Chromogenic Reactions of Starch and Dextrin and Comparative Study of Thin-Layer Chromatography of Oligosaccharides in 35 Batches of Jiulongteng Honey

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Abstract [**Objectives**] To explore the methods for identifying pure honey. [**Methods**] Using 35 batches of Jiulongteng honey sourced from various production areas in Guangxi as the research subjects, this study investigated the chromogenic reactions of starch and dextrin, as well as the comparative study of thin-layer chromatography of oligosaccharides present in Jiulongteng honey. [**Results**] None of the 35 batches of Jiulongteng honey samples exhibited blue (indicating starch), green, or reddish-brown (indicating dextrin) coloration, suggesting that no adulterants such as artificially added starch, dextrin, or sugar were present in these samples. Furthermore, none of the 35 batches displayed additional spots below the corresponding positions of the control, indicating that the sugar composition was consistent with the oligosaccharide profile of natural honey. No components inconsistent with the oligosaccharide profile of natural honey were detected. Therefore, it can be concluded that the Jiulongteng honey samples in this experiment were pure and free from adulteration with starch, dextrin, or other sugar substances. [**Conclusions**] The method employed in this experiment is straightforward and quick to implement, effectively preventing adulterated honey from entering the market. It enhances the efficiency of quality control for Jiulongteng honey and promotes the healthy development of the Jiulongteng honey industry.

Key words Jiulongteng honey, Chromogenic reaction, Thin-layer chromatography, Starch, Dextrin

1 Introduction

Bauhinia championii (Benth.) is a wild plant endemic to karst landscapes, exhibiting a broad distribution in the northern region of Guangxi[1]. Notably, it is most prevalent in areas such as Yangshuo, Pingle, and Gongcheng in Guilin. This species is recognized as a high-quality wild nectar plant in Guangxi in autumn[2-4]. Yangshuo Jiulongteng honey source base encompasses an area of 100 000 ha and produces an annual honey output exceeding 5 000 t[5-6]. Jiulongteng honey is known for its properties in clearing heat and detoxifying, demonstrating beneficial therapeutic effects for conditions such as pharyngitis, cough associated with lung heat, and constipation. In January 2017, Jiulongteng honey from Yangshuo was recognized as a national geographical indication agricultural product[7].

Jiulongteng honey is produced by bees that gather nectar from the medicinal plant *B. championii*. This honey exhibits a deep amber hue, is crystal clear, and possesses a distinctive fresh fragrance. Initially, it has a bitter taste that subsequently transitions to a mellow sweetness, which is why it is also referred to as bitter honey. The color of Jiulongteng honey transitions from light amber to dark amber over time during storage. At room temperature, it exhibits a viscous consistency and is susceptible to crystallization when stored at lower temperatures. Following crystallization, the resulting particles are fine and soft, allowing for easy dissolution when manipulated by hand. To ensure the quality of Jiulongteng honey, this experiment utilized the initial honey produced by the bees after their introduction to the environment, rather than using it directly as a honey product. This approach was implemented to clearly identify the source of the honey powder and prevent contamination of the honey products with nectar from other sources. The current market for Jiulongteng honey is characterized by significant variability and disorder, resulting in a substantial presence of adulterated honey. Certain merchants, seeking to reduce costs, have been known to incorporate additives such as white sugar, high fructose corn syrup, and dextrin into their honey products. This practice complicates the ability of consumers to differentiate between authentic and counterfeit honey, consequently diminishing their trust in domestic honey offerings. As a result, a portion of consumers prefers to procure honey from international suppliers rather than purchasing domestic honey products. This article presents a series of studies aimed at assisting consumers in accurately assessing the quality of Jiulongteng honey.

2 Materials and methods

2.1 Materials and reagents The Jiulongteng honey samples utilized in this experiment were sourced from various locations in Guangxi, including Guilin, Hezhou, Chongzuo, Liuzhou, and

Received: February 25, 2025 Accepted: July 29, 2025

Supported by Enhancement Project of Basic Scientific Research Ability of Young and Middle-aged Teachers in Guangxi Universities (2020KY07040); School-level Scientific Research Project of Guangxi University of Chinese Medicine (2024QN022); Self-financed Scientific Research Project of Guangxi Zhuang Autonomous Region Administration of Traditional Chinese Medicine (GXZYL20240818).

