Determination of Total Flavonoids in Pickled Mustard

Zhuang Li, Xiao Chen, Fuqing Wang, Xuejiian Bai and Yin Wei*

Jining Medical College, Jining 272013, China.

Abstract

Objective: Determination of total flavonoids in pickled mustard tuber by Spectrophotometry MethodsThe samples were extracted with ethanol, adsorbed with polyamide powder, eluted with benzene, and then eluted with methanol. Flavonoids at the wavelength of 360nm were determined by Spectrophotometry. Results: Benzene was used as eluent and total yellow.The concentration of ketone in the range of 0~2010 g/ml is in good linear relationship with the absorbance value (r=0.9998). Conclusion: The method is simple, reproducible and stable, and has a wide linear range. It can be used for the determination of total flavonoids in health foods. The total flavonoids content in preserved mustard tuber is 142ug/100g.

Keywords

Mustard tuber; Total flavones; Spectrophotometry.

1. Introduction

Flavonoids (English: Flavonoid, also known as flavonoids) are flavonoids based on the skeleton of 2-phenylchromone-4-one (2-phenyl-1-benzopyran-4-one). The parent structure is 2-Phenylchromone compounds are now generally referred to as a series of compounds with two phenolic hydroxyl groups connected to each other through a central carbon atom. They may come from fruits, vegetables, tea, wine, seeds and plant roots. Although it is not considered a vitamin, it is considered to have nutritional functions in the internal reactions of the organism. It was once called "vitamin P": for example, it has anti-oxidant or anti-inflammatory effects. It is also believed to resist or slow down the occurrence of tumors. Flavonoids (especially the ratio of flavonoids and catechins) are the most common combination in the human diet, and are commonly found in plants. Dietary flavonoids are naturally occurring fruits, vegetables, chocolate, and beverages like wine and tea. Flavonoids are widely distributed in plants and perform many functions. Flavonoids are important plant pigments for flower coloring, producing petals yellow or red, and blue pigments are intended to attract pollinators. In higher plants, flavonoids can participate in ultraviolet filtering, symbiotic nitrogen fixation and flower pigmentation. They can also act as chemical messengers, physiological regulators and cell cycle inhibitors. The flavonoids secreted by the roots of host plants help rhizobia at the infection stage like pea, broad bean, alfalfa, soybeans and legumes in symbiosis with rhizobia. Rhizobia living in the soil can sense flavonoids and trigger the secretion of nodulation factors, which in turn are recognized by the host plant and may cause root hair deformation and several cellular responses such as ion flux and nodule formation. In addition, some flavonoids have inhibitory activity against microorganisms, causing plant diseases, such as Fusarium oxysporum. There has been great interest in the potential health benefits of flavonoids associated with a diet rich in processed fruits and vegetables. There are many analysis methods. For the mutual separation of flavonoids and the quantitative analysis of single components, high performance liquid chromatography (HPLC) is often used; for the determination of total flavonoids, spectrophotometry is mainly used. This article will use spectrophotometry to determine the total flavonoids in mustard tuber. In the "Technical Specification for Health Food Inspection and Evaluation", direct ultraviolet spectrophotometry is used. In the determination, benzene is required to elute impurities. However, benzene is very toxic and in a large amount, which can easily pollute the environment and cause harm to the experimenter. Therefore, great care should be taken during the elution process.

2. Determination of total flavonoids in samples

1. Wavelength-selective flavonoids are the general term for a class of compounds whose parent structure is diphenylchromone, including: flavonoids, flavonols, isoflavones, dehydroflavonoids and chalcones, etc. The flavonoids have two UV absorption regions. One, namely 240~280nm, 300~400nm, the flavonoids in the absorption region of 300~400nm measured by spectrophotometry, there are some non-absorbing flavonoids such as isoflavones in this wavelength range, the results are not included.

2. Solvent choice Flavonoids including glycosides and glycosides will have strong polarity and are easily soluble in organic solvents such as methanol and ethanol. Use ethanol as the extractant to extract in a water bath at 80°C for 2 hours to complete the extraction. In this experiment, ethanol was used as the extractant, and ultrasonic extraction was sufficient for 20 minutes. Ultrasonic-assisted extraction mainly uses ultrasonic cavitation between the solvent and the extracted sample solution. Under this action, a strong shock wave and scattering flow are generated inside the solution, which generates high temperature and high pressure locally, which will cause multiple secondary Effects, such as emulsification, crushing, diffusion, and strong mechanical shock, will accelerate the mass transfer and heat transfer rate of the system.

3. Respectively, accurately pipet 5 mL of the sample into a 25 mL volumetric flask, and measure the absorbance. The reference is the reagent blank. The same method is used to calculate the content according to the regression equation. The results are shown in Table 1.

