The Production of Lime Honey Wine

By

Ms. Rungnattakan Ploenkutham

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A special project submitted to the School of Biotechnology, [A]ssumption University In part of fulfillment of the requirement of The Degree of Master of Science in Biotechnology

2015

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By : Ms. Rungnattakan Ploenkutham

Advisor : Dr. Churdchai Cheowtirakul

Level of study : Master of Science

School : Biotechnology

Academic Year : 2016

______________________ Advisor

(Dr. Churdchai Cheowtirakul)

Instructor, School of Biotechnology

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Assumption University

Acknowledgement

This project could not be completed without help of many people. I would like to express my appreciation to my advisor, Dr.Churdchai Cheowtirakul for all his valuable suggestion and guidance throughout this project.

I would like to thank all the teachers, students and the laboratory technicians in the Biotechnology Faculty, for their help and encouragement during my working on this project. I also would like to extend my thanks to all the panelists who patiently help to participate in the consumer tests for this project.

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Ms.Rungnattakan Ploenkutham

August 2016

The production of Lime Honey Wine (Mead)

Key Words: Honey Wine, Mead, Lime honey wine, Lime wine, Lime mead

By	: Ms. Rungnattakan Ploenkutham
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Abstract

The objective of this project is to study the method to formulate the consumer prefer recipe of lime honey wine. The project was conducted by vary the amount of lime juice added to the mead making formulation. Adding di-ammonium phosphate (DAP) in mead to enhance fermentation was also explored. The quality of finish products were analyzed by measuring the consumer preference test of the following attributes: color, clarity, aroma, sweetness, acidity, honey flavor, alcohol, after taste and overall liking. The number of panelist participated in the consumer test were 30 people.

The consumer prefer recipe of lime honey wine was: 10 % of lime juice and 1,000 ppm di-ammonium phosphate (DAP) were added. The fermentation period was 10 days at room temperature. Physical and chemical analysis was also studied in the final products. Physical and chemical properties of the lime mead were analyzed. Formula achieved the highest score from sensory analysis had the following properties: pH 2.87, TTS 11.5 °Brix, color 5Y 8.5/8 form munsell book, 11.25 % in alcohol content, total acidity 2.36%, reducing sugar 3.45mg/ml.

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Introduction

Wine is an alcoholic beverage made through the fermentation of sugar or fruit juice. The fermentation process to change sugar into alcohol is conducted by yeast. The main ingredient in fruit juice is sugar which contributes to the sweetness or total soluble solid (°Brix). We can measure the Degree brix to indicate the amount of sugar in the starting fruit juice and the completeness of fermentation.

Mead is wine made from honey. It is the oldest alcoholic beverage before fruit wine. The amount of alcohol in mead normally is limited by the capacity of the yeast adding into must during fermentation. Yeast consumes sugar in honey and converts into ethanol and carbon dioxide. Honey wine generally containing alcohol content of 6 to 17% v/v (Steinkraus, 1983). Fruit juice can be added to mead to create specific flavor. There are mead added with different kinds of fruit juice made by different countries and are called in different names. For example: Morat (blending honey with mulberries), Capsicumel (mead flavored with chili peppers), etc.

Lime is very common and can be found in many kinds of foods and drinks in Thailand. It is a main citrus fruit which contains more vitamin c than lemons. Limes are strongly acidic and can be harmonically complemented into the taste of in mead.

Normally, Thai people consume honey with lime juice as their herbal medicine from the nature. From this reason we would like to study the formulation of lime honey wine to explore the preference of the consumers.

Objective

1. To study the formulation of lime honey wine
2. To explore the preference of consumer of this wine

Literature Review

Mead Wine

The origins of mead can be traced back to the African bush more than 20,000 years ago. It was later produced throughout the Mediterranean basin and Europe, playing an important role in the early ancient civilizations. In fact, mead was an important part of the rituals of the Celts, Anglo-Saxons and Vikings, referred to as the drink of nobles and gods, providing immortality and believed to have magical and healing powers even capable of increasing strength, virility and fertility. The empirical use of honey for the production of mead and provided a detailed description of the procedure used for obtaining the traditional beverage. Fermented drinks obtained from honey, among which mead, are tough to be the oldest alcoholic beverages known to man, made thousands of years before either wine or beer were produced. Evidence regarding the collection of honey dates back to at least 8000 BCE and is thought to date back into the Paleolithic period.

It is important to mention that the production of mead in the southern Europe declined when the grapes were discovered as a less expensive and more predictable source for the production of alcoholic beverages. Honey was prized throughout history; it was often available only to royalty. Somewhere about 1300 A.D., the Italian voyager Marco Polo returned from the Spice Islands with sugar cane. This inexpensive source of sugar became dominant and honey went underground

Despite this, in the northern countries, where these fruits were less available, the popularity of mead persisted. The scientific conducted on mead include the development of additive formulations and fermentation conditions, processing improvements. Actually, mead seems to be a good option for increasing the income of honey producers, allowing the development of a beverage little known in some countries but possessing great commercial potential. This is also in line with the present situation of consumers demanding more options and a willingness to try new products. Traditionally the raw materials of honey wine fermentation are honey, water and yeast. Makers can add other ingredients which can effect to flavor, color, carbonation, alcohol content and other properties changes in wine.

Figure 1 : Mead wine

Main ingredients

1. Honey

Figure 2 : Honey

Honey is a nutritious food which is mainly a supersaturated sugar solution with more than 95% of its mass consisting of sugar and water. Honey is composed primarily of carbohydrate and a few oligosaccharides, also different valuable nutrients such as vitamins, minerals, enzymes, flavoring organic compounds, free amino acids and numerous volatile compounds constitute minor components.

Most of all natural honey contains flavonoides (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin), phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids), ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), Millard reaction products and peptides. Most of those compound works together to provide a synergistic antioxidant effect. However, this small fraction of the overall composition that is responsible for honey's organoleptic and nutritional properties. The composition of honey can associate its nutrition source and also from soil and weather determines compositions. Honey could be classified as floral when it is derived from the nectar of flowering plant when it is derived from sweet deposits secreted by living parts of plants or excreted onto them by sucking pollen.

