Preparation of Low-Sugar Herbal Buccal Tablet and its Antioxidant Activity

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The incidence of obesity, dental caries, hypertension, hyperglycemia, hyperlipidemia and other diseases is on the rise due to excessive intake of high-sugar foods. However, candy has always been a popular traditional food due to people's fascination with sweets for thousands of years. In recent years, low-sugar candies, especially those favored by health benefits are gradually replacing high-sugar candies under the background of advocating a healthy diet (Liu, 2008). Among them, raw material powder pressed candy is more beneficial to the protection, absorption and sustained release of functional factors (Liu, 2018; Liu et al., 2019). Compared with the heat boiled candies, low-sugar functional tablet candy is one of the most important concerned issues in the food and healthcare industry and also has bright prospects for the development of the candy industry.

Excessive accumulation of oxygen free radicals can damage structure and function of cells which leads to the occurrence and development of many diseases (Burlaka et al., 2019). Excessive production of oxygen-centered free radicals (also known as Reactive Oxygen Species-ROS) and the imbalance of antioxidant protection may induce oxidative stress, inhibit the normal functions of cell lipids, proteins, DNA and RNA (Gülcin, 2012) and participate in the pathological process of more than 100 diseases (such as chronic inflammation, atherosclerosis, diabetes and some types of cancer, etc.) (Valko et al., 2006; Li et al., 2016). Antioxidants may remove excess reactive oxygen free radicals and alleviate their damage to targeted tissue (Valko et al., 2006), inhibit the production and activity of inflammatory mediators (Benavente-Garcia et al., 2008) and directly reduce the gene expression pattern of pro-inflammatory cytokines (TNF-a and IL-1b) (Sridharan et al., 2016), thereby reducing the incidence of related diseases. As a result, antioxidant function is the backbone of the treatment of inflammation, tumors and other diseases (Gülcin, 2012; Li et al., 2016). Compared with chemically derived antioxidants, plant-derived antioxidants are gradually attracting people's attention because of their unique effects and good safety (Ferlazzo et al., 2015).

Abstract: In this study, in order to prepare a low-sugar Herbal Buccal Tablet (HBT), Response Surface Methodology (RSM) was used to optimize the formula of HBT. HBT was prepared by wet granulation and tableting process on the basis of formula optimization. The in vitro antioxidant activity, glycemic index and particle microstructure of HBT were evaluated. The results showed that the optimal formula for HBT was Siraitia grosvenorii fruit powder of 15%, canarium album fruit powder of 16%, lily bulb powder of 7.1%, erythritol of 55% and citric acid of 0.45%. HBT was prepared by adding 0.3% x glycosides, 5% Arabic gum, 1% magnesium stearate and 0.15% menthol on the basis of optimal formula. Under the optimal formula, the content of total flavonoids and saponins in HBT was 10.08±0.05 mg/g and 11.28±0.03 mg/g, respectively. HBT had an advantage over Commercial Sugar-Sweetened Confectionery (CSC) because of its higher antioxidant activity. The results showed that HBT may have the potential to become a traditional candy substitute in food industry due to its high content of active ingredients, low sugar content and good antioxidant activity in the future.

Keywords: Siraitia Grosvenorii Fruit, Canarium Album Fruit, Lily Bulb, Herbal Buccal Tablet (HBT), Antioxidant Activity
Fig. 1: The research flowchart of this study

Compound products of multiple ingredients can often get synergistic effects. The formula design based on traditional and modern evidence-based medicine is expected to achieve better raw material ratio in terms of functionality and safety, providing inspiration for the design and development of plant-derived antioxidant low-sugar tablet candy.

Some natural biologically active substances such as total flavonoids and total saponins are recognized as antioxidant substances in plants (Wang et al., 2018; Yi et al., 2017), which constitute the main component of antioxidant plant raw materials such as *Siraitia grosvenorii* fruit (Tang et al., 2020; Zhang et al., 2014), *Canarium album* Fruit (He et al., 2006) and Lily bulb (Lei et al., 2015). The above plant materials are listed in the classical prescription of Traditional Chinese Medicine (TCM) as throat-moistening, cough-relieving, heat-clearing and anti-inflammatory drugs (Gao et al., 2015). They have been identified as raw materials for both medicine and food which has good safety by national departments and experts recently (Yu, 2017). However, to the best of our knowledge, there is little information on pressed candy with these raw materials power for both medicine and food.

