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Effect of High Fructose Syrup (HFS) Addition on Chemical and Organoleptic Properties of Green Coconut Water Kefir

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Abstract

This research aims to determine the effect of High Fructose Syrup (HFS) addition to carbon dioxide (CO_2) content, ethanol content and organoleptic properties of green coconut water kefir, and to determine the ideal HFS concentration for green coconut water kefir. Complete randomized design was used in this research with 5 treatments and 4 replications i.e. T0 (0% v/v HFS), T1 (2.5% v/v HFS), T2 (5% v/v HFS), T3 (7.5% v/v HFS), and T4 (10% v/v HFS). The CO_2 content was measured by sodium carbonate (Na_2CO_3) titration, ethanol content was measured by distillation, while the organoleptic properties that included level of sourness, level of sweetness, soda sensation, sour aroma, and viscosity were done by 25 panelists. The results show that the addition of HFS was statistically gave significant effect to the CO_2 content and organoleptic properties ($P<0.05$). However, the ethanol content, which analyzed using empirical model of quadratic polynomial regression, show that the addition of HFS was incompatible to the ethanol content of green coconut water kefir. The most optimal HFS concentration was 7.5% v/v, resulting CO_2 content of 0.096%; ethanol content 1.545%; and desirable organoleptic properties, which were low level of sourness, high level of sweetness, very high soda sensation, low sour aroma, and high viscosity.

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Introduction

Carbonated beverage is a drink that is injected by carbon dioxide (CO_2) gas to improve its flavor (Wiradona *et al*., 2017). The CO_2 content in the carbonated beverage gives a refreshing, burning, and biting impression on the tongue (Kappes *et al*., 2007), which is the main reason why it is highly preferred by consumers, even the level of consumption increases by 48.57% every year. Indonesia is known as the fifth largest country that consumes carbonated beverages instead of mineral water. Unfortunately, excessive consumption of carbonated beverages affect some health problems, such as caries and toothache (Wiradona *et al.,* 2017), reducing the effectiveness of enzymes, and affecting the digestive system (Fatriawan, 2014). Therefore, it is necessary to find alternatives to carbonated beverages that have good functional properties for health.

Kefir is a refreshing fermented drink with a slightly sour taste and contains a small amount of ethanol and carbon dioxide gas (Montibeller *et al.,* 2018). Ethanol, together with carbon dioxide gives kefir its stimulating and effervescent characteristics, which is similar to carbonated drink (Liu and Lin, 2000). Kefir also has good functional properties for health because it contains vitamins, minerals and essential amino acids (Hosono *et al.,* 1990). Several studies have investigated the presence of antitumor activity (Furukawa *et al.,* 1990; Cevikbas *et al.,* 1994) and antimicrobials on kefir (Cevikbas *et al.,* 1994; Zacconi *et al.,* 1995). Kefir is generally made from dairy milk, but the high intensity of sourness makes it less attractive to consumers. According to Al-Baarri and Murti (2003), about 3 out of 10 people do not like kefir because of its sourness and undesired aroma. Moreover, several consumers are allergic to dairy milk protein. To overcome these problems, a non-dairy kefir was made, called as water kefir (Mubin and Zubaidah, 2006). Water kefir is water-based kefir prepared with a sucrose solution with or without fruit extracts (Schneedorf, 2012) fermented by water kefir grains, consisting of several yeast, that are *Kluyveromyces*, *Candida* and *Saccharomyces*, and lactic acid bacteria (LAB), including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptoccus*. All these microorganisms are embedded in irregular, cloudy-pellucid granules called water kefir grains matrix. This matrix consists of dextran, a glucose polymer bound to α 1-6 chains (Waldherr *et al.,* 2010).

There have been several studies on water kefir made from fruit juice or sugary solution (Koutinas *et al.,* 2009; Magalhaes *et al.,* 2010). Indeed, fruit juice contains several nutrition that is good for microbial growth (Randazzo *et al.,* 2016). One of the ingredient that also suitable for microbial growth due to its high nutrition value is green coconut water. Green coconut water contains vitamins, minerals, especially nitrogen, phosphorus and potassium (Kristina and Syahid, 2012). It also contains growth regulator substances (phytohormones) and various enzymes (Prado *et al.,* 2015). Water kefir made from coconut water so far has been studied by Lestari *et al.* (2018). However, the shortcomings of previous research was the fermentation process did not go well due to lack of sugar content in coconut water. According to Kristina and Syahid (2012), green coconut water contains only 3.45 mg/ml sucrose. Therefore, the addition of sugar is needed to enhance the carbon and nutrition value for kefir grains growth and fermentation process.