Chemical composition of natural honey

Natural honey contains about 200 substances, including amino acids, vitamins, minerals and enzymes. The principal carbohydrate constituents of honey are fructose (32.56 to 38.2%) and glucose (28.54 to 31.3 %), which represents 85–95% of total sugars that are readily absorbed in the gastrointestinal tract.Other sugars include disaccharides such as maltose, sucrose, isomaltoseturanose, nigerose, meli-biose, panose, maltotriose, melezitose. A few oligosaccharides are also present. Honey contains 4-5% fructo-oligosaccharides, which serve as probiotic agents. Water is the second most important component of honey. Organic acids constitute 0.57% of honey and include gluconic acid which is a byproduct of enzymatic digestion of glucose which organic acids are contribute largely to its characteristic taste. Mineral compounds ranges from 0.1% to 1.0 %. Potassium is the major metal, calcium, magnesium, sodium, sulphur and phosphorus. Trace elements include iron, copper, zinc and manganese. Nitrogenous compounds, vitamins C, B_1 (thiamine) and B_2 complex vitamins like riboflavin, nicotinic acid, B_6 and panthothenic acid are also found. Honey contains proteins only in minute, 0.1–0.5 percent quantities.

Physical properties of natural honey

Honey has several important qualities to composition and taste. Freshly extracted honey is a viscous liquid. Its viscosity depends on large variety of its composition and particularly with its water content. Hygroscopicity is another property of honey and describes the ability of honey to absorb and hold moisture from environment. Normal honey with water content of 21% or less will absorb moisture from air of a relative humidity of above 60%. The surface tension of honey varies with the origin of the honey and is probably due to colloidal substances. The color of honey should be clear and shades of yellow and amber. Color varies depend on their origin pollen, age, and storage conditions, but transparency or clarity depends on the amount of suspended particles such as pollen. Once crystallized, honey turns lighter in color because the glucose crystals are white. Honey crystallization results from the formation of monohydrate glucose crystals, which vary in number, shape, dimension, and quality with the honey composition and storage conditions. The lower the water and the higher the glucose content of honey, the faster the crystallization.

Benefits of Honey

1. Wound healing

Honey improves wound healing by rendering it sterile in lesser duration of time; wounds thus treated have a better outcome in terms of hypertrophic scarring and post-burn contractures.

2. Energy Source

Honey contains about 64 calories per tablespoon. On the other hand, one tablespoon of sugar will give you about 15 calories. Furthermore, the carbohydrates in honey are monosaccharide that can be easily use by even the most sensitive stomachs, since it is very easy for the body to digest.

3. Source of Vitamins and Minerals

Honey contains a variety of vitamins and minerals. The type of vitamins and minerals and their quantity depends on the type of flowers. Commonly, honey contains Vitamin C, Calcium and Iron.

4. Antioxidants

Honey contains nutraceuticals, which are very effective for the removal of free radicals from the body. As a result, our body immunity is improved against many conditions, even potentially fatal ones like cancer or heart disease.

5. Weight loss

Honey has more calories than sugar when honey is consumed with warm water; it helps in digesting the fat stored in your body. Similarly, honey with lemon juice or cinnamon help in reducing weight.

2. Water

Most of ingredient are used in honey is water which contain 50-60% of finish honey. Water require microorganism pure, clear, free from metal and odorless. The most common use is tap water which contain elements such as mineral that important for fermentation. There are two components in water that yeast will use as nutrition is calcium and magnesium. Magnesium will help yeast to produce enzyme that require for fermentation.

3. Yeasts (K1-V1116)

The most common yeast associated is *Saccharomyces cerevisiae* which has been favored due to its tolerance of relatively high levels of alcohol and sulfur dioxide as well as its ability in normal wine pH range 3 - 4 and tolerate alcohol levels of 17-20%. It is often used in fortified wine production at high Brix sugar levels.

The primary role of yeast is to convert the sugars (glucose) in the must into alcohol and release CO_2. In the absence of oxygen (Anaerobic) the cell will continue metabolic functions but will rely on other pathways such as reduction of acetaldehyde into ethanol (alcohol). It is through this process of fermentation that ethanol is released by the yeast cells. The *Saccharomyces* yeast genus is favored for winemaking because of the generally reliable and positive attributes it can bring to the wine. These yeasts will usually readily ferment glucose, sucrose. It is metabolize glucose, sucrose, raffinose, maltose and ethanol.

Key Lime

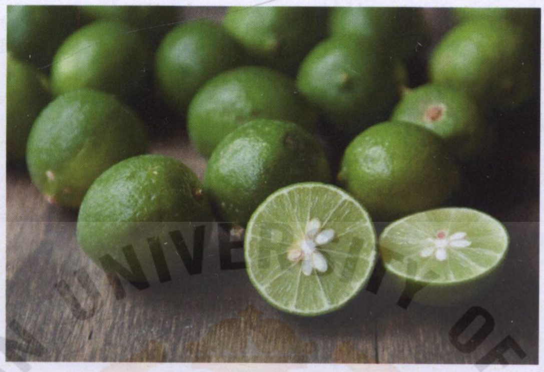

Figure 3 : Key Lime

Lime is native to Southeast Asiawhich it scientific classification name is Citrus aurantiifolia (Christm.) Swingle,it is orange family (rutaceae). Lime is different from lemon because lemon is the orange family as same as with lime but it has the big size with yellow color. Lime is smaller and seedier, with a higher acidity, a stronger aroma, and a thinner rind. Shelf life of limes is an important. The lime still ripens after harvesting, and it is usually stored between 12.5 °C to 15.5 °C at a relative humidity of 75–85%. Lime has a lot of benefits that is relieve the headache and vomit. It is high vitamin C fruit and citric acid is the main properties that can help to reduce calcium in blood vessel and activate enzyme. Lime contains flavonoid which improves capacity of vitamin c, control blood steam and reduce inflammatory. It also contains limonoid(oxygenated triterpenoid) and modified pectin that can resist and prevent cancer cell. The composition of the citric fruits is generally composed of 90% terpenes, 5% oxygenated compounds, and less than 1% non-volatile compounds such as waxes and pigments. Limonene, the most abundant terpene has antimicrobial properties, primarily the exhibition of antibacterial activity against Gram positive bacteria, and also increases the effectiveness of sodium benzoate as a preservative

