THE IMPACTS OF FORMULATION AND STORAGE ON α-GLUCOSIDASE INHIBITORY ACTIVITY OF LEMONGRASS, GINGER, AND BLACK TEA FUNCTIONAL BEVERAGES

Filiana Santoso, Jennifer Sunardi, Florence Ignatia, Maria Dewi Puspitasari Tirtaningtyas Gunawan-Puteri[*]

Faculty of Life Sciences and Technology, Swiss German University, Tangerang, Indonesia

ABSTRACT

Functional beverages from lemongrass (*Cymbopogon citratus*), white ginger (*Zingiber officinale* Roscoe) and black tea (*Camelia sinensis*) were developed based on their α-glucosidase inhibitory (AGI) activities and sensory acceptance. The AGI was evaluated using in vitro enzymatic assay, while sensory acceptance was tested using affective sensory tests. The evaluation of their aqueous extracts showed that dried lemongrass and ginger possessed higher extraction yield (3.4 %, 2.7 %, respectively), though not necessarily accompanied with a better AGI activity (IC_{50} 24.50 mg/mL, IC_{50} 16.61 mg/mL) than the fresh lemongrass and ginger (2.1 %, 1.8 %, IC_{50} 17.93 mg/ml, IC_{50} >47.00 mg/mL, respectively). Meanwhile, the evaluation of the combined extract showed additive and synergistic effects. The extract combination formula was selected based on the sensory acceptance, resulting in the beverages containing 4.29 mg/mL of lemongrass, 0.71 mg/mL of ginger, and 1.05 mg/mL of black tea with a total phenolic content of 636.45 mg/L Gallic Acid Equivalent (GAE). The selected formula showed the stability of AGI activity andthe pH value at 4 °C were in accordance with the growth of microbial count that was lower than those stored at 25 °C in a 50-day period. Changes in color and °Brix value were not significantly observed in the samples stored at 25 °C and 4 °C. Lime juice was selected as the additional flavoring agent, which could increase both the palatability and AGI activity of the beverages.

Keywords: alpha glucosidase inhibitor; diabetes; functional food; ginger; lemongrass.

INTRODUCTION

International Diabetes Federation (2019) reported Indonesia as the 7th country with the highest diabetes patients in the world, and 90% of them are suffering type 2 diabetes mellitus (T2DM). The management of T2DM employed α-glucosidase inhibitors (AGI) as one of the safest oral antidiabetic agents (Mun'im *et al.,* 2013), which becomes the focus of this paper. They work by inhibiting the enzyme α-glucosidase, which delays the passage of carbohydrates into the bloodstream, thus decreases the postprandial blood glucose level (Amiri *et al.*, 2015). The AGI can naturally be found in many Indonesian plants, such as lemongrass (Gunawan-Puteri *et al.*, 2017), ginger (Oboh *et al.,* 2010), and black tea (Christianty *et al.,*2016),

Lemongrass (*Cymbopogon citratus*) is a prevalent Indonesian food ingredient, which is commonly used in tea and traditional concoctions (Togatorop *et al.*, 2015). The anti-hyperglycemic activity of spray dried aqueous extract of lemongrass was confirmed through both in-vitro and in-vivo methods (Gunawan-Puteri *et al.*, 2018). Several studies were conducted to observe the impacts of pretreatment, such as washing and various drying techniques to the AGI activity of lemongrass extract (Widiputri *et al.*, 2017).

*Corresponding author: Maria DPT Gunawan-Putri
Email: *maria.gunawanputeri@sgu.ac.id*

Various parameters including the extraction solvents, temperature, and post-treatment were found to affect the activities of AGI in lemongrass (Gunawan-Puteri *et al.*, 2017). Lemongrass exhibited anti-hyperglycemic activity in the form of various products, such as tea (Garba *et al.*, 2020) and essential oils (Bharti *et al.*, 2013). The hypoglycemic effect of plant extracts was studied to be correlated with the presence of tannic acids and polyphenols, which stimulates glucose-transport activity and inhibits the alpha-glucosidase enzyme while promoting pancreatic insulin release, respectively (Ademuwiya *et al.,* 2015). Powdered and aqueous extract of lemongrass added in yogurt and ice cream showed AGI activity. However, the heating and cooling processes decreased the inhibiting activity of these models (Santoso *et al.*, 2018). In addition, an industrial approach to lemongrass extract as an antidiabetic food ingredient was also studied (Widiputri *et al.*, 2018).

The use of white ginger (*Zingiber officinale* Roscoe) is popular since the medieval period. It is not only used for culinary purposes, but also studied to exhibit the anti-inflammatory, cholesterol-lowering, anti-thrombotic, and antiemetic properties (Abeysekera *et al.*, 2007). The observation of the anti-hyperglycemia of white ginger extract was also conducted through in-vitro and in-vivo methods. The white and red ginger aqueous extracts indicated an inhibition to the carbohydrate metabolism enzymes such as α-glucosidase and α-amylase (Oboh *et al.*, 2010). Both ethanolic extract of ginger and dried ginger powder consumed by diabetic human patients showed potential for the hyperglycemia treatment (Andallu *et al.*, 2003; Yu *et al*, 2011). The antidiabetic activity of white ginger was studied as the effect of chemical compounds such as gingerol and shogaol (Singh *et al.*, 2009; Priyarani *et al.*, 2011).

