International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 3, 2024

Original Article

OPTIMIZATION OF SUGARCANE (*SACCHARUM OFFICINARUM l.*) JUICE MICROFILTRATION FOR STORAGE STABILITY AND ANTIHYPERGLYCEMIC ACTIVITY

TETI INDRAWATI[1], ZAINUR RAHMAN HAKIM[2*], RAHMI HUTABARAT[1], RATNA DJAMIL[2], SISTER SIANTURI[1], MEGAH INDAH DWITA[1], IMANULLAH[1]

[1]Department of Pharmacy, Faculty of Pharmacy, National Institute of Science and Technology, Moch. Kahfi II St., Bhumi Srengseng Indah No.13, Jagakarsa-12630, South Jakarta, Indonesia. [2]Pharmacognosy and Phytochemistry laboratory, Faculty of Pharmacy, Universitas Pancasila, Srengseng Sawah, Jagakarsa-12640, South Jakarta, Indonesia

[*]Corresponding author: Zainur Rahman Hakim; [*]Email: zainurrahmanhakim@univpancasila.ac.id

Received: 05 Dec 2023, Revised and Accepted: 12 Jun 2024

ABSTRACT

Objective: Sugarcane juice (*Saccharum officinarum l.*) is a natural source of liquid carbohydrates traditionally used for health and help with diabetes. The content of flavonoids, octacosanol, and saccharic compounds in sugarcane juice is proven to have antihyperglycemic activity, so it is safe to use by diabetics. Unfortunately, sugarcane juice is only stable for 4 h at room temperature. The study aimed to improve sugarcane juice's stability and obtain data on its antihyperglycemic effect.

Methods: This research was conducted by making sugarcane juice, which was then carried out sterilization by microfiltration. The sugarcane juice is then packaged in a special sterile plastic. The results of sugarcane juice storage were then tested for stability at a certain time and antihyperglycemic effectiveness in mice.

Results: The results showed that the stability of sugarcane juice can be extended up to 168 h. Sugarcane juice (*Saccharum officinarum l.*) at a dose of 5.6 mg/20 g Bodyweight, a dose of 11.2 mg/20 g Bodyweight, and a dose of 16.8 mg/20 g Bodyweight has an antihyperglycemic effect.

Conclusion: Microfiltration techniques can be used to increase the shelf life of sugarcane juice and maintain its qualities as an antihyperglycemic for quite a long time.

Keywords: Antihyperglycemic, Microfiltration, *Saccharum officinarum l.*, Stability

 DOI: https://dx.doi.org/10.22159/ijap.2024.v16s3.06 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Sugarcane (*Saccharum officinarum l.*) is a plant that grows well in Indonesia and is known as an ingredient to produce white sugar. Sugarcane plantations in Indonesia cover an area of around 321 thousand hectares [1]. In addition to white sugar, other uses of sugar cane are used as refreshing drinks in the form of cane juice. Sugarcane juice (*Saccharum officinarum l.*) is a liquid extracted from sugarcane that has a greenish-brown color. Sugarcane juice is a natural liquid as a source of carbohydrates that is often consumed by people in tropical and subtropical regions. Sugarcane juice contains carbohydrates, flavonoids, tannins, vitamins, and minerals [2]. Sugarcane juice (*Saccharum officinarum l.*) has been reported to contain antioxidant properties of phenol and flavonoids that can prevent fat peroxidation, prevent iron oxidation, and capture free radicals due to the phenol and flavonoid content [3, 4].

Sugarcane juice is also beneficial for health that is used to help treat the lungs, and some tumors and heal wounds [2]. In addition, the use of sugarcane juice is also intended for the treatment of hyperglycemia [5]. Meanwhile, hyperglycemia is a condition in which blood sugar levels increase or are excessive, which affects the initiation of diabetes [5]. The combination of sugarcane juice and noni at a dose of 400 mg/kg was able to reduce blood glucose levels in rats [5]. The flavonoid content is thought to have antihyperglycemic activity. Flavonoids act as antihyperglycemic by regenerating damaged pancreatic beta cells and stimulating pancreatic beta cells to produce insulin [4, 6]. The various contains of fatty acid, alcohol, phytosterols, higher terpenoids, flavonoids, -O- and -C-glycosides, and phenolic acids antioxidants that can prevent pancreatic cell damage due to alloxan induction [3, 5, 7].

Unfortunately, sugarcane juice has the property of not being stored for long at room temperature; this compound is damaged when the heating process becomes a disaccharide, namely sucrose. When stored at room temperature, the pH drops within 4 h. This is due to the fermentation process by yeast and within 4 h the pH decreases [8]. If sugarcane juice is pasteurized, it will change its quality because the pasteurization process reduces the pH of the juice to 3-4 and this pH range is not suitable for microbial development associated with most foodborne diseases [9].

The stability of sugarcane juice can be maintained by adding preservatives as has been done in other ingredients, namely sap water. Preservative sodium benzoate drink (NaC7H5O2) with code E11. Sodium benzoate preservative can maintain a high palm sap pH value at pH 5.59 and 0.2 g/l quicklime can maintain a high palm sap pH value at pH 5.32 [10, 11]. However, this study wants to get product quality without preservatives, one of which is by microfiltration method. Microfiltration technology is a cold sterilization technique so that it can withstand all microorganisms and spores [12]. This technique has been successfully applied by the World Agricultural Organization, FAO in processing isotonic drinks from water. This technique has the advantage of short processing time, and the content, aroma, and taste of the product can be maintained. Based on the above background, the purpose of this study time was to optimize the stability of sugarcane juice storage by microfiltration techniques and see the effectiveness of antihyperglycemic in test animals.

