

Proximate composition, antioxidant activity, total phenol and vitamin C content of pineapple combined with orange juice

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ABSTRACT

Background: The development of antioxidant-rich beverages has gained increasing attention because of their potential health benefits. Pineapple and orange are readily available fruits that are naturally high in antioxidants.

Methods: Three juice formulations were prepared: F1 (100g orange juice without honey), F2 (50g orange juice + 50g pineapple juice + honey), and F3 (75g orange juice + 25g pineapple juice + honey). UV-Vis spectrophotometric analysis was used to assess the proximate composition, total phenolic content (TPC), vitamin C content (titration technique), and antioxidant activity (DPPH test). Data were tabulated and descriptively analyzed to compare the nutritional and functional properties among the formulations.

Results: Proximate analysis showed that the addition of pineapple and orange juice decreased moisture, protein, and fat contents, but increased ash, carbohydrate, and total acidity levels. Formula F3 exhibited the highest acid and ash contents, while F2 had the highest carbohydrate level among the formulations. F2 demonstrated the highest antioxidant activity (31.17%), whereas F1 had the highest total phenolic content (161.67 mg GAE/100 g). Both F1 and F2 displayed equally high vitamin C levels (0.88%). These results indicate that blending pineapple and orange with honey can synergistically improve the nutritional and bioactive properties of the beverages.

Conclusion: Formulation F2 showed the strongest antioxidant activity, with 31.17% inhibition, whereas F1 contained the highest total phenolic content at 161.67 mg GAE/100 g. Both F1 and F2 demonstrated similarly high levels of vitamin C (0.88%). These findings suggest a synergistic interaction between pineapple, orange, and honey in improving both the nutritional composition and antioxidant potential.

INTRODUCTION

The creation of antioxidant-rich drinks has garnered more attention in recent years because of the possible health advantages they may offer^{1,2}. Since oxidative stress from free radicals may cause chronic and degenerative illnesses including cancer, autoimmune disorders, aging, cataracts, rheumatoid arthritis, cardiovascular, and neurological diseases, antioxidants are essential for defending the body against this threat^{3,4}. The tropical fruit pineapple (*Ananas comosus*) is prized for its unique scent and sweet taste⁵. It is regarded as tasty because of the intricate combinations of several volatile chemicals present in trace concentrations⁶. Pineapple also has several health advantages since it is a strong source of vitamins and minerals⁷.

The tropical fruit known as pineapple (*Ananas comosus*) is prized for its unique scent and sweet taste, which are ascribed to the intricate interactions between a variety of volatile chemicals present in trace levels⁵. Pineapple has several health-promoting vitamins and minerals in addition to its organoleptic qualities⁸. Additionally, bromelain, a proteolytic enzyme with antioxidant and anti-inflammatory qualities, is present in it⁹. Contrarily, oranges (*Citrus sinensis*) are known for their high levels of vitamin C and hesperidin, flavonoids that boost immunity and provide other health advantages¹⁰.

Combining pineapple and orange in a single beverage may produce a synergistic effect, enhancing the overall antioxidant

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capacity⁸. Such a combination not only offers a refreshing taste, with pineapple's natural sweetness and mild acidity complementing the bright sweetness of oranges, but also provides multiple functional benefits. Both fruits contribute to immune support, while bromelain in pineapple aids digestion and exhibits anti-inflammatory effects¹¹. Furthermore, the antioxidants present in this combination help neutralize free radicals and promote skin health¹².

This study aims to evaluate the proximate composition, antioxidant activity, vitamin C content and total phenol content of a beverage formulated from pineapple and orange juice.

METHODS

Design, Place and Time

The development of Pineapple Combined with Orange Juice used an experimental design. Each formula was prepared once. Formula 1 represented orange juice without the addition of honey and pineapple juice. Formula 2 included 50g pineapple juice, 50g of orange juice and 5g of honey, while Formula 3 included 75 g pineapple juice, 25g orange juice and 5g of honey. The additional ingredients used in F2 and F3. The additional ingredients used in the juice included 5 grams of honey. This research was conducted in January - June 2024. Analysis of the Proximate Composition, Antioxidant Activity, Vitamin C and Total Phenolic Content of Pineapple Combined with Orange Juice was conducted at Central Instrumentation Laboratory of Agricultural Technology Faculty, Andalas University, Padang.