Therefore, in the present study, the powder of *Siraitia grosvenorii* fruit, *Canarium album* fruit and lily bulb was used as the main raw materials. Response Surface Methodology (RSM) was used to optimize the formula of HBT. HBT was prepared by wet granulation and tableting process on the basis of formula optimization. The antioxidant activity in vitro, glycemic index and particle microstructure of HBT were also investigated to reveal the effect of formulation technology on its function (Fig. 1).

Materials and Methods

Materials and Reagents

The dried powder of lily bulb was purchased from Jiangxi WanZai Qiannian Food Co., Ltd (Jiangxi, China). The dried powder of *Siraitia grosvenorii* fruit was purchased from Shanghai Jinliang Food Technology Co., Ltd (Shanghai, China). The dried powder of *Canarium album* fruit was purchased from Tao jia Xiang Electronic Commerce Co., Ltd (Chongqing, China). The menthol was purchased from Henan Junheng Biological Technology Co., Ltd (Henan, China), the stevial glycosides was purchased from Shenzhen Xingmu Biological Engineering Co., Ltd (Guangdong, China). The erythritol, citric acid, Arabic gum and magnesium stearate were all purchased from Shangqiu Jianing Trading Co., Ltd (Henan, China). The above raw materials were of food grade and were sheared, sieved to 80-mesh and then sealed at low temperature before the experiment began.

Ginsenoside standard substance, rutin standard substance, vanillin and other reagents involved were of analytical grades and purchased from Shanghai Tengzhun Biotechnology Co., Ltd (Shanghai, China).

Preparation Method and Evaluation Index of HBT

HBT was prepared according to the reported method with minor modifications (Yu et al., 2020). The key raw materials (*Siraitia grosvenorii* fruit powder, *Canarium album* fruit powder, erythritol, citric acid) was weighed according to the designed composition ratio. The auxiliary materials were 5% of Arabic gum, 0.3% of steviol glycosides, 1% of lubricant (magnesium stearate) and 0.15% cooling agent (menthol). The rest was the amount of lily bulb powder (the total amount was 100%). The weighed raw and auxiliary materials were mixed
evenly and sprayed with 75% ethanol solution to prepare the soft material. The soft material was kneaded into dough by hand. After kneading, the dispersion was good and the prepared soft material was granulated through an 18-mesh sieve. After granulating, the wet granules were dried at 50°C until the moisture was less than 3%. Then the dried granules were sieved through 18-mesh and 80-mesh successively to make the particle size uniformly. Then 1% lubricant was added evenly to the particles under the sieve. The refreshing agent (menthol-ethanol mixed solution) (0.15%) was sprayed on the particles by atomization, finally mixed evenly and pressed for HBT. Each group of HBT were vacuum packaged to evaluate the total flavonoids, total saponins, sensory score and their synthesis score.

**Determination of Total Saponins**

The content of saponin was determined by perchloric acid-vanillin color method described by Lin et al. (2009). Methanol was used to dissolve the ginsenoside standard product to a final concentration of 2 mg/mL. Standard solutions (0, 25, 50, 75, 100, 125 μL) were placed in 10 mL test tubes, respectively. The solvent was evaporated under vacuum to constant weight. 0.2 mL vanillin-glacial acetic acid solution (5%) and 0.8 mL perchloric acid were added into tubes and mixed in water bath at 60°C for 15 min. After cooling to room temperature, 5 mL glacial acetic acid was added to tubes, mixed well and stood for 10 min (Lei et al., 2015). Standard solution was used as blank. The standard curve was obtained by measuring the absorbance of standard solutions at 560 nm. The content of saponin in colorimetric solution (mg). The absorbance value (A) was used as ordinate. The amount of ginsenoside (m) was used as abscissa. The regression equation was obtained as following:

\[ A = 3.5177m - 0.041 \left( R^2 = 0.9911, 0 \sim 0.25mg \right) \]  

(1)

The samples were ground into powder according to the method of the NPC (2005). 1 g powder was dissolved in 50 mL methanol. The absorbance of 1 mL supernatant was measured based on the method described above. The average value of three replicates was substituted into the above Eq. (1) to calculate the content of total saponins in the HBT according to the following formula:

\[ TSC \ (mg \ / \ g) = \frac{m + v \times V}{M} \]  

(2)

where, \( TSC \) was total saponins content in the sample. \( m \) was the content of saponin in colorimetric solution (mg). \( v \) was the amount of reaction solution (1 mL). \( V \) was 50 mL. \( M \) was the sample powder weight (1 g).

