Study on the in Vitro Inhibitory Activity of Peony Seed Blended Oil on α-Amylase and α-Glucosidase

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Abstract: In recent years, China has been suffering from an increasing number of patients with chronic diseases such as diabetes, hypertension, and heart disease, which are gradually showing a "younger" trend. As health problems become more prominent, choosing good cooking oil has become crucial. People are beginning to realize the significance of a balanced diet and healthy eating habits in maintaining good health. Peony seed oil is rich in nutrients, and its content of unsaturated fatty acids can reach over 85%, with the highest content of α-linolenic acid, which effectively lowers blood sugar. Suppose soybean oil and rapeseed oil are combined and added. In that case, the original efficacy of peony seed oil can be complemented and enhanced, its mechanism of action network can be sound, and its nutritional composition can be enriched. We can obtain an optimal ratio for mixing the above several oils by studying their effects and roles. This paper explored the inhibitory effects of peony seed oil, soybean oil, and rapeseed oil on α-amylase activity and α-glucosidase activity, and their optimal ratios were determined by a one-way test and response surface method. The results showed that the optimal ratio was peony seed oil: soybean oil: rapeseed oil = 60:27:13. The results provide specific technology and reference for the development and utilization of peony and provide a particular theoretical basis for the development of hypoglycemic drugs or health food.
***Keywords*:** peony seed oil, soybean oil, rapeseed oil, inhibition rate, α-glucosidase, α-amylase, hypoglycemia

1. Introduction

Peony (Paeonia suffruticosa Andr.) is a deciduous shrub of the Ranunculaceae family (Paeoniaceae). It has many colors and flower shapes. It is known as the "national flower and the king of flowers"[1]. It is widely distributed in Henan, Anhui, and other places. Peony is not only ornamental but also has high medicinal value. Among traditional Chinese medicines, it is called "Paeonia", which reduces blood and relieves blood stasis. [2] The peony seeds are pressed; the golden transparent oily liquid obtained after decolorization and deodorization is peony seed oil. According to the Chinese Ministry of Health Document No. 9 of 2011, this oil has been approved as a new resource food. It has high unsaturated fatty acid content, including α-linolenic acid, linoleic acid, oleic acid, eicosenoic acid, etc., accounting for 89.19% of the total oil. Linolenic acid has the highest content, accounting for 37.84%, linoleic acid for 25.72%, and oleic acid for 21.41% [3]. In addition, peony seeds also contain a variety of pharmacologically active ingredients such as flavonoids and sterols, such as resveratrol, oleanolic acid, luteolin, kaempferol, apigenin, β-carotene, β-sitosterol, etc., which have high nutritional and medicinal value. [4-5] Peony seed oil is rich in omega-3 polyunsaturated fatty acids: α-linolenic acid. α-linolenic acid is an essential fatty acid for the human body, and it cannot synthesize this substance. It can only be taken in from the outside. α-linolenic acid has the functions of regulating blood lipids [6], reducing blood viscosity, increasing blood oxygen carrying capacity, antioxidants [7], and sun protection [8]. α-glucosidase and α-Amylase can degrade glucose in the small intestinal digestive tract and play an important role. By inhibiting its activity, it can effectively inhibit the increase in blood sugar levels after meals [9]. The third-generation oral hypoglycemic drug acarbose can reduce postprandial blood sugar. The effect of concentration [10] is a commonly used α-glucosidase inhibitor in clinical practice. This article compares the inhibition rate of the blended oil on α-glucosidase and α-amylase with that of acarbose on α-glucosidase — And α-amylase inhibition rate to explore the blood sugar-lowering effect of peony seed blend oil.

