Natural fermentation of bitter gourd (*Momordica charantia*) and noni fruit (*Morinda citrifolia*) in honey increases total phenol, total flavonoid, and antioxidant activity

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Abstract. It is well known that bitter gourd and noni fruit contain high levels of beneficial chemicals, but tastes and flavour are less appealing. Due to enzymatic transformation, fermentation can change bioactive compounds that minimize bad taste and unpleasant odor. This study aimed to assess the phenol and flavonoid content and antioxidant activity of bitter gourd (*Momordica charantia* L.) and noni fruit (*Morinda citrifolia*) during fermentation in honey. Blendered-bitter gourd (BG) or noni fruit (NF) each is mixed with honey and distilled water in a ratio of 3:1:10, then fermented for 90 days at room temperature and anaerobic conditions. Total phenol (TPC), total flavonoid content (TFC), and antioxidant activity were measured every two weeks. TPC in fermented NF increased from 56.62 mg GAE/gr to 156.59 GAE in the 6-week. TPC in BG increased from 56.96 GAE/g to 146.39 GAE/g during 12-week fermentation. TFC of fermented NF increased from 4.55 mg QC/g to 12.92 mg QC/g in 12-week fermentation. TFC of fermented BG increased from 9.86 QC/g to 41.69 QC/g in 12 weeks of fermentation. The antioxidant activity of fermented BM and BP showed the highest antioxidant activity in the 6-week (0,574 mg TEAC/g and 0.528 mg TEAC/g). The results indicate that fermentation in honey can increase total phenol and flavonoid content, and the antioxidant activity of bitter gourd and noni fruit.

1 Introduction

Bitter gourd (*Momordia charantia* L.) is a plant cultivated as one of the medicinal plants in India, China, and Southeast Asia. All parts of bitter gourd can be used as a medicinal plant because it contains bioactive compounds that can relieve several diseases, such as diabetes, constipation, cholesterol, and cancer. The secondary metabolite compounds contained in bitter gourd fruit are phenol, carotenoid, triterpenoid, alkaloid, and saponin [1–3]. Bitter gourd has an unpleasant taste, so people choose another alternative to consuming bitter gourd,

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such as cooking the bitter gourd as a vegetable. Alkaloid momordicine is the compound that made the bitter taste of the bitter gourd. In an effort to reduce the bitter taste of bitter gourd, the usually bitter gourd was soaked in salt water [4–6]. Noni fruit also has an unpleasant aroma and a bitter taste caused by capric acid, caprylic acid, and caproat acid components. The alternative processed to reduce the unpleasant taste and aroma is processed noni fruit into pudding [7].

Noni fruit is commonly consumed as a juice through various processes, such as fermentation, non-fermentation, squeezing the noni fruit directly, and performing drop extraction at room temperature. However, the juice of noni fruit that is consumed still has a bitter taste and an undesirable sour smell [8]. Noni fruit has many bioactive compounds used as raw material for traditional medicine in the treatment of high blood pressure, intestinal inflammation, liver disease, diabetes, constipation, dysentery, and gallbladder inflammation. Ethanol extract of noni fruit is known to contain anthraquinone, alkaloid, tannin, flavonoid, steroid, triterpenoid, saponin, and phenolic compounds [9].

Fermentation can help increase the bioactive compounds in medicinal plants. Microorganisms in the fermentation process can destroy cell walls in plants so that they can deliver compounds that can be digested by the body both physically and chemically. Bioactive compounds could be enhanced for their beneficial properties and also for improving the sensory characteristics of medicinal plants [10, 11]. For example, microorganisms will hydrolyze polyphenol molecules that generally have a molecular weight of >500 kDa into compounds with smaller molecular sizes, such as aglycones, ellagic acid, and catechins. During the fermentation process that occurs in pomegranates that are rich in ellagic tannins, microorganisms will break down ellagic tannin compounds into intermediates such as punicalin and gallagic acid, which eventually become ellagic acid [12, 13]. The media for fermentation is unlimited because fermentation can be carried out with sugar water media [14], coconut water [15], fruit juice [16], or honey [17]. Honey has around 200 bioactive compounds that have biological benefits. It also contains glucose, fructose, fructooligosaccharides, minerals, vitamins, amino acids, and enzymes. These compounds act as antioxidant effects of natural honey products [18–20]. This research aims to assess the phenol and flavonoid content and antioxidant activity of bitter gourd (*Momordica charantia* L.) and noni fruit (*Morinda citrifolia*) during fermentation in honey.

