CHEMICAL ENGINEERING TRANSACTIONS
VOL. 59, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Zhuo Yang, Junjie Ba, Jing Pan

ISBN 978-88-95608- 49-5; **ISSN** 2283-9216
DOI: 10.3303/CET1759130

Determination of Total Flavonoids in Leek by $AlCl_3$ Colorimetric Assay

Hua Liu*, Yinghua Song, Xiaojuan Zhang

School of Chemistry and Chemical Engineering, Anshun College, Anshun 561000, China
lzyh1235@126.com

Rutin was taken as reference substance, and the conditions of aluminum chloride colorimetric assay for determining total flavonoids in leek extract were optimized. The effects of several parameters, such as dosage of color developing agent, buffer dosage, pH and reaction temperature and time, on the color development reaction of total flavonoids were evaluated. The optimum conditions were as follows: 1 mL appropriate concentrationor of leek extract, added 1 mL of HAc-NaAc buffer (pH4.8), 2mL of 0.1mol·$L^{-1}AlCl_3$ solution, volume calibration with 70% ethanol to 10 mL and mixing, and 12 min of water bath at 40°C. Under the optimum conditions, the maximum absorption wavelengths of standard rutin solution and leek extract coincided (407nm), with the absorbances reaching maxima also. The method has high stability, reproducibility and recovery. Compared with the commonly used $NaNO_2$-Al $(NO_3)_3$-NaOH colorimetric method, it is considered that the method of determination of total flavonoids in leek by $AlCl_3$ colorimetric assay is more suitable when rutin was taken as reference substance

1. Introduction

Allium tuberosum is a perennial herb in the genus Allium (Amaryllidaceae family), with a special and strong leek odor. The leaves are commonly known as leek. With abundant nutrients, leek contains trace elements beneficial to human body as well as highly volatile sulfur compounds and flavonoids of medicinal values. Until now, the volatile oilsin leek have been extensively studied (Wei and Wan, 1996; Wei and Ren, 2003; Wang and Feng, 2002), but the flavonoids therein remain largely unknown. Total flavonoids in plants has a wide range of physiological activity, such as anti-oxidation, anti-cancer and anti-inflammatory and antimicrobial effects (Joyeux et al, 1995; Balasubramanian et al., 2007; Reinwalds, 2006); They are generally determined by direct spectrophotometry, colorimetry and high-performance liquid chromatography (HPLC) (Wang et al., 2012; Zeraik and Yariwak, 2012; Fu et al., 2012; Marques et al., 2013; Wang et al., 2012). Direct spectrophotometry has low sensitivity. HPLC results are accurate, but the equipment is expensive, failing to meet the general requirements of industrialization. Total flavonoids have most commonly been detected by colorimetric methods, especially that using $NaNO_2$-$Al(NO_3)_3$-NaOH (Jia et al., 2015). Huang et al. also determined the total flavonoid content of leek by this method (Huang et al., 2007), but they did not systematically assess its feasibility. However, the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method cannot exclude the interference of non-flavonoids such aso-dihydric phenols (Guo et al., 2002; Li and Zhang, 2010; Qiu et al., 2013). Therefore, this method is not highly specific to total flavonoid determination. In this study, an $AlCl_3$ colorimetric method was employed to explore the factors influencing the determination of total flavonoids in leek, with rut in as the reference substance. Finally, the conditions for determination were optimized. This simple, feasible, reproducible and stable method can be used as one of the preferred strategies for determining total flavonoids in leek (Sun et al., 2016).

2. Methods

2.1 Solution configuration

Rutin and $AlCl_3$ configuration with 70% ethanol volume, the buffer solution with distilled water.

Please cite this article as: Hua Liu, Yinghua Song, Xiaojuan Zhang, 2017, Determination of total flavonoids in leek by alcl3 colorimetric assay, Chemical Engineering Transactions, 59, 775-780 DOI:10.3303/CET1759130

2.2 Preparation of leek extract

Clean leek leaves were dried, chopped, fully ground into 10g, added 70% ethanol at the sample/liquid ratio of 1/10 (g/mL), and subjected to ultrasonic extraction (temperature: 40°C; time: 15 minutes). After suction filtration, the filtrate was volume-calibrated to 100mL with70% ethanol, as thesample extract.