Nutrient data for: Lime juice				
Nutrient	Unit	1Value per 100 g	1 cup = 242.0g	1 fl oz = 30.8g
Proximates				
Water	g	90.79	219.71	27.96
Energy	kcal	25	60	8
Protein	g	0.42	1.02	0.13
Total lipid (fat)	g	0.07	0.17	0.02
Carbohydrate, by difference	g	8.42	20.38	2.59
Fiber, total dietary	g	0.4	1	0.1
Sugars, total	g	1.69	4.09	0.52
Minerals				
Calcium, Ca	mg	14	34	4
Iron, Fe	mg	0.09	0.22	0.03
Magnesium, Mg	mg	8	19	2
Phosphorus, P	mg	14	34	4
Potassium, K	mg	117	283	36
Sodium, Na	mg	2	5	1
Zinc, Zn	mg	0.08	0.19	0.02
Vitamins				
Vitamin C, total ascorbic acid	mg	30	72.6	9.2
Thiamin	mg	0.025	0.061	0.008
Riboflavin	mg	0.015	0.036	0.005
Niacin	mg	0.142	0.344	0.044
Vitamin B-6	mg	0.038	0.092	0.012
Folate, DFE	mg	10	24	3
Vitamin B-12	mg	0	0	0
Vitamin A, RAE	mg	2	5	1
Vitamin A, IU	IU	50	121	15
Vitamin E (alpha-tocopherol)	mg	0.22	0.53	0.07
Vitamin D (D2 + D3)	mg	0	0	0
Vitamin D	IU	0	0	0
Vitamin K (phylloquinone)	mg	0.6	1.5	0.2
Lipids				
Fatty acids, total saturated	g	0.008	0.019	0.002
Fatty acids, total monounsaturated	g	0.008	0.019	0.002
Fatty acids, total polyunsaturated	g	0.023	0.056	0.007
Fatty acids, total trans	g	0	0	0
Cholesterol	mg	0	0	0
Amino Acids				
Other				
Caffeine	mg	0	0	0

Table 1 : Proximate value of nutrient data for Lime juice

Health benefits of lime

1. Dietary Vitamin C

Drinking lime juice helps to meet the body's daily requirement for vitamin C. A cup of freshly squeezed lime juice contains 72.6 milligrams of Vitamin C. Vitamin C is an essential nutrient that is needed for the growth and repair of tissues, as well as the repair and maintenance of our bones and teeth (University of Maryland Medical Center). It helps the body to produce collagen, a protein that plays a vital role in the production of blood vessels, skin, tendons, ligaments and cartilage.

2. Antibiotic Properties

Limes contain flavonol glycosides, which can anti-cancer and antioxidant benefits and antibiotic properties. The antibiotic properties of these compounds have been shown to protect against the contraction of cholera, a disease caused a bacteria called Vibrio cholera. (Research conducted in West Africa, published in "Tropical Medicine and International Health,") Drinking lime juice with the main meal protects participants against this disease.

3. Anti-carcinogenic

Lime juice has potent anti-carcinogenic properties. (J. Robert Hatherill Ph.D., a research scientist) limes and other citrus fruit contain a variety of cancer-fighting compounds called flavonoids. Flavonoids are a family of naturally-occurring compounds found in many fruit and vegetables. The biological activity of citrus juice flavonoids has anti-cancer effects that prevent the invasion of cancer cells (book "Eat To Beat Cancer,"). These compounds also powerfully inhibit the growth of tumor cells. He notes that the juice has most cancer-fighting clout when it is drunk fresh.

4. Detoxification

Limes contain eight different kind liminoids, which are compounds that promote the activity of an enzyme in the liver. This liver enzyme detoxifies a variety of cancer-causing chemicals. In effect, it deactivates these harmful chemicals, turning them into harmless chemicals that are then removed from the body in your urine.

5. Removes Dead Skin

Acid peels off dead skin cells. Limes contain citric acid. In addition to adding flavor and aroma, the citric acid found in limes helps remove dead skin from your face. Without dead skin hiding the healthy skin cells underneath, your face looks more vibrant and clear. The prompt removal of dead skin also prevents the sloughed cells from clogging up your pores which can help reduce flare-ups of acne and blemishes.

Acid Found in Limes

1. Citric Acid

Citric acid is a weak organic acid that is found in concentrated amounts in citrus fruits, especially lemons and limes. Citric acid is often used as an additive in food preparation, to add a tart flavor and as a preservative. For example, citric acid helps prevent browning of fresh vegetables and maintains the color of meat during storage. It also prevents the crystallization of sucrose in candy and promotes flexibility and separation of cheese slices.

2. Ascorbic Acid (Vitamin C)

Citric acid is not a necessary part of the human diet, but it may have a role to play in your health. It can be beneficial for people who have kidney stones because it inhibits their formation and helps break up stones that are starting to form. (Journal of Biochemistry and Clinical Nutrition in November 2007). Citric acid can help reduce physical fatigue. Study participants were given either a citric acid supplement or placebo and made to perform a strenuous physical task. Physiological stress markers were lower in the citric acid group, as was the subjective feeling of fatigue. The one lime juice provides almost 13 milligrams of vitamin C, which is 20 percent of the daily value.

Health Benefits of Ascorbic Acid

Vitamin C's main functions include forming a crucial protein that is used to make skin, tendons, ligaments and blood vessels; helping wounds heal and scar tissue form; and repairing and maintaining cartilage, bones and teeth. Vitamin C is also an important antioxidant, a nutrient that prevents damage to your body by free radicals. Free radicals are formed by your body during the metabolism of food and when you are exposed to pollutants such as cigarette smoke, and they contribute to the aging process and the development of cancer, heart disease and arthritis.

3. Malic Acid

Limes contain a small amount of malic acid, which is tart but enhances the sweetness of sucrose in fruit.(Journal of Experimental Botany in March 2006). Malic acid may have therapeutic benefits for individuals with fibromyalgia, a condition that causes pain and tenderness throughout the soft tissues of the body. People with fibromyalgia may have trouble using or producing malic acid, which can affect normal muscle function.

Di-Ammonium phosphate (DAP)

Yeast nutrition is an essential factor in success of fermentation and allows enhancing sensory quality. DAP can improve fermentation kinetics has been attempted by a number of researchers (see review by Bell and Henschke 2005) Chemical formula: (NH4)2HPO4, water solubility (20 °C): 588 g/L and pH: 7.5 to 8

Composition

1. Nitrogen 18%

Nitrogen is the most important yeast nutrient. It is a key factor that has a significant impact on wine fermentation. Nitrogen is metabolized by yeast to synthetize proteins. It stimulates yeast multiplication, keeps yeast metabolism active, prevents Hydrogen sulfide aroma.

