academicJournals

Vol. 9(27), pp. 1692-1698, 8 July, 2015
DOI: 10.5897/AJMR2015.7494
Article Number: FF86A1454163
ISSN 1996-0808
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Quality of honey sold in the state of Alagoas, Brasil

Thaís Patrícia Alves, Tania Marta Carvalho dos Santos*, Cícero Cerqueira Cavalcanti Neto, Roger Nicolas Beelen, Sybelle Georgia Mesquita da Silva and Yamina Coentro Montaldo

Universidade Federal de Alagoas. Centro de Ciências Agrárias, BR 104 Norte, Km 85, Rio Largo, Alagoas, Brasil.

Received 23 March, 2015; Accepted 6 July, 2015

The present study aimed to determine the quality of honey marketed in the State of Alagoas, Brazil. Fifteen samples of *Apis mellifera* L. honey sold in supermarkets, free trade, and cooperative located in the State of Alagoas were acquired. Microbiological and physical-chemical analyzes were carried out to establish a standard microbiology condition and check for possible tampering. The physico-chemical analyzes showed that all the samples studied presented acid pH values ranging between 2.3 and 4.4. For diastase activity and reaction, Lugol which are indicative of the presence of starch and dextrin, and reaction Fiehe, which is a qualitative indicator of HMF, all samples were negative for at least the parameters. As the microbiological standard, 26.6% of all samples showed high standard count mesophilic aerobic bacteria, 20% had counts of molds and yeasts above the quality standards established by Brazilian law. For enumeration of coliforms at 35 and 45°C, it was found that most samples were contaminated (86.7%). It is the presence of sporulated bacteria in 13.3% of the samples, which were 15.26 and 84.64% genus *Clostridium* of the genus *Bacillus.*

Key words: Apiculture products, contamination, physico-chemistry, microbiology, *Clostridium botulinum.*

INTRODUCTION

Honey is a complex mixture of sugars (35% glucose, 40% fructose, and 5% sucrose) and highly concentrated organic acids, enzymes, vitamins, flavonoids, mineral and a wide variety of organic compounds that contribute to its characteristics sensory and nutritional (Serrano, 1994). Its composition depends on the nectar of the components of the production plant which it gives the product its specific characteristics.

Honey is an acid food, with low humidity and water activity. Its viscosity is high due to high concentrations of sugars, and osmotic pressure. These conditions make honey slightly favorable substrate for microbial development. However, it may be caused by the bee microflora itself, lack of hygiene in the extraction and processing, including pollen, floral nectar, dust, dirt and the body itself and bee digestive tract, as well as fungi and some bacteria (Snowdon and Cliver, 1996; Bogdanov, 2006; Rissato et al., 2007; Rial-Otero et al, 2007; Kujawski and Namiesnik, 2008).

Another factor rarely considered is the length of the production cycle. The time of flowering station can interfere with the microbiological quality of honey since in

*Corresponding author. E-mail: tesfalem2002@gmail.com.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

low food availability, bees can forage in fungal colonies (Snowdon, 1999) or even feces and other sources of organic matter (Nogueira Neto, 1997).

The osmophilics microorganisms comprise those able to grow and multiply in honey (Ward and Trueman, 2001); other microbial groups which can be found in honey are spore-forming bacteria. These microorganisms can be directly related to the deterioration of the product, production of enzymes, toxins, metabolic conversion of food, the production of growth factors (vitamins and amino acids) and inhibition factors of competing microorganisms (Silva et al., 2008). Usually acidic, high water activity and high humidity are the main factors responsible for the development of these microorganisms (Bogdanov, 2009). The microbiological analysis to determine which and how many microorganisms are present are of fundamental importance to know the hygiene conditions in which food was prepared, the risks that food can offer the consumer health and life span required. This analysis is necessary also to verify that standards and microbiological specifications for foods, domestic or international, are being met adequately.

Honey is subject to variations in its aroma, taste, color, viscosity and medicinal properties. However, these features can also be modified by tampering the generation by unreliable sources who misuse the product, adding in composition lower commercial substances and nutritional value (Ribeiro et al., 2009). Tampering is generally carried out with the addition of other carbohydrates, particularly sugars such as commercial glucose solution or sucrose syrup and invert sucrose solution, from cane or corn (Rossi et al., 1999).

