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Investigation into the glucose-regulating acitivity and mechanisms of Siraitia grosvenorii extracts and their application in the innovation of functional biscuits

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Table 1 to 5 are available in the Supplementary Files section.

Investigation into the glucose-regulating acitivity and mechanisms of *Siraitia grosvenorii* extracts and their application in the innovation of functional biscuits

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Abstract

Siraitia grosvenorii (Swingle) C. Jeffrey, a plant belonging to the Cucurbitaceae family, which is commonly used as a food-medicine homologous species in southern China. In this study, bioactive compounds from *Siraitia grosvenorii* were investigated, and a functional biscuit with blood sugar-lowering properties was developed. Five triterpenoids were isolated from the 70% ethanol extract: 11-oxomogroside V (**1**), mogroside VIB (**2**), 11-oxomogroside VI (**3**), mogroside V (**4**), and mogrol (**5**). Among them，mogroside V exhibited the strongest inhibition of α-amylase, with an IC_{50} value of 0.44±0.009 μM/L, showing significant blood sugar-lowering effects. Kinetic studies revealed that compounds **1**-**5** are complex inhibitors of PPA, binding both to PPA and the PPA-starch complex. Compound **1** was a non-competitive inhibitor, while the others acted as competitive inhibitors. CD spectroscopy showed that compound **4** most strongly inhibited α-amylase by altering its secondary structure. A functional biscuit with hypoglycemic properties was then developed. TPA analysis showed that *Siraitia grosvenorii* extract had little effect on the texture of the biscuits. Notably, *Siraitia*

grosvenorii serves as a natural sweetener with blood glucose-lowering effects, making it an innovative ingredient for enhancing the functional value of biscuits.

Keywords: *Siraitia grosvenorii*, Hypoglycemic activity, *α*-Amylase inhibition kinetics, Functional biscuits

Introduction

Biscuits, as a traditional snack food, are widely favored by consumers due to their convenience, simple production process, and long shelf life. However, conventional biscuits are typically high in sugar and calories and are classified as high glycemic index (GI) products, making them unsuitable for individuals with hypertension, diabetes, or obesity. With the advancement of the economy and the improvement of living standards, consumer focus has shifted from mere satiety to nutritional balance and health. Consequently, recent research and development in the biscuit industry have increasingly emphasized functionality to cater to the evolving dietary needs of the population. According to data published by the International Diabetes Federation, the number of adult diabetes patients increased from 151 million in 2000 to 451 million in 2017, more than doubling, and is expected to reach 693 million by 2045 [1]. As a potential intervention, food products that assist in lowering blood sugar have gradually gained attention [2]. Research has shown that functional foods rich in natural plant ingredients, which can effectively regulate blood sugar, have become an important supplement for the daily management of diabetes patients [3]. Based on this, the present study seeks to develop a healthy, low-sugar, hypoglycemic functional biscuit by replacing traditional sucrose with mogroside, a natural sweetener derived from *Siraitia*

grosvenorii, aiming to provide a more suitable nutritional option for individuals with specific dietary needs.

Siraitia grosvenorii, the mature fruit of a plant in the Cucurbitaceae family, is predominantly distributed in Guangxi Province, China [4]. It has been a commonly used medicinal and edible resource in southern regions, known for its hypoglycemic activity, antioxidant capacity, and anticancer potential [5-8]. Mogrosides, which belong to triterpenoid saponins, are the main active ingredients of *Siraitia grosvenorii*. These compounds exhibit a sweetness significantly greater than that of sucrose, while providing only one-fiftieth of its caloric content [9-11]. As natural low-calorie sweeteners, they are suitable for individuals with diabetes or obesity [12]. Studies have demonstrated that mogrosides can enhance postprandial insulin secretion, helping to maintain glycemic homeostasis and offering potential therapeutic benefits for both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) [13, 14]. Therefore, *Siraitia grosvenorii*, which contains a variety of functionally active components, holds considerable promise for the prevention and adjunctive management of diabetes.

To further expand the application of *Siraitia grosvenorii* in the food industry, this study examined the hypoglycemic activity and mechanism of its bioactive components and developed a functional biscuit based on the results. The findings of this study provide a comprehensive approach to the utilization of *Siraitia grosvenorii* and the development of functional foods targeting blood glucose regulation.

Materials and methods

Plant material

Siraitia grosvenorii, purchased from Guilin Xing'an Dingkang Food Co., Ltd., has its plant specimen stored in Room 504. Department of Functional Foods and Wine, Shenyang Pharmaceutical University.

Extraction and isolation

The 5 kg of pulverized *Siraitia grosvenorii*, which underwent three consecutive 3 hours reflux extractions with eight volumes of 50% ethanol, was filtered and concentrated under reduced pressure to yield 2444 g of 50% ethanol crude extract. The crude extract, which was uniformly suspended in an appropriate amount of distilled water, was fractionated on a D101 macroporous resin column using water, 50% ethanol, 70% ethanol, and 95% ethanol in sequence, to afford eluates of 120 g (50% ethanol), 160 g (70% ethanol), and 6 g (95% ethanol), respectively.

The 70% ethanol extract (160g) was subjected to gradient separation on a silica gel column chromatography (CC) using DCM-MeOH (100:0 to 0:100, v/v, gradient) as the elution solvent, and 26 fractions (Fr. 1-26) were collected. Thin-layer chromatography (TLC) was used to characterize the different components eluted during the gradient process. Fr. 18 (DCM-MeOH, 100:24) was further purified on an ODS column using a gradient of MeOH-H_2O (20:80, 40:60, 60:40, 70:30, 80:20, 90:10, 100:0, v/v), resulting in the isolation of compound **5**. Fr. 23 (DCM-MeOH, 100:45) was also purified on the ODS column with a gradient of MeOH-H_2O (20:80, 40:60, 60:40, 70:30, 80:20, 90:10, 100:0, v/v), and the MeOH-H_2O (55%) fraction was separated, which was further purified using HPLC with a mobile phase consisting of MeOH-H_2O (50%), yielding compounds **1**-**4**.

Inhibition of *α*-Amylase activity

The inhibition ability of different extracts and compounds **1**-**5** on *α*-amylase was determined. 80 μL of *α*-amylase (1 mg/mL) was mixed with various extracts and compounds **1**-**5** (0.1, 0.2, 0.4, 0.8, and 1.6 mg/mL) or acarbose (0.1, 0.2, 0.4, 0.8, and 1.6 mg/mL, positive control) and incubated at 37 ℃ for 30 minutes in a water bath. Subsequently, 80 μL of completely dextrinized soluble starch (1 mg/mL) was added to the solution, and the mixture was incubated for an additional 15 minutes under the same conditions. After incubation, 120 μL of DNS reagent was added, followed by boiling in a water bath for 5 minutes to terminate the reaction. The mixture was then cooled to room temperature, diluted to 4 mL with deionized water, and the absorbance was measured at 540 nm (multifunctional microplate reader, Molecular Devices). The experimental results are expressed as the half-maximal inhibitory concentration (IC_{50}; μg/mL) value for each subject. All experiments were conducted in triplicate. The inhibition rate (IR) of *α*-amylase was calculated as follows:

$$\text{Inhibition (\%)} = [1-(A_{\text{Sample}} - A_{\text{Sample control}})/(A_{\text{Blank}} - A_{\text{Blank control}})] \times 100$$

Inhibition kinetic analysis

The inhibition mode was evaluated based on inhibition kinetics using the Lineweaver-Burk plot double- reciprocal equation. Inhibition kinetics were assessed by incubating *α*-amylase (1.0 mg/mL) with various concentrations of soluble starch (8, 9, 10, 11, and 12 mg/mL), which served as the substrate, and compounds **1**-**5** (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL), which acted as the inhibitor. The double-reciprocal curve, in which the reciprocal of the substrate concentration (1/[S]) was plotted on the X-axis

and the reciprocal of the reaction velocity (1/V) on the Y-axis, were used to determine the type of inhibition. All experiments were conducted in triplicate.

