FORMULATION OF READY TO DRINK (RTD) MADE FROM ROSELLE (HIBISCUS SABDARIFFA. L) TEA AND STEVIA (STEVIA REBAUDIANA) LEAF SAFE FOR DIABETICS

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Abstract: Diabetic is a degenerative disease that has become a public concern. Several studies have shown that roselle tea extracts can reduce blood glucose levels by improving the pancreatic glands, and Stevia rebaudiana leaf has zero calorie with a sweetness coming from diterpene glycosides (stevioside and rebaudioside-A) which are safely metabolized in body, so that it can be used as sweetener for diabetics. The purpose of this study was to determine the formula of roselle tea and stevia leaf that is safe for diabetics. Three formulas of ready to drink (RTD) were prepared using 2% (w/v) rosella tea with 0.1, 0.3, and 0.5% (w/v) stevia leaf, then the sensory qualities were analyzed including the red color, sweetness, bitterness, off flavor. The selected formula was RTD made from 2% (w/v) roselle tea and 0.3% (w/v) stevia leaf. The RTD had a little bright red color, pretty sweet, no bitter taste, quite off flavor, and total microbes < 2.5 EAPC (compromise with Indonesian Standard Regulation for fruit juice). Carbohydrate content (Anthrone method) of RTD roselle-stevia and roselle-sugar was 0.1507% and 1.3293%. Effect analysis of RTD roselle-stevia and roselle-sugar on blood glucose levels of healthy patients based on ISO 26642:2010 measured every 30 minutes for 90 minutes and using t-test showed that RTD roselle-stevia did not affect ($p>0.05$) the increase in blood glucose levels, whereas RTD roselle-sugar did ($p<0.05$). It can be concluded that RTD containing 2% (w/v) roselle tea and 0.3% (w/v) stevia leaf is safe for diabetics.

Keywords: blood glucose, diabetics, *Stevia rebaudiana*, *Hibiscus sabdariffa L.*

1. INTRODUCTION

Diabetes is one of the degenerative diseases that has become the public's attention. The International Diabetes Federation (2013) details the countries with the most diabetics, Indonesia's position is at number seven with 8.5 million sufferers. Meanwhile, China (98.4 million), India (65.1 million), and America (24.4 million). At present, in the world, there are 382 million diabetics with a mortality rate reaching 5.1 million (Anonymous, 2013).

The food industry is increasing rapidly in line with technological innovation and human science. This changes the way of the human view of consuming snacks, from just to meet the needs when hungry, to prioritize the content of nutrients in it, as functional food and even alternative food for health therapy. Natural foods that begin to be seen its functional value for diabetics, including rosella and stevia. Both of these snacks are believed to have good ingredients for diabetics so they are safe for consumption.

Roselle extract has been proven to reduce blood sugar levels of diabetics by improving the

pancreas gland (Mardiah, 2014). The sour taste of ascorbic acid, citric acid and glycolic acid (Wiyarsi, 2011) contained in it causes less demand for consumption, meanwhile the addition of sucrose as a sweetener can be dangerous for diabetics so that other sweeteners are needed, *Stevia rebaudiana* leaves have 0 calories with flavor sweetness derived from diterpene-glycosides (steviosida and rebaudiosida-A) whose extract has 300 times the sweetness of sucrose, high solubility in water, acceptable sweetness and safe metabolism in the body (Soejarto et al., 1982; Megeji et al. ., 2005; Genus et al., 2007) made potential stevia leaves for further processing as a substitute for sweet snacks sourced from sucrose sugar and other chemical sweetener additives which can cause chronic diseases such as diabetes and obesity (Burke et al., 2006).

Consumer needs for ready-to-drink beverages, also known as ready to drink (RTD), increase along with the shift in the function of drinks, lifestyle changes, the increased need for additional nutrients or supplements and consumer desires that are fast and practical. In most urban communities that have a lot of activities and a little time causes a change in the pattern of consumption of beverage products that are practically a necessity, in addition to practicality also to get the benefits provided from a beverage product (Paramita, 2011).

