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Characteristic of physical, chemical, and microbiological kombucha from various varieties of apples

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Abstract. Kombucha is a fermented tea beverage with the addition of kombucha starter also called SCOBY (Symbiotic Culture Of Bacteria and Yeast). The purpose of this research was to know the physical, chemical and microbiological characteristics of kombucha from various varieties of apple kombucha. The study used Randomized Block Design (RAK) with one factor of apple varieties (Anna, Fuji, Granny Smith, Manalagi, Red Delicious, Rome Beauty, Royal Gala). Each treatment was repeated three times. Data was analyzed with ANOVA (Analysis of Variance). The best treatment was selected using Multiple Attribute method. Data of hedonic test was analysed using Friedman Test. The best treatment was obtained on Fuji varieties of kombucha apple with the characteristics as follows: total acid 1.33%; pH 2.95; Total phenol 268.57 µg/ml GAE; Total sugar 6.74%; Antibacterial activity against *S.aureus* 21.30 mm; Antibacterial activity *E.coli* 21.20 mm; Antioxidant activity 35.62%; Organoleptic aroma 3.55, taste 3.3; Color 3.4 (on a scale of 1-5)

1. Introduction

Kombucha is a functional beverages produced by fermenting tea using a SCOBY (Symbiotic Culture of Bacteria and Yeast). SCOBY consists of symbiotic interaction between Bacteria (*Acetobacter xylinum*) and Yeasts (*Brettanomyces, Zygosaccharomyces, Saccharomyces,* and *Pichia*) [1]. The symbiotic interaction during tea fermentation converts sugar into short-chained organic acid, vitamins, and alcohols [2].

Kombucha contains some minerals, vitamins, enzymes, and organic acids which is considered as functional beverages. Kombucha is effective to treat constipation, increasing microflora inside the guts, increasing immune system, reducing blood pressure and cholesterol, eliminating cancer cell, has an antidote effect, antioxidant activity, and also antibacterial effect [3]. Symbiotic interaction in kombucha breaks down the substrate in aerobic environment, for seven to ten days, to produce fresh, acidic carbonated drinks [4].

In general, kombucha is made from black tea and sugar. Many researches have been conducted to increase its functional value of kombucha by substituting the black tea with other materials that contain high amount of natural antioxidant compounds. Apple is one of the fruit that is highly consumed in Indonesia. Three of the most common apple variety cultivated in Indonesia are the Rome Beauty, Manalagi, and Anna. Another varieties of Apples like Fuji, Red Delicious, Roya Gala, and

Granny Smith are largely imported from various countries. Apples are often processed into apple-based products such as apple cider, apple chips, apple toffee, apple vinegar, and many more. Malang, East Java is one of the biggest apple supplier in Indonesia with the apple cropping area reaching 2.500 m^2 which produces up to 10 tons of apples. These apples have not been entirely utilized, therefore more apple-based products innovation is necessary to be developed.

Apple-based kombucha fermentation is expected to increase the functional value and increase the organoleptic value of kombucha. Apple is used to substitute tea as the main ingredients to produce kombucha. Fermentation will increase the bioavailability of several nutritions from raw material such as antioxidant and antibacteria which will make it easier for the substance to be absorbed into digestive system. Therefore, a study is necessary to determine the characteristics of kombucha made from various kinds of apple to obtain kombucha with high quality.

2.Materials and Methods

2.1 Experimental design

This research used Randomized Block Design using single factor with three duplicates of each treatment. The factor were apples varieties (Anna, Manalagi, Fuji, Granny Smith, Red Delicious, Rome beauty and Royal Gala). Black Tea was used as the comparative control. The data were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with 5% of confidence range. Multiple Attribute Method was used to determine the best treatment.

2.2. Apple juice preparation and Kombucha fermentation

Apple was peeled, washed and cut into small size. Each apples were carefully weighed using the analytical balance until 300 g and added with water at a ratio of 1 :2. The mixture was blended for 30 seconds and then filtered with filtering cloth to obtain the apple juice. Apple juice was added with 10% cane sugar and then pasteurized at 65 ^oC for 30 minutes and cooled at room temperature. The pasteurized apple juice was put into sterilized glass jar. Kombucha starter were added for 10% (v/v) and then covering with cloth. The fermentation process was maintained for 14 days at room temperature. Microbial growth and chemical changes were monitored during the fermentation.

