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Limit Test and pH Determination of 5-Hydroxymethylfurfural in Jiulongteng Honey

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Abstract [**Objectives**] To explore the effect of storage time on pH and 5-hydroxymethylfurfural content in Jiulongteng honey. [**Methods**] The pH of Jiulongteng honey was determined by neutralization titration with sodium hydroxide standard solution. The content of 5-hydroxymethylfurfural in Jiulongteng honey was determined by HPLC. Chromatographic conditions: ZORBAX SB-C_{18} column (250 mm × 4.6 mm, 5 μm) from Agilent Co., Ltd., acetonitrile-0.1% formic acid solution (5 : 95) as mobile phase, flow rate of 0.8 mL/min, 5-hydroxymethylfurfural detection wavelength of 284 nm, guanosine detection wavelength of 254 nm. [**Results**] The pH of 12 batches of Jiulongteng honey was 3.70 - 3.84 in the new honey stage, 3.92 - 4.05 in the old honey stage 1, and 4.25 - 4.53 in the old honey stage 2; 5-hydroxymethylfurfural was not detected in the new honey stage, 5-hydroxymethylfurfural was detected in FM-001 in the old honey stage 1, and 5-hydroxymethylfurfural was detected in most samples in the old honey stage 2. [**Conclusions**] The pH and 5-hydroxymethylfurfural content of 12 batches of Jiulongteng honey met the requirements within 3 years of storage. There was no 5-hydroxymethylfurfural in Jiulongteng honey, but with the extension of storage time, the detection amount of 5-hydroxymethylfurfural increased significantly even if Jiulongteng honey was stored at low temperature. Therefore, 5-hydroxymethylfurfural can be used as an important indicator of honey freshness.

Key words Jiulongteng honey, 5-hydroxymethylfurfural, pH, HPLC

1 Introduction

Honey is a mature natural sweet substance[1], brewed by honeybees after collecting nectar, honeydew secreted by insects from bee-derived plants[2], and then fully mixing with their own salivary gland secretions in the hive through a variety of transformations and interactions. Honey is rich in essential nutrients for human body, and has great therapeutic value and medicinal function. According to records of *Herbal Classic of Shennong*, honey can calm the five internal organs, eliminate all diseases, harmonize all medicines, benefit qi and tonify the middle, relieve pain and detoxify, and it can strengthen the mind and lighten the body without hunger and aging[3-4]. *Bauhinia championii* (Benth.) Benth. belongs to the family Legumes. The roots (Jiulonggen), leaves (Jiulongteng), and seeds (Guojianglongzi) of this plant are also used for medicinal purposes. It mainly grows on the edge of ditches, valleys, riversides, under sparse forests or in shrubs, and distributed in Guangdong, Guangxi, Fujian, and other places in China. Jiulongteng honey has the effect of clearing away heat and toxic materials, and has good curative effect on pharyngolaryngitis, cough due to lung heat and constipation, and the excellent quality of Jiulongteng honey in Yangshuo was approved as a national geographic indication (GI) agricultural product in January 2017[5-6].

Studies have shown that 5-hydroxymethylfurfural has antioxidant, anti-tumor, anti-tissue ischemia and other pharmacological effects, as well as nephrotoxicity and potential genotoxicity[7], and sensitization[8]. For the purpose of medication safety, the *Chinese Pharmacopoeia* stipulates that the limit of 5-hydroxymethylfurfural in honey should not exceed 0.004%[9]. 5-Hydroxymethylfurfural is an aldehyde compound with furan ring structure produced by dehydration of monosaccharide compounds such as glucose and fructose under high temperature or weak acid conditions, and is a classical product of Maillard reaction[10]. Therefore, we studied the limit test and pH determination of 5-hydroxymethylfurfural in Jiulongteng honey.