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Laibin, amounting to a total of 35 batches. All samples were unprocessed raw honey supplied by Yangshuo Yichun Bee Industry Co., Ltd. The collection of these samples occurred between October and December 2021 (Table 1).

Following the collection of samples, they were stored in a refrigerator at a temperature range of 5 – 7 ℃ for subsequent use. The 35 batches of samples were categorized based on their storage duration: those stored for one year were classified as the fresh honey stage, those stored for more than one year but less than two years were designated as the aged honey stage I, and those stored for more than two years were identified as the aged honey stage II.

Table 1 Information on 35 batches of Jiulongteng honey samples

Sample No.	Collection location	Origin of bee species	Batch	Collection time
FM-001	Yangdi Township, Yangshuo County, Guilin City	*Apis mellifera ligustica*	1	December 2021
FM-002	Puyi Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	1	December 2021
FM-003	Putao Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	1	December 2021
FM-004	Baisha Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	1	December 2021
FM-005	Puyi Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-006	Jinbao Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-007	Baisha Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-008	Putao Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-009	Yangdi Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-010	Xingping Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-011	Fuli Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-012	Pingle County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-013	Gongcheng County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-014	Quanzhou County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-015	Lingchuan County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-016	Xing'an County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-017	Guanyang County, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-018	Lingui District, Guilin City	*A. mellifera ligustica*	2	November 2021
FM-019	Fuchuan Yao Autonomous County, Hezhou City	*A. mellifera ligustica*	2	November 2021
FM-020	Fusui County, Chongzuo City	*A. mellifera ligustica*	2	November 2021
FM-021	Liuzhou City, Liujiang County	*A. mellifera ligustica*	2	November 2021
FM-022	Xingbin District, Laibin City	*A. mellifera ligustica*	2	November 2021
FM-023	Puyi Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-024	Yangshuo Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-025	Jinbao Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-026	Baisha Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-027	Putao Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-028	Yangdi Township, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-029	Xingping Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-030	Fuli Town, Yangshuo County, Guilin City	*A. mellifera ligustica*	3	December 2021
FM-031	Yangdi Township, Yangshuo County, Guilin City	*A. cerana cerana*	2	November 2021
FM-032	Xingping Town, Yangshuo County, Guilin City	*A. cerana cerana*	2	November 2021
FM-033	Xingping Town, Yangshuo County, Guilin City	*A. cerana cerana*	3	December 2021
FM-034	Xing'an County, Guilin City	*A. cerana cerana*	2	November 2021
FM-035	Lingui District, Guilin City	*A. cerana cerana*	2	November 2021

Starch and dextrin utilized in this study were of food grade and commercially available. The iodine test solution, diatomaceous earth, ethanol, n-propanol, triethylamine, diphenylamine, aniline, phosphoric acid, and acetone were obtained as analytically pure reagents from Sinopharm Chemical Reagent Co., Ltd. The maltopentaose control, with lot No.: 112017-201602 and a purity of 99.5%, was sourced from the China National Institutes for Food and Drug Control. A high-efficiency silica gel G thin layer plate, lot No.: HC41642055, with dimensions of 20 cm × 20 cm, was procured from Merck Chemicals (Shanghai) Co., Ltd. Additionally, the water used in the experiments was ultrapure water prepared in the laboratory.

2.2 Instruments and equipment The instruments and equipment employed in this study comprised the EX225DZH Electronic Analytical Balance from Ohaus Instrument (Changzhou) Co., Ltd.; the UPC-II-10T Laboratory Ultrapure Water Purifier from Sichuan ULUPURE Ultrapure Technology Co., Ltd.; the SZB Vacuum Pump from Zhejiang Yangzijiang Pump Co., Ltd.; and the RE-52A Rotary Evaporator from Shanghai Yarong Biochemical Instrument Factory.

2.3 Chromogenic reaction of starch and dextrin Starch and dextrin were quantified in accordance with the method in the *Chinese Pharmacopoeia* (Volume I, 2020 edition)[8]. Approximately 2 g of the sample were weighed and dissolved in 10 mL of water. The mixture was then heated to boiling, allowed to cool, and subsequently treated with one drop of iodine test solution to observe the resulting color of the sample solution.

