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Production Of Ready-To-Drink Cascara Kombucha Fortified With Coconut Water (*Cocos Nucifera L.*) and Red Dragon Fruit (*Hylocereus Polyrhizus*)

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ABSTRACT – Cascara is coffee pulp which contains caffeine, chlorogenic acid and phenolic compounds, but has not been utilised optimally. Cascara can be processed into ready-to-drink cascara kombucha products, which are beneficial for the body. This research aims to determine the effect of adding coconut water (*Cocos nucifera* L.) and red dragon fruit (*Hylocereus polyrhizus*) on the alcohol content, total phenolics, caffeine, minerals, antioxidant activity and sensory hedonic content of ready-to-drink cascara kombucha. This research method was carried out in 3 stages including optimizing fermentation time and SCOBY starter concentration based on pH value; fortification ratio optimization; and product analysis includes alcohol content by dichromate method, total phenolics by Folin-Ciocalteu method, caffeine by UV-VIS spectrophotometry method, minerals by AAS spectrophotometry method, antioxidant activity by DPPH method, and sensory hedonic. The research results showed that the best time was 12 days, pH value 3.42 at 10% SCOBY starter concentration. The ratio of cascara kombucha : fortificant is 1:1 resulting in an alcohol content of 0.23–0.43%. The highest levels for total phenolics, caffeine, and minerals were 656.32 mg/kg; 35.53 ppm; and 732.83 mg/100g respectively. The highest antioxidant activity was obtained from the red dragon fruit fortified cascara kombucha product with a yield of 89.69%. The highest color and aroma attributes were obtained from the cascara kombucha product fortified with a mixture of coconut water and red dragon fruit, while the highest value for the taste attribute was obtained from the cascara kombucha product fortified with red dragon fruit.

KEYWORDS: cascara kombucha, chemical analysis, coconut water (*Cocos nucifera* L.), dragon fruit (*Hylocereus polyrhizus*), sensory hedonic.

INTRODUCTION

Statistics Indonesia shows that Indonesia's coffee production reached 794,800 ton in 2022, an increase of about 1.10% from the previous year. The increase in coffee production is proportional to the increase in the amount of coffee pulp (Kayaputri et al., 2022). Coffee pulp is the largest waste from coffee processing, with an amount reaching 35–40% of the total weight of fresh coffee beans. Based on these data, coffee pulp in Indonesia reaches around 317,920 ton per year and has not been optimally utilised, even though coffee skin can be processed into food products that have high economic value, one of which is cascara. Processing cascara into food products can create zero waste coffee production and can optimally utilise the potential of functional foods (Rosidah et al., 2021).

Compounds contained in cascara, such as caffeine of 0.14–0.45% which is useful for improving mood and concentration (Arpi et al., 2021), phenolics which have the potential as antioxidants (Nafisah & Widyaningsih, 2018), and chlorogenic acid which has the potential to delay cell damage and prevent premature aging (Firdayeni & Sari, 2023). Based on this content, cascara can be utilised as a medium for making cascara kombucha (Muzaifa et al., 2023). Kombucha was chosen as a cascara processed product because kombucha is a fermented drink that has a refreshing sour taste and has many health benefits (Mahadewi et al., 2022). Based on research conducted by Muzaifa et al. (2022), cascara kombucha fortified with red dragon fruit (*Hylocereus polyrhizus*) showed an alcohol content of 0.60% for red dragon fruit in the form of pieces and 0.63% for red dragon fruit in the form of juice. The alcohol content of the cascara kombucha product has not met the requirements of MUI No. 10 of 2018 (<0.5%) (MUI, 2021). Cascara kombucha made at fermentation times of 4, 8, and 12 days has the highest total phenolic content at the 12-day fermentation time (Rohaya & Sulaiman, 2022), while cascara kombucha made with different SCOBY starter concentration variations can produce different pH values and sensory quality (Agustien et al., 2022).