MATERIALS AND METHODS

Materials

Sugarcane plants *(Saccharum officinarum l.*) were obtained in Cipedak, Jagakarsa, Jakarta, Indonesia, and determined at the Plant Conservation Research Center, Bogor Botanical Gardens, West Java, Indonesia by specimen number B-2449/IPH.3/KS/VII/2018. 70% ethanol, 25% ammonia (NH3), chloroform (CHCl3), hydrochloric acid 2 N (HCl), Mayer's reagent, Dragendorff reagent, Bauchardat reagent, 15% sodium nitrite solution (NaNO2), aluminium chloride (AlCl3), sodium hydroxide (NaOH) 1 N, methanol, ethyl acetate, water, 5% solution of AlCl3 in methanol, 1% gelatin in NaCl, acetic acid anhydride, iron (III) chloride (FeCl3) 1%, ether, concentrated sulfuric acid (H2SO4), iodine 0.1 N. The ingredients used for the

anti-diabetic effect test are: Glibenclamide, distilled water, CMC Na 0.5%, pure sugarcane juice (*Saccharum officinarum l.*), distilled water, concentrated sulfuric acid, phenol 5%, sodium hydroxide, HCL 10%, phenolphthalein, plate count agar (PCA), and standard glucose. All chemicals were purchased from Brataco Chemical, Jakarta, Indonesia. All reagents and chemicals used were of analytical grade. pH meter (PHH221 model, Omega USA), Plastic container.

Animals

The animals used in this study were male white mice (*Mus musculus* l.) Dutche Danken Yoken (DDY) strain aged 2-3 mo and weighing 20-30 g. Experimental animals were certified and obtained from Animal Farm, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Indonesia. This study has been approved by the research ethics committee, faculty of Medicine, University of Indonesia with number 1110/UN2. F1/ETIK/2018.

Production of sugarcane juice (*Saccharum officinarum l.*)

The stems of the sugarcane plant are cleaned until all the skins are peeled off. The sample is washed and ground in a sugarcane grinder until water or sugarcane juice comes out, then collected and sterilized by microfiltration and immediately placed in a sterile plastic container [13, 14].

Phytochemical screening

This screening was due to identified different classes of phytoconstituents that are present naturally in plants. This examination was carried out based on a method carried out by previous studies on sugarcane consisting of the identification of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids [3, 15].

Identification of alkaloids

As much as 2 g of sugarcane juice is moistened with 5 ml of 25% ammonia in a beaker glass, then 20 ml of chloroform is added until the mass is submerged, stirred, and heated over a water bath, then filtered. The filtrate evaporated by half. The remaining evaporation was poured into a test tube, and 3 drops of 2 N hydrochloric acid were added, then shaken and allowed to form 2 layers, the clear layer formed was put into 3 test tubes with the same amount. Then Mayer's reagent was added to tube 1, Bouchardat's reagent to tube 2, and Dragendorff's reagent to tube 3. The positive result contained alkaloids which were indicated by the formation of a white precipitate on Mayer's reagent, a brown precipitate on Bouchardat's reagent, and a red precipitate on Dragendorff's reagent [15].

Identification of flavonoids

As much as 1 g of sugarcane juice is extracted with 100 ml of hot water and then filtered. Take 5 ml of the filtrate, put it in a test tube, add 1 ml of 5% sodium nitrite solution and 1 ml of 10% aluminium chloride, shake, and then add 2 ml of 1 N sodium hydroxide through the tube wall. The formation of red, yellow, or orange in the lower layer indicates the presence of flavonoids [15].

Identification of saponins

As much as 1 g of sugarcane juice is extracted with 100 ml of hot water and then filtered. 10 ml of filtrate was put into a test tube and shaken vertically for 10 s. The presence of saponins was indicated by the formation of firm froth as high as 1 to 10 cm. On the addition of 1 drop of 2 N hydrochloric acid the foam did not disappear, so this indicated the presence of saponins.

Identification of tannins

Add 1 g of sugarcane juice to 100 ml of hot water and then strain. Take 5 ml of each filtrate and put it in a test tube; then in the first test tube add a few drops of 1% ferric (III) chloride solution so that a green or black color is formed if it is positive for tannins while in the second test tube, 3 drops of salt solution are added 1% gelatine in NaCl. If a white precipitate forms, this indicates the presence of the tannin group.

Identification of steroids/triterpenoids

As much as 1 g of sugarcane juice was macerated with 20 ml of ether for 2 h, then filtered and evaporated in an evaporating cup to obtain a residue; add 2 drops of acetic anhydride and 2 ml of chloroform into the residue, then transfer it into a test tube. Then, slowly add 1 ml of concentrated sulfuric acid (Lieberman-Buchard) through the tube wall. Look at the layers of the ring that are formed. The formation of a purple ring indicates the presence of triterpenoids, whereas a green color indicates the presence of steroids.

Total sugar test

Put 1 ml of clear liquid into a test tube, add 3 ml of 3,5-dinitrosalicylic acid (DNS) reagent, then heat it for 5 min in a water bath and let it cool down to measure the absorbance value with a spectrophotometer (λ = 550 nm) [16]. The standard curve was made using a standard glucose solution in the range of 0.2 – 5 mg/ml.

% total sugar = 0.95 x % sugar after inversion (as sucrose) …. (1)

% sucrose = 0.95 x % sugar (after – before inversion) … (2)

Total acid analysis

The material is weighed as much as 10 g, put into a beaker glass, and added distilled water to a volume of 100 ml. Stir until evenly distributed and filtered with filter paper. Take the filtrate as much as 10 ml and put it in an Erlenmeyer and add Phenolphthalein 1% 2-3 drops. Then titrate using 0.1N NaOH. The titration was stopped when a stable pink color appeared [17].

$$\text{Total acid} = \frac{\text{ml NaOH x N NaOH x MW of dominant acid x FP x 100 \%}}{\text{Sample weight (g) x 1000 x valence of acid}} \ldots (3)$$

FP = Dilution Factor

Analysis of the pH of the solution using a pH meter

As much as 20 ml of sugarcane juice was taken into a beaker glass; then, the pH was measured using a pH meter (PHH221 model, Omega USA), which was first calibrated at pH 4 with a pH 4 buffer [18].

Determination of ash content

Each sample and glass were weighed as much as 10 g with a porcelain cup and put into the oven at 50 °C until constant drying. Then it is put into the muffle furnace and burned at 100 °C for 1 h, followed by a temperature of 300 °C for 2 h, and followed by a temperature of 550 °C for 2 h [19, 20]. The ash obtained is then cooled and weighed. The formula obtains ash content:

$$\text{Percent(\%)Ash (Wb)x} = \frac{W2-W0}{W1} \text{x } 10 \ldots (4)$$

W0= weight of crucible, W1= weight of sample, W2= weight of crucible+ash

$$\text{Percent (\%)Ash (DWb)x} = \frac{\text{Percent Ash}}{(100-\text{Percent Moiture})} \text{ x100 } \ldots . (5)$$

Note: Wb (Wet basis), DWb (Dry weight basis)

Total dissolved solids

The material is diluted first. Then it is dripped onto the lens of the hand refractometer. The number that is read between the light and dark limits is the TSS of the material multiplied by the dilution factor in Brix [21].