2.3 Factors affecting $AlCl_3$ colorimetric determination of total flavonoids in leek

The results are shown in Table 1-5.

2.4 Plotting of standard curvefor rutin

Rutin reference solutions (0.5, 1.0, 1.5, 2.0 and 2.5mL) were pipetted into 10mL colorimeter tubes with stopper, followed by addition of 1mL of pH 4.8 HAc-NaAc buffer and 2mL of $AlCl_3$ solution (0.1 $mol \cdot L^{-1}$) sequentially. After volume calibration with 70% ethanol and mixing, the tubes were then left in a 40°C water bath for 12 min. The absorbance (A) at 407 nm was linearly regressed with the concentration (C, $mg \cdot mL^{-1}$) of rutin sample solution, and the standard curve was plotted.

2.5 Evaluation of the method

The stability and reproducibility of the method and the spiked recoveries were measured, and the results were calculated (Yan et al., 2015) and shown in Table 6-8

2.6 Comparison by $AlCl_3$ and $NaNO_2$-Al $(NO_3)_3$-NaOH Colorimetric

Follow-up experiment was referred to the literature (Chen et al., 2016) by $NaNO_2$-Al $(NO_3)_3$-NaOH Colorimetric to determine total flavonoids in leek simply. Three times in parallel. And the results of determination of $AlCl_3$ colorimetric were compared. The results are shown in Table 9.

3. Results and discussion

Rutin reference substance and leekextract both have maximum absorption peaks at about 280nm and 400nm (Figure 1). Considering peak shape and excluding interference of proteins during leek extraction (Tian and Zhang, 2008), we finally selected the absorption peak at approximately 400nm. The results are shown in Table 1.

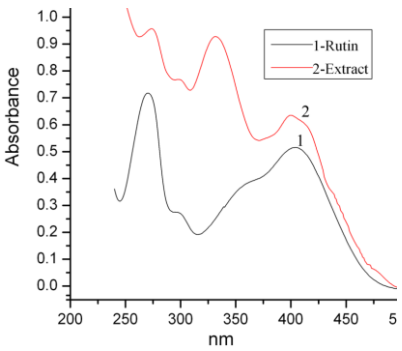

Figure 1: Ultraviolet Scanning Spectra of Rutin Standard and Extract

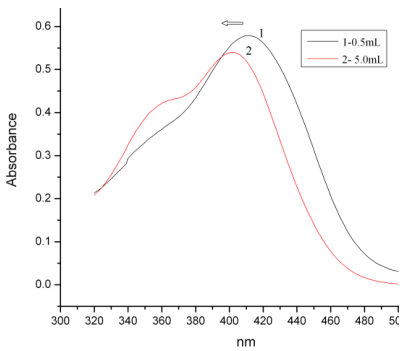

Figure 2: Ultraviolet Spectrum of Rutin

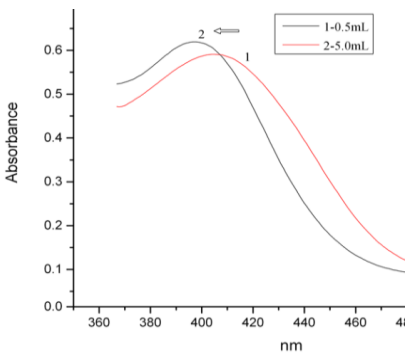

Figure 3: Ultraviolet Spectrum of Extract

The standard curve is shown in Figure 4. The linear regression equation of absorbance (A) versus concentration of rutin sample solution (C, mg·mL^{-1}) was A = 25.45C+0.003(R^2 = 0.9993), suggesting that the linear relationship was good when this concentration ranged from 0.0075 mg/mL to 0.0375 mg/mL.

