



## Analysis of chemical characteristics, flavonoids, and organoleptics on shallot skin (*Allium cepa*) kombucha



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### ABSTRACT

Shallots (*Allium cepa*) is an important aromatic plant, with a level of consumerity that is directly proportional to the increase in shallot skin waste. Whereas the skin of shallots contains flavonoids (quercetin) that are 3-5 times higher than the tubers. This research focuses on the use of shallot skin ingredients fermented by *Symbiotic Culture of Bacteria and Yeast* (SCOBY) into kombucha drinks. With the aim to explore the influence of long fermentation of shallot skin kombucha on the parameters of the chemical characteristics of pH and Total Titrable Acid (TTA); the presence of flavonoids; and organoleptic flavors, colors, aromas. The result is the length of fermentation affecting the decrease in the pH value, and had a very noticeable effect on the increase in the Total Titrable Acid value. Flavonoids were qualitatively tested by 3 reagents (10% NaOH, AlCl<sub>3</sub> 1%, H<sub>2</sub>SO<sub>4</sub> judging by discoloration. At show all samples show positive results of flavonoid presence. The length of fermentation for organoleptic assessment has a significant effect on taste and aromas, but has no significant effect on the kombucha color. The recommendation for a good fermentation time with high acceptance is 8 days of fermentation.

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### INTRODUCTION

Shallots (*Allium cepa*) are an annual herbaceous plant with many benefits, and are one of the richest natural sources of flavonoids. But utilization is limited to the tuber part, while the skin part has not been utilized. The public does not know that the skin of shallots are usually discarded, containing flavonoids such as quercetin higher than the tubers. Previous research has shown that the amount of phenolic & quercetin compounds in shallot skin is 3-5 times higher than tubers (Bardos et al., 2018). Shallot skin extract has been widely researched and has many beneficial

activities, including ace inhibition activity (Angiotensin Converting Enzyme) that regulates blood pressure, antioxidant effects, and hepatoprotector activity (Bardos et al., 2018; Kimoto-Nira et al., 2019).

Kombucha tea made from black tea, green tea, and oolong tea added water and sugar then fermented using kombucha culture. The tea fungus (*Medusomyces gisevii*) or the kombucha culture is called *Symbiotic Culture of Bacteria and Yeast* (SCOBY), is a collection of bacteria and yeast symbiotic in the thick jelly membrane (zooglear mat), which is widely used to produce kombucha tea (Jayabalan et al., 2010). The bacterial and fungal species that make up SCOBY usually include acetic acid bacteria (*Acetobacter*, *Gluconobacter*), lactic acid bacteria (*Lactobacillus*, *Lactococcus*) and yeast (*Saccharomyces*, *Zygosaccharomyces*) (Dimidi et al., 2019). The fermentation process in kombucha uses the main source of carbon in the form of sucrose, which involves the activity of yeast invertase enzymes present in the kombucha consortium. This homemade product produces acetic acid, ethanol and CO<sub>2</sub> in small amounts and tastes of the product slightly sour, carbonated and sweet (Ivanisova et al., 2019).

There is a lot of research on the benefits of shallot skin content, example shallot skin as alternative colorants in food and textiles (Al-qadri, 2018), water extract of shallot skin for the synthesis of bisindolylmethanes (Chia et al., 2019), and fermentation of shallot skin by lactic acid bacteria for production of functional food (Kimoto-Nira et al., 2019), but few studies focus on the development of healthy drinks. Results from the composition of proximate revealed that shallot skin extract produced 98.52 µg QUE ml<sup>-1</sup> total flavonoids, 664.30 µg ml<sup>-1</sup> GAE total phenol (Ifesan, 2017). The high content of flavonoids & phenol indicates that shallot skin can be used as a fermentation substrate by SCOBY into kombucha drink.

Currently consumers evaluate beverage products not only in terms of nutritional needs, but also related to health improvements, one of which is met by healthy drinks such as kombucha. So that shallot skin can be a new innovation in the manufacture of kombucha, which further increases the content of compounds such as flavonoids. The goal of the study was to explore SCOBY's activity in fermenting shallot skin kombucga while monitoring changes in chemical characteristics: pH, total acid, and the presence of flavonoids. And also organoleptic analysis related to taste, color, and aroma parameters. So that it can determine the right fermentation length to produce a shallot skin kombucha that is worth consuming, and can be applied in the field of food in producing functional drinks.