2.3. Data Analysis

The post-fermentation characteristics determination of kombucha included analysis of total microbial cells (TPC method) [5], pH[6], total acids content [7], total sugars content [7], total phenols content [8], antioxidant activity (DPPH method) [9], antibacterial activity analysis [10] and organoleptic analysis [11].

3.Result and discussion

3.1 Total bacteria

Figure 1 shows that total microbial cells in apple kombucha increase from day 0 to 7 days of fermentation, and then decreased after 14 days fermentation. Microbes utilize glucose as the energy source which is abundantly present in the fermentation medium from added sugar and apple itself. The increasing number of total microbes occurred because microbes utilize sugars to replicate cells and grow. A part of the sugars are also converted into metabolites such as alcohols. The number of microbes increased until 7 days of fermentation then reaching stationary phase [12]. Total microbial decreased after 14 days due to the production of various metabolite compounds that inhibit microbial growth. The optimum pH for *Acetobacter sp*. is between 5.4 - 6.3. While, yeast has an optimum pH at 4.0-5.0. In the acid state, the growth of microbes is inhibited.

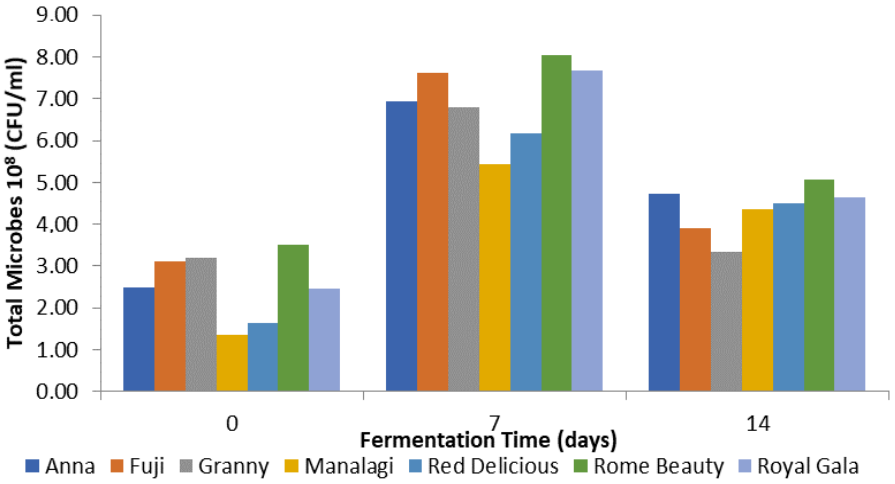

Figure 1. The growth of microbes from various apples varieties during kombucha fermentation

3.2 Total acid and pH kombucha

Figure 2 and Figure 3 show the total acid and pH of kombucha during fermentation, respectively. Total acids increased during fermentation. During fermentation, glucose is metabolized by microbes to produce organic acids. Therefore, the longer the fermentation, the more organic acids are being produced. Kombucha fermentation is initiated by the enzimatic hydrolisis of sucrose resulting glucose and fructose. The yeasts (*Saccharomyces cereviseae*) metabolize the glucose resulting ethanol and carbon dioxide. The ethanol is then oxidized into acetaldehyde by the acetic bacteria (*Acetobacter*) which is later converted into acetic acid. The acetic acid concentration in kombucha increases during fermentation. *Acetobacter* also breaks down glucose into various low molecular weight organic acids. Fermentation of kombucha also produce the other kind of acids such as glucanoic acid, glucoronic acid, and lactic acid. Invertase enzyme activity from the yeasts allows sucrose breakdown, while the acetic acid bacteria do not have the similar ability because of its lack of hydrolase and kinase enzyme [3]. The result of variance analysis showed that the treatment of apple variety was not significantly different ($\alpha = 0.05$). The result of raw material analysis showed that there was no significant difference between apple varieties to sugar content, total acid, pH, and total phenol.