2 Materials and methods

2.1 Materials

2.1.1 Materials and reagents. Jiulongteng honey samples were collected from Guilin, Hezhou, Chongzuo, Liuzhou, Laibin and other places in Guangxi, and were identified by Lu Hailin, senior experimenter of Guangxi University of Chinese Medicine; there were 12 batches of materials in total, and they were collected from October to December 2021. The sample information was given in Table 1. Standard substance: 5-hydroxymethylfurfural (Chengdu Nakeli Biotechnology Co., Ltd., batch No.: nk1211201009, purity: 99.01%), guanosine (Chengdu Nakeli Biotechnology Co., Ltd., batch No.: nk1211201008, purity: 99.34%); sodium hydroxide, potassium hydrogen phthalate (analytically pure, Sinopharm Chemical Reagent Co., Ltd.); acetonitrile, methanol, formic acid (chromatography pure, Sinopharm Chemical Reagent Co., Ltd.), other reagents were analytical pure, and water was ultrapure water. Jiulongteng honey samples were collected and stored in a refrigerator (5 - 7 ℃) for later use, and 12 batches of samples were defined as follows: new honey stage 1 for storage less than 1 year, old honey stage 1 for storage longer than 1 year but less than 2 years, and old honey stage 2 for storage longer than 2 years.

2.1.2 Instruments. Agilent 1260 high performance liquid chromatograph (equipped with ultraviolet detector); Agilent ZORBAX SB-C_{18} chromatographic column (250 mm × 4.6 mm, 5 μm);

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Ohaus Instrument (Changzhou) Co., Ltd. EX225DZH electronic analytical balance.

Table 1 Sample information of Jiulongteng honey

Sample No.	Collection place	Source of bee species	Collection time (Year – Month)
FM-001	Yangshuo County, Guilin City	Italian bees	2021 – 10
FM-002	Pingle County, Guilin City	Italian bees	2021 – 11
FM-003	Gongcheng County, Guilin City	Italian bees	2021 – 11
FM-004	Quanzhou County, Guilin City	Italian bees	2021 – 11
FM-005	Lingchuan County, Guilin City	Italian bees	2021 – 11
FM-006	Xing'an County, Guilin City	Italian bees	2021 – 11
FM-007	Guanyang County, Guilin City	Italian bees	2021 – 11
FM-008	Lingui District, Guilin City	Italian bees	2021 – 11
FM-009	Fuchuan Yao Autonomous County, Hezhou City	Italian bees	2021 – 11
FM-010	Fusui County, Chongzuo City	Italian bees	2021 – 11
FM-011	Liujiang County, Liuzhou City	Italian bees	2021 – 11
FM-012	Lingui District, Guilin City	Chinese bees	2021 – 11

2.2 Methods

2.2.1 Determination of pH. With reference to the method of Ma Chang *et al.* [32], the pH of honey was determined by neutralization titration with NaOH standard solution.

(i) Preparation of NaOH solution. Weighed 4 g of NaOH sample, added water to dissolve it, transferred it to a 250 mL volumetric flask, added water to dilute it to the scale mark, and shook it up for later use. Weighed 1 g of potassium hydrogen phthalate sample dried to constant weight, put it into a 250 mL conical flask, and added 50 mL of distilled water to dissolve it for later use.

(ii) Standardization of NaOH solution. Added 2 – 3 drops of phenolphthalein indicator into the prepared potassium hydrogen phthalate standard solution, titrated with 0.1 mol/L NaOH solution until the solution turned light pink and did not fade for 30 sec, stopped titration, and recorded the reading V_{NaOH}.

According to the formula $N = G_{potassium\ hydrogen\ phthalate}/V_{NaOH} \times 0.2042$, calculated the concentration of NaOH solution to be 0.4 mol/L.

(iii) Determination of samples. Took about 10 g of honey sample, weighed it accurately, put it into a 250 mL conical flask, added 50 mL of distilled water to dissolve it, added 2 – 3 drops of phenolphthalein indicator, titrated it with NaOH standard solution until it turned light pink and did not fade in 30 sec, stopped titration, recorded the reading, and recorded the volume of NaOH consumed. Neutralized and titrated with NaOH standard solution, and the reaction was:

$$NaOH + R-COOH = R-COONa + H_2O$$

Sample pH calculation formula:

$$pH = VN/m$$

where V denotes the volume of NaOH consumed, N represents the concentration of the NaOH solution, and m is the mass of the sample.

2.2.2 Limit test of 5-hydroxymethylfurfural. (i) Preparation of reference substance solution. Took a proper amount of guanosine reference substance, weighed 0.014 g accurately, put it into a 50 mL volumetric flask, added 10% methanol to the scale, and prepared a solution containing 0.2 mg of guanosine per mL for later use. Took a proper amount of 5-hydroxymethylfurfural reference substance, weighed 0.0972 g accurately, added 10% methanol to prepare a solution containing 4 μg/mL for positioning.

