

Optimization of Extraction Parameters of Total Flavonoids from Persimmon Leaves

Fahu Yuan, Kai Liu, Lifeng Gao, Ruiqi Ba and Jiangyuan Chen*

School of medicine, Jiangnan University, Wuhan, 430056, China

*Corresponding author: chenjiangyuan@hotmail.com

Abstract

In order to obtain the optimum conditions for the extraction of flavonoids from persimmon leaves by ethanol extraction, the basic technological parameters were obtained by single factor experiment as follows: extraction time 2h, solid-liquid ratio 1:30, ethanol concentration 70%, and extraction temperature 65°C. On this basis, orthogonal experiment was used to optimize the extraction conditions. The optimal extraction parameters were as follows: extraction time 2h, solid-liquid ratio 1:25, ethanol concentration 70%, extraction temperature 70°C. Under these conditions, the yield of total flavonoids in persimmon leaves was 5.02%.

Keywords

Persimmon leaf; Ethanol extraction; Flavonoids; Orthogonal design.

1. Introduction

Persimmon leaves are rich in active ingredients such as polyphenols, triterpenoids, flavonoids and vitamin C, which have a variety of pharmacological effects such as anti-oxidation, anti-cancer transformation, heat clearing and detoxification [1]. Flavonoids have a variety of health care functions, such as antibacterial and anti-inflammatory, thirst quenching, lung moistening and heart strengthening, anti-cancer and cancer prevention [2]. The vast majority of persimmon leaves are treated as fuel or waste, with very low resource utilization. Oils or foods rich in oils are prone to oxidation reactions that lead to deterioration of quality, and antioxidants are usually added. In recent years, natural antioxidants have become more and more popular due to their safety and non-toxicity [3], and the good antioxidant properties of flavonoids have also attracted extensive attention [4]. The ethanol extraction technology and antioxidant activity of flavonoids from persimmon leaves were studied in this study.

2. Materials and Methods

2.1. Reagent

Persimmon leaves: harvested on the campus of Jiangnan University; Rutin: Shanghai Yuanye Biotechnology Co., LTD. Sodium nitrite, aluminum nitrate, hydrogen peroxide, salicylic acid, etc., were purchased to Sinophanate Reagent Co., LTD., for analytical pure grade.

2.2. Rutin Standard Curve

Refer to sodium nitrite - aluminum nitrate - sodium hydroxide color method. Standard rutin solutions of 0.00mL, 1.00mL, 2.00mL, 3.00mL, 4.00mL and 5.00mL at the concentration of 0.10mg·mL⁻¹ were precisely taken and placed in a 10mL volumetric flask. Add 0.50mL 5%NaNO₂ solution, shake well and stand for 6min. Add 0.50mL 10% Aluminum nitrate solution, shake well and stand for 6min; 4.00mL 4%NaOH solution was added, and then 75% ethanol solution was placed in constant volume for 15min, and the absorbance was measured at 510nm

wavelength. The standard curve was made with rutin concentration as abscissa and absorbance value as ordinate.

2.3. Extraction Process Of Flavonoids From Persimmon Leaves

The process is as follows: Persimmon leaves → cleaning → drying (65 °C) → crushing → sieving (100 mesh) → ethanol impregnation → vacuum filtration → extraction liquid.

Select fresh, no corruption, no damage persimmon leaves, wash, drain, drying at 65 °C, crushed, 100 mesh sieve, sealed storage. 2g persimmon leaf powder was accurately weighed in a 50mL iodine measuring flask, and 75% ethanol solution was added. After 2h water bath in a constant temperature water bath at 60 °C, the filtrate was vacuumized and filtered to a constant volume of 50mL volumetric flask. Add 1mL sample solution in 25mL volumetric flask, add 0.5ml 5% NaNO₂ solution, and place for 6min after shaking; 0.5 ml 10% Al(NO₃)₃ solution was added, and set aside for 6min after shaking; Add 4% NaOH solution 4.00ml, then add 75% ethanol constant volume, set aside for 15min. The absorbance value was measured at 510nm, and the yield of flavonoids was calculated.

2.4. Single Factor Experiment

Four factors were selected: extraction time (1h, 2h, 3h, 4h, 5h), solid-liquid ratio (1: 20, 1: 25, 1: 30, 1: 35, 1: 40), ethanol concentration (50%, 60%, 70%, 80%, 90%), and extraction temperature (60°C, 65°C, 70°C, 75°C, 80°C), with five levels for each factor. Each level was repeated for 3 times to study the influence of each factor on the yield of flavonoids. The experimental data were analyzed by USING SPSS 18.0 statistics software for variance analysis, and the results were expressed as mean ± standard deviation.

