Transformación y agroindustria

Artículo de investigación científica y tecnológica

Physicochemical characteristics of commercial eucalyptus honeys from Southwest Casanare

Características fisicoquímicas de mieles comerciales de eucalipto del suroeste de Casanare

Características físico-químicas de meles comerciais de eucalipto do sudoeste do Casanare

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Fecha de recepción: 08/07/2015
Fecha de aceptación: 13/10/2015

Para citar este artículo: Ortega-Bonilla RA, Chito-Trujillo DM, Suárez-Ramos CA. Physicochemical characteristics of commercial eucalyptus honeys from Southwest Casanare. Corpoica Cienc Tecnol Agropecuaria. 17(1):73-80

Summary

There are few characterization studies of Colombian honeys in the literature. It is important to know different aspects of national honeys in order to verify the compliance with the existing Colombian requirements and International regulations, but also to increase its exploitation considering its quality and distinctive features. Physicochemical parameters are basic factors that determine the final quality of honey. The objective of this research was to assess commercial eucalyptus honeys developed with *Apis mellifera* in the region of Villanueva (Colombia). Water activity (0.54), pH (5.02), free acidity (14.58 meq kg^{-1}), moisture (16.45 %), color (91.65 mm PFund), electrical conductivity (81.3 mS cm^{-1}), minerals (ash, 0.81 %), reducing sugars (69.95 %), sucrose (1.48 %), hydroxymethylfurfural (HMF, 67.29 mg kg^{-1}), diastase activity (3.21 °Gothe), phenolic content (76.57 mg Quercetin/100 g), flavonoids (5.96 mg Gallic acid/100 g) and proline (180.68 mg kg^{-1}) were determined in 24 honey samples following standard and usual procedures. The samples analyzed meet both national and international regulations for free acidity, moisture and HMF. No adulteration treatments were evidenced from proline content. However, the low diastase activity suggests that the honey went through some kind of overheating, probably during its storage under the high temperatures typical in the area of production. Usual estimations of phenolic and flavonoids content were detected. In conclusion, the tested samples fulfill some of the requirements for their commercialization. Hence, it would be convenient to develop a better control of the honey production process in the Southwest Casanare.

Keywords: Honey, Quality, Phenols, Flavonoids, Proline

Resumen

Existen pocos estudios de caracterización de mieles colombianas reportados en la literatura. Es importante conocer los diferentes aspectos de las mieles nacionales para verificar el cumplimiento de los requerimientos colombianos existentes y de las regulaciones internacionales, pero también para incrementar su explotación a partir de su calidad y características distintivas. Los parámetros fisicoquímicos son factores básicos que influencian la calidad final de la miel. El objetivo de esta investigación fue evaluar la calidad de mieles comerciales de eucalipto producidas con *Apis mellifera* en la región de Villanueva (Colombia). Se determinaron algunos parámetros fisicoquímico, tales como: actividad acuosa (0,54), pH (5,02), acidez libre (14,58 meq kg^{-1}), humedad (16,45 %), color (91,65 mm PFund), conductividad eléctrica (81,3 mS cm^{-1}), minerales (cenizas, 0,81 %), azúcares reductores (69,95 %), sacarosa (1,48 %), hidroximetilfurfural (HMF, 67,29 mg kg^{-1}), actividad diastásica (3,21 °Gothe), contenido fenólico (76,57 mg Quercetin/100 g), flavonoides (5,96 mg Gallic acid/100 g) y prolina (180,68 mg kg^{-1}), en 24 muestras de miel siguiendo los procedimientos estandarizados. Las muestras analizadas cumplieron las regulaciones nacionales e internacionales para acidez libre, humedad y HMF. No se evidenció tratamientos de adulteración a partir del contenido de prolina, sin embargo, la baja actividad diastásica sugiere que la miel fue sometida a algún tipo calentamiento, podría ser durante su almacenamiento debido a las altas temperaturas típicas de la región de producción. Se detectaron estimaciones convencionales en los contenidos de fenólicos y flavonoides. En conclusión, las mieles evaluadas cumplen con algunos criterios de calidad requeridos para su comercialización, por lo cual es conveniente tener un mayor control del proceso de producción de mieles en el suroeste de Casanare.