2. Experimental instruments and reagents

2.1 Instrumentation

RAININ pipet-lite SL-2 (0.1-2μl), SL-10 (0.5-10μl), SL-200 (20-200μl), SL-1000 (100-1000μl); Microplate Readers: Agilent BioTek SMATBCD, microplate 96 wells, transparent, flat bottom; SPECORD 250 PLUS Double Beam UV-Vis Spectrophotometer, with 5 Variable Spectral Bandwidths and Double Monochromator, Analytik Jena; H1650 Benchtop High-Speed Centrifuge Machine, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.; AXTD5A low-speed large-

capacity centrifuge, Shanghai Zhaodi Biotechnology Co., Ltd.; AXTD5A low-speed large-capacity centrifuge, Shanghai Zhaodi Biotechnology Co., Ltd.; SQP Electronic Balance, Sartorius Scientific Instruments Beijing Co., Ltd.; DT series electronic balance, Changshu Yiou Instrumentation Co., Ltd., Jiangsu Province; HH-S6 digital display constant temperature water bath, Jintan Medical Equipment Factory; Pipettes; beakers; glass rods; cuvettes; rubber-tipped droppers; several volumetric flasks.

2.2 Reagents

α-glucosidase 5KU, food-grade α-amylase edible enzyme preparation, medium-temperature biological enzyme 100,000 enzyme activity powder hydrolase; p-nitrophenyl-α-D-glucopyranoside PNPG 99%, Macklin Inc.; Peony Seed Oil; Rapeseed Oil, Yihai Kerry Golden Arowana Cereals, Oils and Food Co., Ltd.; Acarbose ≥98%; Non-GMO Third-grade Soybean Oil, Jiusan Food Co., Ltd.; DNS Chromogenic Agent; Soluble Starch from Potato, Sinopharm Chemical Reagent Co., Ltd.; Ethanol, Tianjin Fuyu Fine Chemical Co., Ltd.; Sodium Phosphate Monobasic Dihydrate, Tianjin Kaitong Chemical Reagent Co., Ltd.; Purified Water 500ml, Hangzhou Wahaha Group Co., Ltd.; Potassium Sodium Tartrate Tetrahydrate, Tianjin Kaitong Chemical Reagent Co., Ltd.; Sodium Hydroxide, Tianjin Dongli District Tianda Chemical Reagent Factory; Methanol, Tianjin Tianli Chemical Reagent Co., Ltd.; Sodium Carbonate Anhydrous; Ethanol 95%; Hydrogen Phosphate Disodium.

3. Experimental methods

3.1 Preparation of solution

(1) Preparation of acarbose reference substance solution: Take about 100 mg of acarbose reference substance and weigh it accurately, place it in a 10mL volumetric flask, dissolve it with a small amount of distilled water and set it to volume, shake well, and prepare the acarbose reference substance. Reserve solution and refrigerate until use. [11]

(2) Prepare 0.1mol/L phosphate buffer solution (pH=6.8): Weigh 17.4g of dipotassium hydrogen phosphate, place it in a 1L volumetric flask, dissolve it in distilled water, and dilute to volume to obtain liquid A. Weigh 13.6g of potassium diphosphate, dissolve it in distilled water and set it to a 1L volumetric flask to obtain liquid B. Mix liquid A and liquid B in a particular proportion until the pH reaches 6.8.

(3) 10mg/mL glucose stock solution: Take about 100mg of glucose, weigh it accurately, dissolve it with 0.1mol/L phosphate buffer (pH=6.8), set it to a 10mL volumetric flask, shake well, and get it.

(4) 1% soluble starch solution: Weigh 1g of soluble starch, dissolve it in 0.1mol/L phosphate buffer (pH=6.8), heat and boil until clear and transparent, calm, adjust to a 100mL volumetric flask, shake well and get it.

(5) 25mmol/LPNPG mother solution: Take 0.3766g of PNPG, dissolve it in 0.1mol/L phosphate buffer (pH=6.8), set it to a 50mL volumetric flask, shake well, and get it.[12]

(6) 1mol/LNa2CO3 solution: Weigh 26.5g of sodium carbonate, dissolve it in distilled water, and set it to a 250mlL volumetric flask, shake well, and get it.