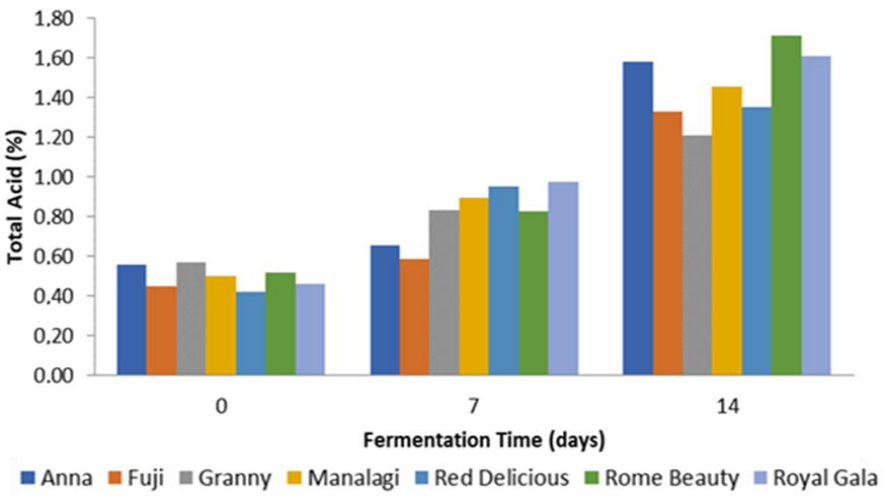

Figure 2. Total Acids of kombucha during fermentation

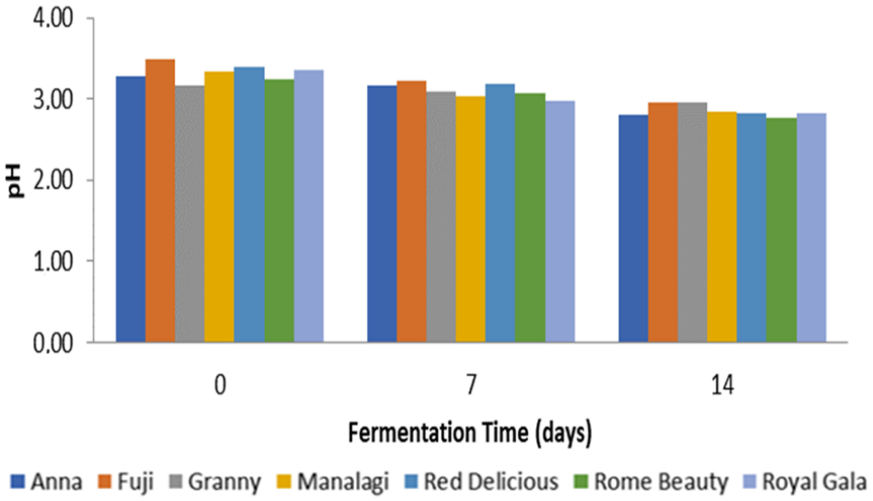

Figure 3. pH of kombucha during fermentation

3.3 Total sugar

Figure 4 shows that total sugars of each of apple kombucha varieties decreased during fermentation. Microbes utilizes glucose to produce new cells during fermentation that produce organic acids as the metabolites. *Saccharomyces cereviseae* metabolizes glucose as its energy sources. Yeasts utilize glucose and fructose during fermentation as their energy sources although they mostly break down glucose to produce ethanol and carbon dioxide, while fructose is metabolized by another minor microorganisms. Sugars are the source of glucose which can be metabolized by the microorganisms to grow their cells and to produce acetic acid. During the fermentation the yeasts metabolize glucose in the media to produce alcohols and other substances simultanously, continued by alcohol oxidation by the *Acetobacter xylinum* producing acetic acid. According to Aditiwati and Kusnadi [3], *Acetobacter xylinum* also oxidize glucose into glucoronic acids and other organic acids at the same time.