Palabras clave: miel, calidad, fenol, flavonoides, prolina

Resumo

Existem poucos estudos de caracterização de meles colombianas reportados na literatura. É importante conhecer os diferentes aspectos dos meles nacionais para verificar o cumprimento dos requerimentos colombianos existentes e das regulamentações internacionais, mas também para incrementar a sua exploração a partir da sua qualidade e características distintivas. Os parâmetros físico-químicos são fatores básicos que influenciam a qualidade final do mel. O objetivo desta pesquisa foi avaliar a qualidade de meles comerciais de eucalipto produzidas com *Apis mellifera* na região de Villanueva (Colômbia). Determinaram-se alguns parâmetros físico-químicos, tais como: atividade aquosa (0,54), pH (5,02), acidez livre (14,58 meq kg^{-1}), umidade (16,45 %), cor (91,65 mm PFund), condutividade elétrica (81,3 mS cm^{-1}), minerais (cinzas, 0,81 %), açúcares redutores (69,95 %), sacarose (1,48 %), hidroximetilfurfural (HMF, 67,29 mg kg^{-1}), atividade diastásica (3,21 °Gothe), conteúdo fenólico (76,57 mg Quercetin/100 g), flavonoides (5,96 mg Gallic acid/100 g) e prolina (180,68 mg kg^{-1}), em 24 amostras de mel seguindo os procedimentos padronizados. As amostras analisadas cumpriram as regulamentações nacionais e internacionais para acidez livre, umidade e HMF. Não se evidenciou tratamentos de adulteração a partir do conteúdo de prolina, embora, a baixa atividade diastásica sugere que o mel foi submetido a algum tipo esquentamento, poderia ser durante o seu armazenamento devido às altas temperaturas típicas da região de produção. Detectaram-se estimações convencionais nos conteúdos de fenólicos e flavonoides. Em conclusão, os meles avaliados cumprem com alguns critérios de qualidade requeridos para a sua comercialização, pelo qual é conveniente ter um maior controle do processo de produção de meles no sudoeste do Casanare.

Palavras claves: mel, qualidade, fenol, flavonoides, prolina

Introduction

Honey is a natural food produced in almost every region of the world, with recognized uses -mainly as a sweetener (Chaven 2014) and an alternative medicine for the treatment of various diseases (Saxena et al. 2014; Stewart et al. 2014). The composition of a specific honey depends on the floral richness in the surroundings of the hive, but it mainly contains sugars, water, amino acids, organic acids, phenolic compounds, enzymes, pigments, waxes, volatile substances, traces of pollen, other secondary metabolites, etc. Moreover, it shows specific properties that are directly related to the origin of its components and can be assessed to determine its botanical and geographical origin and quality. Among the most common types of floral honeys commercialized worldwide, there are *Manuka*, *Eucalyptus, Citrus*, *Chestnut* and *Rosemary* (Piazza and Persano et al. 2004; Stephens et al. 2015). In particular, *Eucalyptus* honeys have been widely promoted because of the global interest on them for apitherapy as an alternative branch of medicine, involving the use of honey and honey-based products (Dor and Mahomoodally 2014) especially in the control of diseases derived from microbial infections (Ghalem and Mohamed 2008).

Colombia is a suitable country for beekeeping due to its high diversity in plants and shrubs (Betancur et al. 1997). At least five species of *Eucalyptus* plantations have been successfully established along its geography (Nates-Parra et al. 2013). One of the ways of exploiting this resource has been the introduction of beekeeping, which has taken place for many years in our country and has increasingly grown in recent years. In fact, this trend is supported by the recognition of the bee and beekeeping production chain by means of the regulation 282/2012 of the Colombian government (Colombia 2012), and by the constitution of rural associations devoted to this activity in diverse regions with different geographical and climatic conditions. In Colombia, honey is mainly produced by Africanized bees (*Apis mellifera* sp.). Villanueva is a city located in the South of Casanare in Colombia with high predominance of plantations of the specie *Eucalyptus camaldulensis*. The development of forest projects using several species of the *Eucalyptus* gender is an evidence of their good adaptation and fast growing in this region (Fernández et al. 2012), and the optimal environment for beekeeping.

