Extraction of Quercetin from Avocado Meal and Its Antioxidant Activity

Xuehua Feng, Ali Tao, Zurong Song
Anhui Xinhua College, Pharmaceutical College, Hefei 230088
Email: fxh77@126.com

Abstract: Response surface methodology (RSM) was used to optimize the extraction process of quercetin from pear meal and to investigate its antioxidant capacity. The solvent ratio, ultrasonic power and extraction time were taken as independent variables, and the percentage of quercetin was taken as dependent variable. Through multiple linear regression and binomial fitting of each level of independent variable, the better extraction process was selected by response surface methodology and predicted and analyzed. The antioxidant activity of quercetin was evaluated by scavenging 1.1-diphenylpicrylhydrazine free radical (DPPH) and reducing ability of iron ions (FRAP). The optimum extraction process was obtained as follows: the ratio of liquid to material was 35 mL/g, the ultrasonic power was 550 W, and the extraction time was 30 min. The content of quercetin was 1.585%. Quercetin in avocado meal has strong DPPH scavenging and iron ion reduction ability. When the concentration is 30 mg/mL, DPPH scavenging rate reaches 70%, and iron ion reduction ability almost equals 6 mmol/L FeSO₄ concentration, and has a dose-effect relationship.

1. Introduction
Persea Americana Mill., also known as avocado, Butyrospermum parkii Kotschy and e li (in Chinese), is a woody oil tree native to tropical humid areas or plateaus of Central America and Mexico[1]. Studies have shown that avocado has good antiviral, antioxidant, hepatoprotective and other biological activities. It is rich in a variety of natural active ingredients, including terpenoids, flavonoids (such as quercetin), alkaloids, steroids, carotenoids, higher fatty acids and derivatives[2]. Quercetin, also known as Meletin, Quercetin as flavonoids, mostly exists in the form of glycosides. Quercetin can be obtained by acid hydrolysis, soluble in cold ethanol (1:290), easily soluble in hot ethanol (1:23), soluble in methanol, ethyl acetate, glacial acetic acid, pyridine, acetone and so on. It has the functions of expectoration, cough relieving, asthma relieving, antioxidation, blood lipid lowering and coronary artery dilation. A wide range of pharmacological effects such as increasing coronary blood flow [3].

Response Surface Methodology (RSM) is a comprehensive application of experimental design, mathematical statistics and optimization techniques. It combines traditional mathematical methods with statistical methods. By modeling and analyzing the effects of multiple variables, RSM is a means to optimize the response values[4]. In recent years, RSM has been widely used in chemical industry, food biology, medicine and pharmaceuticals. Compared with the traditional single variable optimization test, RSM has the advantages of less test times, shorter cycle and higher accuracy. Pear meal is a by-product of pear oil extraction. At present, there are few studies on the extraction of Flavonoids from pear meal in China. In this paper, the percentage content of quercetin in Pear meal was used as the index, and the response surface methodology was used to optimize the extraction process. The optimum extraction process of quercetin in Pear meal was obtained and its antioxidant
capacity was studied, in order to provide reference for the comprehensive development and utilization of pear meal.

2. Materials and methods

2.1 Materials and Instruments
Pear meal: Hefei Kenong Biotechnology Co., Ltd; Quercetin standard (batch number 100081-200907): China Food and Drug Research Institute; 1,1-diphenylpicrylphenyldrazine (DPPH): Sigma Aldrich Company of the United States; other reagents are analytical pure.

Instruments: ZNHW-II Intelligent Constant Temperature Electric Heating Jacket (500mL, Gongyi Kerui Instrument Co., Ltd.); R-201 Rotary Evaporator (Shanghai Shensheng Biotechnology Co., Ltd.); Circulating Water Vacuum Pump (Henan Gongyi Yingyu Instrument Factory); Electronic Balance AL204-IC (Swiss Maitre-Toledo Instrument (China) Co., Ltd.); Dian Ultimate 3000 High Performance Liquid Chromatograph (Dai’an Ultimate 3000) Ann, USA); Chromeleon 6.8 Chromatography Workstation.

2.2 Extraction and determination of Quercetin
Degreasing: The dried pear meal cake was crushed through 40 mesh sieve. Petroleum ether (30-60 C) was used as solvent. After 24 hours of Soxhlet extraction, it was dried and crushed at 40-50 C. After 50 mesh sieve, it was sealed and reserved.