2 Materials and methods

Bitter gourd and noni fruit were obtained from traditional markets, and the honey was obtained from Best Honey Indonesia products. Bitter gourd and noni fruit were cut into three parts and separated between the flesh and seeds. Each flesh of bitter gourd and noni fruit was cleaned using running tap water and soaked in 20% salt water for 2 hours. After that, bittter gourd and noni fruit were roughly blended. Each blend was mixed with honey and water (3:1:10). The mixture was tightly closed in a transparent container. The lid of the container will be perforated to provide a hose connected to a bottle filled with tap water in an effort to control oxygen in the fermentation mixture. The fermentation container is placed at room temperature, away from direct sunlight. The fermentation process is carried out for 90 days. pH measurements were carried out using pH meters every 2 weeks. Measurements were carried out twice (duplo), and the results were averaged [21].

Total flavonoid content was conducted every two weeks using the aluminium chloride colourimetric method. A sample of 0.1 mL was dissolved in 1 mL of deionized water. A total of 0.5 mL of the solution was then mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10% aluminium chloride ($AlCl_3$); and 2.8 mL of deionized water. The mixture was incubated at room temperature for 40 minutes and was absorbed at a wavelength of 415 nm using a UV-Vis spectrophotometer with deionized water as a blank solution. Quercetin (0 - 50 mg / L)

was used as a standard solution. The resulting data are expressed in milligrams of quercetin per 100 grams of sample [22].

Total phenolic content was conducted every two weeks using the Folin-Ciocalteau method. A 1 ml aliquot or standard solution of gallic acid (20, 40, 60, 80, 100 mg/l) was added to a 25 ml volumetric flask containing 9 ml of deionized distilled water (dd H_2O). The blank solution used was dd H_2O. A 1 ml Folin-Coicalteu phenol reagent was added and stirred until evenly distributed. After 5 minutes, 10 ml of 7% Na_2CO_3 was added to the mixed solution. Furthermore, the solution was diluted to 25 ml with dd H_2O and stirred. The mixed solution was incubated for 90 minutes at room temperature, and its absorbance was measured against the blank solution at a wavelength of 750 nm using a UV-Vis spectrophotometer. The total phenol produced was expressed in milligrams of gallic acid per 100 grams of sample [23].

Trolox as the stock solution was prepared by dissolving 0.0125 g of Trolox powder in 50 mL of distilled water to obtain the 1 mM concentration of Trolox. After that, 1 mL aliquot of 1 mM concentration of Trolox was diluted in 10 mL distilled water to obtain the 0,1 mM concentration of Trolox. For the working solution of Trolox, 0,1 mL stock solution was diluted with distilled water to make the variation concentration of working solution (0; 0.02; 0.04; 0.04; 0.06; 0.08; and 0.1 mM) [24]. DPPH stock solution was prepared by dissolving 24 mg of DPPH powder in 100 mL of methanol. Furthermore, the DPPH working solution was prepared by dissolving 25 mL of stock solution in 200 mL of methanol. The absorbance of the DPPH working solution was measured at a wavelength of 517 nm with an absorbance value of 0,751. Antioxidant activity is expressed in Trolox Equivalent Antioxidant Capacity (TEAC) [25].

3 Results and discussions

The fermentation process in bitter gourd and noni fruit leads to changes in their aromas. Before fermentation, bitter gourd has a mild scent, but it develops a more sour aroma after fermentation. Additionally, the fermented bitter gourd exhibits bubbles after the initial two weeks of fermentation. On the other hand, noni fruit has a distinctive and strong aroma before fermentation, which intensifies and becomes even sharper and more pungent after fermentation. The fermented bitter gourd with *Lactobacillus plantarum* resulted in the modification of taste and a decreased content of quite obvious bitterness. This occurs by the reaction of some bitter-tasting alkaloid glycosides and saponins. One of the *L. plantarum* bacterial enzymatic hydrolysis was reported to be that which released β-glucosidase enzyme, breaking down momordicoside into various aglycones. This affected the flavour of bitter gourd juice. This wonder has a nice taste compared to an unfermented one [26].

The changes in the aroma after the fermentation are induced by the changes in the pH value. Noni fruit and bitter gourd show changes in pH value after the fermentation (Figure 1). pH value of noni fruit tends to decrease slowly from the second week until the twelfth week, from 2.96 to 2.65, respectively. Meanwhile, the pH value of bitter gourd shows fluctuative changes. The highest pH value of the fermented bitter gourd is shown in the fourth week, and it obtains 3.69. The longer fermentation time resulted in a lower pH value for the samples. The longer fermentation gave the microorganisms more time to do their metabolic activities. The changes in the pH value can affect the taste of the samples [26].