High Fructose Syrup (HFS) is a highly valued liquid sweetener that is often use in beverages, confectionery and processed food industry, owing to its special attributes like high solubility and non-crystalline nature (Johnson *et al.,* 2009). HFS in generally made from cassava flour that heated and added by α-amylase and glucoamylase enzyme, resulting glucose syrup. After that, the glucose syrup is added by glucose isomerase enzyme to produce High Fructose Syrup (Parker *et al.,* 2010). Based on the previous research by Prastiwi *et al.* (2018), it was known that the addition of HFS into milk kefir can increase the total lactic acid bacteria, total yeast and can improve kefir's organoleptic properties. The sugar content in HFS is mostly in the form of monosaccharaides, which are fructose and glucose (Gunam and Wrasiati, 2009) so it could directly use by kefir grains for fermentation. Moreover, HFS also has lower glycemic index and 2.5 sweeter than sucrose (Richana, 2012).

This research aims to determine the effect of High Fructose Syrup (HFS) addition to carbon dioxide (CO_2) content, ethanol content and organoleptic properties of green coconut water kefir, and also to determine the ideal HFS concentration for green coconut water kefir. Green coconut water kefir with High Fructose Syrup has the potential to be an alternative substitute for carbonated beverages due to its refreshing and desirable characteristics, also good functional properties for health.

Materials and Methods

The materials used in this research were 5 liters of coconut water that were obtained from Tembalang, Semarang, water kefir grains that were obtained from online (Beadsnik Store, South Denpasar, Bali), HFS-55 that was obtained from CV. Inovasindo Berkah Mandiri, Kediri, Jawa Timur, Na_2CO_3 0.0454 N, Phenolphthalein (PP) and aquadest. Burette, distillation set, pycnometer, Erlenmeyer, Kjeldahl flask, analytical scale, plastic cup and organoleptic form were used for analysis.

Carbon dioxide measurement

Carbon dioxide content was measured using the titration method (Jawa *et al.* 2014) with Na_2CO_3 0.0454 N solution as the titrant. The titration process was performed by taking 20 ml sample of green coconut water kefir, and then the sample was dripped by ±1 ml Phenolphthalein. Next, the sample was dripped by titrant until the colour of sample turned into pink.

Ethanol content was measured by distillation and pycnometer method (AOAC, 2013). First, Kjeldahl flask was filled by 50 ml of samples and 100 ml of aquadest sequentially. Then, put the Kjeldahl flask into the distillation set. The distillation process was performed at 80^{o}C. Next, the distillate was collected in the erlenmeyer until 50 ml, after that poured the distillate into the pycnometer. The pycnometer and distillate was weighed. Same procedure was repeated for aquadest. The density of ethanol calculated and converted using the conversion table of ethanol.

The organoleptic properties of green coconut water kefir were tested by ranking test (Safitri and Swarastuti, 2013). Twenty-five panelists were given a questionnaire containing panelists' information (name of the panelist, the test date and the sample being tested), instructions and panelists' responses. Sensory attributes for this test were level of sourness, level of sweetness, soda sensation, sour aroma, and viscosity. The panelists had to assay five samples and ranked each attribute with scale 1-5 for analysis. They were also instructed to cleanse their palate with mineral water between each samples.

Data obtained from the carbon dioxide content and organoleptic test were statistically analysed with SPSS 22.0 for Windows 10 software using ANOVA at 5% significance level to evaluate the effect HFS addition. If the effect was significant then the analysis was continued with Duncan test to find out the difference effect of concentration HFS added. The result of organoleptic test were analysed using non-parametric Kruskal Wallis test and then followed by Mann Whitney test. Meanwhile, data that obtained from ethanol content were analysed descriptively using empirical model of quadratic polynomial regression. The data was analysed by Microsoft Excel 2013.