The coffee pulp or cascara needs to be utilised optimally, so further research needs to be done on the optimisation of fermentation time and the addition of SCOBY starter concentration in making kombucha. Optimisation of the ratio of red dragon fruit (*Hylocereus polyrhizus*) addition also needs to be done to compare the alcohol content produced, so that the best ratio with the lowest alcohol content that meets the regulations can be known. The addition of red dragon fruit is not only a comparison of alcohol content, but also as a fortificant substance that has the potential to increase antioxidants in cascara kombucha (Arivalagan et al., 2021). Another variation of adding fortifying substances is done by adding coconut water (*Cocos nucifera L.*) which is known to have a high magnesium mineral content, 31.65 mg/100 g (Camargo Prado et al., 2015). This study aims to determine the alcohol content, total phenolics, caffeine, minerals, antioxidant activity, and hedonic sensory in various variants of ready-to-drink cascara kombucha products fortified with coconut water (*Cocos nucifera L.*) and red dragon fruit (*Hylocereus polyrhizus*).

METHODS

The equipments used in the production of cascara kombucha include grinder/crusher, thermometer, and glass jars. The equipments used in the analysis pH meter, measuring pipette, test tube, vortex mixer, water bath, centrifuge, analytical balance, measuring flask, beaker, measuring cup, incubator, separating funnel, hotplate, UV-VIS spectrophotometer, and AAS. Materials used in the production of cascara kombucha include cascara, SCOBY starter, coconut water, red dragon fruit, sugar, and water. The materials used in the analysis, namely 1,1-diphenyl-2-pycrylhydrazyl (DPPH), technical CH_3OH, absolute C_2H_5OH, $K_2Cr_2O_7$, H_2SO_4, Na_2CO_3, $FeCl_3$, $CHCl_3$, Mg powder, HCl, Mayer reagent, and Folin-Ciocalteu reagent.

Cascara as much as 20 g was mashed and put into 1 L of water, then heated (T = 90 ℃) for 5 minutes. The cascara was filtered to obtain the filtrate, then mixed with 100 g of sugar, stirred until homogeneous, then cooled to room temperature. SCOBY starter was added into the cascara brew with different concentrations (5%, 10%, and 15%), then covered with porous sterile cloth. Fermentation was carried out at 0, 4, 8, and 12 days, then after obtaining the best pH, further optimised to determine the ratio of the use of fortificants. Optimisation of the ratio of the use of fortificants was carried out with cascara kombucha product (C) with the addition of red dragon fruit (B) with the ratio of C:B is 9:1, 3:1, and 1:1 which is done by testing the alcohol content. Variations in the addition of coconut water and red dragon fruit fortificants were carried out after obtaining the best alcohol content data.

The pH measurement was carried out using a pH meter following the method of (Rosyada et al., 2021). Alcohol content was determined by the dichromate method following the method of (Michałowska-Kaczmarczyk & Michałowski, 2019). Phytochemical analysis was performed following the method of (Kancherla et al., 2019). Antioxidant activity was determined by DPPH

following the method of (Abduh et al., 2023). Total phenolic content was determined using the Folin-Ciocalteu method following the method (SNI 3143, 2011). Caffeine content was determined following the method of (Vuletić et al., 2021). Mineral content was determined using Atomic Absorption Spectrophotometer (AAS). Sensory analysis was carried out by hedonic method based on the level of liking which includes colour, aroma, and taste on a scale of 1-5 (strongly dislike - strongly like) following the method of (Simanungkalit et al., 2018). Statistical analysis was performed with Analysis of Variance (ANOVA) and further tested with Duncan Multiple Range Test (DMRT) at 5% probability level following the method (Lee et al., 2023).

RESULT AND DISCUSSION

pH Optimisation of Cascara Kombucha

pH analysis is carried out to determine products that have a pH value in the range of 2.50–4.20. The pH value of cascara kombucha varies from 2.36–4.60. Based on Figure 1, it is known that 5% starter concentration is not used as a control product because it has a pH value of 4.60 which does not meet the quality requirements of kombucha products. At pH values > 4.20 it is also known to have the potential for the growth of *Clostridium botulinum* bacteria which has the potential to produce toxins that cause disease or infection (WHO, 2023).