2.4 Comparative study of thin-layer chromatography of oligosaccharide[8]

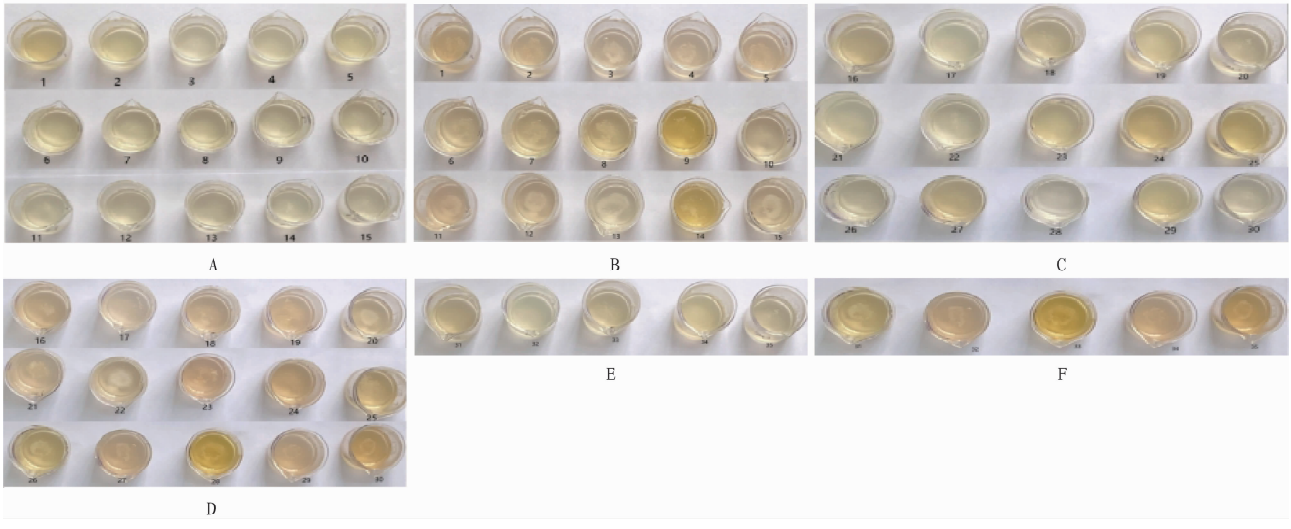
2.4.1 Preparation of test solutions. A total of 2 g of Jiulongteng honey samples were individually measured and placed into separate beakers. Subsequently, 10 mL of water was added to each beaker to facilitate dissolution. The resulting solution was then gradually introduced into an activated charcoal solid-phase extraction column, which had a sieve plate securely positioned at the bottom of the empty solid-phase extraction tube. The assembly was then placed onto the solid-phase extraction device. A total of 0.2 g of diatomaceous earth was accurately weighed and dissolved in an appropriate volume of water to ensure thorough mixing. This mixture was subsequently transferred to a solid phase extraction column tube using a pipette, allowing it to settle naturally and form a 3 mm thick layer of diatomaceous earth. The vacuum pump was then activated to create suction. Following this, 0.5 g of activated carbon was added to 10 mL of water and stirred to achieve a homogeneous mixture. This mixture was also introduced into the vacuum pump via a pipette to facilitate the precipitation of activated carbon through the suction generated by the vacuum pump. When the water level approached the activated carbon layer, an additional 0.2 g of diatomaceous earth, previously mixed with water, was injected. Utilizing the suction generated by the vacuum pump, the solution underwent a pre-washing process with 25 mL of water at a rate of 1 drop/sec. The piston was deactivated when the liquid level rose to 2 mm above the surface of the column, at which point the sieve plate was pressed and maintained in a standby position. The piston was then opened, allowing the solution to flow through the column under the suction of the vacuum pump. When the liquid level decreased to 2 mm above the column surface, the solution was eluted with 25 mL of 7% ethanol, and the eluent was discarded. The solution was subsequently eluted with 10 mL of 50% ethanol. The eluent was collected and placed in a water bath at 65 ℃, where it was concentrated to dryness under reduced pressure. The resulting residue was then dissolved in 1 mL of 30% ethanol to prepare the solution.

2.4.2 Preparation of control solutions. The maltopentaose control was dissolved in 30% ethanol to prepare a solution with a concentration of 1 mg/mL.

