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False Honeys from Ecuador can be Detected by a Simple Test

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Abstract

Objective: Fake honeys from the Ecuadorian market were collected and analyzed with the quality indicators of the Venezuelan honey norm and a simple test.

Method: Ash, free acidity, moisture, reducing sugars, apparent sucrose, qualitative diastase activity, qualitative hydroxymethylfurfural, and a simple test based on the number of phases formed after shaking honey water dilutions with diethyl ether were used to study Ecuadorian fake honeys. A sensory kit is mentioned as an option to differentiate genuine and fake honeys.

Result: The results obtained for 21 fake Ecuadorian honeys from 12 provinces, show that the simple test could detect all of them, by the difference of two phases instead of three phases characteristic for genuine *Apis mellifera* honey. Likewise the qualitative diastase activity and hydroxymethylfurfural. On the other hand, ash and moisture honey standards were easily fulfilled by fake honeys.

Conclusion: The hydroxymethylfurfural and diastase activity are the golden standard to detect Ecuadorian fake honeys, because ash, free acidity, moisture, reducing sugars and apparent sucrose standards can be met by most fake honeys. The simple diethyl ether test also detected 100% of the fake honeys, and it is recommended as a non-official approach to confirm honey authenticity.

Key words:

Ecuador; Honey norm; Fake honey; Quality factors; Authenticity test

Introduction

Adulteration of honey by less expensive sweeteners is a frequent authenticity issue to increase benefits of a highly valued product [1]. A term was coined to refer to economically motivated adulteration (EMA) as "fraudulent, intentional substitution or addition of a substance for the purpose of increasing the apparent value of the product or reducing the cost of its production, i.e., for economic gain" [2].

The honey standard of Codex Alimentarius set in 2001 [3] was adapted by the International Honey Commission (IHC) [4]. Honey standards are the official reference for authenticity testing. However, more specialized methods such as NMR spectroscopy [5,6] and the ratio of the isotopes carbon-12/carbon-13 [7] systematically used to control fraudulent honeys, are not available in developing countries.

Honey falsification is a common practice in markets of countries without official sanctions (VP, personal observation). Fort the expert scientist and beekeeper, the sensory difference between genuine honey and fake honey in the tropics is clear, but not for the consumer. A method to detect false honey with a kit was explained [8]. Later, a sensory training with a set of genuine and false honeys was suggested to have the sensory reference for wise choices to recognize genuine honey (Figure 1).

The name of "Love Honey" was derived from the acquainted expression in Ecuador "Love Life". Training on sensory smell-aroma and taste of honey is perhaps the best protection for the consumer (VP unpublished data), but governments are not prepared to take that action. The wide sensory variations (aroma, color, smell, taste, visual viscosity) of genuine honey according to the entomological and botanical origin did not prevent a naive panel of native Huottujas from Venezuela to differentiate genuine and fake honeys by Free-Choice Profile [9].

In this work, the usefulness of a simple test to detect fake honeys from Ecuador is investigated in contrast with the physico-chemical quality standards in the Venezuelan honey regulation [10] (ash, free acidity, diastase activity, hydroxymethylfurfural, moisture, reducing sugars, and apparent sucrose).

Figure 1: Sensory Kit "Love Honey".

Materials and Methods

Honeys

Twenty-one fake honeys were purchased in local markets from 12 Ecuadorian provinces: Bolívar, Cañar, Carchi, Cochabamba, Guayas, Imbabura, Los Ríos, Morona Santiago, Pastaza, Pichincha, Santo Domingo, and Zamora Chinchipe. A genuine Ecuadorian control honey was collected directly from the apiary by Mr. Themis Hernández. The samples were transported at environment temperature but kept frozen until analysis.

Test to detect fake honey

The simple test to detect fake honey consists in measuring 0.5 mL volume of honey plus the same volume of distilled water, mixed to make a honey dilution in a disposable 5 mL syringe. Then 2 mL of diethyl ether were added and strongly shaked, let stand for 1 min to observe how many phases are formed; genuine honey produce three phases and fake honeys two phases [8]. The 21 fake honeys and the genuine honey were tested with this method.