2. phosphorus anhydride 46% (P_2O_5)
   Total Phosphate 10%, Phosphate available 9.95%, total Calcium 4.01%, Total Mg 1.2 % and other mineral such as Zn, Mg, Cu
3. Ammonium 21%

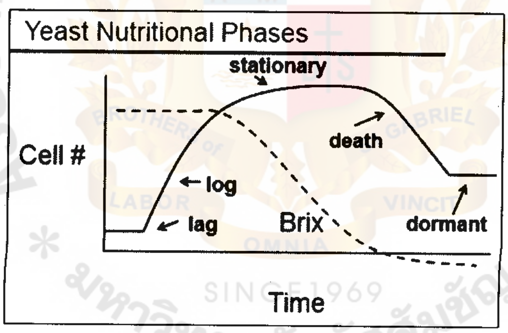

Figure 4 : Yeast phases

A. Lag Phase: yeast are adapting to their environment. Only then do they start to grow.
B. Log Phase: yeast undergo exponential growth to build up their biomass. They especially need complex nutrients as well as just DAP.
C. Stationary Phase: yeast are at maximum concentration. Only then are they triggered to convert sugar to alcohol. After a sugar reading drop of 3°- 4° Brix, the yeast have used up the nutrients you added at the Lag Phase. Now you need more complex nutrients to feed the yeast and also to build "survival factors".
D. Death Phase: yeast is inhibited by alcohol buildup, eventually ending in cell disruption/death and the end of alcohol production. They need some Nitrogen reserves to complete the ferment.

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What happens if the nutrition is not enough?

- Thiamine may reduce yeast growth, slow fermentation and promote the accumulation of pyruvic acid and acetaldehyde, components responsible for oxidation and binding SO_2.
- Low levels of sterols, oxygen or unsaturated fatty acids may shut down sugar consumption (stuck fermentation) and increase volatile acidity. A lack of oxygen will increase the need to add these nutrients.
- Without proper nutrition: yeast will produce undesirable characteristics: off-flavors (hydrogen sulfide, oxidation), high bound SO2, stuck or sluggish fermentations.

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Materials

1. Raw materials
   1.1 Honey
   1.2 Water
   1.3 Lime
   1.4 Di-ammonium phosphate (DAP)
   1.5 Active Dry Yeast (K1-
2. Equipments of wine process
   2.1 Plastic bottles
   2.2 Refractometer
   2.3 Handle ladle
   2.4 Stove
   2.5 Measuring meter : cup scale
   2.6 Pot
   2.7 Whatman filter paper No.4
   2.8 Vacuum pump
3. Equipment for wine analysis
   3.1 Ebulliometer (Dujardin Salleron)
   3.2 pH meter
   3.3 Refractometer
   3.4 Thermometer
   3.5 Munsell book
   3.6 Pipette 10 ml
   3.7 Burette
   3.8 Erlenmeyer flask
   3.9 Hot plate
   3.10 Volumetric Flask

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4. Reagent for chemical measurement

    4.1 Diatomaceous earth (DE)

    4.2 0.1 N sodium hydroxide (NaOH)

    4.3 0.1 N Potassium Hydrogen Phthalate (KHP)

    4.4 Phenolphthalein

    4.5 Copper (II) sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$)

    4.6 Potassium sodium tartrate tetrahydrate

    4.7 Potassium ferrocyanide trihydrate or *Potassium hexacyanoferrate* (II) *trihydrate*

    4.8 Zinc acetate dehydrate

    4.9 Glacial acetic acid

    4.10 Methylene blue

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Methods

Lime honey wine making process

The experiment was designed into 3 separated parts. In the first part: different ratios of lime juice are added together with 500 ppm of Di-ammonium phosphate in to the must. All other factors are remained the same. In the second part, different ratios of lime juice are added together with 1,000 ppm of Di-ammonium phosphate.

Lime juice preparation

Wash and dry the fresh whole limes. Slice the lime into half with a sharp knife. Press or squeeze by using hand or machine. The fresh lime juice can be stored by keeping in the bottle and freeze in the refrigerator. (William A. Hardwick, 1995)

1. Rehydrate active dry yeast

Dry powder yeast was used at the ratio of 0.5 g per liter. The yeast was activated by putting into warm water at 40°C for 15 minutes. A pitch of sugar was added to help accelerate the yeast active.

2. Wine Fermentation

    1) Boiling

In this experiment, we used commercial longan honey due to the availability. The honey was dissolved in hot water to adjust the degree of sugar in must to 24 Brix

Figure 5 : Longan Honey

2) Cooling

After honey mixed with water the solution was called must. Then the must was cool down to room temperature before lime juice and Di-ammonium phosphate (DAP) at the appropriate ratio was added.

3) Add Lime juice and DAP

Lime juice and DAP were added to the must in different quantity. according to the experiment design.

Degree of brix measurement are needed for adjust to 24 °Brix by adding honey and water little by little until the volume of must reach to final volume.

4) Add active dry yeast in fermentation bottle

Yeast was added to the must at a ratio of .05 g per liter. The must is stirred and the container is closed.

5) Fermentation

The fermentation duration of lime honey wine for this experiment was 10 days at room temperature.

6) Filtration and packaging

Diatomaceous earth (DE) was added into the must as a filter aid and the must was filter with What man filter paper no.4. After filtration, the wine was transferred into glass bottle and kept in the refrigerated room at 15 C for 3 days before preference test was conducted.

Experimental determination

The process of lime honey wine production has several steps. In general, the first step involves equipment sterilization, must preparation, must pasteurization, Brix adjustment, adding yeast, fermentation and post-fermentation. Finally, the mead is filtration to remove undesired material (died yeast).

The three parts of experimental design can be concludeas:

1. Determine suitable percentage of lime juice with 500 ppm DAP

Ingredient	Formulation A	Formulation B
Starting °Brix	24	24
Lime juice	5 %	10 %
Yeast	0.5 g/L	0.5 g/L
DAP	500 ppm	500 ppm

Table 2 : The different percent of lime juice in experiment 1.

2. Determine suitable percentage of lime juice With 1,000 ppm DAP

Ingredient	A	B
Starting Brix	24	24
Lime juice	5 %	10 %
Yeast	0.5 g/L	0.5 g/L
DAP	1,000 ppm	1,000 ppm

Table 3 : Increasing Di-ammonium phosphate to 1,000 ppm other factors are same as experiment 1.

3. Determine more different percentage of lime juice at 1,000 ppm DAP

Ingredient	A	B	C
Starting Brix	24	24	24
Lime juice	10 %	12 %	15%
Yeast	0.5 g/L	0.5 g/L	0.5 g/L
DAP	1,000 ppm	1,000 ppm	1,000 ppm

Table 4: Variation of percentage of lime juice other factors are same as experiment 2

Analysis of Lime Honey Wine

4.1 Alcohol Content

Alcohol content is measured by using Ebulliometer (Dujardin Salleron) methods

4.1.1 Determination temperature of boiled water

4.1.2 Determination temperature of boiled wine

4.2 pH

The pH is measured by using pH meter.

4.3 Total Soluble Solid (°Brix)

The total soluble solid (°Brix) is measured by using refractometer.