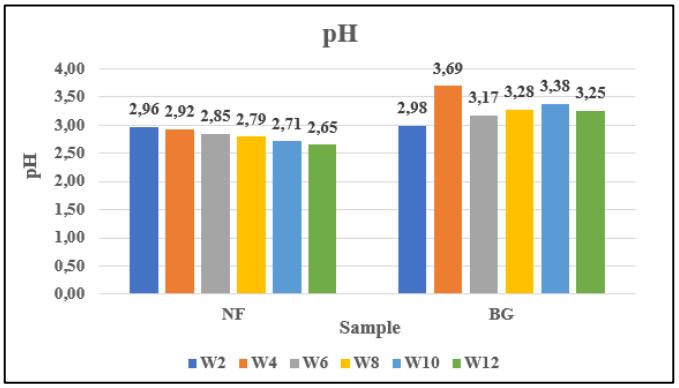

Fig. 1. Changes in pH during fermentation. NF: Noni Fruit; BG: Bitter Gourd; W2-W12: Second - Twelfth week.

Total flavonoid content was measured using aluminium chloride ($AlCl_3$) colourimetry. $AlCl_3$ is capable of identifying the flavone and flavonol compounds, including quercetin. $AlCl_3$ will interact with the C-4 keto and C-3 or C-5 hydroxyl groups of flavones and flavonoids to form a stable acid complex. The reaction in the AlCl3 and flavonol complex occurs within 2-60 minutes with a maximum wavelength at 415 nm [27-28].

The total flavonoid content of noni fruit and bitter gourd was increased as long as the fermentation time (Figure 2). The lowest flavonoid content was shown in the second week for noni fruit and bitter gourd, 0.67 mg QE/gr and 1.90 mg QE/g, respectively. Total flavonoids increased on day 63 of fermentation at laboratory and industrial scales. The total flavonoid content difference was already significant after 35 days of fermentation. The highest total flavonoids were obtained on day 63 of laboratory-scale fermentation, which was 36.73 ± 2.51 mg RE/100 mL. During fermentation, microorganisms affect the changes of the bioactive compounds. Some microorganisms will carry out metabolism that will use the main fermentation material as an energy source, which will then change polyphenols into simpler ones through a complex enzymatic system [29].

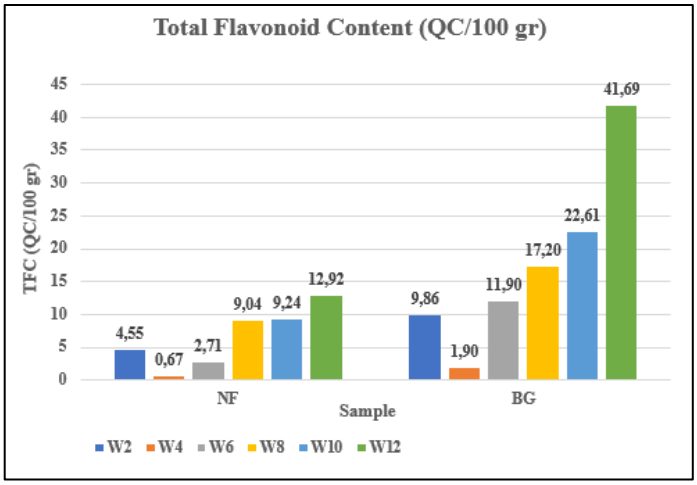

Fig. 2. Total flavonoid content during fermentation. NF: Noni Fruit; BG: Bitter Gourd; W2-W12: Second - Twelfth week.

Gallic acid was used as the standard solution in the 0-80 ppm range. Gallic acid is known as a derivative of hydroxybenzoic that is classified as a stable, simple phenolic acid [8]. The results of absorbance measurements on the gallic acid standard solution. Total phenolic content was determined using the regression equation derived from the plotted standard curve. The total phenolic content was established by the Folin-Ciocalteu reagent and expressed in mg gallic acid equivalent/g of extract (mg GAE/g extract). The Hydroxyl group of phenolic content can interact with the Folin-Ciocalteu reagent and develop a blue solution colour. The greater phenolic compound concentration makes the darker colour of blue colour. The blue colour can be detected at a wavelength of 760 nm [30].

Phenol is an effective hydrogen donor component and has good antioxidant properties [31]. The highest total phenol content of noni fruit was in the sixth week at 156.59 mg GAE/gr. Meanwhile, the highest total phenol content of bitter gourd was in the twelfth week at 146.39 mg GAE/g. The total phenol content of bitter gourd kept increasing from the eighth to the twelfth week (Figure 3). The fermentation process can increase the total phenolic content because of the metabolism activity of the microorganisms, which can increase the antioxidant activity, too. The common microorganism found in the fermentation process is L. plantarum, which can produce some enzymes that can release aglycones containing phenolic groups, thereby increasing the total phenolic content in an extract [26].

