The Production of Krachai Beer

Mr. Chowkrit Banjongnitipat

A Special Project Submitted in Partial Fulfillment of the Requirement for

the Degree of Bachelor of Science in Biotechnology

Department of Food Technology

Assumption University

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Title: The Production of Krachai Beer

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Project's Advisor

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Abstract

Krachai *(Boesenbergia rotunda* (L) Mansf.), traditionally used in Thai cuisine, is used as flavoring agent and eaten as a vegetable. Krachai has varieties of medicinal properties including antioxidant, antibacterial, antifungal, anti-inflammatory, antitumor, and anti-tuberculosis activities. Beer is the most popular alcoholic beverage worldwide, and nowadays flavored beer market is expanding. Adding of Krachai in beers could provide unique flavor and consumer health benefits. In this study, Krachai was added into brewing process at different amount (10, 20, 30 grams) to obtain the most preferred formulation in order to develop a beverage with desirable sensory characteristic. Physical and chemical properties of Krachai beer were determined including pH, alcohol content, acidity and color measurement. Krachai beer with 10 grams of Krachai in formulation had the highest perception score and was accepted by panelists. In conclusion, the addition of Krachai to brewing process created a new perspective to expand variety of beer.

Keywords: Krachai, *Boesenbergia rotunda* (L) Mansf., Beer, Flavored beer, Craft beer

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Introduction

Beer is one of the most popular alcoholic beverages around the world. It has a very long history, even beyond the record of civilization. Yeast, malt, hops, and water are the main ingredients for beer brewing. The brewing process of beer contains several steps, including mashing, fermentation, filtration, and filling. Converting malt through fermentation can produce a favorable beverage. Hops are indispensable in beer, as they supply beer with bitterness and flavor compounds, antibacterial ability, and health effects. Amylases (a-amylase, (3-amylase, a-1 ,4-glucan glucohydrolase, and deramifying enzyme) and proteases are two main enzymes used in beer brewing. Yeast is the soul of beer brewing, as it plays a decisive role in beer quality. (Q. Li *et al.,* 2017) Recently, many flavored beers were developed to meet market's demand. There are beers flavored with either conventional or sometimes unconventional ingredients. The base beer may be related to almost any classic beer style, and the union of the base beer's character with the flavoring element is a critical aspect of the beer's success. These flavoring created variety of beers, also sometimes provide health benefit. This study aims to develop Krachai *(Boesenbergia rotunda* (L) Mansf.) beer that having good aroma, taste, and health benefits.

Boesenbergia rotunda (L) Mansf. is a perennial herb of the family Zingiberaceae, commonly found in the Southeast Asian countries and tropical rainforests including Thailand. It is used in Thai cuisine, commonly called Krachai in Thai. It is used as flavoring agent and eaten as a vegetable, as well as having a variety of medicinal properties. The main bioactive compounds from the rhizome extract of *B. rotunda* consist of boesenbergin, cardamonin, pinostrobin, pinocembrin, panduratin A and 4-hydroxypanduratin A. *B. rotunda* have antioxidant, antibacterial, antifungal, anti-inflammatory, antitumour and anti-tuberculosis activities (Kiat *et al.,* 2006). Moreover, *B. rotunda* has been used for the treatment of gastrointestinal disorders including peptic ulcer (Abdelwahab *et* al.,201 1). These health beneficial properties and flavoring agents were made *B. rotunda* to be interested of applying with beer formulation.

Previously, the development of Krachai Dum *(Kaempferia parviflora)* beer formulation was studied. Many research works found that Krachai also has good medicinal properties and more available in Thailand market. Therefore, this study changed to use Krachai instead of Krachai Dum in beer production. The development of Krachai beer formulation were studied to create new

variety of beer. An aroma and taste of Krachai could enhance the flavored beer. Chemical properties of the beer were tested. The sensory and product acceptance of the selected formulation were done by panelist. This study is the next step to innovate the new alcoholic beverage that contains many health benefits from herbal ingredient.

Objectives

I. To develop the formulation of Krachai beer.

2. To determine physical and chemical properties of Krachai beer by using pH, alcohol content, acidity and color measurement.

3. To determine sensory attribute of Krachai beer.

Literature Reviews

1. Beer

Beer brewing and drinking are activities that have been part of the human experience seemingly since the dawn of civilization. Around 10,000 years ago, mankind began to move away from living life as nomadic hunter gatherers, and began settling down in one spot to farm the land. Grain, a vital ingredient in beer making, was cultivated by these new agricultural societies. Beer is one of the oldest known beverages in the world, and is still a staple low-alcohol product. It is the most popular alcoholic beverage worldwide by volume, with global production steadily increasing from approximately 1.30 billion hectolitres (34.3 billion U.S. gallons) in 1998 to 1.96 billion hectolitres (51.8 billion U.S. gallons) in 2014 (Statista, 2016).

Beer is essentially a fermented drink made from four primary ingredients: grain, hops, yeast and water. Each plays a significant role in its creation of beers as following;

Grain

The chosen grain in beer making determines the beer's color, flavor, sugar content, protein content and dextrin levels. Each grain influences the taste of a particular beer.

- Barley: One of the foundation stones of beer is barley, which is transformed into brew-ready malt by taking a bath in hot water. This causes the grain to create the enzymes that transform proteins and starches into fermentable sugars, which yeast will later feast on to create alcohol. With brewing, top billing on the grain bill usually is reserved for barley malts. This is due mainly to an evolutionary advantage: barley contains husks, which keep the mash (the grains steeped in boiling water) loose and permit drainage of the wort -the broth that becomes beer. For flavor, brewers often blend the lead grain barley with a host of supporting fermentable grains (such as rye and wheat). There's no global system for classifying the hundreds of varieties of barley, but they can be condensed into several broad categories.
- Base malts: These compose the bulk of the grain bill. Typically, lighter-colored, these workhorse malts provide the majority of the proteins, fermentable sugars, and minerals required to create beer.