These changes are detected by domel physical-chemical analysis, as in the case of qualitative analysis of hydroxymethylfurfural (Reaçãode Fiehe) which, when in high concentration shows the heating of honey, or addition of sugar syrups or artificial feeding of bees honey. Bodganov et al. (1997) reported that honey damage caused by heating can be evidenced by determining the HMF content and activity of the diastase, since these parameters together are used as indicators for intensive heating (Ramirez et al., 2000). According to Wiese (2000), the lugol test reaction indicates the adulteration of starch and dextrin which does not occur in pure honey. Another analysis is pH, which when below or above the level permitted, can favor the growth of bacteria, which can spoil the honey and affect the quality, as well as the acidity analysis when at high level.

The objective of this study was to evaluate the *Apis mellifera* bee honey quality marketed in the state of Alagoas- Brazil through analysis of microbiological and physical-chemical parameters.

MATERIALS AND METHODS

The experiment was conducted in the microbiology laboratory at the Academic Unit Centre for Agricultural Sciences (CECA-UFAL), located on Rio Largo district, Zona da Mata Alagoas (9 27' latitude 54.8'' S and longitude 35° 49' 59 7" W), from January to May 2013. The city is situated at an altitude of 127 m, with average maximum temperatures of 29°C and minimum of 21°C and average annual rainfall of 1,268 mm.

Honey samples

The samples were acquired at collection points such as supermarkets, grocery stores, and cooperative located in the State of Alagoas. From November to December 2012, we obtained 15 samples of honey from *A. mellifera* L., where five were acquired in own commercial packaging of independent apiaries produced in this state, settled (had some inspection seal) or not; bee different regions of the State of Alagoas (MM1, MM2, MM3, MM4 andd MM5) and another 10 provided by coopmel (Mel Cooperative State MC6, MC7, MC8, MC9, MC10, MC11, MC12, MC13, MC14 and MC15). All samples were taken to the Academic Unit of Microbiology Laboratory Centre of Agricultural Sciences, Federal University of Alagoas, where they were examined.

Processing of samples

Twenty five grams of each sample (were aseptically collected and added with 225 mL of 0.1% sterile peptone water (SPW), that had 1:10 dilution, were homogenized in shaker orbital at 2,000 rpm for 30 min. The total mesophilic aerobic and psychrotrophic bacteria counts were carried out in pour plate using plate count agar (PCA) followed by incubation at 35°C for 48 h for mesophilic bacteria.

Coliforms at 35 and 45°C were counted through the most probable number (MPN), with three sets of three tubes. Lauryl sulfate tryptose broth (LST) was used as a presumptive medium and incubated at 35°C for 24-48 h. After reading, the positive tubes were transferred to brilliant green bile broth (2%, GB) and EC broth. Then was incubated at 35°C for 24-48 h; for confirmation of total coliforms and EC broth tubes, they were incubated in a water bath at 45°C for 24 h for confirmation of thermotolerant coliforms.

The homogenate used for microbiological characterization was subsequently used for the isolation of bacteria. Isolation of *Clostridium* was performed by seeding 1 ml each decimal serial dilution in triplicate in 10 mL of Cooked Meat Medium (CMM); the tubes were immediately moved to a water bath at 65°C for 30 min in order to inactivate the microorganisms spore. The samples were incubated at 35°C for seven days.

After the incubation period, the cultures were observed for turbidity, gas production, and digestion of meat particles in broth. Cultures with insignificant growth were reincubated in the oven at the same temperature previously used for three days, completing a maximum period of ten days. Cultures still without growth were discarded because they were considered negative.

The positive samples were subjected to Gram's method for detection of Gram-positive bacilli sporulated or not. Positive cultures were seeded to Petri plates containing Anaerobic Egg Yolk Agar (AEY) and incubated anaerobically in Colorina pot, at 35°C for seven days. Typical obtained colonies were re-isolated in plate in duplicate in medium containing AEY and each incubated aerobically and anaerobically at Colorina pot; both at 35°C for 48 h. Later blades were made for the plates for staining by the gram method to detect Gram-positive bacilli.