Determination of total microbes with the total plate count method

The material was taken as much as 1 ml and put into a test tube then added 9 ml of sterile aquadest and stirred until evenly distributed. The results of this dilution were taken 1 ml with a volume pipette then added 9 ml of aquadest. This dilution was carried out to 10^{-3}. From the results of the dilution in the last test tube, 1 ml was taken and spread on the prepared PCA agar medium on a Petri dish plate, then incubated for 2x24 h at 32 °C in an inverted position [22]. The number of existing colonies is counted with a colony counter.

Total colonies = Calculated number of colonies x 1 DF..... (6)

Note: DF= Dilution Factor.

Stability test

The stability test was carried out by storing sterilized cane juice by filtering using a 0.2 µm (Millipore, USA) membrane and putting it in sterile plastic (Javamed, Indonesia), then storing it at 25 °C and 5 °C for 4 d and evaluating organoleptic, sugar content, and acid content every day. Total ash content, pH, total dissolved solids, and total microbes [23–26]. The results obtained were analyzed statistically using the 3-way Analysis of Variance technique (Three-way ANOVA).

Antihyperglycemic test

The antihyperglycemic effectiveness test was carried out by administering 125 mg/kg of alloxan orally to 25 male mice (divided into 5 groups) to increase blood sugar levels; after 48 h, each group of mice was given CMC Na 0.5%, Glibenclamide 0.013 mg/20 g Bodyweight, sugar cane juice 5.6 mg/20 g Bodyweight, 11.2 mg/20 g Bodyweight and 16.8 mg/20 g Bodyweight. Blood sugar levels of mice were determined on days 1, 3, 7, and 14. Data on decreasing blood sugar levels were analysed using the ANOVA statistical test followed by lSD at a 95% confidence level [27, 28].

RESULTS AND DISCUSSION

Sugarcane juice (*Saccharum officinarum l.*) has the characteristics is light brown in color, odorless, and sweet (table 1). The characteristics of sugarcane juice obtained from this research are almost the same as the characteristics of sugarcane juice obtained by Singh [7]; the total sugar was 12-18%, the total dissolved solids between 10.0-18.5 °Brix. The results of these studies showed that the pH was more acidic (5.13 and 5.18). This result difference can occur due to the presence of microorganisms in the juice that is not sterilized. The shape, taste, and smell of cane juice from sterilized cane juice are the same that is liquid, odorless, and sweet. The color of cane juice that is directly sterilized is yellow-brown, while that which is not sterilized is light brown. This is possible because the unsterilized sugar cane juice is influenced by bacteria (3.65 logs CFU/ml) so that apart from the color changing to light brown, the total sugar is greater, the total acid value decreases as well as the lower the pH and the pH is more acidic (5.13) than the pH that was sterilized [29].

Table 1: The characteristics of sugar cane juice and sterile

Characteristics	Sugarcane juice	Sterile sugarcane juice
Form	Liquid	Liquid
Color	Light brown	Yellowish brown
Smell	Odorless	Odorless
Flavor	Sweet	sweet
Total Sugar (%)	16.18	16.5
Total Acid (%)	0.98	1.01
Ash Content (%)	0.17	0.16
Total Dissolved Solids (o Brix)	10.68	10.86
Total Microbes (Log CFU/ml)	3.65	0
pH	5.13	5.18

The characteristics of the sugarcane juice obtained from this study are like the characteristics of the sugarcane juice used by a previous study [30], namely, the total sugar obtained during the study ranged from 12-18%, the total dissolved solids between 10.0-18.5 °Brix, and for a pH of 5.25. Slight differences occur allegedly due to the location of sampling and harvesting age of the samples. The test results for the effect of reducing blood glucose levels of sugarcane juice (*Saccharum officinarum l.*) on male mice using the alloxan induction method can be seen in table 6. Observation of the increase and decrease in blood glucose levels (KGD) in the administration of the test material, namely sugarcane juice (*Saccharum officinarum l.*) with a variation of the dose of 5.6 mg/20 g Bodyweight; 11.2 mg/20 g Bodyweight and 16.8 mg/20 g Bodyweight. Observation time after administration of alloxan on days 1, 3, 7, and 14.

Table 2: Sugar cane phytochemical screening

No	Identification	Observation results	Remarks
1	Alkaloids	Mayer's: white precipitate is formed	(+)
		Bouchardat: brown precipitate formed	(+)
		Dragendorff: brick red precipitate formed	(+)
2	Flavonoids	Reddish-orange color is formed	(+)
3	Saponins	Stable foam is formed	(+)
4	Tanin	FeCL3 1%: green color is formed	(+)
		Gelatin 1%: white precipitate is formed	(+)
5	Triterpenoid	A red ring is formed	(+)

The results of the phytochemical screening qualitatively showed that sugarcane juice contains alkaloids, flavonoids, saponins, tannins, and triterpenoids. The results can be seen in table 2. In testing the alkaloids, a precipitation reaction occurred due to the replacement of the ligand. Nitrogen atoms that have lone pairs of electrons in alkaloids can replace iodine ions in Dragendorff's reagent and Mayer's reagent. In the flavonoid test, there was a red color change due to the formation of flavylium salts. Saponins contain glycosyl groups, which act as polar groups and steroid and triterpenoid groups, which function as non-polar groups. Compounds that have polar and non-polar groups will be surface active so that when shaken with water, saponins can form micelles, where the polar structure will face outward while the nonpolar group will face inward. In this condition, saponins will form like foam. The color change that occurs in the tannin test occurs when $FeCl_3$ is added, which reacts with one of the hydroxyl groups present in the tannin compound. In testing steroids and triterpenoids, the analysis of compounds is based on their ability to form a color with concentrated H2SO4 in acetic anhydride solvent [7, 15].