Tea (*Camellia sinensis*) is available in various varieties and types based on the degree of fermentation, such as unfermented (green tea), semi-fermented (oolong tea), and fully fermented (black tea) types of tea. Among these three types, black tea is the most widely consumed and produced worldwide (Striegel *et al.*, 2015). Tea has been acknowledged for its medicinal properties, such as preventing heart disease, cancer, and diabetes mellitus (Gardner *et al.*, 2007; Christianty *et al.*, 2016). Both catechins and theaflavins in tea are believed to possess AGI effect. The interaction between flavonoids and yeast alpha-glucosidases was studied and showed that the hydroxyl groups play a major role in the inhibition of such enzymes by binding them to their active sites (Christianty *et al.*, 2016). A study comparing black tea, oolong tea, and green tea discovered that black tea possesses the highest AGI activity of all (Lee *et al.*, 2010).

Different from black tea, which is usually found in a form of dried loose leaf or tea bag, lemongrass and ginger as common herbs and spices can be easily found in fresh and dried forms. For that reason, commercial dried black tea leaves were used in this experiment. As for both lemongrass and ginger, it is important to compare which form resulted in more efficiency. Due to the importance of comparing which form resulted in more efficiency for both the lemongrass and ginger.

The combination of these ingredients is expected to lower the postprandial blood glucose level, in order to be served as a functional beverage. However, when several ingredients are combined, some interactions might happen, i.e., the antagonistic, synergistic or additive effects (Wang *et al.,* 2011). Addition of herbs to the tea was also found to enhance the palatability and consumer acceptability of a beverage (Malongane *et al.*, 2017). A combination of lemongrass and ginger essential oils presented a better antimicrobial property (Sahamastuti *et al.*, 2019). Synergistic effect in lowering postprandial blood glucose was also found in the combination of green tea, ginger, and cinnamon extracts (Azzeh, 2013). On the other hand, the mixtures of lemongrass and ginger aqueous extract exhibited lower antioxidant activity compared to the single plant extract (Poh *et al.*, 2018). Thus, it is important to

analyze the combination effects. Aside from their interaction effects, suitable formulation of such beverages remains unidentified.

One important parameter in developing functional beverages is its function stability against storage, especially when the product contains degradable bioactive compounds. To ensure that the product is in optimum condition by the time it reaches the consumers, a shelf-life testing is conducted to provide an insight into the possible chemical changes of the product during the storage.

In this study, the AGI activities of lemongrass and ginger in fresh and dried form were compared and selected for the beverage formulation. Essentially, the alteration of AGI affected by combination, formulation, flavorings, and storage were observed.

METHODS
Materials

The formulation materials consisted of lemongrass (CV. Sekar Utami, Indonesia), white ginger (CV. Sekar Utami, Indonesia), *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (Laresolo, Indonesia), lime (Tangerang Selatan, Indonesia), pandanus leaves (Tangerang Selatan, Indonesia), cinnamon powder (Koepoe-koepoe, Indonesia), sodium benzoate (Purox® S Grains, Holland), Stevia (Tropicana Slim, Indonesia), and non-dairy creamer Fiber Crème (Ellenka, Indonesia).

Analytical grade chemicals were used in this study, including sucrose (Merck, Germany), maltose (Merck, Germany), potassium phosphate (Merck, Germany), potassium dihydrogen phosphate (Sinopharm Chemical Reagent Co., Shanghai), dimethyl sulfoxide (DMSO) (Merck, Germany), ethylenediaminetetraacetic acid (EDTA) (Merck, Germany), Triton-X100 (Merck, Germany), rat intestinal acetone powder (Sigma-Aldrich,Germany),tris(hydroxylmethyl) aminomethane (Merck, Germany), hydrochloric acid 37% (HCl) (PT. Smartlab, Indonesia), glucose kit (Wako Pure Chem. Co., Japan), Folin's reagent (Merck,

Germany), sodium carbonate (Merck, Germany), gallic acid (Merck, Germany), and plate count agar (Merck, Germany).

Extracts preparation

First, fresh lemongrass and ginger were washed, cut, and oven-dried at 40 °C for 72 and 48 h, respectively, until the moisture content was less than 10%. The extraction for both lemongrass and ginger was done at optimum conditions as described in Gunawan-Puteri *et al.,* (2017). The lemongrass and ginger were macerated for 40 min at 70 °C with continuous stirring, and the ratios of them to the water were 3:10 (w/v) and 2:10 (w/v), respectively. Dried black tea leaves were directly macerated in hot water at 80 °C for 3 min, with the extraction ratio of 1:100 (w/v). All extracts were filtered using Whatman filter no.1 and centrifuged for 20 min in 6000 rpm at 4 °C. The obtained supernatant was freeze-dried at –40 °C for ±16 h. Freeze-dried samples were dissolved in DMSO 50% until the desired concentration was obtained and stored in a glass bottle at 4 °C. The extracts underwent rat intestinal sucrase and maltase inhibitory activity assays.

In the evaluation of the effect of extract combination to the AGI activities, black tea, dried lemongrass and dried ginger extracts were diluted into their IC_{50} concentration and combined with the same ratio. C1 consisted of lemongrass and black tea (1:1 v/v), C2 ginger and black tea in the ratio of 1:1 v/v, whereas, C3 contained lemongrass and ginger (1:1 v/v), and C4 was a combination of all ingredients (1:1:1 v/v). Then, the rat intestinal sucrase and maltase inhibitory activity assays were conducted to the extract combinations.

