Characteristics and Anti-Diabetics Activity of Jelly Drink Okra Mucus (*Abelmoschus Esculentus* L.)

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Abstract. Okra mucus is mucilage that contains food fibre, tannin, alkaloids, steroids, and flavonoids, so it has the potential as an anti-diabetic agent, which can be processed into an anti-diabetic functional beverage product in the form of a jelly drink. However, it still needs the addition of a gelling agent to form a jelly, for example carrageenan, while sucralose and stevia were used to add sweetness. The purpose of this study was to determine the characteristics and activities of anti-diabetic jelly drink okra mucus with the formula of adding carrageenan and low calorie sugar. The study used a completely randomized design of two factors: carrageenan (0.10; 0.20 and 0.30%) and sugar (sucralose; stevia) with a concentration of 0.02; 0.03 and 0.04%. Each formulation was repeated 2 times, the difference in the formulation was used Duncan's further test (DMRT 5%). The results showed that formula with the addition of carrageenan 0.3% and stevia sugar 0.04% which produced okra mucus jelly drink with viscosity characteristics 279.50 cps, total soluble solids 15.00% Brix, gel strength 4.53 N, total phenol 76.63 mgTAE / g), anti-diabetic test (IC₅₀) 32.60 mg / ml, and food fibre 1.29 g / 100g. Organoleptic test results showed an average taste score of 2.8 (not favoured - favoured), texture 3.7 (favoured - favoured), aroma 2.75 (not favoured), and colour 2.95 (not favoured - favoured).

Keywords: Jelly drink, Okra mucus, Anti-diabetic, Carrageenan, Low calorie sugar

1. Introduction

Diabetes mellitus (DM) is a disease characterized by blood glucose levels exceeding normal and impaired carbohydrate, fat and protein metabolism caused by a deficiency of the hormone insulin [1]. DM treatment can be done by utilizing compounds from plants such as polyphenol compounds, including flavonoids. Polyphenol compounds also have the ability to bind to proteins so that they can inhibit carbohydrate decomposing enzymes such as α-glucosidase which contribute to postprandial hyperglycemic [2]. Sugara [3] reported that jelly drinks a food product that uses a gelling agent to form a jelly texture that can be consumed using a straw. Novelina [4] state that is a gel-shaped beverage product and has the characteristics of a thick liquid that is consistent and easy to suck. The gel from a jelly drink is softer or smoother and the texture is not as sturdy as jelly, so it can be sucked in its consumption, but when it is still in the mouth the gel texture can still be felt. Jelly drinks can be made from okra mucus (*Abelmoschus esculentus*) with a high content of polyphenol compounds and food fibre so that it has the potential as an anti-diabetic agent [5-6].

Okra mucus has a mucilage texture that is less viscous when processed into jelly drinks, so it is necessary to add gelling agents for making jelly drinks, such as carrageenan [7]. Carrageenan has the characteristics of being soluble in hot water (70°C), easily available on the market, and relatively inexpensive. Previous research showed that 0.3% of carrageenan significantly affected jelly drink and
sweeteners (sucralose and stevia) needed to improve the taste of okra mucus, which are anti-diabetic and low in calories. Stevia sucralose sweetener does not affect the blood glucose level [8]. While stevia has sweetness level of 300 times that of sucrose [9].

Therefore, this study aims to determine the characteristics and activities of anti-diabetic jelly drink okra mucus with a formulation of carrageenan and low-calorie sugar and find the best treatment formulation in the manufacture of okra mucilage jelly drink so that it can be produced as an anti-diabetic product.

2. Material and methods
The okra fruit was obtained from plantations in Batu, Malang, while the carrageenan, sucralose, and stevia sugar merck "Tropicana Slim", corn powder 0.004% were obtained from the local market in Rungkut Surabaya. Protease, amylloglucosidase, ethanol 95%, methanol, α-glucosidase enzyme (Sigma Aldrich, USA), PNPG substrate (p-nitrophenyl-α-glucopyranoside) (methanol, α-glucosidase (Sigma Aldrich, USA), sodium phosphate buffer (pH 6.9).