The results of organoleptic tests on the stability test of sugarcane juice can be seen in tables 3, 4, and 5, as well as fig. 1, 2 and 3. In non-sterile samples stored both at room temperature and cold temperatures, changes in shape, color, and odor occurred. The changes that occur in sugarcane juice are caused by the activity of microorganisms in non-sterile sugarcane juice. This activity is included in the fermentation process. As a result, there is a decrease in the physicochemical quality of sugarcane juice, one of which is the

color component of sugarcane juice. The decline in the physicochemical and microbiological quality of sugar cane juice is mainly due to the microbial content [8]. Sugarcane juice is an excellent living medium for microbes, bacteria, yeast, and mold. These microbes utilize sucrose and other chemical components to live and reproduce [29].

Table 3: Organoleptic profiles of sugarcane juice stored at 25 °C and 5 °C

No	Treatment	Time (Day)			
		0	3	5	7
1	Non-sterile storage 25 °C				
2	Non-sterile storage 5 °C Non Steril				
3	Sterile storage 25 °C				
4	Sterile storage 5 °C				

Table 4: Sugar cane juice organoleptic test

Sample	Temperature (°C)	Time(H)	Form	Color	Odor	Taste
Non-sterile	25	0	Liquid	Light brown	Odorless	Sweet
		3	Liquid	Cloudy Brown	Odor	Sour Odor
		5	Liquid	Dark brown	Odor	Sour
		7	Liquid	Dark brown	Odor	Sour
	5	0	Liquid	Light brown	Odorless	Sweet
		3	Liquid	Cloudy brown	Odor	Sweet sour
		5	Liquid	Dark brown	Odor	Sour
		7	Liquid	Dark brown	Odor	Sour
Sterile	25	0	Liquid	Liquid Yellowish	Odorless	Sweet
		3	Liquid	Liquid Yellowish	Odorless	Sweet
		5	Liquid	Liquid Yellowish	Odorless	Sweet
		7	Liquid	Liquid Yellowish	Odorless	Sweet
	5	0	Liquid	Liquid Yellowish	Odorless	Sweet
		3	Liquid	Liquid Yellowish	Odorless	Sweet
		5	Liquid	Liquid Yellowish	Odorless	Sweet
		7	Liquid	Liquid Yellowish	Odorless	Sweet

In sterilized cane juice, there is no change in shape, color, smell, or taste in the organoleptic test (table 4). This is because, in sterile cane juice, no microbes are found, thus, there is no decrease in quality both physicochemical and microbiological.

Table 5: Sugar cane juice stability test

Sample	Storage temp (°C)	Storage time (days)	Total sugar (%)	Total acid (%)	Ash content (%)	Total dissolved solids (%)	pH	Total microbes (Log CFU/ml)
Non-sterile	25	0	16.82±11.894	0.98±7.717	#±7.887	10.68±4.688	5.13±3.729	3.64±0.063
		3	10.12±5.035	1.38±2.584	#±1.707	11.55±6.960	3.87±4.270	3.74±0.374
		5	7.28±1.612	1.68±0.048	#±0.079	12.12±8.514	3.68±5.297	3.70±1.129
		7	3.93±2.171	2.15±0.015	#±0.103	13.05±9.155	3.35±5.796	3.66±1.510
	5	0	16.44±11.625	0.99±7.520	#±5.197	10.72±3.905	5.16±3.043	3.60±0.394
		3	11.22±5.812	1.28±3.205	#±2.146	11.56±6.657	4.66±3.989	3.64±0.247
		5	9.13±2.920	1.43±1.054	#±0.625	12.20±8.185	3.98±5.031	3.60±1.012
		7	7.14±0.099	1.72±1.146	#±0.697	12.88±8.614	3.56±5.400	3.57±1.294
Sterile	25	0	16.50±11.667	1.01±7.536	#±0.000	10.86±7.679	5.18±4.586	0±2.541
		3	15.45±8.803	1.12±5.433	#±3.729	11.85±5.743	5.12±3.593	0±2.541
		5	14.38±6.633	1.23±3.820	#±2.588	12.27±6.846	5.06±4.109	0±3.400
		7	13.43±4.547	1.32±2.282	#±1.507	12.85±8.021	4.99±4.809	0±3.400
	5	0	17.12±12.106	1.03±7.832	#±5.425	10.95±3.907	5.20±3.125	0±2.425
		3	16.55±9.581	1.08±6.011	#±4.138	11.65±5.312	5.16±3.430	0±2.425
		5	15.96±7.750	1.12±4.688	#±3.202	11.98±6.207	5.12±3.779	0±3.045
		7	15.39±5.933	1.17±3.368	#±2.275	12.46±7.202	5.08±4.306	0±3.045

Data are expressed mean±SD ($p<0.05$).

Table 5 and fig. 1 to 6 show the interaction of temperature and time storage Factors on Total sugar, Total Acid, ash, pH, Total dissolved solids, and Total Microbes. Based on fig. 1, sterilization can maintain the total sugar content of the cane juice during storage. This shows

that the sterilization process can inhibit the factors that can cause a decrease in the quality of cane juice during the shelf life. The inhibition of the decrease in total sugar content is caused by the sterilization process of sugar cane juice, the microbes that cause damage to the cane juice are filtered using a Millipore membrane with a diameter of 0.2 µm so that the juice certainly that the sugar cane does not contain microbes.

The total sugar produced is not much different from the total value of sugar cane juice obtained by Filianty [29] who obtained a total sugar content of 19.29% before storage and experienced a smaller decrease during storage at 5 °C when compared to the total sugar content in cane juice stored at 25 °C. This is because the temperature of 25 °C is the optimum temperature for microbial growth; thus, the decrease in total sugar content will be drastically more significant.