2.4.3 Test methods. A total of 3 μL of each test solution, along with the control solution, was pipetted and subsequently applied to the same high-performance silica gel G thin-layer chromatography plate. The plate was developed using a solvent mixture of n-propanol, water, and triethylamine in a ratio of 60∶30∶0.7. Following development, the plate was removed, dried, and then sprayed with a solution composed of aniline, diphenylamine, and phosphoric acid (specifically, 1 g of diphenylamine, 1 mL of aniline, and 5 mL of phosphoric acid, with acetone added to a final volume of 50 mL). The plate was then heated until the color of the spots became distinct and was subsequently placed under sunlight for examination.

3 Results and analysis

3.1 Chromogenic reaction of starch and dextrin The test results of starch and dextrin conducted on 35 batches of Jiulongteng honey samples are illustrated in Fig. 1. Due to the absence of significant differences in color change among the samples from the fresh honey stage, aged honey stage I, and aged honey stage II, only the experimental results from the fresh honey stage are presented herein.

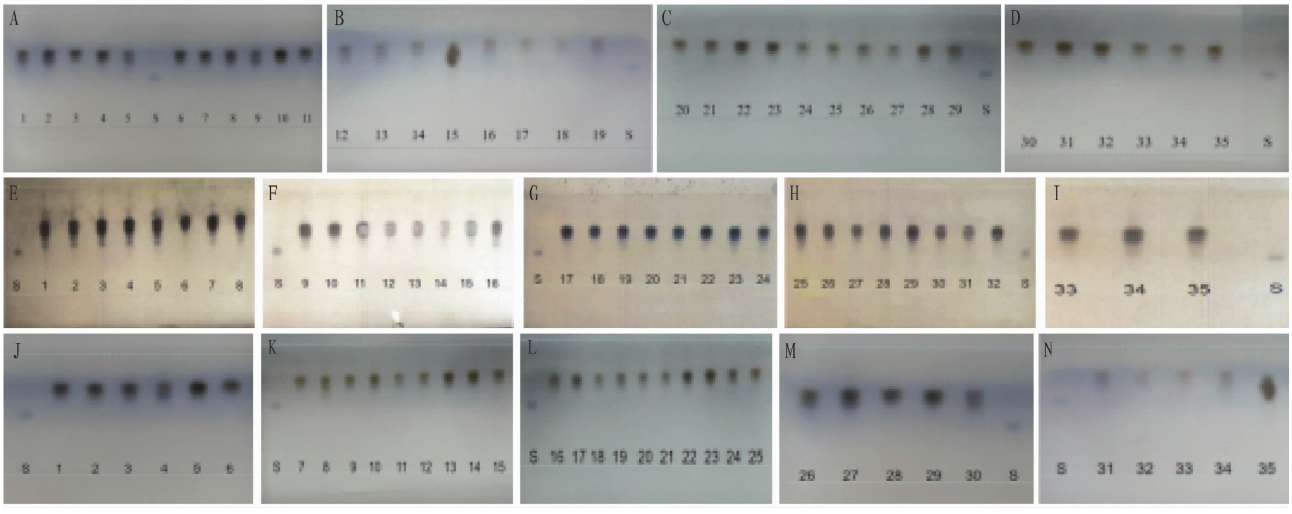


NOTE A and B correspond to the color development before and after the starch and dextrin tests for samples FM-001 to FM-015; C and D represent the color development before and after the starch and dextrin tests for samples FM-016 to FM-030; E and F illustrate the color development before and after the starch and dextrin tests for samples FM-031 to FM-035.

Fig. 1 Starch and dextrin test results of Jiulongteng honey samples

The addition of starch, dextrin, sugar, and other substances to honey for the purpose of modulation is a prevalent method of honey adulteration. In contrast, natural honey contains only a minimal quantity of dextrin, which can not be detected using the experimental method in Section **2.3**. In instances of adulterated honey, the presence of a greater quantity of added starch or dextrin results in more pronounced experimental outcomes[9]. Among the 35 batches of Jiulongteng honey samples, which included those at the fresh honey stage, aged honey stage I, and aged honey stage II, testing was performed using the method in Section **2.3**. The results, illustrated in Fig. 1, did not exhibit blue, green, or reddish-brown coloration, indicating that all 35 batches of Jiulongteng honey samples are classified as natural honey.