2.1 Jelly drink formulation
The okra fruit was sorted, washed, drained, and cut small pieces, soaked in water (1: 3) for 12 hours at 5°C and filtered to obtain okra mucus. Okra mucus were analyse the yield, viscosity, dietary fibre, anti-diabetic (in vitro), and analysis of phytochemical compounds (screening for alkaloids, flavonoids, tannins, saponin, steroids). 100 ml of okra slime mixed with a predetermined formulation. The mixture was heated for 3 minutes at 70°C and cooled at 40°C for further packaging. Jelly drinks formed were analysed using anti-diabetic (in vitro), total phenol, gel strength, viscosity, total soluble solids, and organoleptic tests (colour, taste, aroma, texture).

The simple Completely Randomized Design used in this research, there are 18 formulations of jelly drink consists of carrageenan (0.10; 0.20 and 0.30%), sucralose and stevia (0.02; 0.03 and 0.04%). Data were analysed by Minitab 1.6 and DMRT 5%.

3. Results and discussion
3.1. Raw material of okra mucus
The results analysis of okra mucus viscosity of 7.33 cPs, anti-diabetic in vitro amounted to 31.31 mg / ml, dietary fibre of 0.49 grams, and 65.39% of yield. The phytochemical showed that the okra mucus contained alkaloid, flavonoid, tannin, saponin and steroid.

3.2. Viscosity, total soluble solids, gel strength, total phenol and IC50
Based on the analysis of variance showed that 18 formulations okra jelly drink gave a significant difference (p ≤ 0.05) on viscosity, total soluble solids and gel strength, total phenol and IC50 can be seen in Table 1.

Table 1 shows the viscosity of jelly drink ranging from 67.00 - 279.50 cPs, it was seen that the viscosity of jelly drink increases with the increase in the addition of carrageenan, because carrageenan has water-binding properties on the okra mucus so that it becomes thicker, the carrageenan is able to form a gel in which polymer chains form a continuous three-dimensional mesh. then this mesh captures or mobilizes water in it and forms a strong and rigid structure, as reported in Saha and Bhattacharya [10]. The addition of sucralose or stevia had no significant effect on the viscosity of the okra mucus jelly drink but showed an increased in the viscosity of the jelly drink. This is due to the sweetener has hydrophilic properties that can bind water [11]. Viscosity is increasing because the water in the okra mucilage jelly drink is bound by sucralose or stevia sugar even in small amounts. This sugar stable at high temperatures and not react chemically with other compounds so that toxic compounds not formed [12-13].

The total soluble solids of jelly drink ranged from 8.5% - 16% (Table 1), the carrageenan one of the factors which influenced the total soluble solids. The increased total soluble solids, because more and more particles are bound by carrageenan and reduce the sediment formed by trapped particles suspended in the system and do not precipitate by the influence of gravity [14]. The addition of sucralose or stevia had no significant effect on the total soluble solids of jelly drink, however, it
causes an increase in the total soluble solids, due to the binding of water to the okra mucilage jelly drink even though in small amounts [13].

The gel strength of the jelly drink ranges from 2.25-4.53 N. It is known that carrageenan can strengthen the formation of gel in the product. The gel forming of carrageenan is thermo-reversible, that the gel can melt during heating and reshape the gel during cooling [15]. The addition of sucralose and stevia also affects the gel strength because the water in the okra mucilage jelly drink is bound by the sugar [11].