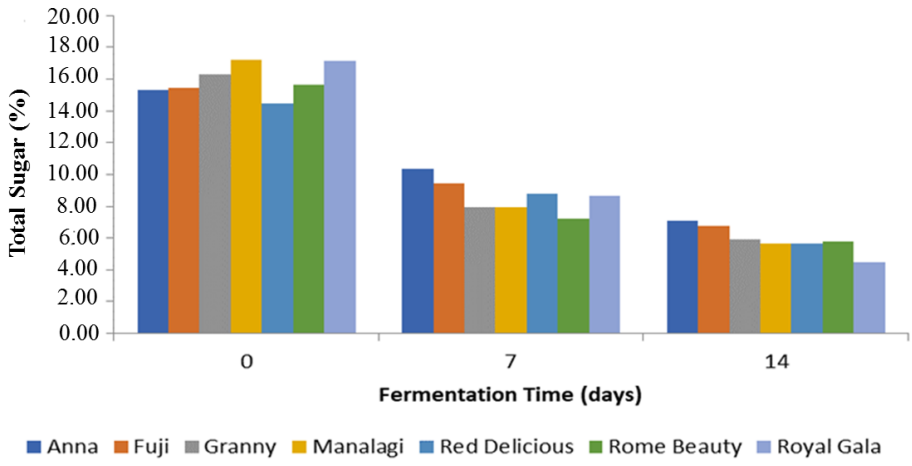

Figure 4. Total sugar content during kombucha fermentaion

3.4 Total phenols and antioxidant activity

Figure 5 and Figure 6 show total phenol and antioxidant activity during kombucha fermentation, respectively. Total phenols content and antioxidant activity each varieties of apple kombucha increased during fermentation. Yeasts and bacteria both have the ability to produces the enzyme that

convert polyphenolic complex into its less complex phenolic components during fermentation. During fermentation, the phenols content increases because of biotransformation. Biotransformation is a process that changes particular functional group into its composing substances mediated by enzymes [12]. Biotransformation utilizes enzymes from a certain plant to increase particular biological activity [13]. According to Bhanja et al. [14], phenol is one of many organic compounds that have antioxidant activity, therefore when more phenols are produced as metabolites, the higher the antioxidant activity.