The isolation of yeasts and molds was carried out using 0.1 mL of seeding on the surface of each agar dilution dicloran Rose Bengal Chloramphenicol, followed by incubation at 25°C for five days. The colony forming units were calculated using the following formula:

CFU g = X.DF/ V

Where, X = average of each dilution, DF = dilution Factor and V =

Table 1. Microbiological parameters of honey of *Apis mellifera* obtained from independent beekeepers and cooperative in the state of Alagoas-Brazil.

Sample	Aerobic mesophilic bacteria	Molds and yeasts	Coliforms	
			35°C	45°C
		CFU.g^{-1}	MPN.g^{-1}	
MM1	1.5x10^{7}	-	0.20	0.15
MM2	-	-	0.16	0.09
MM3	-	-	>24.00	0.53
MM4	-	-	>24.00	0.44
MM5	-	2.2x10^{7}	<0.03	<0.03
MC6	-	-	0.04	0.04
MC7	-	-	0.09	0.03
MC8	-	3.4x10^{7}	0.04	0.07
MC9	-	-	<0.03	<0.03
MC10	7.4x10^{5}	2.5x10^{7}	>24.00	0.44
MC11	-	-	0.19	0.12
MC12	-	-	>24.00	0.75
MC13	4.2x10^{4}	-	0.19	0.03
MC14	1.7x10^{6}	-	>24.00	<0.03
MC15	-	-	0.03	0.06

volume dilution added to the Petri dish

Determination of pH, qualitative test HMF (Fiehe reaction); lugol reaction and determination of diastase activity were performed according to the methods proposed in the standards of the Institute (Adolfo Lutz, 2008). All analyses were done in triplicate and the mean values were used for the statistical evaluation.

The results were submitted to descriptive statistics (mean, standard deviation and coefficient of variation). For statistical analysis, logarithmic transformation (log10) was used for mesophilic microorganisms count, MPN of coliforms, molds and yeast to in order to normalize the distribution frequency.

RESULTS AND DISCUSSION

Table 1 shows the results of the microbiological analyzes of the samples. The presence of the mesophilic aerobic bacteria on four samples were detected which corresponds to 26.6%. The maximum and minimum values MM1 and MC13 samples were respectively 7.4x10^{5} and 4.2x10^{4} CFU.g^{-1}. Presence of yeasts and molds was observed in samples MC5, MM8 and MM10 (20% of the samples); the values obtained were 2.2x10^{7}, 3.4x10^{7} and 2.5x10^{7} CFUg^{-1} respectively. With respect to coliforms at 35°C and coliforms at 45°C, it was observed that only 2 (13.3%) samples had lower results than 3.0 MPNg^{-1}, that is absence in 86.7% of samples; a high rate of contamination was detected in four of them and the presence of coliforms at 35°C was observed higher than the level 24.0 MPNg^{-1}.

Chemical and physical properties of honey can inhibit or destroy the microorganisms. Several authors report showed a strong antibacterial activity, including human and animal pathogens (Iurlina and Fritz, 2005; Kačaniová et al., 2009; Adenakan et al., 2010).

However, the honey production and processing involves different steps through which some microorganisms can survive or even multiply. Primary sources of microbial contamination probably include the pollen, the digestive tracts of honeybees, dust, air, earth and nectar - sources that are very difficult to control. The same secondary (post-harvest) sources that influence other food products are also sources of contamination for honey. These include air, food handlers, cross-contamination, equipment and buildings. Secondary sources of contamination are controlled by good manufacturing practices (Kačániová, 2004; Olaitan et al., 2007).

The Brazilian legislation (Brazil, 2000) does not set values for mesophilic aerobic bacteria in honey but establishes only that you follow good hygiene practices in handling and processing of this product because entire microbial load in honey can indicate the possible presence of pathogens. Therefore, the default score has been used as an indicator of hygienic quality of food, including the cleaning, disinfection and control of environmental health during processing, transport and storage, and providing also of idea about its useful shelf life.

The results were superior to those obtained by Malika et al. (2005) and Schlabitz et al. (2010) and lower than those presented by Melo (2013). According to Snowdon and Cliver (1996) variation in the number of bacteria seems to depend on the type of sample, the age and the honey harvest time. These vegetative forms can be made by secondary contamination which would also explain the high counts sometimes found in honey.