The pear meal powder after degreasing is weighed accurately about 2g. It is put into a plugged conical bottle and added with methanol-25% hydrochloric acid solution (4:1) according to a certain liquid-solid ratio. After ultrasonic extraction for a period of time, the pear meal powder is filtered while it is heated. After repeated extraction, the filtrate was merged, the water bath was steamed and dried, the residue was dissolved in a 50 mL measuring bottle with methanol, shake well, the solution was filtered with 0.45μm filter membrane and injected into the HPLC. Quercetin was used as the reference substance to determine the content of quercetin in the extract according to the external standard method[5].

2.2.1 Chromatographic conditions. Chromatographic column C18 (Sapphire C18, 4.6 mm x 250 mm, 5μm); mobile phase: methanol-0.2% phosphoric acid solution (50:50); flow rate: 1.0 ml/min; detection wavelength: 360 nm; column temperature: 25 C; injection volume: 10μL.

2.2.2 Single factor experimental method. Using dried pear meal powder as raw material, the effects of ultrasonic extraction times, extraction time, ultrasonic power and liquid-to-material ratio on the yield of quercetin from pear meal were studied by ultrasonic extraction method.

2.2.3 Box-Behnken. experimental design According to Box-Behnken's central combination experiment principle, response surface analysis was used to determine the main influencing factors and the appropriate range of each factor by single factor experiment. With liquid-to-material ratio, ultrasonic power and extraction time as independent variables and quercetin yield in avocado meal as dependent variables, Design-Expert.8.0.5 software was used. Three-factor and three-level experiments were used to analyze the level of factors as shown in Table 1.

| Code | Liquid-to-material ratio (mL/g) | Ultrasound power (W) | Extraction time (min) |
|------|-------------------------------|---------------------|----------------------|
| -1   | 25; 1                         | 350                 | 30                   |
| 0    | 30; 1                         | 450                 | 40                   |
| 1    | 35; 1                         | 550                 | 50                   |
2.3 Study on Antioxidant Activity of Quercetin

DPPH scavenging capacity and iron reduction capacity (FRAP) were used to evaluate the antioxidant capacity of quercetin, and vitamin C was used as control[6].

2.3.1 Evaluation of DPPH scavenging capacity. The DPPH scavenging capacity of quercetin was evaluated by Spectrophotometric Determination of OD value. DPPH Clearance ($\eta$) is calculated according to the following formula

$$\eta = \frac{(A_{DPPH} - A_{Sample addition})}{A_{DPPH}} \times 100\%$$

Formula: $A_{DPPH}$: $A_{Sample addition}$ is the OD value without sample, $A_{Sample addition}$ is the OD value after adding sample.

2.3.2 Evaluation of Fe$^{2+}$ Reduction Ability. The reduction ability of iron ion was evaluated by spectrophotometry. The antioxidant capacity was characterized by the concentration of FeSO$_4$: 1 mmol/L FeSO$_4$ was 1 FRAP unit, that is, the antioxidant capacity was equivalent to the number of mmol/L of FeSO$_4$. Calculate the concentration of FeSO$_4$ by standard curve, and calculate the reduction capacity according to the following formula.

$$C = C_0 \times V_0 \times D/V_s$$

In the formula: $C$: FeSO$_4$ concentration (mmol/L); $C_0$: FeSO$_4$ concentration (mmol/L) calculated according to standard curve; $V_0$: extraction liquid volume (mL); $D$: dilution multiple; $V_s$: extraction liquid volume (mL) at the time of determination.

3. Results and Discussions

3.1 Analysis of Quercetin in Avocado Meal by High Performance Liquid Chromatography

The chromatogram of methanol-25% hydrochloric acid (4:1) extract from avocado meal is shown in Fig. 1. Comparing sample 1 (a) with standard quercetin 1 (b), it was found that the peak 1 of extract chromatogram with retention time of 7.869 min was quercetin. Calculating Quercetin Content in Pear Meal Extract by Determining Chromatographic Peak Area of Sample and Standard.
3.2 Single factor experiment

3.2.1 Effect of ultrasonic power on extraction efficiency. The effect of different ultrasound power on the yield of quercetin is shown in Fig. 2.

Figure 2 shows that the yield of quercetin increases first and then decreases with the increase of ultrasonic power. When the ultrasonic power is 450W, the yield of quercetin is the best. When the power is increased, the yield decreases, because the power is too high, the water molecule in the solution vibrates violently, the temperature rises sharply, and the internal structure of quercetin is damaged, resulting in a decrease in the yield[7-8].