Kinetic parameters were calculated from the linear Weaver Burk double reciprocal equation [15] as follows:

$$1/V = 1/V_{max} + K_m/[V_{max}[S]]$$

The competitive inhibition constant (K_{ic}) was calculated using the Dixon plot, based on the following equation [16]:

$$v = V_{max}[S]/[Km(1+[I]/K_{ic})+[S]]$$

The uncompetitive inhibition constant (K_{iu}) was confirmed by the following Cornish-Bowden equation [17]:

$$v/[S] = V_{max}/(K_m+[S])(1+[I]/K_{iu})$$

Where v, v_{max}, Km, [I], [S], represent initial reaction rate, maximum reaction rate, substrate concentration, inhibitor concentration, respectively.

CD measurement

The circular dichroism (CD) spectra were recorded using a 1 mm pathlength cuvette over a wavelength range of 200-260 nm, with a scanning speed of 60 nm/min at 37 °C. The slit width was set to 1 nm, and the response time was 1 second. The measurements were performed in the presence of PPA at a fixed concentration of 0.4 mg/mL. Compounds **1**-**4** were tested at a concentration of 0.3 μmol/L, while compound **5** was tested at 0.8 μmol/L. Each spectrum was obtained as the average of three consecutive scans. PBS buffer (20 mmol/L) was used as the blank control. The experimental data were analyzed and processed using the CDPro software package.

Optimization of *Siraitia Grosvenorii* biscuit manufacturing process

Based on 100 g of low-gluten flour, the study systematically investigates the effects of 70% ethanol extract of *Siraitia grosvenorii* (0.5, 1.0, 1.5, 2.0, 2.5 g), butter (25, 30, 35, 40, 45 g), milk powder amount (2, 4, 6, 8, 10 g), baking soda amount (0.20, 0.25, 0.30, 0.35, 0.40 g), baking time (10, 15, 20, 25, 30 min) and baking temperature (100, 110, 120, 130, 140 ℃) on the quality of cookies. The optimal conditions for each factor were determined by adjusting these variables individually.

Sensory evaluation

The sensory evaluation was conducted by 10 individuals based on five factors: appearance, organization structure, taste, gloss, smell with the scoring criteria shown in **Table 1**. The degree of preference is as follows: a score below 60 indicates "very disliked," a score between 61 and 80 indicates "acceptable," and a score between 81 and 100 indicates "very liked". Biscuits that receive at least 80% of the "very liked" ratings are considered successful.

Measurement of texture properties

The *Siraitia Grosvenorii* extract was added in concentrations of 0%, 0.05%, 0.10%, 0.15%, 0.20%, and 0.25%. A 10 g sample of dough was accurately weighed and shaped into cubes with dimensions of 1 cm in length, width, and height. Then, five cookies with a diameter of 5 cm were selected, cooled to room temperature, and set aside. After resting for 10 minutes, the texture of both the dough and the cookies was measured using a TPA texture analyzer. The testing parameters were as follows: cylindrical probe TA/10, testing speed: 2 mm/s, testing mode: Distance, testing distance: 15 mm, trigger

force: 5 g, testing time: 5 s. Each sample was tested in duplicate, with three parallel measurements for each repetition, and the average value was taken.

Determination of total saponin content in *Siraitia Grosvenorii* biscuits

Weigh 1.0 g of biscuits, grind and sieve, dissolve in methanol, and centrifuge to collect the supernatant. Then, dilute to a final volume of 10 mL to prepare the sample solution. Take 1 mL of the sample solution, transfer it to a 10 mL colorimetric tube, evaporate the solvent to dryness, and add 0.5 mL of 8% vanillin-acetic acid reagent. Heat the mixture at 60 ℃ for 15 minutes, then immediately place it in an ice-water bath for 2 minutes. Add 5 mL of concentrated sulfuric acid, shake well, and use the reagent as a blank control. After ensuring no bubbles are present, measure the absorbance at 540 nm using a UV-visible spectrophotometer.

Results and Discussion

Identification of compounds 1-5 from *Siraitia grosvenorii*

Five triterpenoid compounds were isolated from the 70% ethanol extract of *Siraitia grosvenorii* as 11-oxomogroside V (**1**) [18], mogroside VIB (**2**) [19], 11-oxomogroside VI (**3**) [20], mogroside V (**4**) [21], and mogrol (**5**) [22] (**Fig. 1**) based on ^{1}H and ^{13}C NMR spectroscopic data by comparison with previous literature.

11-oxomogroside V (1): ^{1}H-NMR (400 MHz, Pydine-d_5) δ_H: 5.51 (1H, d, J=6.4Hz, H-6), 5.51 (1H, d, J=7.6Hz, H-G$_I$1), 5.15 (1H, d, J=7.4Hz, H-G$_{IV}$1), 4.94 (1H, d, J=7.6Hz, H-G$_{II}$1), 4.88 (1H, d, J=7.7Hz, H-G$_V$1), 4.83 (1H, d, J=7.6Hz, H-G$_{III}$1), 1.52 (3H, s, H-29), 1.47 (3H, s, H-26), 1.36 (3H, s, H-27), 1.26 (3H, s, H-19), 1.18 (3H, s, H-28), 1.01 (3H, s, H-30), 1.00 (3H, d, J=6.4Hz, H-21), 0.74 (3H, s, H-18). ^{13}C-NMR

(100 MHz, Pydine-d_5) δ_C: 213.69 (C-11), 141.01 (C-5), 118.20 (C-6), 106.72 (C-G_{IV}1), 105.98 (C-G_{II}1), 105.24 (C-G_V1), 104.64 (C-G_{III}1), 103.46 (C-G_I1), 28.32 (C-2), 28.00 (C-16), 26.81 (C-27), 25.55 (C-29), 20.09 (C-19), 18.48 (C-21), 18.12 (C-30), 16.80 (C-18).

mogroside VIB (2): ^{1}H-NMR (400 MHz, Pydine-d_5) δ_H: 5.18 (1H, d, J=6.4Hz, H-6), 5.47 (1H, d, J=8.0Hz, H-G_{IV}1), 5.18 (1H, d, J=7.3Hz, H-G_{VI}1), 4.88 (1H, d, J=7.4Hz, H-G_{II}1), 4.79 (1H, d, J=7.8Hz, H-G_I1), 4.52 (1H, d, J=7.7Hz, H-G_V1), 4.24 (1H, d, J=6.7Hz, H-G_{III}1), 1.52 (3H, s, H-29), 1.46 (3H, s, H-26), 1.34 (3H, s, H-27), 1.33 (3H, s, H-19), 1.16 (3H, s, H-28), 1.09 (3H, d, J=6.3Hz, H-21), 0.92 (3H, s, H-30), 0.91 (3H, s, H-18). ^{13}C-NMR (100 MHz, Pydine-d_5) δ_C: 145.31 (C-5), 119.43 (C-6), 106.58 (C-G_{VI}1), 104.44 (C-G_{III}1), 88.45 (C-G_{IV}2), 83.53 (C-G_{III}2), 71.27 (C-G_I6), 71.11(C-G_{III}6), 28.75 (C-28), 28.01 (C-27), 27.20 (C-29), 25.66 (C-19), 25.43 (C-7), 20.31 (C-30), 20.26 (C-21), 18.04 (C-18).