Physico-chemical analysis

The methods used to analyze fake honeys were those indicated in the Venezuelan honey norms COVENIN 2136-84 [11] Ash was measured by a gravimetric method after incineration. Free acidity by the titrimetric method. Moisture by the refractometric method. Reducing sugars and apparent sucrose were measured by the Lane & Eynon method. Diastase activity and hydroxymethylfurfural were measured by qualitative methods with a color reaction. For the diastase, a iodine indicator reveals the presence of added starch to honey with a blue/brownish color, if not digested by the enzyme in old, overheated and fake honeys. High concentrations of hydroxymethylfurfural are revealed by a red color reaction of the resorcinol reagent and the ether extract of honey.

Statistical analysis

Descriptive statistics was used to estimate average ± SEM, using SPSS 12.0 [12].

Results and Discussion

Number of phases in the test

The number of phases produced with honey dilutions during the test are shown in Figure 2. The genuine *Apis mellifera* honey used as control produced three phases (2a), but all the fake honeys produced two phases (2b) in the test based on the number of phases observed after shaking water honey dilutions with diethyl ether. Fake honeys (2b) lack of the intermediate phase visible in the genuine honey (2a).

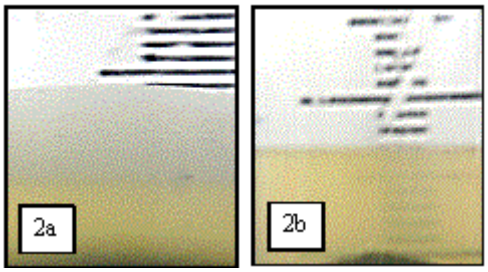

Figure 2: Number of Phases Formed In the Test: Three Phases for Genuine Honey Dilutions (1a), and Two Phases for Fake Honeys, without the Intermediate Phase (1b).

Physico-chemical composition

The results of the physico-chemical analysis (ash, free acidity, diastase activity, hydroxymethylfurfural, moisture, reducing sugars, apparent sucrose) measured in 21 Ecuadorian fake honeys are informed in Table 1, besides the Venezuelan [10] and the Ecuadorian [13] Honey Standards.

Following the analysis of honey quality indicators observed in Table 1, it is relevant that qualitative diastase activity and hydroxymethylfurfural did not fulfill the standards in any of the 21 Ecuadorian fake honeys analyzed. There is no diastase activity in the sugar syrups prepared to imitate honey, with a characteristic bluish color indicating presence of the starch indicator not degraded by the diastase. The fact that sugar syrups are prepared by heating, increases the hydroxymethylfurfural which is always positive resulting in a distinctive red color qualitative response. Similarly, in a study with 500 commercial honeys from Venezuela collected between 1985 and 1987, fake honeys never fulfilled the hydroxymethylfurfural and diastase activity standards [14]. However, old and heated genuine honeys also may fail for these two quality factors.

Interestingly, ash and moisture contents honey standards were easily fulfilled, therefore these measurements do not help to detect fake honeys. Indeed, genuine honeys with higher moisture will ferment, and do not cope with the standard if the honey was not mature before harvesting [15], while more than 70% of Venezuelan fake honeys had a moisture of 14-18 g/100 g and less than 5% were above the maximum 20 g/100 g [14].

Intermediate indicators, valid to detect a set of fake honeys but not others, were free acidity, reducing sugars and apparent sucrose. The averages of these three indicators fulfill the honey standard. However, few samples have a free acidity higher than the permitted 40 meq/kg, or a lower content of reducing sugars than the mínimum of 65 g/100 g.

Surprisingly, a fake honey reached 23.89 g apparent sucrose/100 g, so distant from the máximum limit of 5 g/100 g. These values are highlighted in Table 1, besides the hydroxymethylfurfural and diastase activity that out of standards in all samples.