Ingredients and Equipment

The ingredients used in the preparation of pineapple combined with orange juice include Sunpride brand pineapple (*Ananas comosus*), Pasaman orange (*Citrus sinensis*), and Pure Honey (TJ Brand) which are mostly available in traditional markets in west sumatera. The equipment used in the research of Pineapple Combined with Orange Juice product development are digital scales, blender and stainless container.

The Development of Pineapple and Orange Juice Blend

The process of making a refreshing pineapple and orange juice blend, begin by peeling and coring a ripe pineapple, then cutting it into small pieces. The process of making pineapple and orange juice is shown in Figure 1. It can be seen that the process of making pineapple and orange juice includes several stages; (1) Pineapple fruits were peeled, blanched at 80 °C for 1 minute, cut into small pieces, and blended with water at a ratio of 100 g pineapple pulp to 100 mL water. (2) Oranges were cut in half, manually squeezed to obtain the juice, and filtered to remove seeds. (3) Three product formulations were prepared, **Formula 1:** 100 g pineapple juice without

honey; **Formula 2:** 50 g pineapple juice + 50 g orange juice + 5 g honey; **Formula 3:** 75 g pineapple juice + 25 g orange juice + 5 g honey. 5) To formula 2 & 3, **5 g of pure honey was added per 100 g of juice** and mixed thoroughly. (4) Blend the mixture until it achieves a smooth and homogeneous texture.

Proximate Analysis

Standard procedures were used to determine the samples' proximate analysis (AOAC, 2005)¹³. Drying at 100°C for at least 24 hours produced a steady weight, which was used to calculate the moisture content. By digesting for four hours at 550°C in a muffle furnace, the amount of ash and minerals was ascertained. An enzymatic gravimetric technique was used to measure the total amount of dietary fiber.

Analysis of Antioxidant Activity of Pineapple Combined with Orange Juice

The DPPH test, as outlined by Huang et al. (2005), was used to assess the sample's antioxidant activity¹⁴. To create a solution with a 1000 ppm concentration, 10 mg of the sample extract was first dissolved in 10 ml of methanol. After that, the solution was vortexed and ultrasonicated to guarantee adequate mixing. After that, 1 ml of the DPPH solution was added to a test tube containing 2 ml of the prepared solution that had been pipetted there. The mixture stood for half an hour in the dark. A spectrophotometer set at 517 nm wavelength was then used to test the sample's absorbance. For comparison, a blank solution comprising 1 milliliter of DPPH and 2 milliliters of methanol was utilized. Using the following formula, the sample's antioxidant activity was represented as the percentage inhibition of DPPH radicals: % Inhibition = ((Abs control – Abs sample) / Abs control) × 100%. In this case, Abs sample denotes the absorbance of the sample containing DPPH at 517 nm, whereas Abs control denotes the absorbance of the DPPH solution.

Analysis of Total Phenol of Pineapple Combined with Orange Juice

A spectrophotometer and the Folin–Ciocalteu technique can be used to measure a sample's total phenolic content (TPC). The sample extract should first be prepared by dissolving it in distilled water to the proper concentration, such as 1 mg/mL. Next, combine 2.5 mL of Folin–Ciocalteu reagent (10% of the total volume) with 0.5 mL of the sample, and let it react for five minutes. After that, carefully stir in 2 mL of a 7–10% sodium carbonate solution, and let the mixture sit at room temperature for 30–60 minutes, shielded from the sun. A spectrophotometer should be used to measure the absorbance at 765 nm following incubation. Gallic acid (0–200 µg/mL) is used to create a standard calibration curve for quantifying TPC. The phenolic content of the sample is then calculated

