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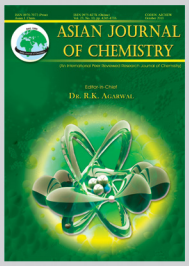

Analysis and Structural Identification of the Flavonoid Compounds in Fruit of *Citrus reticulata* Blanco *cv. Ougan* from China

YU-XIAN LI[1], XIANG-TAO CHEN[2], HUA-LIANG LIU[2] and KE YUAN[2,*]

[1]College of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450008, P.R. China
[2]The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Lin'an 311300, P.R. China

*Corresponding author: Tel: + 86 571 63743607; E-mail: yuan_ke001@163.com

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Using the fruit of *Citrus reticulata* Blanco *cv. Ougan* from the different regions of Zhejiang Province, China, as object, this research separated and identified the compounds of flavonoid in *C. ougan* fruit, meanwhile, we also determined the content of the three glycosylflavones in *C. ougan* fruit by the method of HPLC. This study used the method of column chromatography of sephadex LH-20, RP 18 and silica gel to separate and purify and thus elucidated 8 kinds of chemical compounds by the physicochemical properties and spectral data and identify the structures of the compounds. The 8 compounds were named as: eugenol (**1**), hesperetin (**2**), β-sitosterol (**3**), succinic acid (**4**), neohesperidin (**5**), hesperidin (**6**), naringin (**7**), naringenin (**8**). The above compounds are obtained for the first time by separation from the new species of *C. ougan* fruit. Using the above separated three monomer compounds *i.e.*, hesperidin, neohesperidin and naringin as the reference substances, the research made a content determination of the three glycosylflavones in *C. ougan* fruit produced in different regions of Zhejiang Province by HPLC. The experimental results show that the content of the three glycosylflavones in the *C. ougan* fruit of different regions vary remarkably. The content of neohesperidin in the *C. ougan* fruit is the highest, the content of neohesperidin is more than naringin, over 10 times almost. The content of hesperidin is the lowerest in *C. ougan* fruit. The content of the three glycosylflavones in pericarp are all higher than those in pulp. From these studies, it is suggested that there are rich glycosylflavones in the *C. ougan* fruit from China.

Key Words: *Citrus reticulata* Blanco *cv. Ougan*, The flavonoid, Separation, HPLC.

INTRODUCTION

Citrus ougan fruit is a cultivar of citrus genus from the *Citrus reticulata* Blanco var. *Suavissima* fruit. It is the recently cultivated new variety which has the distinctive local features, widely grown along the coast of river in southern Zhejiang Province[1]. *C. ougan* fruit is sweet and juicy with a taste of bitterness. It has a high nutritional value, containing rich vitamins, amino acids, sugar and citric acids[2-6]. In addition, it has much flavonoids with strong antioxidation activity[7-9]. *C. ougan* fruit has the special effects of reducing fever and promoting the production of saliva, eliminating phlegm and stopping cough and clearing away heat and toxic material. From the initial clinic observation, eating *C. ougan* fruit can obviously improve the cerebral blood circulation of the primary high-blood pressure patients[10].

Through literature survey, there is not any report on the separation and determination of *C. ougan* active ingredients. Still less reported on the research of the flavonoids component by HPLC method. This paper chooses *C. ougan* fruit as the object of study, separating and purifying its main chemical component produced along the coast of river, Zhejiang Province and uses spectral technology to make a structural identification, meanwhile takes HPLC method to determine the content of three glycosylflavones of *C. ougan* fruit in 7 different producing regions of Wenzhou and Lishui counties, Zhejiang Province. This paper provides the scientific data for its generalizing and planting, further development and its efficient utilization.