(ii) Preparation of the test solution. Took 1 g of Jiulongteng honey, weighed it accurately, put it into a beaker, added 10% methanol to dissolve it, transferred it to a 50 mL volumetric flask, added 1 mL of guanosine reference solution accurately, added 10% methanol to the scale, and shook it up, to obtain the test solution.

(iii) Preparation of blank solution. Added a proper amount of 10% methanol into a 50 mL volumetric flask, precisely added 1 mL of guanosine reference solution, added 10% methanol to the scale, and shook up to obtain the blank solution.

(iv) Determination method. Accurately pipetted 10 μL of the test solution, guanosine reference solution and 5-hydroxymethylfurfural reference solution separately, and injected them into the liquid chromatograph for determination to determine the chromatographic peaks of 5-hydroxymethylfurfural and guanosine in the chromatogram of the test solution; the content was calculated with the guanosine reference substance and corrected by multiplying by a correction factor of 0.340.

(v) Chromatographic conditions and system suitability test. Acetonitrile-0.1% formic acid solution (5 : 95) was used as the mobile phase at a flow rate of 1 mL/min, and the detection wavelength of 5-hydroxymethylfurfural was 284 nm, and the detection wavelength of guanosine was 254 nm.

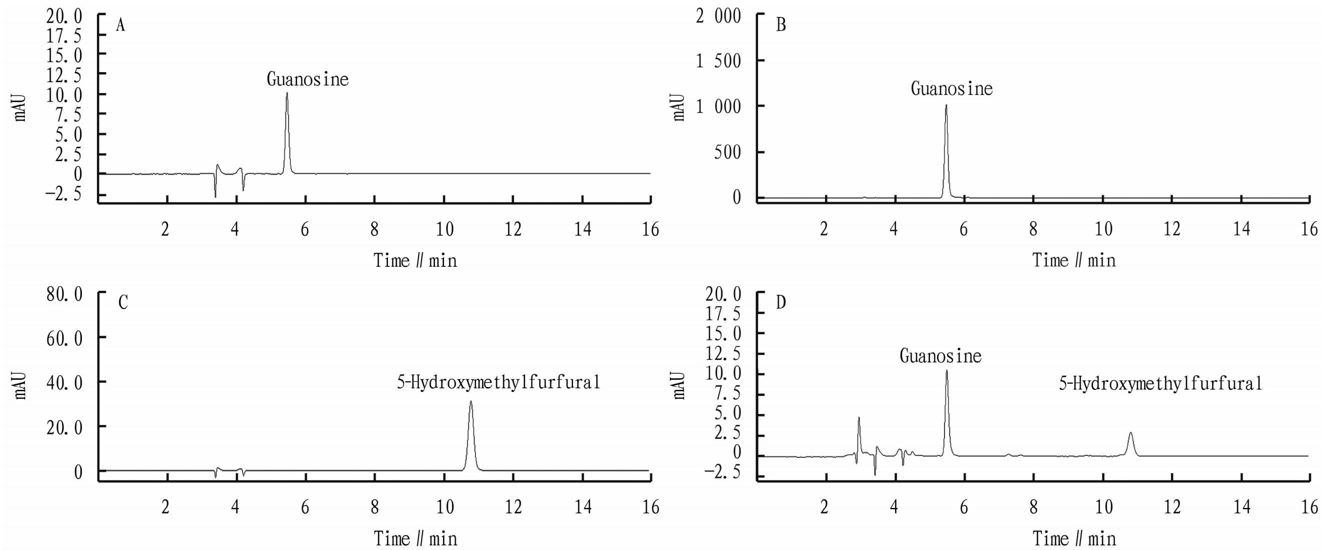
3 Results and analysis

3.1 Results of pH determination The pH determination results of 12 batches of Jiulongteng honey samples at different storage times are shown in Table 2. The pH of new honey stage was 3.70 – 3.84, the pH of old honey stage 1 was 3.92 – 4.05, and the pH of old honey stage 2 was 4.25 – 4.53.