The combination of stevia with rosella which is safe and efficacious for diabetics in a ready-to-drink beverage package can be one alternative for beverage consumption for diabetics. The drink formulation must not reduce the taste value but still contain the functional value as desired.

2. METHODS

This research consists of preparation for the RTD formula continued by the analysis of chosen formula. The preparation includes the formulations of rosella-stevia with various concentration (Rosela 2% (w/v) with Stevia leaves 0.1% (w/v); 0.3% (w/v); 0.5% (w/v)) above the water bath with a temperature of 100^{o}C for 1 hour. This composition based on trial and error experiment before supported by conversion data on the use of stevia leaves and stevia extracts against sugar in food. Then filtering is carried out to obtain a clean extract of dissolved solids.

The product then evaluated by sensory analysis with the line scale of Hedonic method to find out the most preferred quality based on consumers wants. The selected product then analyzed by TPC (Total Plate Count) for microbe contaminations, total sugar content, and its effect on normal patient's blood sugar level.

3. RESULTS AND DISCUSSION

Results

3.1. Sensory Quality Test of Formula

This test is carried out to determine the best quality of products made. The treatment concentrations of products made were A1 ((Rosela 2% (w/v) and Stevia 0.1% (w/v)), A2 ((Rosela 2% (w/v) and Stevia 0.3% (w/v)), and A3 ((Roselle 2% (w/v) and Stevia 0.5% (w/v)). The test parameters are the red color, sweetness, bitterness, and the off flavor from the product. This test was carried out with 30 untrained panelists with variations in age and education. The data obtained from this sensory quality test were then processed using Microsoft Excel 2007 software in the Analysis of variance (ANOVA) and carried out the Least Significant Difference (LSD) test. The result of sensory quality test can be seen in Table 1. Based on the data shown, the selected formula is A2. The formulation A2 red color is considered the best (bright) and different from other formulations, and has a sweet taste that is almost the same as formulation A 3 with less mixture of stevia leaves or more economical. There are not significant different between the formulation of the bitterness taste and off flavor. The

formulations still have an enough off flavor (scale 5-6 from 10) and a slightly bitterness taste (scale 3 from 10). Comparison of selected formulations with standard formulas using glucose can be seen in the Figure 1.

Table 1 Result of sensory quality test

No.	Test parameter	Variance		
		A 1	A 2	A 3
1.	Red color	4.17[a]	5.76[b]	4.59[a]
2.	Sweetness	2.09[a]	4.26[b]	4.43[b]
3.	Bitterness	3.48[a]	3.90[a]	3.24[a]
4.	Off flavor	6.66[a]	5.71[a]	6.42[a]

Note : Different notation on each line determines the difference between treatments at the 95% confidence level and p <0.05.

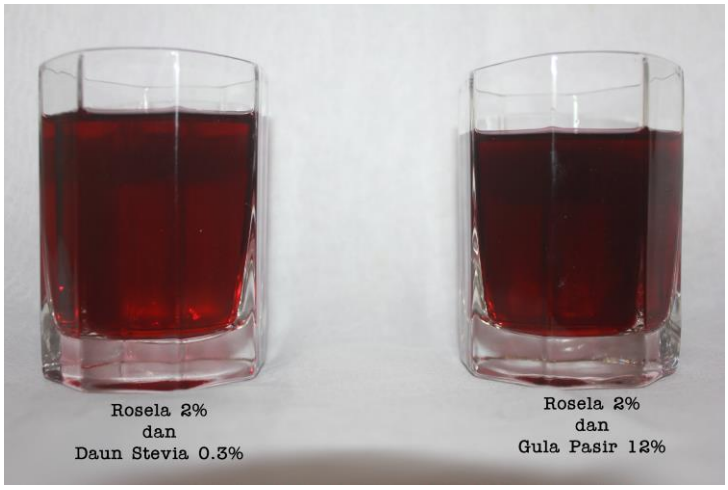

Figure 1 Comparison between chosen formula with stevia and standar formula with sugar

3.2. Blood Sugar Test of Formula on Healthy Patient

The analysis of blood sugar levels carried out had obtained permission from the Faculty of Medicine, University of Indonesia for using human patients as research subjects, based on Letter Number 433 / UN2.F1 / ETIK / 2015 on May 25, 2015. This analysis used 9 patients, blood drawn before the intervention and after the intervention with an interval of 30 minutes for 90 minutes. The patient is a Bogor Chemical Analyst Vocational High School student aged 16-17 years with a healthy condition that is known from interviews with researchers, taking blood using a different sterile lancet each patient, and measured with a calibrated Accucheck Active® brand glucometer.