3.2.2 Effect of Extraction Time on Extraction Effect. The effect of different extraction time on the yield of quercetin is shown in Figure 3.
Fig. 3 shows that the yield of quercetin increases first and then decreases with the increase of time. The highest yield is 1.69% at 40 minutes. When the extraction time was between 10 min and 30 min, the yield of quercetin increased significantly, but it did not change significantly between 30 min and 40 min. After 40 min, the yield began to decrease with the extension of extraction time. Probably because quercetin did not completely infiltrate into the solvent at the beginning, the yield of quercetin increased with the increase of time, and the saturated extraction rate of quercetin in the solution did not change after a certain time. Therefore, 30, 40 and 50 minutes were selected as three levels for response interview.

3.2.3 Effect of liquid-to-material ratio on extraction efficiency. The effect of different liquid-to-material ratios on the yield of quercetin is shown in Fig. 4.

Fig. 4 shows that the yield of quercetin increases with the increase of liquid-to-material ratio. When the liquid-to-material ratio reaches 30:1, the highest yield is 1.45%. After that, the yield of quercetin decreases with the increase of liquid-to-material ratio. It may increase the heat load and the time required for the extraction of quercetin due to the excessive amount of solvent, thus reducing the concentration of quercetin in the extracted solution.

3.2.4 Analysis of the influence of extraction times on extraction effect. The effect of ultrasonic extraction times on the yield of quercetin is shown in Fig. 5.
Fig. 5 Effect of extraction times on quercetin yield.

Fig. 5 shows that the effect of extraction times on the yield of quercetin is almost non-existent. Combined with actual production and cost savings, ultrasound extraction is selected once.

3.3 Response Surface Experiment

3.3.1 Design and results. The experimental design and results are shown in Table 2.

| Serial number | Liquid-to-material ratio (mL/g) | Ultrasound power (W) | Extraction time (min) | Yield (%) |
|---------------|-------------------------------|---------------------|-----------------------|-----------|
| 1             | 1                             | 0                   | 1                     | 1.29      |
| 2             | 0                             | 0                   | 0                     | 0.99      |
| 3             | 1                             | -1                  | 0                     | 1.38      |
| 4             | 0                             | 1                   | -1                    | 1.51      |
| 5             | 1                             | 1                   | 0                     | 1.48      |
| 6             | -1                            | 1                   | 0                     | 1.45      |
| 7             | -1                            | 0                   | 1                     | 0.98      |
| 8             | 1                             | 0                   | -1                    | 1.35      |
| 9             | 0                             | 1                   | 1                     | 1.53      |
| 10            | 0                             | -1                  | 1                     | 1.17      |
| 11            | 0                             | 0                   | 0                     | 0.95      |
| 12            | 0                             | 0                   | 0                     | 1.28      |
| 13            | -1                            | 0                   | -1                    | 0.93      |
| 14            | 0                             | 0                   | 0                     | 1         |
| 15            | 0                             | -1                  | -1                    | 1.26      |
| 16            | -1                            | -1                  | 0                     | 1.03      |
| 17            | 0                             | 0                   | 0                     | 1.23      |

The analysis of variance is shown in Table 3.

| Type              | SS     | MS     | F value | P value (Prob > F) | Df | Significance |
|-------------------|--------|--------|---------|--------------------|----|--------------|
| Model             | 0.6    | 0.066  | 4.22    | 0.0355             | 9  | Remarkable   |
| A (Material-liquid ratio) | 0.15  | 0.15   | 9.79    | 0.0167             | 1  | Remarkable   |
| B (Ultrasound power) | 0.16  | 0.16   | 10.14   | 0.0154             | 1  | Remarkable   |
| C (Extraction time) | 8.000×10^4 | 8.000×10^4 | 0.051   | 0.8281             | 1  | REMARKABLE   |
| AB                | 0.026  | 0.026  | 1.63    | 0.2429             | 1  |              |
| AC                | 3.025×10^3 | 3.025×10^3 | 0.19    | 0.6743             | 1  |              |
| BC                | 3.025×10^3 | 3.025×10^3 | 0.19    | 0.6743             | 1  |              |
| A^2               | 2.368×10^4 | 2.368×10^4 | 0.015   | 0.9058             | 1  |              |
| B^2               | 0.24   | 0.24   | 15.09   | 0.0060             | 1  | Remarkable   |
| C^2               | 6.737×10^3 | 6.737×10^3 | 0.43    | 0.5339             | 1  |              |
Table 3 shows that the regression model is significant (p < 0.05), the missing items are not significant (p = 0.8650 > 0.05), and R² = 0.8443 and Adeq Precision = 5.82, greater than 4. It is known that the regression equation has a high fitting degree and reliability, and can predict the yield of quercetin well. The results of regression model coefficient significance test showed that the linear effect of the first item A (p =0.0167<0.05), the first item B (p =0.0154<0.05) on quercetin extraction was significant, while the extraction time C had no significant effect on the yield (p =0.8285>0.05); the second item B² (p =0.0060<0.05) had significant surface effect on quercetin extraction, while A² (p =0.9058>0.05), C² (p =0.5339 > 0.05) had significant effect on quercetin extraction. were not significant; the interaction terms BC, AB and AC were not significant; it indicated that the influence of various factors on quercetin yield was not a simple linear relationship[9-10] . The regression equation obtained by model fitting is:

