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Effect of Various Types of Sugars on Antioxidant Activity and Physico-chemical Properties of Kombucha Fermented Whey

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ABSTRACT. *Kombucha or known as SCOBY (Symbiotic Culture of Bacteria and Yeast) is a fermented tea beverage with the addition of sugar then fermented for 7-14 days. Recent studies showed kombucha conceivably used for milk fermentation, such as fermented whey, yogurt, and fermented milks. Sugar is added to kombucha as carbon source for microorganisms in kombucha. Type of sugar, sugar concentration, tea concentration, temperature and incubation time are factors that affect kombucha fermentation. The aims of this study were to determine the effect of various types of sugars on antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH)) and physicochemical properties (pH, titratable acidity, total dissolved solid, and CIE L* a* b* color space value). The various types of sugars were granulated white sugar (G1), coconut sugar (G2), palm sugar (G3) and no sugar addition as control (G0). Fermented whey using 10% kombucha liquor, 3% kombucha cellulose and 10% sugar addition, whey then fermented at 37oC for 14 hours. Result demonstrated that using of various types of sugars significantly affected antioxidant activity, CIE L*a*b* color space value, pH, titratable acidity, total dissolved solid and vitamin C content. The highest vitamin C content was G3 (0.91±0.05 mg/100ml), kombucha fermented whey using palm sugar. Any type of sugar could be used on kombucha fermented whey, and the highest antioxidant activity (DPPH) was fermented whey kombucha with the addition of coconut sugar (G2) (86.79±10.94%).*

Keywords: Antioxidant, fermented whey, kombucha, kombucha fermented whey

INTRODUCTION

Technology has enhanced and changed the lifestyle of humans and urge to low physical activities. Furthermore, unhealthy lifestyles such as smoking, poor eating habits, and busy work can lead to stress. These conditions commonly led to degenerative diseases such as hypertension, diabetes, cardiovascular diseases, and other degenerative diseases. The increase of degenerative diseases urges to improve healthy lifestyle through consuming functional foods and nutraceuticals. One of the beverages that potentially as functional beverages is kombucha.

Kombucha is a beverage obtained from fermented tea to be expected originated from Manchurian (Villarreal-Soto et al., 2018). Kombucha or SCOBY is a symbiotic culture between bacteria (acetic acid bacteria (AAB) and lactic acid bacteria (LAB))and yeast in the form of thick cellulose membrane (Kumar & Joshi, 2016). Kombucha is made of a sweetened tea inoculated with kombucha sour liquid and kombucha cellulose membrane and further fermented for 7-14 days. Sugar is used as substrate for microorganism metabolism of SCOBY. Organic acids are the main metabolites that are produced by kombucha. Sucrose (from sugarcane) is commonly used in kombucha as carbon source for microorganisms in kombucha fermentation. Research conducted by several researchers showed that source carbon of kombucha might be varied, coconut sugar, palm

sugar, molasses sugar, and sorbitol can be used in kombucha making (Kluz et al., 2022; Muhialdin et al., 2019a). The use of different sugar sources in kombucha affected the properties of kombucha, such as biomass formation, glucose, sucrose, organic acid content, antioxidant activity and total phenolic content. Therefore, the sugar source or type of sugar affected kombucha fermentation system (Muhialdin et al., 2019a). In addition, kombucha fermentation is also affected by tea concentration, temperature, and incubation time (Al-Kalifawi & Hassan, 2014).

Kombucha tea fermentation uses tea and sugar as their substrate, implicated bacteria, and yeast in their fermentation system. During fermentation, cellulose membrane was formed by *Acetobacter xylinum* and the substrate concentrations gradually declined (Laureys et al., 2020). It is adequate to improve bacteria and yeast interactions. The different yeast and bacteria species act in parallel to produce fermented tea and the cellulose membrane (biofilm). At the beginning of the fermentation, yeast hydrolyzed sucrose into glucose and fructose, and glucose is converted to ethanol. Then, acetic acid bacteria (AAB) transform ethanol into acetic acid (Villarreal-Soto et al., 2018). Acetic acid stimulates yeast to produce ethanol and supports acetic acid bacteria to grow and produce acetic acid. Acetic acid and ethanol production are adequate to protect kombucha from contamination of pathogenic bacteria (Liu et al., 1996).