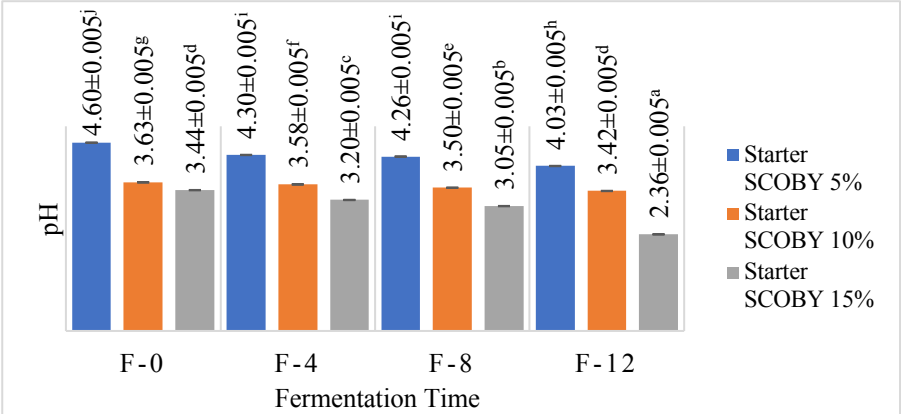

Figure 1. Changes in pH of cascara kombucha at fermentation times of 0 days (F-0), 4 days (F-4), 8 days (F-8), and 12 days (F-12) with starter concentrations of 5%, 10%, and 15%.

The best fermentation time used is 12 days because the longer the fermentation, the higher the content of phenolic compounds obtained, so that the potential as an antioxidant substance will be better. The pH value on the 12th day of fermentation with a starter concentration of 10% was 3.42, these results were very close to the best cascara kombucha pH obtained by (Nurhayati et al., 2020). The pH of 3.42 was chosen because at that pH *Lactobacillus acidophilus* bacteria can grow well which is beneficial for improving the immune system (immunomodulator) because it can stimulate the formation of antibodies that prevent the growth of harmful bacteria (Rusli et al., 2018). Therefore, a fermentation time of 12 days with a starter concentration of 10% was chosen as the control product for further variations in the form of adding coconut water and red dragon fruit.

Alcohol Content of Cascara Kombucha

Alcohol content is determined based on the redox titration method using potassium dichromate ($K_2Cr_2O_7$). $Cr_2O_7^{2-}$ ions act as an oxidising agent that will oxidise alcohol into carboxylic acid compounds. The reaction that occurs is as follows.

$$2K_2Cr_2O_7 + 8H_2SO_4 + 3C_2H_5OH \rightarrow 2Cr_2(SO_4)_3 + 11H_2O + 3CH_3COOH + 2K_2SO_4$$

(Michałowska-Kaczmarczyk & Michałowski, 2019)

The alcohol content of cascara kombucha with variations in the addition of red dragon fruit is presented in a bar chart in Figure 2 below.

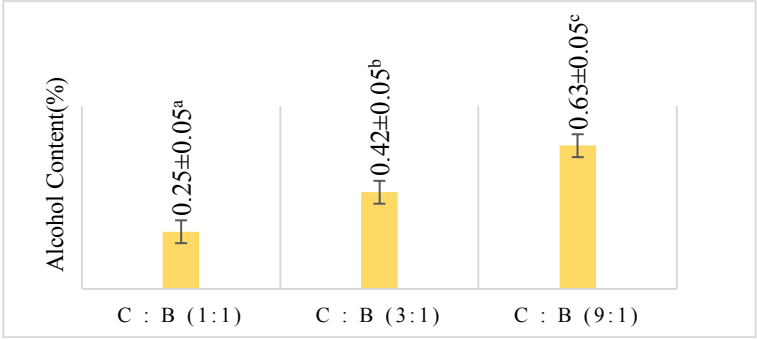

Figure 2. Alcohol content of cascara kombucha (C) fortified with red dragon fruit (B) at the ratio of C:B = 1:1, 3:1, and 9:1.