11-oxomogroside VI (3): ^{1}H-NMR (400 MHz, Pydine-d_5) δ_H: 5.50 (1H, d, J=7.4Hz, H-6), 5.16 (1H, d, J=7.7Hz, H-G_V1), 4.91 (1H, d, J=7.2Hz, H-G_{IV}1), 4.88 (1H, d, J=7.6Hz, H-G_{VI}1), 4.81 (1H, d, J=7.8Hz, H-G_{III}1), 4.50 (1H, d, J=7.6Hz, H-G_{II}1), 4.26 (1H, d, J=7.0Hz, H-G_I1), 1.52 (3H, s, H-29), 1.46 (3H, s, H-26), 1.35 (3H, s, H-27), 1.18 (3H, s, H-19), 1.00 (3H, d, J=6.7Hz, H-21), 1.03 (3H, s, H-28), 1.02 (3H, s, H-30), 0.75 (3H, s, H-18). ^{13}C-NMR (100 MHz, Pydine-d_5) δ_C: 213.66 (C-11), 141.01 (C-5), 118.26 (C-6), 70.25 (C-G_{IV}6), 69.97 (C-G_I6), 63.22 (C-G_{VI}6), 62.53 (C-G_{III}6), 62.31 (C-G_{II}6), 60.79 (C-G_V6), 28.09 (C-28), 27.99 (C-27), 26.80 (C-29), 25.56 (C-26), 20.10 (C-19), 19.25 (C-21), 18.82 (C-30), 18.11 (C-18).

mogroside V (4): ^{1}H-NMR (400 MHz, Pydine-d_5) δ_H: 5.74 (1H, d, J=7.5Hz, H-6), 5.49 (1H, d, J=7.5Hz, H-G$_I$1), 5.19 (1H, d, J=7.5Hz, H-G$_{IV}$1), 4.93 (1H, d, J=7.0Hz, H-G$_{II}$1), 4.88 (1H, d, J=7.5Hz, H-G$_V$1), 4.82 (1H, d, J=8.0Hz, H-G$_{III}$1), 1.61 (3H, s, H-29), 1.53 (3H, s, H-26), 1.47 (3H, s, H-27), 1.35 (3H, s, H-19), 1.15 (3H, s, H-28), 1.13 (3H, s, H-30), 1.08 (3H, d, J=6.7Hz), 0.92 (3H, s, H-18). ^{13}C-NMR (100 MHz, Pydine-d_5) δ_C: 144.09 (C-5), 118.15 (C-6), 106.80 (C-G$_I$1), 105.37 (C-G$_{II}$1), 105.22 (C-G$_V$1), 104.65 (C-G$_{IV}$1), 103.46 (C-G$_{III}$1), 29.73 (C-2), 27.35 (C-16), 26.80 (C-29), 26.60 (C-27), 26.04 (C-1), 19.15 (C-30), 18.82 (C-21), 16.89 (C-18).

mogrol (5): ^{1}H-NMR (400 MHz, Pydine-d_5) δ_H: 5.83 (1H, d, J=4.5Hz, 3-OH), 5.73 (1H, brs, 24-OH), 5.68 (1H, d, J=5.5Hz, 11-OH), 5.55 (1H, s, 25-OH), 5.35 (1H, d, J=7.0Hz, H-6), 4.21 (1H, td, J=4.5, 4.5, 11.5Hz, H-3), 3.77 (1H, td, J=5.5, 11.0Hz, H-11), 3.62 (1H, brs, H-24), 1.57 (3H, s, H-26), 1.54 (3H, s, H-27), 1.45 (3H, s, H-29), 1.39 (3H, s, H-19), 1.33 (3H, s, H-28), 1.03 (3H, d, J=6.5Hz, H-21), 1.00 (3H, s, H-30), 0.97 (3H, s, H-18). ^{13}C-NMR (100 MHz, Pydine-d_5) δ_C: 145.17 (C-5), 120.09 (C-6), 80.00 (C-24), 78.78 (C-11), 77.21 (C-3), 73.64 (C-25), 28.16 (C-16), 27.66 (C-22), 27.07 (C-26), 26.86 (C-19), 25.56 (C-7), 20.22 (C-28), 19.85 (C-30), 17.96 (C-18).

The *α*-Amylase inhibition activity

Significant differences were observed in the inhibitory activity against porcine pancreatic *α*-amylase (PPA) among the 50% ethanol extract of *Siraitia grosvenorii* and its macroporous resin eluates obtained with 50%, 70%, and 95% ethanol, respectively (**Fig. 2A**). The 50% and 70% ethanol macroporous resin eluate exhibited the strongest inhibitory effects, with IC_{50} values of 0.58 ± 0.02 mg/mL and 0.56 ± 0.04 mg/mL (**Table**

214 **2**), respectively. These results preliminarily suggest that the major bioactive
215 components with hypoglycemic activity were primarily enriched in the 50% and 70%
216 ethanol eluates of *Siraitia grosvenorii*, which were fractions that contain the compounds
217 responsible for α-amylase inhibition. Compounds **1**-**5** exhibited varying degrees of
218 porcine pancreatic amylase (PPA) inhibitory activity (**Fig. 2B**). Among them,
219 compound **4** showed the strongest inhibitory effect and significantly stronger than the
220 positive control at high dose ($p < 0.05$). The IC_{50} value of compound **4** was 0.44 ± 0.009
221 μmol/L (**Table 2**), slightly lower than the IC_{50} value of acarbose (0.48 ± 0.008 μmol/L).

222 **α-Amylase inhibition kinetics**

223 The Lineweaver-Burk double reciprocal plots for compounds **1**-**5** (**Fig. 3**) passed
224 through the x-axis and y-axis and intersected at a point in the first or second quadrant.
225 Further analysis of the Dixon and Cornish-Bowden plots for compounds **1**-**5** (**Fig. 4**)
226 revealed that they all intersect at a single point, confirming that compounds **1**-**5** acted
227 as mixed-type α-amylase inhibitors. These results suggested that compounds **1**-**5** could
228 form a starch-inhibitor-α-amylase complex by binding to both α-amylase and its α-
229 amylase-starch complex and the inhibitory effect of was independent of substrate
230 concentration.