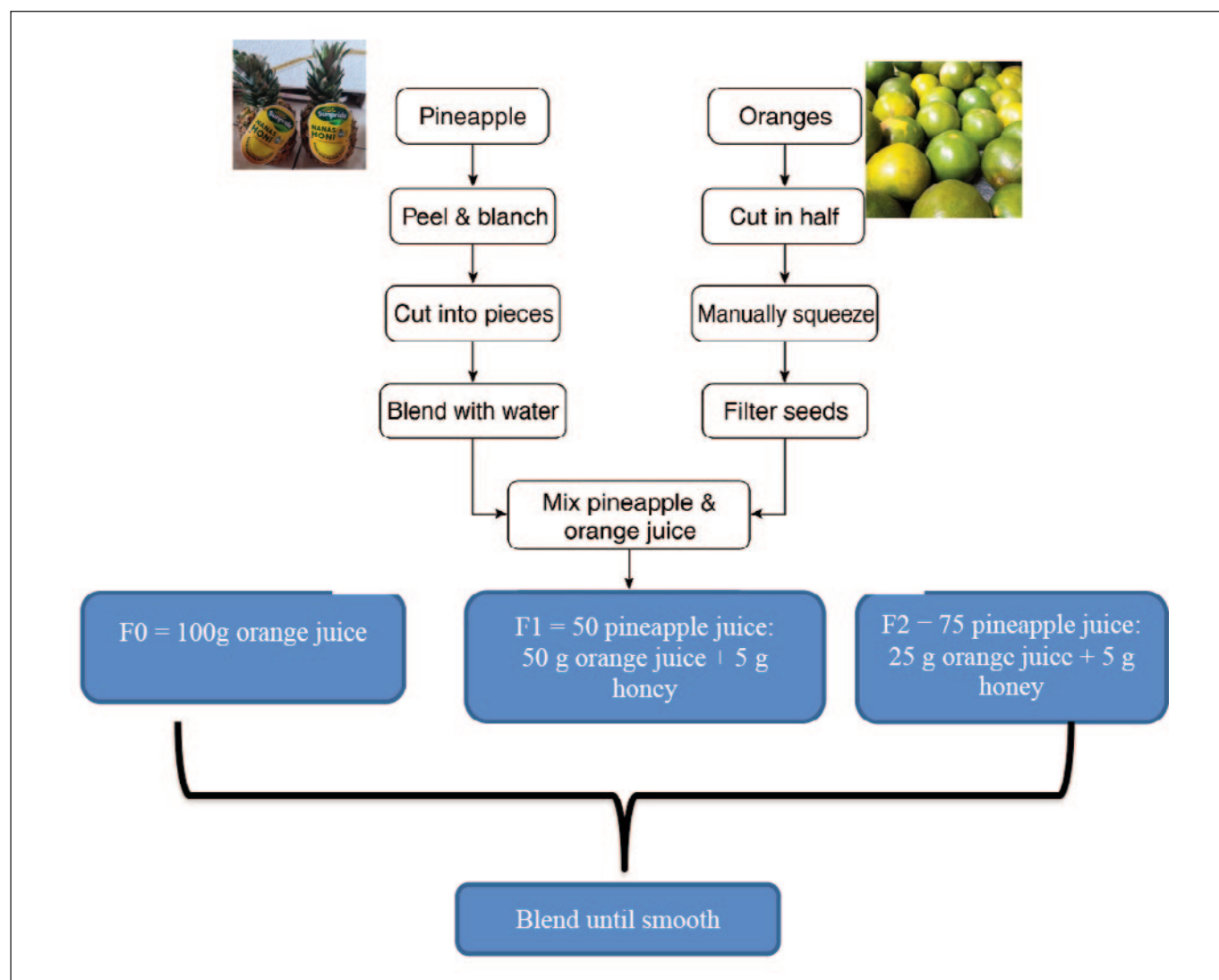


Figure 1. Procedure for making pineapple and orange juice blend

and usually represented as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

Analysis of Vitamin C of Pineapple Combined with Orange Juice

The analysis of total ascorbic acid (vitamin C) begins with sample preparation. A specific amount of the sample, such as 10 g of food or beverage, is placed in a volumetric flask and diluted with distilled water up to the mark to dissolve the vitamin C. The solution is then separated from any solid residues by filtration or centrifugation to obtain a clear filtrate. Next, a standard ascorbic acid solution with a known concentration is prepared, along with the titrant, typically an iodine solution, to be used in the titration. For the iodometric titration, a measured portion of the sample filtrate is

transferred to an Erlenmeyer flask, and a suitable indicator, such as starch solution, is added. The solution is titrated with iodine from a burette, gently mixing until the endpoint is reached, indicated by a persistent dark blue-black color. The volume of iodine titrant used is recorded, and the amount of ascorbic acid in the sample is calculated using the appropriate stoichiometric formula. Finally, the total content of ascorbic acid (vitamin C) in the sample is determined.