The results obtained for standard counting of molds

and yeasts showed that 20% of samples had values above the maximum established by the Brazilian technical standards for food, RDC 012 (Brazil, 2001), being considered unfit for direct human consumption.

Snowdon and Cliver (1996) found that yeast is one of the most important microorganisms that interfere with the quality of honey. Typically this yeast presence in the samples, can be detected in high concentrations; they survive under acidic conditions and are not inhibited by sucrose. These osmophilic yeasts (tolerant sugar) represent a problem in honey industry because they have the ability to grow at low water activity.

The contamination in honey may occur naturally, where the fungi are brought to the hive by bees or by the absence of the use of good apicultural practices during handling of the hives; it is worth emphasizing the importance of continuous monitoring throughout the honey processing, to ensure the marketing of a reliable food.

The presence of yeasts and molds is generally accepted for all honey, however the biggest problem is related to fermentation of the product, resulting in the hydrolysis of sugars to produce alcohol and carbon dioxide, changing the taste and the flavour of honey (White Jr, 1978).

In fresh honey, the number of yeasts and molds is generally low, but under certain conditions these organisms are able to multiply in honey during storage, especially in honeys with high moisture content and water activity (Martins et al., 2003; Iurlina and Fritz, 2009; Kačaniová et al., 2009; Carvalho et al., 2010; Różańska and Osek, 2012). Jimenez et al. (1994) observed a significant increase in the number of yeasts and molds with storage time.

Other work related to the quantification of microorganisms in honey found similar results. In Cameroon honey samples, Tchoumboue et al. (2007) found the presence of contamination by microorganisms in more than 73.4% of the samples, attributing this contamination to post-harvest processing or tampering of the product, since their witness honey sample did not show these levels of contamination. Finola et al. (2007) determined that lower count of 1.0x10 $CFUg^{-1}$ in molds and yeasts in all samples.

The results observed for coliforms at 35°C, suggest a failure to follow good practices of manipulation of honey and that the presence of these microorganisms also constitutes an indicator of the possible presence of other pathogenic microorganisms that are more difficult to detect. The presence of enterobacteria in total honey originates from fecal contamination which is evidence of poor condition of extraction and processing and their own marketing.

The results coincide with those obtained by other authors. Gomes et al. (2010) isolated *Salmonella* spp., *Coliforms* and *E. coli* in Portugal at a 34% rate; Kokubo et al. (1984) analyzed 70 samples of honey and isolated coliforms at a rate of 95.7%. Dumen et al. (2013) studied the honey produced in Istanbul and verified the presence of coliforms in 18% of 80 samples.

Table 2. Gram positive confirmation in culture medium AEY.

Sample	Anaerobic organism	Aerobic organism
MC4	+	+
MM9	+	+

The major quantitative indicators of microorganisms can be related to the collection period of pollen by bees. According to Barth (2004) when there is shortage of flowers, bees can forage in the most diverse substrates, from fungal colonies through soil, clay and even matter organic fecal origin. Based on this, it is desirable that areas close to breeding sites are free from other ranchers activities such as the creation of other animals. Matos et al. (2011) found that honey samples collected from hives that had potential contamination sources in the environment such as cattle dung, showed high counts of these microorganisms.

For the detection of *Clostridium* sulfite reducers in the samples, analyzes were performed by means of cooked meat; after the incubation period, 13 samples were discarded by negative results, they were: MC1, MC2, MC3, MC5, MM6, MM7, MM8 , MC10, MC11, MC12, MC13, MC14, MC15; the cultures in which they observed turbidity, gas production, digestion of meat particles in the broth represented a total of 13.3% of the samples and these were subjected to Gram's method for detection of Gram-positive bacilli sporulated or not.

The two positive samples were stained by the Gram method, and the presence of Gram-positive bacilli were detected and then were passed to the Petri dishes containing the AEY, incubated aerobically and anaerobically and submitted again to the Gram stain for confirmation of Gram-positive bacilli. The results are shown in Table 2.