231 Lower K_{ic} and K_{iu} values generally indicate stronger inhibitory effects. In competitive
232 inhibition, a lower K_{ic} value means that the inhibitor was more likely to bind to the free
233 enzyme, while in non-competitive inhibition, a lower K_{iu} value suggested that the
234 inhibitor was more likely to bind to the enzyme-substrate complex. The K_{ic} and K_{iu}
235 values of compounds **1**-**5** were determined from the Dixon and Cornish-Bowden plots,

respectively, as the absolute values of the abscissas corresponding to the intersection points in the plots (**Fig. 4**). As shown in **Table 3**, for compound **1**, the K_{ic} value was greater than the K_{iu} value, suggesting that it was a non-competitive inhibitor of α-amylase and tends to bind to the α-amylase-starch complex. In contrast, the K_{ic} values for the remaining compounds were lower than their respective K_{iu} values, indicating that they exhibit competitive inhibition and bind more tightly to free α-amylase.

CD spectra

Circular dichroism (CD), an intuitive and effective technique, enabled the detection of changes in the secondary structure of α-amylase treated with inhibitors by comparing it to the characteristic spectrum of α-amylase. Two typical negative bands at 209 nm and 220 nm can be clearly observed from the CD spectra, which were mainly attributed to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the amide group, representing the characteristics of the α-helix. The result showed (**Fig. 5**) that the degree of the double negative bands of α-amylase reacted with each compound varies significantly, which was attributed to the covalent bond between the inhibitors and the amino group of α-amylase. Among these compounds, compound **4** exhibited significant α-amylase inhibitory activity. In the presence of compound **4**, the contents of α-helix, β-turn, and random coil increase from 26.8% to 27.8%, 22.3% to 22.8%, and 23.5% to 29.8%, respectively, while the β-sheet decreases from 28.5% to 19.1% (**Table 4**). This result suggested that compound **4** could tightly bind to α-amylase, altering its secondary structure through a hydrogen bond network, which in turn hinderd substrate entry into the catalytic site, leading to α-amylase inactivation.

Optimal manufacturing process of *Siraitia Grosvenorii* Biscuits

Additive amount of *Siraitia grosvenorii* extract, butter, milk powder and baking soda. The addition of *Siraitia grosvenorii* extract, butter, milk powder, and baking soda significantly affects the quality of *Siraitia grosvenorii* biscuits. The result (**Fig. 6**) showed that when the *Siraitia grosvenorii* extract content was 1.5 g, the biscuits had a strong *Siraitia grosvenorii* aroma, a golden color, and an even texture. However, an excessive amount of *Siraitia grosvenorii* extract resulted in a noticeable color change, while too little extract led to an absence of distinct *Siraitia grosvenorii* aroma and a whitish color. When the butter content was 35 g, the flavor and texture of the biscuits were enhanced, making them crisp and non-greasy. Conversely, excessive addition of butter not only led to the greasy taste of biscuits, but also had the negative effects on health. When the milk powder content was 6 g, the biscuits had a rich taste with a suitable milk aroma, whereas too much milk powder can overshadow the biscuits natural flavor. When the baking soda content was 0.20 g, the biscuits were crisp with a well-defined structure, while an excess of baking soda resulted in undesirable reactions on the surface and a rough texture. In conclusion, the optimal additions of *Siraitia grosvenorii* extract, butter, milk powder, and baking soda were 1.5 g, 35 g, 6 g, and 0.20 g, respectively.

Baking time and baking temperature. Baking time and temperature significantly affect the quality of *Siraitia grosvenorii* biscuits, directly influencing their taste, internal texture, and surface color. The result showed (**Fig. 6**) that when the baking time was 20 minutes, the biscuits, which were crisp, had a complete surface and a golden

color. However, when the time was either too long or too short, the texture of the biscuits becomes either soft or hard. When the baking temperature is 120°C, the biscuits, which exhibit a golden color, a crisp texture, and an even structure, show optimal quality. Conversely, when the temperature is lower or higher than this range, the biscuits color may become too pale or overly browned, resulting in an undesirable taste. In conclusion, a baking time of 20 minutes and a temperature of 120°C are the optimal conditions.

Texture Profile Analysis

Texture properties such as hardness, elasticity, and chewiness of the dough and biscuits were measured through Texture Profile Analysis (TPA). As shown in **Table 5**, the addition of *Siraitia grosvenorii* extract had minimal effect on the texture properties of the dough. After high-temperature baking, the addition of *Siraitia grosvenorii* extract had little effect on the hardness, elasticity, and chewiness of the biscuits, indicating that the use of *Siraitia grosvenorii* extract at the current concentration was feasible for developing functional products without compromising the texture properties.

Total saponin content of *Siraitia grosvenorii* biscuits

The total saponin content in *Siraitia grosvenorii* functional biscuits showed (**Fig. 7**) minimal change before and after baking. Moreover, with the increasing addition of *Siraitia grosvenorii* extract, the total saponin content in the biscuits continuously increases. When the addition amounts of *Siraitia grosvenorii* extract are 0.05%, 0.10%, 0.15%, 0.20%, and 0.25%, the total saponin contents in the functional biscuits are 0.0298, 0.0579, 0.0917, 0.1218, and 0.1497 mg/g, respectively. These results indicated that *Siraitia grosvenorii* saponins was stable and high temperature resistant, which was

suitable for the preparation of functional biscuits.

Conclusions

This study represents the first application of *Siraitia grosvenorii* extract in the development of functional biscuits. Rich in triterpenoid saponins, which confer exceptionally high sweetness, low caloric content, and substantial nutritional value, *Siraitia grosvenorii* is highly suitable for incorporation into health-oriented food products. The inhibitory activity of *Siraitia grosvenorii* extract and its monomeric compounds against pancreatic α-amylase (PPA) was systematically evaluated. The fractions eluted with 50% and 70% ethanol from macroporous resin exhibited notable hypoglycemic activity, among which compound **4** demonstrated the strongest inhibitory effect. This compound acts as a mixed-type inhibitor of PPA, exerting its effect through modulation of the enzyme's secondary structure. Furthermore, sensory evaluation results indicated that 1.5 g was the optimal amount of *Siraitia grosvenorii* extract to be added to the biscuits. Textural Profile Analysis (TPA) was employed to assess the dough's hardness, elasticity, and chewiness. The results showed that the addition of *Siraitia grosvenorii* extract had minimal impact on the textural properties of both the dough and the final biscuit product. In conclusion, *Siraitia grosvenorii* extract possesses significant antihyperglycemic potential and can be effectively utilized in the formulation of functional biscuits. As a natural ingredient that is not only low in calories but also rich in nutrients, it enhances the health value of biscuits and offers a novel and promising strategy for the development of antidiabetic functional foods.

Ethics statement

This study involved sensory evaluations conducted by a trained panel, which was approved by the Ethics Review Committee of the Department of Functional Food and Wine at Shenyang Pharmaceutical University (approval date: September 5, 2024). Prior to the experiments, all participants were given written information about the study and provided written, voluntary consent to participate. They were assured that their personal information and responses would remain confidential and that they could withdraw from the study at any time.