**Determination of Total Flavonoid Content**

Total flavonoid was measured according to the method of Peñarrieta et al. (2007) with slight modifications. The standard curve was drawn before samples test. Rutin standard solution with a concentration of 0.14 mg/mL was prepared. 0.3 mL NaNO\(_2\) (5%) was mixed with 0, 1, 2, 3, 4, 5 and 6 mL standard solution and stood for 6 min, respectively. Then 0.3 mL 10% Al(NO\(_3\))\(_3\) solution was added to the mixture and stood for 6 min. 2 mL NaOH solution (1 mol/L) was added to the volumetric flask and made up to volume with 60% methanol. The mixture was blended and stood for 15 min. The standard solution was used to zero setting at the absorbance of 510 nm. Standard curve was drawn using rutin Concentration (C) and Absorbance (A). The regression equation was obtained as following:

\[ A = 6.6046C + 0.014 \left( R^2 = 0.9932, 0 \sim 0.15mg / mL \right) \]  

(3)

1 g sample powder was dissolved in 10 mL ethanol (60%). Then, 1 mL supernatant was used for absorbance determination based on the above method. The measurement was replicated for three times. The content of total flavonoid in HBT was calculated based on the Eq. (3) and formula (4).

\[ HBT - TFC \ (mg \ / \ g) = \frac{c \times n \times V}{m} \]  

(4)

where, \( TFC \) is total flavonoids content in samples. \( C \) is the concentration of total flavonoids in the sample reaction liquid (mg/mL). \( n \) is diluted multiple (10). \( V \) is a sample raw liquid to accumulate volume (10 mL). \( m \) is sample powder weight (1 g).

**Sensory Evaluation**

Sensory evaluation was conducted according to the method of (Zeeshan et al., 2017) with slight modifications. The specific evaluation standard was listed in Table 1. Ten evaluators were selected to evaluate the sensory qualities of HBT in optimization tests from flavor, appearance and taste. Among them, 40 points were assigned to flavor, 30 points were assigned to appearance. The remaining 30 points were given to taste. The product was scored with the average score after removal of the highest score and the minimum score.

**Comprehensive Evaluation Score**

The comprehensive evaluation score of tablets were
calculated based on the formula:

\[
Y = (G_i / G_{max}) \times 0.40 + (H_i / H_{max}) \\
\times 0.30 + (Z_i / Z_{max}) \times 0.30
\]

(5)

where \( Y \) was synthesis score. \( G_i \) was each group of sensory evaluation. \( G_{max} \) was CCD design maximum value rating in 30 groups; \( H_i \) was total flavonoid content measurement value per group. \( H_{max} \) was CCD design maximum value of total flavonoid content in 30 groups. \( Z_i \) was total saponin content measurement value per group. \( Z_{max} \) was CCD design maximum value of total saponin content in 30 groups.

**Experimental Design for Response Surface Optimization**

Functional ingredients and flavoring agents are important factors that affect the nutritional and flavor quality of HBT (Shen et al., 2019). Therefore, in this study, the synthesis score of the total saponins content, total flavonoids content and sensory score was the response value. The addition ratio of *Siraitia grosvenorii* fruit powder \((X_1)\), *Canarium album* fruit powder \((X_2)\), erythritol \((X_3)\) and citric acid \((X_4)\) were chosen as key independent variables to optimize formula of HBT using a four-factor and five-level Central Combination Design (CCD). The experimental scheme was shown in Table 2.

**Verification Indicator: In Vitro Antioxidant Activity, Glycemic Index, Sem of Microstructure**

**In vitro Antioxidant Activity of HBT**

**DPPH Radical Scavenging Capacity**

The DPPH free radical cleaning ability of samples were measured according to the method of (Pio-León et al., 2018) with slight modifications. The optimized HBT was ground into powder and dissolved in 60% ethanol to obtain sample solution with different concentrations \((0.05, 0.50, 1.00 \text{ mg/mL})\). 2.0 mL sample solutions and 2.0 mL DPPH (0.1 mmol/L, dissolved in 95% ethanol) were mixed together and reacted for 30 min in dark. Then, the mixture was centrifuged at 8000 r/min for 10 min. The absorbance \(A_1\) of the supernatant was determined at 517 nm. At the same time, the absorbance \(A_2\) was detected for reaction system of DPPH solution (2 mL) and sample solvent (2 mL). Vc and sugar-Sweetened Confectionery (CSC) solutions with different concentrations \((0.05, 0.5, 1.0 \text{ mg/mL})\) were used as the control. Each test was repeated for 3 times. The average value was used to calculate scavenging rate on DPPH as well as the half inhibitory concentration (IC_{50}) of the samples. DPPH scavenging rate was calculated as following.