(7) α-Amylase mother liquor with an initial concentration of 10U/mL: Take 10mg of α-amylase and weigh it accurately, dissolve it in 0.1mol/L phosphate buffer (pH=6.8) and set it to a 50mL volumetric flask, shake well and get it.

(8) The initial concentration is 25U/mL α-glucosidase mother solution: Take 5mg of α-glucosidase, weigh it accurately, dissolve it in 0.1mol/L phosphate buffer (PH=6.8), and dilute it to a 10mL volumetric flask, shake well and get it.

(9) 3,5-Dinitrosalicylic acid (DNS) reagent: Prepare 500 mL of hot water solution containing 185 g of potassium sodium tartrate; prepare 262 mL of 2 mol/L NaOH solution; weigh 6.3 g of 3,5- Dinitrosalicylic acid; weigh 5.0g crystallized phenol; weigh 5.0g sodium sulfite; add the above NaOH solution, 3,5-dinitro salicylic acid, phenol, and sodium sulfite to the potassium sodium tartrate solution, stir until of dissolution. After cooling, transfer it to a 1000mL passenger volume bottle, dilute to volume with distilled water, and store it in a brown bottle for later use. Cap the bottle tightly to prevent CO2 from entering. If the solution is turbid, it can be filtered before use [13].

3.2 Determination of hypoglycemic activity in vitro

3.2.1 α-amylase activity inhibition experiment

α-Amylase hydrolyzes α-1,4-glycosidic bonds and breaks down starch to produce reducing sugars. Reducing sugars and DNS can undergo a redox reaction under alkaline conditions to produce 3-amino-5-nitrosalicylic acid, the color of which, after boiling, is directly proportional to the reducing sugar content.

3.2.1.1 Experiment on the inhibitory effect of a single oil on α-amylase activity

Using the single-factor investigation method, the experiment was divided into three groups. Each group of experiments

consisted of a blank group, a blank control group, a sample group, a sample control group, an acarbose group, and an acarbose group. The sample groups were peony seed oil, soybean oil, and rapeseed oil. The order and amount of addition of each reactant are shown in Table 1. The mass concentrations of acarbose are 2.5, 5, 7.5, 10, 12.5, and 15 mg/mL, respectively; the concentration of the α-amylase solution is 2U/mL, the configured sample solution is centrifuged at 40000r/min for 5 minutes, and the supernatant is taken for measurement. There are three sets of parallel experiments, and the measurement is repeated three times. The sample solution calculates the inhibition rates of different concentrations of samples and acarbose according to Formula 1 and calculates the sample's half inhibitory concentration (IC50) [14-16].

The calculation formula for the inhibition rate of the sample solution is shown in Formula 1.

$$\text{Inhibition rate} = [1- (At-At0)/(Ac-Ac0)]*100\% \text{ [17]} \quad (1)$$

In Formula 1:

At: The absorbance of the sample control group at 491nm

At0: absorbance of sample group at 491nm

Ac: Absorbance of the blank group at 491nm

Ac0: Absorbance of the blank control group at 491nm

Table 1. α-amylase inhibitory activity assay (Unit: μL)

Reagent	Blank group	Blank control group	Sample group	Sample control group	Acarbose group	Acarbose control group
PBS solution	1000	2000	0	1000	0	1000
Sample solution	0	0	1000	1000	0	0
Acarbose	0	0	0	0	1000	1000
α-amylase solution	1000	0	1000	0	1000	0
Mix well and react in a 37°C constant temperature water bath for 10 minutes.						
soluble starch	2000	2000	2000	2000	2000	2000
Mix well and react in a 37°C constant temperature water bath for 6 minutes.						
DNS solution	1000	1000	1000	1000	1000	1000
Mix well, react in a boiling water bath for 5 minutes, then cool, dilute to volume with a 25mL volumetric flask, and measure the absorbance value at 491nm.						