CRediT authorship contribution statement

Qing Yang and Wei Li wrote the manuscript; Wei Li funded the experiments; Qianqian Yang and Xuejin Wang contributed to the determination of bioactive components and activity testing; Qing Yang carried out the process optimization and quality assessment of the functional biscuits; the final manuscript was read and approved by all authors.

Declaration of competing interest

The authors declare that there are no competing financial interests or personal relationships that could be perceived as influencing the research and conclusions presented in this paper.

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Data availability

Data will be made available on request.

Consent to Publish

Not applicable.

Abbreviations

IC_{50}: half maximal inhibitory concentraion

PPA: Porcine Pancreatic Alpha-Amylase

CD: circular dichroism

TPA: texture profile analysis

References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ (2022) IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice 183: 13, Article 109119. https://doi.org/10.1016/j.diabres.2021.109119

2. Zheng Y, Yang W, Sun W, Chen S, Ye X (2020) Inhibition of porcine pancreatic α-amylase activity by chlorogenic acid. Journal of Functional Foods 64: 7, Article 103587. https://doi.org/10.1016/j.jff.2019.103587

3. Riccardi G, Capaldo B, Vaccaro O (2005) Functional foods in the management of obesity and type 2 diabetes. 8(6): 630-635. https://doi.org/10.1097/01.mco.0000171126.98783.0c

4. Wang Q, Qin HH, Wang W, Qiu SP (2010) The pharmacological research progress of Siraitia grosvenorii. Journal of Guangxi University of Chinese Medicine 13(03): 75-76. https://doi.org/10.3969/j.issn.1008-7486.2010.03.040

5. Liu ZB, Zhu XF, Mohsin A, Yin ZP, Zhuang YP, Zhou B, Du LX, Yin XJ, Liu N, Wang ZJ, Guo MJ (2022) Embryogenic callus induction, cell suspension culture, and spectrum-effect relationship between antioxidant activity and polyphenols composition of Siraitia grosvenorii cultured cells. Industrial Crops and Products 176: 15, Article 114380. https://doi.org/10.1016/j.indcrop.2021.114380

6. Wang L, Li ZY, Zhong YY, An H, Ye Y (2022) The mechanism of the effective ingredients of Siraitia grosvenorii in the treatment of lung cancer based on network pharmacology. Journal of Guangxi University of Science and Technology 33(2): 107-115,125. https://doi.org/10.16375/j.cnki.cn45-1395/t.2022.02.016

7. Suzuki YA, Tomoda M, Murata Y, Inui H, Sugiura M, Nakano Y (2007) Antidiabetic effect of long-term supplementation with Siraitia grosvenori on the spontaneously diabetic Goto-Kakizaki rat. British Journal of Nutrition 97(4): 770-775. https://doi.org/10.1017/s0007114507381300

8. Suzuki YA, Yuji M, Hiroshi I, Masaki S, Yoshihisa N (2005) Triterpene glycosides of Siraitia

grosvenori inhibit rat intestinal maltase and suppress the rise in blood glucose level after a single oral administration of maltose in rats. Journal of Agricultural & Food Chemistry 53(8): 2941-2946. https://doi.org/10.1021/jf0478105
9. Takemoto T, Arihara S, Nakajima T, Okuhira M (1983) Studies on the constituents of fructus Momordicae. I. On the sweet princple. Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan 103(11): 1151-1154. https://doi.org/10.1248/yakushi1947.103.11_1151
10. Takemoto T, Arihara S, Nakajima T, Okuhira M (1983) Studies on the constituents of fructus Momordicae. II. Structure of sapogenin. Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan 103(11): 1155-1166. https://doi.org/10.1248/yakushi1947.103.11_1155
11. Takemoto T, Arihara S, Nakajima T, Okuhira M (1983) Studies on the constituents of fructus Momordicae. III. Structure of mogrosides. Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan 103(11): 1167-1173. https://doi.org/10.1248/yakushi1947.103.11_1167
12. Li HB, Zhang M, Wang Y, Guo BJ, Li L (2006) Colorimetric determination of triterpenoid saponin in Luohanguo. Food Science 27(06): 171-173. https://doi.org/10.3321/j.issn:1002-6630.2006.06.037
13. Chen WJ, Wang J, Qi XY, Xie BJ (2007) The antioxidant activities of natural sweeteners, mogrosides, from fruits of Siraitia grosvenori. International Journal of Food Sciences and Nutrition 58(7): 548-556. https://doi.org/10.1080/09637480701336360
14. Liu XY, Cao HW, Lai JB, Fang YJ, Huang XX, Li XM (2020) Clinical Observation of Empagliflozin Combined with Metformin Therapy in Treatment of Patients with Newly Diagnosed Overweight and Obese Type 2 Diabetes Mellitus. Medical & Pharmaceutical Journal of Chinese People's Liberation Army 32(9): 26-29. https://doi.org/10.3969/j.issn.2095-140X.2020.09.007
15. Lineweaver H, Burk D (1934) The Determination of Enzyme Dissociation Constants. Journal of the American Chemical Society 56(3): 658–666. https://doi.org/10.1021/ja01318a036
16. Dixon M (1953) The determination of enzyme inhibitor constants. Biochemical journal 55(1): 170-171. https://doi.org/10.1016/0003-9861(53)90381-6
17. Cornish-Bowden A, Eisenthal R (1974) Statistical considerations in the estimation of enzyme kinetic parameters by the direct linear plot andother methods. The Biochemical journal 139(3): 721-730. https://doi.org/10.1042/bj1390721
18. Chaturvedulasupa/Sup VSP, Prakashsupa/Sup I (2011) Cucurbitane Glycosides from Siraitia grosvenorii. Journal of Carbohydrate Chemistry 30(1): 16-26. https://doi.org/10.1080/07328303.2011.583511
19. Niu B, Ke CQ, Li BH, Li YY, Yi YJ, Luo YW, Shuai L, Yao S, Lin LG, Li J, Ye Y (2017) Cucurbitane Glucosides from the Crude Extract of Siraitia grosvenorii with Moderate Effects on PGC-1α Promoter Activity. Journal of Natural Products 80(5): 1428-1435. https://doi.org/10.1021/acs.jnatprod.6b01086
20. Li F, Yang FM, Liu X, Wang L, Chen B, Li LH, Wang MK (2017) Cucurbitane glycosides from the fruit of Siraitia grosvenori and their effects on glucose uptake in human HepG2 cells in vitro. Food Chemistry 228: 567-573. https://doi.org/10.1016/j.foodchem.2017.02.018
21. Li C, Lin LM, Luo M, Ma CF, Wang ZM (2011) A new natural saponin from fruits of Siraitia grosvenorii. China Journal of Chinese Materia Medica 36(6): 721-724. https://doi.org/10.4268/cjcmm20110616
22. Yang XW, Zhang JY (2007) NMR Structural Elucidation of Mogrol and Its Glycosides. Chinese Journal of Magnetic Resonance 24(3): 249-260. https://doi.org/10.1016/S1872-2067(07)60066-7

425 **Figure Caption**

426 **Fig.1 The compounds isolated from *Siraitia grosvenorii***

427 **Fig. 2 Inhibitory effect of different concentration of 50% ethanol extract of *Siraitia***

