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Identification of Anthocyanins and Rutin in Black Goji, Blueberry, and Goji

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Abstract

Objective: The current market supply of black goji berries is limited, leading to high prices and a shortage in availability. This situation has resulted in an increase in counterfeit products, primarily using goji berries, blueberries, and other similar fruits to impersonate black goji berries. Most people cannot distinguish with the naked eye, this method utilizes two distinct standard products to identify black goji berry products and blueberries. It observes the patterns of polyphenols and flavonoids at various wavelengths. **Methods:** HPLC was used to establish the fingerprint of 6 flavonoids and 9 Fingerprints of polyphenols. **Results:** The fingerprints of black goji berries and blueberries were analyzed by high-performance liquid chromatography, and the fingerprints were obtained and analyzed. **Conclusion:** By analyzing the fingerprints of polyphenols and flavonoids in black goji berries and blueberries, we can establish a liquid-phase identification method to differentiate between black goji berry products and the anthocyanins and rutin found in blueberries.

Keywords

Black goji; adulteration; anthocyanins; flavonoids; HPLC identification

Black goji berries belonging to the genus Lycium in the Solanaceae family. Black fruit goji berry is a sweet and flat food, rich in protein, lycium barbarum polysaccharide, amino acids, vitamins, minerals, trace elements, and other nutrients. It is mainly distributed in China's Xinjiang, Mongolia, Qinghai, Ningxia, Eastern Shanxi, and other places. Its main components are anthocyanin substances and flavonoids. Anthocyanin is a heat-sensitive active substance, afraid of high temperatures, too high temperature will destroy anthocyanin, its stability is greatly affected by PH value, and the separation effect is best under strong acid conditions [1]. Plenty of Experimental studies have shown that anthocyanins in black goji have the health care effect of antioxidant, anti-aging, cardiovascular protection and regulation of blood lipids, anti-atherosclerosis, anti-inflammation, anti-Alzheimer's, anti-fatigue, liver protection, and anti-cancer effects [2-4], and are effective in the treatment of diabetes mellitus and hypertensive retinopathy [5]. Recent studies have shown that the nutritional value of black goji is much higher than that of goji and blueberry. Black goji contains plenty of anthocyanin ingredients [6-9], which can be used in food, health food, cosmetics, and drug products. However, there are many fake black goji berries in the market such as goji berries and blueberries. Therefore, it is very meaningful to use suitable methods and techniques to study and identify whether the black goji is adulterated. At present, the main detection ways of anthocyanins include UV-VIS spectroscopy, HPLC, UPLC, NIR, NMRS, infrared absorption spectrometry, near-infrared absorption spectrometry, paper chromatography, and thin layer chromatography, etc. [10-19]. Each of these methods has its own advantages and limitations. Based on the short analysis time and high resolution, HPLC has become the most popular analytical method for the detection of anthocyanins.

In this paper, the multi-wavelength channel of the HPLC-PDA detector was used to identify anthocyanins and flavonoids with Cyanidin-3-O-Glucoside Chloride and rutin. The chromatographic characteristic peak of anthocyanins and

flavonoids in black goji was observed, which made the identification of this method more specific and more applicable [20, 21].

1. Materials and Method

1.1 Standards and reagents

Cyanidin-3-O-Glucoside Chloride, American Chinese Herbal Medicine Inc; Rutin, Anpel; Methanol and acetonitrile are HPLC grades, and phosphoric acid and hydrochloric acid are AR grades.

1.2 Plant material

Black fruit goji berries and black fruit goji berry powder are from Ningxia Haohan Biological Co., Ltd., concentrated black fruit goji berry juice is from Qinghai CommScope Biotechnology Co., Ltd., and blueberry dried fruit and goji berries are from NIFDC.

1.3 Instrument

Waters2695 HPLC with PDA 2998; Snoicator, Shanghai Kedao; oscillator, Burrell Scientific, Inc. Balance (0.01 mg), Mettler Toledo.

1.4 HPLC condition

Column: Phenomenex C-18, 4 x 3.0 mm; Wavelength scan: 200 nm to 600 nm; Mobile phase A: Combination 2 ml of phosphoric acid to 1000 ml of water and mix well; Mobile phase B: 100% Aacetonitrile; Column Temperature: 35 ℃; Flow: 1 mL/min; Inject: 20 μL.