**Hydroxyl Radical (•OH) Scavenging Capacity**

The measurement was conducted according to the method of Li (2013) with slight modifications. 2 mL sample solutions \((0.05, 0.50, 1.00 \text{ mg/mL})\) was added in the test tube, respectively. 2 mL H₂O₂ solution \((6 \text{ mmol/L})\) and 2 mL FeSO₄ solution \((6 \text{ mmol/L})\) were added to 2 mL sample solutions. The mixture was blended and stood for 10 min. 2 mL salicylic acid \((6 \text{ mmol/L})\) was added to the mixture and kept at 37°C for 30 min. After that, the mixture was centrifuged at 5000 r/min for 5 min. The supernatant was used to determine absorbance at 510 nM, which was A1. The sample solution was replaced with 2 mL 60% ethanol (sample solvent) and its absorbance \((A0)\) was determined. Finally, the salicylic acid solution was replaced with 2 mL anhydrous ethanol (salicylic acid solvent) and its absorbance \((A2)\) was determined.

Different concentrations of Vc solutions \((0.05, 0.5 \text{ and } 1.0 \text{ mg/mL})\) and different concentrations of CSC solutions \((0.05, 0.5 \text{ and } 1.0 \text{ mg/mL})\) were used as the control. The experiment was replicated for three times. The hydroxyl free radical scavenging rate was calculated by the following formula:

**Determination of Total Reducing Capacity**

The total reducing capacity of samples was determined based on the method of Wang (Wang et al., 2021). 1 mL sample solutions with different concentrations \((0.05, 0.50 \text{ and } 1.00 \text{ mg/mL})\), 2.5 mL phosphate buffer \(0.2 \text{ mol/L, pH 6.6})\) and 2.5 mL potassium ferricyanide solution \((1%, \text{ w/v})\) were mixed and incubated at 50°C for 20 min. Then, 2.5 mL trichloroacetic acid solution \((10%, \text{ w/v})\) was added into the test tube and centrifuged at 3000 r/min for 10 min. 5 mL supernatant, 5 mL distilled water and 1 mL ferric trichloride solution \((0.1%, \text{ w/v})\) were mixed together and blended. After standing for 10 min, the absorbance \((OD)\) value of homogenate was determined at 700 nM. The larger the OD value, the
stronger the total weight of the reaction measurement. Vc solutions (0.05, 0.5, 1.0 mg/mL) and CSC solutions (0.05, 0.5, 1.0mg/mL) acted as the controls. Each sample was repeated three times for analysis.

**Glycemic Index of HBT in vitro**

The Glycemic index was measured according to a method described by Englyst *et al.* (1999) and Goñi *et al.* (1997) with slight modifications. The digestion rate of starch in low-sugar Buccal Tablets (HBT) was measured by hydrolysis in vitro with complex enzymes to predict the glycemic index. The hydrolysis rate of HBT was calculated based on the following formula:

\[
HBT\text{ (or} \text{ RF}W\text{B)} \text{ starch hydrolysis rate(%) } = \frac{[\text{Sampling time point hydrolyzed glucose amount} \times 0.90]}{\text{Total quality of samples}}
\]

(8)

In this experiment, CSC with sucrose acted as control. Digestibility of sucrose in vitro by hydrochloric acid hydrolysis was determined to predict the glycemic index. (CNSMC, 2016). The polysaccharide hydrate rate of CSC was calculated according to the following formula:

\[
\text{Hydrolysis rate(%) } = \frac{[\text{Sampling time point hydrolyzed glucose amount} \times 0.95]}{\text{Total quality of control samples}}
\]

(9)

The Hydrolysis Index (HI) of sample (HBT and CSC) was calculated based on the following formula:

\[
HI = \text{the AUC of sample / the AUC of reference food (white bread)}
\]

(10)

where, the AUC are the areas under hydrolysis curves (0-180 min) for all products (HBT, CSC and white bread) respectively.

The glycemic index (GI) of HBT and CSC was calculated based on the following equation (Xue *et al.*, 2018):

\[
GI = 39.71 + 0.549HI
\]

(11)

where HI was the hydrolysis index of the HBT and CSC respectively.

**Microstructure Scanning of HBT**

The microscopic morphology of HBT was performed by Scanning Electron Microscopy (SEM) (Correia *et al.*, 2013). The HBT was crushed into powder and dried at 40°C for 12 h. The powder was evenly coated on the dissociated mica slide sprayed with metal and observed by SEM.