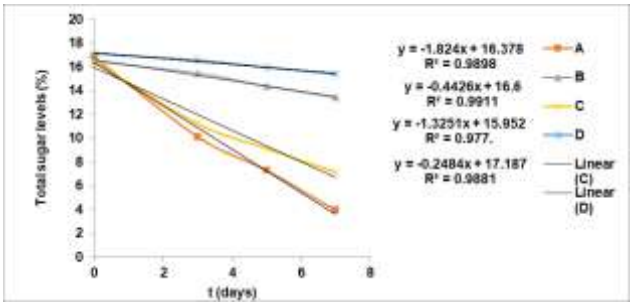

Fig. 1: Interaction of temperature and storage factors on total sugar. A = total sugar of non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = Total sugar of sterile cane juice at a storage temperature of 25 °C for 7 d; C = Total sugar of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = Total sugar of sterile cane juice at a storage temperature of 5 °C for 7 d

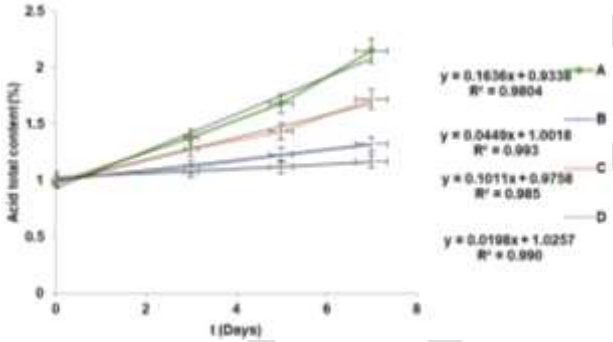

Fig. 2: Interaction of temperature and storage factors on total acid. A = total acid of non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = total acid of sterile cane juice at a storage temperature of 25 °C for 7 d; C = total acid of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = total acid of sterile cane juice at a storage temperature of 5 °C for 7 d

The increase of total acid in sugarcane juice occurred during the storage process. The highest increase in total acid was found in non-sterile cane juice stored at 25 °C. This shows that the activity of microorganisms in sugar cane juice has changed the sugars in cane juice into organic acids. The increase in total acid is a result of the fermentation process of microorganisms. The cause of the increase in total acid levels in unsterilized sugar cane juice is the absence of barriers for microbes to move. This contaminated sugarcane juice can undergo further fermentation reactions to produce alcohol and organic acids [8, 29].

The increase in total acid value was followed by a decrease in the pH value of sugar cane juice. As seen from fig. 2, a very drastic increase in total acid occurred in unsterilized cane juice; this also shows that the pH of non-sterilized cane juice is also low. The increase in total acid and decrease in pH are the results given from the activity of microorganisms in the fermentation process in high sugarcane juice. This is by the study of Irawan, which states that sap has properties that are not resistant to storage; after 4 h there will be a decrease in pH, this is due to the fermentation process by yeast [29]. To prevent the fermentation process from occurring during storage, it is necessary to find the best way to maintain the quality of sugar cane juice [8, 29].

There is no significant difference in the ash content of non-sterile sugarcane juice or the ash content of sterile sugarcane juice, whether stored at 25 °C or 5 °C. Physical treatment and storage time had very significantly different effects on all parameters except for ash content, which was not significantly different [29].

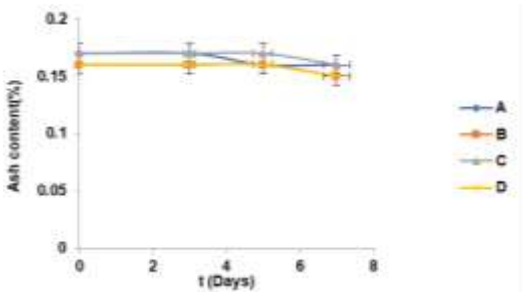

Fig. 3: Interaction of temperature and storage factors on ash content sugarcane, A = ash content non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = ash content of sterile cane juice at a storage temperature of 25 °C for 7 d; C = ash content of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = ash content of sterile cane juice at a storage temperature of 5 °C for 7 d

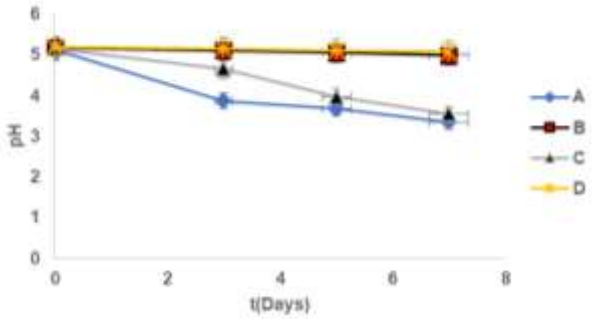

Fig. 4: Interaction of temperature and storage factors on pH content of sugarcane. pH non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = pH of sterile cane juice at a storage temperature of 25 °C for 7 d; C = pH of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = pH of sterile cane juice at a storage temperature of 5 °C for 7 d

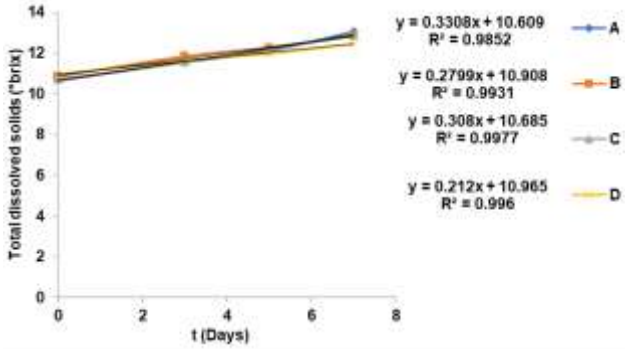

Fig. 5: Interaction of temperature and storage factors on sugarcane total dissolved solid content, A= total dissolve solid of non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = total dissolve solid of sterile cane juice at a storage temperature of 25 °C for 7 d; C = total dissolve solid of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = total dissolve solid of sterile cane juice at a storage temperature of 5 °C for 7 d

The characteristics of the sugarcane juice obtained from this study are like the characteristics of the sugarcane juice used in a previous study, namely the total sugar obtained during the study ranges from 12-18%, the total dissolved solids between 10.0-18.5 °Brix, and for a pH of 5.25. Slight differences occur allegedly due to the location of sampling and harvesting age of the samples [25, 29].