4.4 Color measurement

The color of wine is measured by using munsell book

Figure 6 : Compared color of honey with Munsell book

4.5 Total Titration Acidity

Citrus fruit juices contain citric acid as the dominant acid. The citric acid content can be determined through a titration. This process involves a certain volume of juice having known concentration added to citric acid until reached a neutralized point (known as the endpoint). Citric acid is a weak acid, its best titrated using a strong base, such as sodium hydroxide.

Calculation

1. Concentration of NaOH

$$N_{NaOH} = \frac{N_{KHP} \times V_{KHP}}{V_{NaOH}}$$

2. Total titrate acid

$$\%\text{Total acid (w/v)} = \frac{V_{NaOH} \times N_{NaOH} \times MW \times 100}{V_{sample} \times 1000}$$

Where MW = molecular weight predominant acid

4.5 Reducing Sugar

Reducing sugar is measured by using Lane and Eynon method

Lane and Eynon method is the most common method that using Fehling's agent. The cupric ion (Cu^{+}) is reduced by reducing sugar to cuprous ion (Cu^{+}) and precipitated in the form of cuprous oxide (red precipitant). The amount of cuprous oxide is determined by titration method. This reaction used methylene blue as the indicator and the solution must be boiling during titration.

4.6 Sensory test

This consumer test was used 9 henodic scale to scoring in all attributes with Randomized Complete Block Design (RCBD) and Analyzed by SAS program.

Result and Discussion

Determine suitable percentage of lime juice

Experiment 1

Formulation	5 % lime	10 % lime
Starting brix	24	24
Final brix	18	17

Table 5: Comparison of degree brix between 2 Formulations of lime with 500 ppm DAP

The result from table 5 shown that the decreased of degree brix of 2 formulations from 24 brix to 18 and 17 brix respectively. These result may be caused by the insufficient nutrient in must for the yeast to further utilize sugar. Therefore in the next experiment we increase the amount of DAP to 1,000 ppm.

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Experiment 2

Determine suitable percentage of lime juice at 1,000 ppm DAP

Formulation	5 % lime	10 % lime
Starting brix	24	24
Final brix	10.5	11

Table 6: Comparison of degree brix between 2 Formulations of lime with 1,000 ppm

From table 6 shown that the degree brix of lime honey wine was decreased obviously with the increase amount of DAP from 500 ppm to 1,000 ppm. It means that 1,000 ppm of DAP can increase nutrition source for yeast during fermentation.

2.1 Chemical and physical properties analysis

Analysis	5 % of lime	10 % of lime
pH	3.33	2.85
Total Soluble Solid (°Brix)	10.5	11
Color	5Y 8/8	5Y 8/8
Alcohol content	11.05%	10.43%
Total Acidity	1.72%	2.12%
Reducing Sugar	2.80 mg/ml	3.24 mg/ml

Table 7: Proximate analysis of honey wine in 5% of lime juice and 10% of lime juice

The results of chemical and physical properties analysis of 2 different lime honey wines were shown in table 7. Alcohol content of the wine added with 5% and 10% lime juice were 11.05% and 10.43% respectively. Total acidity and reducing sugar of 5% of lime juice were lower than 10% of lime juice added.

Consumer test

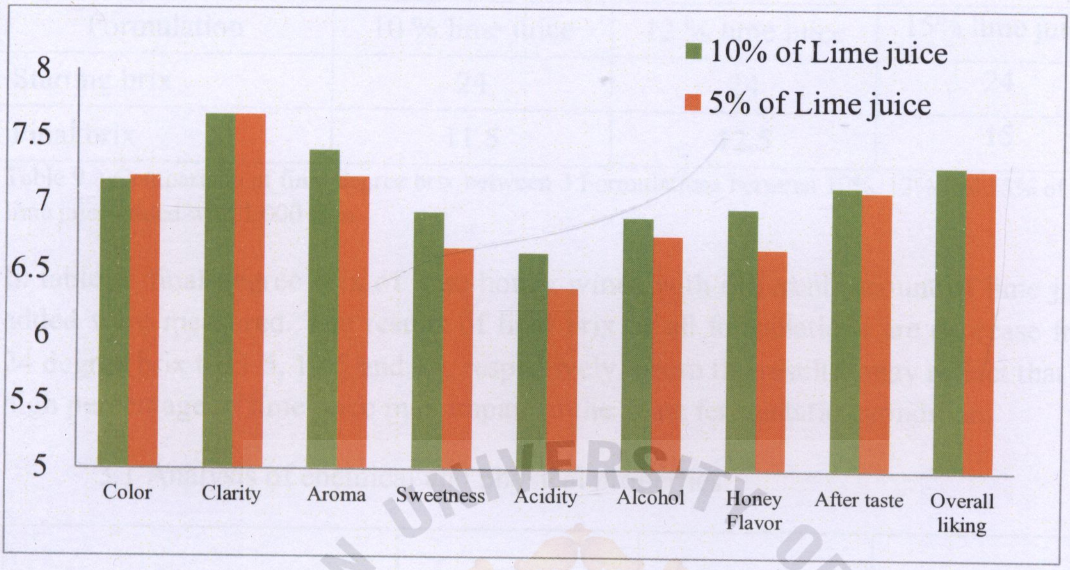

Figure 7: the comparison of highest mean score in all attributes between 5% of lime juice and 10% of lime juice added from 30 panelists

Attribute	5% (925)	10% (361)
Color	7.20 ± 1.13 [a]	7.167 ± 1.05 [a]
Clarity	7.67 ± 1.27 [a]	7.67 ± 0.92 [a]
Aroma	7.20 ± 0.99 [a]	7.40 ± 0.89 [a]
Sweetness	6.67 ± 1.56 [a]	6.93 ± 1.34 [a]
Acidity	6.37 ± 1.73 [a]	6.63 ± 1.42 [a]
Alcohol	6.77 ± 1.63 [a]	6.90 ± 1.35 [a]
Honey Flavor	6.67 ± 1.54 [a]	6.97 ± 1.63 [a]
After taste	7.10 ± 1.18 [a]	7.13 ± 1.57 [a]
Overall liking	7.23 ± 1.17 [a]	7.26 ± 1.08 [a]

Table 8: Sensory analysis from SAS program between 5% of lime juice and 10% of lime juice added from 30 panelists

In the 10 % lime juice added wine has the higher mean score in the following attributes: aroma, sweetness, acidity, alcohol, honey flavor, after taste and overall liking. There are no significantly differences between two formulations. Both of their attributes are same.