Table 1. Gradient elution procedure

Time	Flow (ml/min)	A%	B%
0	1.00	95	5
30	1.00	75	25
45	1.00	10	90
55	1.00	10	90
56	1.00	95	5
60	1.00	95	5

1.5 Procedure

(1) Solvent preparation: Mixed 600 ml of methanol and 400 ml of water, adjust PH = 1 with hydrochloric acid.
(2) Standard solution preparation: Accurately weigh approximately 1.0 mg of cyanidin 3-O-glucoside and approximately 1.0 mg of rutin into a 25 mL volumetric flask, add 15 mL of solvent, and sonicate for about 5 minutes. After cooling, add solvent and other volumetric flasks and mix well. (Store in 2-8 °C refrigerator for 3 months).
(3) Sample preparation: Weigh 2 g of black fruit goji berry, 7 g of concentrated black goji berry juice, weigh 10 g of black goji powder, and put it into a 50 mL screw cap test tube (the sample can be weighed appropriately according to the sample content); Add 20 mL of solvent, place the tube on a shaker, and set the speed to 150 rpm for 10 min. Then sonicate for about 30 minutes to cool and filter through 0.45 μ PVDF filter.

2. Results

2.1 254 nm (Characteristic peaks of Flavonoids)

The chromatogram of black goji and its products showed that there were 6 characteristic peaks in 19.0 minutes, 19.2 minutes, 19.4 minutes, 24.3 minutes, 25.3 minutes, 25.8 minutes (rutin); There are 6 characteristic peaks in black goji; 1 characteristic peak for blueberry and 1 characteristic peak for goji.

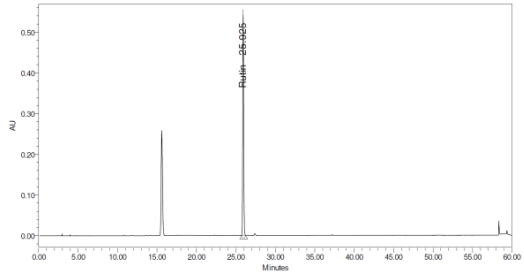

Figure 1a. Rutin in standard solution.

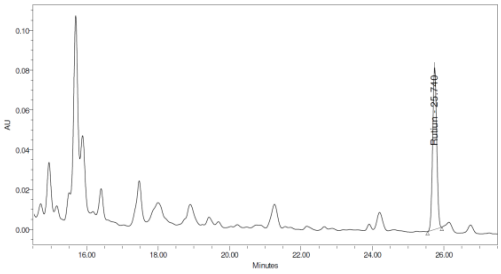

Figure 1b. Chromatogram of goji.

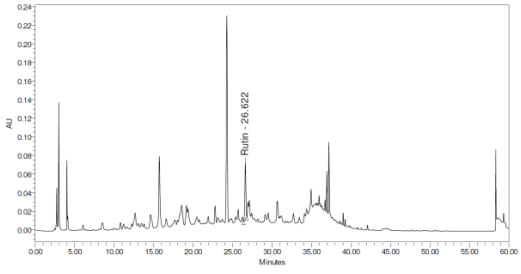

Figure 1c. Chromatogram of blueberry.

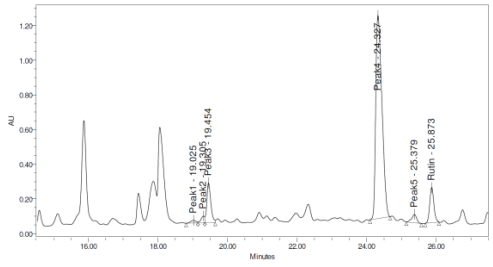

Figure 1d. Chromatogram of black goji.

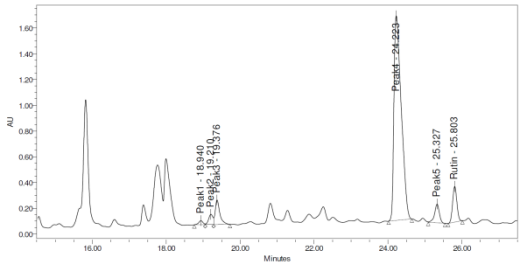

Figure 1e. Chromatogram of black goji juice.

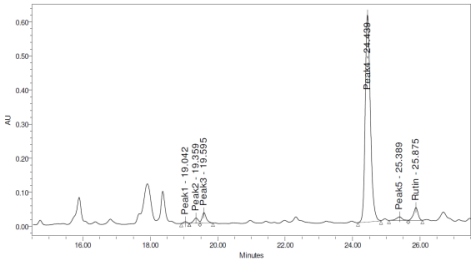

Figure 1f. Chromatogram of black goji powder.