| Table 1. Total viscosity soluble solids, gel strength, total phenol and IC₅₀ of jelly drink. |
|---------------------------------|-----------------|----------------|-----------------|-----------------|-----------------|
| **Formulation Jelly drink**     | **Viscosity**   | **Total**      | **Gel**         | **Phenol total**| **IC₅₀**        |
| Carragenan (%)                  | Sugar low calorie | soluble solids | strength (N)    | (mgTAE/100g)    | (mg/ml)         |
| 0.1%                            | Sucralosa       | 0.02           | 74.00⁺          | 8.50⁺           | 2.37⁺           | 60.28⁺           | 43.75⁺           |
|                                 |                 | 0.03           | 67.75⁺          | 9.00⁺           | 2.45⁺           | 60.51⁺           | 42.59⁺           |
|                                 |                 | 0.04           | 69.00⁺          | 9.00⁺           | 2.35⁺           | 60.74⁺           | 42.38⁺           |
|                                 | Stevia          | 0.02           | 67.00⁺          | 10.50⁺          | 2.25⁺           | 68.45⁺           | 38.43⁺           |
|                                 |                 | 0.03           | 71.00⁺          | 9.00⁺           | 2.50⁺           | 73.36⁺           | 36.77⁺           |
| 0.2%                            | Sucralosa       | 0.02           | 108.00⁺         | 12.50⁺          | 4.15⁺           | 60.51⁺           | 42.21⁺           |
|                                 |                 | 0.04           | 112.00⁺         | 12.00⁺          | 4.07⁺           | 60.74⁺           | 42.26⁺           |
|                                 | Stevia          | 0.02           | 114.40⁺         | 12.50⁺          | 4.12⁺           | 67.75⁺           | 38.62⁺           |
| 0.3%                            | Sucralosa       | 0.03           | 125.15⁺         | 12.00⁺          | 4.13⁺           | 73.59⁺           | 36.22⁺           |
|                                 |                 | 0.04           | 127.25⁺         | 13.50⁺          | 4.15⁺           | 76.40⁺           | 33.45⁺           |
|                                 | Stevia          | 0.02           | 275.75⁺         | 14.50⁺          | 4.45⁺           | 60.04⁺           | 43.55⁺           |
|                                 |                 | 0.03           | 276.50⁺         | 15.00⁺          | 4.43⁺           | 60.28⁺           | 41.87⁺           |
|                                 |                 | 0.04           | 274.50⁺         | 16.00⁺          | 4.46⁺           | 59.57⁺           | 42.11⁺           |
|                                 | Stevia          | 0.02           | 258.25⁺         | 15.00⁺          | 4.42⁺           | 68.69⁺           | 38.67⁺           |
|                                 |                 | 0.03           | 287.50⁺         | 16.00⁺          | 4.39⁺           | 72.42⁺           | 35.78⁺           |
|                                 |                 | 0.04           | 279.50⁺         | 15.00⁺          | 4.53⁺           | 76.63⁺           | 32.60⁺           |

Alphabetic describe average value each parameters that means significantly different (p<0.05)

Table 1 also showed that the total phenol jelly drink ranged from 60.28 - 76.63 mgTAE / 100 g. The addition 0.1% carrageenan and 0.02% sucralose gave the lowest while 0.3% carrageenan and 0.04% stevia the highest total phenol. Stevia contains several compounds of steviol glycosides, stevioside and some phenol compounds such as flavonoids, saponin, sterols, and tannins [9,16]. Meanwhile, sucralose is an artificial sweetener that does not contain phenols [17]. Therefore, products with stevia formulations have a higher total phenol value than sucralose. The addition of carrageenan to the okra mucilage jelly drink had no significant effect on testing the total phenol content because carrageenan does not have phenol compounds [18].

Anti-diabetic properties of the jelly drink were determined in vitro by measuring IC₅₀ values, shown in Table 2. It appears that IC₅₀ jelly drink ranged from 32.60 to 43.60 mg / L. The lower IC50 value indicates the higher α-glucosidase inhibition. Carrageenan contains water-soluble fibers that can inhibit the work of the α-glucosidase enzyme and its substrate due to the formation of lattice such as nets that can trap the enzymes and substrate of the water so that the two do not react with each other as reported by Sabita [6].

The sucralose has no effect on the inhibition of α-glucosidase because there are no phytochemical compounds can inhibit the work of enzymes. According to Tandel [19], sucralose is not used by the body as an energy source because it cannot be broken down. Therefore, sucralose is useful as a sugar substitute for diabetics. While the stevia has a significant effect on the inhibition of α-glucosidase because stevia has phytochemical compounds that can inhibit α-glucosidase [20].
The stevia can be increased in the IC$_{50}$ because there are phytochemical compounds that have the ability to inhibit the enzyme $\alpha$-glucosidase [20-22]. According to Kim [23], most $\alpha$-glucosidase inhibitors work by imitating the transition position of the pyranosidic unit from the natural glucosidase substrate, so it is suspected that the inhibitory mechanism is in the form of competitive inhibition.