## RESEARCH METHODS

### Research Design

This type of research is experimental using a Completely Randomized Design with one factor. Which aims to analyze the influence of independent variables i.e. various fermentation lengths (8 days, 12 days, 16 days, and 20 days) on dependent variables i.e. chemical characteristics of pH & total titrable acids, organoleptics (taste, color, aroma), and the presence of flavonoids.

**Table I.** Completely Randomized Design with one factor

Replication	Treatment (fermentation lengths / days)				
	0 (A)	8 (B)	12 (C)	16 (D)	20 (E)
1	A1	B1	C1	D1	E1
2	A2	B2	C2	D2	E2
3	A3	B3	C3	D3	E3
4	A4	B4	C4	D4	E4
5	A5	B5	C5	D5	E5

**Description :** Sample A (0 days/control), B (8 days), C (12 days), D (16 days), E (20 days)



## Population and Samples

Population used in this study are all waste shallot skin (*Allium cepa*), obtained from shallot traders in Pasar Baru, Kuningan Regency, West Java. For the sample is the skin of shallots that have gone through the selection stage, dried and then made brewed red shallot skin tea fermented using *Symbiotic Culture of Bacteria and Yeast* (SCOBY) into Kombucha drink.

## Instruments

Materials needed and tools used in this study for first step preparation of test materials are shallot skin, aluminum foil, containers, ovens. For Sterilization tools are clear glass jars, Autoclave. For making culture starter *Symbiotic Culture of Bacteria and Yeast* (SCOBY) & making Shallot Skin kombucha are water, SCOBY Starter, sugar, black tea, kombucha shallot skin, Measuring Glass, Pan, Plastic Knife, Spoon, Clamp, Scales, Stove, Cover cloth, Rubber band, Sieve. For pH Test are Universal PH Paper (*Merck*), test tube + rack. For Test of Total Titrable Acid are Glacial Acetic Acid, aquadest, Indicator PP 1%, , Erlenmeyer Flask 250 ml, Burette, Measuring Glass 50 ml, Funnel, Beker Glass, Statif & Clamp, Spray Bottle, Propypet, Pipette Volum, Watch Glass, Pumpkin Measure. For Flavonoid Test are  $AlCl_3$ ,  $H_2SO_4$ ,  $NaOH$ , Water Bather, drip pipette, volumetric flask, test tube + rack. For organoleptic test are plastic cup, plastic pipette, questionnaire sheet.

## Procedures

Research began from the stage of Preparation of Test Materials, Sterilization Tools, Culture Starter *Symbiotic Culture of Bacteria and Yeast* (SCOBY) (Jayabalan et al., 2010), Making Shallot Skin Kombucha, pH Test, Test of Total Titrable Acid (Dwiputri & Feroniasanti, 2019), Flavonoid Test (Marpaung & Wahyuni, 2018; T et al., 2019), Organoleptic Test with Hedonic Scale (1 : very dislike; 2 : dislike; 3 : rather dislike; 4 : rather like; 5 : like; 6 : very like). The procedure of this research can show in Figure I.