The results of this study demonstrate the presence of sporulated bacteria in 13.3% of the samples identified by smear slide and stained by the Gram method, both under aerobic and anaerobic conditions. Biochemical tests showed that 15.26% were genus *Clostridium* and 84.64% of the genus *Bacillus*.

Although honey is a hostile environment for the growth of food-borne pathogenic bacteria, spores and vegetative latent forms may be present due to primary and secondary contamination. Spore-forming bacteria such as *Bacillus cereus* and *Clostridium* spp. are regularly found in honey. Pucciarelli et al. (2014) found the incidence of *Clostridium* and *Bacillus* (42.85 and 39% respectively) in yateí honey, Argentina. Ragazani et al. (2008) studying honey marketed in several Brazilian states found 39% sulfite-reducing bacteria, and 11% were *Clostridium* genus and 28% of the genus *Bacillus*.

Table 3. pH, diastatic activity, lugol reaction and qualitative analysis of hydroxymethylfurfural (Fiehe reaction) in honey bees *Apis mellifera* L. marketed in Alagoas-Brazil.

Sample	pH	Diastase activity	Lugol's iodine reaction	Fiehe reaction
MM1	2.4	-	+	-
MM2	2.7	-	-	+
MM3	3.0	-	-	+
MM4	2.5	-	+	+
MM5	2.3	-	+	-
MC6	2.8	-	-	-
MC7	3.5	+	-	+
MC8	3.1	-	-	+
MC9	3.6	-	-	+
MC10	4.4	+	-	+
MC11	3.3	-	-	-
MC12	3.6	-	-	+
MC13	3.0	-	-	+
MC14	3.0	-	-	+
MC15	3.4	-	-	+

The presence of bacteria of the genus *Bacillus* spp. honey would be expected, since there is a symbiotic relationship between these microorganisms and insects including bees (Nicholson, 2002). *C. botulinum* is a bacterium of the bacterial type, straight or semi-curved, gram-positive spore, mobile, strictly anaerobic and has sulfite-reducing activity that is common in soil, air and environmental waters and can be found in various foods. This bacterium produces toxins that cause digestive and neurological disorders in the patient; the disease known as botulism is a very serious disease.

The incidence of *C. botulinum* spores in honey has been estimated in several studies. Sugiyama et al. (1978), using the dialysis method of 241 samples of honey in USA, reported the presence of *C. botulinum* spores in samples originating from 18 States: California, Florida, Iowa, Michigan, Minnesota, Nebraska, Tennessee, Texas and Washington etc. In experiment conducted by Midura et al. (1979), *C. botulinum* were isolated from nine samples from 90 honey samples analyzed. Among these, six samples were provided to infants, who developed the disease.

Küplülü et al. (2006) isolated *C. botulinum* from 12.5% of the retail market honey samples in Ankara, Turkey. Ragazani et al. (2008) isolated bacteria from 11% of the samples; Schocken-Hurrino et al. (1999) detected *C. botulinum* in 7% of Brazilian honey samples.

The evidence of tampering were carried out according to the Analytical Standards Institute Adolfo Lutz. All analyzes were performed in triplicate. The results of the physical-chemical analysis are presented in Table 3.

All samples had pH values ranging between 2.3 and 4.4; for enzymatic activity only two honey (13.3%) MC7 and MC10 showed positive result. Three samples (20%) showed a positive reaction to lugol. Regarding the Fiehe reaction, 73.3% of samples (11) were salmon-colored red cherry, that is positive reaction.

There is no national or international rules setting limits for pH (Silva et al., 2004) but it is a very important parameter for obtaining and honey storage for its influence on the development of microorganisms and enzymes. It also affects the physical properties of the product such as a texture, stability and resistance.

Variations in pH observed, according to Crane (1990) are probably due to peculiarities of the composition Floristics collection areas, since the pH of the honey can be influenced by the pH of nectar. In addition, differences in soil composition, or the association of plant species for final composition of honey, can also influence the pH of this product.

Substances present in the jaw bees are added during transport to the hive which can change this factor. All samples showed acid pH; the acids added by bees contribute to the taste of the honey and stability against microbial growth, and the main gluconic acid resulting from oxidation of glucose by glucose oxidase (Bogdanov et al., 2004).