Results and Discussion

Carbon dioxide content

Based on Table 1, it is known that the addition of HFS with different concentration was statistically provided significant effect ($P<0.05$) to the CO_2 content of green coconut water kefir. Carbon dioxide is one of the metabolite product that produced by water kefir grains. The mechanism process of metabolism first begin with the conversion of fructose to glucose by the glucose isomerase enzyme. *Streptomyces sp., Bacillus spp., Acetobacter cloacae, Lactobacillus* sp.*, Escherichia coli,* and *Candida* sp. are known as the microorganisms that can produce that enzyme (Vongsuvanlert and Tani, 1988), while *Candida sp.* and *Lactobacillus sp.* are also found in water kefir grains (Waldherr *et al.,* 2010). Glucose that has been formed then used by LAB to produce lactic acid, while yeast used glucose for cell

Table 1. The result of carbon dioxide content of green coconut water kefir with HFS

Parameter	Samples				
	T0	T1	T2	T3	T4
Carbon dioxide content (CO_2)	0.073±0.004[a]	0.081±0.009[ab]	0.090±0.002[bc]	0.096±0.008[c]	0.091±0.007[bc]

Results are mean±standard deviation; Different superscript letters in the same column indicates the significant differences (p<0.05).

Table 2. The results of organoleptic test of green coconut water kefir with HFS

Organoleptic Attributes	Treatments				
	T0	T1	T2	T3	T4
Level of Sourness	1.36±0.638[a]	2.04±0.351[b]	2.52±0.653[c]	3.64±0.952[d]	4.84±0.374[e]
Level of Sweetness	4.72±0.542[a]	3.84±0.746[b]	2.88±0.833[c]	2.24±0.970[d]	1.16±0.374[e]
Soda Sensation	3.88±1.563[a]	3.12±1.364[b]	2.56±1.083[bd]	1.24±0.523[c]	2.32±0.557[d]
Sour Aroma	1.28±0.458[a]	1.56±0.651[a]	2.64±0.700[b]	3.92±1.077[c]	4.52±0.823[d]
Viscosity	3.52±1.388[a]	3.66±1.180[a]	2.56±0.870[b]	2.12±1.092[c]	1.28±0.614[d]

Results are mean±standard deviation; Different superscript letters in the same column indicates the significant differences (p < 0.05)

metabolism to produce ethanol and carbon dioxide gas (Musdholifah and Zubaidah, 2016).

The results in Table 1 show that the CO_2 content of green coconut water kefir tended to increase due to the increasing of HFS concentration, where the CO_2 content of T0-T3 were 0.073%; 0.081%; 0.090%; 0.096%, respectively, however the CO_2 content of T4 was decreased compared to T3, which was only 0.091%. This caused by the excessive level of fructose in T4, which could slow down sugar conversion process, resulting lower level of metabolite products (Lin and Tanaka, 2006). Petry *et al.* (2000) also stated that microorganism could only convert 2.0-3.5 g/l sugar during the adaptation phase and 8.0 g/l during the log phase.

Green coconut water kefir with 7.5% v/v HFS was the best treatment because it has the highest amount of CO_2 content. Carbon dioxide, together with ethanol gives effervescent characteristics, which is similar to carbonated drink (Liu and Lin, 2000). Carbonation effect on beverages give a refreshing sensation, which is highly preferred by consumers (Sandrasari and Abidin, 2006). However, green coconut water kefir cannot be categorized as carbonated beverages, because according to standard set by SNI 3709:2015 about carbonated beverages, the CO_2 content of carbonated beverages must be not less than 0.589-0.900%. The CO_2 content in the green coconut water kefir was lower because it was obtained naturally through yeast fermentation and metabolism process, whereas in the carbonated beverages were obtained by the injection of CO_2 gas (Wiradona *et al.,* 2017).

Ethanol Content

The model of quadratic polynomial regression of ethanol content of green coconut water kefir with High Fructose Syrup was shown in Figure 1. The equation of ethanol content was $Y= -0.0179X^2+0.2035X+0.9524$ with a very strong positive correlation (r=0.98), which showed that the addition of HFS is significant to the ethanol content of green coconut water kefir.

