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# Phytochemical Profile and Bioactive Compounds of Pineapple Infused Arak Bali

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## *Abstrak*

*Seiring dengan perkembangan industri pariwisata, budaya lokal berupa produk dan aktivitas menjadi salah satu komoditas utama untuk menarik wisatawan. Salah satu budaya lokal yang saat ini dikembangkan sebagai minuman gastronomi di Bali adalah Arak Bali. Pengembangan formulasi Arak Bali sebagai minuman gastronomi dilakukan dengan pemanfaatan nanas sebagai bahan infusa sehingga dihasilkan Arak Bali infused nanas. Penelitian ini merupakan jenis true experimental dengan rancangan Posttest Only Control Group Design. Sampel yang digunakan dalam penelitian ini adalah Arak Bali infused nanas formula FU dan FD. Penelitian ini dilakukan untuk mengetahui profil fitokimia dan kandungan senyawa bioaktif dalam sampel. Penentuan profil fitokimia dilakukan dengan uji kualitatif sedangkan uji kuantitatif kandungan senyawa bioaktif antara lain tanin, total fenol dan flavonoid dilakukan dengan menggunakan Metode Folin-Denis, Folin-Ciocalteu dan $AlCl_3$ secara spektrofometri visibel. Berdasarkan hasil penelitian diketahui bahwa Arak Bali infused nanas mengandung senyawa fitokimia antara lain alkaloid, tanin, flavonoid, fenol dan terpenoid. Hasil uji kuantitatif menunjukkan bahwa sampel Arak Bali infused nanas memiliki kadar tanin, total fenol dan flavonoid adalah sebesar 52,9545; 42,005 dan 6,8995 mg/100g. Hasil penelitian menunjukkan bahwa penggunaan nanas dalam formulasi Arak Bali infused nanas mampu meningkatkan jenis senyawa fitokimia dan kadar senyawa bioaktif dibandingkan dengan formulasi awalnya yaitu Arak Bali.*

***Kata kunci*:** *Arak Bali infused nanas, profil fitokimia, senyawa bioaktif.*

## Abstract

Along with the development of the tourism industry, local culture has become valuable as a product and activity to attract tourists. The development of the Arak Bali formulation as a gastronomic drink was carried out by using pineapple as an infusion ingredient to produce Pineapple Infused Arak Bali. This type of research is a true experimental with a Post-test Only Control Group Design. The sample in this study was Pineapple Infused Arak Bali with FU and FD formulas. The purpose of this research was to determine the phytochemical profile and the concentration of bioactive compounds of the sample of Pineapple Infused Arak Bali. The determining of the phytochemical profile was carried out by qualitative tests while the quantitative analysis of bioactive compounds such as tannins, total phenols, and flavonoids was carried out by using the Folin-Denis, Folin-Ciocalteu, and $AlCl_3$ methods by visible spectrophotometry. The results showed that Pineapple Infused Arak Bali contains phytochemical compounds, such as alkaloids, tannins, flavonoids, phenols, and terpenoids. The quantitative analysis results showed that the Pineapple Infused Arak Bali had tannin, total phenol, and flavonoid level of 52.9545, 42.005, and 6.8995 mg/100g. The results showed that the use of pineapple in the formulation of Pineapple Infused Arak Bali was able to increase the types of phytochemical compounds and the bioactive compounds levels compared to the original formulation, Arak Bali.

**Keywords:** Bioactive compounds, Phytochemical profile, Pineapple Infused Arak Bali.

## 1. INTRODUCTION

Bali is one of the most famous tourist destinations because of its natural beauty, diversity of arts and culture, and also traditions (Jaya et al., 2019; Pratiwi et al., 2017). Along with the development of the tourism industry, there is competition between tourist destinations in various regions. Various efforts continue to attract tourists, one of which is by introducing local culture in the form of products and activities to become one of the commodities to attract tourists (Benur & Bramwell, 2015; Rodzi et al., 2013; Sucitawathi et al., 2019). One of the local cultures is gastronomic food and drink. Gastronomic tourism is becoming popular due to the unique experience that does not only come from natural beauty but also traditional food or beverage products in a tourist area. One type of gastronomic drink