In addition to tea, recently fruit juice also use as substrate of kombucha, such as grape juice, pineapple juice, apple juice and pomegranate juice, using fruit juice influenced microbial composition of kombucha, pH, organic acids and also influence the antioxidant activity of kombucha (Osiripun & Apisittiwong, 2021). Kombucha can also be applied in milk fermentation and its derivatives (Suciati et al., 2019), milk fermentation using kombucha has been researched by several researchers (Hrnjez et al., 2014; Vitas et al., 2013). Traditional kombucha and kombucha fermented milk-based beverages have different characteristics, including chemical composition such as different disaccharides. Whey is a co-product of cheese and casein manufacturing in the dairy industry. Whey is a soluble fraction of milk, rich in proteins, minerals, and lactose. The main constituents of the cheese whey are β-lactoglobulin, α-lactalbumin, two globular proteins that account for 70-80 percent of total whey. Minor components include immunoglobulin, bovine serum albumin, glycomacropeptide, lactoferrin, lactoperoxidase and numerous endogenous enzymes (AMS, USDA, 2015).

The presence of lactose and type of sugars addition will affect fermentation system of kombucha. Fermented milk drinks using kombucha inoculum have different characteristics compared to traditional kombucha, kombucha microorganism and the kombucha cellulose membrane could adapt to environmental conditions of milk. In traditional kombucha, AAB are microorganism present dominantly over LAB and yeast., while in fermented drinks using kombucha as inoculum, this the percentage of the LAB and yeast increased, and the AAB decreased (Kruk et al., 2021).

The studies of kombucha using different substrates like fruit juice or different tea leaves and with various types of sugar might be varied. However, studies of fermented milks using kombucha and its derivatives are still limited. This work considers manufacturing kombucha fermented whey using various types of sugars (granulated white sugar, coconut sugar, and palm sugar) aiming to determine the antioxidant activity and physico-chemical properties.

MATERIALS AND METHODS

The research was conducted at the Laboratorium Teknologi Pengolahan Hasil

Pertanian dan Laboratorium Kimia, Jurusan Agroindustri, Politeknik Negeri Subang. The research material used was Whey Protein Concentrate (WPC) 34, granulated white sugar, coconut sugar, palm sugar, kombucha, tea, aquadest, pH buffer 4 and 7, amylum 1%, iodine 0,01 N, NaOH 0,1 N, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol p.a., and phenolphthalein (PP) 1%. Research equipment used were incubator, pH meter (Mettler Toledo F20), chromameter (Konica Minolta (CR-400), analytical balance, refractometer (Atago Pocket Refractometer PAL-1), spectrophotometer UV/Vis, burette, Erlenmeyer flask, volumetric flask, pipettes, and graduated cylinder flask.

Research Design

This research used was experiment method with a Completely Randomized Design (CRD) with 4 treatments and 3 replications for each treatment. The treatments were the use of various sugars namely G0 (no sugar addition), G1 (sugarcane), G2 (coconut sugar), and G3 (palm sugar). The addition of sugar is 7% (w/v) respectively. The data obtained were analyzed using one-way Analysis of Variance (ANOVA). If the results showed significant differences at 5%, then further statistically analyzed using Dunnet Test to compare each of a number of treatments with a single control, namely G0 (no sugar addition).

Kombucha Preparation

The procedure of making kombucha started with dissolving 2.25 g/L black tea and 7% sucrose in 1 L hot tap water, then cooled in room temperature. Thereafter 10% of kombucha liquor and 2.5% (w/v) kombucha cellulose added and transferred to a jar then covered with cheesecloth then incubated for 5 days in room temperature (Srihari & Satyanarayana, 2012; Vitas et al., 2013).

