Journal of Polyphenols

2024, 6 (3): 98–105

journal homepage: http://jpolyph.syphu.edu.cn

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

Determination of total flavonoids content in buckwheat and inhibition of α-amylase activity

Huijing Chao [†], Rong Yang [†], Ming Yang, Beijie Xu, Xu Zhao [*]

Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract Diabetes is one of the most difficult chronic diseases to cure in the world, which seriously affects people's health and quality of life. Flavonoids in buckwheat can regulate blood glucose levels by inhibiting *α*-amylase activity. Therefore, sweet buckwheat produced in Inner Mongolia was used as the research object, and buckwheat flavonoids were extracted by ultrasonic-assisted extraction method. Total flavonoids content was determined by ultraviolet-visible spectrophotometry. With acarbose as the positive control, the inhibition test of *α*-amylase was carried out by DNS colorimetry to study the inhibition behavior of flavonoids on *α*-amylase activity. The results showed that the extraction process of flavonoids was stable and reliable, and the established method for the determination of flavonoids was simple, accurate and reproducible. The total flavonoids content of buckwheat samples was 2.706 mg/g, buckwheat total flavonoids extraction solution had an inhibitory effect on *α*-amylase, and its median inhibition concentration (IC_{50}) was 38.53 mg/mL. The results of this experiment provide a technical reference for the development and utilization of flavonoids in Inner Mongolia sweet buckwheat, and provide a theoretical reference for the development and application of flavonoid-rich hypoglycemic food.

Keywords: buckwheat; total flavonoids content; *α*-amylase; activity inhibition

1 Introduction

Buckwheat belongs to the Polygonaceae family and is a medicinal and edible plant that is rich in nutrients and also has health benefits. As a food crop, it is an important food source for China and some Asian countries [1]. Meanwhile, buckwheat has various medicinal functions such as lowering blood sugar [2], lowering lipids, and lowering blood pressure, making it a functional food worth accelerating its development and utilization.

Related studies have shown that most of the pharmacological effects of buckwheat are related to the phenolic compounds it contains, among which the most important phenolic compounds are flavonoids. Flavonoids are natural compounds formed by connecting two benzene rings with phenolic hydroxyl and blood lipids, anti-cancer and anti-oxidation, so they are also used as an important evaluation index of buckwheat and its products [3].

With the improvement of living standards, diabetes has gradually become one of the diseases endangering human health. The incidence rate of diabetes will increase year by year [4], and its mortality rate ranks the third among various diseases. Among the deaths caused by diabetes, more than 70% of the deaths caused by diabetes complications [5].

* Corresponding author: Xu Zhao (zhaoxu_1010@163.com).

† These authors contributed equally to this work;

These authors have no conflict of interest to declare.

α-amylase is a key enzyme that hydrolyzes starch to produce glucose in digestive system. Inhibiting the physiological activity of *α*-amylase can effectively control the rise of postprandial blood glucose level [6]. Therefore, the development of functional food to prevent diabetes and effective antidiabetic drugs with no side effects has a broad application prospect. Flavonoids are an effective class of compounds as *α*-amylase inhibitors [7]. It can be seen that the food made from buckwheat rich in flavonoids has potential use for patients with diabetes.

The content of buckwheat flavonoids is related to the variety and origin, and different treatment methods will also affect the extraction effect and content of buckwheat flavonoids. In this experiment, sweet buckwheat produced in Inner Mongolia was selected and ultrasonic-assisted extraction method was adopted. The traditional solvent extraction method has long extraction time and low yield. Ultrasonic-assisted extraction method has the advantages of simple operation, fast extraction, high extraction rate, environmental protection and no pollution. It is widely used in the extraction of natural active components of plants, and is most suitable for the extraction of flavonoids.

The content of total flavonoids and the inhibition of *α*-amylase in Inner Mongolia sweet buckwheat were determined and studied in this experiment (Fig. 1), which provide theoretical basis for further study and utilization of buckwheat.