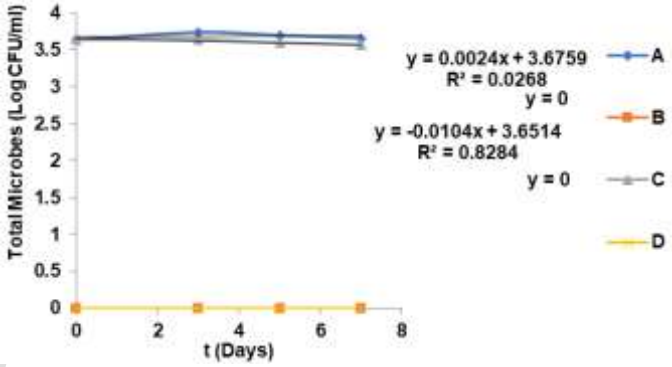

Fig. 6: Interaction of temperature and storage factors on total microbes (Log CFU/ml) content. A= total microbes of non-sterile cane sugar at a storage temperature of 25 °C for 7 d; B = total microbes of sterile cane juice at a storage temperature of 25 °C for 7 d; C = total microbes of non-sterile cane juice at a storage temperature of 5 °C for 7 d; D = total microbes of sterile cane juice at a storage temperature of 5 °C for 7 d

Fig. 6 shows the fluctuation in the number of microbes from sterile and non-sterile sugarcane juice from day 0 to 7. In unsterilized sugarcane juice at a storage temperature of 25 °C (from 3.64 to 3.66) and 5 °C (from 3.64 to 3.57 on the 7th d, there was a decrease in the number of microbes, which shows that the microbes have gone through all phases of their life. Starting from the lag phase (adaptation phase), which occurred on day 0 and the optimum phase on day 3, then on day 5 the number of microbes began to decrease until 7 d. This shows that the substrate on day 5 has been used up so that the microbes gradually decrease. Meanwhile, sugar cane juice has been sterilized using a filtration method using a Millipore membrane with a diameter of 0.2 µm. showed that there was no microbial growth in the sugarcane juice.

This is because the existing microbes are trapped on the Millipore membrane, making the sugarcane juice sterile. The results of this stability test showed that sterilization using the filtration method can prevent microbial growth in sugar cane and storage at 25 °C and 5 °C can be used to maintain sugarcane stability.

The test results for the effect of reducing blood glucose levels of sugarcane juice (*Saccharum officinarum l.*) on male mice using the alloxan induction method can be seen in table 6. Show the result of the increase and decrease in blood glucose levels in the administration of the test material, namely sugarcane juice (*Saccharum officinarum l.*) with a variation of the dose of 5.6 mg/20 g Bodyweight; 11.2 mg/20 g Bodyweight and 16.8 mg/20 g Bodyweight. Observation time after administration of alloxan on days 1, 3, 7, and 14.

Table 6: Blood sugar levels in mice after alloxan induction

No	Treatment	Blood sugar levels (mg/dl)					
		Random	Fast	1 d	3 d	7 d	14 d
1	Na. CMC	126.6±10.0	88.0±5.34	234.0±15.81	194.6±13.89	161.4±12.68	124.6±6.69
2	Glibenclamide	131.4±7.14	88.2±7.31	236.4±10.78	172.0±12.20	146.0±10.75	127.6±9.22
3	Sugarcane 5.6 mg/20gbw	130.0±6.78	86.2±7.79	235.4±8.76	173.6±8.02	147.0±6.89	131.0±6.78
4	Sugarcane 11.2 mg/20gbw	130.0±6.56	91.4±5.55	225.0±7.97	163.8±8.07	140.6±9.90	124.2±9.01
5	Sugarcane 16.8 mg/20gbw	125.2±5.12	85.6±6.03	225.6±5.60	161.4±5.77	136.6±4.04	119.8±3.49

The effect of reducing blood glucose levels of sugarcane juice. Data are expressed mean±SD, (n=3).

Based on table 6, during fasting and fasting glucose levels, the mice were in normal condition. On 1 d there was an increase for each group, while on 3 d, 7 d, and 14 d there was a decrease in Mice Blood Sugar levels (MBSL). The average MBSL of mice between groups after treatment can be seen in fig. 7. The MBSL of mice on the 3rd, 7th, and 14th d after administration of the MBSL test substance decreased to normal. Each treatment has a different value that can reduce the MBSL of mice.

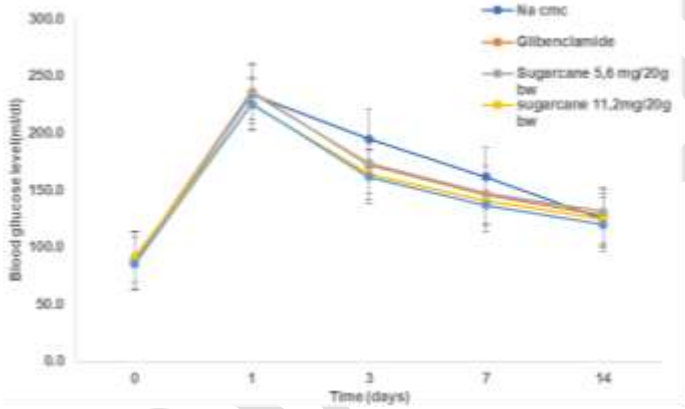

Fig. 7: Profile of mice blood sugar levels after alloxan induction and treatment, data are expressed mean±SD (n=3)

Compounds containing natural ingredients or phytochemicals often have activity in medicine, especially in correcting body disorders associated with oxidative stress [31]. Oxidative stress is proven to be one of the causes of increased blood sugar until the occurrence of diabetes [32]. The presence of alloxan, which is the main trigger of diabetes through oxidative stress pathways [33]. Bioactive compounds from plants are reported to show control of blood sugar levels by various mechanisms such as modulation of antioxidant enzymes [34], lipid homeostasis [35], stimulating insulin secretion [36], increased liver glutathione [37], alpha-glucosidase inhibitors [39], dipeptidyl peptidase IV inhibitors [39], pancreatic lipase [40] and cholesterol esterase [41]. The effect of sugarcane juice in lowering blood sugar is due to its phytochemical content, such as various fatty acids, alcohol, phytosterols, higher terpenoids, flavonoids,-O-and-C-glycosides, and phenolic acids [42].

Studies have shown that naturally concentrated polyphenols from sugarcane extract have inhibitory effects on glucose and fructose absorption, transporter expression, and insulin restoration ability in cell culture. This is due to the role of polyphenol extract from sugarcane juice as an antioxidant [43]. The higher levels of polyphenols in sugar cane juice are closely related to the decrease in the glucose index value [44]. The quality of sugar cane juice can be maintained by microfiltration sterilization without any damage to the phytochemical components. This is very beneficial for diabetes sufferers to get drinks while protecting their condition from rising glucose indexes, which can trigger the bad effects of diabetes [45]. This is very beneficial for diabetes sufferers to get drinks while protecting their condition from rising glucose indexes, which can trigger the bad effects of diabetes.