428 ***grosvenorii* and eluates of 50%、70%、95% on *α*-amylase, and compounds 1-5.**

429 **$^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$ versus positive control**

430 **Fig. 3 Lineweaver-Burk plots for *α*-amylase inhibition by compounds 1-5**

431 **Fig. 4 Dixon and Cornish-Bowden plots for *α*-amylase inhibition by compounds 1-**

432 **5**

433 **Fig. 5 CD spectra of *α*-amylase with compounds 1-5**

434 **Fig. 6 Overall view and sensory rating of the biscuits. (A1) *Siraitia grosvenorii***

435 **extract addition amounts: 0.5, 1.0, 1.5, 2.0, 2.5 g, (B1) Butter addition amounts: 25,**

436 **30, 35, 40, 45 g, (C1) Milk powder addition amounts: 2, 4, 6, 8, 10 g, (D1) Baking**

437 **soda addition amounts: 0.20, 0.25, 0.30, 0.35, 0.40 g, (E1) Baking time: 10, 15, 20,**

438 **25, 30 min, (F1) Baking temperature: 100, 110, 120, 130, 140 ℃, (A2) Effect of**

439 ***Siraitia grosvenorii* extract addition on sensory rating, (B2) Effect of butter**

440 **addition amount on sensory rating, (C2) Effect of milk powder addition amount**

441 **on sensory rating, (D2) Effect of baking soda addition amount on sensory rating,**

442 **(E2) Effect of baking time on sensory rating, (F2) Effect of baking temperature on**

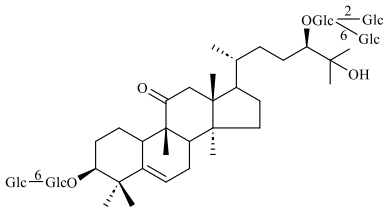
443 **sensory rating**

444 **Fig. 7 Total saponin content of *Siraitia grosvenorii* functional biscuits**

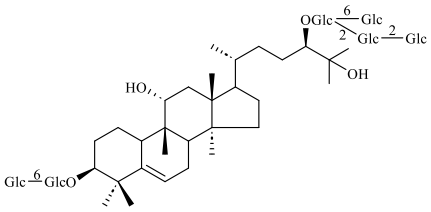
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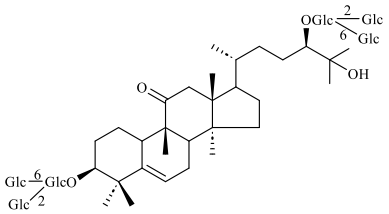
Fig. 1



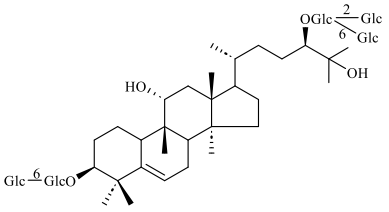
11-oxomogrosideV (1)



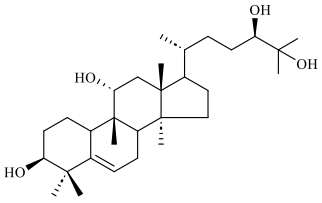
mogrosideVIB (2)



11-oxomogrosideVI (3)

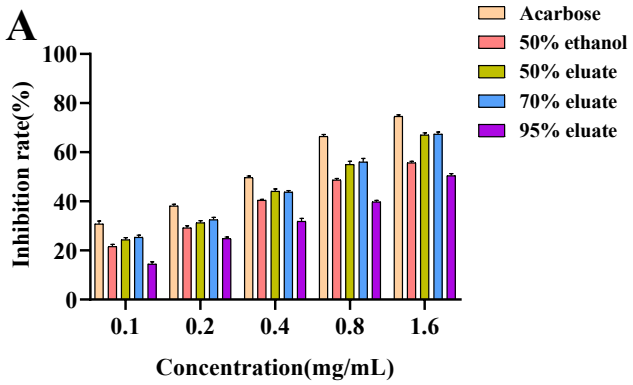


mogrosideV (4)

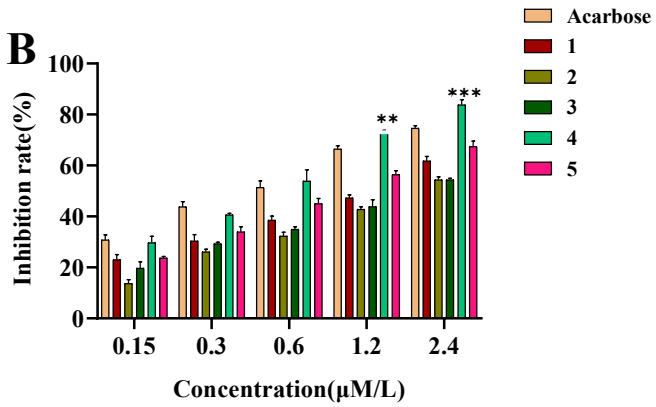


mogrol (5)

463 **Fig.2**



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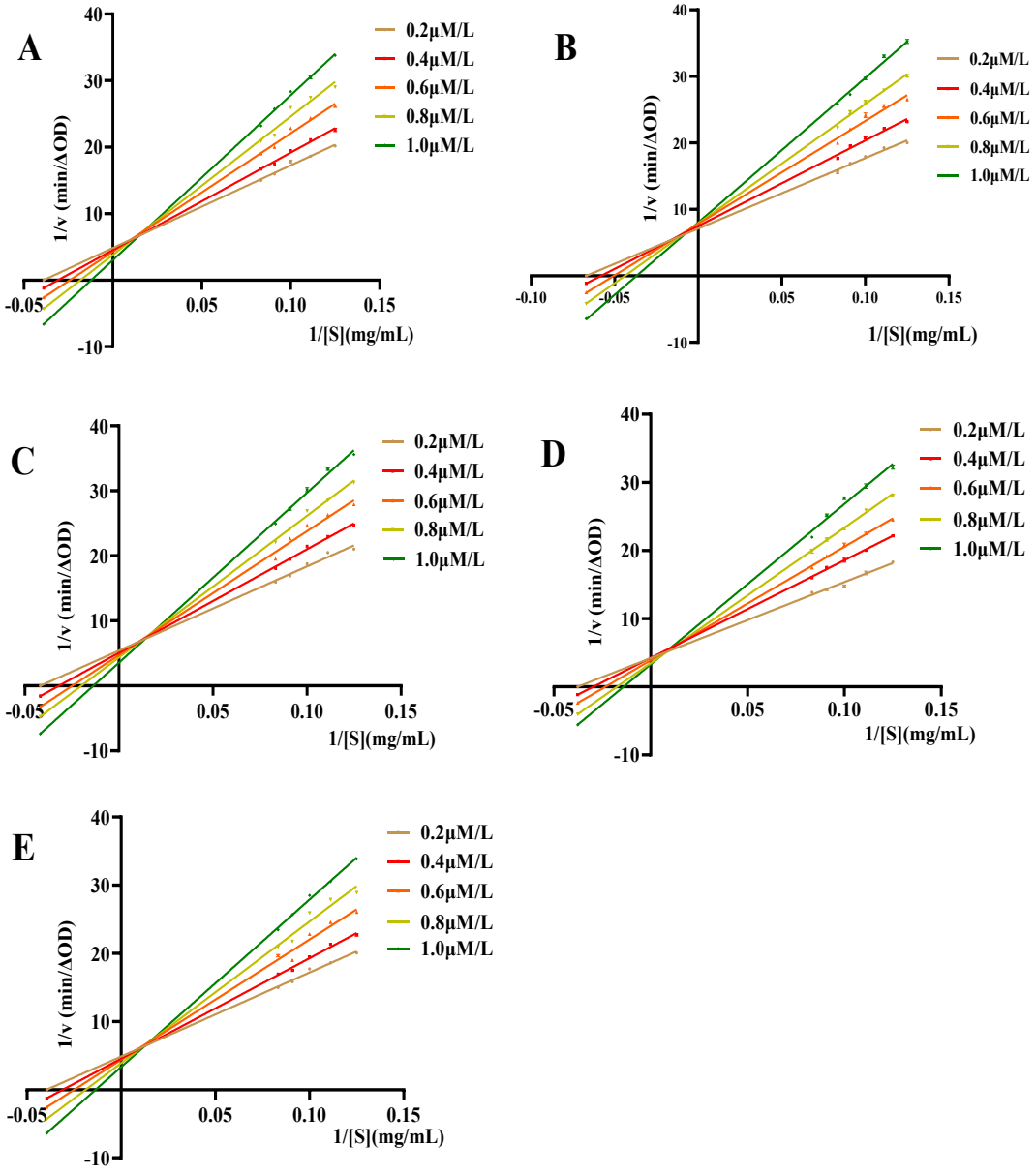
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476 **Fig.3**