Although beekeeping is being strengthened, the beekeepers must guarantee the quality criteria of the product from their beehives as a strategy to keep the product in the national market and have access to new markets. The requirements of honey for human consumption are defined in the national Regulation 1057/2010 (Colombia 2010) that provides the physicochemical parameters that must be met. Nonetheless, there is a lack of studies on characterization and quality of Colombian honeys. Therefore, this study aimed at analyzing the standard physicochemical parameters (water activity, pH, free acidity, moisture, color, electrical conductivity, ash content, reducing sugars, sucrose), HMF (which is a water-soluble heterocyclic organic compound derived from sugars), the content of phenolic compounds and flavonoids, diastase activity (as an indicator of excessive heat-treatment and possible adulteration with other sugars), and the content of proline of 24 commercial *Eucalyptus* honeys from the region of Villanueva (Casanare-Colombia), in order to increase the scientific information useful for the industrial community.

Materials and methods

Honey samples and geographical production area

Twenty four commercial honey samples (*Eucalyptus camaldulensis*) were obtained from several apiaries located in *Villanueva*, a village in the Southwest Casanare in Colombia (4° 36' 31.45" N, 72° 55' 43.65" W). All samples were between 6 and 8 months old according to the manufacturing data. The honey samples showed no signs of fermentation and crystallization. All samples were packed in glass bottles and kept away from light at a temperature below 20 °C until they were analyzed.

Physicochemical parameters

Water activity was determined using the Rotronic Hygropalm AW1, equipped with a temperature-controlled system that allows a temperature stable sampling environment; each honey sample was placed inside the sample-holder, and measurements were performed at a temperature of 20.0 ± 0.2 °C.

The pH was measured with a combined glass electrode attached to the pH meter Basic 20, in a solution containing 10 g of honey in 75 ml of distilled water. The free acidity was estimated by acid-base titration and computed as 10 times the volume of 0.05 M NaOH used in titration, and stopped at pH 8.5 (Bogdanov et al. 1997).

Moisture in honey (Association of Official Analytical Chemists 1990) was determined with an Abbe refractometer (Digital refractometer Atago model Master-Honey/Bx) at 20 °C.

The color determination was conducted by the spectrophotometric assay (Montenegro et al. 2005) on aqueous honey solutions obtained by mixing 5 g of honey and 10 ml of distilled water. This solution was kept quiescent for 15 minutes and then absorbance was determined at 650 nm. The color was expressed as mm PFund.

Electrical conductivity and ash contents were assessed by conductimetry (WTW Inolab conductivemeter, Weilheim) (Sancho et al. 1991). The ash content was determined from the following equation:

(Equation 1)

Ash content (%) = 0.083 × conductivity - 0.092"

Reducing sugars and apparent sucrose were analyzed involving the reduction of Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey (2 g), using methylene blue as an internal indicator (Bogdanov et al. 1997).

Hydroxymethylfurfural (HMF) was assessed by the application of Winkler's original method of Winkler (Bogdanov et al. 1997): a honey solution was mixed with solutions of p-toluidine and barbituric acid and the resultant color was measured against a blank at 550 nm.

The diastase activity was determined by monitoring the absorbance at 660 nm (Varian Cary Scan model 1998) to reach 0.235. The time required was estimated with absorbance measurements at 5 and 10, or 5, 15, and 20 min, depending on the activity (Association of Official Analytical Chemists 1990). The results were expressed (as Gothe degrees) as ml of 1% starch hydrolyzed by an enzyme in 1 g of honey in 1 h.

The total phenolic content was determined by means of the Folin-Ciocalteu method (Singleton et al. 1999). Honey solutions (0.5 ml) were mixed with 0.5 ml of the Folin-Ciocalteau reagent (Kanto Chemicals, Tokyo, Japan) and 0.5 ml of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h incubation at room temperature. Honey samples were evaluated at the final concentration of 20 μg/ml. The total phenolic content was expressed as mg/100 g (Gallic acid equivalents).

The flavonoids content was assayed by the Kumazawa's method (Kumazawa et al. 2004):0. 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of honey solution. After 1 h at room temperature, the absorbance was measured at 420 nm. The honey samples were evaluated at the final concentration of 20 μg/ml. The total flavonoid content was calculated as quercetin (mg/100 g) from a calibration curve.

Proline was determined according to the IHC method based on the reaction of the proline with ninhydrin in an acidic medium, and measurement of the resulting product by the absorbance at 520 nm (Bogdanov et al. 1997).