Comparison data of increase in blood sugar to the formulation of rosella-stevia with rosella-granulated sugar was calculated using a T-Test with a confidence level of 95%, α 0.05, or p <0.05 to determine its effect on the increase in the patient's blood sugar level. Roselle-sugar formulation has an average t-value at 0-30 (8,380), 0-60 (3,169), and 0-90 (4,562) minutes which is greater than t-table (2,306) so that it can be concluded that the formulation of rosella- granulated sugar has a significant effect on increasing the patient's blood sugar level. While the rosella-stevia formulation has a t-value at 0-30 (0.699), 0-60 (0.068), and 0-90 (1,826) minutes which is lower than the t-table

(2,306) so that it can be said that the rosela-formulation stevia has no significant effect on the increase in patients' blood sugar levels.

3.3. Microbes Contaminate

Microbial contamination analysis is an important analysis that must be done on every food product that is ready for consumption, because food is directly related to the body's metabolism and consumer health. One method that can be used to analyze microbial contamination of food products is the Total Plate Number, this method calculates the total colonies of microbes in the product including bacteria, fungi, and yeast. The media used is a standard media PCA (Plate Count Agar) consisting of glucose and yeast extract (BAM, 2001). The results of the analysis of the number of colonies at a 10^{-0} simplo dilution are <3 CFU.

3.4. Total Carbohydrate of Formulation with Anthrone Method

In this study the total carbohydrate analysis was used to determine the carbohydrates available in reference foods (granulated sugar), selected products from a sensory quality test, and positive control (rosella + sugar) to estimate the effect of carbohydrates on increasing blood glucose levels in normal patients. The carbohydrate content in the positive control of roselle and sugar used was 13.1848g / g, and in the A2 formulation was 1.507g / g. The carbohydrate content affects calories and blood sugar levels after consumption by the patient.

Discussion

The extraction process for making the RTD formulation carried out in this study was on a 100ºC water heater for 1 hour, previously boiled and produced a very red color of very rosy rosé, very bitter and languid stevia leaves, this was due to the direct heating process with boiling all the components of the rosé compound and especially the stevia leaves in 1 hour in a solution so that they produce a cohesive and unpleasant taste. The extraction process was then performed by indirect heating on a water heater at 100ºC for 1 hour as done by Isdianti (2007).

The red color formed in the product comes from the main pigment, hibiscin. The pigment has been identified by the name daphniphylline. Hibiscin is included in the anthocyanin compound which is an antioxidant, the more anthocyanin content, the thicker red color will be formed (Maryani et.al, 2010). Stevia leaf extraction itself has a brown color (Isdianti, 2011) due to the content of chlorophyll pigments in dried stevia leaves (Esmat, 2010). The two main colors of this formulated product affect the final result, in theory because roselle petals are formulated in the same composition, only the addition of stevia leaves plays a role in the different colors of the product formulation, the more stevia leaves added will make the red color darker. The selected formulation seen from this data is formulation A 2 with better quality values in the direction of bright red (5.76).

Stevia leaf contains seven diterpene glycosides that cause sweetness, namely rebaudioside A, rebaudioside B, rebaudioside C, rubusoside, dulcoside A, steviolibioside, and stevioside. The highest content in dried stevia leaves is steviosida (3.8%) and rebaudiosida A (9.1%) (Goyal et al. 2010). Rebaudioside A is the sweetest and most stable glycoside-stevia and no more bitter than steviosida, while rebaudioside E has the same sweetness as stevioside, and rebaudioside D has the same sweetness as rebaudioside A, and other glycosides have sweetness under stevioside (Cramer and Fish) 1987 in Goyal et al. 2010). This sweet nature can make stevia leaves as an alternative sweetener for rosella drinks so that they can be consumed as functional drinks with acceptable sweetness.

