AOAC SMPR 2020.XXX; Draft AOAC Standard Method Performance Requirements (SMPRs) for Targeted Testing (TT) of Barley and Malt Extract, Beet Sugar Syrup, Corn and Cane Sugar Syrup, C-4 Plant Sugar and High Fructose Corn Sugar for Adulteration of Floral and Acacia Honey; Version 3; February 13, 2020

Intended Use

AOACI *SMPRs*® describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study.

SMPRs are written and adopted by AOACI using the consensus of stakeholders representing the industry, government, and academic and/or research institutions. AOACI SMPRs are used by AOACI expert review panels (ERPs) in their evaluation of validation study data for method being considered for *Performance Tested Methods*^{SM} or AOACI *Official Methods of Analysis*^{SM} and can be used as acceptance criteria for verification at user laboratories.

1. Applicability

This document contains assessment parameters on the performance of Targeted Testing methods to monitor honey for the detected presence of the following Economically Motivated Adulterants (EMA) ***barley and malt extract, beet sugar syrup, corn and cane sugar syrup, C-4 plant sugar and high fructose corn sugar in Floral and Acacia Honey***.

2. Analytical Technique

A Targeted method to be used to monitor and enforce regulatory requirements for foods and ingredients for detected and identified EMAs. Any method capable of detecting, identifying the presence of a defined adulterating ingredient and quantifying the amount (proportion/ concentration) present in the food item will be considered.

The scope of the TT method will be defined by the available authentic honey samples used in validating the method.

3. Definitions

Applicability Statement – a general statement about the intended purpose and scope of the method entailing key aspects of expected achievements for the specific situation and circumstances. Key points to cover are the intended matrix, the purpose, and an indication of sensitivity, selectivity, enforcement and action levels where available

Authentic Samples – Samples representative of the genuine commodity. ~~Ideally~~ these samples should represent the food's or ingredient's variability seen naturally in the commodity. The authentic samples and/or standard reference materials used to validate the method will be used to properly define the TT method testing scope.

Economically Motivated Adulteration – The fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain of the seller.

False Origin – Honeys containing mislabelled geographic and botanical sources.

Authentic Honey – The type(s) of honey used to generate the baseline fingerprint. The method's scope of authenticity is defined by the honey(s) used in generating the baseline fingerprint.

Single Laboratory Validation – Demonstration by one laboratory of method performance on the validation samples described in Table 1.

Multi-laboratory Validation – Demonstration between laboratories using adulterated samples created by a third-party group and supplied blindly to the participating laboratories.

4. Method Performance Requirements

(Table 1: Method Performance Requirements for Barley and Malt extract in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	10 – 50% (w/w) OF EVOO
LOQ	≥ 10 %
Recovery	80 – 120 %
Accuracy	± 20%
NOTES: Check for publication and analytical range	

(Table 2: Method Performance Requirements for Beet Sugar Syrup in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	10 – 50 (w/w) % of EVOO
LOQ	≥ 10%
Recovery	80 – 120 %
Accuracy	± 20%
NOTES: Detection of honey adulteration with beet sugar using stable isotope methodology. Food Chemistry, Volume 61, Issue 3, 31 March 1998, Pages 281-286. González Martín, E Marqués Macías, J Sánchez Sánchez, B González Rivera. https://doi.org/10.1016/S0308-8146(97)00101-5 A usual aspect of our work involves the analysis of honey samples for later sale, following current Spanish legislation. Such analyses essentially consist of studying pollen sediments, and sensory and physicochemical analyses. With this background, it seemed appropriate to investigate possible adulterations due to the addition of sugar (beet and cane). To do this, we selected 49 samples of honey obtained from 14 floral types and used them for pollinic and sensory analyses and to detect possible adulterations due to the addition of beet sugar products (treating the oligosaccharide fraction contained in the honey with the galactose oxidase reaction) or due to corn syrup addition (with normal δ13C stable carbon isotope ratios). After classifying the samples according to the results of the pollen and sensory analyses, further assays were conducted. From the results it was concluded that 15% of the samples had been adulterated with beet sugar and 4% with cane sugar. The implementation of many analyses for each sample means that the results can be intercorrelated very well.	

