Jurnal Pertanian Terapan of JURNAL ROCE (AGROSCIENCE)
https://jurnal.rocewisdomaceh.com/index.php/roce e-ISSN : 3032-4505
Vol. 3, No. 1, Thn. 2026
https://doi.org/10.71275/roce.v3i1.162

INNOVATION IN FERMENTED FUNCTIONAL BEVERAGES BASED ON NUTMEG SEEDS (*Myristica Fragrans* Houtt.) AND ACACIA HONEY AS A SOURCE OF NATURAL ANTIOXIDANTS

INOVASI DALAM MINUMAN FUNGSIONAL FERMENTASI BERBASIS BIJI PALA (*Myristica Fragrans* Houtt.) DAN MADU AKASIA SEBAGAI SUMBER ANTIOKSIDAN ALAMI

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Abstract

Nutmeg seeds (*Myristica fragrans* Houtt.) are rich in bioactive compounds and have strong potential for development as functional beverages. This study evaluated the effects of formulation ratio and fermentation duration on the physicochemical characteristics and antioxidant activity of a fermented nutmeg seed beverage supplemented with acacia honey. Three formulations with different nutmeg seed–acacia honey ratios were subjected to anaerobic fermentation for 15 and 30 days, followed by analysis of moisture content, sugar content, total acidity, insoluble solids, ash content, and antioxidant activity using the DPPH method (IC_{50}), with data evaluated by analysis of variance (ANOVA). The results showed that fermentation duration significantly affected moisture content, total acidity, ash content, and antioxidant activity ($p < 0.05$), while formulation ratio significantly influenced insoluble solid content and antioxidant performance. Moisture content ranged from 0.27% to 0.91%, sugar content from 0.64% to 0.75%, total acidity from 165.26 to 179.21, insoluble solids from 1.46% to 5.63%, and ash content from 0.17% to 0.93%. Antioxidant activity showed IC_{50} values ranging from 56.85 to 148.59 µg/mL, with the highest activity observed in the formulation containing equal proportions of nutmeg seeds and acacia honey fermented for 30 days. These findings indicate that fermented nutmeg–acacia honey beverages possess promising functional properties, with formulation composition and fermentation time playing critical roles in optimizing antioxidant activity.

Keywords: *Nutmeg seeds; Acacia honey; Fermentation; Antioxidant activity*

Abstrak

Biji pala (*Myristica fragrans* Houtt.) kaya akan senyawa bioaktif dan memiliki potensi besar untuk dikembangkan sebagai minuman fungsional. Studi ini mengevaluasi pengaruh rasio formulasi dan durasi fermentasi terhadap karakteristik fisikokimia dan aktivitas antioksidan minuman biji pala fermentasi yang ditambahkan madu akasia. Tiga formulasi dengan rasio biji pala–madu akasia yang berbeda difermentasi secara anaerobik selama 15 dan 30 hari, kemudian dianalisis kadar air, kadar gula, total keasaman, padatan tak larut, kadar abu, dan aktivitas antioksidan menggunakan metode DPPH (IC_{50}), dengan data dievaluasi menggunakan analisis varians (ANOVA). Hasil menunjukkan bahwa durasi fermentasi secara signifikan memengaruhi kadar air, total keasaman, kadar abu, dan aktivitas antioksidan ($p < 0,05$), sedangkan rasio formulasi secara signifikan memengaruhi kadar padatan tak larut dan kinerja antioksidan. Kadar air berkisar antara 0,27% hingga 0,91%, kadar gula antara 0,64% hingga 0,75%, total keasaman antara 165,26 hingga 179,21, padatan tidak larut antara 1,46% hingga 5,63%, dan kadar abu antara 0,17% hingga 0,93%. Aktivitas antioksidan menunjukkan nilai IC_{50} antara 56,85 dan 148,59 µg/mL, dengan aktivitas terkuat diamati pada formulasi yang mengandung proporsi yang sama antara biji pala dan madu akasia yang difermentasi selama 30 hari.

https://jurnal.rocewisdomaceh.com/index.php/roce e-ISSN : 3032-4505
Vol. 3, No. 1, Thn. 2026
https://doi.org/10.71275/roce.v3i1.162

Temuan ini menunjukkan bahwa minuman pala-madu akasia fermentasi memiliki sifat fungsional yang menjanjikan, dengan komposisi formulasi dan waktu fermentasi memainkan peran penting dalam mengoptimalkan aktivitas antioksidan.