Experiment 3

Formulation	10 % lime juice	12 % lime juice	15% lime juice
Starting brix	24	24	24
Final brix	11.5	12.5	15

Table 9 : Comparison of final degree brix between 3 Formulations between 10%, 12% and 15% of lime juice added with 1,000 ppm

In table 9: final degree brix of lime honey wines with different amount of lime juice added were measured. The results of final brix of all formulations are decrease from 24 degree brix to11.5, 12.5 and 15, respectively. From the result it may reflect that the high percentage of lime juice may impair to the yeast fermentation condition.

3.1 Analysis of chemical and physical properties

Analysis	10 % lime juice	12 % lime juice	15% lime juice
pH	2.87	2.85	2.81
Total Soluble Solid (°Brix)	11.5	12.5	15
Color	5Y 8.5/8	5Y 8.5/8	5Y 8.5/8
Alcohol content	10.25%	7.60%	5.90%
Total Acidity	2.36%	2.75%	2.91%
Reducing Sugar	3.45 mg/ml	3.98 mg/ml	4.32 mg/ml

Table 10: The analysis result between 10%, 12% and 15% of lime juice added.

The results of chemical and physical properties analysis of 3 different percentages of lime juice added; 10%, 12% and 15% were shown in the table 10. Increasing the percentage of lime juice also increasing the following factors: Total soluble solid, total acidity, and reducing sugar, and reducing the value of pH and alcohol content. But there is no change in color measuring which is 5Y 8/8.

3.2 Consumer test

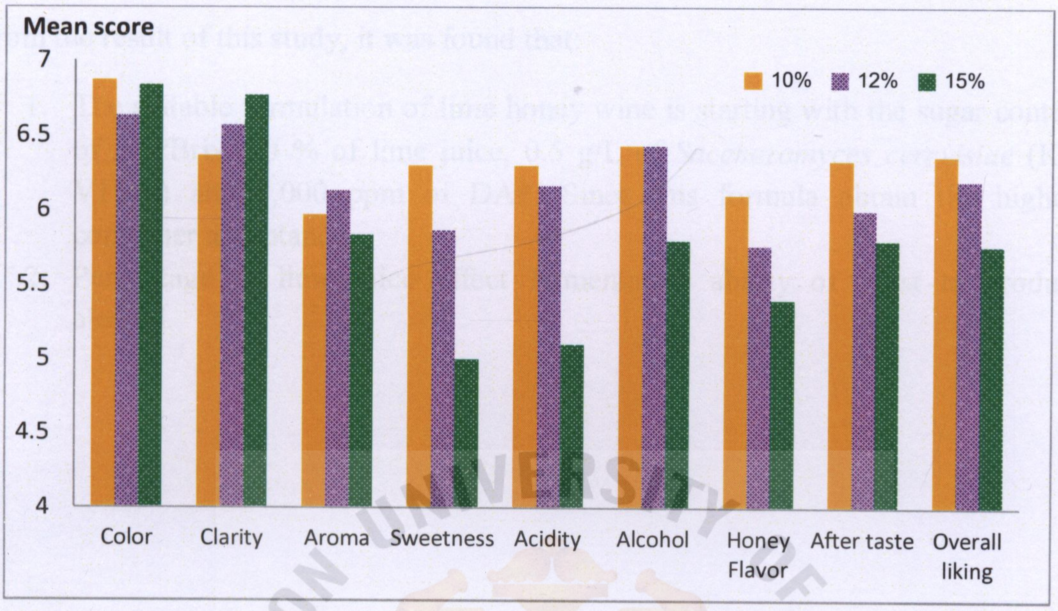

Figure 8: the comparison of highest mean score in all attributes from 30 consumers between 10%, 12% and 15% of lime juice added.

Attribute	10% (653)	12% (487)	15% (192)
Color	6.87 ± 1.20 [a]	6.63 ± 0.96 [a]	6.83 ± 0.99 [a]
Clarity	6.37 ± 1.03 [a]	6.57 ± 0.94 [a]	6.77 ± 1.17 [a]
Aroma	5.97 ± 1.50 [a]	6.13 ± 1.63 [a]	5.83 ± 1.49 [a]
Sweetness	6.30 ± 1.37 [a]	5.87 ± 1.87 [a]	5.00 ± 2.21 [b]
Acidity	6.30 ± 1.18 [a]	6.17 ± 1.82 [a]	5.10 ± 2.47 [b]
Alcohol	6.43 ± 1.52 [a]	6.43 ± 1.70 [a]	5.80 ± 2.27 [b]
Honey Flavor	6.10 ± 1.77 [a]	5.77 ± 1.20 [ab]	5.40 ± 2.08 [b]
After taste	6.33 ± 1.63 [a]	6.00 ± 1.78 [ab]	5.80 ± 2.35 [b]
Overall liking	6.37 ± 1.22 [a]	6.20 ± 1.73 [ab]	5.77 ± 1.61 [b]

Table 11: Sensory analysis from SAS program from 30 consumers between 10%, 12% and 15% of lime juice added.

The highest mean score is the wine contains 10 % of lime juice added.

There are no significantly differences between three formulations in color, clarity, aroma, honey flavor, after taste and overall liking. There are significantly different in sweetness, acidity and alcohol.

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Conclusion

From the result of this study, it was found that:

1. The suitable formulation of lime honey wine is starting with the sugar content of 24 °Brix, 10 % of lime juice, 0.5 g/L of *Saccharomyces cerevisiae* (K1-V1116) and 1,000 ppm of DAP. Since this formula obtain the highest consumer acceptance.
2. Percentage of lime juice effect fermentation ability of yeast to produce alcohol.

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Appendix

APPENDIX A

Analysis method

1. Measuring pH by using a pH meter
    1. Switch on the power supply
    2. Rinse the electrode with distilled water
    3. Select using the mode botton the desired measurement mode
    4. Calibrade the meter using at least 2 buffers
    5. rinse the electrode with distilled water
    6. Carry out the measurement by dipping the electrode in the sample
    7. Rinse the electrode with distilled water before change sample
    8. After finish rinse the electrode and storage.
    9. switch off the meter by pressing the "power"

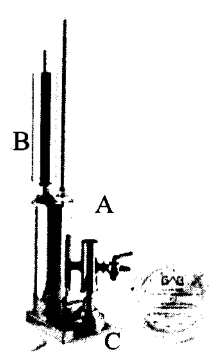
2. Measuring total soluble solid by refractometer

This method is determines by measuring the refractive index of the solution. Refractive indices vary with composition as well as wavelengths of light by sending light through a fluid sample. Refractometer measures the percent solid, the concentration of a solid substance in water or other fluids.