(Table 3: Method Performance Requirements for Corn and Cane Sugar Syrup in honey)

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	5 – 95 % (w/w) OF SUGAR to EVOO
LOQ	≥ 10 %
LOD	
Recovery	80 – 120 %
Accuracy	± 20%

NOTES:

1. *Fingerprint targeted compounds in authenticity of sugarcane honey - An approach based on chromatographic and statistical data.* LWT, Volume 96, October 2018, Pages 82-89. Pedro Silva, Catarina L. Silva, Rosa Perestrelo, Fernando M. Nunes, José S. Câmara. https://doi.org/10.1016/j.lwt.2018.04.076

Sugarcane honey (SCH) is a black syrup recognized by its excellent quality, being produced in Madeira Island using the regional sugarcane cultivars and following a traditional and peculiar manufacturing and storage processes. However, some low-quality commercial products have been labeled as SCH but do not respect its criteria, revealing the need of develop powerful strategies in order to detect and prevent adulterations. The knowledge of furanic derivatives (FDs) profile, produced during browning reactions that occurs during food processing and storage, emerged as a promising strategy in food quality and fraud prevention. Therefore, the aim of this study was to establish the FDs profiling of typical SCH produced by certified and non-certified producers, in different geographical regions (Madeira and Brazil), based on microextraction by packed sorbent (MEPS) combined with ultra-high performance liquid chromatography (UHPLC) as a useful approach to define its typicality and authenticity. These parameters are defined through the differentiation and discrimination of FDs profiles among other sugarcane-derived products using multivariate statistical analysis (ANOVA with post-hoc Tukey, principal components analysis, partial least square, linear discriminant analysis and hierarchical clustering). The results demonstrated that SCH samples from non-certified producers present the highest levels of FDs. In addition, SCH samples from Brazil present higher levels of FDs than samples from Madeira region. The obtained results revealed that the proposed approach is a valuable strategy to establish the typicality of SCH, ensuring its quality, authenticity, safety control and a useful support regarding the application of SCH from Madeira Island to EU certification.

2. *Thermal properties of honey as affected by the addition of sugar syrup.* Journal of Food Engineering, Volume 213, November 2017, Pages 69-75. Lara Sobrino-Gregorio, María Vargas, Amparo Chiralt, Isabel Escriche.

https://doi.org/10.1016/j.jfoodeng.2017.02.014

Ensuring the authenticity of honey is a priority for producers and regulatory authorities. The aim of this work was to evaluate the thermal properties (using a Differential Scanning Calorimeter "DSC") of ten types of sugar syrup, six types of honey and mixtures of sunflower honey with all these syrups at different proportions simulating the adulteration of honey (ratio honey/syrup: 80/20; 90/10; 95/05). The glass transition temperature (Tg midpoint) ranged from 60.2 °C to 67.3 °C in honey samples and from 32.8 °C to 95.8 °C in syrup samples. The differences in sugar composition of the syrups mainly affect their thermal properties. In the adulterated samples, the glass transition temperature was affected by the type of syrup, proportionally to the adulteration level. These results offer compelling evidence that the DSC can be used for the identification of addition of syrup to honey, although to be conclusive a greater number of honey types must be considered.

3. *Detection of adulteration in honey samples added various sugar syrups with 13C/12C isotope ratio analysis method.* Food Chemistry, Volume 138, Issues 2–3, 1 June 2013, Pages 1629-1632, Murat Tosun

https://doi.org/10.1016/j.foodchem.2012.11.068

Honey can be adulterated in various ways. One of the adulteration methods is the addition of different sugar syrups during or after honey production. Starch-based sugar syrups, high fructose corn syrup (HFCS), glucose syrup (GS) and saccharose syrups (SS), which are produced from beet or canes, can be used for adulterating honey. In this study, adulterated honey samples were prepared with the addition of HFCS, GS and SS (beet sugar) at a ratio of 0%, 10%, 20%, 40% and 50% by weight. 13C/12C analysis was conducted on these adulterated honey samples using an isotope ratio mass spectrometer in combination with an elemental analyser (EA–IRMS). As a result, adulteration using C4 sugar syrups (HFCS and GS) could be detected to a certain extent while adulteration of honey using C3 sugar syrups (beet sugar) could not be detected. Adulteration by using SS (beet sugar) still has a serious detection problem, especially in countries in which beet is used in manufacturing sugar. For this reason, practice and analysis methods are needed to meet this deficit and to detect the adulterations precisely in the studies that will be conducted.