\[ Y=1.09+0.14A+0.14B-0.001C-0.08AB-0.027AC+0.028BC+0.0075A^2+0.24B^2+0.04C^2. \]

3.3.2 Conditional optimization and prediction. The response surface diagram of each factor to quercetin yield is shown in Fig. 6. Design-Expert 8.0.5 software was used to derive the derivative. When the liquid-solid ratio was 35 mL/g, the ultrasonic power was 550 W and the extraction time was 30 minutes, the maximum value of response value (yield) was 1.585%[11].

![Response Surface Diagram](image-url)
3.3.3 Verification experiment. According to the results of the quadratic regression model analysis, the optimum conditions are as follows: the liquid-solid ratio is 35 mL/g, the ultrasonic power is 550 W, and the extraction time is 30 min. The maximum predicted response value (yield) is 1.585%. According to the above optimized conditions, three pieces of pear meal were extracted in parallel, and the average value of quercetin was 1.576%. The calculated deviation was 0.57% compared with the maximum value predicted by binomial fitting equation. The deviation was small, which indicated that the model was effective.

3.4 Study on antioxidant activity of quercetin from avocado meal

3.4.1 Determination of scavenging capacity of quercetin to DPPH. From Figure 7, it can be seen that with the increase of quercetin concentration, its ability to scavenge DPPH free radicals is gradually enhanced. At the concentration of 30mg/mL, the DPPH scavenging rate reaches 70%. It can be seen that the ability of quercetin to scavenge DPPH free radicals has a good dose-effect relationship with its concentration. Although quercetin from avocado meal is less effective than vitamin C in scavenging
3.4.2 Evaluation of Ferric Ion Reduction Ability (FRAP). From Fig. 7, it can be seen that the FRAP value of quercetin increases obviously with the increase of quercetin concentration. At the concentration of 30mg/mL, the reduction ability of iron ions increases remarkably, almost equal to 6mmol/L FeSO4. It can be seen that quercetin from oil pear meal exhibits strong reduction ability, indicating that quercetin from oil pear meal has better antioxidant ability[13].

![Graph](image)

(a) Scavenging rate of quercetin from avocado meal on DPPH

(b) Ferric Reduction Capability Curve

Figure 7. Graph

4. Conclusion

Through single factor and response surface optimization experiments, the optimum extraction conditions of quercetin from avocado meal were determined as follows: liquid-to-material ratio 35 mL/g, ultrasonic power 550 W, extraction time 30 min. Under these conditions, the maximum predicted response value (yield) was 1.585%, the experimental verification value was 1.576%, and the deviation was small. Therefore, the results of response surface design were accurate and reliable, and could be used to optimize and predict the technological conditions of ultrasonic extraction of quercetin from pear meal.

The study on DPPH scavenging capacity and iron ion reduction capacity showed that quercetin in Pear meal had good antioxidant capacity. When the concentration was 30 mg/mL, DPPH scavenging rate reached 70%, and iron ion reduction capacity increased significantly equivalent to 6 mmol/L FeSO4. Both of them had a good dose-effect relationship with quercetin concentration in pear meal. This experiment provides a theoretical basis for the development of quercetin in avocado meal[14].

Author's Profile

Xuehua Feng (1979-), female, master, associate professor, mainly engaged in pharmaceutical analysis, Email: fxh77@126.com

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