Formulai A 2 and A 3 have a sweeter taste due to the addition of more stevia leaves, whereas

in formulation A 1, stevia leaves are added only slightly so that the sour taste of the rosella extract is more pronounced. The chosen formulation seen from this data is A 2 formulation because it is sufficient to provide a suitable sweetness at a smaller concentration compared to A 3 so that for future production it will be economicaly profitable.

The formulation of rosella and stevia extract, varying the concentration of stevia leaves with the active group content of steviosida which gives the impression of high sweetness, but if used alone as pure sugar for sweetening food and drinks in large doses, can cause the sweetness to be less effective in tongue. This is due to steviosida still has a sense of sepat and unpleasant. Taste sepat and unpleasant apparently not found in other compounds. If the extracts from rebaudiosida A, D and E are combined, the mixture will have a level of sweetness equivalent to steviosida without unpleasant taste (Lutony, 1993). According to Soejarto (1982), the sequiterpen lactone molecule found in stevia leaves causes a bitter after taste during consumption, according to Goyal et al. (2010) that the bitter taste after stevia leaf consumption is due to the presence of essential oils, tannins and flavonoids. The use of stevia leaves as a sweetener needs sensory data representing the unpleasant taste of the leaves that can be felt by panelists, it is expected that the unpleasant taste of leaves can be minimized by the sour taste of rosella extract and sweetness from steviosida itself.

Formula A 1, 2 and 3 have a quality value above 5, or the bitter taste is less tasted. The average value shows that the panelists felt the most bitter taste after taste in A2 formulations with the addition of 0.3% stevia leaves, then in the A1 formulation (0.1% stevia leaves) and finally in the A3 formulation (0.5% stevia leaves) this is because in the formulation A1 Stevia leaves are added a little, so panelists do not really feel it, in the A2 formulation there is a balance after taste that is formed with the sweet taste of stevia leaves so that the panelists feel bitter, and in A3 formulations with 0.5% stevia leaves which is quite a lot, after taste bitter taste is covered by the sweet taste of stevia leaves.

According to the CDC at 2013, aroma is the most important factor determining flavor in food, aroma can be felt due to the presence of nerve receptors in the nasal cavity. So it is important to assess the acceptance of product aroma in the community by sensory quality testing. The content of roselle extract itself has a distinctive rosella aroma like in fruits whose aroma comes from the content of ester compounds in flower petals and is volatile when heated (Winarno, 2009), while stevia leaves have an unpleasant aroma (unpleasant) derived from the content of dry leaves. (not stevioside) such as chlorophyll and other isoflavones (Goyal et al., 2010), so that it can affect the acceptability of product aroma. Roselle formulations with variations of stevia leaves are made, assessed by panelists, the higher the value is given, the better the quality or the distinctive scent of roselle, without leaving the unpleasant scent of leaf chlorophyll.

Treatments A 1, 2 and 3 have a quality value below 5, or the unpleasant aroma still smells in all treatments. If sorted, the highest scent of scent is formulation A 1 with 0.3% stevia, then A 3 with 0.5% stevia and finally A 2 with 0.1% stevia, from this data formulations with the use of stevia have less scent that is less pleasing than the use more stevia, this is due to the use of untrained panelists who have varying ratings of the product.

As the blood glucose analysis, according to a summary of research written in JECFA (2006) because stevia is not metabolized as blood sugar in the body, steviosid or rebaudiosida and other molecules contained in stevia leaves are hydrolyzed into steviol before being absorbed in the small intestine. In addition, steviosid has anti-hyperglycemic effects by increasing insulin response and suppressing glucagon and anti-hypertension levels, significantly reducing systolic and diastolic blood pressure in experimental animals and humans. Research on the effects of steviosid in humans by Curi et al. (1986) is widely used as a reference and proof that steviosida reduces blood sugar levels of patients.