Table 2 pH of 12 batches of Jiulongteng honey samples at different storage times

Sample No.	New honey stage	Old honey stage 1	Old honey stage 2
FM-001	3.70 ± 0.05	3.92 ± 0.04	4.25 ± 0.06
FM-002	3.74 ± 0.09	3.98 ± 0.06	4.35 ± 0.08
FM-003	3.76 ± 0.05	3.79 ± 0.07	4.40 ± 0.10
FM-004	3.75 ± 0.07	3.88 ± 0.06	3.99 ± 0.05
FM-005	3.80 ± 0.03	4.01 ± 0.04	4.29 ± 0.27
FM-006	3.84 ± 0.05	4.01 ± 0.06	4.28 ± 0.04
FM-007	3.79 ± 0.08	3.99 ± 0.12	4.19 ± 0.06
FM-008	3.79 ± 0.08	3.98 ± 0.07	4.32 ± 0.05
FM-009	3.82 ± 0.08	4.00 ± 0.07	4.45 ± 0.04
FM-010	3.78 ± 0.08	3.97 ± 0.06	4.09 ± 0.08
FM-011	3.81 ± 0.05	4.05 ± 0.08	4.48 ± 0.07
FM-012	3.80 ± 0.05	3.90 ± 0.10	4.42 ± 0.08

3.2 Chromatographic conditions and system suitability test results Chromatographic conditions and system suitability test results are shown in Fig. 1. $T = 10.8$ min for 5-hydroxymethylfurfural and $T = 5.4$ min for guanosine.



NOTE A. Blank solution; B. Guanosine reference solution; C. Reference solution; D. Jiulongteng honey sample solution.

Fig. 1 HPLC chromatogram

3.3 Results of 5-hydroxymethylfurfural limit test The quantitative determination results of 5-hydroxymethylfurfural of 12 batches of Jiulongteng honey with different storage time are shown in Table 3. The 5-hydroxymethylfurfural was not detected in the new honey stage, 5-hydroxymethylfurfural was detected in FM-001 in the old honey stage 1, and 5-hydroxymethylfurfural was detected in most samples in the old honey stage 2.

Table 3 Determination results of 5-hydroxymethylfurfural content in Jiulongteng honey with different storage times %

Sample No.	New honey stage	Old honey stage 1	Old honey stage 2	Standard in *Chinese Pharmacopoeia* (≤0.004)
FM-001	–	0.000 1 ±0.000 0	0.000 2 ±0.000 3	Qualified
FM-002	–	–	0.000 5 ±0.000 5	Qualified
FM-003	–	–	0.001 0 ±0.000 0	Qualified
FM-004	–	–	0.000 4 ±0.000 2	Qualified
FM-005	–	–	0.002 0 ±0.000 0	Qualified
FM-006	–	–	–	Qualified
FM-007	–	–	0.001 0 ±0.000 5	Qualified
FM-008	–	–	0.000 6 ±0.000 2	Qualified
FM-009	–	–	0.001 0 ±0.000 0	Qualified
FM-010	–	–	0.000 1 ±0.000 0	Qualified
FM-011	–	–	0.002 0 ±0.001 7	Qualified
FM-012	–	–	0.001 0 ±0.000 0	Qualified

NOTE " – " denotes not detected.

4 Discussion and conclusions

(i) The acidity of honey. The pH of honey can prove the adulteration and freshness of honey, so it is one of the important indicators to measure the quality of honey. The lower the acidity of honey, the better the quality and taste[11–12]. Therefore, after knowing the results of acidity color test of Jiulongteng honey, we further determined the pH of Jiulongteng honey. According to the EU standard for honey, the pH of honey should be between 3.40 and 6.10[13]. The results showed that the pH of 12 batches of Jiulongteng honey samples met the European Union standard in different storage time.

(ii) 5-hydroxymethylfurfural. In the test, the content of 5-hydroxymethylfurfural in Jiulongteng honey was not detected in the new honey stage, and the content of 5-hydroxymethylfurfural in the old honey stage 1 and the old honey stage 2 did not exceed the limit standard requirement of 0.004% in *Chinese Pharmacopoeia*. The results showed that the 5-hydroxymethylfurfural limit of Jiulongteng honey was in line with the regulations within 3 years of storage. In addition, 5-hydroxymethylfurfural was not detected in 12 batches of new Jiulongteng honey, and 5-hydroxymethylfurfural was detected in only one batch of 12 batches of old Jiulongteng honey stored for the second year; 5-hydroxymethylfurfural was not detected in only one batch of 12 batches of Jiulongteng honey in the third year of storage, and 5-hydroxymethylfurfural was detected in other batches.