- Specialty malts: These auxiliary grains are great for increasing body, improving head retention, and adding color, aroma, and flavor, such as coffee, chocolate, biscuit, and caramel. Specialty grains are blended to achieve unique flavor profiles and characteristics. Popular varieties include the following: Crystal (or caramel) malts, specially stewed to create crystalline sugar structures within the grain's hull. They add sweetness to beer. Roasted malts, kilned or roasted at high temperatures to impart certain flavor characteristics. Coffee beans undergo a similar transformation. Dark malts, highly roasted to achieve the robust flavors associated with stouts, Schwarz biers, bocks, and black IPAs.
- Unmalted barley: This imparts a rich, grainy character to beer, a key characteristic of styles such as dry stout. Unmalted barley helps head retention, but it will make a beer hazier than Los Angeles smog.
- Corn: When used in beer, corn provides a smooth, somewhat neutral sweetness. It is utilized to lighten a beer's body, decrease haziness, and stabilize flavor.
- Oats: Used in conjunction with barley, oats create a creamy, full-bodied brew that's as smooth as satin. Stouts are a natural fit.
- Rice: As a beer ingredient, rice imparts little or no discernible taste. Instead, the grain helps create snappy flavors and a dry profile as well as lighten a beer's body.
- Rye: Working in conjunction with barley, rye can sharpen flavors and add complexity, crispness, and subtle spiciness as well as dry out a beer. The grain also can be kilned to create a chocolate or caramel flavor. Its shortcoming: since rye is hull-less, using large percentages of the grain during brewing can cause it to clump up and turn to concrete.
- Wheat: Packed with proteins, this grain helps create a fuller body and mouthfeel and a foamy head as thick and lasting as Cool Whip. A large proportion of wheat can result in a smooth, hazy brew such as a hefeweizen or a witbier. Wheat can impart a slight tartness.

The color of the grain used to make a specific beer determines its final color, while its flavor is imbued primarily by malted barley, and to a lesser degree by hops and yeast. Maltose, the fermentable sugar derived from malted grain is converted by the yeast component into alcohol, while the proteins in the grain help to create and form the head or foam cap on a poured beer. The dextrins in the grain are responsible for creating "mouthfeel" in a beer. This is the feeling of fullness or the viscosity of the final product.

Hops

A wide variety of hops, the flower of the hop plant, a member of the hemp family was used in the creation of beer. Each adds a different flavor, smell and finish to the beer. While some beers are made using a single type of hop, most use a variety of hops to obtain a specific taste and smell. There are many types and styles of hops.

- Amarillo — Amarillo is an aroma-type cultivar of recent origin, discovered and introduced by Virgil Gamache Farms Inc.
- Cascade — Cascade is an aroma-type cultivar which originated as the first commercial hop from the USDA-ARS breeding program. It was bred in 1956 but not released for cultivation until 1972. It reached its peak in 1975 when it produced 13.3% of the total American crop. It was obtained by crossing an English Fuggle with a male plant, which originated from the Russian variety Serebrianka with a Fuggle male plant. A very popular U.S. variety, with a moderate bitterness level and fragrant, flowery aroma. Cascade is often used in highly hopped West Coast ales that have a citrus-floral hop character.
- Centennial — Centennial is an aroma-type cultivar, bred in 1974 and released in 1990. A relatively new hop on the market, this hop used to be called CFJ90. Described by some as a "Super Cascade" and we tend to agree, but it's not nearly as "citrusy". Some even use it for aroma as well as bittering. Bitterness is quite clean and can have floral notes depending on the boil time.
- Golding — Golding is a group of aroma-type cultivars originating in England. Over the decades, the group has been changed and widened. Mostly they have been named after villages in East Kent, (Petham, Rothersham, Canterbury, Eastwell) or hop farmers, who grew them (Amos's Early Bird, Cobbs). English Goldings grown in East Kent, are a premium hop, called East Kent Golding and should not be confused with U.K. Goldings, which are grown in other parts such as Kent, Worcestershire, Hampshire and Herefordshire. The cultivar grown in the USA (Oregon and Washington State) is a Canterbury Golding. The premier English aroma hop. Superb in English-style ales, and lend a unique character to fine lagers as well. This hop has a unique spicy aroma and refined flavor.

- Nugget — Nugget is a bittering-type cultivar, bred in 1970 from the USDA 65009 female plant and USDA 63015M. Nugget is a great bittering hop with a heavy herbal aroma.
- Tettnang — Tettnang is an aroma-type cultivar which originated in the Tettnang hop growing area of Germany as a land-race hop. It is grown in the U.S.A. in Oregon and Washington State. The original noble hop from the Tettnang region of Germany, ideal for your finest lagers and wheat beers. This limited availability hop has a fine, pure aroma, that is not present in United States grown Tettnanger.
- Willamette — Willamette is a triploid aroma-type hop, which originated in the mid-1970s and is a seedling of Fuggle. It is a very popular aroma hop, contributing in 1998 to 18% of the total USA hop crop. A variation on English Fuggle hops grown in Oregon and Washington. Willamette has a fragrant spicy woody aroma. An excellent American aromatic hops for ales and lagers.

The process to obtain the desired extract from the hops usually takes about two hours. The hop resins contribute flavor, aroma and bitterness to the brew. Once the hops have flavored the brew, they are removed. The hops used determine four characteristics of the beer: they add bitterness to counteract the malty sweetness; they give the beer its flavor and add complexity; essential oils in the hops give beer its distinctive smell, and the acids in hops help to stabilize the beer and give it a shelf life.

Yeast

Yeast is a single-celled organism, a fungus — is the most important ingredient in beer brewing. It is a living organism that metabolizes, reproduces and lives off ingredients in beer. Yeast is responsible for converting sugar to alcohol during the fermentation stage of brewing, and is also a determinant of flavor. Beer consists of many compounds (volatile and non-volatile) that affect beer flavor, many of these aroma compounds are synthesized by yeasts during fermentation, others derive directly from the raw materials. Although there are thousands of yeast strains, only cultivated strains should be used when brewing of beer. Using other yeast strains may cause over-carbonation and unusual flavors. Brewers choose yeast strains based on which style of beer is being made. The two main yeast strains used are *Saccharomyces cerevisiae,* for top-fermenting ales, and *Saccharomyces uvarum,* for bottom-fermenting lagers.

Water

Since it comprises 95% of the finished product, the quality of the water used in the brew is paramount; it must be pure, clean and free from pollutants. Pure water is essential to good beer and brewers pay scrupulous attention to the source and purification of their brewing water. The water used in brewing is purified to rigidly set standards. If it doesn't have the proper calcium or acidic content for maximum activity of the enzymes in the mash, it must be brought up to that standard.