**Statistical Analysis**

Data Analysis was performed according to the reported method (Umana *et al.*, 2020). The software of Design Expert V 8.0.6 was used to optimize and analyze the variance of the results. Each group of experiments was repeated 3 times and the average value was taken for further analysis. The results were expressed as the mean ± SD (standard deviation). Significant differences among samples were determined at p<0.05(Olaoye *et al.*, 2022).

**Table 1:** The sensory evaluation criteria of herbal buccal tablet

<table>
<thead>
<tr>
<th>Scores</th>
<th>Flavor</th>
<th>Appearance</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-40</td>
<td>Suitable sourness and sweetness, no bitterness and astrigency</td>
<td>Complete and smooth surface, natural and uniform color</td>
<td>Smooth, delicate and ungranular</td>
</tr>
<tr>
<td>21-30</td>
<td>Comparative suitable sourness and sweetness, mild astrigency or bitterness</td>
<td>Complete without imperfection, smooth surface, natural and uniform color</td>
<td>smooth, delicate and ungranular</td>
</tr>
<tr>
<td>11-20</td>
<td>More acidic, heavier bitterness and astrigency</td>
<td>Disability and rough surface, slightly uneven color is very uneven</td>
<td>Smooth, slightly rough, slightly grainy</td>
</tr>
<tr>
<td>0-10</td>
<td>More acidic, bitterness and astrigency are heavier</td>
<td>Complete without imperfection, smooth surface, natural and uniform color</td>
<td>Too crisp, rough and grainy</td>
</tr>
</tbody>
</table>

**Table 2:** Levels and codes of four factors in central composite design

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Level</th>
<th>Addition ratio of Siraitia grosvenorii (w/w, %)</th>
<th>Addition ratio of Canarium album (w/w, %)</th>
<th>Addition ratio of erythritol (w/w, %)</th>
<th>Addition ratio of citric acid (w/w, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>19</td>
<td>20</td>
<td>60</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>16</td>
<td>55</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>12</td>
<td>50</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td>7</td>
<td>8</td>
<td>45</td>
<td>0.45</td>
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<tr>
<td>-2</td>
<td>3</td>
<td>4</td>
<td>40</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>
Results

Statistical Analysis and the Model Building

A four-factor five-level test was carried out in accordance with the CCD-RSM. The result was shown in Table 3. Multiple regression fitting on the response values and each factor was performed using Design-Expert 8.0.6.1 software. The quadratic polynomial regression equation was obtained as following:

\[
Y = 0.70 + 0.036X_1 + 0.072X_3 + 0.008X_3 - 0.023X_2 + 0.011X_1X_2 - 0.001X_1X_3 + 0.021X_3 - 0.003X_1X_4 - 0.013X_1X_4 - 0.032X_3X_4 + 0.021X_1^2 - 0.004X_3^2 + 0.007X_4^2 + 0.021X_4^2
\]

where, \(Y\) is the synthesis score of the total saponins content, total flavonoids content and sensory score. \(X_1, X_2, X_3\) and \(X_4\) are the addition ratio of \(Siraitia grosvenorii\) fruit powder, \(Canarium album\) fruit powder, erythritol and citric acid (%), respectively.

The Analysis of Variance (ANOVA) was performed for the regression model according to the method (Sunday 2020; Eissa et al., 2022), which was shown in Table 4. The F test showed that the regression model had a high F value (\(F = 4.84\)) and a low P value (\(P = 0.0022\)), indicating that the model was highly significant (\(P < 0.01\)). The lack of fit of the equation was not significant (\(P > 0.05\)), indicating that the established regression quadratic model could be used to analyze the formula optimization of HBT.