Kata kunci: Biji pala; Madu akasia; Fermentasi; Aktivitas antioksidan

INTRODUCTION

Nutmeg seeds (*Myristica fragrans* Houtt.) are well-suited for development as a functional beverage due to their high content of bioactive compounds. These seeds contain myristicin, elemicin, safrole, and eugenol, which are known to have antioxidant properties that support human health. In addition to their antioxidant capacity, nutmeg seeds have natural relaxing and antidepressant effects, support digestive health, and help counteract free radicals linked to premature aging and chronic diseases. Moreover, nutmeg seeds have a distinctive aroma and flavor, enhancing their potential as a functional beverage ingredient. Fruit-based fermentation has considerable potential for developing functional foods and probiotic beverages, as the natural sugar content provides an ideal substrate for microbial metabolism. Fermentation can improve shelf life, enhance the availability of bioactive compounds, and increase antibacterial activity against pathogenic microorganisms. Furthermore, fermented fruit products may function as probiotic carriers with high nutritional value and associated health benefits. Traditionally, this fermentation has occurred naturally with wild microorganisms, without starter cultures, resulting in a slower process and a more complex flavor profile. Fermentation outcomes are strongly influenced by factors such as temperature, pH, substrate composition, microbial culture, and fermentation duration (Rahmah *et al*., 2023).

The fermentation process used in this functional beverage production is anaerobic, in which microorganisms break down organic compounds in the absence of oxygen. During fermentation, lactic acid bacteria utilize sugars as an energy source and produce lactic acid as the primary metabolite. Compared to chemical synthesis, microbial fermentation yields lactic acid of higher purity and fewer by-products (Nurfauzianti, 2021). Functional beverages are increasingly developed from natural ingredients such as leaves, herbal teas, and spices. Herbal materials may consist of combinations of flowers, leaves, seeds, or dried fruits that are traditionally used to prepare functional or herbal drinks (Sunia Widyantari, 2020).

These beverages are generally rich in antioxidants, including flavonoids, tannins, phenolic compounds, and anthocyanins. Free radicals in the human body can lead to blood thickening and decreased energy levels due to insufficient antioxidant activity (Ryadha *et al*., 2021). Antioxidants play a crucial role in preventing oxidative reactions and reducing physiological stress. Natural antioxidant sources are predominantly derived from plants, which contain phenolics, polyphenols, vitamins, and alkaloids; the latter are nitrogen-containing compounds with smaller molecular structures than phenolics (Carp *et al*., 2021).

Although previous studies on nutmeg fermentation have been conducted, many were not formally published. Therefore, this study proposes an innovative approach to developing functional beverages using nutmeg seeds and acacia honey as primary ingredients. Nutmeg has long been utilized in traditional medicine to treat various ailments, including digestive disorders, rheumatism, and malaria. Scientifically, nutmeg has been shown to have

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cholesterol-lowering, antidepressant, aphrodisiac, antimicrobial, and antioxidant properties, as well as cognitive-enhancing and hepatoprotective effects. Its essential oil has also demonstrated anticancer potential (Okiki *et al*., 2023).

In Indonesia, honey quality is regulated under SNI 8664:2024, titled "Honey," which is a revision of SNI 8664:2018 and was established by the National Standardization Agency (BSN) in 2024. Extrafloral honey derived from *Acacia crassicarpa* is widely produced in industrial plantation forests, particularly in Riau Province. In addition to their economic value as a source of plywood, acacia trees support sustainable agroforestry systems. Farmers can harvest honey year-round because nectar is produced in leaf axils rather than in flowers (Ratri Rahayu *et al*., 2023).

Honey is a naturally sweet substance produced by bees from nectar. Its color influences its sensory characteristics: darker honey has a stronger flavor, while lighter honey has a milder taste. Honey contains essential minerals, such as magnesium and iron, which support red blood cell production and hemoglobin levels (Panjaitan, 2018). The iron present in honey, particularly in heme form, can directly contribute to erythropoiesis by binding with heme and globin to form hemoglobin. Meanwhile, vitamin C in honey enhances the absorption of non-heme iron (ferric) by converting it into the heme (ferrous) form during digestion, particularly in the duodenum (Dahlan & Ardhi, 2021).