64

65 **(Table 4: Method Performance Requirements for C-4 Plant Sugar in honey)**

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	0.20 – 50% (w/w) OF EVOO
LOQ	≥ 38 %
LOD	0.11 %
Recovery	80 – 120 %
Accuracy	± 20%

NOTES:

1. *In-house validation for the determination of honey adulteration with plant sugars (C4) by Isotope Ratio Mass Spectrometry (IR-MS).* LWT – Food Science and Technology, Volume 57, Issue 1, June 2014, Pages 9-15. Mehmet Fatih Cengiz, M. Zeki Durak, Musa Ozturk https://doi.org/10.1016/j.lwt.2013.12.032

The objective of any analytical measurement is to obtain consistent, reliable, and accurate data. Validated analytical methods play a major role in achieving this goal. Although there have been many studies reporting about the isotopic compositions of

honeys, little has been documented regarding the validation of these methods. In this study, an Isotope Ratio Mass Spectrometry (IR-MS) method for the determination of honey adulteration was validated in-house in terms of selectivity, stability, linearity, accuracy, repeatability, sensitivity, and recovery. This study was the first attempt to describe some important method validation parameters, such as the limit of detection (LOD), limit of quantification (LOQ), and recovery for the IR-MS studies. The LOD of the method was 0.11%, and the LOQ was 0.38% based on the percent adulteration ratio. The recovery value for spiked blank honey sample with the in-house standard was 98.57%. To evaluate the usefulness of the method, 13 different brands of honey samples were collected from markets in Turkey and analyzed. The ranges of δ13C values of analyzed honey samples and their protein fractions were from −12.87 ± 0.01 to −25.56 ± 0.08‰ and from −23.77 ± 0.09 to −25.98 ± 0.06‰, respectively. Adulteration was found in one honey sample.

68 **(Table 5: Method Performance Requirements for High Fructose Corn Sugar in honey)**

Analytical Parameter	Acceptance Criteria
Analytical Range (%)	10 – 50 % (w/w)
LOQ	≥ 10 %
Recovery	80 – 120 %
Accuracy	± 20%

NOTES:

1. *Determination of high fructose corn syrup concentration in Uruguayan honey by* ***^{13}C*** *analyses.* LWT, Volume 73, November 2016, Pages 649-653. Verónica Berriel, Carlos Perdomo https://doi.org/10.1016/j.lwt.2016.07.004

The methodology of internal standard which relies on the difference between the isotopic composition in terms of $\delta^{13}C$ of honey and its proteins, has been extensively used in many countries to assess honey adulteration with high fructose corn syrup (HFCS) or other C4-adulterants, but there have been no reports of such studies in Uruguay. To obtain this information, 51 honey samples were collected from different outlets in two Uruguayan regions. The δ13C composition of honey varied between −26.89 and 23.72‰, while that of its proteins ranged between −26.49 and −24.61‰. When the international value of −1.0‰ was used as the maximum accepted difference between the isotopic values of proteins and honeys, it was determined that 5.9% of samples were adulterated with HFCS, but when this limit was replaced by the locally determined threshold of 0.80‰, the proportion of adulterated samples increased to 7.8%. Both values, however, were lower than most of those reported internationally, which suggest that honey fraud is not widespread in Uruguay.

2. *Detection of honey adulteration by high fructose corn syrup and maltose syrup using* ***Raman spectroscopy****.* Journal of Food Compositionand Analysis, Volume 28, Issue 1, November 2012, Pages 69-74 Shuifang Li, Yang Shan, Xiangrong Zhu, Xin Zhang, Guowei Ling https://doi.org/10.1016/j.jfca.2012.07.006

Raman spectroscopy was used to detect adulterants such as high fructose corn syrup (HFCS) and maltose syrup (MS) in honey. HFCS and MS were each mixed with authentic honey samples in the following ratios: 1:10 (10%), 1:5 (20%) and 1:2.5 (40%, w/w). Adaptive iteratively reweighted penalized least squares (airPLS) was chosen to remove background of spectral data. Partial least squares-linear discriminant analysis (PLS-LDA) was used to develop a binary classification model. Classification of honey authenticity using PLS-LDA showed a total accuracy of 91.1% (authentic honey vs. adulterated honey with HFCS), 97.8% authentic honey vs. adulterated honey with MS) and 75.6% (authentic honey vs. adulterated honey with HFCS and MS), respectively. Classification of honey adulterants (e.g. HFCS or MS) using PLS-LDA gave a total accuracy of 84.4%. The results showed that Raman spectroscopy combined with PLS-LDA was a potential technique for detecting adulterants in honey.