Quality factors (units)	Average ± SEM	Minimum	Maximum	COVENIN 2194-84 [10] Standards	NTE INEN 1572 [13]2 Standards
Ash (g/100g)	0.20 ± 0.02	0.08	0.42	Max 0.5	Max 0.5
Free Acidity (meq/kg)	33.85 ± 4.07	15.70	102.00	Max 40	Max 50
Diastase activity (qualitative)	negative	negative	negative	positive	3-83
Hydroxymethylfurfural (qualitative)	positive	positive	positive	negative	404
Moisture (g/100 g)	17.08 ± 0.39	12.90	20.00	Max 20	Max 20
Reducing Sugars (g/100 g)	69.07 ± 1.18	51.80	74.80	Min 65	Min 65
Apparent Sucrose (g/100 g)	3.71 ± 1.33	0.00	23.80	Max 5	Max 5

Table 1: Physicochemical composition of fake honeys from Ecuador (n=21)[1]. Values highlighted in grey do not comply with the honey standards [10, 13] in the last column. 2In the Ecuadorian regulation, besides these Class I honey standards, there is a set of more permissive limits for Class II industrial honey, with maximum 23% moisture, minimum 60% reducing sugars, and maximum 7% apparent sucrose. The quantification of diastase activity and HMF is not done in Ecuadorian laboratories although the standards are quantitative: 3Schade Units, 4 mg HMF/kg. In this regulation the relative density and the water insoluble solids are included, but they are not considered in Table 1 as we did not measured these variables.

Basically, the simple test used here can detect 100% fake honeys like the qualitative hydroxymethylfurfural and diastase activity measurements. It is a fast and easy test to be performed by consumers and beekeepers, but the limitation is that the diethyl ether is highly flammable and a restricted reagent to the public. However, this test is valid to reject fake honeys. Another approach to detect additions of cane sugar to adulterated honey were proposed by microscopic observations of sugar cane residues (single rings, parenchyma cells, epidermal cells) coupled to delta C-13 measurements to confirm C-4 sugars derived either from sugar cane or corn syrups [16].

Besides the fake honeys manufactured with sugar cane, more sophisticated indirect syrup-bee-feeding demand constant analytical developments due to the rise of honey adulterations [1]. The fact that honey is either abundant in the forests but also a scarce commodity in the cities, creates opportunities to satisfy the demand with cheaper sugars imitating honey. The sensory approach by experts in honey tasting is advised to empower consumers on critical assessment to easily reject fake honeys [17].

A Public Health issue for consumers protection and a strategical issue to protect the genuine products of the bee industry are the applications of this research. Detecting fake honeys by a simple method has advantages for the administrative system: 1. To implement a control in situ for a fast response by authorized officers, 2. To transduce the information into sanctions, 3. To remove fake honeys from the shelves, These three steps are needed for adequate vigilance, and can be confirmed by aleatory planning for classic honey quality control for the removed samples. By doing so, savings are predicted in the regulatory process.

From a veterinary science perspective, the economic implications of reducing fake honeys in the market, would benefit the profits of beekeepers by reducing a competitive price caused by imitation honeys not derived from the beehive. Additionally, evidence of apitherapy for veterinarian practice [18,19] considers medical grade honey a better choice [20]. The uses of honey in diverse medical disciplines, is surprisingly effective in oncology because it is postulated as a highly cytotoxic matrix against cancer cells but non-cytotoxic to normal cells [21]. Honey is used alone in cancer treatment, but also in combination with other natural products such as *Aloe arborescens* [22]. Additionally, the entomological origin of honey confers diverse anticancer activity, ranging from IC50 of 2.74 mg/mL *Melipona solani* pot-honey to 24.37 mg/mL *Melipona scutellaris* pot-honey [23] Therefore further labelling could be envisaged for Ecuadorian genuine honey to be prescribed for medicinal use in future.

Conclusion

Honey consumers and beekeepers need protection from fake honeys. In this investigation the detection of fake honeys by a simple test was contrasted with the analytical output of the seven honey quality factors in the Venezuelan Honey Norm. The hydroxymethylfurfural and diastase activity are the golden standard to

detect Ecuadorian fake honeys, because ash, free acidity, moisture, reducing sugars and apparent sucrose standards can be met by most fake honeys. The simple diethyl ether test also detected 100% of the fake honeys, and it is recommended as a non-official approach to confirm honey authenticity.

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