This study is limited to evaluating fermented beverage formulations based on nutmeg seeds, with acacia honey added at varying mass ratios. Three formulation treatments were applied, namely F1 with a ratio of 100 g nutmeg seeds to 60 g honey, F2 with a ratio of 80 g nutmeg seeds to 80 g honey, and F3 with a ratio of 60 g nutmeg seeds to 100 g honey. In addition, the study focuses exclusively on the effect of fermentation duration, specifically 15 and 30 days. The objectives of this study are to identify the fermented nutmeg–acacia honey beverage formulation that exhibits the highest antioxidant activity and to evaluate the influence of different fermentation periods (15 and 30 days) on the physicochemical characteristics of the fermented beverage.

RESEARCH METHODS

Equipment

The equipment used to prepare the fermented functional beverage included basins, knives, cloths, cutting boards, weighing scales, plastic fermentation jars, and muslin cloth for filtering the fermented product. Analytical instruments included an analytical balance, dropper pipettes, a desiccator, an oven, porcelain crucibles, Erlenmeyer flasks, a pH meter, a UV–Vis spectrophotometer, test tubes, a water bath, a high-performance liquid chromatography (HPLC) system, and a centrifuge.

Materials

The raw materials used to produce the functional beverage were nutmeg seeds and acacia honey, each with a total mass of 1000 g. Chemicals employed for the analysis of fermented samples included DPPH solution, distilled water, and deionized water.

Research Procedures

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Determination of Antioxidant Activity

The antioxidant activity of fermented nutmeg beverages supplemented with acacia honey was determined using the DPPH method. This method is based on the ability of antioxidant compounds in the fermented extract to neutralize $DPPH^+$ free radicals, as indicated by a color change from deep purple to pale yellow. Fermentation is assumed to enhance antioxidant activity through enzymatic hydrolysis and the formation of more bioactive secondary metabolites derived from nutmeg. In addition, acacia honey not only acts as a carbon source for microbial growth but also contributes phenolic compounds, thereby creating a synergistic antioxidant effect. Antioxidant activity was quantified by comparing the absorbance of the sample solution with that of the DPPH control at 517 nm after incubation. A decrease in absorbance indicates greater inhibition, which can be quantified as an IC_{50} value to assess antioxidant potency (Ikhlas *et al*., 2023).

Preparation of Fermented Nutmeg Beverage

Nutmeg seeds (1 kg) and acacia honey (1 kg) were prepared along with the required equipment. The nutmeg seeds and all utensils, including bottles, knives, and cutting boards, were thoroughly washed with clean water and air-dried. The nutmeg seeds were then cut into small pieces of approximately 5 mm in size. Clean, airtight containers were prepared, and the chopped nutmeg seeds and honey were added according to the designated formulations: Formula 1 (100 g nutmeg seeds: 60 g honey), Formula 2 (80 g nutmeg seeds: 80 g honey), and Formula 3 (60 g nutmeg seeds: 100 g honey). The total amount of raw materials required was 480 g of nutmeg seeds and 480 g of honey for both fermentation durations. After sealing the containers tightly to prevent oxygen entry, the samples were stored in a low-light environment and allowed to ferment anaerobically for 15 and 30 days. Upon completion of fermentation, the products were pressed and filtered, and the filtrates were transferred into 5 mL or 10 mL sample bottles for further analysis. To ensure simultaneous completion of both fermentation periods, preparation of the 15-day fermentation was initiated on the 14th day of the 30-day fermentation. A total of six samples were obtained: three fermented for 30 days and three for 15 days.

Analytical Procedures

Moisture Content

Moisture content was determined to quantify the water content in the fermented beverage samples. The oven-drying method was used, based on measuring weight loss due to water evaporation when samples were heated at approximately 100 °C. This method is suitable for most food products, except those containing volatile compounds or those susceptible to thermal decomposition at high temperatures (Yerinia, 2015). For analysis, approximately 2 g of sample was weighed and placed in a pre-weighed porcelain crucible, then dried in an oven at 105–110 °C for 2 hours. The sample was subsequently cooled in a desiccator for 10–30 minutes and reweighed. The drying and weighing process was repeated until a constant weight was achieved (Badan Standardisasi Nasional, 1992). Moisture content was calculated using the standard formula. The objective of moisture content analysis is to determine the amount of water in a material or product, as water content directly influences product quality, shelf life, production processes, quality standards, and pricing.