EXPERIMENTAL

Bruker DPX 400 NMR spectrometer with TMS used as internal standard (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR), Melting points were determined with an WRS-1B melting point apparatus and uncorrected (Shanghai Precision Scientific Instrument Co., China), Waters 2695 HPLC system (Waters Company., MA, USA), with a binary solvent manager, an automatic sample-feeding device and Waters 2996 PAD binary lineup examiner (Waters Company, MA, USA), Empower chromatogram workstation, Shumei KQ2200DE ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument

Co., China) was used for extraction, Millipore super-purified water device (Millipore Company, USA), RE-52A rotary evaporator (Shanghai Yarong Biology Technology Ltd., China). Concentration device of vacuum thin film (oneself installation)[11], sephadex LH-20 were used for column chromatography (Pharmacia Bioteck Inc.), RP18(YMC Company, Japan), silica gel GF254 were used for TLC and silica gel G (200-300 mesh) were used for column chromatography (Qingdao Marine Chemical Inc., China), microanalysis balance (EETTLER AE 240 Switzerland).

The sample of *C. ougan* fruit procured from seven different regions of Wenzhou and Lishui, Zhejiang Province (Table-1). These fruit have been identified by Professor Lu-Huan Lou from Zhejiang Agricultural and Forestry University.

The reference samples of hesperidin, neohesperidin and naringin are prepared by separating in this research (identified by NMR spectrometer). For the above reference samples, through the normalization method of peak area by HPLC, we identified its purification degree as 99.28, 98.04 and 98.16 % respectively, the methanol is chromatogram pure, water is super purified water and the other reagents are analytical pure.

Extraction and isolation: The fully-grown *C. ougan* fruit is picked from Lishui county, Zhejiang Province, picking time is in December, 2008. Take 80 kg of its pulp after the *C. ougan* fruit is peeled, then smash it to powder and take its juice and extract by the petroleum ether of 60-90 ºC until the colour of petroleum ether layer becomes nearly colourless, then combine the different fraction of petroleum ether and concentrate it till get 35 g of extract powder. The water fraction after the extracted of petroleum ether was also extracted by EtOAc, till the EtOAc layer becomes colourless. Combine the extracting liquid of EtOAc, concentrate it by vacuum thin film below 50 ºC and get 65 g of the extract powder. The water fraction after the extracted of EtOAc was then extracted repeatedly by *n*-BuOH and you can get the extract powder of 45 g from the extracted fraction of *n*-BuOH using concentration device by vacuum thin film below 50 ºC.

The fraction of petroleum ether were fractionated by repeated column chromatography of silica gel, use petroleum ether, the system of petroleum ether and EtOAc, EtOAc and MeOH system to make a gradient elution and collect them respectively and test the obtained samples by TLC method and then combine the same fraction. Eventually yielded compound **1** (22 mg), compound **2** (145 mg), compound **3** (220 mg), compound **4** (56 mg), compound **5** (238 mg) and compound **8** (160mg).

The fraction of EtOAc were fractionated by repeated column chromatography of Sephadex LH-20, RP 18 and silica gel, use the system of petroleum ether and EtOAc, EtOAc and MeOH, MeOH and H_2O to make a gradient elution and collect them respectively and test the obtained samples by TLC method and then combine the same fraction. Eventually yielded compound **6** (89 mg), compound **7** (270 mg).

Content determination of HPLC

Chromatographic conditions and the experiments of the adaptation of the system: Waters XBridge C_{18} column (4.6 mm × 250 mm, 5 μm), the mobile phase: CH_3CN-H_2O (29:71), the flow rate is 0.8 mL min^{-1}, its testing wavelength is 283 nm. the temperatures of the chromatographic column is 27 ºC. The sensitivity is 0.2 AUFS, sample-feeding mode is automatic. Under the above chromatographic condition, the separation degree between the chromatographic peak and the neighboring peaks of the reference samples from hesperidin, neohesperidin and naringin are all higher than 1.6 and the theoretical number of plate like tower is over 10000.