The addition of HFS up to 7.5% v/v could increase the ethanol content of green coconut water kefir. This is in accordance with the opinion of Gunam and Wrasiati (2009) which stated that the addition of sugar could increase the ethanol levels. On the other hand, the addition of HFS up to 10% v/v could reduce the ethanol content of green coconut water kefir due to incomplete sugar conversion process. This is in accordance with the opinion of Petry *et al.* (2000) which stated that LAB could convert only 2.0-3.5 g/l sugar during the adaptation phase and 8.0 g/l during the log phase. The excessive level of fructose could slow down sugar conversion process, resulting lower level of metabolite products (Lin and Tanaka, 2006). Besides that, excessive level of sugar can caused osmotic shock to water kefir grains, which could damage to cell organelles (Azizah *et al.,* 2014).

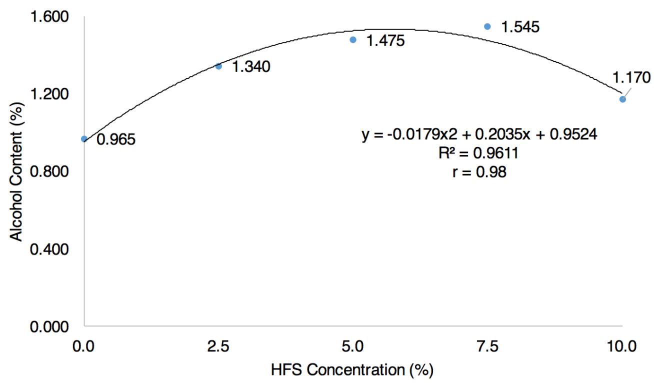

Figure 1. Ethanol content of green coconut water kefir with high fructose syrup

Based on Figure 1, the addition of HFS contributes 96.11% (R^2) to the ethanol content of green coconut water kefir. Meanwhile, the 3.89% other could came from other factors besides HFS, such as the presence of sugar alcohol. Naturally, green coconut water contains sugar alcohol, such as manitol, sorbitol, myo-inositol and scyllo-inositol (Yong *et al.,* 2009). According to standard set by CODEX STAN 243-2003, it was stated that kefir contains ethanol, although it was not clearly stated the minimum/maximum amount of ethanol content. In Indonesia, there was a standard set by FATWA MUI No. 4/2003 that stated that any kind of beverages containing ethanol above 1% should not be consumed freely. Furthermore, according to the research performed by Gunawan (2015), it was stated that the ethanol content found in water kefir was around 0.5-1.0%. Therefore, the green coconut water kefir that meets the requirements of the standards set by CODEX and FATWA MUI is the T0 treatment (0% v/v HFS), which contains ethanol 0.965%.

Organoleptic Properties

The results of the organoleptic test of green coconut water kefir included level of sourness, level of sweetness, soda sensation, sour aroma, and viscosity are presented in Table 2.

Level of Sourness

The addition of HFS give a significant effect to the sourness of green coconut water kefir ($P<0.05$). The higher the concentration of HFS added, the lower intensity of sourness. The panelists rated that the highest to lowest level of sourness of green coconut water kefir sequentially were T0, T1, T2, T3, and T4. The sourness of green coconut water kefir wes occurred due to the presence lactic acid in large amounts and acetic acid in small amounts. Those organic acids are produced from water kefir grains' fermentation (Laureys and Vuyst, 2004). However, the HFS addition into the green coconut water kefir could covered the sour taste, because only 30% of sugar that can be converted into acid. The 70% other are still in the form of sugar (Haryadi *et al.,* 2013). Panelists did not like too strong sour flavor, they tended to like neutral to sweet flavor of kefir (Musdholifah and Zubaidah, 2016). Therefore, the best treatment was T3, because with only 7.5% v/v HFS added, could already produce green coconut water kefir that had neutral to sweet flavor.