2.5. Orthogonal Test

In order to determine the optimal conditions for the extraction of flavonoids from persimmon leaves, extraction time, solid-liquid ratio, ethanol concentration and extraction temperature were selected to conduct L₉(3⁴) orthogonal experiment based on the yield of flavonoids as an index and the results of single factor experiment.

3. Results

3.1. Effects of Extraction Time on the Yield of Flavonoids from Persimmon Leaves

The yield of flavonoids in persimmon leaves increased firstly and then decreased with the increase of extraction time. When the extraction time was 2 h, the yield of flavonoids was the highest. When the extraction time was more than 2 h, the yield of flavonoids decreased significantly. The possible reason is that after long time extraction, part of ethanol volatilizes and the boiling point increases, which leads to the destruction of part of flavonoids and the decline of flavonoid yield. Therefore, the extraction time is 2 h. SPSS software analysis of variance showed that extraction time had a significant effect on the yield of flavonoids from persimmon leaves ($P < 0.05$), see Figure 1.

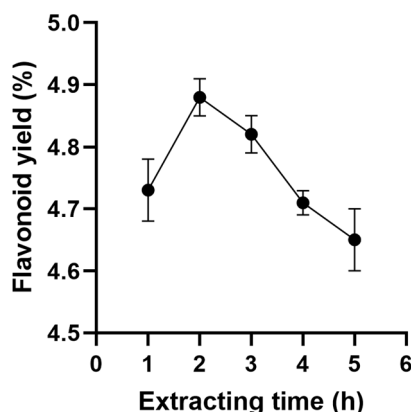


Figure 1. Effects of extraction time on the yield of flavonoids from persimmon leaves

3.2. Effects of Solid-liquid Ratio on the Yield of Flavonoids from Persimmon Leaves

With the increase of solid-liquid ratio, the yield of flavonoids in persimmon leaves increased gradually. When the ratio of solid to liquid was less than 1 : 30, the yield of flavonoids increased greatly. When the ratio of solid to liquid was greater than 1 : 30, the yield of flavonoids increased slowly, indicating that the increase of ethanol dose was conducive to the complete dissolution of flavonoids from persimmon leaves. However, considering the extraction cost, the ratio of solid to liquid was 1 : 30. Anova showed that the ratio of feed to liquid had a significant effect on the yield of flavonoids from persimmon leaves ($P < 0.05$), see Figure 2.

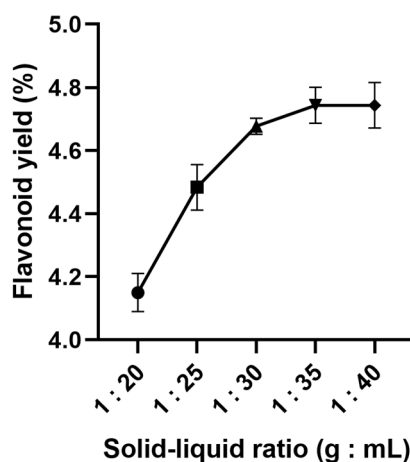


Figure 2. Effects of different solid-liquid ratio on the yield of flavonoids from persimmon leaves

3.3. Effects of Ethanol Concentration on the Yield of Flavonoids from Persimmon Leaves

The yield of flavonoids in persimmon leaves increased first and then decreased with the increase of ethanol concentration. When the concentration of ethanol was 50%-70%, flavonoids were easily dissolved from persimmon leaves, and the yield of flavonoids increased continuously. When the concentration of ethanol continues to increase, some lipid soluble substances and pigments will be leaching out, and the yield of flavonoids will decrease significantly, and these substances will compete with flavonoids in solvent, affecting the dissolution of flavonoids. Therefore, the ethanol concentration was chosen at 70%. Anova showed that ethanol concentration had a significant effect on the yield of flavonoids from persimmon leaves ($P < 0.05$), see Figure 3.