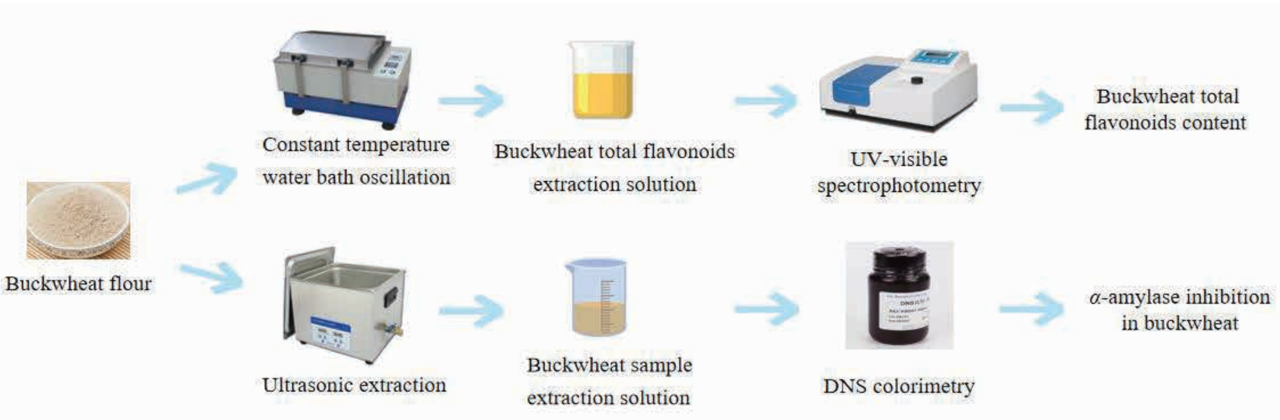

Fig. 1 The experimental process of this study

2 Materials and methods

2.1 Materials

Sweet buckwheat flour was purchased from supermarkets in bags (200 g/bag), rutin standard (purity 99.0%), acarbose standard (purity 99.80%), aluminum chloride, potassium acetate, methanol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, soluble starch were analytically pure.

2.2 Preparation of standard solution

57.3 mg of rutin standard ($C_{27}H_{30}O_{16}\cdot 3H_2O$, purity 95%) was weighed and placed in a beaker, dissolved with methanol solution, transferred to a 1 000 mL volumetric flask, and diluted with methanol solution to the scale, that is, 0.050 0 mg/mL rutin standard solution was obtained.

2.3 Methodology review

2.3.1 Linear range

1.0, 1.5, 2.0, 3.0, 4.0 mL of 0.050 0 mg/mL rutin standard solution were put into 10 mL volumetric flask. Then, 2.0 mL of aluminum trichloride solution and 3.0 mL of potassium acetate solution were added. The volume was fixed to scale with 70% methanol solution and shaken well. They were placed at room temperature for 30 min. The content of rutin in the standard curve was 5.00, 7.50, 10.00, 15.00, 20.00 μg/mL, respectively, and the absorbance was measured at 420 nm wavelength. The standard curve was drawn with the absorbance value as the vertical coordinate and the concentration value as the horizontal coordinate.

2.3.2 Instrument precision

Rutin standard solutions with low, medium and high concentrations of 5.00, 10.00, 20.00 μg/mL were taken, respectively, and the absorbance was determined with blank solution at wavelength 420 nm after being adjusted to zero. The absorbance values of three concentrations of rutin standard were recorded by measuring the solution 6 times.

2.3.3 Stability of sample solution

3 g of buckwheat was extracted and processed into sample solution to be measured. After standing for different time, absorbance was determined at wavelength 420 nm after zero adjustment with blank solution.

2.3.4 Method repeatability

3 g of buckwheat was extracted and processed into sample solution to be measured. 6 samples of buckwheat were accurately weighed and zeroed with blank solution at wavelength 420 nm to record the measured absorbance. The content of total flavonoids in each sample was calculated by using the established standard curve.