Based on these results, it is known that the greater the amount of cascara kombucha in the product, the higher the alcohol content. This is because cascara kombucha contains alcohol as a product of fermentation (Puspaningrum et al., 2022). It was found that the addition of red dragon fruit with a C:B ratio of 1:1 had the lowest alcohol content among the three ratios made. Therefore, the ratio between cascara kombucha and the additive used was 1:1. Further alcohol content testing was carried out on products with variations in the addition of coconut water and red dragon fruit. The alcohol content of cascara kombucha with variations in the addition of coconut water and red dragon fruit is presented in Figure 3 below.

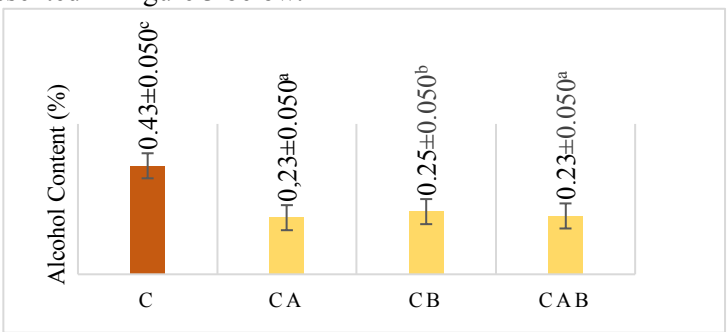

Figure 3. Alcohol content of cascara kombucha (C); cascara kombucha fortified with coconut water (CA); cascara kombucha fortified with red dragon fruit (CB); cascara kombucha fortified with coconut water and red dragon fruit (CAB)

The alcohol content in the fortified product is lower than the control cascara kombucha product. This is due to the composition of cascara kombucha in the product variation of adding fortificant is only half of the control product, so if the amount is less, the resulting alcohol content will be lower. The range of alcohol content obtained was 0.23%–0.43%, still fulfilling MUI regulations (<0.5%), so cascara kombucha products can be consumed by the general public, especially Muslim communities. This halal status is strengthened by not using haram and dangerous ingredients, as well as a simple production process and can be ensured of its halalness, so it has fulfilled MUI Fatwa No. 10 of 2018 concerning Food and Beverage Products Containing Alcohol (MUI, 2021).

Phytochemical Analysis of Cascara Kombucha

Based on the results of the phytochemical testing carried out, in the alkaloid test it is known that none of the samples showed positive results, even though the cascara contains caffeine compounds which are one of the alkaloid compounds. The non-detection of alkaloid compounds indicates that the content of alkaloid compounds or caffeine in the sample is very small, so further quantitative testing is needed to prove the content of alkaloid compounds.

In flavonoids and terpenes testing, positive results were shown by samples with the addition of red dragon fruit. This is because red dragon fruit contains flavonoid compounds such as Myricetin, Quercetin, and Rutin which are beneficial for health (Nishikito et al., 2023). It is also known that dragon fruit contains terpen compounds such as Pinene, Myrcene, and Caryophyllene which greatly affect its aroma, flavour, and therapeutic effects (Patrick, 2024). In the tannin and phenolic tests, only the coconut water sample showed negative results, while the other samples showed positive results. This is because the cascara already contains tannins and phenolic compounds, so when other fortificants are added, it does not make a significant difference.

Antioxidant Activity of Cascara Kombucha

Antioxidant activity analysis was conducted to determine the effect of the addition of coconut water and red dragon fruit on the antioxidant activity of cascara kombucha using the DPPH method. The results of the antioxidant activity of cascara kombucha products are shown in Figure 4 below.