3. Measuring color from Munsell book

Pour 100 ml of sample into clear glass and compare color in munsell book

4. Measuring alcohol content



4.1 Determination temperature of boiled water

- Pipette water for 15 ml into the A
- Fill water(full) in B
- Put thermometer into A

- Boil at C position and record boiling temperature of water.

4.2 Determination temperature of boiled wine

- Pipette wine sample for 40 ml into the A
- Fill water(full) in B
- Put thermometer into A
- Boil at C position and record boiling temperature of wine sample.

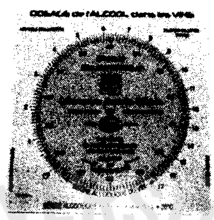


Figure 9 : Ebulliometer conversion scale

5. Measuring Total titrate acidity

The titrating reagent used is a 0.1 N sodium hydroxide (NaOH) solution, which contains 4 g of NaOH per 1000 mL of solution. For titration of drinks, which are clear and colourless (or pale yellow), phenolphthalein is an excellent acid-base indicator substance.

5.1 Standardize sodium hydroxide (NaOH) solution

1. Weigh 0.1 N Potassium Hydrogen Phthalate (KHP) 2.04 g
2. Dissolve 2.04 g (KHP) in water and dilute to 100 ml
3. Pipette 10 ml of Potassium Hydrogen Phthalate (KHP) solution in to 250 Erlenmeyer flask; add a few drop of phenolphthalein.
4. Titrate with 0.1 N sodium hydroxide (NaOH) solution to endpoint

Volume of N_aOH (ml)	Sample
	0.1 N KHP
1	14.6
2	15
Average	14.8

Table12 : The titrate volume of N_aOH with 0.1 N KHP

Concentration of NaOH

$$N_{NaOH} = \frac{N_{KHP} \times V_{KHP}}{V_{NaOH}}$$

$$= \frac{0.1 \times 10}{14.8}$$

$$= 0.0676 \text{ N}$$

5.2 Determination Acidity in sample

1. Pipette 10 ml of Potassium Hydrogen Phthalate (KHP) solution in to 250 Erlenmeyer flask; add a few drop of phenolphthalein.
2. Titrate with 0.1 N sodium hydroxide (NaOH) solution to endpoint

<u>Raw data</u>

Volume of N_aOH	Sample	
	5%	10%
1	13.2	17.1
2	13.3	15.6
Average	13.25	16.35

Table 13 : The titrate volume of N_aOH with 5% and 10% of lime juice added in wine

Volume of N_aOH	Sample		
	10%	12%	15%
1	18.10	21.1	22.6
2	18.20	21.2	22.2
Average	18.15	21.15	22.4

Table 14 : The titrate volume of N_aOH with 10%, 12% and 15% of lime juice added in wine

<u>Calculation</u>

Example: 5% of lime juice use 13.25 ml of N_aOH

$$\%\text{Total acid (w/v)} = \frac{V_{NaOH} \times N_{NaOH} \times MW}{V_{sample} \times 1000} \times 100\%$$

$$= \frac{13.25 \times 0.0676 \times 192.14}{10 \times 1000} \times 100\%$$

$$= 1.721 \ \%$$

Where MW = molecular weight citric acid

6. Measuring reducing sugar

6.1 Reagent preparation (keep in dark bottle)

Fehling's solutions A: dissolve 69.28g Copper (ll) sulfate pentahydrate in 500 ml distilled water and dilute to 1 L. The solution may be stored indefinitely.

Fehling's solutions B: dissolve 34g Potassium sodium tartrate tetrahydrate and 100g NaOH in 500 ml distilled water and dilute to 1 ml.

Carrez reagents 1: 10.6g Potassium ferrocyanide trihydrate per 100 ml.

Carrez reagents 2: 21.9g zinc acetate dehydrate and 2 ml glacial acetic acid distilled water and dilute to 100 ml.

6.2 Sample preparation and analysis

Blend sample about 5g (or ml) with distilled water 10-20 ml. Add 5 ml of clearing reagent Carrez 1 and 2, shake and make up value to 100 ml. Filter and use the filtrate to determine the amount of reducing sugar.

6.3 Preliminary titration

1. Fill the sample solution into 50 ml burette.
2. Pipette 10 ml of Fehling's A and 10 ml of B to 250 ml flask and mix well.
3. Heat until boil and add 2-3 drops of methylene blue.
4. From the burette, titration the sample to mixture of Fehling's solution until blue color of methylene disappears. The volume should be within 15-50 ml.

6.4 Accurate titration

1. Pipette 10 ml of Fehling's solution A and 10 ml of B to 250 ml flask. Add the sample from the burette with the volume that less than the volume obtained from preliminary titration by 2-3 ml.
2. Heat until boil. Drop one of the Methylene blue as indicator and titrate drop by drop until blue color disappears. The titration should finish within 3 min.
3. Calculate the amount of sugar from the conversion factor obtained from the table for a specific sugar.

Factors of ml of sample

$$= \text{ml of sugar solution require} \times \frac{10\ ml\ fehling'ssolution\ mg\ dextrose\ per\ 100\ ml}{100}$$

$$\frac{\text{Factors of milliliters of samples}}{\text{milliliters of sugar solution require}} = \frac{mg}{ml} \text{ of anhydrous dextrose of prepared solution (m)}$$

$$\text{Reducing sugar by mass} = \frac{m}{M} \times 10$$

Where m = mg/ml of anhydrous dextrose of prepared solution (m)

M = Mass in g of the prepared sample used for 100 ml of solution

Times	Volume of titration	
	5%	10%
1	35.5	30
2	36.5	30
Average(ml)	36	30

Table 15 : The titrate volume of 5% and 10% of lime juice with Fehling's solutions

Times	Volume of titration		
	10%	12%	15%
1	30	25	22
2	29	25	24
Average(ml)	29.5	25	23

Table 16 : The titrate volume of 10%,12% and 15% of lime juice with Fehling's solutions

<u>Calculation</u>

Example: 5 % Of lime juice use 36 ml

A. Factors of ml of sample

$$= \text{ml of sugar solution required} \times \frac{10\ ml\ fehling'ssolution\ mg\ dextrose\ per\ 100\ ml}{100}$$

$$= 36 \times \frac{140}{100}$$

= 50.4

B. $$\frac{\text{Factors of milliliters of samples}}{\text{milliliters of sugar solution require}} = \frac{mg}{ml} \text{ of anhydrous dextrose of prepared solution (m)}$$

$$\frac{50.4}{36} = 1.4 \text{ mg}$$

C. Reducing sugar by mass $= \frac{m}{M} \times 10$

$= \frac{1.4\ mg}{5\ ml} \times 10$

$= 2.8$ mg/ml

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Mg of sugar solution require		10 ml of Fehling'ssolution / mg dextrose per 100 ml
15		327
16		307
17		289
18		274
19		260
20		247.4
21		235.8
22		225.5
23		216.1
24		207.4
25		199.3
26		191.8
27		184.9
28		178.5
29		172.5
30		167
31		161.8
32		156.9
33		152.4
34		148
35		143.9
36		140
37		136.4
38		132.9
39		129.6
40		126.5
41		123.6
42		120.8
43		118.1
44		115.5
45		113
46		110.6
47		108.4
48		106.2
49		104.1
50		102.2

Table 17 : Dextrose table

Appendix B

Questionnaire

Product Description: Mead or Honey Wine is the oldest alcoholic drinks known to man. It is made from honey and water via fermentation with yeast. It is known from many sources of ancient history throughout Europe, Africa and Asia.