Kombucha Fermented Whey Making

Whey was pasteurized at 70^{o}C for 30 minutes, then cooled until the temperature reached 37^{o}C following with the addition of 10% kombucha inoculum from previous fermentation, 2.5% (w/v) kombucha cellulose, after that 7% of sugar then added in accordance with the treatments, namely without sugar addition (G0), the addition of granulated white sugar (G1), coconut sugar (G2) and palm sugar (G3)). The products then incubated for 14 hours at 37^{o}C (Suciati et al., 2019).

Measurement

Antioxidant activity

Antioxidant Activity was determined using free radical scavenging activity by DPPH assay. 2 mL of 160 folds diluted whey kombucha samples were mixed with 2 mL of 0,1 mM/L methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH). The mixture was shaken vigorously and allowed to stand in the dark for 20 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer (Fu et al., 2014).

Color

Color is one of the important parameters to consider regarding to quality and perception of consumers acceptance in agro-industrial products (Sitanggang & Machfoedz, 2023). The color notation system used in this research is from the Commission International de I Declaration (CIE) with the measured color components Lightness (L*), redness (a*) and yellowness (b*). The color of fermented whey kombucha sample was measured using Konica Minolta (CR-400) Chromameter. The chromameter was calibrated with white calibration plate (a Minolta standard). The sample was placed in a protective cap, placed on a light project tube and then measured. Data output is in the form of L*, a*, and b* value.

pH

Measuring the pH value of fermented food to assure the minimum pH for fermented dairy products is 4.6 or lower (Chandan, 2006). The pH value was measured using a pH meter. The pH meter calibrated with buffer solution pH 4 and 7, in accordance with range of pH fermented milk products. Measurement was done by whelming

the pH meter electrode to 10 ml of kombucha fermented whey sample.

Titratable acidity

The titratable acidity is a critical parameter in yogurt and fermented milk production. U.S. Standard recommend a standard of 0.6% titratable acidity (Frye, 2013). Titratable acidity also called acidity to measure total acid concentration contained within food (Sadler & Murphy, 2010). Titratable acidity was performed by titrated 10 ml of sample and 2-3 drops of phenolphthalein (PP) 1% was added as an indicator. The titrant was 0.1 N NaOH. The titration endpoint reached when permanent pink color appeared (Hadiwiyoto, 1994).

Total soluble solid

Total soluble solid measured all substances (inorganic and organic) dissolved in food (Zubaidah et al., 2019). Total soluble solid was performed using a refractometer (Atago Pocket Refractometer PAL-1). One drop of sample was placed on the prism then press start; the measurement is displayed on the LCD of the refractometer.

Vitamin C content

Kombucha contains high levels of Vitamin C. Vitamin C is one of water-soluble vitamin that is necessary components for biochemical and physiological process in the human body (Bishop et al., 2022). Vitamin C was performed using iodometric titration. Amylum 1% was added to 10 ml of sample, and the solution was then titrated by Iodine. The titration endpoint was reached when permanent blue color appeared (Sudarmadji & Haryono, 1997).

RESULT AND DISCUSSION

Antioxidant Activity

The antioxidant activity is affected by the prevalence of bioactive compounds within the products, and usually fermented foods and beverages have very high antioxidant activity (Muhialdin et al., 2019a). During kombucha fermentation the microorganism metabolizes vitamins, enzymes, organic acids and polyphenols, which contributie to the antioxidant activity (Villarreal-Soto et al., 2019).

Various chemical compounds in kombucha beverages are organic acids, vitamins, ethanol, proteins, tea polyphenols and mineral (Villarreal-Soto et al., 2018). The DPPH radical scavenging activities of kombucha fermented whey with different types of sugars are shown in Table 1 and statistical analysis are shown in Table 2.

Table 1. Antioxidant activity of kombucha fermented whey using various types of sugars.

Various Type of Sugar	Antioxidant Activity (%)
G0	89.08±2.95
G1	30.90±1.94
G2	86.79±10.94
G3	80.34±6.33

Table 2. Dunnett's test results comparisons between G0 (control) and treatments G1 to G3 Antioxidant activity (%) of kombucha fermented whey using various type of sugars.