CONCLUSION

Sterilization using the microfiltration method can maintain the stability of sugarcane juice (*Saccharum officinarum l.*) from 4 h to 7 ds and increase the effectiveness of hyperglycemia in mice can reduce blood sugar levels to normal.

ACKNOWLEDGMENT

The authors would like to thank of National Institute of Science and Technology for providing facilities and all those who have provided thoughts, suggestions, and attention in guiding and directing the writing of this journal.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Teti Indrawati: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. Zainur Rahman Hakim: Validation, Methodology, Writing–review, Editing. Rahmi Hutabarat: Conceptualization, Investigation, Data curation, Funding acquisition, Resources, Formal analysis, Methodology, Writing–review and editing: Ratna Djamil: Review, Validation, Writing–review and editing, Sister Sianturi: Supervision, Methodology, Review, Validation, Writing–review and editing, Megah Indah Dwita: Methodology, Review, Validation, Writing and

editing, Imanullah: Methodology, Review, Validation, Writing and editing.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Directorate general of estates. Tree crop estate statistics of Indonesia 2018-2020: sugar cane. Secretariat of directorate general of estates. Ministry of Agriculture. 2021. p. 1-68.
2. Arif S, Batool A, Nazir W, Khan RS, Khalid N. Physiochemical characteristics nutritional properties and health benefits of sugarcane juice. Nonalcoholic beverages. Sci Beverages. 2019;6:227-57.
3. Feng S, luo Z, Zhang Y, Zhong Z, Lu B. Phytochemical contents and antioxidant capacities of different parts of two sugarcane (Saccharum officinarum L.) cultivars. Food Chem. 2014;151:452-8. doi: 10.1016/j.foodchem.2013.11.057, PMID 24423556.
4. Ali SE, El Gedaily RA, Mocan A, Farag MA, El-Seedi HR. Profiling metabolites and biological activities of sugarcane (*Saccharum officinarum* linn.) juice and its product molasses via a multiplex metabolomics approach. Molecules. 2019;24(5). doi: 10.3390/molecules24050934, PMID 30866484.
5. Kubota H, Shinohara S, Yokoyama D, Tanaka W, Matsuyama H, Arimura T. Suppressive effects of sugarcane molasses concentrate on starch-induced hyperglycemia in mice. J Funct Foods. 2023;107:105652. doi: 10.1016/j.jff.2023.105652.
6. Ojewunmi O. Evaluation of the anti-diabetic and antioxidant activities of aqueous extracts of *Morinda lucida* and *Saccharum officinarum* leaves in alloxan-induced diabetic rats. IJBCRR. 2013;3(3):266-77. doi: 10.9734/IJBCRR/2013/5043.
7. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Busselberg D. Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. Biomolecules. 2019;9(9). doi: 10.3390/biom9090430, PMID 31480505.
8. Singh A, Lal UR, Mukhtar HM, Singh PS, Shah G, Dhawan RK. Phytochemical profile of sugarcane and its potential health aspects. Pharmacogn Rev. 2015;9(17):45-54. doi: 10.4103/0973-7847.156340, PMID 26009693.
9. Filianty F, Raharja S, Suryadarma P. Change of quality in sugar cane (*Saccharum officinarum*) juice using kawao root (*Millettia* sp.) and mangosteen bark (*Garcinia mangostana* l.) as agent preservatives. J Teknol Ind Pertan. 2010;20(1):57-64.
10. Soccol CR, Vandenberghe lP de S, Spier MR, Medeiros ABP, Yamaguishi CT, De Dea lindner J. The potential of probiotics: a review. Food Technol Biotechnol. 2010;48(4):413–34.
11. Musita N, Saptaningtyas WW. The effect of additional natural preservers on juice palm to quality palm sugar. In: Seminar Nasional Balai Riset & Standarisasi Industri Samarinda; 2017. p. 124-34.
12. Tanra N, Syam H, Sukainah A. The effect of additions natural preservatives to the quality of palm sugar (*Arenga pinnata* Merr.) will be result. Pendidik Teknol Pertan. 2019;5:83-96.
13. Zhang S, Liu L, Pang X, Lu J, Kong F, Lv J. Use of microfiltration to improve quality and shelf life of ultra-high temperature milk. J Food Process Preserv. 2016;40(4):707-14. doi: 10.1111/jfpp.12651.
14. Schianoli Frateschi C, Durigan JF, Omir Marques M, Hojo TD, O Santos L, Cunha Junior LC. Storage of sugarcane stalks (Saccharum officinarum cv. SP 79-1011) in low oxygen atmospheres and the effects on enzymatic browning. Postharvest Biol Technol. 2013;86:154-8. doi: 10.1016/j.postharvbio.2013.06.032.
15. Rosidah A, Irham I, Masyhuri M, Perwitasari H. Sugarcane production efficiency: a case study on PTPN X partner farmers. JMA. 2023;20(1):56-67. doi: 10.17358/jma.20.1.56.
16. Widiawati QUL. Phytochemical analysis and determination of total phenolic content in ethanol extract of red sugar cane and green sugar cane (*Saccharum officinarum l.*). J Farm Tinctura. 2023;4(2):91-102.
17. Nguyen TM, Do TA, Dinh TH, Dang Bao T. Quantification of total sugars and reducing sugars of dragon fruit-derived sugar samples by UV-Vis spectrophotometric method. Lam HH. IOP Conf S Earth Environ Sci; 2021.
18. Krishnakumar T, Thamilselvi C, Devadas CT. Effect of delayed extraction and storage on quality of sugarcane juice. Afr J Agric Res. 2013;8(10):930-5.
19. Zaidan NL, Mohamad Fuzi SF, Hailan IM, Roshidi AA, Kormin F, Abu-Bakar MF. Physicochemical and sensory characteristic of treated sugarcane juice. IOP Conf Ser: Earth Environ Sci. 2021;736(1). doi: 10.1088/1755-1315/736/1/012073.
20. Singels A, Jackson P, Inman Bamber G. Sugarcane crop physiology case histories for major crops. Elsevier; 2021. p. 674-713. doi: 10.1016/b978-0-12-819194-1.00021-9.
21. Jackson PA, Schroeder BL, Rattey AR, Wood A, O'Shea MG. Management of ash/impurity ratio in sugarcane: relative effects of genotypes, and N and K fertiliser rates. Aust J Agric Res. 2008;59(9):795-801. doi: 10.1071/AR07387.
22. Khofifah K, Utami M. Analysis of total dissolved solid (TDS) and total suspended solid (TSS) levels in liquid waste from sugar cane industry. Indones J Chem Res. 2022;7(1):43-9.
23. Issa Zacharia A, Rwabunywenge SP. Evaluation of bacteriological quality and safety of sugarcane juice locally processed and vended in Dar es Salaam City, Tanzania. JFSH. 2023;9(4):282-98. doi: 10.18502/jfsh.v9i4.15003.
24. Akbar ANR RH, Murtini ES. Optimizing of pasteurization time and temperature on color profile of sugarcane juice drink. J Pangan Agroindustri. 2018;6(3):48-57.
25. Nadhari WN, Karim NA, Boon JG, Salleh KM, Mustapha A, Hashim R. Sugarcane (Saccharum officinarium L.) bagasse binderless particleboard: effect of hot pressing time study. Mater Today Proc. 2020;31:313-7. doi: 10.1016/j.matpr.2020.06.016.
26. Dewi shinta. The effect of temperature cooking of sugar juice and stirring speed on the quality of brown sugar cane. JTP. 2014;15(3):149-58. doi: 10.21776/ub.jtp.2014.015.03.01.
27. Destriyani l, Tamrin KMZ. Effect of saving age from sugarcane's water storage life to sweet nest level of sugarcane' water. J Tek Pertan Lampung. 2014;3(2):119-26.
28. Chan MC, Tseng JS, Hsu KH, Shih SJ, Yi CY, Wu CL. A minimum blood glucose value less than or equal to 120 mg/dl under glycemic control is associated with increased 14 d mortality in nondiabetic intensive care unit patients with sepsis and stress hyperglycemia. J Crit Care. 2016;34:69-73. doi: 10.1016/j.jcrc.2016.04.002, PMID 27288613.
29. Markus CR, Rogers PJ. Effects of high and low sucrose-containing beverages on blood glucose and hypoglycemic-like symptoms. Physiol Behav. 2020;222:112916. doi: 10.1016/j.physbeh.2020.112916, PMID 32416157.
30. Irawan SA, Ginting S, Karo Karo T. The effect of physical treatment and storage time on the quality of sugar cane juice. JRekayasa Pangan Dan Pert. 2015;3(3):343-53.
31. Basuki B, Sari VK. Specifications of sugar cane varieties based on land characteristic and typology. JTEP-L. 2024;13(1):132. doi: 10.23960/jtep-l.v13i1.132-146.
32. Hamedi Shahraki S, Jowshan MR, Zolghadrpour MA, Amirkhizi F, Asghari S. Dietary phytochemical index is favorably associated with oxidative stress status and cardiovascular risk factors in adults with obesity. Sci Rep. 2023 Apr;13(1):7035. doi: 10.1038/s41598-023-34064-4, PMID 37120685.
33. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-a concise review. Saudi Pharm J. 2016;24(5):547-53. doi: 10.1016/j.jsps.2015.03.013, PMID 27752226.
34. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas). 2017;53(6):365-74. doi: 10.1016/j.medici.2018.02.001, PMID 29548636.
35. Zandalinas SI, Balfagon D, Arbona V, Gomez Cadenas A. Modulation of antioxidant defense system is associated with combined drought and heat stress tolerance in citrus. Front Plant Sci. 2017;8(1):953. doi: 10.3389/fpls.2017.00953, PMID 28638395.
36. Uehara K, Santoleri D, Whitlock AE, Titchenell PM. Insulin regulation of hepatic lipid homeostasis. Compr Physiol. 2023 Jun;13(3):4785-809. doi: 10.1002/cphy.c220015, PMID 37358513.