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Fig.4

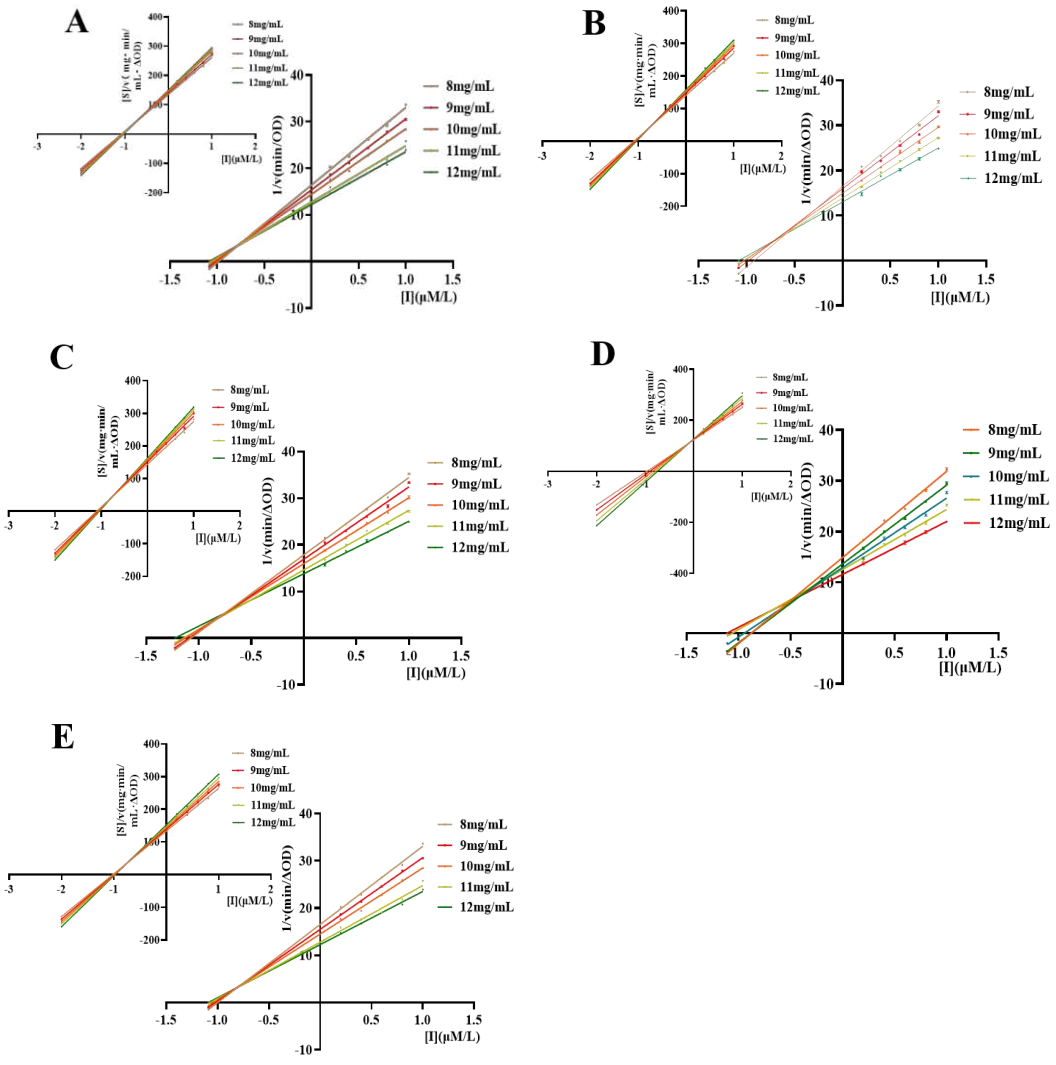

A
B
C
D
E
8mg/mL
9mg/mL
10mg/mL
11mg/mL
12mg/mL
[I](μM/L)
1/v(min/ΔOD)
[S]/v(mg·min/mL·ΔOD)