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in Bali is Arak Bali (Ratih & Habibah, 2022). Arak Bali is a traditional drink made from glutinous rice and coconut water which is simply processed, through a process of fermentation and distillation. Arak Bali is included in category C with an alcohol content of more than 25%. This type of drink has the characteristics of a clear liquid, colourless with a strong alcoholic taste. One of the potential local fruits for the formation and diversification of Arak Bali is pineapple. The use of pineapple as the other ingredient for the formulation and diversification of Arak Bali is to enrich the taste and increase its benefits. The pineapple is a bush plant with the scientific name *Ananas comosus (L.) Merr*. This plant is easy to grow in areas with tropical climates. Various studies have proven that pineapple contains secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, and tannins (Babajide et al., 2013; Hikal et al., 2021; Nurdalilah et al., 2018; Rivera et al., 2022; Rustini et al., 2022; Saleh et al., 2021; Sayago-Ayerdi et al., 2021; VH et al., 2021). The various contents of these secondary metabolites are important factors for the antibacterial activity of pineapple. Various parts of the pineapple plant, such as fruit and tubers, and also peel is proven to have antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* K2044, *Pseudomonas aeruginosa* MTCC4676, *Bacillus subtillis* Py79 *and Xanthomonas axonopodis pv. malvacearum* LMG859 (Juariah & Wati, 2021; Rustini et al., 2022).

Pineapple infused Arak Bali is made by soaking 400 grams of sliced pineapple in Arak Bali for 7 days at room temperature. The optimum formulation is organoleptically acceptable to consumers (Ratih & Habibah, 2022). This immersion process imitates the stages of the extraction process with the maceration method. In the maceration process, the active compounds from the material can be extracted by the solvent used. The type and concentration of the active compound extracted from the material depend on the type and nature of the solvent used (Atun, 2014). In the maceration process, the immersion time of the material in the solvent is the main factor that influences the effectiveness of extraction. Usually, maceration is carried out for 6-7 days so that the process of extracting active compounds from the materials used runs more optimally (Atun, 2014; Gil-Martín et al., 2022; Naviglio et al., 2022; Roda et al., 2013). Pineapple fruit as an infusion ingredient of Arak Bali is reported to be able to increase the taste and consumer preference for the resulting product. Organoleptic tests on Pineapple infused Arak Bali showed higher scores on color, taste, and smell parameters compared to the original product (Ratih & Habibah, 2022).

In addition, the process of soaking pineapples in Arak Bali during the manufacturing process allows the active compounds to dissolve from the pineapples so that it can increase the potential for utilization and development of Pineapple infused Arak Bali as a gastronomic drink. Therefore, in this study, qualitative tests of phytochemical compounds and quantitative analysis were carried out to determine the phytochemical profiles and quantitative content of various active compounds in Pineapple infused Arak Bali.

## 2. METHODS

The materials used in this study based on Figure 1 were samples of pineapple-infused Arak Bali with the basic formula (FD) and the test formula (FU). The basic formula (FD) is the initial formula for Arak Bali which is obtained without the addition of pineapple fruit while the test formula (FU) is pineapple-infused Arak Bali which is made by soaking 400 grams of pineapple fruit in 500 mL of Arak Bali for 7 days at room temperature in a closed container and protected from the direct sunlight. The reagents and solvents used were 1% aluminum chloride, dilute ammonia, concentrated and dilute ammonium hydroxide, acetic anhydride, glacial acetic acid, 1% hydrochloric acid, 0.1 N hydrochloric acid, concentrated sulfuric acid, butanol, phenol, iron ( III) chloride 0.1%, iron (III) chloride 0.1 M, chloroform, olive oil, tannin standard, quercetin standard, gallic acid standard, Mayer's and Wagner's

reagent, Pb-acetate solution, Liebermann-Burchard reagent, Folin-Ciocalteu reagent, phosphate citrate buffer, sodium carbonate, Folin-Dennis reagent, $AlCl_3$ solution, sodium nitrite, N-ammonia-chloroform solution, diethyl-ether, ethanol, ethyl acetate, methanol, distilled water. The apparatus needed in this research include: vials (2 pieces), analytical balance (Radwag) (1 piece), measuring pipettes (Iwaki-Pyrex®) 1 ml and 10 ml (1 piece each), 20µl micropipette–1000µl (Secorex) (1 piece each), ball pipette (D&N ballpipet) (1 piece), measuring cup (Iwaki-Pyrex®) 250ml (1 piece), Erlenmeyer (Iwaki- Pyrex®) 250 ml, 500 ml, 1000 ml (1 each), test tubes (Iwaki-Pyrex®) (12), test tube rack (1), magnetic stirrer hotplate (2), spirit lamp (1), Uv-Vis spectrophotometer.