3.2.1.2 The inhibitory effect of each blended oil on α-amylase activity

The experiment is divided into three groups. Each group of experiments consists of a blank group, a blank control group, a sample group, a sample control group, an acarbose group, and an acarbose control group. The order and amount of addition of each reactant are shown in Table 1. Through reasonable design, the regression equation between test indicators and component proportions can be obtained [18-20]. The formula range was determined by calculation with Matlab software: peony seed oil (59-61%), soybean oil (27-29%), rapeseed oil (11-13%), and then the response surface method was used to design the sample solution combination as shown in Table 2. The mass concentrations of acarbose were 2.5, 5, 7.5, 10, 12.5, and 15 mg/mL, respectively, and the concentration of the α-amylase solution was 2 U/mL. After the preparation, each solution is placed in a low-speed centrifuge at 4000r/min. After 5 minutes, the supernatant is taken for measurement. Carry out three sets of parallel experiments and repeat the measurement three times. According to the formula and calculation of the inhibition rates of samples with different concentrations and acarbose, find the sample's half inhibitory concentration (IC50).

Table 2. Volume mixing ratio of each blended oil

Sample / Sequence	Peony seed oil	Soybean oil	Rapeseed oil
1	60	28	12
2	61	27	12
3	60	27	11
4	59	28	13
5	60	28	12
6	61	28	13
7	61	29	12
8	60	29	11

Sample / Sequence	Peony seed oil	Soybean oil	Rapeseed oil
9	60	29	13
10	60	28	11
11	59	28	11
12	60	28	12
13	61	28	11
14	60	27	13
15	60	28	12
16	59	29	12
17	59	27	12

3.2.2 Inhibition of α-glucosidase activity

3.2.2.1 Experiment on the inhibitory effect of a single oil on α-amylase activity

The experiment is divided into three groups. Each group of experiments is a blank group, a blank control group, a sample group, a sample control group, an acarbose group, and an acarbose control group. The order and amount of addition of each reactant are shown in Table 3. The sample solutions are peony seed oil, soybean oil, and rapeseed oil, while the mass concentrations of acarbose are 2.5, 5, 7.5, 10, 12.5, and 15 mg/mL respectively, and the concentration of the α-glucosidase solution is 0.4U/mL. The prepared sample solution was centrifuged using a high-speed centrifuge at 40,000 r/min for 5 minutes, and then the supernatant was taken for measurement. Three sets of parallel tests were conducted, and the measurement was repeated three times. Calculate the inhibition rates of different samples and acarbose according to Formula 1 and find the sample's half inhibitory concentration (IC50).

Table 3. Determination of α-Glucosidase Inhibition Activity (Unit:μL)

Reagent	Blank Group	Blank Control Group	Sample Group	Sample Control Group	Acarbose Group	Acarbose Control Group
PBS solution	1000	1100	0	100	0	100
Sample solution	0	0	1000	1000	100	100
Acarbose	0	0	0	0	1000	1000
α-Glucosidase	100	0	100	0	100	0
Mix well, maintain a constant temperature water bath at 40°C for 10 minutes.						
PNPG solution	200	200	200	200	200	200
Mix well, maintain a constant temperature water bath at 40 °C for 8 minutes.						
Na2CO3 solution	1000	1000	1000	1000	1000	1000
Mix well, fix the volume to 25mL, and measure the absorbance value at 399nm.						

3.2.2.2 The inhibitory effect of each blended oil on α-glucosidase activity

The experiment was divided into three major groups, each further divided into six subgroups: blank group, blank control group, sample group, sample control group, acarbose group, and acarbose control group. The addition order and amount of each reactant are specified in Table 3. The sample solutions are detailed in Table 2. The mass concentrations of acarbose used were 2.5, 5, 7.5, 10, 12.5, and 15 mg/mL, respectively. The concentration of the α-glucosidase solution was 2U/mL. After preparing the sample solutions, it was centrifuged using a high-speed centrifuge at a speed of 40,000 r/min for 5 minutes, and the supernatant was collected for measurement. Three parallel experiments were conducted, with each experiment repeated three times. Based on Formula 1, the inhibition rates of different samples and acarbose were calculated. Finally, the samples' half-maximal inhibitory concentration (IC50) was determined.