Prototype beverage preparation

The formulation of prototype beverage was made considering the effective dose based on the previous in-vivo studies using rats conducted by Ademuwiya *et al.* (2015) and Abdulrazaq *et al.* (2012). The human effective dose was calculated as presented in Table 1.

Table 1. Human effective dose calculation

Sample	Rat Effective Dose (mg/kg BW)	Conversion Ratio	Human Effective Dose (mg/kg BW)	Human Effective Dose (mg/day)[##]
Lemongrass	200[*]	0.162[#]	32.43	2,010.81
Ginger	100[**]		16.22	1,005.41

[*] Ademuwiya *et al.*, 2015 [**] Abdulrazaq *et al.*, 2012 [#] FDA, 2005
[##] Assuming the average human body weight is 62 kg (Walpole *et al.*, 2012)

The minimum volume of serving was calculated using the IC_{50} result from the rat intestinal sucrase and maltase inhibitory activity assays. The volume used in the formulation was adjusted by considering the beverage palatability. The liquid lemongrass (Total Soluble Solids/TSS=16.7 $^{mg}/_{mL}$), ginger (TSS=8.3 $^{mg}/_{mL}$), and black tea (TSS=1.6 $^{mg}/_{mL}$)

extracts were combined into nine formulations. The ratios of lemongrass and ginger extracts were varied as follows: 1:3 for 13-A, B, C, 1:1 for 11-A, B, C, and 3:1 for 31-A, B, C samples. Black tea was used to fill up the remaining volume and the samples were served into three common serving sizes for RTD tea products (Table 2).

Table 2. Prototype formulation

Sample	Lemongrass Extract (mL)	Lemongrass Concentration (mg/mL)	Ginger Extract (mL)	Ginger Concentration (mg/mL)	Black Tea Concentration (mg/mL)	Serving Size (mL)
13-A		1.43		2.13	1.05	350
13-B	30	1.67	90	2.49	0.96	300
13-C		2.00		2.99	0.83	250
11-A		2.86		1.42	1.05	350
11-B	60	3.34	60	1.66	0.96	300
11-C		4.01		1.99	0.83	250
31-A		4.29		0.71	1.05	350
31-B	90	5.01	30	0.83	0.96	300
31-C		6.01		1.00	0.83	250

Each prototype was mixed with sodium benzoate 1% and sweetener (stevia, sorbitol and erythritol), pasteurized (75 ° C, 1 min), and hot-filled into autoclaved glass bottles. Focus group discussion, followed by hedonic and forced ranking tests were conducted to evaluate the sensory acceptance of the formulations. Prototype with the highest acceptance was further improved with the addition of flavorings: F1 (+0.4% pandanus leaves, 2% non-dairy creamer), F2 (+0.1% cinnamon powder), and F3 (+1% lime juice). The AGI activities and sensory acceptance of these three improved prototypes were evaluated using forced ranking test.

Rat intestinal sucrase and maltase inhibitory activity assay

The evaluation of α-glucosidase inhibitors (AGI) activity was determined using rat intestinal glucosidase inhibitory assay, adjusting to the method of Arsiningtyas *et al.* (2015) and Gunawan-Puteri and Kawabata (2010) with a minor modification. Rat intestinal acetone powder was dissolved in potassium phosphate buffer (0.1 M, pH 7) and the mixture was homogenized in cold mortar. The mixture was centrifuged for 60 min (11,000 rpm, 4 ° C). The precipitation obtained from the centrifugation was solubilized in the same buffer containing 1% Triton-X100 and centrifuged for the second time for 90 min (11,000 rpm, 4 ° C).

Sucrose 56 mM in phosphate buffer (200 μL) was added to the sample and control test tubes while 0.1 M potassium phosphate buffer (400 μL) was added to the sample blank

and control blank test tubes. Working samples diluted in DMSO 50% (100 μL) were added to the sample and sample blank test tubes. Subsequently, DMSO 50% (100 μL) was added to the control and control blank test tubes. The reaction began when crude rat intestinal glucosidase (200 μL) was added to the sample and control test tubes. Procedures for the assay of rat intestinal maltase inhibitory activity were basically similar, except for replacing sucrose 56 mM as the substrate with maltose 3.5 mM in phosphate buffer, and reducing the amount of crude enzyme from 200 μL to 50 μL. The reaction was carried out at 37 ° C for 15 min and stopped by adding Tris-HCl buffer (2 M, pH 9, 750 μL).

The mixtures were then delivered through a column that had been filled with *1* g of aluminum oxide 60. Around 50 μL portion of each mixture was inserted into a 96-wells microplate, and glucose kit solution (200 mL) was added which turned pink when it had reacted with glucose. The incubation was done in an incubator at 37 °C for 15 min. The absorbance was read using a microplate reader at 492 nm wavelength. The inhibition activity was determined using the following formula.

$$\% \ Inhibitory \ Activity = \frac{(Absorbance_{control} - Absorbance_{control \ blank})}{(Absorbance_{sample} - Absorbance_{sample \ blank})} \times 100\%$$

Equation 1. Inhibitory activity calculation

The experiments were conducted twice for each concentration and the resulting sucrase and maltase inhibitory activities were plotted in a curve against the concentration to derive the linear regression mathematical formula. The data were presented as IC_{50}, which was defined as a sample concentration that inhibited 50% of sucrose and maltose hydrolysis into glucose and fructose or to glucose and glucose in the presence of crude extract of rat intestinal acetone powder.