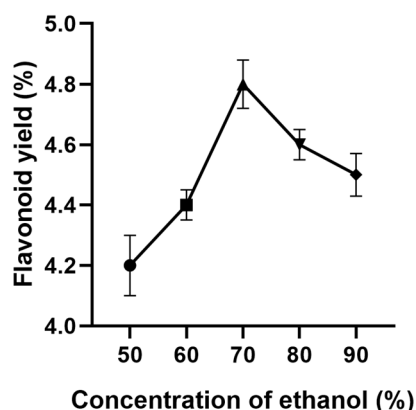


Figure 3. Effects of different ethanol concentration on the yield of flavonoids from persimmon leaves

3.4. Effects of Extraction Temperature on the Yield of Flavonoids From Persimmon Leaves

Figure 4 shows that the yield of flavonoids in persimmon leaf extract increased first and then decreased with the increase of temperature. The increase of temperature accelerated the diffusion of molecules, which was conducive to the dissolution of alcohol-soluble impurities in persimmon leaves. High temperature will also denaturate some soluble proteins, increase the viscosity of the solution, and reduce the yield of flavonoids. Therefore, the most appropriate extraction temperature is 65 °C. Anova showed that extraction temperature had a significant effect on the yield of flavonoids from persimmon leaves ($P < 0.05$).

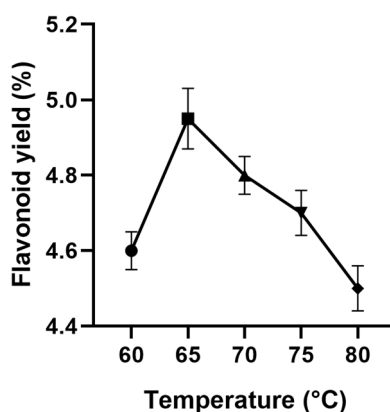


Figure 4. Effects of extraction temperature on the yield of flavonoids from persimmon leaves

3.5. Orthogonal Test

Orthogonal experiments were carried out according to the selected factors and levels. The results showed that the optimal extraction scheme of flavonoids from persimmon leaves was as follows: extraction time 2 h, ratio of persimmon leaves to ethanol 1 : 30, ethanol concentration 70%, and extraction temperature 70 °C. The range analysis showed that the order of factors affecting flavonoid yield was ethanol concentration > extraction temperature > extraction time > solid-liquid ratio.

3.6. Verification Tests

The experiments were repeated for three times respectively. The significant difference analysis showed that the two extraction processes had significant difference. Therefore, the optimal

extraction conditions were as follows: the extraction time was 2 h, the ratio of persimmon leaf to ethanol was 1 : 25, the concentration of ethanol was 70%, and the extraction temperature was 70 °C.

4. Conclusion

There are many kinds of flavonoids, and their physical and chemical properties differ greatly. The flavonoids extracted from the same raw material by different extraction methods differ greatly. At present, the extraction methods of flavonoids mainly include ethanol refluxing method, ultrasonic method and microwave assisted method. In this experiment, the optimal extraction parameters of flavonoids from persimmon leaves were as follows: extraction time was 2 h, the ratio of persimmon leaves to ethanol was 1 : 25, the concentration of ethanol was 70%, and the extraction temperature was 70 °C. Under these conditions, the yield of flavonoids from persimmon leaves was 5.02%. Persimmon tree planting area is wide, persimmon leaf resources are rich, low price. Flavonoids have anti-fat oxidation, anti-aging, antibacterial, treatment of cardiovascular and cerebrovascular diseases and other effects. Therefore, the research on the flavonoids of persimmon leaves can make full use of persimmon leaves and turn waste into treasure, which can provide support for the deep processing of persimmon leaves and the research and development of health products.

References

- [1] Kameda K, Takaku T, Okuda H, et al. Inhibitory Effects of Various Flavonoids Isolated from Leaves of Persimmon on Angiotensin-Converting Enzyme Activity[J]. Journal of Natural Products, 1987, 50(4):680-683.
- [2] Sun L, Zhang J, Fang K, et al. Flavonoids from persimmon (*Diospyros kaki*) leaves (FPL) attenuate H₂O₂-induced apoptosis in MC3T3-E1 cells via the NF-κB pathway[J]. Food & Function, 2014, 5(3):471-479.
- [3] Choi S W. Antioxidative Activity of Flavonoids in Persimmon Leaves[J]. food science & biotechnology, 1996.
- [4] Gao Y F, Gao Y S, Xin X M. Hypoglycemic and hypocholesterolaemia effect of Total flavonoids from persimmon leaf in alloxan-induced diabetic mice and its mechanism[J]. Journal of Taishan Medical College, 2009.