Statistical analysis

The analysis of the parameters analyzed in triplicate was performed using the SPSS Statistical package, version 20 August 2011, to obtain the descriptive analysis for 24 honey samples represented as the mean ± standard deviations.

Results and discussion

A summary of the physicochemical parameters of the 24 samples of honey is shown in Table 1, including the mean ± standard errors, minimum and maximum values. The mean water activity value of the honey samples was 0.54 ± 0.01, ranking in the expected typical interval for honey of 0.5 to 0.65. Although this parameter is not regulated by law, this is an important factor that prevents or reduces microbial growth. Some microbes as osmotolerant yeasts are able to grow at a minimal water activity of 0.60 (Chirife et al. 2006). In this respect, in the analyzed *Eucalyptus* honey samples, the water activity is enough to avoid this type of contamination and make the tested samples less vulnerable to microorganism spoilage than honeys from other departments of Colombia close to Villanueva like Antioquia (0.61-0.65) (Velásquez et al. 2013), Boyacá and Tolima (0.57-0.59) (Salamanca et al. c1995-2004).

Table 1. Physicochemical parameters of the honey samples of *Eucalyptus camaldulensis* under study

Parameter	Mean ± SD (n=24)	Range (Min-Max)
Water Activity	0.54 ± 0.01	0.537 - 0.547
pH	5.02 ± 0.03	4.98 - 5.07
Free Acidity (meq kg-1)	14.58 ± 0.51	14.0 - 15.0
Moisture (%)	16.45 ± 0.18	16.1 - 16.7
Color (mm Pfund)	91.65 ± 1.02	90.54 - 93.88
Electrical Conductivity (10^{-4} S cm^{-1})	8.13 ± 0.03	8.08 - 8.19
Ash Content (%)	0.81 ± 0.01	0.808 - 0.819
Reducing Sugars (%)	69.95 ± 0.44	69.2 - 70.42
Sucrose (%)	1.48 ± 0.03	1.42 - 1.55
HMF (mg kg^{-1})	67.29 ± 5.96	60.48 - 74.30
Diastase Activity (°Gothe)	3.21 ± 0.04	3.14 - 3.27
Phenolics (mg/100 g)	76.57 ± 1.47	74.51 - 78.84
Flavonoids (mg/100 g)	5.96 ± 0.17	5.71 - 6.18
Proline (mg kg^{-1})	180.68 ± 2.58	176.02 - 184.62
SD: standard deviation		

Source: Own preparation

The pH is not a parameter regulated by law yet, but it influences the formation of HMF, texture and stability during honey extraction and storage (Terrab et al. 2004). The tested samples were found to be acid (5.02) and within the common values in floral honeys; nonetheless, they have a higher pH than those determined in similar honeys from other places like Turkey (3.80) (Sorkun et al. 2001) and Iberian Peninsula (4.10) (Rodríguez et al. 2014).

The free acidity value was below 50 meq kg^{-1} (14.58 meq kg^{-1}), meeting the national and international requirements and indicating the absence of undesirable fermentations. This value suggests a less active acidity than honeys of the same species but from different geographical regions whose values even reached the double (Sorkun et al. 2001; Piazza and Persano 2004).

Eucalyptus honeys showed moisture content (16.45 %) below the limit legally established by Colombian and International regulations (≤ 20 %). This is the only standard criterion that has to be fulfilled in world honey trade (Iglesias et al. 2012). It influences honey color and flavor and depends on environmental conditions (Gomes et al. 2010). The result is similar to values found in other honeys (Bath y Singh 1999; Sorkun et al. 2001; Piazza and Persano 2004; Rodríguez et al. 2014).

Color plays a key role in the initial appreciation of consumers. The *Eucalyptus* honeys from Casanare (Colombia) tend to be darker than European, provided that their mean value (91.65 ± 1.02 mm Pfund) is higher than the reported (55-73 mm Pfund); yet, all are within the amber tonalities. This fact can be explained by the content of pigments (carotenoids, flavonoids, etc.) and its association with the content of phenolic compounds, which largely depend on the botanical and geographical origin (Martos et al. 2000; Kumazawa et al. 2004; Bentabol et al. 2014).

The electrical conductivity (0.813 mS cm^{-1}) was slightly higher than the requirements of the Colombian regulations, but still within the limit provided by the European Directive for Eucalyptus honey and in line with other data reported for analogous honeys (Sorkun et al. 2001; Piazza and Persano 2004; Benaziza-Bouchema and Schweitzer 2010; Chakir et al. 2011; Rodríguez et al. 2014).

