	2.62	0.1152
Treatment	2	4.93333333	2.46666667	11.38	0.0046

T- test for Cut Bitter leaf and Smashed Bitter leaf samples.

T-test (2 samples)	Cut	Smashed
Mean	6	5.2
Variance	1.333333333	3.733333333
Observations	10	10
Pearson Correlation	-0.099602384	
Hypothesized Mean Difference	0	
df	9	
t Stat	1.077631812	
P(T<=t) one-tail	0.154616596	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.309233193	
t Critical two-tail	2.262157163	