Fig. 5

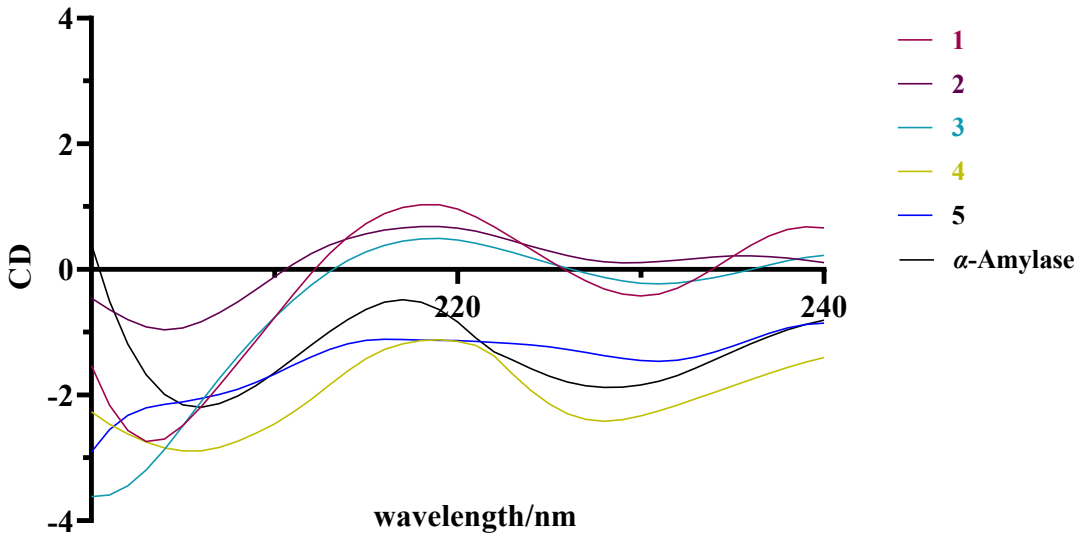
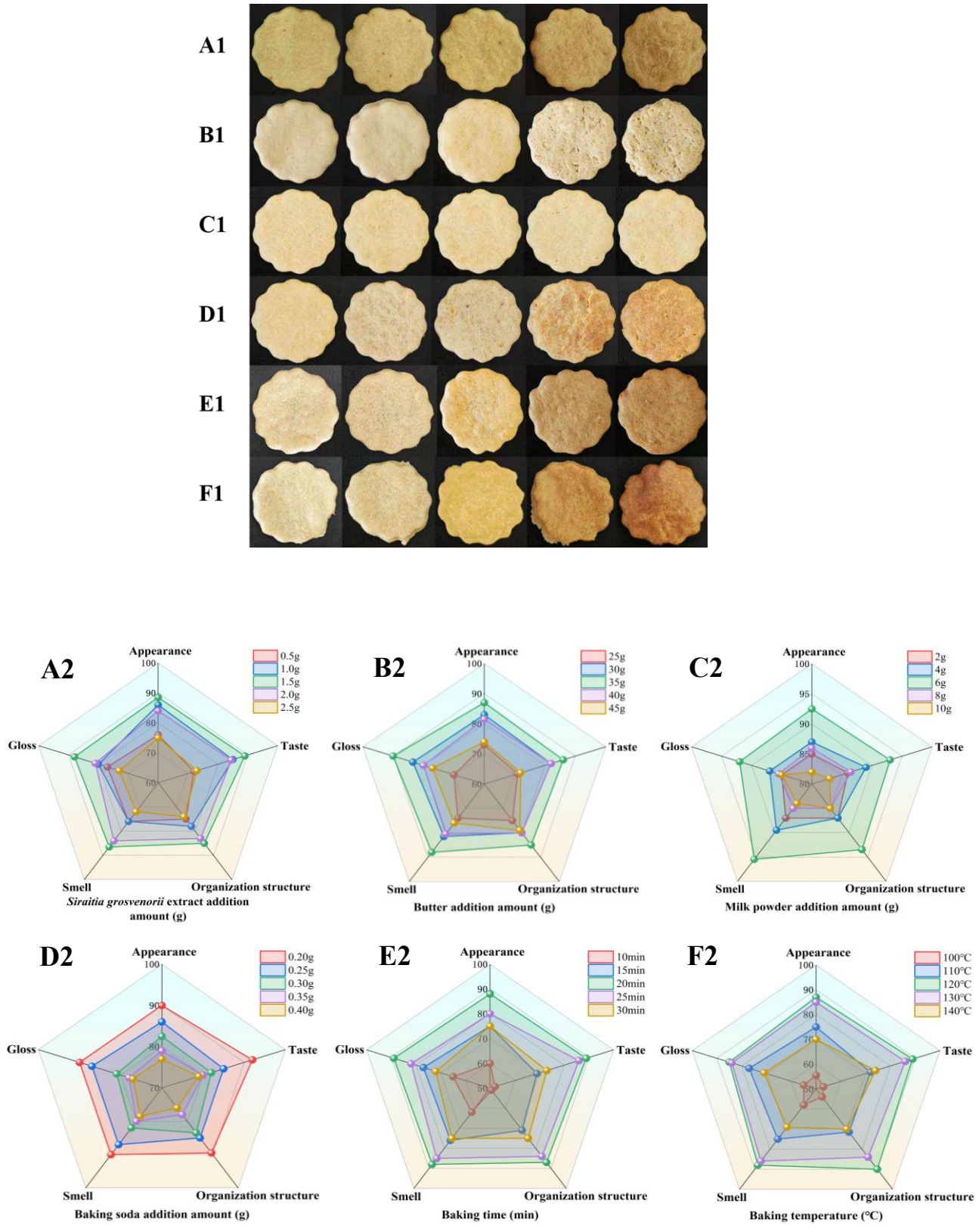
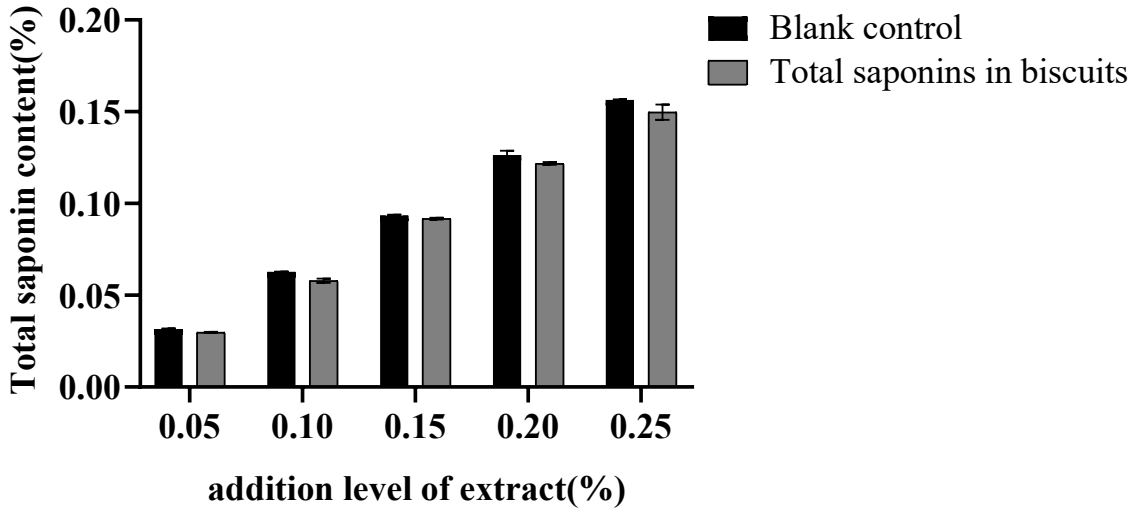


Fig. 6


A1
B1
C1
D1
E1
F1
A2
Appearance
Taste
Organization structure
Smell
Gloss
0.5g
1.0g
1.5g
2.0g
2.5g
Siraitia grosvenorii extract addition amount (g)
B2
25g
30g
35g
40g
45g
Butter addition amount (g)
C2
2g
4g
6g
8g
10g
Milk powder addition amount (g)
D2
0.20g
0.25g
0.30g
0.35g
0.40g
Baking soda addition amount (g)
E2
10min
15min
20min
25min
30min
Baking time (min)
F2
100℃
110℃
120℃
130℃
140℃
Baking temperature (℃)

535 **Fig. 7**


Total saponin content(%)
0.20
0.15
0.10
0.05
0.00
0.05
0.10
0.15
0.20
0.25
addition level of extract(%)
Blank control
Total saponins in biscuits

536

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