Data Analysis

The Social Science Statistics application (SPSS) version 16.0 for Windows and the Microsoft Excel 2016 computer application were used to examine the values of each parameter. Antioxidant activity, vitamin C, total phenol, and data proximate analysis were all tallied, descriptively assessed, and displayed in tables.

RESULTS AND DISCUSSION

Proximate Analysis of Pineapple and Orange Juice

The fresh juice extracted from the Pineapple and Orange fruit showed the three formulas (F1, F2, and F3) exhibit varying proximate characteristics. **Moisture content** is highest in F1 at 91.8%, while F2 and F3 have moisture levels of 87.7% and 87.5%, respectively. **Ash content** in F1 is 1.19%, slightly lower than F2 at 1.2%, with F3 having the highest ash content at 2.1%. Regarding **protein**, F1 has the highest value at 1.3%, while F2 contains none, and F3 has 0.4%. **Lipid (fat)** levels are highest in F1 at 1.2%, followed by F3 at 0.6%, and the lowest in F2 at 0.08%. The **crude fiber** content in F1 and F3 is identical at 0.6%, whereas F2 is slightly lower at 0.5%. **Total acid** in F1 and F2 is the same at 5.9%, but it increases significantly in F3 to 22%. Finally, **carbohydrate** content in F1 is 4.4%, lower than F2 at 10.8%, while F3 contains 9.2%. This data highlights that each formula has distinct nutritional advantages based on its composition as presented in Table 1.

Based on the table 2, the three formulas (F1, F2, and F3) demonstrate varying levels of antioxidant activity, total phenol content, and vitamin C concentration. Antioxidant activity is highest in F2, measuring 31.17%, followed by F3 at 23.95%, and the lowest is F1 at 18.25%. For total phenol content, F1 has the highest value at 161.67 mg, followed by F2 with 147.60 mg, and F3 with the lowest at 129.59 mg. Regarding vitamin C concentration, F1 and F2 share the same value at 0.88 %, while F3 has the lowest concentration at 0.66 %. This data indicates that F2 excels in antioxidant activity, while

Table 1. Proximate Analysis of Pineapple and Orange Juice per 100 g

Proximates	Formulas		
	F1	F2	F3
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Moisture	91,8	87,7	87,5
Ash	1,19	1,2	2,1
Protein	1,3	0	0,4
Lipid (Fat)	1.2	0.08	0.6
Crude Fiber	0,6	0,5	0,6
Total acid	5,9	5,9	22,0
Carbohydrate	4,4	10,8	9,2

F1 = 100 g orange juice.

F2 = 50 g orange juice + 50 g pineapple + 5 g honey.

F3 = 75 g orange juice + 25 g pineapple + 5 g honey.

Table 2. Antioxidant Activity, total phenol and vitamin C of Pineapple and Orange Juice

Parameter	Formulas		
	F1	F2	F3
Antioxidant Activity	18,25	31,17	23,95
Total Phenol	161,67	147,60	129,59
Vitamin C (mg/100g)	0,88	0,88	0,66

F1 is superior in total phenol content and maintains a comparable vitamin C level to F2.

DISCUSSION

Proximate composition refers to the analysis of food components such as moisture, protein, fat, ash, and carbohydrates. These components provide important information about the nutritional value and quality of a food product¹⁵. By analyzing the levels of protein, fat, ash, and carbohydrates in each food sample, we can determine their overall nutritional content and make informed decisions about incorporating them into a balanced diet.

Conversely, the capacity of specific food ingredients to shield cells from harm brought on by free radicals is known as antioxidant activity¹. In order to lower the risk of chronic illnesses including diabetes, heart disease, and cancer, antioxidants are essential^{16–21}. To learn more about the nutritional qualities and health advantages of different food samples, we will examine their proximate composition and antioxidant activity in this study. Additionally, assessing the antioxidant activity of these foods will give us insight into how they can help protect our cells and potentially prevent the development of serious illnesses. Through this research, we hope to provide valuable information that will empower individuals to make healthier food choices and improve their overall well-being.