4. Results and Analysis

4.1 Study on the inhibitory activities of individual oils on α-amylase and α-glucosidase

According to Formula 1, the inhibition rates of peony seed oil, soybean oil, rapeseed oil, and acarbose on α-amylase are 27.98%, 0.37%, 0.244%, and 49.14%, respectively. Linear regression equations were established based on the relationship between the concentration of different sample solutions (X) [Unit: mg/ml] and the inhibition rate (Y) [Unit: mg/ml]. The regression equations were obtained, and the half maximal inhibitory concentration (IC50) of the four samples on α-amylase

was calculated, as shown in Figure 1 and Table 4. The data shows that the half-maximal inhibitory concentration of peony seed oil is the highest compared to the other two oils.

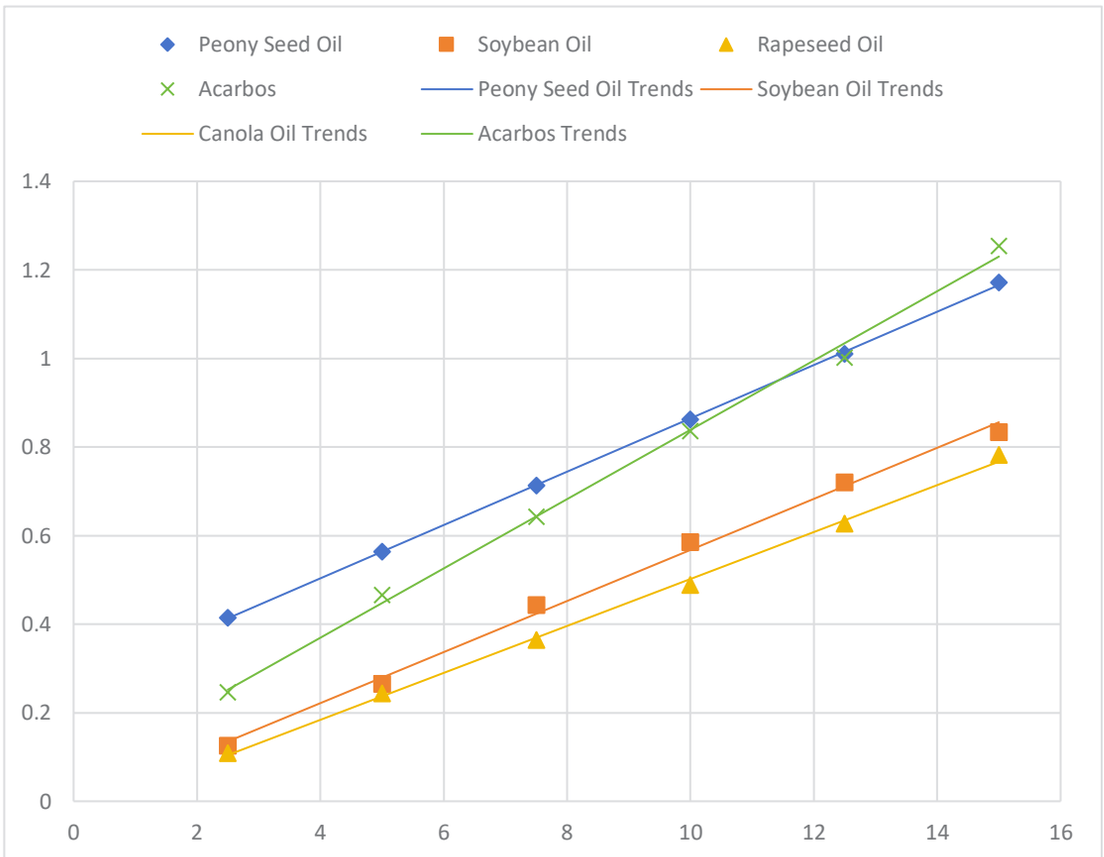

Figure 1. The inhibition rates of α-amylase by different oils at various concentrations