2.3.5 Recovery rate of spiking

3 g of buckwheat was extracted and treated into the sample solution to be measured, and flavonoids content was 75.5 μg. The sample solution was taken from 1 mL to four 10 mL volumetric bottles, and 0.050 0 mg/mL rutin standard solution 0, 1, 2, 3 mL (0.00, 50.00, 87.50, 125.00 μg rutin control product) was added to the volumetric bottles, respectively. Then the solution to be measured was prepared according to the method of flavonoids content determination, the absorbance was measured at wavelength 420 nm, and the blank solution was adjusted to zero.

2.4 Preparation of buckwheat total flavonoids extraction solution

3 g of buckwheat was weighed and 30 mL of 70% methanol solution was added. They were shaken and placed in a 150 mL stopper triangle flask, oscillated in a constant temperature water bath oscillator at 160 r/min for 2 h at 65 ℃, and filtered while hot. The filtrate was transferred to a 50 mL volumetric bottle with a constant volume to the scale, and 1 mL of sample solution was transferred to a 10 mL centrifuge tube, followed by 2 mL of $AlCl_3$ solution and 3 mL of CH_3COOK solution with a constant volume to 10 mL. It was placed at room temperature for 30 min.

2.5 Preparation of buckwheat sample extraction solution for inhibiting α-amylase

2 g of buckwheat was weighed, dissolved in 20 mL of 60% ethanol solution, and extracted by ultrasound at 45 ℃ for 40 min to obtain the buckwheat sample extraction solution.

2.6 Preparation of α-amylase solution

46.3 mL of 1 mol/L dipotassium hydrogen phosphate and 53.7 mL of 1 mol/L potassium dihydrogen phosphate were mixed to a constant volume of 1 L to obtain 0.1 mol/L phosphate buffer with pH = 6.8. The α-amylase solution of 0.5 U/mL was obtained by accurately weighing 0.01 g of 10 000 U/g α-amylase and titrated to 200 mL with phosphate buffer.

2.7 Preparation of 1% starch solution

0.5 g of soluble starch was weighed and a little phosphate buffer was added and stirred well. The solution was slowly poured into 50 mL of boiling phosphate solution and continued to boil for 2 min with constant stirring. After cooling, the supernatant was taken to obtain 1% starch solution.

2.8 Determination of moisture content

Two clean glass flat weighing bottles were taken and placed in a 105 °C drying oven. The bottle cap was obliquely supported on the edge of the bottle for 1.0 h, then the cap was taken out and cooled to room temperature in the dryer. The weight was weighed and dried repeatedly until the mass difference between the two times was not more than 5 mg, which was constant weight. 2 g of buckwheat was taken into a weighing bottle, with a sample thickness not exceeding 5 mm. If the sample was loose, the thickness was not more than 10 mm. The buckwheat sample was dried at 105 °C for 3 h, then removed, covered, and cooled to room temperature in a dryer. After taking out and weighing, redrying according to the above method. Cooling and weighing were taken out every 30 min until the weight difference between the two times did not exceed 5 mg.

2.9 Determination of total flavonoids content in buckwheat

1 mL of buckwheat total flavonoids extraction solution was accurately absorbed into a 10 mL volumetric flask, 2 mL of aluminum trichloride solution and 3 mL of potassium acetate solution were added respectively. And methanol solution was used for constant volume to the scale and shaken well. It was placed at room temperature for 30 min. After centrifugation at 4 000 r/min for 10 min, the absorbance value was measured at the wavelength of 420 nm, and the content of total flavonoids was calculated by substituting it into the standard curve equation [8].

2.10 Determination of the inhibition rate of buckwheat on α-amylase

According to the method in the previous literature [9], the activity of α-amylase was determined and slightly modified. 1.0 mL buckwheat sample extraction solution and 0.3 mL α-amylase were dissolved in phosphate buffer with pH = 6.8 at 37 °C for 5 min, and 0.4 mL 1% starch solution was added to react at 37 °C for 15 min. Another 0.5 mL of DNS reagent was added, mixed and brought to a boiling water bath for 5 min, cooled to room temperature, and a constant volume of 10 mL of deionized water was used to measure the absorbance at 540 nm. Acarbose was used as positive control.