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$$\text{Moisture Content} = \frac{\text{initial sample weight} - \text{final sample weight}}{\text{initial sample weight}} \times 100\%$$

Sugar Content

The total sugar content of honey was measured using a refractometer. The honey sample was applied to the refractometer prism, and the refractive index was then read and expressed in degrees Brix. The Brix value reflects the concentration of soluble solids in the sample and indicates sugar content; one degree Brix corresponds to one gram of sugar per 100 grams of sample. Measurements were obtained directly from the refractometer scale (Khasanah *et al*., 2017).

Acidity Analysis

Certain food products contain acidic compounds that occur naturally or are deliberately introduced during processing. Variations in pH can significantly influence sensory attributes such as flavor, color, and texture, as well as the overall stability of food products during processing. The acid–base properties of food systems are critical for several functions, including regulating microbial growth, suppressing browning reactions, preventing lipid oxidation, promoting emulsification, and enhancing flavor (Yerinia, 2015). Based on the guidelines issued by the Indonesian National Standardization Agency (Badan Standardisasi Nasional, 2013), total acidity is determined by neutralizing the sample's acidic components with a standardized sodium hydroxide (NaOH) solution, with phenolphthalein (PP) as the indicator to identify the titration endpoint. Prior to analysis, all necessary apparatus were prepared, and 10 g of honey was weighed into a 250 mL Erlenmeyer flask. The sample was diluted with 75 mL of distilled water in a separate 250 mL beaker, then 4–5 drops of PP indicator were added. Titration was conducted with 0.05 M NaOH at 5.0 mL/min until the pH reached 8.5, at which point the titration was terminated. A back titration was then performed by pipetting 10 mL of 0.05 M NaOH and titrating with 0.05 M HCl until the pH reached 8.30. An identical procedure was carried out for the blank using 75 mL of distilled water. The volumes of NaOH and HCl consumed during titration were recorded (Badan Standardisasi Nasional, 2013). Total acidity was calculated using the equation presented below.

$$\text{Free Acidity} = \frac{(\text{mL of 0.05 M NaOH} - \text{mL of blank}) \times N_{\text{NaOH}}}{\text{Sample Weight}} \times 1000$$

$$\text{Lactone Content} = \frac{(10\ \text{mL NaOH} - \text{mL HCl}) \times N_{\text{HCl}}}{\text{Sample Weight}} \times 1000$$

$$\text{Total Acidity} = \text{Free Acidity} + \text{Lactone Content}$$

Insoluble Solids Test

According to the Indonesian National Standard (BSN, 2024), water-insoluble matter refers to foreign impurities such as sand particles, leaf fragments, insects, and other extraneous materials. The analysis was conducted by preparing the required apparatus,

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weighing the sample, and dissolving it in hot water. The solution was filtered through a filter paper with a previously determined constant weight. The residue on the filter paper was subsequently rinsed with hot water and dried in an oven at 100–105 °C for two hours. After drying, the filter paper containing the insoluble residue was cooled in a desiccator and weighed using an analytical balance until a constant mass was achieved (BSN, 2024). The content of insoluble solids was calculated using the formula presented below.

$$\text{Insoluble Solids (\%)} = \frac{W_1 - W_2}{W} \times 100\%$$

Where: W: Sample weight
W_1: Weight of the crucible and filter paper containing the insoluble residue after oven drying
W_2: Weight of the empty crucible and filter paper

Antioxidant Activity Test

A total of 2 mL of each sample was transferred into a test tube and mixed with 2 mL of a 0.002% DPPH solution. The mixture was then vortexed until homogeneous and incubated in the dark for 30 minutes. Absorbance was measured at a wavelength of 516 nm using a Perkin Elmer Lambda 25 UV–Vis spectrophotometer. Ascorbic acid at 20-100 ppm was used as the standard and treated under the same conditions as the test samples. The purpose of the antioxidant assay was to evaluate the ability of a substance or product to scavenge or neutralize free radicals that can cause cellular damage.