According to the brewing processes and ingredients the beer varieties are huge. The brewing can be carried on high or low fermentation temperatures. Lager beers, the most popular worldwide, are produced with strains of *Saccharomyces calsbergensis,* being fermented at temperatures from 3.3 to 13.0 °C for 4-12 weeks. On the other hand, ale beers are typically fermented at warmer temperatures between 20 ± 4 °C for shorter periods with the top strain, *Saccharomyces cerevisiae,* being more prevalent in northern countries such as Germany, Belgium, Canada, the United States and Britain.

2. Flavored beer

The world beer market is extremely competitive, and has become more so in recent years as Europe, North America and Japan have witnessed a decrease in beer sales, partially associated with the expansion in these markets of lager beer. In response, many breweries have attempted to widen their offers by developing new, innovative products to meet consumer demand (Carvalho *et al.,* 2009).

Recently, many flavored beers were developed. There are beers flavored with either conventional or sometimes unconventional ingredients. The base beer may be related to almost any classic beer style, and the union of the base beer's character with the flavoring element is a critical aspect of the beer's success. These flavoring created variety of beers, also sometimes provide health benefit. The number of new flavored offerings breweries large and small are rolling out to the marketplace has exploded by a whopping 80% over a five-year period (2010-2015), according to data from market research firm Mintel. In 2010, 15% of the new beers introduced were flavored beers. The growth of the related hard cider category also helps bolster the flavored-

beer boom because it exposes drinkers to other, fruit-forward alcoholic beverage options. Perhaps unsurprisingly, the shift towards sweeter tastes and flavor profiles (chocolate, spices and the ubiquitous pumpkin are especially popular) can be attributed to the burgeoning number of millennial consumers. The rise in flavored beer goes hand-in-hand with the explosive growth in craft beer, which nearly doubled in share between 2010 and 2015 and now makes up more than 10 percent of all beer sales in America.

3. Krachai *(Boesenbergia rotunda* (L) Mansf.)

A new alcoholic beverage, Krachai beer, is produced from Thai herb. It helps to support Thai herbal and alcoholic beverage markets to become a well-known product to the world market. The development of Krachai beer formulation were studied to create new variety of beer. An aroma and taste of Krachai could enhance the flavored beer. It also provides health benefits to consumer.

Boesenhergia rotunda (L) Mansf. is a perennial herb of the family Zingiberaceae, commonly distributes in Southeastern Asian countries and tropical rainforests such as Indonesia, Malaysia and Thailand. It is used in Thai cuisine, commonly called Krachai in Thai. It is used as flavoring agent and eaten as a vegetable, as well as having a variety of medicinal properties. The main bioactive compounds from the rhizome extract of *B. rotunda* consist of boesenbergin, cardamonin, pinostrobin, pinocembrin, panduratin A and 4-hydroxypanduratin A. *B. rotunda* have antioxidant, antibacterial, antifungal, anti-inflammatory, antitumour and anti-tuberculosis activities (Kiat *et al.,* 2006). The rhizomes of *B. rotunda* has been used for the treatment of gastrointestinal disorders including peptic ulcer (Abdelwahab *et* al.,201 I). *B. rotunda* (finger root) is also used to treat colic, oral diseases, urinary disorders, dysentery and inflammation (Saralamp *et al.,* 1996). Several studies suggest that this plant to be neuroprotective, anti-inflammatory, anti-mutagenic, anti-cancer, chemo preventive, anti-dermatophytes, anti-Helicobacter pylori and anti-dengue-2 virus NS3 protease (Bhamarapravati *et al.,* 2003).

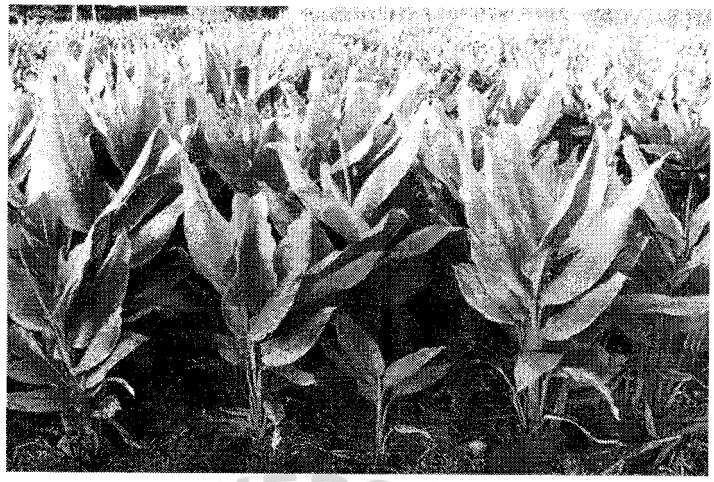


Figure 1 Picture of B. rotunda

Figure 2 Picture of B, rotunda

gun' 3 Picture of B. rotunda

Health benefits of *B. rotunda*

Antimicrobial Activities

Pathogenic bacteria are a group of bacteria that induce diseases in humans and plants. Spoilage bacteria are another group of bacteria that cause food spoilage through fermentation and decomposition of food products. There has been a rising concern pertaining to food safety and diseases caused by these pathogenic microorganisms, and hence, a renewed interest in finding new antimicrobial agents to combat these pathogens. In 2006, Pattaratanawadee *et al.* (2006) studied the antimicrobial activity of different extracts from four Zingiberaceae species, namely, *Zingiber officinale* Rosc (ginger), *Curcuma longa* Linn. (tumeric), *Alpinia galangal* Stuntz (galangal), and *Boesenbergia pandurata* Schltr (fingerroot), on different strains of pathogenic bacteria *(Salmonella enterica* serotype Typhimurium: 2380, 2486, 2576, 2582, *Escherichia coli* **0157** : H7, *Listeria monocytogenes: 101,* 108, 310, Scott A, and V7, *Bacillus cereus,* and *Staphylococcus aureus)* and spoilage bacteria *(Lactobacillus plantarum:* PD26 and PDI 10 and *Lactobacillus cellobiosus:* RE33, PD32, PD40, PD55 and PDI 12). The MIC value for all plant extracts was between 8 and 10% (v/v) against Gram-negative bacteria, while fingerroot showed the highest inhibitory activity against three pathogenic bacteria; *L. monocytogenes, B. cereus,* and *S. Aureus,* with an MIC value of 0.2-0.4% (v/v). Meanwhile, galangal extract showed

strongest inhibition against spoilage bacteria *L. plantarum* and *L. cellobiosus,* with an MIC value of 4% (v/v). Both galangal (at 8% (v/v)) and fingerroot (at 10% (v/v)) exhibited bactericidal effect against *E. coli* population (log cfu/mL) at 36 and 9 hours, respectively, while 8% (v/v) turmeric extract showed bacteriostatic effect. Panduratin A of *B. rotunda* was also found to possess antimicrobial activity against *Staphylococcus* strains with MICso of 0.5 ug/mL and MIC90 of 1 ug/mL, both comparable to the most potent antibiotic, vancomycin.