Among them, the control group was 0.3 mL α-amylase, 0.4 mL starch solution and 0.5 mL DNS. The blank control group was 0.4 mL starch solution and 0.5 mL DNS. The blank sample group was 1 mL sample solution, 0.4 mL starch solution and 0.5 mL DNS. Each group was filled with deionized water to 10 mL.

3 Results

3.1 Methodological validation

3.1.1 Linear range

The standard curve was drawn with the concentration of rutin standard solution as the horizontal coordinate and absorbance as the vertical coordinate. As can be seen from Fig. 2, the regression equation was $y = 0.037\,8x - 1.04 \times 10^{-2}$, and the correlation coefficient was 0.999, indicating that the linear relationship of control product rutin was good in the range of 5.00−20.00 μg/mL.

3.1.2 Instrument precision

As can be seen from Table 1, RSD values of low, medium and high concentrations of rutin standard solution were 1.24%, 1.09%, 1.45%, respectively, which were all less than 2%, indicating good precision of the instrument.

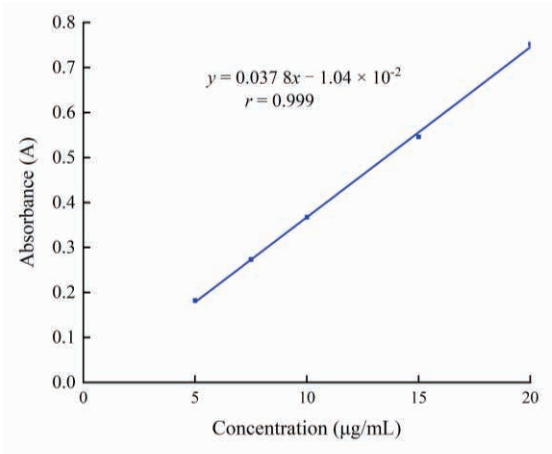

Fig. 2 Rutin standard curve

Table 1 Instrument precision for determination of total flavonoids content in buckwheat

Concentration (μg/mL)	A_1	A_2	A_3	A_4	A_5	A_6	RSD (%)
5.00	0.250	0.246	0.248	0.238	0.245	0.241	1.24
10.00	0.379	0.383	0.391	0.387	0.385	0.388	1.09
20.00	0.714	0.728	0.752	0.729	0.720	0.731	1.45

3.1.3 Stability

After the sample was placed, the absorbance was measured every 1 h, and the six absorbance values were 0.247, 0.245, 0.244, 0.241, 0.242, 0.238, 0.239, respectively. The absorbance of the sample was stable within 6 h, and the RSD value of the sample solution was less than 2%, indicating that the solution was still stable after 6 h.

3.1.4 Method repeatability

The total flavonoids in buckwheat samples were 72.1, 73.1, 73.9, 73.4, 70.7, 72.6 μg, respectively. The RSD value of flavonoids content determination in buckwheat was 1.55%, less than 2%, indicating good repeatability of the method.

3.1.5 Recovery rate

The results of the recovery rate of rutin standard were shown in Table 2. The recovery rates of flavonoids in all groups were between 95% and 105%, and the RSD value was less than 5%. The recovery rate of the method was good.

3.2 Total flavonoids content of buckwheat samples

Buckwheat is rich in nutrients and flavonoids, and rutin content is relatively high among its known flavonoids [10], and it has been reported that its content accounts for 70% to 90% of the total flavonoids. Therefore, rutin can be selected as the standard substance and 420 nm as the detection wavelength to detect the content of total flavonoids in buckwheat samples.

According to GB/T 5497, the content of moisture in buckwheat was 10.99 ± 0.11%.

The total flavonoids content was calculated according to NY/T1295-2007, based on the mass fraction of rutin, and the calculation result of total flavonoids content was (2.706 ± 0.087) mg/g.