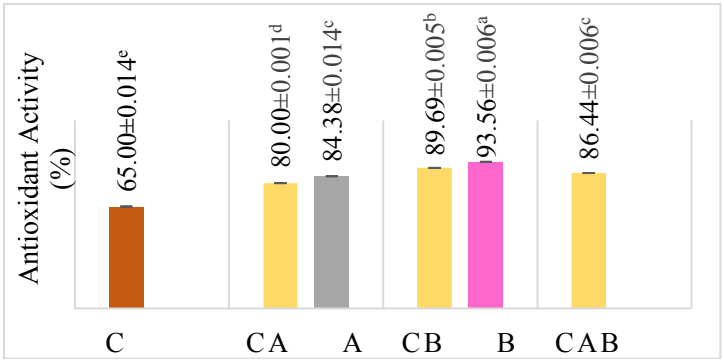

Figure 4. Antioxidant activity of cascara kombucha (C); cascara kombucha fortified with coconut water (CA); cascara kombucha fortified with red dragon fruit (CB); cascara kombucha fortified with coconut water and red dragon fruit (CAB); coconut water (A); B = red dragon fruit (B).

Antioxidant activity in the fortified products showed higher results than the control products, namely 80.00% for CA, 89.69% for CB, and 86.44% for CAB. Antioxidant activity in CA product was 15% higher than C product because coconut water contains catalase enzyme which acts as an antioxidant (Jeeva et al., 2015). The highest antioxidant activity was obtained from CB product with an increase of 24.69% compared to the control product. This is because red dragon fruit contains flavonoid compounds that can act as antioxidant substances (Nishikito et al., 2023). The content of flavonoid compounds can also be proven from the results of phytochemical testing which showed positive results. The CAB product had an antioxidant activity that increased by 21.44% compared to the control product, but showed a decrease of 3.25% compared to the CB product. This is due to the formation of a bond between catalase and flavonoids which makes catalase a non-reactive compound (Krych & Gebicka, 2013).

Total Phenolic Content of Cascara Kombucha

Analysis of total phenolic content was carried out to determine the effect of the addition of coconut water and red dragon fruit on the total phenolic content of cascara kombucha using the Folin-Ciocalteu method. The results of the total phenolic content of cascara kombucha products are in Figure 5 below.

Based on the test results, it is known that the total phenolic content for cascara kombucha product (C) is 501.15 mg/kg. The results obtained are quite high because cascara contains phenolic compounds in the form of chlorogenic acid which undergoes enzymatic activity during the fermentation process, so that the matrix components in kombucha will be degraded and produce more phenolic compounds (Nurhayati et al., 2020).

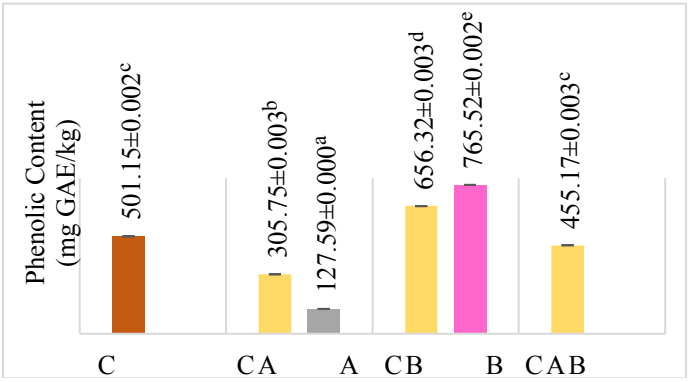

Figure 5. Total phenolic content of cascara kombucha (C); cascara kombucha fortified with coconut water (CA); cascara kombucha fortified with red dragon fruit (CB); cascara kombucha fortified with coconut water and red dragon fruit (CAB); coconut water (A); B = red dragon fruit (B)

There was a decrease in total phenolic content after the addition of coconut water, which was 305.75 mg/kg for CA products, and 455.17 mg/kg for CAB products. This can occur because the total phenolic content in coconut water is very low, so that if the fortificant is mixed into cascara kombucha with a ratio of 1:1, it will certainly produce lower total phenolic content. Different results were shown by the CB product because the total phenolic content increased compared to the control product, resulting in a level of 656.32 mg/kg. The increase in total phenolic content in CB products is due to the fact that red dragon fruit has the highest total phenolic content, so that if the fortificant is mixed into cascara kombucha with a 1:1 ratio, it will produce higher total phenolic content. This is also in accordance with the high antioxidant activity possessed by the product when compared to the control product.