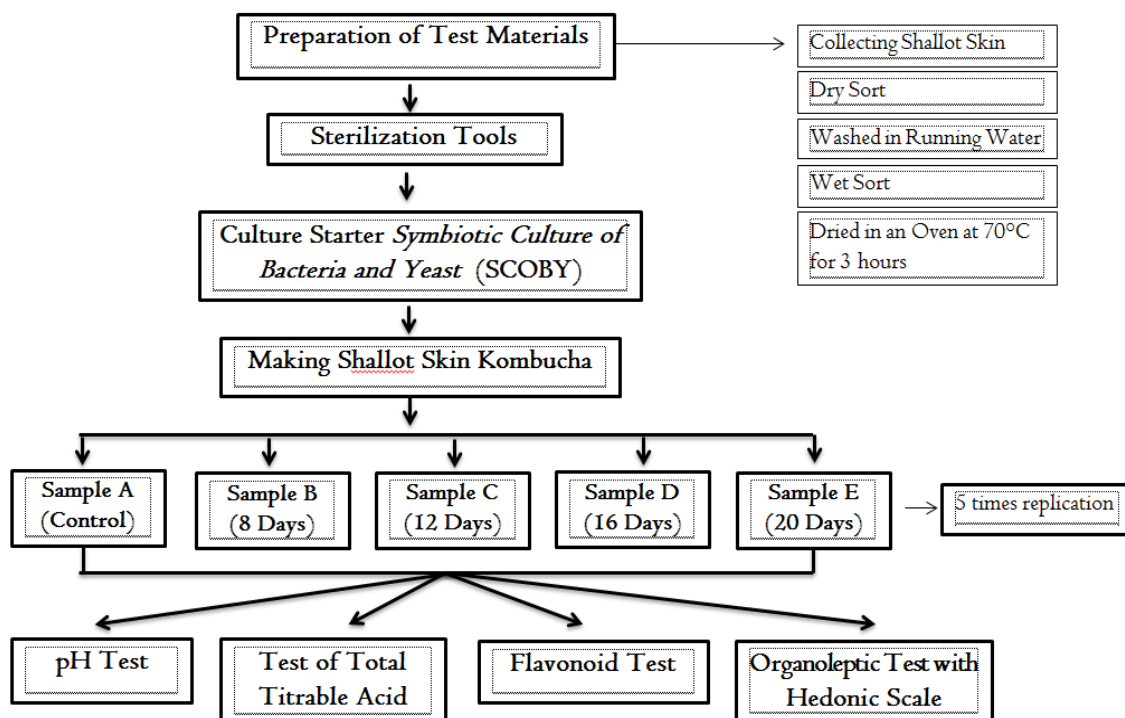


Figure I. Research Flow Chart

## Data Analysis

pH data and the presence of flavonoids are analyzed qualitatively, Total Titrable Acid (TTA) data is analyzed using Completely Randomized Design, Analysis of Variance (ANOVA) with Honest Significantly Difference (HSD) advanced test, organoleptic test data using Kruskal-Wallis test.

## RESULTS

### Chemical Characteristics

#### I. pH

Five samples kombucha shallot skin ie sample A (control without fermentation), sample B (8 days), sample C (12 days), sample D (16 days), sample E (20 days) in a row has a pH result of 5, 3, 2, 2, 2 (Figure 2). Thus the influence of long fermentation is correlated to the decrease in the pH value of Kombucha shallot skin.

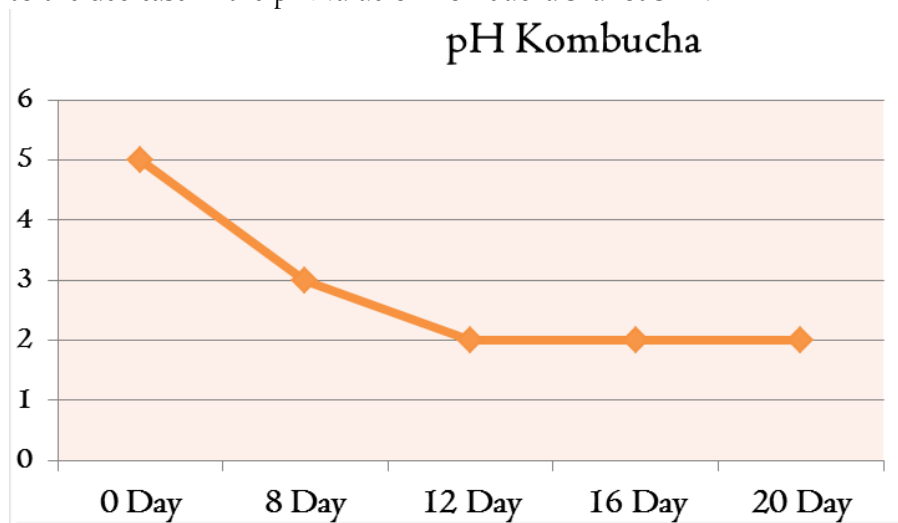


Figure 2. Shallot Skin Kombucha pH Chart

#### 2. Total Titrable Acid (TTA)

Figure 2. shows a chart of consecutive increase in the value of Total Titrable Acid kombucha from 0, 8, 12, 16, 20 days of fermentation resulting in total acid by 0.06%, 0.58%, 0.81%, 1.00%, and 1.14% (Figure 3.). ANOVA calculation result is  $F$  Count (701.6433) >  $F$  Table 1% (4.43) (Table 2.). So that the influence of long fermentation on the value of Total Titrable Acid Shallot Skin Kombucha shows very real different results (Table 3.).