3. Object and method

3.1 Instruments and reagents

1. Apparatus Model 7220 UV-Vis Spectrophotometer; Ultrasonic Extractor; Colorimetric tubes with plugs, 10ml and 25ml; Constant temperature water bath box; Volumetric flask, electric constant temperature blast drying box, electronic balance (1/10th)

2. Reagents (1) methanol analytical purity (2) absolute ethanol (3) polyamide powder (4) standard stock solution of rutin: Weigh 10mg of rutin, add methanol to dissolve and dilute to 25ml, that is, 0.4mg/ ml. (5) Experimental water: tertiary water

3.2 Measurement method

1. Sample pretreatment: Weigh a certain amount of mustard tuber samples into a 25ml colorimetric tube, add absolute ethanol to the volume, shake well, and ultrasonically extract for 20 minutes. Set aside, draw 1.0ml of supernatant to an evaporating dish, then add 1g of polyamide powder for adsorption, evaporate ethanol in a water bath, and turn to the chromatography column. First use 20ml of benzene to elute the magazine; then use methanol to elute the flavonoids, and collect the eluate to a volume of 25ml. Measure the absorbance of the solution at a wavelength of 360nm.

2. Drawing of standard curve (1) Take a 10ml colorimetric tube and draw the standard solution of rutin: 0.00, 0.05, 0.10, 0.20, 0.40, 1.0, 1.50, 2.00ml in the colorimetric tube, add methanol to the volume and shake well. (2) Using a spectrophotometer at 360nm, with methanol as a reference, measure the absorbance of the mustard sample and the standard solution respectively. (3) Carry out a linear regression on the mass by absorbance to find the regression equation. Get the regression equation Y=0.027X+0.0261 (r=0.9998)

3. Data processing

According to the standard working curve, find the content of rutin equivalent to the absorbance of the sample, and find the total flavonoid content as follows:

Table 1. The content of total flavonoids in mustard tuber (n=3)

No.	Absorbance(A)	Total flavonoids (ug/100g)	Average (ug/100g)	RSD (%)
1	0.536	135		
2	0.538	135	142	1.52
3	0.560	158		

4. Precision test Precisely pipette 5.0 mL of the same sample solution 6 times in a 25 mL volumetric flask, dilute it with methanol to volume, and the absorbance is 0.538, 0.550, 0.546, 0.532, 0.531, 0.530, respectively. As a result, the RSD was 1.35%. The results show that the precision is good.

5. Linear range Under the above experimental conditions, the concentration of flavonoids is in the range of 0-20μg/ml, and the linear relationship is good, r=0.9998

3. Stability test Precisely pipet 5.0 mL of the same sample solution 6 times in a 25 mL volumetric flask, dilute it to volume with methanol, and measure the absorbance at 360 nm at 0, 5, 10, 20, 30, and 40 min. recording. Calculate the relative standard deviation (RSD), the result RSD is 1.89%. The results show that the sample is stable within 40 minutes.

4. Repeatability test. Precisely pipet 5.0 mL of the same sample solution 6 times in a 25 mL volumetric flask. After diluting to volume with methanol, the absorbance is 0.532, 0.541, 0.536, 0.538, 0.531, 0.547, and the result RSD is 1.05%. The results show that the sample repeatability is good.

5. Recovery rate test accurately weigh 100mg of the sample with known total flavonoid content, add 10mg of rutin reference substance, prepare the test solution according to the original method, accurately pipette 5.0mL of the same sample solution into a 25mL volumetric flask, and use methanol After diluting to the mark, measure the absorbance and calculate the recovery rate of sample addition. Results The average sample recovery rate was 89%, and the result was an RSD of 1.60%.

4. Discuss

1. Wavelength selection There are two ultraviolet absorption regions of flavonoids, namely 240~280nm, 300~400nm, the flavonoids in the 300~400nm absorption region measured by spectrophotometry, there are some non-absorbed flavonoids in this wavelength range, such as Isoflavones, results are not included.

2. Sample pretreatment The sample should be ground as fine as possible to achieve better extraction results. After the experiment, directly grind the sample of mustard tuber which is not meticulous, and extract the absorbance value measured after the adsorption and elution is 0.485, but the absorbance value measured by the same method after grinding the sample is 0.531. It can be seen that after the sample is finely ground, the extraction effect will be better it is good.

3. Adsorbent particle size The adsorbent used in this experiment is a polyamide adsorbent. This adsorbent has two particle sizes: small particles of 30-60 mesh and large particles of 14-30 mesh. The experiment should take the same specifications of adsorbents. The experimental results of different specifications of adsorbent are different. Take 1ml of adsorbents with different particle sizes for comparative tests, and the results show that the absorbance of large particles will be significantly lower than that of small particles.

4. After the elution, the storage time before the measurement is tested using the same specification of adsorbent eluent, the absorbance value of the sample solution just after elution is relatively high, this is because the sample solution is relatively turbid, and the absorbance will decrease after a period of time. After centrifugation, the effect is not obvious and it is very inconvenient. So consider the measurement after the same time.

5. The use of benzene for elution should pay attention to safety and must be operated in a fume hood. Because benzene is very toxic, it can harm the body and easily cause damage to the environment. In addition, you can also consider using petroleum ether as the eluent. Compared with the measurement results using benzene as the eluent, there is no significant difference between the two. In particular, it reduces the pollution of benzene to the inspectors and the environment. It can be used for food. Determination of total flavonoids in medicines.

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