Antioxidant Activities

Sohn *et al.* (2005) reported the protective effects of panduratin A against t-BHP, an organic hydroperoxidant that initiates lipid peroxidation through its metabolism to free-radical intermediates, causing oxidative damage to cells. MTT cell viability assay showed a decrement in HepG2 cell growth inhibition by t-BHP, whereas fluorometric measurement revealed a dose-dependent reduction in malondialdehyde (MDA) formation and glutathione (GSH) depletion, upon treatment with panduratin A. Intracellular reactive oxygen species (ROS) production was also reduced from 665 ± 11.79 to when treated with 15 uM of the compound, further implying the potential application of this compound as a natural antioxidant.

Antiulcer Effect

B. rotunda is also used as a traditional medicine to treat ulcer by local communities in Thailand and Indonesia. The antiulcer effect of *B. rotunda* methanolic extract and its pure compound, pinostrobin, was recently explored by Abdelwahab *et al.* (2011). *B. rotunda* extract and pinostrobin exhibited cytoprotective effects on ulcer-induced rats, as evidenced by the reduction in ulcer area and mucosal content. In addition, submucosal edema and leukocytes infiltration were significantly reduced or prevented. The antioxidant activity of pinostrobin was proven through its ability to reduce the level of thiobarbituric acid reactive substances (TBARS) and through ferric reducing antioxidant power (FRAP) assay which gave a value of 116.11 ± 0.004.

Obesity Treatment

Obesity is a metabolic disorder that poses a global threat to humans. Caused by fat accumulation due to improper energy balance and lipid metabolism, obesity can cause liver and cardiovascular diseases. Panduratin A, previously determined to be a novel natural AMP-activated protein kinase

(AMPK) activator, was studied in attempts to decipher the regulatory mechanisms involved in AMPK-PPARa/6 signalling. AMPK is an enzyme that regulates cellular energy through activation of LBK1 and Ca^{2+}/calmodulin-dependent protein kinase kinase f3 (CaMKKI3). The activation of AMPK will increase the fatty acid oxidation by activating fatty acid oxidation-related genes. This process will prevent lipid synthesis via reduction of sterol regulatory element-binding protein-1c (SREBP-1c) and PPARy phosphorylation. When 50 mg/kg/day of panduratin A was applied, AMPK signalling was found to be stimulated, nuclear translocation of AMPKa2 induced, followed by activation of PPARa/6, with LKB1 being the key mediator of these effects. Activation of PPARa/6 increased fatty acid oxidation, resulting in weight loss, and reduced fat pad mass as observed in the *in vivo* obese mouse model. Moreover, these mice showed reduction in fatty liver and an improvement in the serum lipid profiles. Myofibre proportion and mitochondria content in muscles were significantly increased, enhancing running endurance. (Kim *et al.,* 2011) Taken together, these results exemplify the usefulness of panduratin A in treating obesity and associated metabolic disorders.

Breast Cancer and Colon Cancer Prevention

Breast and colon cancers are among the leading causes of cancer deaths worldwide. Despite the extensive ongoing research in finding an effective treatment regime or anticancer drug to fight these diseases, researchers are still far from uncovering a breakthrough, due in part to the lack of knowledge on the physiology of these cancers. Nevertheless, many current researches are still focused on natural plant herbs as potential targets in anticancer drug development, with *B. rotunda* being amongst them. Kirana *et al.* (2003) screened through eleven species of Zingiberaceae and found *B. rotunda* and *Zingiber aromaticum* to exhibit the highest inhibition towards MCF-7 breast cancer and human HT-29 colon cancer cell growth, with the IC50 values being 21.3+0.3 ttg/mL and 32.5+1.5 pg/mL, and 20.211.8 ug/mL and 11.8+1.0 ttg/mL, respectively. Morphological studies of the cells suggested death by apoptosis, as evidenced by the appearance of membrane blebs, nuclear condensation, and formation of apoptotic bodies (Kirana *et al.,* 2003). Further evaluation of another compound, panduratin A, on the same cell lines revealed potent inhibitory properties as well. The IC50 values for MCF-7 and HT-29 cells were determined to be 3.75 ttg/mL and 6.56 p.g/mL, respectively. Cell cycle and proliferation studies showed that 71% of the cells were arrested at GO/G1 after treatment with panduratin A, as

compared to 33% for untreated cells. Additionally, animal study showed that this compound was nontoxic to the rats as no obvious weight loss was observed, and the aberrant crypt foci formation, although reduced, was not significantly different as compared to the control (Kirana *et al.,* 2007). In 2004, Zaeoung *et al.* reported cytotoxic activities of B. rotundavolatile oils against breast cancer MCF-7 (IC50 ittg/mL) and LS174T colon cancer (1050 pg/mL) cell lines.

Anti-Inflammatory

Inflammation is a biological process that is activated in response to extracellular stimulants such as pathogens and chemicals, to mitigate the effects or heal the organism. *B. rotunda* has been traditionally used in treating several inflammatory-related diseases such as gout, allergy, and peptic ulcer. In 2009, Tewtrakul *et al.* reported that the extracts of *Kaempferia parvUlora* and B. *rotunda* exhibited anti-inflammatory effects through the inhibition of nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor alpha (TNF-a). NO acts as an inflammatory intermediator within the human metabolic processes, defending against intracellular and extracellular stimulants. Excess of this molecule; however, will induce pathogenesis in cells and form reactive free-radical upon reaction with other radicals. These reactive radicals cause direct damage to the function of normal cells. PGE2 and TNF-a are also inflammatory inter-mediators involved in inflammation and carcinogenesis. Panduratin A and hydroxypanduratin A, which were purified from *B. rotunda* methanolic extract, showed strong inhibitory activity against NO, with IC50 values of 5.3 j_tIVI and 13.31.1M, respectively, compared to the most potent compound of K. parviflora which was 5-hydroxy-3,7,3',4'-tetramethoxyflavone (IC50 16.111M). High inhibition against PGE2 production was observed, whereby both panduratin A and hydroxypanduratin A had IC50 values of 10.5 ttM and 12.31.1M, respectively, which was comparable to that of 5-hydroxy-3,7,3',4'-tetramethoxyflavone (1050 16.311M). Conversely, only a moderate inhibitory activity on TNF-a was observed for all three compounds, panduratin A (1050 60.3 pM), hydroxypanduratin A (IC50 57.3 ttIVI), and 5-hydroxy-3,7,3',4'-tetramethoxyflavone (1050 > 100 ttM).