The inhibition rate of α-amylase (%) =

$$\frac{\Delta A_{\text{Control group}} - \Delta A_{\text{Sample group}}}{\Delta A_{\text{Control group}}}$$

Table 2 Recovery rate of rutin standard products

Number	Flavonoids content in the sample (μg)	The amount of rutinadded (μg)	Measured amount after adding standard material (μg)	Recovery (%)	Average recovery (%)	RSD (%)
1	75.5	50.00	126.83	102.7		
2	75.5	50.00	127.35	104.2		
3	75.5	50.00	126.56	102.1		
4	75.5	87.50	164.66	101.9		
5	75.5	87.50	162.28	99.2	100.6	2.37
6	75.5	87.50	163.86	101.0		
7	75.5	125.00	199.05	98.8		
8	75.5	125.00	196.93	97.1		
9	75.5	125.00	197.99	98.0		

3.3 Inhibition of α-amylase activity

Median inhibition concentration (IC_{50}) is often used to evaluate the inhibitory activity of an inhibitor on α-amylase. The inhibition rate was calculated by the formula below.

$$A_{\text{Control group}} = A_{\text{Control group}} - A_{\text{Blank control group;}}$$
$$A_{\text{Sample group}} = A_{\text{Sample group}} - A_{\text{Blank sample group.}}$$

The results of enzyme inhibition rate of acarbose with different concentration gradients were shown in Fig. 3. In the range of 0.02−1.20 mg/mL, the inhibition of acarbose on α-amylase activity was dose-dependent, and the inhibition rate increased with the increase of dose. When the concentration of acarbose reached 1.20 mg/mL, the inhibition rate of α-amylase reached 90.08%. According to the obtained inhibition rate, the IC_{50} value of the inhibition effect of acarbose on α-amylase was calculated by Graphpad Prism as 0.152 0 mg/mL.

The results of enzyme inhibition rate of buckwheat sample extraction solution with different concentration gradients were shown in Fig. 4. In the range of 1.00−100.00 mg/mL, the inhibition ability of buckwheat on α-amylase was gradually enhanced with the increase of the concentration of the sample extraction solution, indicating that the inhibition effect of buckwheat on α-amylase was dose-dependent with its concentration. When the concentration of buckwheat extraction solution reached 100.00 mg/mL, the inhibition rate of α-amylase could reach 70.78%. According to the obtained inhibition rate, the IC_{50} value of the inhibition effect of buckwheat on α-amylase was calculated by Graphpad Prism as 38.53 mg/mL.

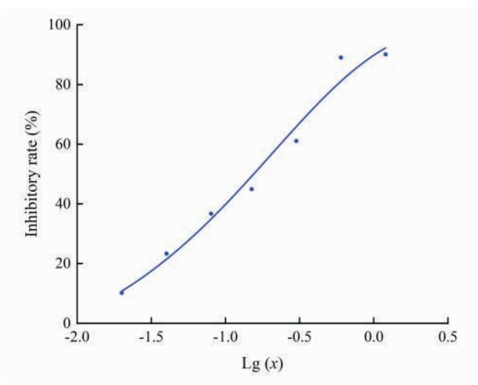

Fig. 3 Dose-effect relationship of acarbose to α-amylase

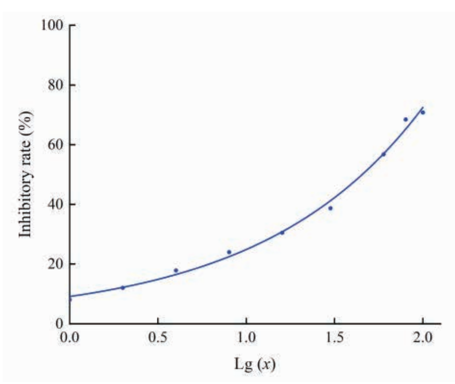

Fig. 4 Dose-effect relationship of buckwheat to α-amylase

4 Discussion

Buckwheat is rich in bioactive substances, such as flavonoids, peptides, protease inhibitors and resistant starch. Flavonoids have always been a hot spot in buckwheat research, and their types and contents have been reported in a large number of literatures. Buckwheat resources in Inner Mongolia are rich, with large output and export volume, but there has been a lack of systematic nutritional data. In this experiment, the content of flavonoids in sweet buckwheat flour was determined, which provided reliable basic data for the study of flavonoids in sweet buckwheat from Inner Mongolia. At the same time, the ultrasonic-assisted extraction method was used to extract flavonoids. This method is simple, fast, and has high extraction efficiency. It is a simple and effective method for extracting flavonoids. Besides, the content of flavonoids in buckwheat was determined in order to guide people to choose and match food reasonably, so as to achieve the purpose of nutritional balance and healthy diet.