Table 4. Regression equations and half maximal inhibitory concentrations (IC50) of different oils on α-amylase

Sample	Linear regression equation	IC50(mg/mL)
Peony seed oil	Y=0.0602X+0.2625 R2=0.9998	3.95
Soybean oil	Y=0.0577X-0.0091 R2=0.9958	8.82
Rapeseed oil	Y=0.053X-0.0281 R2=0.9981	9.96
Acarbose	Y=0.0783X+0.0563 R2=0.9970	5.67

Similarly, based on Formula 1, we can determine that the inhibitory rates of peony seed oil, soybean oil, rapeseed oil, and acarbose on α-glucosidase are 23.32%, 14.61%, 3.04%, and 66.67%, respectively. By establishing linear regression equations to analyze the relationship between the concentration of the sample solution (X) [Unit: mg/ml] and the inhibitory rate (Y) [Unit: mg/ml] across various concentrations, we can derive the regression equations and calculate the half-maximal inhibitory concentrations (IC50) of these four samples against α-glucosidase. As shown in Figure 2 and Table 5, the data indicates that the IC50 of peony seed oil is the highest compared to the other two oils.

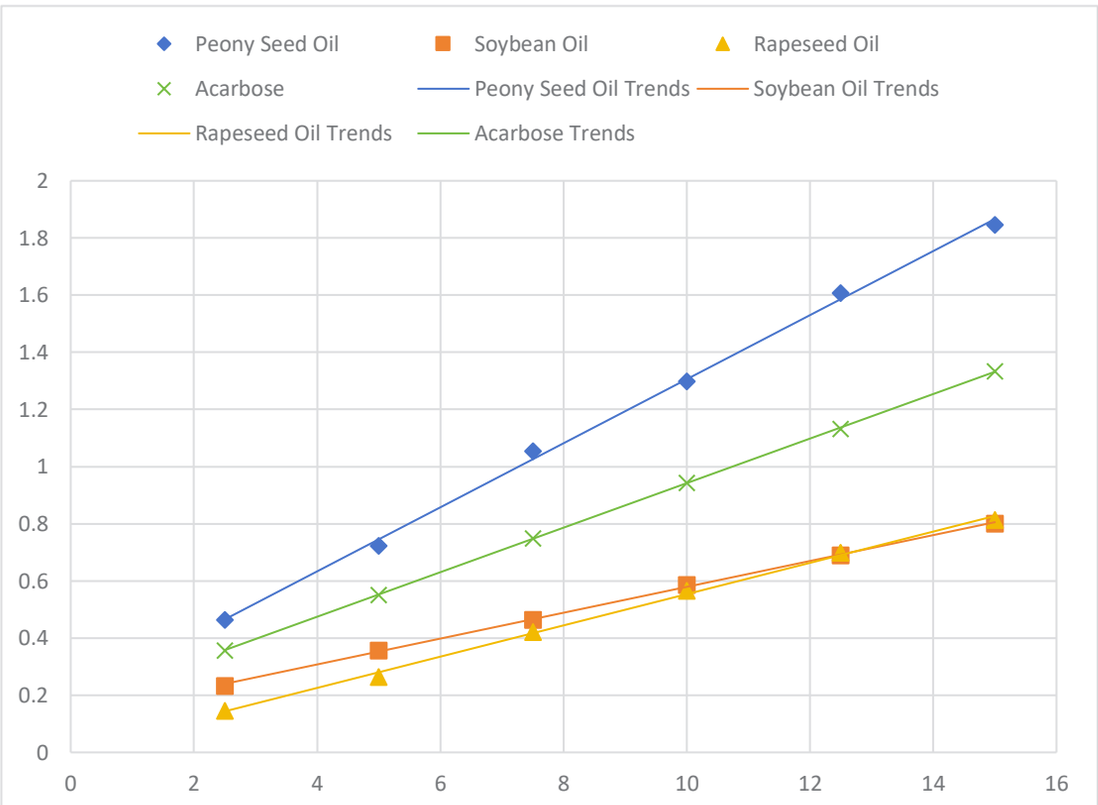

Figure 2. Inhibition rates of α-glucosidase by different oils at different concentrations