Total polyphenol analysis

Total polyphenol was determined using Folin's Ciocalteu reagent with gallic acid (10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm) as the standard. The working samples were made into a 10-fold dilution, and a portion of them (1 mL) were added into a test tube. Folin's Ciocalteu 10% (5 mL) was added into the test tube, and it was incubated for 3-8 minutes. The next step was adding sodium carbonate 7.5 % (4 mL). Then, the mixture was homogenized using a vortex and incubated in a dark place for 2 h. The absorbance was measured at 740 nm wavelength. Total polyphenol was measured using the following formula, where W stands for sample weight (g), *a* stands for the intercept of standard linearity, and *b* stands for slope of standard linearity.

$$Total \ Polyphenol = \frac{[Absorbance - a]/b \ x \ 100}{W}$$

Equation 2. Total polyphenol calculation

Sensory evaluation

A focus group discussion (n panelists = 9) was conducted to the nine prototypes (Table 2) to determine the reactions that can be expected from a larger population, and to obtain qualitative data of different formulations. The acquired information was the descriptions of sensory attributes (aroma, color, flavor, and aftertaste) and the acceptance of each formula (like or dislike).

Three formulations with the highest acceptance rate were analyzed using hedonic and forced ranking tests, with larger population (n panelist = 30). Hedonic test was grounded on the overall acceptance of the product with the degree of preference using 9-point hedonic test where 1 indicates "like extremely" and 9 indicates "dislike extremely". Forced ranking test was conducted following the hedonic test. The panelists were asked to

Filiana Santoso *et al.*

rank the samples from 1 to 3 based on their preferences, in which 1 indicates the most preferred sample, and 3 indicates the least preferred sample. The formula with the highest acceptance rate was further improved using flavorings and evaluated for the forced ranking test using larger population (n panelist = 94).

The results of the sensory affective tests were expressed as mean ± standard deviation (SD). The data were analyzed using Friedman's and Wilcoxon test. The 0.05 probability level was used for all statistical analysis in this research, and p-value >0.05 was considered not statistically significant.

Shelf-life analysis

The selected formulation was stored at 4 and 25 °C for 50 days and its shelf-life was analyzed by observing the AGI activity as described above, °Brix value using refractometer, pH value using pH meter (Horiba, Japan), color intensity using colorimeter (PCE, Germany), and total microbial count. The colors were represented as L, a, and b values. Total microbial count was conducted using total plate count method. The sample was prepared into 4 dilutions (from 10^{-1} to 10^{-4}) using saline solution. Around 1 ml of sample from each dilution was pipetted aseptically to the sterilized petri dishes. Then, about 12-15 ml of PCA (±45 °C) was poured, and the petri dishes were sealed using Parafilm M. The aerobic mesophilic bacteria growth was recorded after the sample was incubated in proper culturing environmentfor 48 hours at the temperature of 37±1 °C.The observed colonies ranging from 25 to 250 were recorded.

Addition of flavoring agents

Three flavors were developed based on the Indonesian tea recipes to increase customer acceptability, divided into sample F1 (+0.4% pandanus Leaves, 2% non-dairy creamer), F2 (+0.1% cinnamon powder), and F3 (+1% lime juice). Rat intestinal sucrase and maltase inhibitory activity assay and forced ranking test (n=94) were conducted to the samples.

RESULTS AND DISCUSSION
Determination of raw ingredients

Drying can significantly reduce food moisture content not only to prolong its shelf life, but also to decrease product mass and volume to ease its transportation and storage. However, the drying process may also diminish the product quality, such as losses of nutrients and functionality (Singh and Heldman, 2014). In this experiment, dried and fresh lemongrass and ginger were compared in terms of practicality, storage stability, and AGI activity. Dried form, as mentioned earlier, possesses a better practicality and storage stability. Inhibitory activity assay conducted on dried lemongrass (DC) resulted in IC_{50} of 24.50 mg/mL (yield = 3.4 %), while fresh lemongrass (FC) produced an IC_{50} of 17.93 mg/mL (yield = 2.1 %). In terms of AGI activity, both DC and FC possessed similar figures. Therefore, since DC is more practical and stable than FC, it was selected as the ingredient of RTD. On the other hand, dried ginger (DZ) was found to possess much higher AGI activity (IC_{50} 16.61 mg/mL) than the fresh extract (FZ) (IC_{50} >47.00 mg/mL). The yield of DZ (2.7%) extract was also higher than FZ (1.8%). As a result, DZ was selected as the raw ingredient.