Table 2. Analysis of Variance (ANOVA)

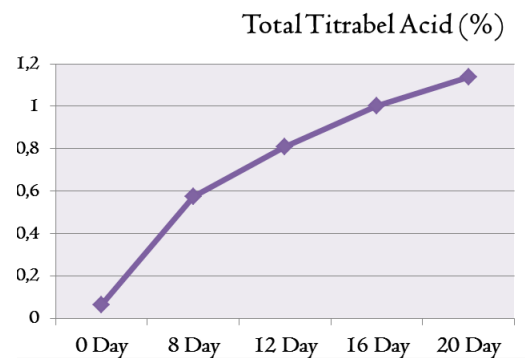
ANOVA						
Source of Variation	SS/ JK	df / DB	MS / KT	F	P-value	F crit
Between Groups	3,57128	4	0,892820023	701,6433121	4,43	2,866081
Within Groups	0,025449	20	0,00127247			
Total	3,596729	24				

**Table 3.** Honest Significantly Difference (HSD)

SD	HSD 1%	HSD
0,0159529	5,02	0,080083396

SAMPLE	MEAN	MEAN + HSD	SYMBOL
A	0,06	0,14	a
B	0,58	0,66	b
C	0,81	0,89	c
D	1	1,08	d
E	1,14	1,22	e



**Description :** SD= Standard Deviation, treatment values not followed by the same letter (symbol) means significantly different.

**Figure 3.** Kombucha Total Titrabel Acid Chart

### Flavonoid

Overall all kombucha sample colors have the same color in the test tube, which is pale pink. When the sample is reacted with NaOH reagents 10% of sample A produces a solid brown color (+), sample B is brown (+), sample C is slightly yellow brown (++), sample D is yellowish-brown (++), and sample E is light brown and solid yellow (+++). The shallot skin kombucha sample was reacted by a 1% AlCl<sub>3</sub> reagent, samples A and B showed pale yellow (+), sample C; D; E is yellow (++). The last reagent is H<sub>2</sub>SO<sub>4</sub>, samples A and B show a pale red color (+), C sample red (++), then sample D, E red which is getting thicker (+++) (Table 4.). So even though the 5 samples of shallot skin kombucha show different color and intensity gradients but all show positive results containing flavonoids and there is an increase in color intensity.

**Table 4.** Qualitative Test of Flavonoids

Reagent	Kombucha Shallot Skin Sample				
	A	B	C	D	E
NaOH 10%	+	+	++	++	+++
AlCl <sub>3</sub> 1%	+	+	++	++	++
H <sub>2</sub> SO <sub>4</sub>	+	+	++	+++	+++

**Description :** +++ : Flavonoids Present in large quantities, ++ : Flavonoids Present , + : Flavonoids Present in small quantities, - : Flavonoids Not Present

### Organoleptic

#### I. Taste

Scale 1 to 6 obtained from the questionnaire was averaged, and obtained the results of sample A (4,3); B (3,4); C (2,75); D (2,75); and E (2,65) (Figure 4). Quantitative data is ranked according to the Kruskal-Wallis test method, calculation results showed H count of 29.1 > 9.49 (Chi square Table 5%), meaning there was a significant difference in taste between the 5 kombucha samples.



Figure 4. Average Organoleptic Value of Shallot Skin Kombucha Taste

## 2. Color

Color of shallot skin kombucha sample A shows a bright brown color like a brew of green tea in general, while in samples B to E has orange color. The hedonic scale of the questionnaire was averaged in sample A (4.45); B (4.1); C (3.85); D (3.65); and E (3.80) (Figure 5.). Kruskal-Wallis calculation results showed H count  $7.27 < 9.49$  (Chi square Table 5%), which means there is no significant color difference between the 5 kombucha samples.