37. Marenah l, Ali l, Rokeya B, Flatt PR, Abdel Wahab YH. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β-cells. Hannan JMA. J Endocrinol. 2006;189(1):127-36.
38. Nurkhasanah LH, Hakim ZR. Effect of rosella (*Hibiscus sabdariffa* l) extract on glutathione-S-transferase activity in rats. Trop J Pharm Res. 2017;16(10):2411-6. doi: 10.4314/tjpr.v16i10.14.
39. Kumar S, Narwal S, Kumar V, Prakash O. α-glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacogn Rev. 2011;5(9):19-29. doi: 10.4103/0973-7847.79096, PMID 22096315.
40. Prasetiyo A, Hakim ZR, Yamin M, Maulinda ES, Megawati SH. The potency of white tea leaf compouds (*Camelia sinensis* l.) as an enzyme inhibitor (dipeptidyl peptidase) DPP 4. Indones Nat Res Pharm J. 2023;8(2):70-9.
41. Grimmelmann I, Momma M, Zimmer L, Hassel JC, Heinzerling L, Pfohler C. Lipase elevation and type 1 diabetes mellitus related to immune checkpoint inhibitor therapy–a multicentre study of 90 patients from the german dermatooncology group. Eur J Cancer. 2021;149:1-10. doi: 10.1016/j.ejca.2021.02.017, PMID 33812141.
42. Bagheri B, Alikhani A, Mokhtari H, Rasouli M. Esterification of HDL-cholesterol is decreased in diabetes mellitus and CAD and enhanced following treatment with statins. Med Arch. 2018 Jun;72(3):197-201. doi: 10.5455/medarh.2018.72.197-201, PMID 30061766.
43. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Res Int. 2020;132:109114. doi: 10.1016/j.foodres.2020.109114, PMID 32331689.
44. Ji J, Yang X, Flavel M, Shields ZP, Kitchen B. Antioxidant and anti-diabetic functions of a polyphenol-rich sugarcane extract. J Am Coll Nutr. 2019;38(8):670-80. doi: 10.1080/07315724.2019.1587323, PMID 31008696.
45. Flavel M, Neoh J, Lim KF. Dose-dependency of the glycemic response to polyphenol-rich sugarcane extract (PRSE). Biologics. 2023;3(4):308-20. doi: 10.3390/biologics3040016.
46. Chapter HCC. 7-membranes for bioseparations. Yang STBTB for VAP from RR, editor. Bioprocessing for value-added products from renewable resources. Amsterdam: Elsevier; 2007. p. 163-83.