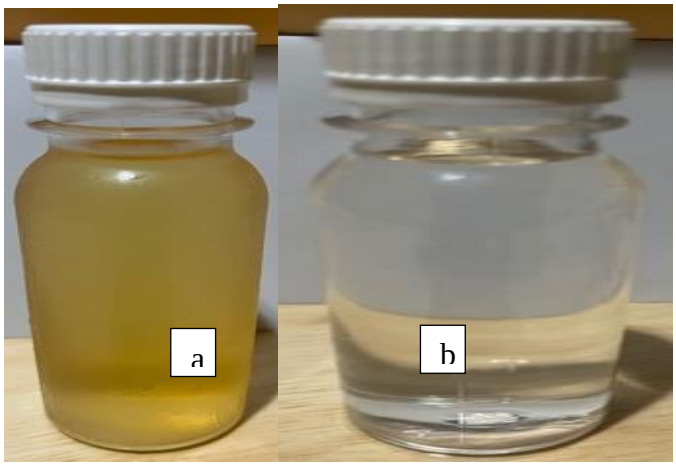


**Figure 1.** a. Basic formula (FD) of sample Arak Bali; b. Test formula (FU) of sample Pineapple Infused Arak Bali

**Qualitative Test**

**Alkaloids**

1 mL of pineapple infused Arak Bali with basic formula (FD) and test formula (FU) added with N-ammonia-chloroform solution. Furthermore, the mixture was shaken for 1 minute and then filtered. Next, 5 mL of $H_2SO_4$ was added and shaken. After settling, separate the aqueous phase and test it by adding Mayer's reagent.

**Flavonoids**

5 mL of the pineapple-infused Arak Bali base formula (FD) and test formula (FU) were put into a glass beaker, then added with 10 mL of ethyl acetate, then boiled and filtered. Furthermore, 0.5 mL of the filtrate was added with 1 mL of dilute ammonia solution, then observed the changes that occurred in the sample.

**Tannins**

A total of 1.6 mL of pineapple-infused Arak Bali basic formula (FD) and test formula (FU) were added to the $FeCl_3$ solution, subsequently, the colour changes were observed.

**Phenol**

A total of 2 mL of pineapple-infused Arak Bali basic formula (FD) and test formula (FU) were pipetted and added a few drops of $FeCl_3$.

**Saponins**

A total of 10 mL of pineapple-infused Arak Bali base formula (FD) and test formula (FU), were added to 5 mL of distilled water and then shaken vigorously until foam formed.

Then 3 drops of olive oil were added, after which it was shaken again and observed for the formation of an emulsion.

**Steroids**

A total of 2 mL of pineapple-infused Arak Bali with basic formula (FD) and test formula (FU) was evaporated in a porcelain cup. The residue is dissolved with 0.5 mL of chloroform, then 0.5 mL of anhydrous acetic acid is added. Then 2 mL of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring at the boundary of the solution indicates the presence of triterpenoids, whereas a greenish-blue ring appears indicating the presence of steroids.

**Terpenoids**

A total of 2 mL of pineapple-infused Arak Bali base formula (FD) and test formula (FU) were added to 10 mL of ethanol, boiled, and filtered. Then 5 mL of the extract was taken and 2 mL of chloroform and 3 mL of concentrated sulfuric acid were added, and the changes observed were observed.

**Quantitative Analysis**

**Total Phenol**

A total of 0.1 mL of pineapple-infused Arak Bali base formula (FD) and test formula (FU) were added to 0.3 ml of 70% ethanol. After that, 0.4 ml of Folin-Ciaocalteu reagent was added and then incubated for 6 minutes. After the incubation process, 4.2 ml of 5% $Na_2CO_3$ was added, then vortexed and incubated for 90 minutes. The absorbance is read at a wavelength of 760 nm. The reading results were compared with a standard curve made using gallic acid.

**Tannin**

A total of 0.25 ml of pineapple-infused Arak Bali basic formula (FD) and test formula (FU) were then added to 0.25 ml of Folin-Denis reagent, then vortexed, and added 2 ml of 5% $Na_2CO_3$. The solution was vortexed and then incubated for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 725 nm. The readings were compared with the standard curve using tannic acid.