The AGI activity of DZ that was higher than those of FZ was predicted to be affected by the fact that drying ginger caused the alteration of one active compound to another structure that is responsible for AGI activity. In this case, it was from gingerols to shogaols (Figure 1). Gingerols are the major constituents in ginger. According to Bhattarai *et al.*, (2001), gingerols have β-hydroxy keto functional group in the structure, which makes them thermally unstable. When exposed to drying, dehydration occurs, which removes the hydroxyl group in gingerols, thus forming shogaol. The presence of double bond in α, β-unsaturated carbonyl in the chemical structure of shogaol identifies shogaol as a possible nucleophile that can donate electrons and form a new covalent bond when there is water addition in the extraction process. Therefore, it was predicted that the reason for a higher sucrase inhibitory activity in the ginger is due

to shogaol that acts as an inhibitor towards sucrase. As a result, sucrase is not able to hydrolyze sucrose into glucose and fructose, thus presenting an inhibition activity.
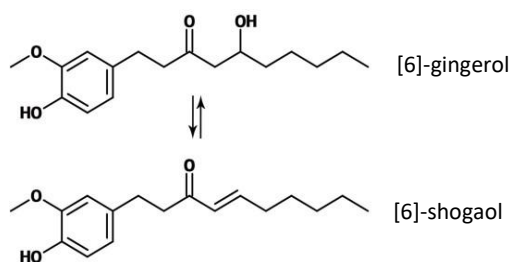

Figure 1. Structure of [6]-gingerol (A) and [6]-shogaol [B]

The synergistic/antagonistic effects in black tea, lemongrass and ginger were assessed. The extracts were prepared into their IC_{50} concentration, and combined as presented in Table 3.

Table 3. Effects of Extracts Combination

Sample[*]	% Inhibitory Activity	
	Sucrase	**Maltase**
C1 (DC[1] + BT[2], 1:1 (v/v))	$56.87 \pm 5.67^{a,b}$	42.52 ± 4.53^{c}
C2 (DZ[3] + BT, 1:1 (v/v))	$55.15 \pm 3.51^{a,b}$	61.76 ± 4.58^{d}
C3 (DC + DZ,1:1 (v/v))	61.74 ± 0.94^{a}	57.77 ± 6.06^{d}
C4 (DC + DZ + BT, 1:1:1 (v/v/v))	51.62 ± 1.75^{b}	63.03 ± 2.29^{d}

[*]All extracts were prepared in their IC_{50} concentration as follows: [1] Sucrase IC_{50}: 24.5 mg/mL; Maltase IC_{50}: 17.5 mg/mL, [2] Sucrase IC_{50}: 12.53 mg/mL; Maltase IC_{50}: 1.95 mg/mL, [3] Sucrase IC_{50}: 19.61 mg/mL; Maltase IC_{50}: 13.38 mg/mL. Lowercase letters (*a,b,c,d*) indicate significant differences between treatments (p<0.05)

The results showed that all combinations did not confirm the synergistic/antagonistic effect on the sucrase nor maltase inhibitory activities. This may occur if the compounds in each ingredient do not interact in any way. However, it can be concluded that they possess additive effects if combined; not only in terms of maltase inhibitory activity, but also in terms of palatability (Togatorop *et al.*, 2015). Since the combination of extract did not adversely affect the AGI activities, the formulation was further selected based on the sensory properties and acceptance. Since tea has been associated with full-bodied, rich, and viscous sensory properties (Ukers, 1935; Ellis, 2002), C4 formula was selected. Furthermore, tea costs cheaper than lemongrass and ginger, thus it reduces the beverage production cost. Then, focus group discussion (n panelists = 9) was conducted to the nine formulations (Table 2) and it resulted in 11-B, 11-A, and 31-A as the three most preferred formulas (Table 4).

Table 4. Focus group discussion result ranked by preferences

Formula	Aroma	Appearance	Flavor	Aftertaste	Preference (%)
11-B	Strong lemongrass	Cloudy, dark-colored	Tea-like, slightly spicy, bitter, sweet	Slightly spicy, sweet	100
11-A	Lemongrass, ginger	Dark-colored, tea-like	Tea-like, sweet, lemongrass, slightly spicy, slightly bitter, sweet	Slightly spicy, sweet	77.8
31-A	Lemongrass	Dark-colored, tea-like	Tea-like, slightly spicy, slightly bitter, sweet	Slightly spicy, sweet	77.8
11-C	Strong lemongrass	Cloudy, tea-like	Tea-like, sweet, slightly spicy, bitter, slightly sweet, slightly astringent	Spicy, slightly astringent, slightly bitter, sweet	66.7
13-C	Strong ginger	Cloudy, concoction-like	Concoction-like, very spicy, very bitter, slightly sweet	Very spicy, astringent, sweet	11.1
13-B	Concoction-like, ginger	Cloudy, tea-like	Concoction-like, very spicy, very bitter, slightly sweet	Very spicy, bitter	0
13-A	Strong ginger, light lemongrass	Cloudy	Concoction-like, spicy	Spicy	0
31-C	Strong lemongrass	Cloudy, tea-like	Tea-like, strong lemongrass, very bitter, sweet	Slightly spicy, bitter, sweet	0
31-B	Strong lemongrass, ginger	Cloudy	Concoction-like, light lemongrass, slightly spicy, bitter, sweet	Slightly spicy, sweet	0

The three most preferred formulas were further tested to a larger population (n=30) to select the best formula using a 9-scale hedonic and confirmed by a ranking test (Table 5). The selected formula in both tests was in agreement with each other, resulting in "like moderately" to "like slightly" for 31-A formula. Therefore, formula 31-A was used for the further research.