Caffeine Content of Cascara Kombucha

Analysis of caffeine content was carried out to determine the amount of caffeine in cascara kombucha products. This analysis was also carried out to determine the amount of cascara kombucha products that can be consumed per day in accordance with BPOM and FDA regulations. The results of the caffeine content test are presented in Figure 6 below.

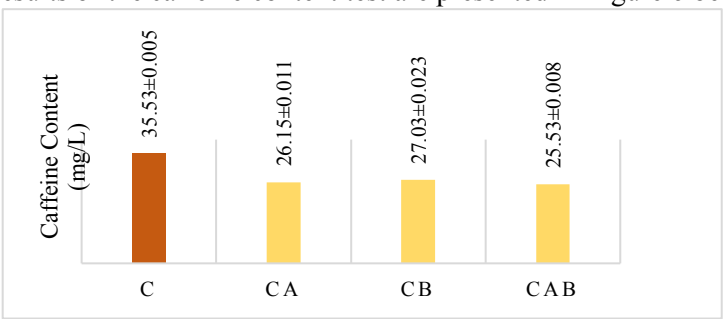

Figure 6. Caffeine content of cascara kombucha (C); cascara kombucha fortified with coconut water (CA); cascara kombucha fortified with red dragon fruit (CB); cascara kombucha fortified with coconut water and red dragon fruit (CAB)

Based on the test results, it is known that all products are still below the maximum limit of caffeine consumption per day. According to BPOM regulation No. HK.00.05.23.3644 of 2004, the maximum amount of caffeine that can be consumed is 150mg/day as a food supplement, while according to the Food and Drug Administration (FDA) the maximum amount of caffeine that can be consumed is 100-200 mg/day (Amalia et al., 2024). The maximum amount of kombucha that is safe to consume per day according to the Centers for Disease Control and Prevention (CDC) is 4–12 oz or about 118-350 ml for adults. Based on the results obtained, all cascara kombucha products have a range of caffeine levels of 25–35 mg/L. It is known that the highest caffeine level

is owned by the control cascara kombucha product at 35.53 mg/L, so that from the results of the average amount of kombucha that is safe to consume per day of about 250 ml, the amount of caffeine content is only about 8.88 mg. This means that cascara kombucha products have caffeine content that are still below the maximum limit, so cascara kombucha products are safe for consumption per day.

Mineral Content of Cascara Kombucha

Analysis of mineral content was carried out to determine the effect of the addition of coconut water and red dragon fruit on magnesium (Mg) content in cascara kombucha products. The test results of magnesium content are presented in Figure 7 below.

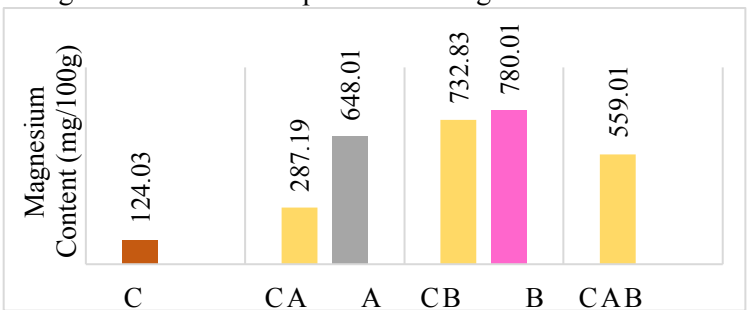

Figure 7. Magnesium content of cascara kombucha (C); cascara kombucha fortified with coconut water (CA); cascara kombucha fortified with red dragon fruit (CB); cascara kombucha fortified with coconut water and red dragon fruit (CAB); coconut water (A); B = red dragon fruit (B).

Based on the test results, it is known that the Mg content per 100 g serving size for cascara kombucha product (C) is 124.03 mg. These results have increased for products fortified with coconut water and red dragon fruit, namely 287.19 mg for CA products, 732.83 mg for CB products, and 559.01 mg for CAB products.