**Flavonoid**

A total of 1 ml of sample was mixed with 4 ml of distilled water and 0.3 ml of $NaNO_2$ (10%) solution was added. After that, it was incubated for 5 minutes and 0.3 ml of $AlCl_3$ solution (10%) and 2 ml of NaOH solution (1%) were added, then immediately tested with a spectrophotometer at a wavelength of 510 nm.

## 3. RESULTS AND DISCUSSION

**Result**

The results of phytochemical qualitative tests on the pineapple infused Arak Bali basic formula (FD) and test formula (FU) showed that the FU samples contained more types of phytochemical compounds than FD. The qualitative test results for the basic formula (FD) and the test formula (FU) are presented in Table 1. Based on the results of phytochemical test, it is known that Arak Bali (FD) contains several phytochemical compounds such as tannins, flavonoids, and phenols, while pineapple infused Arak Bali (FU) contains more phytochemical compounds, such as alkaloids, tannins, flavonoids, phenols, and terpenoids.

**Table 1.** Qualitative Test Result of Phytochemical Compounds on the Pineapple Infused Arak Bali

| Phytochemical compounds | FD | FU |
|---|---|---|
| Alkaloids | - | + |
| Tannin | + | + |
| Saponins | - | - |
| Flavonoid | + | + |
| Phenol | + | + |
| Steroids | - | - |
| Terpenoids | - | + |

The quantitative analysis of bioactive compounds was conducted by using The Folin-Denis, Folin-Ciocalteu, and $AlCl_3$ methods by visible spectrophotometry. The tannin, total phenol, and, flavonoid content was reported as the equivalent of tannic acid, gallic acid, and quercetin, respectively. Among the three of bioactive compounds, the test sample (FU) of pineapple infused Arak Bali was containing the highest levels of tannin followed by total phenol and flavonoids. The results showed that pineapple infused Arak Bali (FU) had a greater level of bioactive compound compared to the FD sample. The quantitative analysis result of bioactive compound is presented in the Table 2.

**Table 2.** Quantitative Analysis Result of Bioactive Compounds on the Pineapple-Infused Arak Bali

| Bioactive compounds (mg/100g) | FD | FU |
|---|---|---|
| Tannin | 29.0255 | 52.9545 |
| Total Phenol | 26.279 | 42.005 |
| Flavonoids | 3.087 | 6.8995 |

## Discussion

### Qualitative Test

#### Alkaloids

Alkaloids are a class of secondary metabolites that are widely distributed and mostly found in various types of plants. Alkaloid compounds are basic compounds that contain one or more nitrogen atoms as part of their cyclic system. Alkaloids are a class of secondary metabolite compounds that have prominent physiological activities and are often used extensively in the field of medicine (Harborne, 1984).

In the qualitative alkaloid test, the alkaloid compounds in the sample were extracted by adding N-ammonia-chloroform. Furthermore, the extracted alkaloid compounds are salted with the addition of sulfuric acid. The results of the alkaloid test with Mayer's reagent showed that the FU sample positively contained alkaloids, while the FD sample did not contain alkaloid compounds. This is indicated by the formation of a white precipitate as a result of the reaction between the alkaloid compounds in the FU sample and Mayer's reagent. The presence of alkaloid group compounds in the FU sample is possible due to the soaking/maceration of pineapple fruit in Arak Bali during the manufacturing process. This is in accordance with previous studies which stated that alkaloids are a class of secondary metabolite compounds contained in pineapple (Babajide et al., 2013; Hikal et al., 2021; Nurdalilah et al., 2018; Rivera et al., 2022; Rustini et al., 2022; Saleh et al., 2021; Sayago-Ayerdi et al., 2021; VH et al., 2021).

**Tannins**

Tannins are a class of secondary metabolite compounds consisting of phenolic compounds found in many woody plants. Chemically, there are two main types of tannins, there are condensed tannins and hydrolyzed tannins (Harborne, 1984). Tannins have bioactivity as an astringent, antibacterial, and antioxidant (Matheus et al., 2022).The qualitative test results for the phytochemical compounds in the FD and FU samples indicated that both formulations contained tannins positively. This is indicated by the formation of blue-black precipitates on hydrolyzed tannins and greenish-black on condensed tannins. Based on the test results, it is known that the precipitate formed in the FU sample is more than the precipitate formed in the FD sample. This may indicate that the amount of tannin compounds in the FU sample is higher compared to the FD sample.