Materials and methods

I. MATERIALS

1. Raw-materials

- Dry yeast *(Saccharomyces cerevisiae)*
- Bitter hop
- Aroma hop
- Malt Extract
- Water
- Krachai

2. Lab analysis equipment

- Refractometer (0-32° Brix)
- Balance
- Ebulliometer
- Thermometer
- pH meter
- Filtration equipment
- Pump

3. Chemical agent

- Liquid Carbon-dioxide
- 95% ethanol
- Diatom

II. METHOD

1. Brewing process

The experiment has been set into 3 formulas by varying the amount of Krachai (10,20, and 30 grams). First, boil 2.5 Liters of water in the pot and keep the water warm constantly. Malt extract was added until 12 ° Brix and Krachai was added into the pot. Mixture was boiled at 40° C for 40 minutes. After that add bitter hops and heat up to 75 ° C for 20 minutes and add aroma hops then stop heating. Cool down the temperature and move to the sterile fermenter tank, then start the yeast and pour into the tank. Close the tank and seal tight and make sure no air can get inside.

Leave the tank at the room temperature and let fermentation begin. Check brix every day until it will not go down anymore then rack, Filter and carbonate the beer.

Formu.r. chui b voduction

Formula	Amount of Krachai (g)	Hops (Bitter) (g)	Hops (Aroma) (g)
1	10	6	6
2	20	6	6
3	30	6	6

2. Physical and Chemical Analysis

2.1 pH measurement

Calibrate the pH meter following the manufacturer specifications. Rinse the probe with clean water before using it. Dry it off with a clean tissue. Collect a sample in a clean container. The sample must be deep enough to cover the tip of the electrode. Let the sample sit for a moment so the temperature can stabilize. Measure the temperature of the sample using a thermocouple. Dip the probe into the sample. Wait for the meter to come to equilibrium. The meter has reached equilibrium when the measurement becomes steady. Record the result.

2.2 Alcohol content

Rinse the boiling chamber of the ebulliometer with some of the beer to be tested and drain through the outlet tap. Fill the boiling chamber with approximately 50 ml of the beer, either using a pipette or the provided measuring cylinder. After that fill the condenser jacket with cold water. Insert and seal the special thermometer into the boiling chamber via the rubber stopper. Apply heat and the temperature will naturally rise as indicated by the thermometer. When the temperature first remains steady for at least 30 seconds record the temperature as Ti. Repeat the above procedure using approximately 20m1 of distilled water instead of the 50m1 of beer. Record this temperature as T2. Then calculate the ebulliometer degree (Ebulliometer degree = T2-T1 (°C)). Read the alcohol

concentration (%v/v) from the ebulliometer degree table and record.

2.3 Total Acidity

Acidity of sample determination by titration method. Put 10 ml of the sample into a conical flask and add 2-3 drops of Phenolphthalein indicator. Titrate with O. IN NaOH until the color turn into pink. Calculate percent Total acidity as following

$$Total\ acidity = \frac{Volume\ of\ NaOH\ x\ N\ of\ NaOH\ x\ MW\ of\ equivalent\ acid\ x\ 1000}{Volume\ of\ sample\ taken}$$

The equivalent acid of Krachai is Chlorogenic acid (354.31g/mol) (Ding *et al.*, 2010).

2.4 Color measurement

The color parameters CIE L*, a*, b* were analyzed with a HunterLab ColorFlex® EZ (Reston, Virginia, USA). The instrument is calibrated by standard black and white tile. The color is expressed under the term L* = lightness, a* = red (+) to green (-) and b* = yellow (+) to blue (-).

3. Sensory Evaluation

The best formula was selected by sensory test using 9-point Hedonic scale from 3 formulas. The experiment was conducted by 30 people and the final sensory was conducted by 50 people.

4. Statistical analysis and Experimental design

All experiments were conducted in duplicate and statistical analysis was accomplished using ANOVA with RCBD and Duncan's multiple range tests ($p < 0.05$) by SAS software version 9.4.

Result and Discussion

The development of Krachai beer formulation were studied to create new variety of beer. An aroma and taste of Krachai could enhance the flavored beer. The amount of Krachai adding to the brewing process were varied at 10,20, and 30 grams in formulas. The physical and chemical properties analysis of Krachai beer were done on °Brix, pH, alcohol content, acidity, and color.

Table 2 Phi: icai slat! Chemical Properties t?I krucha Beer

Physical/Chemical Properties	Formula 1	Formula 2	Formula 3	Final (Formula])
°Brix	8	7.5	7.4	7.8
pH	5.35	4.75	5.42	5.23
Alcohol content	4.24%	4.43%	4.48%	4.39%
Acidity (gChlorogenic acid acid/100m1)	0.36	0.43	0.52	0.39
Color L*A*B	0.80,-0.13,0.53	0.82,-0.24,0.48	0.88,-0.18,0.59	0.79,-0.15,0.61

The results of physical and chemical properties analysis of Krachai beer such as °Brix, pH, alcohol content, acidity, and color are presented in the Table 3. Final °Brix of formulation 1,2, 3, and finalized were 8, 7.5, 7.4, and 7.8 respectively. Normally pH standard of larger beer is range around 3.90 - 4.10. All formulations (1, 2, 3, and finalized) have higher pH than beer standard (5.35, 4.75, 5.42, and 5.23) that may unstable in term of shelf-life of the products. Alcohol content of formulation 1,2, 3, and finalized were 4.24%, 4.43%, 4.48%, and 4.39% respectively. Total acidity, chlorogenic acid is the organic acid found in Krachai and used as equivalent acid. Acidity of formulation 1,2, 3, and finalized were 0.36, 0.43, 0.52, and 0.39 gChlorogenic acid acid/100m1 respectively. Color is an important characteristic of beer, giving the information about its style. The color is expressed under the term L* = lightness, a* = red (+) to green (-) and b* = yellow (+) to blue (-). All formulation provided light-yellow with a little turbid. The alcohol content and pH were similar for all formulas. Formulation 2 has slightly higher alcohol content and slightly lower pH than others as 4.48% and 4.75 respectively.