3. *Adulteration of honey with high-fructose corn syrup: Detection by different methods.* Food Chemistry, Volume 48, Issue 2, 1993, Pages 209-212. E-S. M. Abdel-Aal, H. M. Ziena, M. M. Youssef https://doi.org/10.1016/0308-8146(93)90061-J

Pure honey was deliberately adulterated with high-fructose corn syrup (HFCS) at levels of 10%, 20%, 30%, 40%, and 50% (w/w). Sugar composition as a fingerprint was determined by **HPLC** for all samples. The following compositional properties were determined for pure and adulterated honey: moisture, total soluble solids, nitrogen, apparent viscosity, hydroxymethylfurfural (HMF), ash, sodium, calcium, potassium, proline, refractive index and diastatic activity. Statistical analysis revealed that the following compositional properties were highly significantly negatively correlated with sugar composition: dry matter, apparent viscosity, sodium, potassium, proline, and nitrogen. In contrast, ash, calcium, HMF, and moisture were highly significantly positively correlated with sugar composition for pure and adulterated honey. Accordingly, such simple tests can be applied as good indicators for detecting the adulteration of honey with HFCS at adulteration levels ranging from 10% to 50%.

4. *Determination of Chinese honey adulterated with high fructose corn syrup by* ***near infrared spectroscopy.*** Food Chemistry, Volume 128, Issue 4, 15 October 2011, Pages 1110-1114. Lanzhen Chen, Xiaofeng Xue, Zhihua Ye, Jinghui Zhou, … Jing Zhao

https://doi.org/10.1016/j.foodchem.2010.10.027

The use of fibre optic diffuse reflectance near infrared spectroscopy (NIR) in combination with chemometric techniques has been investigated to discriminate authenticity of honey. NIR spectra of unadulterated honey and adulterated honey samples with high fructose corn syrup were registered within 10,000–4000 cm^{-1}spectral region. Discriminant partial least squares (DPLS) models were constructed to distinguish between unadulterated honey and adulterated honey samples and main bands responsible for the discrimination of samples are in the range of 6000–10,000 cm^{-1}. For these models, the correct classification rate for calibration samples were above 90%. Hundred percentage of unadulterated honey and 95% of adulterated honey samples from test set were correctly classified after appropriate preprocessing of first derivative, 13 smoothing points, followed by mean centering pre-treatment and eight model factors, respectively. Our results showed that NIR spectroscopy data with chemometrics techniques can be applied to rapid detecting honey adulteration with high fructose corn syrup.

5. *Detection of honey adulteration of high fructose corn syrup by Low Field Nuclear Magnetic Resonance (LF 1H NMR).* Journal of Food Engineering, Volume 135, August 2014, Pages 39-43, Roberta de Oliveira Resende Ribeiro, Eliane Teixeira Mársico, Carla da Silva Carneiro, Maria Lúcia Guerra Monteiro, … Edgar Francisco Oliveira de Jesus.

https://doi.org/10.1016/j.jfoodeng.2014.03.009

The effect of honey adulteration by high fructose corn syrup in different concentrations from 0% (pure honey) to 100% (pure high fructose corn syrup) was investigated using Low Field Nuclear Magnetic Resonance spectroscopy (LF 1H NMR) and physicochemical analytical methods. The LF 1H NMR data were analyzed by bi-exponential fitting and compared with physicochemical data. The physicochemical parameters demonstrated that water content, water activity, pH and color differed significantly in honey samples adulterated with different concentrations of high fructose corn syrup. These differences were also observed by transverse relaxation (T2). Bi-exponential fitting of T2 resulted in the observation of two water populations in all samples, T21 and T22, with relaxation times in the range of 1.26–1.60 ms and 3.33–7.38 ms, respectively. Relaxation times increased with higher percentages of high fructose syrup in adulterated honey. Linear correlations were observed between the T2, T21 and T22 parameters and physicochemical data, suggesting that LF 1H NMR can be used to discriminate pure blossom honey from honey adulterated with high fructose corn syrup.

5. System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blanks, and appropriate check standards.

6. Reference Materials

A detailed description of the process used to obtain and evaluate authentic/reference standard materials (sources), and of the test protocol used for validating the method must be provided.

7. Validation Guidance

a. Data demonstrating method performance is required.

b. Samples: Complete documentation for the collection and use of authentic samples must be supplied by the method authors. The scope of “authentic” samples used to validate the method must be applicable to the defined scope of the TT method. Expansion of the scope is possible with the inclusion of additional authentic samples and abbreviated validation using the protocol listed in this SMPR.

c. For single lab validation studies, the method will be evaluated using prescribed adulterated materials as shown in Table 1. Methods approved at this level will proceed to a second level of evaluation (multi-laboratory) where blinded samples containing unknown adulterants will be sent to participating laboratories.

d. Statistical analysis of interlaboratory studies. Sample size needed to meet performance requirement on proportion.

8. Maximum Time-to-Results

None.