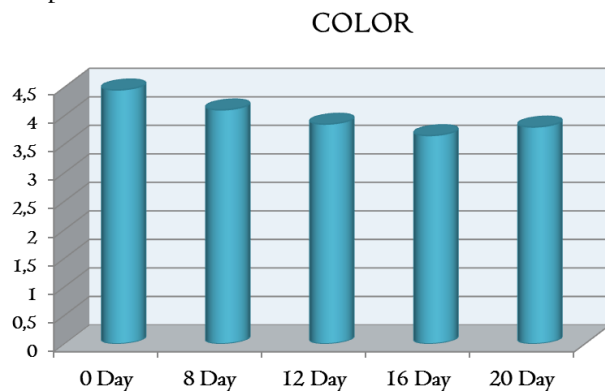


Figure 5. Average Organoleptic Value of Shallot Skin Kombucha Color

## 3. Aroma

Organoleptic data aroma kombucha onion skin from the questionnaire averaged, the results of which are sample A (3.55); B (2.85); C (2.65); D (2.4); and E (2.2) (Figure 6.). Kruskal-Wallis calculation results showed H count  $15.6 > 9.49$  (Chi square Table 5%), meaning there was a significant aroma difference between the 5 kombucha samples.

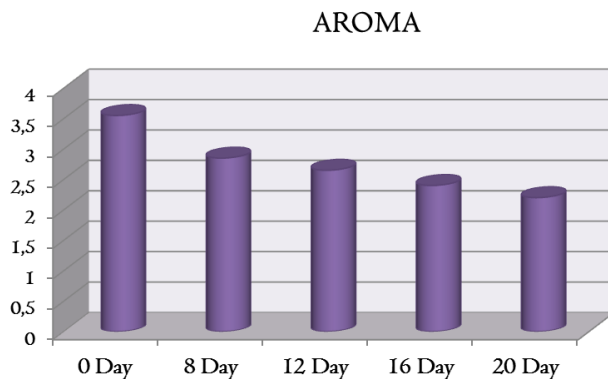


Figure 6. Average Organoleptic Value of Shallot Skin Kombucha Aroma

## DISCUSSION

Chemical characteristic pH of shallot skin kombucha experienced a significant increase in the rate of pH decrease at the beginning of fermentation ie pH 5 to pH 3. In fermentation 8 days is the optimum point of kombucha fermentation, because the bacteria and yeast contained in the starter SCOBY is very active metabolized. Fermentation results in the form of organic acids is what finally lowers the pH kombucha. According to research by Vázquez-cabral et al., (2014) showed that oak leaf kombucha (*Quercus resinosa*) experienced a significant pH decrease at the beginning of 4.15 and after fermentation to pH 2.11 – 2.46.

The decrease in pH during kombucha fermentation has a close relationship also with the total Lactic Acid Bacteria and the number of cells in the kombucha culture. Bacterial growth increases rapidly during the first 6 days followed by a gradual decrease until the end of fermentation (Chen & Liu, 2001). On fermentation day 12, 16 and 20 pH kombucha shallot skin turned out to decrease and remained in the range of pH 2. This is because the nutrient content for fermentation is getting less and less, so the number of microorganism cells is stable and the pH decrease is only a little. The decrease in the number of bacteria and yeast after 9 days of fermentation is likely caused by an acidic shock that is a low pH that affects the multiplication of bacteria and yeast.

Another conjecture related to the rate of decline in kombucha pH is due to CO<sub>2</sub> being released faster at the beginning of fermentation, the buffer properties of dissociated CO<sub>2</sub> produce amphiprotic hydrocarbon anion (HCO<sub>3</sub><sup>-</sup>). This component easily reacts with hydrogen ions (H<sup>+</sup>) from organic acids produced during kombucha fermentation, thus preventing changes in H<sup>+</sup> concentrations in kombucha solutions (Ulusoy & Tamer, 2019). Thus despite the constant increase in the value of organic acids during the fermentation process, no significant change of pH value was observed at the end of fermentation. This may be associated with some buffering effects. This shows the results according to this study that the formation of kombucha organic compounds continues to increase in fermentation 8 days, but the decrease in pH value is very small and is at a constant point for fermentation 12 to 20 days.

Total Titrable Acid (TTA) shallot skin kombucha from 0 to fermentation day 20 experienced a continuous increase. The total content of shallot skin kombucha acid is directly proportional to the duration of fermentation for 20 days, which means that the longer the fermentation, the more total acid is concentrated. In fact each treatment has a real different TTA value. This significant and distinct increase in TTA values occurs because during the fermentation process, the activity of bacteria and yeast in kombucha takes place simultaneously and is directly proportional to the total acid produced. Invertase enzymes derived from yeast catalyze sucrose hydrolysis and produce ethanol through glycolysis pathways, on the other hand gluconobacter *Bacteria. sp* and *Acetobacter sp.* using glucose to produce gluconic acid and ethanol to produce acetic acid (Jayabalan et al., 2014). As fermentation lasts longer, more and more organic acids are produced.