The most important enzyme in honey is invertase, also known as sucrase, whose function is to convert nectar honey, since it acts by hydrolyzing sucrose and generating final products, glucose and fructose (White, 1975).

According to Huidobro and Simal (1984a) there are three very important enzymes for honey: amylase, invertase and glucose oxidase. The diastase activity in honey, usually quantified by α-amylase, is a quality factor that can be changed during processing and storage of honey, so it is used as heating and freshness indicator (Bogdanov et al., 2006).

The diastase activity varies with the botanical origin of

honey; many countries require minimum amounts of diastase or amylase activity, which is easily degraded by aging and the action of heat, disappearing half its content in 17 months at room temperature. However, when interpreting the results of diastase activity, one must consider that some monofloral honeys such as the citrus have a natural low activity (Huidobro and Simal, 1984a), implying an analysis that has limited power as deterioration indicator (Bogdanov et al., 1997).

Lugol reaction yielded positive results indicating the presence of starch and dextrin in three (20%) samples. Honey is formed carbohydrates composed of mono- and oligosaccharides obtained from flower nectar, which does not have in its composition polysaccharides such as starch. The reaction with Lugol's shows the presence of starch (large molecule formed by the union of several hundred glucose molecules / natural energy reserve of the plants) and dextrin (polysaccharide class of low molecular weight) in honey. The positive result is indicative of adulteration of the product with starch and dextrin.

Regarding the reaction Fiehe, 73.3% of the samples had salmon color red cherry, that is positive reaction to the test, being at odds with Brazilian legislation (Brazil, 2000). The hydroxymethylfurfural (HMF) is not a normal component of honey; it is a cyclic aldehyde formed at room temperature by fructose dehydration in acid medium (pH 3.9), a process that is accelerated by heating or storage at elevated temperatures (Huidobro and Simal, 1984b).

The content of HMF, is directly related to the heat that has undergone honey and the degree of aging (Bosch and Serra, 1986). Its presence causes the browning interactions with amino compounds and sugars, undergoing polymerization and rearrangement in the presence or in the absence of oxygen. The results indicate that these samples may have been subjected to overheating conditions, high temperature or stored with addition of sugar syrup or corresponds to an old honey. The HMF concentration is also associated with the existing enzyme activity, so that those honeys with low index diastases possibly have high numbers of hydroxymethylfurfural which would be indicative of improper storage.

Conclusion

At the end of this study, it was observed that none of the samples showed all microbiological and physico-chemical parameters within acceptable limits. With tamper analysis, it was observed that there is a need to identify factors that result in overheating of these samples so that preventative measures can be taken, since in these cases important properties of honey may be lost. The quality of honey can be affected by management during harvest, thus the beekeeper must perform the appropriate procedures from the time of withdrawal of honey from hives to transportation of the extraction unit, in order to interfere as little as possible with the hygienic sanitary quality.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Foundation for Research In Alagoas State (FAPEAL) is acknowledged.