Optimization of Formula Hbt and Validation of the Model

As shown in Table 4, the coefficient evaluation and significance test of the regression model showed that the interaction parameters \(X_2X_4\) was significant (\(P < 0.05\)), suggesting that the interaction between erythritol and citric acid significantly affected the synthesis score of HBT. According to the above established model, the optimal formula of HBT principal component was obtained (15% \(Siraitia grosvenorii\) fruit powder, 16% \(Canarium album\) fruit powder, 55% erythritol, 0.45% citric acid). The comprehensive evaluation score of total flavonoids, total saponins content and sensory value was 0.9175. Validation tests were carried out under these conditions. The above formula ingredients and other ingredients (0.3% stevia glycoside, 5% Arabic gum and 7.1% lily bulb powder) were mixed evenly to prepare soft material and then the soft material was sieved into particles (18 mesh). The particles were dried at 50°C until moisture was less than or equal to 3%. The dried particles were added into 1% magnesium stearate and 0.15% menthol. Finally, HBT was prepared by pressing the above ingredients into tablets. The actual total flavonoids content of HBT was 10.08±0.05 mg/g. The total saponins content was 11.28±0.03 mg/g. The sensory score was 87 points and synthesis score were 0.9319 points. The actual result was a 1.57% deviation compared with the predicted data by the regression model. These results indicated that the model had reliable predictability in optimizing the formula parameters of HBT. The microcapsule powder of xanthyl ester, blueberry powder, isomaltitol, sorbitol and other auxiliary materials were mixed to prepare pressed candy by tableting technology. The optimum formula was determined using RSM as follows: Blueberry powder was 3.19%, peppermint essence was 4.26%, sucralose taste was 0.19%, lutein ester microcapsule powder was 9%, isomaltose ketositol was 55%, sorbitol was 30%, magnesium stearate was 1% and arabic gum was 0.75%. After wet granulation, drying, sizing, blending and tableting. Smooth tableted candy was obtained (Liu et al., 2019).

In Vitro Antioxidant Analysis of HBT

In vitro antioxidant activity of HBT was seen in Fig. 2. As shown in Fig. 2(A), the scavenging capacities of different samples on DPPH free radicals increased when the concentration increased gradually. The scavenging capacities of HBT against DPPH free radicals was lower than the positive control Vc at the same concentration but higher than CSC significantly (\(P < 0.05\)). The half inhibitory concentration (\(IC_{50}\)) values of HBT, CSC and Vc against DPPH were 0.4398, 1.108 and 0.1131 mg/mL, respectively. Lower \(IC_{50}\) values means a higher antioxidant activity. These results indicated that the effect of HBT on DDPH free radicals was always larger than CSC, although it was smaller than positive control Vc.

As shown in Fig. 2(B), the samples with different concentrations had the effect of scavenging hydroxyl free radicals (\(-\cdot OH\)). The scavenging rate of HBT, CSC and Vc had an obvious dose-effect relationship in the concentration range from 0.05 to 1.00 mg/mL. The \(IC_{50}\) of HBT, CSC and Vc against \(-\cdot OH\) were 0.3856, 1.7121 and 0.1650 mg/mL, respectively. The \(-\cdot OH\) scavenging rate of HBT was superior to CSC significantly (\(P < 0.05\)), lower than positive control Vc overall. The \(-\cdot OH\) scavenging rate of HBT was closer to that of Vc and the scavenging rate reached 90.41% when the concentration was 1.00 mg/mL.

Reducing force of antioxidants are often measured by the capacity of reducing potassium ferricyanide (Fe(III) to Fe(II)). The absorbance at 700 nM was determined to calculate the reducing force (Han et al., 2019). As shown in Fig. 2(C), the reducing power of HBT was lower than that of the positive control Vc, but was larger than that of CSC at the same concentration significantly (\(P < 0.05\)). As the concentration increased, the reducing power of all samples generally improved in a dose-effect manner within the concentrations of 0.05–1.00 mg/mL. The median Effect Concentration (\(EC_{50}\)) of HBT, CSC and Vc was 0.661, 1.494 and 0.454 mg/mL, respectively. The results showed that the total reducing power of HBT was larger than that of CSC. Based on the formula of HBT.
principal component, HBT contained man component of *Siraitia grosvenorii* and *Canarium album*. *S. grosvenorii* is a Chinese perennial that grows in southern China (Li et al., 2009). For the past two centuries, its fruit has been used to treat dry coughs, sore throats and severe thirst (Lu et al., 2012), which contains morgosides, polysaccharides, polyphenols, vitamins, etc. (Abdel-Hamid et al., 2020). *S. grosvenorii* has antitussive, anti-asthmatic, antioxidant and anti-diabetic activities (Liu et al., 2018). In China, the ripe fruit of *C. album* is also used as food and traditional medicine to treat swelling and sore throat, polydipsia, cough, etc. with antioxidant activity (Chang et al., 2017). The results of antioxidant analysis revealed that HBT had the higher antioxidant activity than CSC, which may be due to the antioxidant components of *S. grosvenorii* and *C. album* in HBT.