A sensory profile analysis was performed to describe the best Krachai beers formula. To select the best formulation, a total of 30 judges of a panel participated in the tests. Water was provided for mouth rinsing between the beer samples tastings. The panelists evaluated the intensity of 8 attributes of Krachai beer which are foam stability, color, clarity, Krachai aroma, beer taste, bitterness, alcohol and overall liking were described by using a 9-point Hedonic scale.

TaIble .3 Se L i Krachai Beer on 8 At hutes by 9-point Hedonic scale by 30 Consumer.

Attributes	Average scores (mean ± S.D.)		
	Formula 1	Formula 2	Formula 3
Foam stability	7.27 1 0.94 a	6.90 1 0.96 a	7.101 1.09 a
Color	7.17 ± 1.23 a	7.1011.03 a	7.1711.18 a
Clarity	7.1311.20 a	6.67+1.47 [ab]	6.6011.65 [b]
Krachai aroma	6.8711.04 a	6.50+1.20 [ab]	6.1711.51 [b]
beer taste	7.0011.08 [a]	6.7311.17 [ab]	6.3711.40 [b]
bitterness	6.9610.89 a	6.8311.18 a	6.50±1.17 [a]
alcohol	6.8311.02 a	6.97±1.07 [a]	6.7711.50 [a]
overall liking	7.1710.95 [a]	6.8010.85 [ab]	6.3311.21 [b]

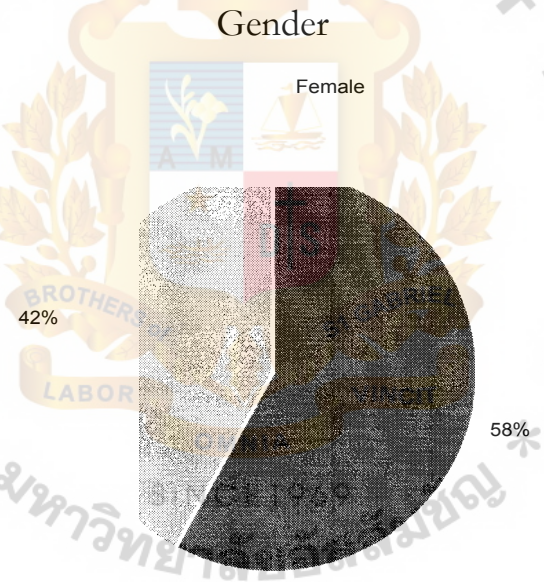
Remark: a b means with superscripts in a row and those without a common superscript were significantly different at $p<0.05$.

All 8 attributes of Krachai beer which are foam stability, color, clarity, Krachai aroma, beer taste, bitterness, alcohol and overall liking were described. Table 2 showed the score of each attributes indicated by using a 9-point Hedonic scale. Formula 1 shows significantly higher score in all attribute, foam stability 7.27 1 0.94, color 7.17 1 1.23, clarity 7.13±1.20' Krachai aroma 6.8711.04, beer taste 7.0011.08, bitterness 6.9610.89, alcohol 6.83±1.02, and overall liking 7.1710.95. Formula 3 shows significantly lower score in clarity, Krachai aroma, beer taste and overall liking as 6.60±1.65, 6.17±1.51, 6.3711.40, and 6.3311.21 respectively. Therefore, formula 1 seem to be the best formula of Krachai beer. It was selected to perform consumer's test.

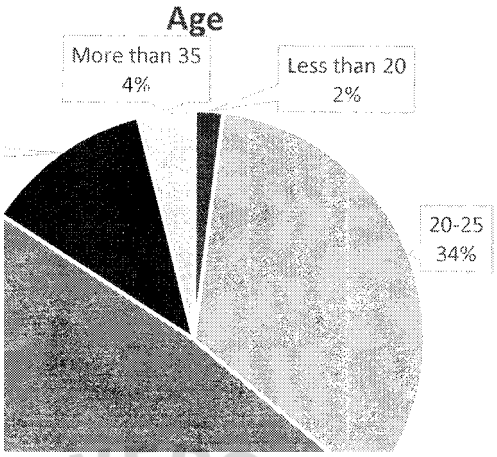
Consumer's Test of Krachai Beer

According to sensory profile analysis, formula I showed the highest score on overall liking and other attributes. A consumer's test was performed with the best Krachai beers formula 1. A total of 50 judges of a panel participated in the tests. Water was provided for mouth rinsing between the beer samples tastings. The panelists evaluated the intensity of overall liking using a 9-point Hedonic scale. All panel participated were given a questionnaire consists of 3 parts; demographic information, consumer behavior, and product acceptance.