REFERENCES

Adenekan MO, Amusa NA, Lawal AO, Okpeze VE (2010). Physico-chemical and microbiological properties of honey samples obtained from Ibadan. J. Microbiol. Antimicrob. 2(8): 100-104.
Barth OM (2004). Melissopalynology in Brazil: a review of honeys, propolis an pollen loads of bees. Sci. Agric. 61(3): 342-350.
Bogdanov S (2006). Contaminants of bee products. Apidologie 37(1):1-18.
Bogdanov S (2009). Harmonised methods of the International Honey. Commission. IHC: 1-61.
Bogdanov S, Martin P, Lüllmann C (1997). Harmonised methods of the European Honey Commission. Apidologie, extra issue: 1-59.
Bogdanov S, Ruoff K, Oddo L (2004). Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie 35:S4-S17.
Bosch J, Serra J (1986). Variations of the contents of HMF in processed honeys on the Spanish market. Alimentaria 23: 59-61
Brazil (2001). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 12 de 02 de janeiro de 2001. Approves the Technical Regulation on Microbiological Standards for Food. Diário Oficial da República Federativa do Brasil, Seção 1: 45.
Brazil (2000). Ministério da Agricultura e do Abastecimento. Instrução normativa n° 11, de 20 de outubro de 2000. Technical regulation for identity and quality of honey. Diário Oficial da República Federativa do Brasil. Seção 1:16-17.
Carvalho CM, Meirinho S, Estevinho MLF, Choupina A (2010). Yeast species associated with honey: different identification methods. Arch. Zootec. 59(1):103-113.
Crane E (1990). Bees and beekeeping-science, practice and world resources. London: Neinemann Newnes. 614 p.
Dumen E, Akkaya H, Öz GM, Sezgin FH (2013). Microbiological and parasitological quality of honey produced in İstanbul. Turk. J. Vet. Anim. Sci. 37(5): 602-607.
Finola MS, Lasagno MC, Marioli JM (2007). Microbiological and chemical characterization of honeys from central Argentina. Food Chem. 100(4):1649-1653.
Gomes S, Dias L, Moreira L, Rodrigues P, Estevinho L (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food Chem. Toxicol. 48(2):544-548
Huidobro JF, Simal J (1984a). Parametros de Calidad de la miel V: índice de diastasas. Offarm. 3(11):705.
Huidobro JF, Simal J (1984b). Parámetros de Calidad de la miel VI: hidroximetilfurfural. Offarm. 3(12):767.
Institute Adolfo Lutz (2008). Physical and chemical methods for food analysis. Coordinators Odair Zenebon, Neus Sadocco Pascuet and Paulo Tiglea: Instituto Adolfo Lutz, IV ed. São Paulo. pp. 330-332.
Iurlina MO, Fritz R (2005) Characterization of microorganisms in Argentinian honeys from different sources. Int. J. Food Microbiol. 105(3):297-304.
Jimenez M, Mateo JJ, Huerta T, Mateo R (1994) Influence of the storage conditions on some physicochemical and mycological