Ash Content Determination

Ash represents the inorganic residue remaining after the combustion of organic matter at high temperatures (>450 °C) and/or the destruction of organic components using strong acids. This inorganic residue consists of various minerals, the composition and concentration of which depend on the type of food material and the analytical method applied (Yerinia, 2015). According to the Indonesian National Standard (BSN, 1992), during the ashing process, organic substances are decomposed into water and carbon dioxide, while inorganic constituents remain unaffected. Prior to analysis, all necessary equipment was prepared. Approximately 3 g of the sample was weighed and placed in a porcelain crucible of known mass. The sample was dried in an oven at 105–110 °C for 1 hour, then cooled in a desiccator for 30 minutes and reweighed. Subsequently, the crucible was transferred to a muffle furnace and ashed at 500–600 °C for 8 hours. After ashing, the sample was cooled to approximately 120 °C, placed in a desiccator, and weighed repeatedly until a constant mass was obtained (BSN, 1992). The ash content was calculated using the following formula:

$$\text{Ash Content (\%)} = \frac{W_1 - W_2}{W} \times 100\%$$

Where:
W : Weight of the sample (g)
W_1: Weight of the empty crucible (g)
W_2: Weight of the crucible containing ash after ashing (g)

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https://doi.org/10.71275/roce.v3i1.162

The purpose of the ash content analysis is to determine the total amount of minerals or inorganic residue remaining after complete combustion of organic components in the sample. In addition, this analysis provides information on mineral content, product purity, and nutritional value, and serves as a basis for evaluating raw materials and processed products.

Data Analysis

Observational data were statistically evaluated using Analysis of Variance (ANOVA). When statistically significant differences were observed, further comparisons were conducted using a post hoc test at a 5% level of significance. The study used a Completely Randomized Design (CRD) with treatment factors based on different ratios of nutmeg seeds to acacia honey, comprising three formulations: F1 (100 g: 60 g), F2 (80 g: 80 g), and F3 (60 g: 100 g). These formulations were assessed at two fermentation periods, namely 15 and 30 days. The experimental layout for fermentation characteristics is summarized in Table 1.

Table 1. Experimental Design for Fermentation Characteristics

Sample Code	Nutmeg Seeds (g)	Acacia Honey (g)	Fermentation Time (days)
F1	100	60	15
F2	80	80	15
F3	60	100	15
F1	100	60	30
F2	80	80	30
F3	60	100	30

RESULTS AND DISCUSSION

Table 2. Moisture Content (Percentage)

Sample Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Total	Average	SD (Standard Deviation)
F1H15	0.323	0.344	0.277	0.286	0.345	1.58	0.31	0.03
F1H30	0.199	0.466	0.469	0.741	0.582	2.46	0.47	0.2
F2H15	0.291	0.186	0.288	0.3	0.212	1.28	0.27	0.05
F2H30	0.217	0.409	0.442	0.479	0.443	1.99	0.39	0.1
F3H15	0.25	0.214	0.415	0.509	0.654	2.04	0.35	0.18
F3H30	0.439	0.211	0.449	2.543	0.208	3.85	0.91	1
Total	1.719	1.83	2.34	4.858	2.444			

Table 3. Sugar Content (%)

Sample Code	Replications (1-5)	Total	Mean	SD
F1H15	0.64, 0.64, 0.64, 0.64, 0.64	3.2	0.64	0
F1H30	0.72, 0.72, 0.72, 0.72, 0.72	3.6	0.72	0
F2H15	0.75, 0.75, 0.75, 0.75, 0.75	3.75	0.75	0
F2H30	0.74, 0.74, 0.74, 0.74, 0.74	3.7	0.74	0
F3H15	0.75, 0.75, 0.75, 0.75, 0.75	3.75	0.75	0
F3H30	0.75, 0.75, 0.75, 0.75, 0.75	3.75	0.75	0
Total	4, 4, 4, 4, 4			

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https://doi.org/10.71275/roce.v3i1.162