Demographic information of panelists



rre 4 Gentler Pane



48%

Figure 5A

Income per Month

More 5« 40"00
20%

1

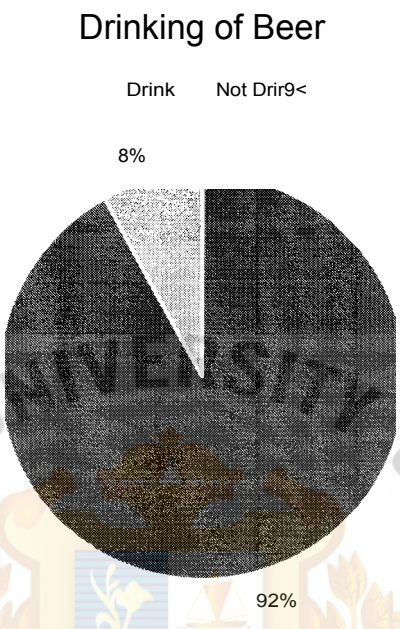
22%

0,001-40,0(
gs

20,001-30,000
46%

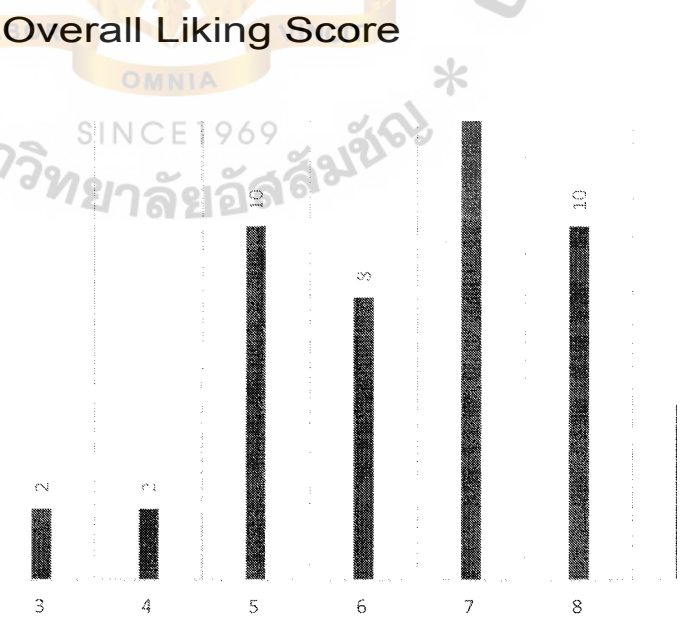
igure 6 *ell:*

Consumer behavior



7 *Drink'* hehavk of

Product acceptance



Score "roc

on.sltmer s "keep ce o rad hai Bee

According to all questions, the formulation of Krachai beer is concluded. The formulation that made from adding 10 grams of Krachai in brewing process is the best formula. Krachai beer was further perform consumer's test. From the test, it could be concluding that the product acceptance is 80% and average of overall liking score is 6.56. In addition, there is several information of consumer got from the test. The group of consumer are both male (58%) and female (42%). Most of them are age between 25-30 years old (48%) and 20-25 years old (34%), and 40% of all consumer having income between 20,001-30,000 Bath per month. 92% of consumer in the test drink beer.

Conclusion

From this study, the formulation of Krachai beer was developed. An amount of Krachai were varied at 10, 20, and 30 grams in formulations. The physical and chemical properties, sensory analysis of all formulations were done. Physical and chemical properties of Krachai beer were determined by using pH, alcohol content, acidity and color measurement. All beer shows similar in physical and chemical properties such as color (light-yellow with a little turbid), alcohol content and pH. The formulation that made from adding 10 grams of Krachai in brewing process is the best formula. It shows significantly higher in a 9-point Hedonic scale in all attribute, foam ability 7.27 ± 0.94, color 7.17 ± 1.23, clarity 7.1311.20, Krachai aroma 6.8711.04, beer taste 7.0011.08, bitterness 6.9610.89, alcohol 6.8311.02, and overall liking 7.1710.95. From consumer's test, product acceptance of Krachai beer is 80% and average of overall liking score is 6.56. Thus, an aroma and taste of Krachai could enhance the flavored beer. It also provides health benefits to consumer. To conclude, the addition of Krachai to brewing process creates a perspective to enlarge variety of beer.

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Appendix A

Consumer's testing form

Please score the samples base on your preference on a 9-point hedonic scale.

(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 code				
Foam ability					
Color					
Clarity					
Krachai aroma					
Beer taste					
Bitterness					
Alcohol					
Overall liking					

Krachai Beer Consumer Testing

I'm a student from School of Biotechnology, Assumption university conducting this product survey as my special project for my Bachelor's degree to learn about consuming behavior attitudes and need toward the product developed product with the goal to determine the consumer acceptance of the developed product. The important information from your participation will be further used to complete my project.

Please kindly complete the question below and carefully give mark on the answer base on your opinion. Your personal data will be kept confidential. Thank you for your participation.

I. Consumer's behavior

1. Do you drink beer?

o Yes O No

II. Attitude on their need toward the developed product

1. Please score the samples base on your preference on a 9-point hedonic scale. (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)

Overall liking _____

2. Do you accept this product?

O Accept O Not accept

III. General information

1. Gender

O Male 0 Female

2. Age

- O Less than 20
- O 20-25
- O 25-30
- O 30-35
- O More than 35

3. Income per month

- O Less than 10,000
- O 10,001-20,000
- O 20,001-30,000
- O 30,001-40,000
- O More than 40,000

Thank you

Appendix B

SAS output

The SAS System

The ANOVA Procedure
Foam ability

		Class Level: nformation
Class	Levels	Values
Treatment	3	P I P2 P3
rep	30	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Num her of Observations Used	90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	55.97777778	1.80573477	3.14	<.0001
Error	58	33.31111111	0.57432950		
Corrected Total	89	89.28888889			

R-Square	Coeff Varl Root MSE		Score Mean
0.626929	10.690611	0.757845	7.088889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	2.02222222	1.01111111	1.76	0.1810
rep	29	53.95555556	1.86053640	3.24	<.0001

Level of Treatment	N	Score	
		Mean	Std Dev
P1	30	7.26666667	0.94443318
P2	30	6.90000000	0.95952574
P3	30	7.10000000	1.09387007

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.57433

Number of Means	2	
Critical Range	.3917	.4120

IN eans with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	7.2667	30	P1
A			
A	7.1000	30	P3
A			
A	6.9000	30	P2

The SAS System

The ANOVA Procedure'
Color

Class Level Information		
Class	Levels	Values
Treatment	3	P I P2 l'3
rep	30	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Number of Observations Used	90

Source	DF	Sum of Squares	Nlean Square	F Value	Pr.> F
Model	31	79.2111111	2.5551971	4.13	<.0001
Error	58	35.9111111	0.6191571		
Corrected Total	89	115.1222222			

R-Square	Coeff Var	Root MSE	Score Mean
0.688061	11.01367	0.786865	7.144444

Source	OF	Anov a SS	Mean Square	F Value	Pr > F
Treatment	2	0.08888889	0.04444444	0.07	0.9308
rep	29	79.12222222	2.72835249	4.41	<.0001

Level of Treatment	Score		
	N	Mean	Std DeN
P1	30	7.16666667	1.23409420
P2	30	7.10000000	1.02889294
p3	30	7.16666667	1.17688465

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type 1 comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square.	0.619157

Number of Means	2	3
Critical Range	.4067	.4278

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	7.1667	30	P I
A			
A	7.1667	30	P3
A			
A	7.1000	30	P2

The SAS System

The ANOVA Procedure
Clarity

Class Level Information	
Class	Levels I Values
Treatment	31 P1 P2 P3
rep	30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Number of Observations. Used	90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	135.4666667	4.3698925	4.79	<.0001
Error	58	52.9333333	0.9126437		
Corrected Total	89	188.4000000			

R-Square	CocIT Var	Root MSE Score Mean	
0.719038	14.04888	0.955324	6.800000

Source	OF	A nova SS	Mean Square	F Value	Pr > F
Treatment	2	5.0666667	2.5333333	2.78	0.0706
rep	29	130.4000000	4.4965517	4.93	<.0001

Level of Treatment	N	Score Mean	Score Std Dev
PI	30	7.13333333	1.19577801
P2	30	6.66666667	1.47000665
p3	30	6.60000000	1.65258418

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type 1 comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.912644

Number of Means	2	3
Critical Range	.4938	.5194

Means with the same letter are not significantly different.