**In Vitro Glycemic Index Evaluation of Hbt**

**In Vitro Total Sugar Hydrolysis Rate**

The rate of total sugar digestion such as starch or sucrose, etc., was expressed as the percentage of total sugar hydrolysed in the product at different times (30, 60, 90, 120 and 180 min) (Goñi et al., 1997). The curve was shown in Fig. 3. The white bread was used as the reference food. The in vitro hydrolysis rate of total sugar between groups of products increased from 0 to 90 min obviously and then reached a maximal plateau level from 90 to 180 min slowly. The hydrolysis value of total sugar for the Reference Food White Bread (RFWB) was 80.42% within 180 min. The hydrolysis value for CSC was 37.66%. Compared with these control groups, HBT generated least percentage of hydrolysis, which was only 21.95%. The reason may be the different ingredient contents in HBT and CSC. There are many factors that affect starch digestion, such as the source and processing of starch, which can significantly affect the glycemic index of starch (Hu et al., 2004). HBT incorporated non-sugar sweetener (erythritol, stevia) in the hydrolysis to reduce the total sugar content and its digestibility compared with white bread with high starch content and CSC with sucrose as sweetener, resulting in reduction of total sugar hydrolysis rate.

**In Vitro Glycemic Index**

The GI refers to the relative ability of sugary foods to raise blood glucose level, compared to the postprandial blood glucose response of a reference food (glucose or white bread). The GI of hyperglycemic food is greater than or equal to 70. The GI of medium-glycemic food is in the range of 56-69 and the hypoglycemic food is less than or equal to 55 (Zhang et al., 2013). As shown in Table 5, the total sugar Glycemic Index (HI) for HBT was 22.50 and lower than the control CSC significantly (P<0.05) which was 88.45.

**Table 3**: Central composite design with response values for formula the optimization of HBT

<table>
<thead>
<tr>
<th>run</th>
<th>Xi(%)</th>
<th>Xj(%)</th>
<th>Xk(%)</th>
<th>Xl(%)</th>
<th>Sensory Score</th>
<th>Total Flavonoids (mg/g)</th>
<th>Total Saponins (mg/g)</th>
<th>Y (synthesis score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>72</td>
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<td>6.23</td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
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DOI: 10.3844/ajbbsp.2022.87.99
Table 4: Analysis of variance for the fitted quadratic polynomial model

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<th>Variance source</th>
<th>Sum of square</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F-value</th>
<th>P-value</th>
<th>Significant test</th>
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<td>Model</td>
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Table 5: Hydrolysis index (HI) and glycemic index of different buccal tablets

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<th>GI</th>
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<td>CSC</td>
<td>88.45±0.72a</td>
<td>88.26±0.65a</td>
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<tr>
<td>HBT</td>
<td>22.50±0.61b</td>
<td>52.06±0.252b</td>
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Fig. 2: The in vitro antioxidant activities of HBT and CSC using Vc as the positive control. (A) DPPH· scavenging capabilities; (B) ·OH scavenging capabilities; (C) Reducing power. Different lower-case letters for the same concentration represented sign if
Fig. 3: Total sugar hydrolysis rate of different products in vitro

Fig. 4: The image of HBT and raw material mixed powder (A is HBT, B is a raw material mixed powder)

Fig. 5: SEM images of microstructure of HBT (A: 500times; B: 1000times; C: 2000times; D: 10000times)
The predictive GI for HBT was 52.06 by Eq. (11), which may be classified as a food with low-glycemic index (GI≤55), also lower than CSC by 41.01% significantly (P<0.05). The GI value of CSC was as high as 88.26, which belonged to hyperglycemic food (GI>75). (Zhang et al., 2013) found that the hydrolysis index and glycemic index of resistant starch biscuits were 49.19 and 66.72, respectively. Meanwhile, the resistant starch biscuits had the advantages of uniform color and intact appearance with moderate glycemic index (Zhang et al., 2013). Our results suggested that HBT belonged to food with low-glycemic index, which was different from the resistant starch biscuits (Zhang et al. 2013). The main reason may be that the type and content of starch were different. The results indicated that HBT may be suitable for people with diabetes and those who want to control blood sugar.

**Evaluation of the Appearance and Microstructure**

Four kinds of spray dried fruit powder (pineapple, dragon fruit, guava and mango), maltodextrin and stevia were mixed to prepare mixed fruit and vegetable powder tablet by tableting technology. SEM image showed irregular particle shape on the surface of fruit powder tablet (Saifullah et al., 2014). The HBT obtained by plant powder tableting process was light yellow, which was closer to white with a smooth surface (Fig. 4A). It matched the color of the raw mixed material powder before processing (Fig. 4B), which was mainly composed of white lily bulb powder, a small amount of brown Siraitia grosvenorii fruit powder and Canarium album fruit powder. The result showed that the plant powder tableting process had better color protection effect on candy products.