Table 5. Regression equations and half-maximal inhibitory concentrations (IC50) of different oils on α-glucosidase

Sample	Linear regression equation	IC50(mg/mL)
Peony seed oil	Y=0.1121X+0.185 R2=0.9984	2.81
Soybean oil	Y=0.0453X+0.1267 R2=0.9995	8.24
Rapeseed oil	Y=0.0547X+0.0069 R2=0.9979	9.01
Acarbose	Y=0.0776X+0.1643 R2=0.9988	4.33

4.2 Study on the inhibitory activities of each blended oil against α-amylase and α-glucosidase

The inhibition rates of α-amylase activity by each blended oil are shown in Figure 3. Among them, the third group of samples exhibited the highest inhibition rate of 80.47%, higher than the 49.14% inhibition rate of acarbose. Similarly, each blended oil's inhibition rates of α-glucosidase activity are presented in Figure 4. Here, the third group of samples again demonstrated the highest inhibition rate of 69.39%, surpassing the 66.67% inhibition rate of acarbose. When considering the hypoglycemic activities of the three ingredients alone or in combination, the order is as follows: compound (peony seed oil + soybean oil + rapeseed oil) > (acarbose) > (peony seed oil > (soybean oil) > (rapeseed oil). There is a specific synergistic effect among the three compounds in the compound. Based on these results, the optimal ratio for the third group is peony seed oil: soybean oil: rapeseed oil = 60:27:11.