Table 5. Hedonic test results

Sample	Overall Acceptance Mean*	Overall Acceptance	Forced Ranking Test Mean**
31-A	3.83 ± 1.15[a]	Like moderately – Like slightly	1.33 ± 0.61[c]
11-A	5.16 ± 1.64[b]	Neither like nor dislike	2.23 ± 0.73[d]
11-B	5.20 ± 1.40[b]	Neither like nor dislike	2.43 ± 0.68[d]

*1 = like extremely, 9 = dislike extremely ** 1 = most preferable, 3 = least preferable
Lowercase letters (*a,b,c,d*) indicate significant differences between treatments (p<0.05)

Antioxidant is one of the responsible chemical compounds exhibiting AGI activity. Furthermore, phenolic compounds such as monoterpenes, sesquiterpenes, and oxygenic derivatives in plant extracts were observed to possess carbohydrate hydrolyzing enzymes inhibiting activity (Jumepaeng *et al.*, 2012). Therefore, the total polyphenol content of the selected beverage formulation 31-A was measured, which resulted in 636.45 mg/L GAE (Gallic Acid Equivalent). The total polyphenol content in this formula exceeded the Indonesian National Standard of Tea Beverage (SNI 3134:2011) with a minimum of 400 mg/kg.

The overall acceptance results showed "like moderately" to "like slightly" for 31-A formula. Therefore, the flavor development was required to enhance the beverage acceptance. Based on the world-wide tea recipes, three flavor profiles were chosen: *bandrek* (Indonesian traditional concoction using coconut cream and pandanus leaves), cinnamon tea, and lime tea. The ranking test

was used to determine the most preferable flavor. Moreover, their AGI activities were also taken into consideration. Flavoring agent addition affected the AGI activity of the beverage (Table 6). F1 contained non-dairy creamer made of oligosaccharides that can be degraded into simpler sugars and retarded the AGI activity of the beverage. The F2 and F3 formulations possessed higher AGI activities than the flavorless formulation. Cinnamon, as added in F2, was found to potentially possess anti-hyperglycemic activity due to the cinnamtannin B1 that affects the insulin receptors phosphorylation (Al-Samydai *et al.*, 2018). Whereas, lime juice in F3 contains chemical compounds with antioxidant properties, such as vitamin C, that was found to inhibit the aldose reductase enzyme. Other antioxidants in the lime juice reduced the advanced glycation end products (AGEs) formation in sorbitol-aldolase reductase pathway and affected the glucose metabolism-related enzyme gene expression (Mawarti *et al.*, 2018). Moreover, the addition of lime juice increased the acidity of the formula, which promoted freshness, enhanced the perception of other tastes and might prolong the shelf life of a beverage (Marcus, 2019). The ranking test showed that both F1 and F3 were more preferred than F2. However, F1 was found to have no AGI activity towards sucrase; therefore, F3 formulation was considered to be developed further.

Table 6. Effect of flavoring agents to the AGI and ranking test results

Samples	Sucrase Inhibitory Activity (%)	Maltase Inhibitory Activity (%)	Ranking Test Mean[*]
F1	No inhibition	48.95%	1.86 ± 0.78^a
F2	60.36%	57.11%	2.19 ± 0.79^b
F3	69.20%	68.77%	1.94 ± 0.85^a

[*]1 = most preferable, 3 = least preferable, Lowercase letters (*a, b*) indicate significant differences between treatments ($p < 0.05$)

Stability of the product

Stability of 31-A was tested in a 50-day period at 4 °C and 25 °C. The results are presented in Table 7. Apparently, the samples stored at 25 °C showed significant microbial growth compared to those stored at 4 °C. On the 5th day of storage, microbial growth of the samples stored at 25 °C exceeded 2.4 log CFU/mL, while microbial growth of samples stored at 4 °C exceeded 2.4 log CFU/mL only on the 50th day with 2.5 log CFU/mL. The temperature-dependent stability also showed the same trend in pH value, in which the samples stored at 25 °C showed more apparent pH decline (from 5.81 to 4.16) than those stored at 4 °C (final pH 4.73). The °Brix value was stable at 2.0 throughout 50 days of storage period (no significant difference at $p > 0.05$). Higher stability of AGI activities for the samples stored at 4 °C was also coherent with the evaluation of microbial growth and pH value. The L, a, and b values, which represent colors, of samples stored at both temperatures after 50 days were decreased, but not statistically significant at $p > 0.05$. The experiment showed that 4 °C was a more preferable storage temperature for this functional beverage. However, novel preservation techniques should be studied to prolong the shelf life of the beverage.

Filiana Santoso *et al.*

Table 7. Results of stability test at 4 °C and 25 °C for 50 ays

Days	Microbial Growth (log CFU/mL)		Color Alteration									
			pH		Brix°		L		A		b	
	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C	4°C
0	<2.40	<2.40	5.81	5.81	2.05	2.05	43.85	43.85	11.18	11.18	12.33	12.33
5	3.78	<2.40	5.09	5.41	1.95	1.95	42.86	44.20	10.45	12.34	14.14	13.87
10	4.04	<2.40	4.63	5.16	2.05	2.05	43.53	42.69	11.12	10.55	13.70	11.13
14	4.17	<2.40	4.56	5.06	2.05	2.05	41.93	41.33	10.94	10.45	15.14	10.79
20	4.26	<2.40	4.53	5.05	2.00	1.85	41.16	40.82	8.90	8.76	13.42	8.58
30	4.46	<2.40	4.50	4.96	1.90	1.85	40.57	39.54	8.77	8.40	10.48	7.28
40	4.60	<2.40	4.38	4.89	1.90	1.85	39.72	38.93	8.37	7.55	6.51	6.01
50	4.60	2.50	4.16	4.73	1.95	1.90	38.80	37.99	7.49	7.59	6.00	5.60