This experiment also showed that buckwheat sample extraction solution had a significant inhibitory effect on *α*-amylase. *α*-amylase is one of the enzymes closely related to glucose and lipid metabolism. Inhibiting the activity of *α*-amylase can effectively hinder the digestion and hydrolysis of carbohydrates in food in the body, so as to reduce sugar intake and significantly reduce blood sugar content. It is also often used as one of the effective means to explore the mechanism of hypoglycemic and lipid-lowering. Studies have shown that buckwheat contains flavonoids, which can obtain amylase resistance properties by heating, because polyphenols can form complexes not only with starch [11, 12], but also with various non-covalent polysaccharides [13-18]. In addition, polyphenols, including flavonoids, have been reported as inhibitors of *α*-amylase activity [19-24]. It can be seen that the inhibition of *α*-amylase activity of buckwheat in this experiment can be attributed to the flavonoid compounds contained in buckwheat. Although the inhibitory ability of flavonoids on *α*-amylase was lower than that of acarbose, its natural characteristics and the results of enzyme inhibition still show that flavonoids as functional active ingredients can assist acarbose to better play the role of lowering blood glucose. Therefore, buckwheat can inhibit the activity of *α*-amylase and thus hinder the rapid digestion and hydrolysis of carbohydrates in food, and has no toxic side effects, which has certain potential value in lowering blood sugar and can be used to develop functional foods.

5 Conclusion

The content of total flavonoids in buckwheat and its inhibitory effect on *α*-amylase were studied in this paper. The results showed that buckwheat was rich in flavonoids, which could inhibit *α*-amylase activity in a dose-dependent manner, indicating that buckwheat could hinder the effective decomposition of carbohydrates by affecting the activity of *α*-amylase. This indicates that flavonoids in buckwheat, as natural active components, have good potential in hypoglycemia, providing a reference for the comprehensive development of buckwheat resources. However, the hypoglycemia effect and mechanism *in vivo* need to be further studied.

References

[1] Zheng B, Zhang H, Wang L, et al. Characterization of 16-kDa major allergen with *α*-amylase inhibitor domain in tartary buckwheat seeds [J]. Molecular Immunology, 2018, 94: 121-130.

[2] Skrabanja V, Liljeberg Elmsthl Helena G, Kreft I, et al. Nutritional properties of starch in buckwheat products: studies *in vitro* and *in vivo* [J]. Journal of Agricultural and Food Chemistry, 2001, 49 (1): 490-496.

[3] Wang MT, Shi JY, Wang L, et al. Inhibitory kinetics and mechanism of flavonoids from lotus (*Nelumbo nucifera* Gaertn.) leaf against pancreatic *α*-amylase [J]. International Journal of Biological Macromolecules, 2018, 120 (Pt B): 2589-2596.

[4] Thanh MT, MinhHoang P, Thi TT, et al. Discovery of novel flavonoid derivatives as potential dual inhibitors against *α*-glucosidase and *α*-amylase: Virtual screening,

synthesis, and biological evaluation [J]. Molecular Diversity, 2024, 28 (3): 1629-1650.

[5] Vang T, Bottini N, Cucca F, et al. Role of PTPN22 in type 1 diabetes and other autoimmune diseases [J]. Seminars in Immunology, 2006, 18 (4): 207-213.

[6] Lam TP, Tran NVN, Pham LHD, et al. Flavonoids as dual-target inhibitors against *α*-glucosidase and *α*-amylase: A systematic review of *in vitro* studies [J]. Natural Products and Bioprospecting, 2024, 14 (1): 4.

[7] Hassan R, Mohammad-Bagher H-GS, Hadi A, et al. Differential *α*-amylase/*α*-glucosidase inhibitory activities of plant-derived phenolic compounds: A virtual screening perspective for the treatment of obesity and diabetes [J]. Food & Function, 2017, 8 (5): 1942-1954.