Treatments Comparison	I	J	Mean difference	Standard Error	p^c
G1 vs G0	30.90	89.08	-58.18333	5.360	0.000
G2 vs G0	86.79	89.08	-2.30000	5.360	0.948
G3 vs G0	80.34	89.08	-8.74667	5.360	0.305

The antioxidant activity of kombucha fermented whey using various types of sugar showed significant differences, G0 compared to G1 had significant differences, while G2 and G3 had no differences compared to G0. Kombucha fermented whey with granulated white sugar

addition (G1) antioxidant activity was lower compared to G0 as control, while G1 had two types of sugar, namely sucrose (granulated white sugar) and lactose from the whey protein concentrate and G0 only had lactose from the whey protein concentrate.

Traditional kombucha using low-cost green tea, black tea and tea powder had different antioxidant activity. Low-cost green tea kombucha reached 95.30% dan tea powder kombucha had the lowest antioxidant activity, with the value of 38.7% (Fu et al., 2014). Compared to traditional kombucha, kombucha whey fermented, the antioxidant activity was similar. The antioxidant activity depends on the substances contained in kombucha products. The chemical composition of kombucha were varied depending on the inoculum source, the sugar and tea concentration, the fermentation time, and the temperature used (Villarreal-Soto et al., 2018).

Kombucha tea fermented with different sugar sources, namely white refined sugar, molasses, and coconut palm sugar had antioxidant activity 29,08, 32,93 and 49,26%, respectively (Muhialdin et al., 2019a). The addition of various types of sugars on kombucha fermented whey showed the different antioxidant activity, this is indicated that various types of sugar affect the antioxidant activity of kombucha fermented whey.

Naturally coconut sap, sugar palm juice and sugarcane juice has antioxidant properties (DPPH scavenging ability), namely 23.42%, 19,82% and 12,40%, respectively (Asghar et al., 2020). Types of sugar have different nutritional content. The content of sucrose, glucose and fructose in those sugars are different, palm sugar contains 89.94% of sucrose, 3.61% of glucose and 3.50% of fructose, while coconut sugar contains 86.86% of sucrose, 4.64% of glucose and 3.70 % of fructose, and sugar cane or white sugar contains 94.75% of sucrose, fructose and glucose were not detected. Sucrose is disaccharide, while glucose and fructose are monosaccharides (Maryani et al., 2021).

Besides natural antioxidants contained in those sugars, changes in substances during fermentation also affected the antioxidant activity of kombucha fermented whey. The main antioxidants in fermented kombucha beverages are polyphenols and vitamins and organic acids. Vitamins and organic acids are metabolites that synthesized during fermentation (Ivanišová et al., 2020). These are related with the sugar addition which was the primary carbon source for microorganism (Muhialdin et al., 2019). Furthermore milk and dairy products possess antioxidant activity due to the presence of caseins, whey proteins, oligosaccharides, vitamin A, E, C, selenium, zinc, antioxidant enzymes and bioactive peptides that are generated during milk fermentation (Khan et al., 2019).

During fermentation Acetic Acid Bacteria (AAB) transform ethanol into acetic acid, nonetheless production gluconic and glucuronic acids, while yeast hydrolyzed sucrose into glucose and fructose(Villarreal-Soto et al., 2018). This indicated that the low activity of antioxidant in G1 compared to G0, due to the fermentation condition. G1 contained sucrose that need longer fermentation time to break down the sucrose into glucose and fructose to further transform into organic acids. On the contrary, G2 and G3 had different compositions of glucose and sucrose. Vitamin C in whey kombucha fermented is also expected to contribute as antioxidant.

Color Measurement of Kombucha Fermented Whey Using Various Types of Sugars

The color of the kombucha fermented whey using various types of sugars is shown in Table 3 and statistical analysis of colormeasurement of kombucha fermented whey is shown in Table 4.

Table 3. L*, a*, b* value of kombucha fermented whey using various type of sugars.