Preparation of reference sample and the sample solution: Weigh precisely 3.20 mg of hesperdin, 6.64 mg of neohesperidin and 2.78 mg of naringin and use chromatographic methanol to dissolve it by supersonic method and move it to a volumetric flask of 10 mL and use methanol again to dilute it and then make it a constant volume. The mixed contrast solutions with different concentration were obtained with the concentration: 6.672×10^{-3}, 3.328×10^{-3} and 61.088×10^{-3} mg mL^{-1}. After filtration through 0.45 μm microporous filtering membrane, the filtered reference sample was analyzed by HPLC.

Weigh precisely 1 g of each powdered samples and filter them through a 60-mesh sieve. And use 30 mL of methanol to extract by supersonic method for 0.5 h and then filter them. Move the extracted liquid to 50 mL volumetric flask and use MeOH to make it a constant volume, shake it evenly and filtered it through 0.45 μm filtration membrane and the obtained sample solution was used for the analysis of HPLC.

Methodology examination of the content determination

Examination of linear relation: Weigh precisely the mixed reference solution in turn 2, 5, 10, 15, 20, 25 μL and feed the samples respectively and then determine them according to the above chromatographic conditions. The regression equation is obtained, using mass of reference substance X (μg) as the abscissa axis and the peak area Y (μg) as the longitudinal coordinates for linear regression. The regression equation, correlation quotiety and linear range of the three reference substances can be seen in Table-2.

Precision experiment: Precisely imbibed the mixed solution of contrast sample, continually feed the samples for 5 times according to the above chromatographic condition, 10 μL each time and record respectively the peak area of the

TABLE-1
THE SAMPLE SOURCE OF C. *ougan* FRUIT

No.	Locality of collection	Collecting time	Altitude/m	Side condition
1	Tianqingshan	08.12.01	270	Mountain
2	Baikou	08.11.20	100	Warp Land
3	Biaoqiao	08.11.23	80	Warp Land
4	Taipingxiang	08.12.01	500	Mountain
5	Kaitan	08.11.30	90	Mountain
6	Jiukeng	08.11.30	250	Mountain
7	Sanyang	08.12.02	50	Field Land

TABLE-2
REGRESSION EQUATION AND THEIR CORRELATION COEFFICIENTS OF THREE COMPONENTS

Component	Regression equation	r	Linear range (μg)
Hesperidin	$Y = 2.15 \times 10^6X - 4.89 \times 10^3$	0.999 5	0.67×10^{-2}~8.32×10^{-2}
Neohesperidin	$Y = 2.16 \times 10^6X - 2.83 \times 10^4$	0.999 9	12.22×10^{-2}~152.72×10^{-2}
Naringin	$Y = 4.32 \times 10^6X + 2.04 \times 10^3$	0.999 9	1.33×10^{-2}~16.68×10^{-2}

three glycosylflavones. The calculation results show that the RSD of the peak area of hesperidin, neohesperidin and naringin are 1.40, 1.394 and 1.53 % respectively. which shows a high accuracy.

Experiment of reproducibility: Take 5 of the samples from the same batch of the samples in parallel, prepare the sample solution according to the sample-solution preparation method and determine them 3 times according to the above chromatographic condition, with 10 μL each time in sample-feeding and then get its average value. Calculate the content of each component by the determined peak value. The RSD of relative peak area of hesperidin, neohesperidin and naringin are 2.01, 1.42, 2.3 and 2.16 %, respectively. This shows that this experiment has a good reproducibility

Experiment of stability: Take the same sample solution and feeding with 10 μL at different time 0, 2, 4, 6, 8, 12 h respectively, determine them according the above chromatographic condition and record the peak areas. The RSD of the relative peak area of hesperidin, neohesperidin and naringin are 2.52, 1.26 and 2.25 %, respectively. which shows a good stability.

Experiment of sample-adding recovery rate: Weigh precisely six samples of the same group of the determined content and put in reference substances of hesperidin, neohesperidin and naringin into above sample in proper quantities respectively. Then prepare them according to the preparation method of the sample solutions and determine them with the feeding amount of 10 μL and calculate its recovery rate. As a result, the average sample-adding recovery rates are 99.29, 99.02 and 101.01 % and the RSD are 1.82, 1.98 and 1.24 %, respectively.