parameters of honey. J. Sci. Food Agric. 64(1):67-74.
Kačániová M, Chlebo R, Kopernický M, Trakovická A (2004). Microflora of the honeybee gastrointestinal tract. Folia Microbiol. 49(2):169-172.
Kačaniová M, Melich M, Kňazovická V, Haščik P, Sudzinova J, Pavličova S, Čuboň J (2009). The indicator microorganisms value in relations to primary contamination of honey. Lucrări Stiintifice Zootehnie Biotehnol. 42 (2): 159-163.
Kokubo Y, Jinbo K, Kaneko S, Matsumoto M (1984). Prevalence of spore forming bacteria in commercial honey. Ann. Rep. Tokyo Metr. Res. Lab. Public Health 35: 192-196.
Kujawski MW, Namiesnik J (2008). Challenges in preparing honey samples for chromatographic determination of contaminants and trace residues. Trends Anal. Chem. 27(9): 785-793.
Küplülü O, Göncüoðlu M, Özdemir H, Koluman A (2006). Incidence os *Clostridium botulinum* spores in honey in Turkey. Food Control 17(3):222-224.
Malika N, Mohammed F, Chaucib E, (2005). Microbiological and physicohemical properties of Morrocan honey. Int. J. Agric. Biol. 7(5):773-776.
Martins HM, Martins ML, Bernardo FMA (2003). *Bacillaceace* spores, fungi and aflatoxins determination in honey. Rev Port Cien Vet. 98(1): 85-88.
Matos ITSR, Nunes MT, Mota DA, Laureano MMM, Hoshiba MA (2011). Microbiological quality of honey from *Melipona* sp. produced in Central Amazon rainforest (Parintins-AM-Brazil) Rev. Verde Agroecologia Desenvolv. Sustent. 6(4):91- 95.
Melo LM (2013). Microbiological Quality of honeys marketed Bees in Maceió. Monography degree in animal science. Center of Agrarian Sciences Federal University of Alagoas.67p
Midura TF, Snowden S, Wood RM, Arnon SS(1979). Isolation of Clostridium botulinum from Honey. J. Clin. Microbiol. 9(2): 282-283.
Nicholson WL (2002). Roles of Bacillus endospores in the environment. Cell Mol. Life Sci. 59(3):410-416.
Nogueira-Neto P (1997). Life and creation of indigenous stingless bees. São Paulo: Nogueirapis. 446p.
Olaitan PB, Adeleke OE, Ola IO (2007). Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr. Health Sci. 7(3):159-165.
Pucciarelli AB, Schapovaloff ME, Kummritz S, Señuk IA, Brumovsky LA, Dallagnol AM (2014). Microbiological and physicochemical analysis of yateí (*Tetragonisca angustula*) honey for assessing quality standards and commercialization. Rev. Argent. Microbiol. 46(4):325-332.
Ragazani AVF, Schoken-Iturrino RP, Garcia GR, Delfino TPC, Poiatti ML, Berchielli SP (2008). *Clostridium botulinum* spores in honey commercialized in São Paulo and other Brazilian states. Cienc. Rural 38(2):396-399.
Ramírez S, Dressler RL, Ospina M (2002). Abejas euglossinas (Hymenoptera: Apidae) de la región Neotropical: lista de especies con notas sobre su biología. Biota Columbia. 3(1): 7-118.
Rial-Otero R, Gaspar EM, Moura I, Capelo JL (2007). Chromatographic-based methods for pesticide determination in honey: An overview. Talanta71(2): p.503-514.
Ribeiro ROR, Silva C, Monteiro ML, Baptista R, Guimarães CF, Mársico ET, Mano SB, Pardi HS (2009). Comparative evaluation of physicochemical properties of different types of honey, selec ted from inspected and non-inspected samples, on sale in Rio de Janeiro state, Brazil. Rev. Bras. Ciênc. Vet. 16(1): 3-7.
Rissato SR, Galhiane MS, Almeida MV, Gerenuti M, Apon BM (2007). Multiresidue determination of pesticides in honey samples by gas chromatography-mass spectrometry and application in environmental contamination, Food Chem. 101(4): 1719-1726.
Rossi NF, Martinelli LA, Lacerda THM, Camargo PB, Victória RL (1999). Honey adulteration analysis by commercial sugars additions using the stable carbon isotope composition. Food Sci. Technol. 19(2):199-204.
Różańska H, Osek J (2012). Effect of storage on microbiological quality of honey. Bull. Vet. Inst. Pulawy. 56(2): 161-163.
Schlabitz, C, Silva SAF, Souza CFV (2010). Evaluation of physical-chemical and microbiological parameters in honey. Rev. Bras. Prod. Agroind. 4(1): 80-90.
Schocken-Hurrino RP, Carneiro MC, Kato E, Sorbara JOB, Rossi OD, Gerbasi LER (1999). Study of the presence of the spores of *Clostridium botulinum* in honey in Brazil. FEMS Immunol. Med. Microbiol. 24(3): 379-382.
Serrano RB, Villanueva MTO, Marquina AD (1994). Honey. Natural sweetener par excellence II . Composition , production and legislation. Alimentaria 253(9): 29-38.
Silva CL, Queiroz AJM, Figueiredo RMF (2004). Physical and chemical characterization of honeys produced in the State of Piauí - Brazil. Rev. bras. Eng. Agríc. Ambient. 8(2-3): 260-265.
Silva MBL, Chaves JBP, Message D, Gomes JC, Gonçalves MM, Oliveira GL (2008). Microbiological quality of honey produced by small beekeepers and honey warehouses registered in the Federal Inspection Service in State of Minas Gerais. Braz. J. Food Nutr. 19(4):417-420
Snowdon JA(1999). The microbiology of honey-meeting your buyers' specifications (Why they do what they do). Am Bee J. 1(1):51-60.
Snowdon, JA, Cliver DO (1996). Microorganisms in honey. Int. J. Food Microbiol. 31(1-3): 1-26.
Sugiyama H, Mills DC, Kuo LJC (1978). Number of *Clostridium botulinum* spores in honey. J. Food Prot. 41(11): 848 – 850.
Tchoumboue J, Awah-Ndukum J, Fonteh FA, Dongock ND, Pinta J, Mvondo ZA (2007). Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon. Afr. J. Biotechnol. 6 (7):908–913.
Ward WH, Trueman KF (2001). Microbiological quality y of Australian honeys. RIRDC.01/049.
White Júnior JW (1978). Honey. Adv. Food Res. 24: 287-374.
Wiese H (2000). Apicultura: Novos Tempos Guaíba. Agropecuária LTDA. 424p.