Mg levels contained in kombucha according to the USDA are 0 mg/100g, while based on the test results is 124.03 mg/100g. Factor that can affect Mg content in cascara kombucha is the use of different type of kombucha tea media. Tea media commonly used in making kombucha are black tea and green tea, while in this study cascara brew was used as a medium for making kombucha. This means that the Mg content in cascara is higher than the Mg content in black tea and green tea. This is in accordance with research conducted by (Brzezicha-Cirocka et al., 2016), the Mg content in green tea and black tea is only 1.26 mg/100g and 2.84 mg/100g, respectively.

The magnesium requirement per day according to the National Institute of Health for adult men is 400-420 mg, for adult women is 310-320 mg, while for pregnant women is 350-360 mg (NIH, 2021). This amount may vary depending on gender, age, and physical activity, so it is necessary to pay attention to the maximum amount when consuming this cascara kombucha. Magnesium is thought to play an important role in the prevention and treatment of several chronic diseases, such as Alzheimer's, asthma, hypertension, type 2 diabetes mellitus, cardiovascular disease, and osteoporosis (Chondro et al., 2022).

Sensory Analysis of Cascara Kombucha

Sensory hedonic analysis was conducted to determine the level of panelist preference for cascara kombucha products with the addition of coconut water and red dragon fruit. The hedonic test results are presented in the form of a radar diagram in Figure 8 below.

Based on the results of the hedonic test, product acceptability can be seen from the results of the favorability score. The colour and aroma attributes obtained the highest scores of 4.07 and 3.50 for CAB products, respectively. The most preferred colour of CAB products is due to the addition of red dragon fruit, the product becomes more attractive with the presence of betacyanin pigments in red dragon fruit (Luu et al., 2021). The most preferred aroma of CAB products is due to the addition of coconut water and red dragon fruit, resulting in a less pungent sour aroma, when

compared to product C, and is still well accepted by panelists. The pungent sour aroma in product C is obtained from organic acid compounds produced during the fermentation process, while the addition of coconut water and red dragon fruit, which have their own distinctive aroma and fruity flavour, reduces the sour aroma, so that the CAB product becomes more preferred.

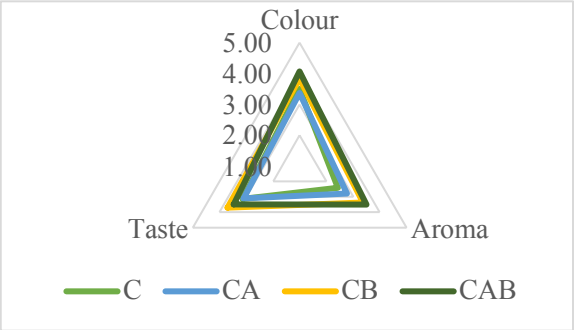

Figure 8. Average hedonic test results on colour, aroma and taste attributes of cascara kombucha (C); coconut water fortified cascara kombucha (CA); red dragon fruit fortified cascara kombucha (CB); coconut water and red dragon fruit fortified cascara kombucha (CAB).

In the taste attribute, the highest score of 3.70 was obtained for product CB. This is due to the addition of red dragon fruit in CB products making the product much sweeter when compared to the control cascara kombucha in product C. Overall, the addition of coconut water and red dragon fruit affects the hedonic sensory test results and gives better results on the colour, aroma and taste attributes of cascara kombucha. This means that panelists prefer cascara kombucha as a result of variations in the addition of coconut water and red dragon fruit.

CONCLUSIONS

The results of the analysis of cascara kombucha in various variants showed that the best alcohol content, total phenolic, caffeine, mineral, and antioxidant activity were obtained in the CB variant (C:B = 1:1) with consecutive results 0.25%; 656.32 mg GAE/kg; 27.03 mg/L; 732.83 mg/100 g; and 89.69%. Hedonic sensory results showed that the CAB variant (C:A:B = 2:1:1) produced the highest average score on colour and aroma attributes with scores of 4.07 and 3.05, while the CB variant (C:B = 1:1) produced the highest average score on taste attributes with a score of 3.70.

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