RESULTS AND DISCUSSION

Structural identification of monomer compounds: Compound **1** (122 mg), was obtained as a colourless liquid, which can be dissolved in $CHCl_3$, $FeCl_3$-$K_3[Fe(CN_6)]$ showed blue reaction, prove the presence of phenolic hydroxy group. ^{1}H NMR (400 MHz, in $CDCl_3$) δ: 6.83(1H, d, *J* = 8.5 Hz, H-6), 6.68 (1H, d, *J* = 2.1 Hz, H-2) and 6.67 (1H, d, *J* = 1.7 Hz, H-5) formed an ABX-type coupling system in the B-ring. 3.31 (2H, d, *J* = 6.7 Hz, H-7), δ: 5.95 (1H, m), 5.06 (2H, m), 3.88 (3H, s, 3-OMe). ^{13}C NMR (100 MHz, in $CDCl_3$) δ: 131.9 (C-1), 115.5 (C-2), 146.4 (C-3), 143.9 (C-4), 114.2 (C-5), 111.1 (C-6), 39.9 (C-7), 137.8 (C-8), 121.1 (C-9). In the TLC plate as contrast with reference substance, R_f values are the same, value of mixed melting point does not decrease of sample and reference substance, identified as eugenol.

Compound **2** (145 mg), was obtained as a white solid, which can be dissolved in MeOH, $FeCl_3$-$K_3[Fe(CN_6)]$ showed blue reaction, prove the presence of phenolic hydroxy group. Hydrochloric acid-magnesium powder showed positive reaction. ^{1}H NMR(400 MHz, $CDCl_3$) δ: 5.35 (1H, dd, *J* = 12.9, 3.0 Hz, H-2), 3.10 (1H, dd, *J* = 17.1, 13.1 Hz, H-3a), 2.82 (1H, dd, *J* = 17.1, 9.0 Hz, H-3b), 3.98 (3H, s, 4'-OMe), 6.01(1H, d, *J* =1.8 Hz, H-8), 5.86 (1H,d, *J* = 1.8 Hz, H-6), 7.06 (1H, d, *J* = 1.6 Hz, H-2'), 6.96 (1H, d, *J* = 8.0 Hz, H-5') and 6.91 (1H, d, *J* = 8.0 Hz, H-6') formed an ABX-type coupling system in the B-ring. ^{13}C NMR (100 MHz, in $CDCl^3$) δ: 78.9 (C-2), 43.1 (C-3), 195.9 (C-4), 164.5 (C-5), 96.6 (C-6), 164.3 (C-7), 95.4 (C-8), 163.1 (C-9), 103.2 (C-10), 118.1 (C-1'), 110.6 (C-2'), 145.9 (C-3'), 146.9 (C-4'), 112.6 (C-5'), 131.4 (C-6'), 56.0 (4'-OCH_3). The spectral data showed complete agreement with the literature[12], identified as hesperetin.

Compound **3** (220 mg), was obtained as a white crystalline solid, which can be dissolved in $CHCl_3$, 10 % H_2SO_4-EtOH showed red reaction. ^{13}C NMR(100 MHz, in $CDCl_3$) δ: 37.3 (C-1), 26.1 (C-2), 71.7 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 33.9 (C-7), 31.6 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.1 (C-17), 19.4 (C-18), 11.9 (C-19), 36.1 (C-20), 18.8 (C-21), 31.9 (C-22), 28.2 (C-23), 45.8 (C-24), 29.2 (C-25), 11.9 (C-26), 19.8 (C-27), 23.0 (C-28), 19.0 (C-29). The spectral data showed complete agreement with the literature[13], In the TLC plate as contrast with reference substance, R_f values are the same, value of mixed melting point does not decrease of sample and reference substance, identified as β-sitosterol.

Compound **4** (56 mg), was obtained as a white crystalline solid, which can be dissolved in MeOH and $CHCl_3$. ^{13}C NMR (100 MHz, DMSO) δ:175.4 (C-1), 29.4 (C-2). The spectral data showed complete agreement with the literature[14], In the TLC plate as contrast with reference substance, R_f values are the same, identified as succinic acid.