For some other cases if the fermentation process lasts longer, the value of TTA will not continue to increase. There is a point where the value of kombucha TTA begins to decrease, because the concentration of acetic acid increases at a limited rate, then it will decrease gradually. So in accordance with the results of this study that the fermentation of shallot skin kombucha total acid continues to increase until the 20th day, because it is still on the verge of the availability of glucose and fructose for its metabolic processes, so that microorganisms in the kombucha consortium are still actively metabolizing.

Flavonoid test compounds are said to contain flavonoids positively if the reaction that occurs will produce a discoloration on the amye layer of alcohol, namely the color red / yellow / orange (Harborne, 1987). The color difference seen by the shallot skin kombucha due to the aqueous

NaOH solution is highly alkaline fully dissociated into  $\text{Na}^+$  and  $\text{OH}^-$ , sodium cations will be attracted by oxygen ion phenoxide (Olabiyi et al., 2008). This causes at the beginning of the formation of sodium phenoxide such as colorless globules, but after some time yellow deposits will accumulate. The results of the flavonoid shallot skin kombucha test by  $\text{AlCl}_3$  reagents all samples showed pale yellow to yellow color. It shows a positive test of the presence of flavonoids in the sample, due to the formation of complex compounds between flavonoids and  $\text{AlCl}_3$ .  $\text{AlCl}_3$  reagents function in forming  $\text{AlCl}_3$  reactions with ketone groups in C4 and OH clusters in C3 or C5 in flavon and flavonol compounds in forming complex and stable yellow compounds (Nurmila et al., 2019).

The last reagent is  $\text{H}_2\text{SO}_4$ , The addition of concentrated sulfuric acid aims for the formation of flavilium salts which are flavonoid compounds, indicated by orange color in the test samples (Puspa et al., 2017). The red color produced by the shallot skin kombucha means that there is an oxidation reduction reaction between sulfuric acid and flavonoids that form complex compounds. Thus resulting in a discoloration that is red due to electrophilic substitution reaction (T et al., 2019). The presence of flavonoids in the shallot skin kombucha is appropriate because the main flavonoid in the skin of shallots is kuersetin which is present in its conjugated form. Such as quercetin 4'-O- $\beta$ - glycopyranoside, quercetin 3,4'-O- $\beta$ -diglycopyranoside, and quercetin 3,7,4'-O- $\beta$ -triglycopyranoside (Sellappan and Akoh, 2002 in Lee et al., 2014). Flavonoid compounds can increase due to the degradation of flavonoids and complex polyphenols in kombucha into smaller and simpler molecules. Chakravorty et al., 2016 research showed that the total concentration of polyphenols and kombucha flavonoids increased progressively over time of fermentation, it is also known that *Candida tropicalis* microorganisms are able to degrade polyphenols and flavonoid compounds resulting in an increase in the overall content observed.

Organoleptic Taste is the result of the panelist's response by using the taster sense (tongue) to the sample of shallot skin kombucha. According to Naland (2008), the pH for kombucha is good and safe for consumption ranging from pH 3.00. If kombucha has a pH value below 3.00 then the kombucha needs to be diluted first in order to be safe to consume. kombucha pH for samples C, D, E has a pH of 2. That means before taste testing by panelists, kombucha samples must be diluted first. Sample B with an average rating of 59.075 is the highest value when compared to other fermented samples. Sucrose sugar content decreases during fermentation as a result of conversion into glucose and fructose by yeast cells into organic acids. The result of the average organoleptic taste value in samples C, D, E decreases with the duration of fermentation. This result is also influenced by the process of dilution of kombucha by water. So the longer the fermentation of kombucha causes higher levels of organic acids that give a strong sour taste and less positive response from panelists. It has been observed that kombucha with a higher concentration of acetic acid produces a more acidic taste, while when gluconic acid is higher it will produce a lighter taste (Leal et al., 2018). It is this acetic acid that is responsible for the taste and smell produced by kombucha.