The results showed that the pH of Jiulongteng honey was acidic. It can be seen that 5-hydroxymethylfurfural is a decomposition product formed by monosaccharides under acidic conditions, and generally there is no 5-hydroxymethylfurfural in new honey, but with the extension of storage time, even if Jiulongteng honey is stored at low temperature, the detection amount of 5-hydroxymethylfurfural was significantly increased. Therefore, 5-hydroxymethylfurfural can be used as an important indicator of honey freshness[14–15].

References

[1] BAI QM. Nectar, ganoderma, honey[J]. Journal of Bee, 1984(1): 49–50. (in Chinese).
[2] Ministry of Health of the People's Republic of China. GB 14963-2011 National Standard for Food Safety Honey[S]. Beijing: China Standard Press, 2011. (in Chinese).
[3] CRANE E. The world history of beekeeping and honey hunting[M]. Taylor and Francis, 1999.

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weight of the indicator. It has the characteristics of strong practicability and adaptability, and is objective and scientific[12]. In recent years, it has been widely used in the weight calculation of the comprehensive evaluation of multi-indicator components of traditional Chinese medicines[13-16]. In this study, the extraction rate of epicatechin and the comprehensive score obtained by the standard weight of dry extract rate were determined by entropy weight method, and the amount of extraction solvent, extraction time and extraction times were investigated by orthogonal experimental design. The extraction process of Fagopyri Dibotryis Rhizoma was optimized. The optimized process is stable and reliable, and is expected to provide a reference for the further development and utilization of Fagopyri Dibotryis Rhizoma.

References

[1] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China[M]. Beijing: China Pharmaceutical Science and Technology Press, 2020: 228. (in Chinese).

[2] WANG LY, HUANG J, CHEN QF, *et al.* Research progress of *Fagopyrum dibotrys*[J]. Journal of Chinese Medicinal Materials, 2019, 42(9): 2206-2208. (in Chinese).

[3] YANG XW, ZHANG Y, LI LY. Advances in studies on medicinal plant of *Fagopyrum dibotrys*[J]. Modern Chinese Medicine, 2019, 21(6): 837-846. (in Chinese).

[4] YAN J, YUAN JJ, LIU LN, *et al.* Progress of research on pharmacological effects and clinical application of *Fagopyrum dibotrys*[J]. Shandong Journal of Traditional Chinese Medicine, 2017, 36(7): 621-624. (in Chinese).

[5] SHENG HG, ZHU LQ, LIN GT. Progress of research on chemical composition and pharmacological effects of *Fagopyrum dibotrys*[J]. Northwest Pharmaceutical Journal, 2011, 26(2): 156-158. (in Chinese).

[6] ZHANG XL, ZHOU YB, LIU CW. (-)-Epicatechin pharmacological activity and its mechanism progress research[J]. Tea in Fujian, 2018, 40(9): 3-6. (in Chinese).

[7] TONG GZ, FU XP, YANG Y, *et al.* Advances in research on the distribution and pharmacological activities of epicatechin[J]. Journal of Yunnan Agricultural University (Natural Science), 2018, 33(2): 343-349. (in Chinese).

[8] CAO TW, ZHAO L, LUO H, *et al.* Study on the extraction and purification technology for active ingredients of JinQiaoMai by taking epicatechin as the marker[J]. Western Journal of Traditional Chinese Medicine, 2019, 32(3): 36-40. (in Chinese).

[9] RUAN HS, CAO L, CHEN ZB, *et al.* Optimization of extraction technology for *Fagopyrum dibotrys* by central composite design and response surface methodology[J]. Herald of Medicine, 2013, 32(2): 226-229. (in Chinese).

[10] WANG WZ, TIAN Y, WU X, *et al.* Optimisation of the extraction process of total flavonoids from *Fagopyrum dibotrys* by quadratic regression orthogonal design method[J]. Journal of Chinese Medicinal Materials, 2012, 35(11): 1861-1863. (in Chinese).