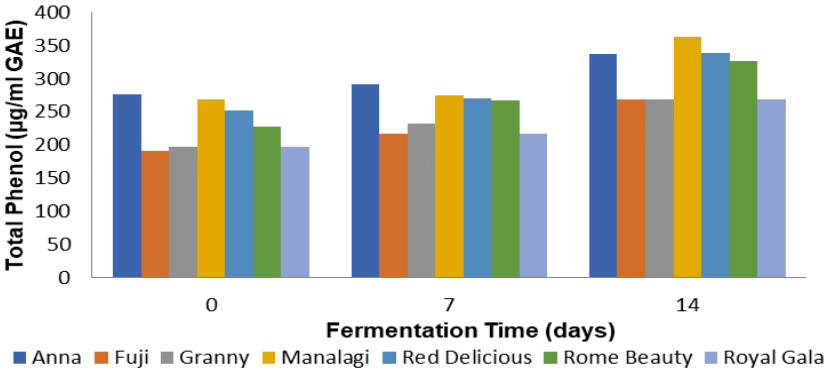

Figure 5. Total Phenols content during kombucha fermentation

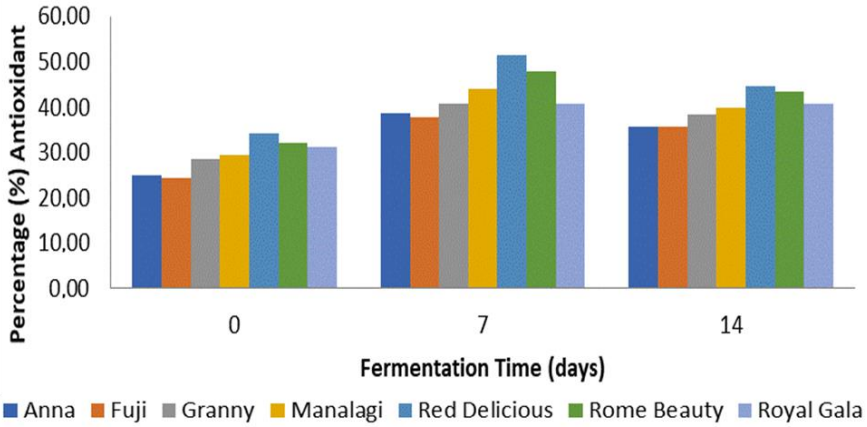

Figure 6. Antioxidant activity during apple kombucha fermentation

3.5 Antibacterial activity against Staphylococcus aureus and E.coli

Figure 7 and Figure 8 show antibacterial activity against *Staphylococcus aureus and E.coli* during kombucha fermentation, respectively. During fermentation, the antibacterial activities of apple kombucha against *Staphylococcus aureus* increased. The bigger inhibition zone is likely caused by the organic acids produced during kombucha fermentation. Kombucha itself has already demonstrated its antibacterial activity against pathogenic microorganism [15]. Acetic acid is lipophilic that makes it penetrates easily into the gram positive bacteria cell compared to gram negative ones. Antimicrobial activity is also determined by the value of pKa which defined as the amount of un-dissociated acid molecule in water. The lower the pKa, the higher the antimicrobial activity. Naidu and Clemens [16] studied that acetic acid dissosiates and releases protons resulting lower the pH value. These protons released by the acetic acid disturb the membrane cell function of the bacteria. Acids cause enzyme denaturation and interfere the membrane cell's permeability. The antibacterial activity of kombucha is

highly caused by the presence of acetic acid produced from the glucose metabolism by bacteria and yeast, and also polyphenols that did not undergo biotransformation during the fermentation [15].

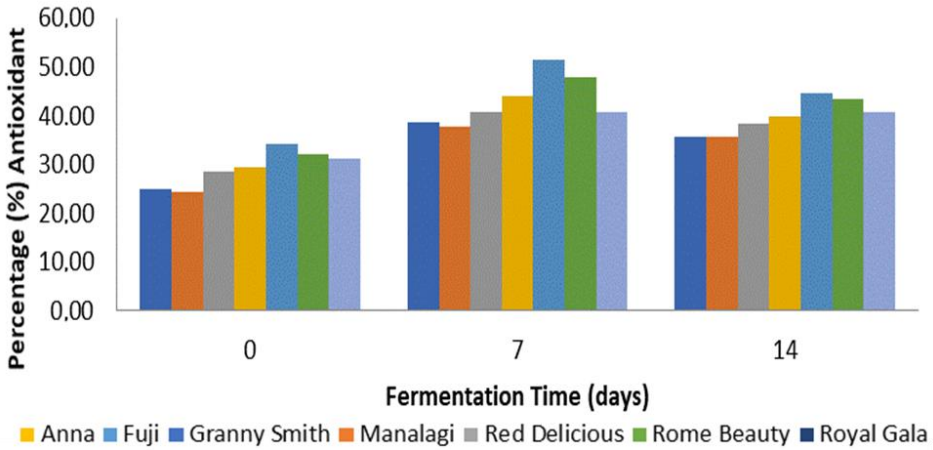

Figure 7. Antibacterial activity of apple kombucha against *Staphylococcus aureus*

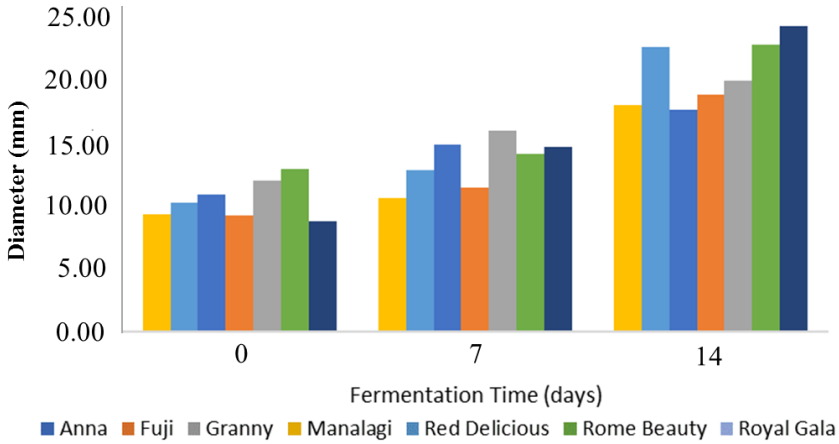

Figure 8. Antibacterial activity of apple kombucha against *E.coli* during kombucha fermentation