Free radicals are unstable chemicals that can cause oxidative stress and contribute to the development of chronic illnesses including cancer, kidney disease, heart disease, and Alzheimer's disease. Antioxidants are essential for defending our cells against these destructive agents. Including foods high in antioxidants in our diet can strengthen our body's defenses against these dangerous compounds²². We may encourage longevity, energy, and general health by realizing the significance of antioxidants and including them in our regular diets. Antioxidants are abundant in fruits such as oranges, pineapple, and honey^{5,23}. A healthy and more vibrant existence may be achieved by consciously choosing to eat a range of foods high in antioxidants, which can help lower inflammation, strengthen our immune system, and shield our cells from harm²⁴.

Pineapple and orange juice both contain large amounts of antioxidants, including vitamin C and other polyphenols, their combination is very interesting²⁵. These compounds have been shown to work synergistically in the body, enhancing their overall antioxidant effects. Furthermore, the anti-inflammatory qualities of both fruits are well-known and can promote general health and wellbeing. The interaction between vitamin C and phenolic compounds as antioxidants is synergistic, meaning they work together to enhance overall antioxidant activity. As a water-soluble antioxidant, vitamin C provides electrons to counteract free radicals, primarily in aqueous phases, while phenolic compounds, which can be both water- and lipid-soluble, use their hydroxyl (-OH) groups to scavenge free radicals, preventing lipid oxidation and cellular damage^{24,26–28}. In combination, vitamin C can regenerate oxidized phenolics, restoring their antioxidant capacity, while phenolics can stabilize the ascorbate radical, preventing the rapid degradation of vitamin C.

The combined effects of pineapple and orange juice as antioxidants may provide valuable insights into how certain food combinations can optimize our health and vitality. For example, a study could be conducted where participants consume a daily smoothie containing both pineapple and orange juice for a certain period of time, with regular blood tests to measure changes in antioxidant levels²⁹. The results could help illustrate how these fruits work together to promote better health outcomes and potentially inform dietary recommendations for improving antioxidant intake. Additionally, researchers could also explore how the combination of pineapple and orange juice affects other aspects of health, such as inflammation and immune function³⁰. By gaining a better understanding of the synergistic effects of these two fruits, we may be able to recommend specific fruit combinations to help individuals achieve optimal health benefits. This research could pave the way for personalized nutrition plans that incorporate specific food pairings to maximize the protective effects of antioxidants and promote overall well-being.

Furthermore, this study also used Madu TJ (Tj Pure Honey) as one of the ingredients, which provides an additional source of carbohydrates and natural sugars that may influence the overall nutritional composition of the samples. According to the nutrition label on the Madu TJ bottle, every 20 grams of pure honey contains 16 grams of total carbohydrates, 12 grams of total sugars, and 0 grams of protein, saturated fat, and total fat. These values indicate that honey mainly serves as a source of natural energy through simple sugars such as fructose and glucose. Although it contains no protein or fat, honey is known to possess natural antioxidant compounds such as flavonoids and phenolic acids, which contribute to its ability to protect cells from oxidative stress. Therefore, the relatively high antioxidant activity observed in Formula 2 compared to the other formulations may also be attributed to the presence of honey,

which enhances the overall antioxidant potential of the blend, followed by Formula 3, which also contained honey and showed moderately high antioxidant activity compare with Formula 1 without adding honey.

CONCLUSION

This study concludes that blending pineapple and orange juice with honey can enhance both nutritional composition and antioxidant activity, with F1 (100% orange juice without honey) showing the highest phenolic content, F2 (50 g orange juice + 50 g pineapple juice + honey) demonstrating the strongest antioxidant activity and comparable vitamin C levels to F1, and F3 (75 g orange juice + 25 g pineapple juice + honey) presenting higher ash and acidity values. The results indicate a synergistic effect between pineapple and orange, producing a functional beverage with potential health benefits. It is suggested that future research should include sensory evaluation and consumer acceptability testing, clinical validation of health effects, and optimization of F2 as the most promising formulation for product development, while also exploring the incorporation of other fruits or bioactive ingredients to further improve antioxidant properties and diversify applications. For further research, it is recommended to include at least one juice formulation without honey as a control sample for comparison and to provide a detailed analysis of the honey's nutritional composition.

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