Compound **5** (238 mg), was obtained as a white crystalline solid, which can be dissolved in MeOH and EtOH. Hydrochloric acid-magnesium powder and Molish reagent showed positive reaction. ^{1}H NMR (400 MHz, DMSO): δ 6.95 (1H, d, *J* = 2.0 Hz, H-2'), 7.05 (1H, dd, *J* = 8.0, 2.0 Hz, H-6') and 6.56 (1H, d, *J* = 8.0 Hz, H-5') formed an ABX-type coupling system in the B-ring, 6.13 (2H, d, *J* = 2.0 Hz, H-6, 8), 5.51 (1H, dd, *J* = 12.0, 3.2 Hz, H-2), 3.16 (1H, m, H-3a), 2.78 (1H, m, H-3β), 5.10 (1H, d, *J* = 2.9 Hz, glu-1), 3.77 (3H, m, -OCH_3). ^{13}C NMR (100 MHz, DMSO) δ: 77.0 (C-2), 42.1 (C-3), 196.9 (C-4), 162.8 (C-5), 96.2 (C-6), 162.5 (C-7), 95.0 (C-8), 164.7 (C-9), 103.2 (C-10), 130.8 (C-1'), 114.0 (C-2'), 146.4 (C-3'), 147.9 (C-4'), 111.8 (C-5'), 117.7 (C-6'), 100.3 (C-1''), 75.9 (C-2''), 76.8 (C-3''), 71.7 (C-4''), 78.3 (C-5''), 60.3 (C-6''), 97.3 (C-1'''), 70.3 (C-2'''), 69.4 (C-3'''), 76.0 (C-4'''), 68.2 (C-5'''), 17.9 (C-6'''), 55.6 (-OCH_3). After acid hydrolysis, TLC method check out the glucose and rhamnose in the water. The spectral data showed complete agreement with the literature[15-17], identified as neohesperidin.

Compound **6** (89 mg), was obtained as a white amorphous powder, which can be dissolved in MeOH and EtOH. Hydro-

TABLE-3
CONTENT OF HESPERIDIN, NEHOESPERIDIN AND NARINGIN IN C. *ougan* FRUIT

Locality of collection	Pericarp of *C. Ougan* (%)			Pulp of *Citrus. Ougan* (%)		
	Hesperidin	Neohesperidin	Naringin	Hesperidin	Neohesperidin	Naringin
Tianqingshan	0.075	4.885	0.250	0.077	1.675	0.114
Baikou	0.074	3.155	0.277	0.059	1.224	0.094
Biaoqiao	0.058	3.702	0.205	0.071	1.995	0.096
Taipingxiang	0.045	3.344	0.215	0.064	1.420	0.126
Kaitan	0.062	4.784	0.224	0.085	1.965	0.112
Jiukeng	0.050	4.116	0.237	0.069	1.728	0.108
Sanyang	0.112	4.185	0.295	0.122	2.196	0.156