Inhibition of IC$_{50}$ $\alpha$-glucosidase by rootbose in the research by Cetto and Heinrich [24] showed a value of 12.80 mg / ml lower than the results of the lowest okra mucilage jelly drink of 32.60 mg / ml in the formulation of carrageenan 0.3% and stevia sugar 0.04%. This is because the process of making jelly drink okra slime underwent a heating process so that a lot of phenol content is reduced in the activity. Flavonoid compounds are not heat resistant and are easily oxidized at high temperatures [25], so the ability to inhibit the activity of $\alpha$-glucosidase will be reduced. Acarbose is a competitive $\alpha$-glucosidase oligosaccharide compound used as an oral treatment for patients with type 2 anti-diabetic mellitus.

3.3. Organoleptic test
The organoleptic test is carried out by using the hedonic scale method, including taste, texture, the jelly aroma and colour (Table 1), which shows that carrageenan formula 0.3% and stevia sugar 0.04% are jelly drink products that are preferred by panelists, because they have the highest score, taste, texture, aroma and colour with taste criteria (favoured), texture (such as commercial jelly drinks), aroma (favoured) and colour (no influence).

| Formulation Jelly drink | Ranking of Carrageenan (%) | Sugar low calorie | Taste | Texture | Flavor | Colour |
|-------------------------|---------------------------|------------------|-------|---------|--------|--------|
|                         |                           |                  |       |         |        |        |
| 0.1%                    | Sucralose                 | 0.02             | 188.50| 139.50  | 188.50 | 197.00 |
|                         |                           | 0.03             | 133.50| 150.20  | 133.50 | 174.30 |
|                         | Stevia                    | 0.04             | 154.00| 128.30  | 154.00 | 196.00 |
|                         |                           | 0.02             | 164.00| 219.00  | 164.00 | 201.50 |
| 0.2%                    | Sucralose                 | 0.03             | 195.00| 217.20  | 195.00 | 197.00 |
|                         |                           | 0.04             | 210.00| 204.20  | 210.00 | 203.00 |
|                         | Stevia                    | 0.02             | 223.00| 231.60  | 223.00 | 233.00 |
| 0.3%                    | Sucralose                 | 0.03             | 228.00| 247.70  | 228.00 | 202.00 |
|                         |                           | 0.02             | 227.00| 271.70  | 227.00 | 194.00 |
|                         | Stevia                    | 0.03             | 196.00| 130.30  | 196.00 | 164.80 |
|                         |                           | 0.04             | 153.00| 123.70  | 153.00 | 168.00 |
|                         |                           | 0.02             | 167.50| 113.20  | 167.50 | 209.50 |
|                         |                           | 0.04             | 158.50| 180.20  | 180.00 | 167.00 |
|                         | Stevia                    | 0.03             | 176.00| 163.60  | 144.00 | 203.00 |
|                         |                           | 0.02             | 225.60| 217.40  | 217.40 | 235.00 |
|                         |                           | 0.04             | 242.50| 246.00  | 197.00 | 189.00 |
|                         | Stevia                    | 0.03             | 190.00| 225.60  | 118.00 | 197.00 |
|                         |                           | 0.04             | 256.00| 258.20  | 235.00 | 215.50 |

4. Conclusions
The okra mucous jelly drink with a formulation of 0.3% carrageenan and 0.04% stevia sugar is the best formulation, viscosity results obtained 279.50 cPs, total soluble solids 15.00%, gel strength 4.53 N, total phenol 76.63 mgTAE / g, antidiabetic test (IC$_{50}$) result was 32.60 mg / ml and food fibre 1.29 g / 100g. Organoleptic jelly drink test results that have the highest value are the treatment of addition of carrageenan 0.3% and stevia 0.04% with an average score of taste 2.8 (un-favored), texture 3.7 (favoured), aroma 2.75 (un-favoured), and colour 2.95 (un-favoured).
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