Organoleptic Color is the result of the panelist's response using the sense of vision (eyes) to the sample of shallot skin kombucha. The reddish purple color of the shallot skin has been associated with anthocyanin pigments such as glycosides from peonidin (Pn) and or cyanidin (Cy), then flavonol (quercetin) which can contribute to the production of yellow and brown compounds (Al-qadri, 2018; Ifesan, 2017). So the color that appears on the shallot skin kombucha is brownish orange. During kombucha storage, anthocyanins are removed from and color properties are negatively affected (Ulusoy & Tamer, 2019). With this long fermentation treatment, the longer it will produce a lighter kombucha color. Microbial transformation of polyphenols and discoloration due to chemical modifications of phenolics and carotenoids during fermentation and storage that will lead to decreased kombucha color (Ayed et al., 2016; Watawana et al., 2015). In line with



Widyasari (2016) research that kombucha moringa leaves color fades with the duration of fermentation. This fading color is less favored by some people because it is considered less attention-grabbing. Thus the panelist's assessment was reduced on longer fermentation samples, but some panelists were less able to see the noticeable color differences of all the shallot skin kombucha samples.

Organoleptic Aroma is the result of panelist response by using sense of smell (nose) to sample shallot skin kombucha. The aroma produced by the shallot skin kombucha in addition to derived from the fermentation process, also comes from compounds contained in the skin of shallots. Organic acids formed plus volatile compounds will produce a distinctive aroma of the shallot skin kombucha, and the oxidation results will increase again the intensity of the aroma of kombucha. Volatile compounds in question include acetic acid, alcohol, and other organic acids that are formed and eventually give rise to a distinctive concentrated acid aroma (Purnami et al., 2018). So the panelist's assessment of organoleptic aroma of shallot skin kombucha is decreasing often increasing the duration of fermentation. The decrease in the favorability level of kombucha aroma is also evident from Nasir & Rahmadani (2015) research, which suggests that panelists prefer fermented kombucha at 7 days compared to 10 days. This can also be attributed to a decrease in pH and a total increase in acid over the duration of fermentation. The low pH produced by kombucha can contribute to a general decline in sensory quality to unacceptable levels, but the general quality at the end of fermentation is quite high (Neffe-skocińska et al., 2017). Apart from the biological potential of processed products kombucha, consumer acceptance is low related to the high astringent taste of the brew of kombucha material that will reduce the overall organoleptic quality.

Based on the results of the analysis of the influence of long fermentation shallot skin kombucha is thorough, then the best fermentation duration to be recommended is fermentation 8 days (sample B). This is because in the analysis of chemical characteristics shows the pH value and total acid that is at the safe level of consumption, namely pH 3 and total acid by 0.58%. Analysis of the presence of flavonoid sample B showed brown color + (NaOH 10%), yellow + (AlCl<sub>3</sub> 1%), and pale red color + (H<sub>2</sub>SO<sub>4</sub>). So from the 3 reagents showed a positive reaction to the presence of flavonoids. Organoleptic analysis showed a high average rank in sample B, with taste results (58,075); color (53.6); and aroma (54,225). A sizable organoleptic assessment of this B sample showed that the panelist's acceptability was higher for an 8-day fermented kombucha sample. In line with Vázquez-cabral et al., (2014) research that sensory reception showed a high negative correlation (-0.55) with respect to time, which meant greater reception at shorter fermentation times. Because the longer fermentation although improving the quality of microbiology & physicochemicals but will decrease the sensory quality significantly to a level that is unacceptable to humans.

## CONCLUSION

Analysis of chemical characteristics shows there is a longer fermentation influence in the form of a decrease in pH in a row has a result of 5, 3, 2, 2, 2. Total Titrable Acid increase in red shallot skin kombucha ANOVA calculation result is F Count (701.6433) > F Table 1% (4.43), with Honest Significantly Difference for all sample means significantly different. Analysis of flavonoids shows even though the 5 samples of shallot skin kombucha show different color and intensity gradients but all show positive results containing flavonoids, and the longer the fermentation occurs the increase in color intensity. Organoleptic analysis of shallot skin kombucha for taste showed H count of 29.1 > 9.49 (Chi square Table 5%) and aroma showed H count 15.6 > 9.49 (Chi square Table 5%), there is a significant influence on fermentation duration. But for color organoleptics showed H count 7.27 < 9.49 (Chi square Table 5%), there is no significant

difference from the influence of long fermentation. The recommendation of right fermentation time to produce shallot skin kombucha with total acid and pH safe consumption, there are flavonoids and organoleptic values with high acceptability that is for 8 days.

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