chloric acid-magnesium powder and Molish reagent showed positive reaction. ^{1}H NMR(400 MHz, DMSO): δ: 6.92 (1H, d, *J* = 2.0 Hz, H-2'), 6.94 (1H, dd, *J* = 8.4, 2.0 Hz, H-6') and 6.83 (1H, d, *J* = 8.4 Hz, H-5') formed an ABX-type coupling system in the B-ring, 6.14 (2H, d, *J* = 2.0 Hz, H-6), 6.11(2H, d, *J* = 2.0 Hz, H-8), 5.49 (1H, dd, *J* = 12.0, 3.0 Hz, H-2), 3.20 (1H, m, H-3a), 2.78 (1H, m, H-3β), 5.53 (1H, d, *J* = 2.9 Hz, glu-1), 3.70(3H, m, -OCH_3). ^{13}C NMR (100 MHz, DMSO): δ 78.6 (C-2), 42.3 (C-3), 197.3 (C-4), 165.3 (C-5), 96.6 (C-6), 162.7 (C-7), 95.7 (C-8), 165.3 (C-9), 103.5 (C-10), 131.1 (C-1'), 114.4 (C-2'), 146.6 (C-3'), 148.2 (C-4'), 112.2 (C-5'), 112.3 (C-6'), 103.5 (C-1''), 75.7 (C-2''), 76.5 (C-3''), 70.9 (C-4''), 78.6 (C-5''), 66.3 (C-6''), 96.6 (C-1'''), 70.5 (C-2'''), 69.8 (C-3'''), 73.2 (C-4'''), 68.5 (C-5'''), 18.1 (C-6'''), 55.9 (-OCH_3). After acid hydrolysis, TLC method check out the glucose and rhamnose in the water. The spectral data showed complete agreement with the literature[18], identified as hesperidin.

Compound **7** (270 mg), was obtained as a white amorphous powder, which can be dissolved in MeOH and EtOH. Hydrochloric acid-magnesium powder and Molish reagent showed positive reaction. ^{13}C NMR (100 MHz, DMSO): δ: 80.7 (C-2), 43.9 (C-3), 198.5 (C-4), 165.0 (C-5), 97.8 (C-6), 166.5 (C-7), 96.7 (C-8), 164.6 (C-9), 104.9 (C-10), 130.8 (C-1'), 129.2 (C-2'), 116.3 (C-3'), 159.1 (C-4'), 116.3 (C-5'), 129.1 (C-6'), 99.3 (C-1''), 79.2 (C-2''), 79.9 (C-3''), 71.2 (C-4''), 78.1 (C-5''), 62.2 (C-6''), 102.5 (C-1'''), 71.2 (C-2'''), 72.2 (C-3'''), 73.8 (C-4'''), 69.9 (C-5'''), 18.2 (C-6'''). After acid hydrolysis, TLC method check out the glucose and rhamnose in the water. The spectral data showed complete agreement with the literature[19], identified as naringin.

Compound **8** (160 mg), was obtained as a white amorphous powder, which can be dissolved in MeOH and EtOH, mp 242-243 ºC. Hydrochloric acid-magnesium powder and Molish reagent showed positive reaction. ^{1}H NMR (400 MHz, DMSO): δ: 6.94 (2H, d, *J* = 8.4 Hz, H-2',6'), 6.68 (2H, d, *J* = 8.0 Hz, H-3',5'), 5.92 (1H, d, *J* = 2.4 Hz, H-8), 5.84 (1H, d, *J* = 2.4 Hz, H-6), 5.23 (1H, dd, *J* = 12.8, 2.2Hz, H-2), 3.22 (1H, m, H-3a), 2.79 (1H, m, H-3β). ^{13}C NMR (100 MHz, in DMSO) δ: 79.9 (C-2), 43.6 (C-3), 196.8 (C-4), 165.2 (C-5), 96.8 (C-6), 166.7 (C-7), 95.8 (C-8), 163.7 (C-9), 103.6 (C-10), 130.2 (C-1'), 126.6 (C-2',6'), 117.4 (C-3', 5'), 157.9 (C-4'). The spectral data showed complete agreement with the literature[20], identified as naringenin.

Results and analysis of the content determination of the samples: The results incorporated into the standard curves and calculate the average content of hesperidin, neohesperidin and naringin (Table-3). The chromatographic figure of the reference substance and sample can be seen in Fig. 1.