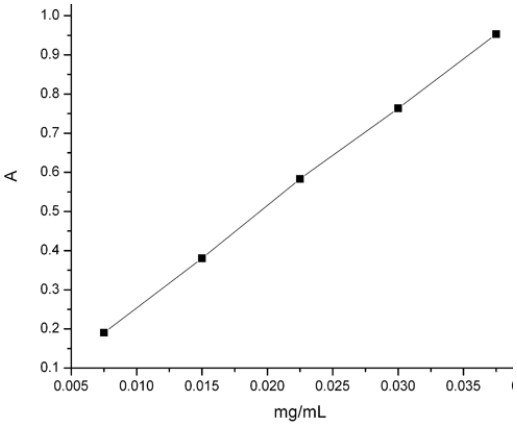

Figure 4: The standard curve for rutin

Table 1: Effect of the dosages of aluminum chloride

Sample	dosages of aluminum chloride (mL)	Λmax(nm)	A
Rutin	0.5	408	0.5708
	1.0	408	0.5722
	1.5	407	0.5753
	2.0	407	0.5832
	2.5	406	0.5696
	3.0	404	0.5535
	4.0	403	0.5529
	5.0	401	0.5451
Leek extract	0.5	410	0.5825
	1.0	408	0.6250
	1.5	407	0.6336
	2.0	407	0.6502
	2.5	406	0.6371
	3.0	404	0.6285
	4.0	403	0.6170
	5.0	396	0.6096

Table 2: Effect of pH of HAc-NaAc buffer system

Sample	pH	Λmax(nm)	A
Rutin	4.0	399	0.5587
	4.4	405	0.5662
	4.8	407	0.5821
	5.2	408	0.5689
	5.6	410	0.5546
	6.0	410	0.5429
Leek extract	4.0	406	0.6020
	4.4	406	0.6365
	4.8	407	0.6508
	5.2	407	0.6287
	5.6	408	0.6155
	6.0	408	0.6086

Table 3: Effect of dosage of HAc-NaAc buffer system

Sample	dosage of buffer (mL)	Λmax(nm)	A
Rutin	0.5	406	0.5565
	1.0	407	0.5763
	1.5	409	0.5689
	2.0	409	0.5523
	2.5	412	0.5629
	3.0	413	0.5667
	5.0	415	0.5626
Leek extract	0.5	410	0.6023
	1.0	407	0.6359
	1.5	407	0.6117
	2.0	405	0.6068
	2.5	403	0.5910
	3.0	399	0.5775
	5.0	395	0.5531

Table 4: Effect of reaction temperature

Sample	Temperature (°C)	Λmax(nm)	A
Rutin	20	407	0.5501
	30	407	0.5632
	40	407	0.5712
	50	407	0.5585
	60	407	0.5458
Leek extract	20	407	0.6075
	30	407	0.6229
	40	407	0.6426
	50	407	0.6272
	60	407	0.6168

Table 5: Effect of reaction time

Sample	Time (min)	Λmax(nm)	A
Rutin	4	407	0.5580
	8	407	0.5658
	12	407	0.5826
	16	407	0.5673
	20	407	0.5531
Leek extract	4	407	0.6075
	8	407	0.6302
	12	407	0.6451
	16	407	0.6343
	20	407	0.6135

Table 6: Stability tests of the determination results

Leek extract	A_1	A_2	A_3	A_4	A_5	A	SD	RSD/%
1mL	0.6502	0.6488	0.6506	0.6471	0.6505	0.6494	0.0015	0.2310

Table 7: Reproducibility tests of the determination results

Leek extract	A_1	A_2	A_3	A_4	A_5	A	SD	RSD/%
1mL	0.6508	0.6491	0.6477	0.6435	0.6429	0.6468	0.0035	0.5411

Table 8: Recovery tests of total flavonoid

The original number of total flavonoids(mg)	The amount of rutin added(mg)	Final measurement of total Flavonoids(mg)	Recovery rate (%)
0.1412	0. 05	0.1913	100.2
0.1412	0.1	0.2411	99.9
0.1412	0.15	0.2913	100.1

Under the same conditions, the results showed that the extract had no obvious maximum absorption peak at 500-550 nm, while the rutin reference substance had the maximum absorption at 510 nm (Figure 5). It was taken 510nm as comparison to determine total flavonoids in Leek by $NaNO_2$-Al $(NO_3)_3$-NaOH colorimetric. And the results were compared with the method of $AlCl_3$ colorimetric. The results are shown in Table 9.