[8] Siddhu N, Saxena J. Quantification of total phenolic and total flavonoid content of extracts of tagetes *Erecta* flowers [J]. Asian Journal of Pharmaceutical and Clinical Research, 2017, 10 (6): 328-330.

[9] Zhang HL, Wu QX, Qin XM. Camellia nitidissima Chi flower extracts inhibit *α*-amylase and *α*-glucosidase: *In vitro* by analysis of optimization of addition methods, inhibitory kinetics and mechanisms [J]. Process Biochemistry, 2019, 86: 177-185.

[10] Jiang P, Burczynski F, Campbell C, et al. Rutin and flavonoid contents in three buckwheat species Fagopyrum esculentum, F. tataricum, and F. homotropicum and their protective effects against lipid peroxidation [J]. Food Research International, 2006, 40 (3): 356-364.

[11] Davis AB, Hoseney RC. Grain sorghum condensed tannins. I. Isolation, estimation, and selective adsorption by starch [J]. Cereal Chemistry, 1979, 56: 310-314.

[12] Cohen R, Orlova Y, Kovalev M, et al. Structural and functional properties of amylose complexes with genistein [J]. Journal of Agricultural and Food Chemistry, 2008, 56 (11): 4212-4218.

[13] Renard CMGC, Baron A, Guyot S, et al. Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences [J]. International Journal of Biological Macromolecules, 2001, 29 (2): 115-125.

[14] Bourvellec CL, Guyot S, Renard CMGC. Non-covalent interaction between procyanidins and apple cell wall material [J]. BBA - General Subjects, 2004, 1672 (3): 192-202.

[15] Cai Y, Gaffney SH, Lilley TH, et al. Cheminform abstract: Polyphenol interactions. Part 4. Model studies with caffeine and cyclodextrins [J]. Cheminform, 1991, 22 (11): 51.

[16] Fayad N, Marchal L, Billaud C, et al. Comparison of *β*-cyclodextrin effect on polyphenol oxidation catalyzed by purified polyphenol oxidase from different sources [J]. Journal of Agricultural and Food Chemistry, 1997, 45 (7): 2442-2446.

[17] Ishizu T, Kintsu K, Yamamoto H. NMR study of the solution structures of the inclusion complexes of *β*-cyclodextrin with (+)-catechin and (-)-epicatechin [J]. The Journal of Physical Chemistry B, 1999, 103 (42): 8992-8997.

[18] Kriz Z, Koca J, Imberty A, et al. Investigation of the complexation of (+)-catechin by beta-cyclodextrin by a combination of NMR, microcalorimetry and molecular modeling techniques [J]. Organic & Biomolecular Chemistry, 2003, 1 (14): 2590-2595.

[19] Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid [J]. Bioscience, Biotechnology, and Biochemistry, 2000, 64 (11): 2458-2461.

[20] Lee YA, Cho EJ, Tanaka T, et al. Inhibitory activities of proanthocyanidins from persimmon against oxidative stress and digestive enzymes related to diabetes [J]. Journal of Nutritional Science and Vitaminology, 2007, 53 (3): 287-292.

[21] Li Y, Gao F, Gao F, et al. Study on the interaction between 3 flavonoid compounds and alpha-amylase by fluorescence spectroscopy and enzymatic kinetics [J]. Journal of Food Science, 2009, 74 (3): C199-C203.

[22] Kim HM, Kim JK, Kang LW, et al. Docking and scoring of quercetin and quercetin glycosides against *α*-amylase receptor [J]. Bulletin of the Korean Chemical Society, 2010, 31 (2): 461-463.

[23] Narita Y, Inouye K. Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas alpha-amylase isozymes I and II [J]. Journal of Agricultural and Food Chemistry, 2009, 57 (19): 9218-9225.

[24] He Q, Lv YP, Yao K. Effects of tea polyphenols on the activities of *α*-amylase, pepsin, trypsin and lipase [J]. Food Chemistry, 2006, 101 (3): 1178-1182.