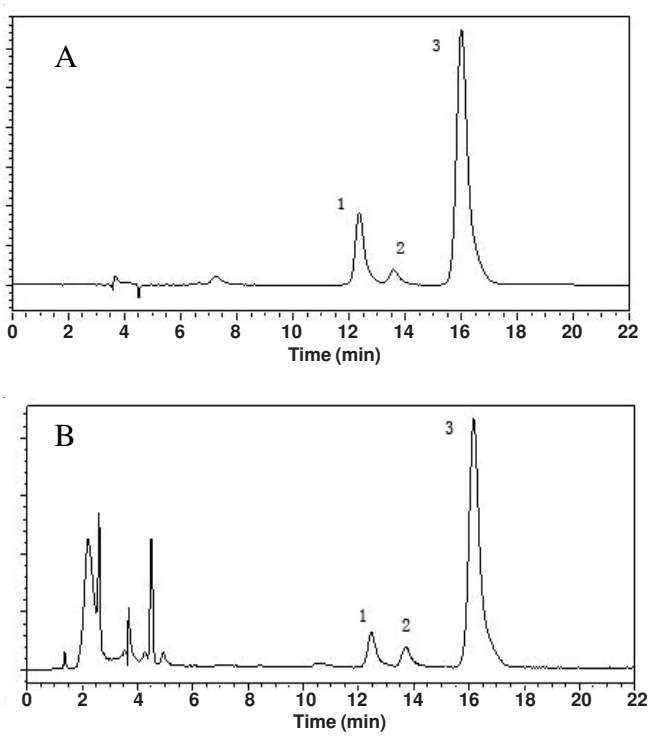

Fig. 1. HPLC chromatogram of reference substances (A) and sample (B) 1. Naringin 2. Hesperidin 3. Neohesperidin

This experiment got the best extracting method by comparing the extracting rate of total flavonoids in the obtained extracts between Soxhlet extraction, reflux extraction and ultrasonic extraction. The result shows that the extracting rate of total flavones by the supersonic extracting method is the highest, suggesting that heat-extracting may harm the structures of the effective components. During the supersonic extracting, we also compared the influence of extracting time, dosage of solution and extracting frequency on extracting effect. The result shows that the extracting rate of total flavones is the highest when extracted one time by supersonic method for 0.5 h with MeOH as solvent.

This experiment chooses the mobile phase system of MeOH-H_2O and MeOH-H_3PO_4. Under this mobile phase, the chromatographic peak shape of the three components were not good and it is difficult to reach the point of the baseline separating. So, we change it to CH_3CN-H_2O (29:71) as the mobile phase, the three components can have a good separation and the separation degree between the chromatographic peak and the neighboring peaks of the three compounds from hesperidin, neohesperidin and naringin are all greater than 1.6 and the theoretical number of plate like tower is over 10000.

The determination of the wavelength Use UV-visible spectrophotometer to scan hesperidin, neohesperidin, naringin within the scope 250-300 nm, we know that hesperidin, neohesperidin have the greatest absorption value at 282.8 nm; while naringin has the greatest absorption value at 284.0 nm. Considering the absorption wavelength of the three compounds, we choose 283.0 nm as the determination wavelength to determine the content of the three compounds and it has a accurate sensitivity.

The results shows that the content difference of glycosylflavones in pericarp and pulp of three different producing areas of *C. ougan* fruit is quite different. In the *C. ougan* pulp of seven different regions, the content of neohesperidin is the highest, next is naringin and the lowest is hesperidin. Among these, the content of the three glycosylflavones in the pulp of *C. ougan* fruit grown in Sanyang region is relatively highest. The content in neohesperidin is the highest, reaching 2.196 %, next is naringin, reaching 0.1566 %. The content of hesperidin is the lowerest, only reaching 0.122 %. The content of the three glycosylflavones in *C. ougan* pericarp are all higher than those in the pulp. For example, the content of the three glycosylflavones in the pericarp of *C. ougan* fruit grown in Tianqingshan region is relatively the highest. The content in neohesperidin is the highest, reaching 4.024 %. The content in naringin is 0.243 %, the content in hesperidin is the lowerest, only 0.068 %.

From this research, it is suggested that the percarp and pulp of the *C. ougan* fruit from China contains rich flavonoid components with strong antioxidation activity, the fruit is a excellent health food with nutritional value. The results of this research provides the reference data for the further development, popularization of growing and its efficient utilization.

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