**Flavonoids**

Flavonoids are a class of secondary metabolite compounds which are derivatives of the main flavone compounds, which are water-soluble and can be extracted using 70% ethanol. Flavonoids are one of the largest groups of phenolic compounds found in plants (Harborne, 1984). Based on the qualitative test results, it was found that the FD and FU samples positively contained flavonoids. This is indicated by the formation of a yellowish colour in the sample. The change in the colour of the sample is due to the fact that the flavonoids in the form of phenolic compounds can change colour when added with a base or ammonia as a result of the conjugation system of the aromatic group (Indarto, 2015; Kharismawati et al., 2009).

**Phenol**

Phenols are a class of secondary metabolites that are mostly found in plants. Phenol compounds have the characteristic of having an aromatic ring containing one or more hydroxyl groups in their structure. Most groups of phenolic compounds bind to other compounds, such as sugar, so they tend to dissolve easily in water (Harborne, 1984). From the results of the qualitative test, it was found that the FD and FU samples positively contained phenol. This was indicated by the formation of a colour change in the sample after the addition of $FeCl_3$ solution. This colour change to green, red, purple, blue, or black occurs because a complex compound is formed between the phenolic compound and the added $FeCl_3$ reagent (Harborne, 1984).

**Terpenoids**

Terpenoids are a class of secondary metabolite compounds derived from isoprene molecules (CH2=C(CH3)-CH=CH2) which are further classified based on the number of units present in the compound. Terpenoid compounds are found in the cytoplasm of plant cells and are a class of fat-soluble compounds so that the extraction process can be carried out using non-polar solvents such as ether, chloroform, or other types of non-polar solvents (Harborne, 1984).

The terpenoid test results showed that the FU sample positively contained terpenoids, while the FD sample did not contain terpenoid compounds. The presence of terpenoid group in the FU sample is due to the soaking/maceration process of pineapple fruit in Bali Arak during the manufacturing process. This is in accordance with previous research which stated that terpenoids are a class of secondary metabolite compounds contained in pineapple (Babajide et al., 2013; Hikal et al., 2021; Nurdalilah et al., 2018; Rivera et al., 2022; Rustini et al., 2022; Saleh et al., 2021; Sayago-Ayerdi et al., 2021; VH et al., 2021).

## Quantitative Analysis

### Tannins

Tannins are secondary metabolites which are widely distributed, especially in vascular plants, and are found in wood tissue. Tannins are secondary metabolites that are reported to have several bioactivities, such as astringent, anti-diarrheal, antibacterial, and antioxidant (Bansod et al., 2023; Indarto, 2015). The quantitative analysis of tannins was carried out spectrophotometrically using the Folin-Denis reagent with standard tannic acid as a reference. The quantification of the tannin levels in the sample is based on the reduction reaction of phosphomolybdic acid present in the reagent by the tannin compound in the sample to become blue molybdenum. The intensity of the blue color formed is proportional to the tannin content in the sample (Kharismawati et al., 2009). Theoretically, the absorbance of the molybdenum blue complex can be measured in the wavelength range of 700-800nm. In this study, the absorbance of the molybdenum blue complex compound in FD and FU samples and the reference solution was measured for its absorbance at a wavelength of 725nm.

Based on the test results, it is known that the tannin content in the FD and FU samples is 29.0255 and 52.9545 mg/100g samples. From the results of the tannin test, it was found that there was an increase in the average tannin levels in the FU samples compared to the FD samples. This is because the soaking/maceration process of the pineapple during the process of making the FU sample allows the tannin compounds to be extracted from the pineapple and dissolved in the pineapple infused Arak Bali. This is consistent with the results of previous studies which proved that pineapple contains various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, and terpenoids (Babajide et al., 2013; Hikal et al., 2021; Nurdalilah et al., 2018; Rivera et al., 2022; Rustini et al., 2022; Saleh et al., 2021; Sayago-Ayerdi et al., 2021; VH et al., 2021).

### Total Phenol

Phenols are a class of secondary metabolites which are mostly found in plants and have an aromatic ring structure with one or more hydroxyl groups. The largest group of phenolic compounds is flavonoids. Some previous report was proved that phenolic compounds contribute to the antioxidant activity of the materials (Ferreira et al., 2016; Hossain & Rahman, 2011).