Various Type of Sugar	L*	a*	b*
G0	61.23±1.88	3.09±0.08	1.26±0.16
G1	57.16±0.91	3.07±0.14	1.13±0.17
G2	54.87±1.15	2.25±0.13	12.00±0.20
G3	46.17±0.79	0.81±0.09	18.37±0.51

Table 4. Dunnett's test results comparisons between G0 (control) and treatments G1 to G3

Treatments Comparison	I	J	Mean difference	Standard Error	p^c
L*					
G1 vs G0	57.16	61.28	-4.1133	1.026	0.010
G2 vs G0	54.87	61.28	-6.4100	1.026	0.001
G3 vs G0	46.17	61.28	-15.1066	1.026	0.000
a*					
G1 vs G0	3.07	3.09	-0.0200	0.9117	0.992
G2 vs G0	2.25	3.09	-0.8367	0.9117	0.000
G3 vs G0	0.81	3.09	-2.2767	0.9117	0.000
b*					
G1 vs G0	1.13	1.45	-0.3200	0.2712	0.534
G2 vs G0	12.00	1.45	10.5567	0.2712	0.000
G3 vs G0	18.37	1.45	16.9233	0.2712	0.000

The color of the food is the parameter of quality evaluated by consumers. Color is the first property of food that is evaluated by consumers. Various types of sugars affected the lightness (L*), red-green (a*), and yellow-blue (b*) of kombucha fermented whey. The L* values of G1, G2 and G3 were different compared to G0, G0 as control has the highest L* value. The a* and b* values of G2 and G3 were significantly different compared to G0, where the a* and b* values of G1 were not significantly different compared to G0. The results indicate that various types of sugar have different impacts on the color of kombucha fermented whey.

The color of each sugar is different, granulated white sugar (G1) commonly comes from refined sugarcane, and it is white in color. The use of granulated white sugar in kombucha fermented whey did not give different appearance compared to control (G0). On the other hand, the a* and b* values of G2 and G3 are significantly different compared to G0, in view of fact that G2 and G3 have brownish color. The brownish color comes from the origin color of each sugar, where the coconut sugar and palm sugar are brown in color, yet palm sugar has darker color compared to coconut sugar. The brownish color comes from the Maillard reaction and caramelization that occur during the heating process of palm and coconut sap (Srikaeo & Thongta, 2015). The original color of sugars affected the color of kombucha fermented whey. This is in accordance with the present study that reported the use of different types of sugar at different concentrations significantly affected the appearance of meringues (Lekjing et al., 2022). The L* value of all samples are lower than kombucha fermented milks (goat's milk, cow's milk, and soy's milk), its indicated that the color of the origin substrate influenced the color of the products and the color value of beverages changed during fermentation (Özyurt, 2020).

pH Value, Titratable Acidity, Total Soluble Solid, and Vitamin C of Kombucha Fermented Whey

Food properties play a large role in determining palatability and nutrient assimilation. The chemical properties of Kombucha Fermented Whey is shown in Table 5 and the statistical analysis is shown in Table 6.

Table 5. pH value, titratable acidity, total soluble solid and vitamin C content of kombucha fermented whey using various types of sugar.

Various Type of Sugar	pH	Titratable Acidity (%)	Total Soluble Solid (%Brix)	Vitamin C Content (mg/100g)
G0	4.71±0.02	0.49±0.01	6.37±0.16	0.47±0.05
G1	4.55±0.12	0.55±0.06	13.60±0.40	0.56±0.10
G2	4.29±0.05	0.65±0.04	13.00±0.20	0.65±0.05
G3	4.36±0.12	0.79±0.05	12.60±0.44	0.91±0.05

Table 6. Dunnett's test results comparisons between G0 (control) and treatments G1 to G3.

Treatments Comparison	I	J	Mean difference	Standard Error	p^c
pH					
G1 vs G0	4.55	4.71	-0.1600	0.0706	0.124
G2 vs G0	4.29	4.71	-0.4200	0.0706	0.001
G3 vs G0	4.36	4.71	-0.3500	0.0706	0.003
Titratable Acidity					
G1 vs G0	0.55	0.49	0.0600	0.0364	0.298
G2 vs G0	0.65	0.49	0.1560	0.0364	0.007
G3 vs G0	0.79	0.49	0.2940	0.0364	0.000
Total Soluble Solid					
G1 vs G0	13.60	3.67	7.2333	0.2981	0.000
G2 vs G0	13.00	3.67	6.6333	0.2981	0.000
G3 vs G0	12.60	3.67	6.2333	0.2981	0.000
Vitamin C Content					
G1 vs G0	0.56	0.47	0.0880	0.0548	0.317
G2 vs G0	0.65	0.47	0.1760	0.0548	0.031
G3 vs G0	0.91	0.47	0.4400	0.0548	0.000