SEM image of microstructure of HBT powder was shown in Figure under SEM magnified 500-10000 times. As shown in Fig. 5, the microscopic particle is irregular in shape and vary in size. Most of the small particles (except for a few scattered) are attached to the large particles, indicating that HBT by plant powder tableting process were rich in components, easy to interact and cross-link between molecules, which made the particles easy bond.

**Discussion**

In this study, four factors that have a greater impact on the efficacy ingredients and taste of HBT components were selected, including Siraitia grosvenorii fruit powder, Canarium album fruit powder, erythritol and citric acid, which act as independent variables. The CCD-RSM was used for a four-factor five-level test to optimize the optimal HBT formula based on the comprehensive evaluation of the content of functional ingredients (total saponins, total flavonoids) and sensory scores. Under the predicted formula conditions, the actual comprehensive scores of total flavonoids, saponins content and sensory scores in HBT were almost equivalent to the predicted values, indicating that the CCD-RSM could be a feasible way to optimize the formula parameters of HBT.

The results showed that the antioxidant capacity of optimized HBT formula was higher than that of CSC and lower than the positive control VC (Fig. 1). When the mass concentration of HBT increased to 1 mg/mL, its scavenging rate against •OH was close to VC (Fig. 1(B)), which was probably due to the combination of VC at the high concentration •OH electrons was close to saturation. •OH was in equilibrium, so the radicals scavenging rate was difficult to improve and close to HBT at the same concentration (Akinmoladun et al., 2007). The antioxidant capacity of HBT was higher than that of CSC, which was due to the strong antioxidant activity of total saponins and flavonoids in Siraitia grosvenorii fruit (Qi et al., 2006; Shao et al., 2019), Canarium album fruit (Xu et al., 2017) and lily bulb powder (Su et al., 2021; Lei et al., 2015). The verification test showed that content of total flavonoids and total saponins in HBT reached 10.08±0.05 mg/g and 11.28±0.03 mg/g respectively. These ingredients may serve as the material basis of HBT antioxidant function. Precise structural analysis is necessary to investigate in further study.

The in vitro hydrolysis rate of total sugar and predictive glycemic index in HBT were lower than those of CSC (Fig. 2 and Table 5), suggesting that non-sugar sweeteners (erythritol, stevioside) instead of traditional sugars (such as sucrose) used in HBT could effectively reduce the sugar content and glycemic index. In summary, the HBT prepared may be a foodstuff suitable for obese and diabetic people.

**Conclusion**

In the present work, the formula optimization, in vitro antioxidant capacity and glycemic index of HBT were investigated. The optimal formula of HBT by RSM was obtained. The HBT prepared by optimal formula had a cool taste with suitable sweet and sour taste. HBT had a higher antioxidant activity than the control CSC. In addition, the GI of HBT was lower than that of CSC. HBT was more suitable for people suffering from inflammation caused by free radicals such as pharyngitis as well as high blood sugar.

The present study might provide a reference for the development of low-sugar health foods. In the future, other bioactive components involved in antioxidant function in HBT still need to be further identified. Meanwhile, precise structural analysis of antioxidant functional components in HBT and their structure-activity relationship need to be further investigated.

**Nomenclature**

HBT  low-sugar herbal buccal tablet  
RSM  Response surface methodology  
CSC  Commercial sugar-sweetened confectionery  
TCM  Traditional Chinese Medicine
Acknowledgement

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Author’s Contributions

Yuqing Wang, Mingjie Sun, Rui Xu and Xuan Hu: Performed the preparation, Antioxidant and Glycemic Index Evaluation of HBT.

Yao Chen, Jieru Zhang and Gaixia QU: Carried out the formulation optimization of HBT based on comprehensive evaluation of total saponins, total flavonoids and sensory scores.

Yang Zhang, Hongfang Cai: Took Photographs of product appearance and electron microscope scanning of microstructure.

Hongfang Cai and Xinyi Chen: Revised and polish the manuscript.

Dongxing Zhu: Designed the experiments, wrote and polish the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References


Sridharan, B., Mehra, Y., Ganesh, R. N., & Viswanathan, P. (2016). Regulation of urinary crystal inhibiting proteins and inflammatory genes by lemon peel extract and formulated citrus bioflavonoids on ethylene glycol induced urolithic rats. Food and Chemical Toxicology, 94, 75-84. doi:10.1016/j.fct.2016.05.013


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