In this study, the total phenol levels were analysed using the Folin-Ciocalteu method with gallic acid as a reference standard. Quantitative analysis of total phenol is based on the principle of the oxidation reaction of phenolic hydroxyl groups. The Folin-Ciocalteu reagent will oxidize the phenolic group and reduce the hetero-poly acids (phosphomolybdic-phosphotungstic) into molybdenum-tungsten complexes. During the reaction process presented in Figure 2, the phenolic hydroxyl group will react with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex (Hapsari et al., 2018; Hossain & Rahman, 2011; Marjoni et al., 2015; Safitri et al., 2018). The reaction of phenolic compounds with these reagents occurs in alkaline conditions. In alkaline conditions with the addition of sodium carbonate, proton dissociation occurs in phenolic compounds to become phenolic ions (Hapsari et al., 2018). Furthermore, the intensity of the blue colour formed was measured spectrophotometrically at a wavelength of 760 nm. The intensity of the blue colour proportionally to the total phenolic compound in the sample.

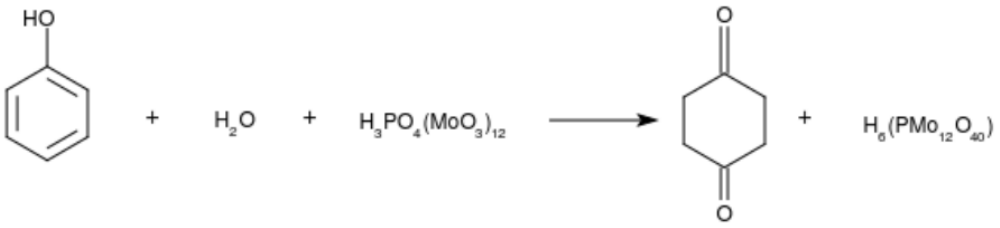


**Figure 2.** Reaction Of Complex Formation Between Phenol and The Folin-Ciocalteu Reagent

In line with the tannin, the average of total phenol levels in the FU sample also showed higher results, which was 42.005 mg/100g when compared to the total phenol levels in the FD sample, which was 26.279 mg/100g. This is due to the addition of pineapples in the process of making pineapple-infused Arak Bali which causes the active compounds in pineapples, especially the phenol compound group, to dissolve in Arak Bali. Arak Bali, which has an ethanol content of up to 34.22%, is an appropriate solvent for extracting phenolic compounds from pineapples (Ratih & Habibah, 2022). Previous studies have also proven that the solubility of phenolic compounds is affected by the type of solvent. The phenolic compound group has a higher solubility in polar solvents compared to other solvents (Hapsari et al., 2018; Nurdalilah et al., 2018).

**Flavonoids**

Flavonoids are a class of phenolic compounds that are widely distributed in nature and found in many types of plants. Based on the quantitative test results of the FD and FU samples, it was found that the levels of flavonoids contained in each sample were 3.087 and 6.8995 mg/100g.

Quantitative analysis of flavonoid levels in the samples was carried out using the $AlCl_3$ method (Hossain & Rahman, 2011). Quantification of flavonoid content using the $AlCl_3$ method is based on the principle of the reaction of forming a complex compound between $AlCl_3$ and flavonoids to produce a yellow complex compound whose absorbance can be measured spectrophotometrically in the visible wavelength region (Hossain & Rahman, 2011; Safitri et al., 2018). In this study, the absorbance of the complex compound formed was measured at a wavelength of 520nm. The levels of flavonoids is closely related to the antioxidant capacity of a substance (Ferreira et al., 2016; Hossain & Rahman, 2011). Flavonoids can neutralize free radicals because they have hydroxy groups and double bonds in their structure (Hossain & Rahman, 2011; Septiani et al., 2018). Flavonoid levels in the FU sample in this study were higher when compared to the FD sample. This is because the process of adding pineapple fruit to the process of making the FU formulation was able to increase the levels of bioactive compounds in the sample compared to the initial formulation. As has been reported in previous studies that one of the compounds contained in secondary metabolites in pineapple fruit is flavonoids.

## 4. CONCLUSION

The result of this research proved that pineapple infused Arak Bali has several phytochemical compounds such as alkaloids, tannins, flavonoids, phenols, and terpenoids. The quantitative analysis result showed that the pineapple infused Arak Bali had tannin, total phenol, and flavonoid. Among the three bioactive compounds, the FU sample was containing the highest levels of the bioactive compound followed by FD sample. From these results, it can be concluded that the use of pineapple in the formulation of Pineapple Infused Arak Bali

was able to increase the types of phytochemical compounds and the levels of bioactive compounds compared to the original formulation.

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