Level of Sweetness

The variation in HFS concentration provided a significant effect on the sweetness of green coconut water kefir ($P<0.05$). The level of sweetness was inversely proportional to the sourness of green coconut water kefir, which indicated by panelists' rating. According to panelists, the highest to lowest level of sweetness of green coconut water kefir sequentially were T4, T3, T2, T1, and T0. This was occurred due to HFS was 2.5 times sweeter than sucrose (Richana, 2012). It is also supported by the opinion of Gunam and Wrasiati (2009) which stated that according to panelists, the sweetness level of HFS tends to be higher than the other sugar. Kefir is a refreshing fermented drink with a slightly sour taste (Montibeller *et al.,* 2018), but also taste sweet and alcoholic (Gulitz et al., 2011). Therefore, the best treatment was T3, because with only 7.5% v/v HFS added could already produce green coconut water kefir with the sweet flavor.

Soda Sensation

Soda sensation is a sensation of refreshing, burning, and biting on the tongue (Kappes *et al.,* 2007). This kind of sensation occurs due to CO_2 and ethanol content from kefir grains' fermentation and metabolism process (Lestari *et al.,* 2018). Carbonated effect gives the refreshing impression so it is highly preferred by consumers (Sandrasari and Abidin, 2006). Data on Table 2 shows significant effect of the addition of HFS to the soda sensation ($P<0.05$), yet the T1, T2, and T4 tended to be indistinguishable by panelists. According to panelists, the intensity of soda sensation of T0 was low, T1, T2, T4 was neutral, and T3 was very high. The intensity of soda sensation is supposedly increase along with the increasing of HFS concentration, because HFS is a source of nutrition that supports yeast metabolism and fermentation process to produce CO_2 and ethanol (Musdholifah and Zubaidah, 2016). However, this was not applicable to T4, which had lower intensity of soda sensation compared to T3. This caused by the excessive level of fructose in T4, that could slow down sugar conversion process, resulting lower level of metabolite products (Lin and Tanaka, 2006). The addition of 7.5% v/v HFS was the ideal concentration for yeast to convert fructose and glucose into CO_2 and ethanol.

Sour Aroma

Based on Table 2, it was shown that the difference concentration of HFS give significant effect on the sour aroma of green coconut water kefir ($P<0.05$). This was in accordance with Rumeen *et al.* (2018) which stated that the sugar concentration has a significantly different effect on the aroma of kefir. According to panelists, both T0 and T1 had very strong sour aroma, T2 was rather strong, T3 was not strong, and T4 was very not strong. The sour aroma of kefir is caused by the presence of volatile compounds, such as acetaldehyde, acetone, ethyl acetate, 2-butanone, diacetyl, and ethanol (Beshkova *et al.,* 2003). Those volatile compounds are produced by yeast, but high yeast activity caused an overpowering sour aroma, which was undesirable (Musdholifah and Zubaidah). The addition of HFS could cover the undesirable aroma, the more HFS is added, the lower the intensity of sour aroma produced (Qonitah *et al.,* 2016). Therefore, the best treatment was T3, because with only 7.5% v/v HFS added could already produce green coconut water kefir with low intensity of sour aroma.

Viscosity

The result of the viscosity of green coconut water kefir as presented in Table 3, shows that the addition of HFS was statistically significant to viscosity ($P<0.05$). This is in accordance with Rumeen *et al.* (2018) which stated that the sugar concentration has a significantly different effect on the viscosity of kefir. The viscosity of green coconut water kefir was increased along with the increasing of HFS concentration. According to panelists, T0 and T1 both was not viscous, T2 was rather viscous, T3 was viscous, and T4 was very viscous. The increasing of viscosity is occurred due to amount of total dissolved solids. The higher concentration of sugar, the higher amount of total dissolved solids, resulting the increasing of viscosity (Gunam and Wrasiati, 2009). The treatment T3 was a good quality green coconut water kefir, because it had a viscous texture, yet still pourable (Alsayadi *et al.,* 2014).

Conclusion

The addition of HFS gave significant effect to the CO_2 content and organoleptic properties. However, the addition of HFS was incompatible to the ethanol content of green coconut water kefir. The most optimal HFS concentration was 7.5% v/v, resulting CO_2 content of 0.096%; ethanol content 1.545%; and desirable organoleptic properties, which were low level of sourness, high level of sweetness, very high soda sensation, low sour aroma, and high viscosity.

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