Duncan Grouping	Mean	*N*	Treatment
A	7.1333	30	P1
A			
A	6.6667	30	P2
B	6.6000	30	P3

The SAS System

The ANOVA Procedure
Krachai aroma

Class Level Information		
Class	Levels	Values
Treatment	3	P I P2 P3
rep	30	I 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Number of Observations Used	90

Source	OF	Sum of Squares	Mean Square	F Value	Pr> F
Model	31	96.5111111	3.1132616	3.61	<.0001
Error	58	49.9777778	0.8616858		
Corrected Total	89	146.4888889			

R-Square	Coeff Var	Root MSE	Score Mean
0.658829	14,25671	0.928270	6.511111

Source	DF	Anova SS	Mean Square	F Value	Pr> F
Treatment	2	7.35555556	3.67777778	4.27	0.0187
rep	29	89.15555556	3.07432950	3.57	<.0001

Level of Treatment	N	Score Mean	Score Std Dev
PI	30	6.86666667	1.04166092
P2	30	6.50000000	1.19625854
P3	30	6.16666667	1.51049965

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error.Deg rees.of Freedom	58
Error Mean Square:.	0.861686

Number of Means	2	3
Critical Range	.4798	.5047

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	6.8667	30	P1
	A			
B	A	6.5000	30	P2
B				
13		6.1667	30	P3

The SAS System

The ANOVA Procedure
Beer Taste

Class Level Information		
Class	Levels	Values
Treatment	3	P1 P2 P3
rep	30	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Obsen ations Read	90
Number of Observations Used	90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	75.6333333	2.4397849	2.31	0.0029
Error	58	61.2666667	1.0563218		
Corrected Total	89	136.9000000			

R-square	Coeff Var	Root MSE	Score Mean
0.552471	15.33993	1.027775	6.700000

Source	iM	Anova SS	Mean Sothic	F Value	Pr > F
Treatment	2	6.06666667	3.03333333	2.87	0.0647
rep	29	69.56666667	2.39885057	2.27	0.0040

Level of. Treatment	N	Score Mean!	Score Std Dev
PI	30	7.00000000	1.08278058
P2	30	6.73333333	1.17248140
P3	30	6.36666667	1.40155907

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type] comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	1.056322

Number of Means	2	3
Critical Range	.5312	.5588

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	7.0000	30	P I
A			
B A	6.7333	30	P2
B			
13	6.3667	30	P3

The SAS System

The ANOVA Procedure
Bitterness

Class Level Information		
Class	Levels	Values
Treatment	3	I3 1 P2 P3
rep	30	1 2345678910 11 1213 1415 16 17 18192021 222324252627282930

Number of Observations Read	90
Number of Observations Used	90

Source	DF	Sum of Squares	Mean Square	17 Value	Pr> F
Model	31	56.2333333	1.8139785	2.11	0.0070
Error	58	49.8666667	0.8597701		
Corrected Total	89	106.1000000			

R-Square	C'oeff Var	Root MSE	Score Mean
0.530003	13.70302	0.927238	6.766667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	3.46666667	1.73333333	2.02	0.1424
rep	29	52.76666667	1.81954023	2.12	0.0076

Level of Treatment	N	Score	
		Mean	Std Dev
PI	30	6.96666667	0.88991799
P2	30	6.83333333	1.17688465
P3	30	6.50000000	1.16707710

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom ,	58
Error Mean Square	0.85977

Number of Mea	2	3
Critical Range	.4792	.5041

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	6.9667	30	P1
A			
A	6.8333	30	P2
A			
A	6.5000	30	P3

The SAS System

The ANOVA Procedure
Alcohol

Class Level Information	
Class	Levels Values
Treatment	3 P1 P2 P3
rep	30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Number of Observations Used	90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	81.7444444	2.6369176	3.23	<.0001
Error	58	47.3777778	0.8168582		
Corrected Total	89	129.1222222			

R-Square	Coeff Var	Root MSG	Score Mean
0.633078	13.18350	0.903802	6.855556

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	2	0.62222222	0.31111111	0.38	0.6850
rep	29	81.12222222	2.79731801	3.42	<.0001

Level of Treatment	Score		
	N	Mean	Std Dev
P1	30	6.83333333	1.01991661
P2	30	6.96666667	1.06619961
P3	30	6.76666667	1.50134040

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.816858

Number of Means	2	3
Critical Range	.4671	.4914

Means with the same letter are aot significantly difterent.

Duncan Grouping	Mean.	N	Treatment
A	6.9667	30	P2
A			
A	6.8333	30	PI
A			
A	6.7667	30	P3

The SAS System

The ANOVA Procedure
Overall liking

Class Level Information		
Class	Levels	Values
Treatment	3	P I P2 P3
rep	30	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Number of Observations Read	90
Number of Observations Used	90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	49.2333333	1.5881720	1.81	0.0254
Error	58	50.8666667	0.8770115		
Corrected Total	89	100.1000000			

R-Square	Coeff Var	Root MSE	Score Mean
0.491841	13.83974	0.936489	6.766667

Source	DF	Anova SS	Mean Square	F Value	I > F
Treatment	2	10.46666667	5.23333333	5.97	0.0044
rep	29	38.76666667	1.33678161	1.52	0.0861

Level of Treatment	IN	Score Mean	Score Std Dev
PI	30	7.16666667	0.94989413
P2	30	6.80000000	0.84690104
P3	30	6.33333333	1.21295687

The SAS System

The ANOVA Procedure

Duncan's Multiple Range Test for Score

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	58
Error Mean Square	0.877011

Number of Means		3
Critical Range	.4840	.5092

Means with the sante.letter are not • significantly different.,•

.Duncan Grouping		Mean	N	Treatment
	A	7.1667	30	P I
	A			
B	A	6.8000	30	P2
B				
B		6.3333	30	P3