3.2 Comparative study of thin-layer chromatography of oligosaccharide The results of the oligosaccharide tests conducted on 35 batches of Jiulongteng honey, each with varying storage durations, are presented in Fig. 2 where S represents the maltopentaose control. The fresh honey stage is illustrated in Fig. 2A-D, the aged honey stage I is depicted in Fig. 2E-I, and the aged honey stage II is shown in Fig. 2J-N.



NOTE S. Maltopentaose control; A-D. Samples at fresh honey stage; E-I. Samples at aged honey stage I; J-N. Samples at aged honey stage II.

Fig. 2 TLC plots of oligosaccharides tests on honey samples

Oligosaccharides are carbohydrates composed of 2 to 10 monosaccharide units. Honey is particularly rich in oligosaccharides, and the identification of these compounds can serve as an indicator of honey purity. This method is one of several approaches used to assess the authenticity of honey[10]. Oligosaccharide identification was conducted on 35 batches of Jiulongteng honey samples across various storage periods. The results, illustrated in Fig. 2, indicated that no spots appeared in the chromatograms of the samples at positions corresponding to the control. Consequently, the samples met the criteria for oligosaccharide verification. Therefore, the experimental samples were determined to be pure Jiulongteng honey.

4 Conclusions

In adulterated honey, substances such as starch, dextrin, and sugar are often added to honey for the purpose of financial profit. Natural honey typically contains minimal amounts of dextrin, which poses challenges for detection through conventional testing methods. When a significant quantity of dextrin or starch is introduced into adulterated honey, a reaction occurs. If the solution in the test tube exhibits a brown, purple, or brownish-purple coloration, it indicates the presence of dextrin. Conversely, if the solution turns gray or gray-blue, or if a gray or gray-blue precipitate forms, this suggests the presence of starch. Thus, these observations can be instrumental in identifying adulterated honey. Honey is abundant in oligosaccharides, the type and concentration of which serve as significant indicators of honey quality. By employing thin layer chromatography to isolate and analyze the oligosaccharide components present in honey, and subsequently comparing these with standard oligosaccharide controls, it is possible to accurately determine the purity of the honey and ascertain whether it has been adulterated with other sugar substances. The method employed in this experiment is characterized by its simplicity and rapid execution, requiring neither complex instrumentation nor cumbersome pre-treatment processes. This approach facilitates preliminary on-site rapid testing and screening of a substantial number of samples pertaining to various stages of honey production, processing, and sales within a condensed timeframe. Consequently, it serves as an effective measure to prevent adulterated honey from entering the market, enhances the efficiency of quality control for Jiulongteng honey, and reduces associated costs. Furthermore, when integrated with additional testing parameters, this method can yield a more comprehensive and accurate assessment of honey quality, thereby providing robust technical support for quality supervision and the standardization of honey in the marketplace.

In conclusion, the chromogenic reaction of starch and dextrin, along with the identification of oligosaccharides through thin-layer chromatography, are two widely utilized testing methods in the quality control of honey. These methods are instrumental in detecting adulteration and ensuring quality control, thereby safe-

guarding the quality and safety of Jiulongteng honey. Furthermore, they contribute to the enhancement of regulatory measures within the honey market, protect the legitimate rights and interests of consumers, and promote the sustainable development of the Jiulongteng honey industry.

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(From page 23)

plaining traditional "Heat-Clearing" efficacy of *S. baicalensi*[1,11].

4.3 Flavonoid ensemble advantage Despite structural similarities, baicalin (lower predicted targets) showed strongest $PTGS_2$ binding, while baicalein/wogonin modulate broader targets (such as TNF, $ABCB_1$). This suggests functional complementarity-where baicalin potently inhibits prostaglandin synthesis, other flavonoids concurrently regulate inflammation and calcium signaling[7,12].

5 Conclusions

S. baicalensis alleviates PD through a flavonoid ensemble (baicalein, baicalin, wogonin, *etc.*) that synergistically targets $PTGS_2$-mediated prostaglandin synthesis, ESR_1-linked hormonal regulation, and calcium signaling pathways. Network pharmacology and molecular docking validate: $PTGS_2$ inhibition as the central mechanism, with baicalin exhibiting optimal binding affinity. Co-regulation of ovarian steroidogenesis and ABC transporters plays as key therapeutic pathways. These findings will provide a mechanistic foundation for developing *S. baicalensis* based multi-target therapeutics against PD.

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