Instruction: You are receiving the sample. Please test the sample and rate the sample base on your preference on 9 point hedonic scale. Please rinse your mouth with water before and after test each sample. Take a good sip - swirl and slosh around your whole mouth.

1 – Extremely dislike
2 – Dislike very much
3 – Moderately dislike
4 – Slightly dislike
5 – Neither like or dislike
6 – Slightly like
7 – Moderately like
8 – Like very much
9 – Extremely like

Attributes	Sample ______	Sample ______	Sample ______
Overall liking			
Color			
Clarity			
Aroma (smell)			
Sweetness			
Acidity			
Alcohol			
Honey flavor			
After taste			

Preference Test

Which sample do you prefer? ______________

Comments

____

____

____

____

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Appendix C

Experiment 2

ANOVA table of lime honey wine with varying 2 different percentages of lime in each attribute

1. Color

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.01666667	0.01666667	0.02	0.8759
Rep	29	49.4833333	1.70632184	2.54	0.0072

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	7.2000	30	925
A	7.1667	30	361

Note : The same letter are not significantly different.

2. Clarity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.00000000	0.00000000	0.00	1.0000
Rep	29	61.3333333	2.11494253	6.13	<.0001

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	7.6667	30	361
A	7.6667	30	925

Note : The same letter are not significantly different.

3. Aroma

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.60000000	0.60000000	0.74	0.3956
Rep	29	28.6000000	0.98620690	1.22	0.2962

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	7.4000	30	361
A	7.2000	30	925

Note : The same letter are not significantly different.

4. Sweetness

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	1.06666667	1.06666667	0.94	0.3405
Rep	29	89.6000000	3.08965517	2.72	0.0044

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	6.9333	30	361
A	6.6667	30	925

Note : The same letter are not significantly different.

5. Acidity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	1.0666667	1.0666667	0.84	0.3676
Rep	29	109.000000	3.7586207	2.95	0.0024

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	6.6333	30	361
A	6.3667	30	925

Note : The same letter are not significantly different.

6. Alcohol

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.2666667	0.2666667	0.28	0.6015
Rep	29	102.333333	3.5287356	3.69	0.0004

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	6.9000	30	361
A	6.7667	30	925

Note : The same letter are not significantly different.

7. Honey flavor

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	1.3500000	1.3500000	0.95	0.3374
Rep	29	104.483333	3.6028736	2.54	0.0072

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	6.9667	30	361
A	6.6667	30	925

Note : The same letter are not significantly different.

8. After taste

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.01666667	0.01666667	0.01	0.9242
Rep	29	59.6833333	2.05804598	1.14	0.3658

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	7.1333	30	361
A	7.1000	30	925

Note : The same letter are not significantly different.

9. Overall liking

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	1	0.01666667	0.01666667	0.02	0.8935
Rep	29	46.7500000	1.61206897	1.77	0.0659

Duncan's multiple range tests

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Treatment
A	7.2333	30	925
A	7.2667	30	361

Note : The same letter are not significantly different.

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Experiment 3

ANOVA table of lime honey wine with varying 3 different percentages of lime in each attribute

1. Color

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	0.95555556	0.47777778	0.98	0.3827
Rep	29	68.22222222	2.35249042	4.81	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.8667	30	653
A	6.8333	30	192
A	6.6333	30	487

Note : The same letter are not significantly different.

2. Clarity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	2.40000000	1.20000000	1.40	0.2540
Rep	29	46.10000000	1.58965517	1.86	0.0225

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.7667	30	192
A	6.5667	30	487
A	6.3667	30	653

Note : The same letter are not significantly different.

3. Aroma

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	1.3555556	0.6777778	1.07	0.3487
Rep	29	169.9555556	5.8605364	9.28	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.1333	30	487
A	5.9667	30	653
A	5.8333	30	192

Note : The same letter are not significantly different.

4. Sweetness

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	23.4888889	11.7444444	7.58	0.0012
Rep	29	207.7888889	7.1651341	4.63	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.3000	30	653
A	5.8667	30	487
B	5.0667	30	192

Note : The same letter are not significantly different.

5. Acidity

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	25.9555556	12.9777778	7.42	0.0013
Rep	29	211.7888889	7.3030651	4.18	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.3000	30	653
A	6.1667	30	487
B	5.1000	30	192

Note : The same letter are not significantly different.

6. Alcohol

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	8.0222222	4.0111111	3.04	0.0558
Rep	29	222.8888889	7.6858238	5.82	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.			
Duncan Grouping	**Mean**	**N**	**Treatment**
A	6.4333	30	653
A	6.4333	30	487
B	5.8000	30	192

Note : The same letter are not significantly different.

7. Honey Flavor

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	7.3555556	3.6777778	4.78	0.0120
Rep	29	286.6222222	9.8835249	12.84	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.				
Duncan Grouping		Mean	N	Treatment
	A	6.1000	30	653
B	A	5.7667	30	487
B		5.4000	30	192

Note : The same letter are not significantly different.

8. After taste

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	4.3555556	2.1777778	2.38	0.1011
Rep	29	276.4888889	9.5340996	10.44	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.				
Duncan Grouping		Mean	N	Treatment
	A	6.3333	30	653
B	A	6.0000	30	487
B		5.8000	30	192

Note : The same letter are not significantly different.

9. Overall liking

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	5.7555556	2.8777778	2.83	0.0670
Rep	29	146.2222222	5.0421456	4.96	<.0001

Duncan's Multiple Range Test

Means with the same letter are not significantly different.				
Duncan Grouping		**Mean**	**N**	**Treatment**
	A	6.3667	30	653
B	A	6.2000	30	487
B		5.7667	30	192

Note : The same letter are not significantly different.

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