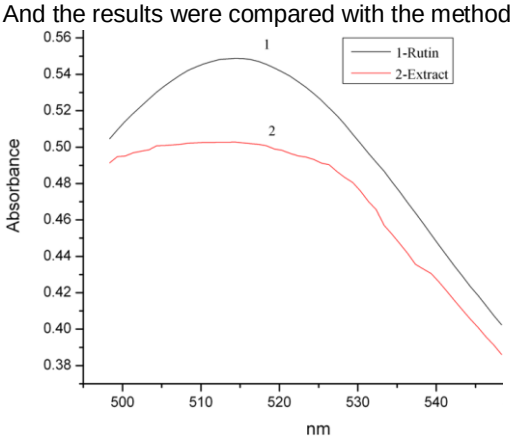

Figure 5: Ultraviolet spectrum by $NaNO_2$-Al $(NO_3)_3$-NaOH Colorimetric

Table 9: The results of flavonoid content by two methods in leek

Method	$AlCl_3$ Method			$NaNO_2$-Al $(NO_3)_3$-NaOH Method		
Number	1	2	3	1	2	3
Flavonoid Content(mg/g)	2.36	2.33	2.35	1.86	1.83	1.88

By comparison, the content of flavonoids by two methods was not consistent when the same amount of extract. $AlCl_3$ method was higher than $NaNO_2$-Al $(NO_3)_3$-NaOH method. This may be that $NaNO_2$-Al $(NO_3)_3$-NaOH method for measuring the specificity of flavonoid content is not strong. The specific composition of the flavonoids in leek is still to be further analyzed and studied.

5. Discussion and Conclusion

In this study, the conditions for determining total flavonoids in leekby $AlCl_3$ colorimetric assaywere optimized. The effects of dosage of color developing agent, systempH, buffer dosage, temperature and time on the color development reaction of $AlCl_3$ were assessed. By changing the factors, the maximum absorption peaks and corresponding absorbances of rutin reference substance and leek extract were altered, but each factor exerted different effects onthe two solutions. Probably, compared with standard rutin solution, the leek extract had more flavonoids. However, their maximum absorption peakscoincidedunder a certain optimumcondition of eachfactor.

When the color development reaction was conducted at 40°C for 12 min after addition of 1 mL of pH 4.8 HAc-NaAc buffer and 2 mL of ACl_3 solution (0.1 mol·L^{-1}) into 1 mL of leek extract at an appropriate concentrationor rutin, volume calibration with 70% ethanol to 10 mL and mixing, the maximum absorption peaks of the two solutions were both located at 407 nm. Hence, the conditions were optimum for determining the total flavonoids in leek using $AlCl_3$ colorimetric method. Thismethod has highstability, reproducibility and recovery.

In addition, this article does not have a systematic study on the determination of total flavonoids in leek by $NaNO_2$-Al $(NO_3)_3$-NaOH colorimetric. However, the results through the simple study were compared with the method by$AlCl_3$ colorimetric assay, it is considered that the method of determination of total flavonoids in leek by $AlCl_3$ colorimetric assay is more suitable when rutin was taken as reference substance.

Reference

Balasubramanian R., Narayanan M., Kedalgovindaram L., Rama K.D., 2007, Cytotoxic activity of flavone glycoside from the stem of indigofera aspalathoides vahl, Journal of Natural Medicines, 61(1), 80-83, DOI: 10.1007/s11418-006-0026-6.