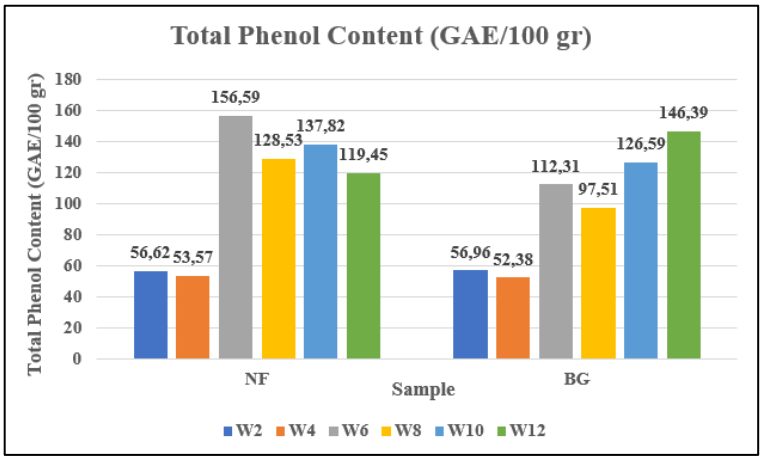

Fig. 3. Total phenol content during fermentation. NF: Noni Fruit; BG: Bitter Gourd; W2-W12: Second - Twelfth week.

Trolox was used as the standard solution in the 0 – 100 ppm range. Trolox is synthesized from a vitamin E derivative and is known as an antioxidant that can easily be soluble in water. Trolox is widely used as a positive control for comparing various antioxidants [32].

Antioxidant activity of noni fruit and bitter gourd increased from the second week until the sixth week, from 1839.67 mg TEAC/g to 2383 mg TEAC/g for noni fruit and 1688 mg TEAC/g to 2193 mg TEAC/g for bitter gourd. Antioxidant activity for noni fruit and bitter gourd was decreased in the eighth week (Figure 4). The result showed that the sixth week of the fermentation is the ideal time for higher antioxidant activity. Phenolic compounds contribute to the antioxidant activity found in bitter gourd and noni fruit. Fermentation can lead to the biotransformation of the bioactive compounds of bitter gourd, including polyphenols, which may enhance the antioxidant activity [11]. The results of this study are also in line with the research of [33], which showed an increase in the total phenol content in *Saccharina japonica* extract after the fifth day of fermentation mediated using *A. oryzae*. Phenolic compounds are one of the large groups of secondary metabolites found in plants.

Phenol has biological activity as an antioxidant compound with its electron donor ability to bind reactive oxygen species. The number and location of hydroxyl groups in phenolic compounds affect the effectiveness of its antioxidant ability [34].

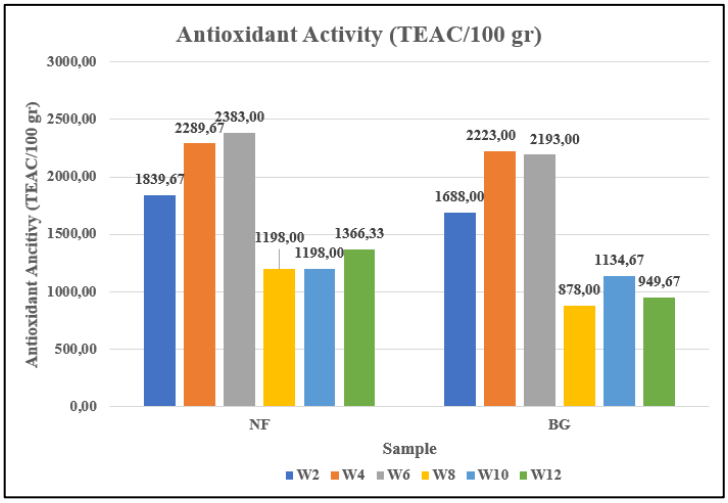

Fig. 4. Antioxidant activity during fermentation. NF: Noni Fruit; BG: Bitter Gourd; W2-W12: Second - Twelfth week.

4 Conclusions

Natural fermentation in honey can affect the total phenolic, total flavonoid, and antioxidant activity of noni fruit and bitter gourd. The longest fermentation time can increase the flavonoid and phenolic content of noni fruit and bitter gourd. Total phenol and flavonoids affect antioxidant activity in fermented products. Increasing total phenolic content and total flavonoid content can increase antioxidant activity. In the sixth week, it was seen that antioxidant activity in both fermented products was the highest activity.

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