[11] LIU QY, WU XN. Review on the weighting methods of indexes in the multi-factor evaluation[J]. Knowledge Management Forum, 2017, 2(6): 500-510. (in Chinese).

[12] WANG R, YANG LY, WU ZF, *et al.* Effect of drying method on quality attribute of benchmark samples of Huagai Powder Powder based on entropy weight method[J]. Chinese Traditional and Herbal Drugs, 2023, 54(3): 768-778. (in Chinese).

[13] FU Q, XIAO XF, ZHOU YT, *et al.* Optimization of extraction process of iridoids from Gardenia based on entropy weight method and multiple indexes[J]. Chinese Journal of New Drugs, 2022, 31(13): 1265-1272. (in Chinese).

[14] ZHENG WW, HUI SY, LIN JD, *et al.* Optimization of extraction process of Jingshi Mixture based on multi-index comprehensive evaluation of UPLC-MS/MS and G1-entropy weight method[J]. Chinese Journal of Hospital Pharmacy, 2022, 42(24): 2581-2588. (in Chinese).

[15] LIU XL, ZHANG R, WANG X, *et al.* Optimization of extraction process for Qushi Qingfei Recipe by combination of central composite design-response surface methodology with entropy weight method and its anti-inflammatory activity[J]. Chinese Journal of Hospital Pharmacy, 2022, 42(24): 2600-2606. (in Chinese).

[16] LI X, LIU W, SONG XL, *et al.* Optimizing the extraction process of Shaoyao Gancao Decoction based on entropy weight method and Box-Behnken response surface method[J]. Asia-Pacific Traditional Medicine, 2022, 18(12): 53-58. (in Chinese).

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[4] JIN LH. The determination of mineral elements in honey by ICP-MS and its application to discriminate Northeast-China black honey and linden honey[D]. Tai'an: Shandong Agricultural University, 2016. (in Chinese).

[5] LI CS, ZHONG N. Yangshuo: Chasing flowers and honey to strengthen the bee industry[N]. Guangxi Daily, 2018-10-11. (in Chinese).

[6] QIN HR, BI ZH, ZHOU DW, *et al.* Investigation and research on honey bees utilising the nectar plant *Bauhinia championii* in Guangxi[J]. Apiculture of China, 2016(67): 40-42. (in Chinese).

[7] LIN L. *In vitro* immunotoxicity evaluation of 5-HMF and its dimer OMBF using RBL-2H3 cells and immunogenicity of three traditional Chinese medicine injections using PLNA[D]. Beijing: Peking Union Medical College, 2018. (in Chinese).

[8] ZHANG XX, TANG YT, TANG L, *et al.* Research progress of processing and quality analysis of *Polygonatum sibiricum*[J]. China Modern Doctor, 2018, 56(22): 165-168. (in Chinese).

[9] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (a)[M]. Beijing: China Pharmaceutical Science and Technology Press, 2020: 374-376. (in Chinese).

[10] FALLICO B, ARENA E, ZAPPALA M. Degradation of 5-Hydroxymethylfurfural in honey[J]. Journal of Food Science, 2008, 73(9): 625-631.

[11] DONG YY, CHI ZZ, CHI YS. Comparative study on the quality of sugar source honey, flower source honey and commercial honey[J]. Food Research and Development, 2021, 42(1): 48-54. (in Chinese).

[12] WANG TH, ZHANG SB, ZHANG HM, *et al.* Determination of volatile components in buckwheat honey and correlation analysis with honey maturity[J]. Food Science, 2020, 41(22): 222-230. (in Chinese).

[13] European Community. Council Directive 2001/110/EC of December 2001 relating to honey[S]. Official Journal of the European Communities, 2002, L10/47-L10/52.

[14] ZHANG LY, CHEN RY, ZHANG M, *et al.* Identification of syrup adulterated honey based on solid phase extraction-thin layer chromatography image analysis[J]. Journal of Chinese Institute of Food Science and Technology, 2021, 21(3): 263-273. (in Chinese).

[15] LU HX, WANG YH, YU YS, *et al.* Research and analysis of Yunnan honey HMF[J]. Journal of Bee, 2016, 36(8): 4-7. (in Chinese).