The evaluated honey samples of Casanare showed a mean value of ash content (0.81 %) higher than expected according to Colombian quality criteria and those reported in the bibliography by Sorkun et al. (2001). This parameter is an indicator of mineral composition and could be useful for the classification of honeys from geographic origin. This fact may lead to presuming a possible adulteration, e.g. a dilution using enriched mineral water. This assumption needs to be confirmed, for which their exact origin (area, beehive) must be established.

Although these two previous parameters exceeded the requirements (≤ 0.8 mS cm^{-1} and 0.6 %, for EC and ashes respectively), the literature reports that some monofloral honeys such as those of chestnuts, strawberry plants, heather, eucalyptus, lime, manuka, tea tree or jelly bush honeys have achieved values above the standard (Persano et al. 1995).

The evaluated honeys thoroughly complied with the HMF limitation for samples of tropical origin, 80 mg kg^{-1}, established in the standard codex for honey (Codex Alimentarius Standard 2001). These results show the samples are fresh, despite their storage time. This value is above the one reported by other authors (Sorkun et al. 2001; Benaziza-Bouchema and Schweitzer 2010; Rodríguez et al. 2014); we can infer that other tested honeys were fresher than the ones analyzed here.

Other research studies with *Eucalyptus camaldulensis* and comparable honeys (Sorkun et al. 2001; Piazza and Persano 2004; Benaziza-Bouchema and Schweitzer 2010; Rodríguez et al. 2014) have reported a diastase mean value higher than 13.2° Gothe, representing a low enzymatic content related to the period of year in which the production is done, and the period of brood feeding. The results show a very low production of enzymes provided that the mean diastase activity (3.21 °Gothe) did not fulfill the minimum value required by law (8 °Gothe). The decrease of this diastase activity is typical in old or heated honeys.

Although the proline content is not defined in Colombian or International regulations, this parameter represents the level of the total amino acids in honey and it is measured as a criterion for estimating its quality and antioxidant properties. A content of proline below 183 mg kg^{-1} indicates sugar adulteration (Bogdanov et al. 1997). The honey samples studied had proline levels close to 183 mg kg^{-1}, indicating absence of adulteration. Sorkun et al. (2001) stated that the average of proline of *Eucalyptus camaldulensis* honeys from Turkey was 82.23 mg/100 g. The value we found is almost four times lower (18.07 mg/100 g). Compared to the European (Piazza and Persano 2004) and Indian *Eucalyptus* (Bath and Singh 1999) honeys, our mean value was also lower. The results are more consistent with those reported for African honey

samples (Meda et al. 2005). The differences between the values reported by other researchers and those found in this study basically reveal a remarkable differentiation in the content of proline in the samples of different origin.

The total phenolic (mg Quercetin/100 g) and flavonoids (mg Gallic acid/100 g) content in the samples analyzed were 76.57 ± 1.47 and 5.96 ± 0.17, respectively. These values were similar to those found by Rodríguez et al. (2014) in honeys of the same genus. The total phenolic content was below the average reported by Ciappini and Stoppani (2014) but the flavonoids content was slightly higher. The content of flavonoids detected in these Colombian eucalyptus honeys was higher than that of European honeys (Meda et al. 2005). These findings could be a start point to tackle a study Gasicrelated to the identification of the identification of regional origin product based on specific phenolic contents but further research is needed (Gašić et al. 2014) compounds as it was intended by Gasic et al. (2014), but further investigations are needed.

Conclusions

The physicochemical parameters of the twenty four commercial honeys labeled as *Eucalyptus camaldulensis* and purchased from Southwest Casanare that were studied satisfactorily met most of the requirements established by the Colombian honey regulations and those reported for similar species from other origins. The proline content resulted closer to that determined for African honeys from regions with comparable altitude to Villanueva. Low Diastase activity values may indicate an inadequate processing or storage of these samples and a certain ageing. The total phenolic and flavonoids contents differ from European *Eucalyptus* honeys. Therefore, in the context of marketability of honeys, beekeepers should give the composition data to the consumers.

Conflicts of interest

The authors declare they have no conflicts of interest in the work presented in this report.

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