Chen A., Wan D., Wan S., 2016, Comparative on the determination method of total flavonoidsin onion, Food science technology, 41(8), 276-279.

Fu Y., Guo H., Shangguan X., 2012, Determination of Isoflavones in Radix Puerariae by HPLC Calibration Spectrophotometry, Food Science and Technology, 39(6), 284-287.

Guo Y., Fan L., Zhang Z., 2002, Discussion on the method of colorimetric determination of total flavonoids, Chinese Journal of Pharmaceutical Analysis, 22(2), 97.

Huang S., Lin D., You T., 2007, Extraction of flavonoids from Chinese chives and their effects on hydroxyl radical scavenging, Lishizhen Guo Yao Guo Yao, 18(11), 2786-2787.

Jia N., Zhao E., Fan J., 2015, Optimization of Flavonoids from Onion by Ultrasonic Wave and Its Antioxidant Activity, Journal of Henan University of Technology (Natural Science), 36(2), 62-65.

Joyeux M., Lobstein A., Anton R., Mortier F., 1995, Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from ginkgo and some flavonoids, Planta Medica, 61(2), 126-129, DOI: 10.1055/s-2006-958030.

Li X., Zhang Y., 2010, Cedrela sinensis leaves flavonoids content determination method research, Journal of Chinese food, 10(5), 243-248.

Marques G.S., Waleska F.L., Magaly A.M., 2012, Comparative evaluation of UV / VIS and HPLC analytical methodologies applied forquantification of flavonoids from leaves of Bauhinia forficate, Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacog-nosy, 21, 51-57, DOI: 10.1590/S0102-695X2012005000143.

National Pharmacopoeia Commission, Chinese Pharmacopoeia, I Department. Beijing: Chemical Industry Press, 2005, 256.

Qiu H., She H., Zeng L., 2013, Study on the Improvement of Flavonoids Determination in Eucommia ulmoides Oliv, Food Research Development, 34(20), 96-100.

Reinwalds W., 2006, Soy Isoflavones and Bone Health: A Double-Edged Sword, Journal of Natural Products, 69(3), 450-459, DOI: 10.1021/np058104g.

Sun J., Li X.H., Yu X.L., 2016, Antioxidant activities, total flavonoids and phenolics content in different parts of silybum marianum l. plants, Chemical Engineering Transactions, 55, 37-42, DOI: 10.3303/CET1655007.

Tian Z., Zhang Y., 2008, Rapid Determination of Protein Content in Liquid Milk and Milk Powder by Ultraviolet Spectrophotometry, Journal of Chinese Journal of Health Laboratory Technology, 02, 263-264

Wang H., Feng J., 2002, Study on Chemical Constituents of Essential Oil from Chinese Chives, Journal of Tianjin Medical University, 8(2), 191-192.

Wang L., Mi S., Pang W., 2012, Determination of Total Flavonoids in Persimmon Leaves by Colorimetric Method, Food Research and Development, 33(6), 122-124.

Wang Q., Ao W., Tai W., 2012, Simultaneous Determination of Seven Flavonoids in Aerial Parts of Artemisia frigid by HPLC, Chinese Herbal Medicines, 4(3), 252- 258.

Wei H., Ren Z., 2003, Chinese chive flower volatile components of gas chromatography - mass spectrometry analysis, Journal of chromatography, 21(1), 96-97.

Wei H., Wan R., 1996, Gas-GC-MS Analysis of the Essential Oil from Leek, Chinese Journal of Analytical Chemistry, 24(2), 192-194.

Yan Y., Xia T., Zhang Y., 2015, Systematic Study on the Determination of Flavonoids in Pyracantha fortuneana by Colorimetric Method of Aluminum Chloride, Food Research and Development, 36(5), 89-93.

Zeraik M.L., Yariwake J.H., 2012, Quantification of isoorientin and total flavonoids in Passiflora edulis fruit pulp by HPLC-UV / DAD, Mi-crochemical Journal, 96, 86-91.