The studies summarized in the 2006 JECFA stated that the mixture of stevioside products did

not increase blood sugar levels as evidenced by the discovery of steviol content in feces collected for 48 hours after consumption and the absence of steviol content in the blood for 24 hours. Experiments using human faeces incubated with steviosida and rebaudiosida did not show a decrease in steviol levels for 10 and 24 hours respectively, so it was concluded that intestinal bacteria could not degrade steviol compounds.

Patient's blood sugar measurement data consists of measurement data for pure glucose, selected formulation (A2) and positive control (roselle + granulated sugar). From the data of blood sugar levels after consumption of pure glucose, it can be seen that patients whose blood is drawn are in normal condition ie blood sugar levels have increased in the first 30 minutes. The data is made in the form of a line curve connecting blood sugar measurements in mg / dL per unit time. Pure glucose measurements were performed to check the condition of the patients used as in the study conducted by Curi et al. (1986) and produce data on blood sugar levels that rise in the first 30 minutes and fall in the next 30 and 60 minutes, this is in accordance with studies for the measurement of the glycemic index using pure glucose as a reference food for patients to consume (Larasati, 2013).

The use of rosella itself in this formulation is due to various studies that have been carried out stating that roselle has a very good effect for sufferers of hypertension, diabetes, and other diseases caused by the body's metabolism which is already poor (Mardiah et al. 2009) and it is proven that the formulation of rosella with stevia can reduce blood sugar levels in normal patients. Graph of blood sugar levels between selected formulations with positive control of rosella with sugar can be seen in Figure 2. The use of stevia as a sweetener in drinks has also been carried out by Harismah et al. in yogurt, organoleptic analysis of the aroma, taste and color showed a high preference for the combination of sugar with stevia compared to the use of stevia alone.

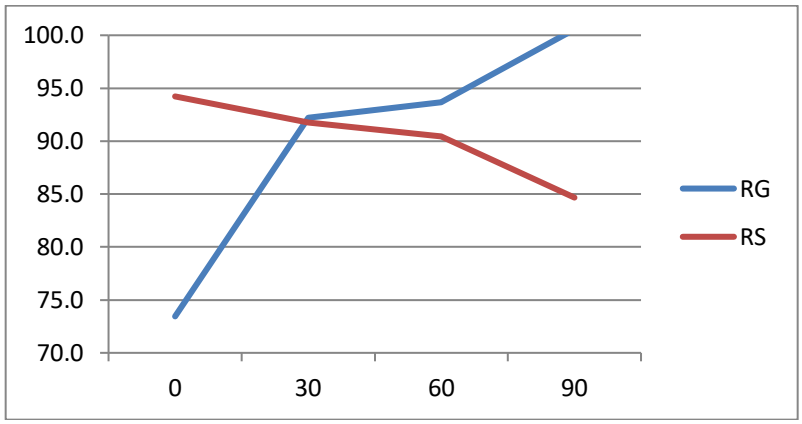

Figure 2 Blood glucose level on healthy patient

4. CONCLUSION

Based on the results of research conducted, the best formulation was selected from sensory quality tests, namely formulation A 2 with 2% (w / v) rosella and 0.3% (w / v) stevia leaves with a rather bright red color, sufficient sweet taste, bitter taste of leaves which does not feel and smells pleasantly felt. Total microbial analysis of the formulation that is <2.5 EAPC included in the SNI standard for fruit drinks, sugar-anthrone showed the formulation carbohydrate content of 0.1507% and 1.3293% in the rosella formulation with granulated sugar, and the formulation did not affect the increase in blood sugar levels of healthy patients. So it can be concluded that this formulation of rosella-stevia is good for diabetics to consume with a taste that is still preferred, as well as a high

antioxidant content in rosella can help cure diabetes.

A conclusion is not merely a re-statement of the data or findings, but a synthesis of key points and, as mentioned in the “Introduction” which eventually produces the "Results and Discussion" chapter so that there is compatibility. In addition, the prospects for developing research results and the prospects for future research applications (based on results and discussion) can also be added.

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