The pH value, TA, TSS and vitamin C content were taken after 14 hours incubation at the end of fermentation. The pH value of kombucha fermented whey ranged from 4.29-4.71. The used of various types of sugar significantly affected ($p<0,05$) pH value of kombucha fermented whey. The pH value of G1, G2 and G3 were different compared to G0 (Table 4). Before fermentation the pH in the range of 6.14-6.36 then decreased to 4.29-4.71 after 14 hours fermentation at 37^{o}C. The pH value of G1 higher than G2 and G3, this pH value obtained in this research agreed with previous studies. Many authors conducted research on the possibility of using kombucha as an inoculum of the production fermented milks. The similar research investigated that the pH value of kombucha fermented milk (lactose milk and lactose-free milk) in the range of 4.53-4.71 (Kruk et al., 2021).

The pH decreased during fermentation indicates that decomposition of the substrates in kombucha fermented whey such as sugar and lactose occurred. During this fermentation process, sugar is converted into alcohol and carbon dioxide. Concurrently, alcohol metabolized by acetic acid bacteria into organic acid. Moreover, acetic acid bacteria also produce cellulose layers (Neffe-Skocińska et al., 2017). The titratable acidity of kombucha fermented whey using various types of sugar in this research varied from 0.49-0.79%. The TA G2 and G3 were different compared to G0, while G1 were not significantly different compared to G0. The TA of kombucha fermented whey in this research was lower than TA of fermented goat milk using combination of kombucha and *Lactobacillus casei* were 1.09-1.06% (Nurliyani et al., 2019).

Total soluble solid varied from 6.37–13.6% brix, TSS value decreased after 14 hours

fermentation. The TSS is an approximate measurement of sugar content (Daji et al., 2022). Before the incubation, TSS of sugared whey inoculated with kombucha around 8.0% brix for G0, 16.1% brix, 15.1% brix, and 15.0% brix for G1, G2 and G3, respectively. It is indicated that it could be associated with substrate decomposed during fermentation, sugar is converted into glucose, then the TSS content decreased (Sinamo et al., 2022). The use of various types of sugar affected the TSS value of kombucha fermented whey.

Vitamin C is a common product of kombucha metabolism in kombucha. It was found that kombucha is capable for the biosynthesis of vitamins during fermentation of milk using kombucha stinging nettle and winter savory (Vitas et al., 2013). Vitamin C content in this research varied ranged from 0.47-0.91 mg/100g. The various type of sugar affected the vitamin C content of kombucha fermented whey in this research found that the vitamin C content of G2 and G3 were significantly different compared to G0, while G1 were not different compared to G1. The highest vitamin C content was G3, kombucha fermented whey using palm sugar. Based on the results, any type of sugar could be used in kombucha whey fermentation.

During fermentation, glucuronic acid is synthesized by AAB. Glucuronic acid is also a precursor in the biosynthesis of vitamin C. Glucuronic acid is one of valuable healthy kombucha components (Neffe-Skocińska et al., 2017). The presence of vitamin C in kombucha fermented whey not only due to the biosynthesis of vitamin C during fermentation, likewise is expected to the vitamin C contained in those sugars. Asghar et al. (2020) reported that coconut sap, sugar palm juice and sugarcane juice contain 116.9, 78.24 and 42.52 mg/L of vitamin C, respectively.

CONCLUSION

Based on this study, it can be concluded that any type of sugar granulated white sugar (G1), coconut sugar (G2) and palm sugar (G3) could be used in kombucha whey fermentation. The use of various types of sugar significantly affected the antioxidant and physicochemical properties of kombucha fermented whey. The highest vitamin C content was G3, kombucha fermented whey using palm sugar and the highest antioxidant activity (DPPH) was fermented whey kombucha with the addition of coconut sugar (G2).

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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