	AGI Activities			
	Sucrase Inhibition (%)		Maltase Inhibition (%)	
Days	25°C	4°C	25°C	4°C
0	16.33	16.33	48.46	48.46
5	17.99	14.63	47.97	45.69
10	14.31	18.79	42.11	49.43
14	15.29	16.41	39.26	45.19
20	14.50	16.97	33.95	46.63
30	14.56	14.84	35.00	42.22
40	14.49	13.79	32.78	40.28
50	8.14	10.52	24.72	36.04

CONCLUSION

Formulation and storage of functional beverages affected the AGI activity and the study found that dried ingredients provided more extraction efficiency and practicality. Combination of dried lemongrass, ginger and black tea resulted in additive effects for maltase inhibitory activity. Formula consisted of 4.29 mg/mL lemongrass, 0.71 mg/mL ginger, and 1.05 mg/mL black tea was preferable and predominantly stable in terms of pH, °Brix value, color, and AGI activity at 4 °C of storage. Lime juice addition could increase both the palatability and AGI activity of the beverages, hence, was selected to be further developed. In addition, more research on beverage preservation is required to prolong the shelf life.

REFERENCES

Abdulrazaq, N.B., Cho, M.M., Win, N.N., Zaman, R., Rahman, M.T., 2012. Beneficial Effects of Ginger (*Zingiber officinale*) on Carbohydrate Metabolism in Streptozotocin-induced Diabetic Rats. *British Journal of Nutrition*, 108(7), 1194–1201.

Ademuyiwa, A.J., Olamide, O.Y., Oluwatosin, O.O., 2015. The Effects of *Cymbopogon citratus* (Lemongrass) on the Blood Sugar Level, Lipid Profiles and Hormonal

Profiles of Wistar Albino Rats. *Merit Research Journal of Medicine and Medical Science*, 3(6), 210–216.

Al-samydai, A., Al-mamoori, F., Shehadeh, M., Hudaib, M., 2018. Anti – Diabetic Activity of Cinnamon: A Review. *International Research Journal of Pharmacy and Medical Sciences*, 1(5), 43–45.

Amiri, A., Azemi, M.E., Khodayar, M.J., Namjoyan, F., 2015. In Vitro α- Amylase and α- Glucosidases Inhibitory Effects of Some Plant Extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 7(2), 315-318.

Andallu, B., Radhika, B., Suryakantham, V., 2003. Effect of Aswagandha, Ginger and Mulberry on Hyperglycemia and Hyperlipidemia. *Plant Foods for Human Nutrition*, 58, 1–7.

Azzeh, F.S., 2013. Synergistic Effect of Green Tea, Cinnamon and Ginger Combination on Enhancing Postprandial Blood Glucose. *Pakistan Journal of Biological Sciences*, 16(2), 74–79.

Bharti, S.K., Kumar, A., Prakash, O., Krishnan, S., Gupta, A.K., 2013. Essential Oil of *Cymbopogon citratus* Against Diabetes: Validation by In vivo Experiments and Computational Studies. *Journal of Bioanalysis and Biomedicine*, 5(5), 194–203.

Christianty, F.M., Holidah, D., Yasmin, 2016. In vitro α-Glucosidase Inhibitory Activity of Various Tea (*Camellia sinensis* L.) Extracts. Proceeding ICMHS, 104–107.

Ellis, H., 2002. Tea: Discovering, Exploring, Enjoying, p 6-60. Ryland Peters & Small, New York.

Food and Drug Administration (FDA), 2005. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Center for Drug Evaluation and Research (CDER), United States.

Garba, H.A., Mohammed, A., Ibrahim, M.A., Shuaibu, M.N., 2020. Effect of Lemongrass (*Cymbopogon citratus* Stapf) Tea in a Type 2 Diabetes Rat Model. *Clynical Phytoscience*, 6(19), 1–10.

Gardner, E.J., Ruxton, C.H.S., Leeds, A.R., 2007. Black tea - Helpful or Harmful? A Review of The Evidence. *European Journal of Clinical Nutrition*, 61(1), 3–18.

Gunawan-Puteri, M.D.P.T., Kartawiria, I.S., Widiputri, D.I., 2017. Aqueous Extraction Optimization of *C. citratus* for Development of Food Ingredients with Alpha Glucosidase Inhibitory. In Sukmana, I. and Ulvan, A. *Integrated Sci-Tech: Interdisciplinary Research Approach*, (2), 55-61.

Gunawan-Puteri, M.D.P.T., Rustandi, F., Hendra, P., 2018. Spray Dried Aqueous Extract of Lemongrass (*Cymbopogon citratus*) Exhibits In-vitro and In-vivo Anti-hyperglycaemic Activities. *Journal of Pharmaceutical Science and Community*, 15(2), 55–61.

International Diabetes Federation. IDF Diabetes Atlas, 9th edn. Brussels, Belgium.