Table 4. Total Acidity

Sample Code	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5	Total	Average	SD
F1H15	193.18	175.54	177.22	172.46	177.63	896.03	179.21	8.07
F1H30	162.77	164.47	151.23	173.79	181.53	833.79	166.76	11.51
F2H15	158.79	162.27	176.97	155.94	174.09	828.06	165.61	9.38
F2H30	164.2	158.87	173.65	162.5	167.06	826.28	165.26	5.55
F3H15	166.68	167.94	172.12	176.22	187.95	870.91	174.18	8.56
F3H30	181.08	161.43	158.69	176.15	174.73	852.08	170.42	9.79
Total	1026.7	990.52	1009.88	1017.06	1062.99			

Table 5. Insoluble Solid

Sample Code	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5	Total	Average	SD
F1H15	1.41	1.31	1.42	1.78	1.89	15.1	1.562	0.2559
F1H30	1.98	1.05	1.21	1.2	1.85	19.21	1.458	0.4245
F2H15	2.38	2.22	2.87	2.35	2.1	27.98	2.384	0.2936
F2H30	3.03	3.16	3.26	3.49	3.12	44.2	3.212	0.176
F3H15	5.59	5.53	5.56	5.71	5.75	39.96	5.628	0.0965
F3H30	2.17	2.72	2.15	2.48	2.3	11.82	2.364	0.2386

Table 6. Ash Content

Sample Code	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5	Total	Average	SD
F1H15	0.1166	0.0614	1.547	1.4719	1.4666	4.6635	0.93	0.77
F1H30	0.2461	0.0906	0.1195	0.1687	0.2215	0.8464	0.17	0.07
F2H15	0.5443	0.1616	0.1692	1.2983	0.9942	3.1676	0.63	0.5
F2H30	0.9326	1.1658	0.7629	0.3107	0.5472	3.7192	0.74	0.33
F3H15	0.6948	0.5897	0.3956	0.1238	0.4156	2.2195	0.44	0.22
F3H30	0.8462	0.5658	0.4951	0.0632	0.7731	2.7434	0.55	0.31
Total	3.3806	2.6349	3.4893	3.4366	4.4182			

Table 7. Antioxidant

Sample Code	IC_{50}	Description / Category
F1H15	85.71	Strong
F2H15	63.97	Strong
F3H15	148.59	Moderate
F1H30	67.48	Strong
F2H30	56.85	Strong
F3H30	99.77	Strong

Discussion

The moisture content of fermented nutmeg seed beverages ranged from 0.27% to 0.91% (Table 1). Generally, samples fermented for 30 days had higher moisture content than those fermented for 15 days. This trend may be associated with extended microbial activity during longer fermentation periods, which influences carbohydrate degradation and enhances water retention within the matrix. Similar fermentation-induced changes in moisture distribution and physicochemical properties have been reported in other fermented functional beverages (Zhang *et al.*, 2024; González & Ortega, 2025).

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ANOVA results indicated that fermentation duration significantly affected moisture content ($p < 0.05$), while formulation ratio showed a less pronounced effect. The high variability observed in F3H30 suggests that increased nutmeg seed content may contribute to inconsistent water binding and matrix interactions during prolonged fermentation, as also reported for plant-based fermented substrates rich in fibrous components (López *et al*., 2022).

Sugar content was relatively stable across all treatments, ranging from 0.64% to 0.75% (Table 2), with a standard deviation of 0% across replicates, indicating highly repeatable measurements. Statistical analysis confirmed that neither formulation ratio nor fermentation duration significantly affected sugar content ($p > 0.05$).

This stability likely reflects a balance between microbial utilization of fermentable sugars and the continuous availability of sugars from acacia honey. Previous studies on honey-based and plant-derived fermented beverages have similarly reported minimal changes in total soluble sugars when sufficient carbohydrate sources are present throughout fermentation (Kim & Park, 2023; Zhang *et al*., 2024).

Total acidity values ranged from 165.26 to 179.21 (Table 3). Samples fermented for 15 days tended to have higher acidity than those fermented for 30 days, suggesting that acid production may peak early in fermentation and then stabilize or slightly decline. ANOVA showed that fermentation time significantly affected total acidity ($p < 0.05$), whereas formulation ratio did not have a significant main effect. This pattern aligns with previous findings that fermentative microorganisms produce organic acids early in fermentation, followed by metabolic conversion or buffering during extended fermentation (Ikhlas *et al*., 2023; González & Ortega, 2025).