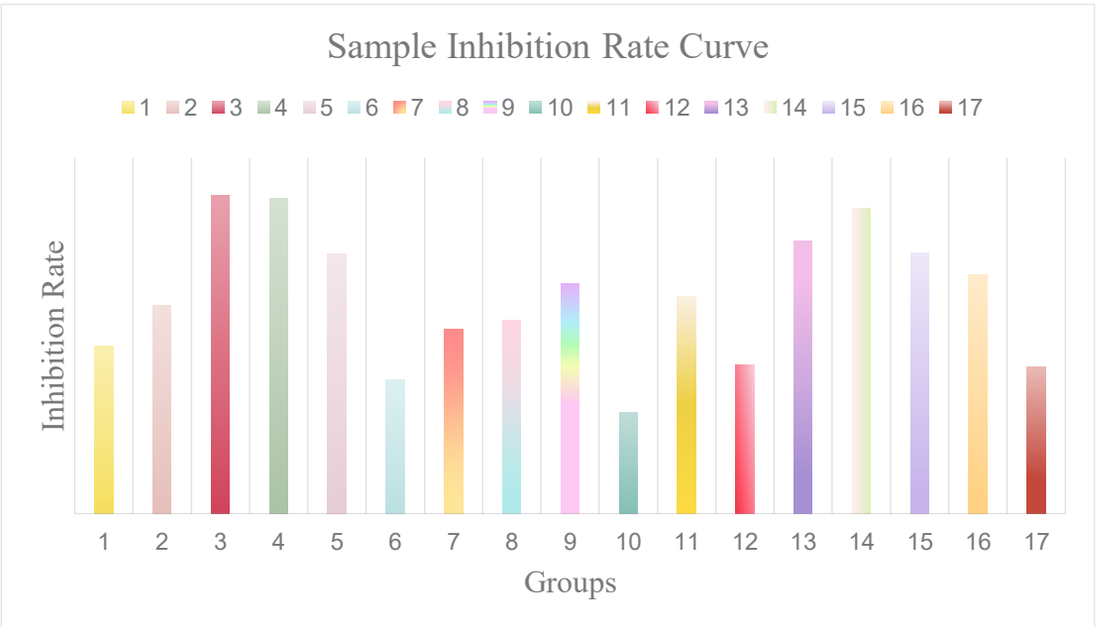

Figure 3. The inhibition rates of different blended oils on α-amylase

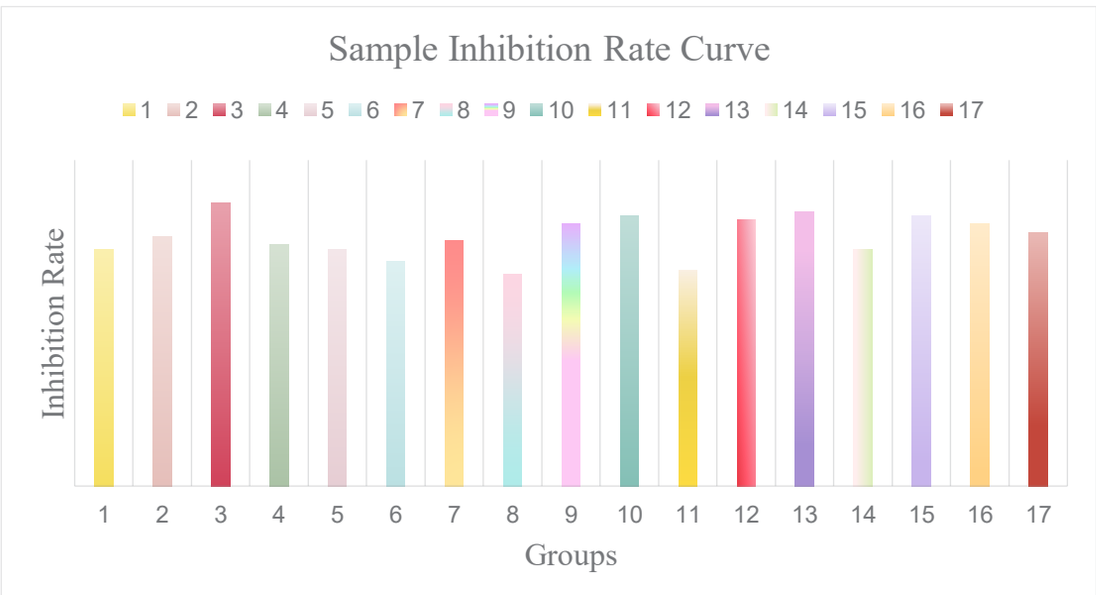

Figure 4. The inhibition rates of different blended oils on α-glucosidase

5. Conclusion

The present study primarily focused on the in vitro hypoglycemic activity of peony seeds, soybeans, rapeseed, acarbose, and blended oils. Evaluation of the hypoglycemic activity in vitro demonstrated that peony seed oil and its blended varieties exhibited notable inhibitory effects on α-amylase and α-glucosidase, with a positive correlation between inhibitory activity and linolenic acid content. The half-maximal inhibitory concentrations (IC50) of peony seed oil, soybean oil, rapeseed oil, and acarbose against α-amylase were 3.95, 8.82, 9.96, and 5.67 mg/mL, respectively, while those against α-glucosidase were 2.81, 8.24, 9.01, and 4.33 mg/mL, respectively. Additionally, the optimal ratio of the blend was determined using response surface methodology, resulting in a proportion of peony seed oil: soybean oil: rapeseed oil = 60:27:11.

In conclusion, the abundant linolenic acid content in peony seed oil confers inhibitory solid effects on α-amylase and α-glucosidase in vitro, revealing the potential hypoglycemic effects of peony seed blended oil. This study provides a

theoretical foundation for developing peony seed oil-related products.

Acknowledgments

Tiantian Sun, and Zirui Chang contributed equally to this work and should be considered co-first authors.

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