Jumepaeng, T., Luthria, D., Chanthai, S., 2012. The Effect of Surfactant on Headspace Single Drop Microextraction for the Determination of Some Volatile Aroma Compounds in Citronella Grass and Lemongrass Leaves by Gas Chromatography. *Analytical Method*, 4, 421-428.

Lee, W.K., Wong, L.L., Loo, Y.Y., Kasapis, S., Huang, D., 2010. Evaluation of different teas against starch digestibility by mammalian glycosidases. *Journal of Agricultural and Food Chemistry*, 58(1), 148–154.

Malongane, F., McGaw, L., Mudau, F.N., 2017. The Synergistic Potential of Various Teas, Herbs and Therapeutic Drugs in Health Improvement. *Journal of the Science of Food and Agriculture*, 97(14), 4679–4689.

Marcus, J.B., 2019. Aging, Nutrition and taste: Nutrition, Food Science and Culinary Perspectives for Aging Tastefully. Academic Press, San Diego.

Mawarti, H., As'ad, Z.M., Rajin, M., 2018. Ameliorative Effect of *Citrus aurantifolia* and *Cinnamomum burmannii* Extracts on Diabetic Complications in A Hyperglycaemic Rat Model. *Tropical Journal of Pharmaceutical Research*, 17(5), 823–829.

Mun'im, A., Katrin, Azizahwati, Andriani, A., Mahmudah, K.F., Mashita, M., 2013. Screening of α- Glucosidase Inhibitory Activity of Some Indonesian Medicinal Plants. *International Journal of Medicinal and Aromatic Plants*, 3(2), 144–150.

Oboh, G., Akinyemi, A.J., Ademiluyi, A.O., Adefegha, S.A., 2010. Inhibitory Effects of Aqueous Extracts of Two Varieties of Ginger on Some Key Enzymes Linked to Type-2 Diabetes In Vitro. *Journal of Food and Nutrition Research*, 49(1), 14-20.

Poh, K.H., Muhammad, N., Abdullah, N., Talip, B.A., 2018. The Evaluation of Antioxidant Activity of Individual and Mixture of Lemongrass, Curry Leaves, Turmeric and Ginger Extracts. *Journal of Science and Technology*, 10(2), 66–70.

Priyarani, M., Padmakumari, P., Sankarikutty, B., Cherian, O.L., Nisha, V.M., Raghu, K.G., 2011. Inhibitory Potential of GingerExtracts Against Enzymes Linked to Type 2 Diabetes, Inflammation and Induced Oxidative Stress Inhibitory. *International Journal of Food Sciences and Nutrition*, 62(2), 106–110.

Sahamastuti, A.A.T., Andre, Foustine, S., Sumarpo, A., Hartiadi, L.Y., 2019. Synergistic Antibacterial Activities of Ginger and Lemongrass Essential Oils as an Alternative Prevention to Food-Borne Disease. *Indonesian Journal of Life Sciences*, 01(02), 54–61.

Santoso, F., 2018. Application of Lemongrass (*Cymbopogon citratus*) as a Functional Food Ingredient with Alpha- Glucosidase Inhibitory Activity. *Advance in Engineering Research*, 172, 205–209.

Singh, A.B., Singh, N., Maurya, R., Kumar, A., 2009. Anti-hyperglycaemic, Lipid Lowering and Anti-oxidant Properties of [6]-gingerol in db/db Mice. *International Journal of Medicine and Medical Sciences*, 1(12), 536–544.

Singh, R.P., and Heldman, D.R., 2014. Dehydration, in: Introduction to Food Engineering. Fifth Edition. Academic Press, San Diego.

Togatorop, D.M., Nainggolan, R.J., Lubis, L.M., 2015. Pengaruh Perbandingan Sari Batang Sereh dengan Sari Jahe Dan Konsentrasi Serbuk Gula Aren Terhadap Mutu Serbuk Minuman Penyegar Sereh. *Jurnal Rekayasa Pangan dan Pertanian*, 3(2), 157–163.

Ukers W.H.,1935. All about tea. The Tea and Coffee Trade Journal Company, New York.

Wang, S., Meckling, K.A., Marcone, M.F., Kakuda, Y., Tsao, R., 2011. Synergistic, Additive, and Antagonistic Effects of Food Mixtures on Total Antioxidant Capacities. *Journal of Agricultural and Food Chemistry*, 59(3), 960–968.

Widiputri, D.I., Gunawan-Puteri, M.D.P.T, Kartawiria, I.S., 2018. Benchmarking Study of Cymbopogon citratus and C. nardus for Its Development of Functional Food Ingredient for Anti-diabetic Treatment. Proceedings of the International Conference on Innovation, Enterpreneurship and Technology, 103–108.

Widiputri, D.I., Mariana, N., Josopandojo, B., Gunawan-Puteri, M.D.P.T., Kartawiria, I.S., 2017. Effect of Pre-treatment Processes and Stability Testing of Lemongrass (Cymbopogon citratus) Extract on α-Glucosidase Inhibitor (AGI) and α-Amylase Inhibitor (AAI) activities.

Proceedings of International Postgraduate Symposium on Food, Agriculture, and Biotechnology, pp. 10-20.

Yu, Y., Zick, S., Li, X., Zou, P., Wright, B., Sun, D., 2011. Examination of the Pharmacokinetics of Active Ingredients of Ginger in Humans. *The AAPS Journal,* 13(3), 417–426.