Insoluble solid content varied widely, ranging from 1.46% to 5.63% (Table 4), with significantly higher values observed in formulations containing a greater proportion of nutmeg seeds. ANOVA results showed that the formulation ratio significantly affected insoluble solid content ($p < 0.05$). Extended fermentation reduced insoluble solids in certain treatments, possibly due to enzymatic degradation of fibrous components by fermentative microorganisms.

Comparable results have been reported in fermented plant-based beverages, where microbial enzymes modify physical structure and reduce insoluble fractions through biotransformation processes (López *et al*., 2022; Ikhlas *et al*., 2023).

Ash content ranged from 0.17% to 0.93% (Table 5). Both fermentation duration and formulation ratio significantly affected ash content ($p < 0.05$). Higher ash values were generally observed at shorter fermentation times, while prolonged fermentation tended to reduce ash content. This reduction may be due to mineral complexation, microbial assimilation, or the redistribution of inorganic components during fermentation. Such changes in mineral content during fermentation have been documented in recent studies investigating mineral bioavailability and inorganic residue dynamics in fermented functional beverages (Zhang *et al*., 2024; González & Ortega, 2025).

Antioxidant activity, expressed as IC_{50} values, ranged from 56.85 to 148.59 µg/mL (Table 6), with lower IC_{50} values indicating stronger antioxidant potential. Samples fermented for 30 days, particularly F2H30, exhibited significantly enhanced antioxidant activity. ANOVA results confirmed that both formulation ratio and fermentation duration significantly influenced antioxidant capacity ($p < 0.05$). The observed enhancement in

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antioxidant activity is consistent with previous studies demonstrating that fermentation improves the bioavailability of phenolic compounds and other antioxidants through microbial biotransformation (López *et al*., 2022; Ikhlas *et al*., 2023). Fermentation has also been shown to increase radical-scavenging activity by modifying phenolic and flavonoid profiles in plant-based substrates (Kim & Park, 2023).

Overall, the combined results demonstrate that fermentation duration and formulation ratio play pivotal roles in determining the physicochemical and functional characteristics of the nutmeg–acacia honey fermented beverage. Fermentation time predominantly influenced moisture content, total acidity, ash content, and antioxidant activity, while formulation ratio exerted a stronger effect on insoluble solids and antioxidant performance. Sugar content remained stable across all treatments, indicating a balanced substrate environment throughout fermentation.

Among all treatments, the F2 formulation fermented for 30 days exhibited the most favorable quality profile, characterized by strong antioxidant activity and balanced physicochemical properties. These findings support the potential application of this formulation as a functional fermented beverage, in agreement with recent developments in plant-based fermentation research (Zhang *et al*., 2024; González & Ortega, 2025).

CONCLUSION

This study demonstrates that fermented functional beverages formulated from nutmeg seeds (*Myristica fragrans* Houtt.) and acacia honey possess promising physicochemical and antioxidant properties. Both fermentation duration and formulation ratio significantly influenced product quality. Fermentation time had a major effect on moisture content, total acidity, ash content, and antioxidant activity, whereas the nutmeg seed–acacia honey ratio predominantly affected insoluble solid content and antioxidant performance. Sugar content remained relatively stable across all treatments, indicating balanced carbohydrate utilization during fermentation.

Antioxidant activity, expressed as IC_{50} values, improved with longer fermentation periods, highlighting the role of microbial biotransformation in enhancing the bioavailability of antioxidant compounds. Among all treatments, the formulation with equal proportions of nutmeg seeds and acacia honey fermented for 30 days showed the strongest antioxidant activity and the most balanced physicochemical characteristics. Overall, these findings confirm that nutmeg–acacia honey fermented beverages have strong potential to be developed as functional drinks, with formulation composition and fermentation duration serving as key factors for optimizing product quality and health-related functionality.

ACKNOWLEDGEMENT

Thank you to the Head of the Chemistry Laboratory of Universitas Tanjungpura for the assistance provided in conducting the antioxidant analysis.

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Vol. 3, No. 1, Thn. 2026
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