3.6 Taste and aroma

The acceptance on the taste and aroma of all the apple kombucha from various types of apples was different (Figure 9 and Figure 10). The result from statistical analysis using Friedman's test showed that the variety of apples gave significant effect (α=0.05) on the degree of likeness in respect to the taste of apple kombucha. The Non-Parametric test showed significant difference between the following varieties; anna-manalagi, anna-royal gala, fuji-manalagi, granny smith-manalagi, granny smith-red delicious, granny smith-royal gala, and granny smith-rome beauty. According to Anugrah [17], kombucha became more acidic as the longer the fermentation. This is most likely caused by the sucrose metabolism which produces organic acids such as acetic acid, glucoronic acid, and gluconic acid by the bacteria and yeasts. The taste of kombucha depends on the ratio of sugar and acids, the variety and amount of flavoring agent, and also vitamins. Friedman's test showed that the variety of apples gave significant effect (α=0.05) on the degree of likeness in respect to the aroma of apple kombucha. Short chain organic acids are aroma source of the apple kombucha. The longer the fermentation, the more organic acids are being produced. More organic acids enhance the aroma of

kombucha, yet decrease the acceptance of the taste. According to Wood [18], khamir has the ability to produce components that are responsible for aroma and taste.

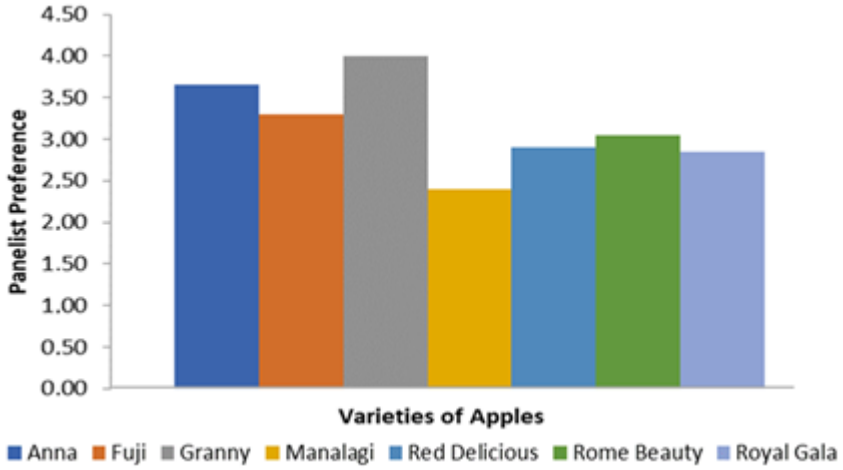

Figure 9. Panel's preference score of the taste of apple kombucha

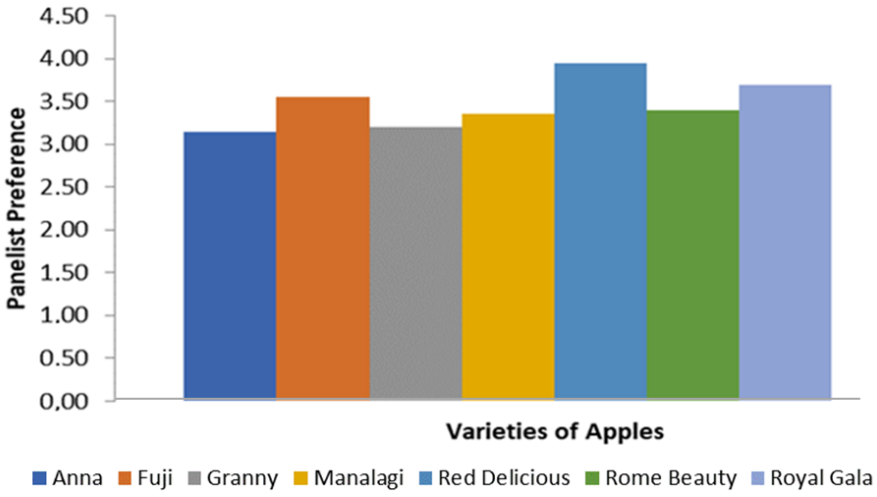

Figure 10. Panel's preference score of aroma of apple kombucha

4. Conclusion

Based on Multiple Attribute Test, Fuji apple variety is the best apple to be processed to kombucha. This apple kombucha has characteristics as follows: degree of likeness in terms of aroma 3.55, taste 3.3, total acid content of 1.33%, pH value of 2.95, antioxidant activity of 35.62 %, total sugars content of 6.74 %, total phenols content of 268. 57 µg/ml GAE, antibacterial activity against *S.aureus* of 21.30 mm, antibacterial activity against 21.20 mm.

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