2.2 520 nm (Characteristic peaks of anthocyanins)

The chromatogram of black goji and its products showed that there were 9 characteristic peaks in 16.0 minutes (Cyanidin-3-O-Glucoside Chloride), 17.7 minutes, 19.2 minutes, 21.8 minutes, 22.2 minutes, 24.2 minutes, 25.2 minutes, 26.5 minutes, 27.3 minutes. There are 9 characteristic peaks in black goji; 1 characteristic peak for blueberry and 0 characteristic peak for goji.

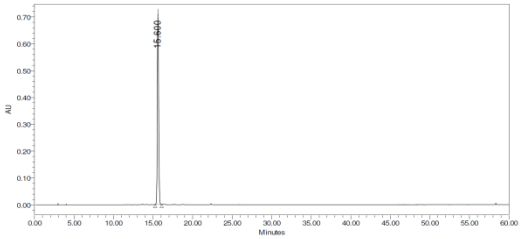

Figure 2a. Cyanidin-3-O-Glucoside Chloride in standard solution.

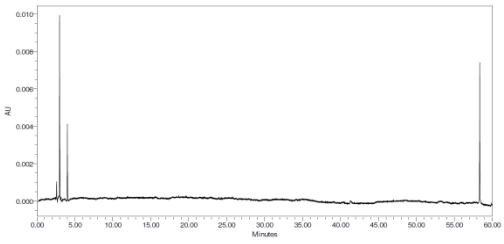

Figure 2b. Chromatogram of goji.

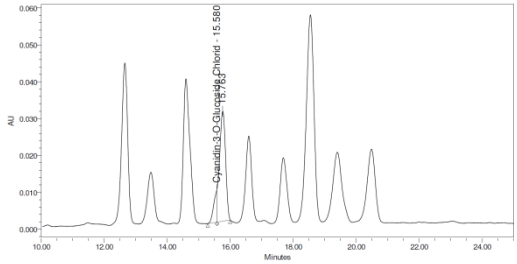

Figure 2c. Chromatogram of blueberry.

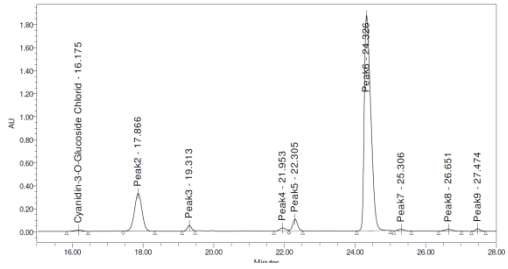

Figure 2d. Chromatogram of black goji.

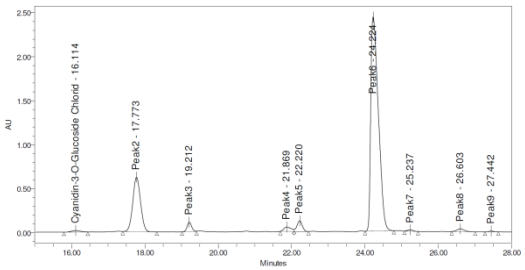

Figure 2e. Chromatogram of black goji juice.

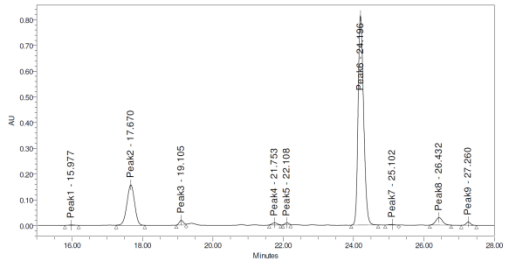

Figure 2f. Chromatogram of goji.

3. Discussion

In this method, the chromatographic characteristic peak with the best separation effect of anthocyanins was obtained under the extraction of a strong acid solution. There was no effect on the chromatogram of flavonoids, and the methanol reagent was used to the maximum. The anthocyanin components in the sample were extracted, and the solvent mixed with methanol and water was used to make the instrument baseline not drift, reduce the heterogeneous peak, and obtain the most intuitive results. The chromatogram meets the requirements of sample identification, and the separation effect of liquid phase conditions is good. The method did not detect anthocyanin content in goji berries, blueberries and black goji berries had different anthocyanin chromatographic characteristic peaks. Black goji berries had the same products' chromatographic characteristic peaks, this liquid phase method has a good effect on the identification of goji berries, blueberries, and black goji berries. Black goji berries contain a large number of anthocyanins, which have high pharmacological research value and wide effects. The research on anthocyanins can be extended to others. The study of various foods provides a route for the subsequent research of an identification method for detecting anthocyanins in all foods and supports the quantification of anthocyanins later.

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