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MICROBIOLOGICAL QUALITY OF HONEY FROM FAMILY AGRICULTURE IN THE DISTRICT OF CANTAGALO-RIO DAS OSTRAS-RJ

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Abstract: Honey is a high-quality food, rich in energy and countless other substances that are beneficial to the balance of the body's biological processes, such as phenolic compounds, amino acids, vitamins, mineral salts, organic acids and enzymes, which attribute therapeutic effects to the product. Honey, along with other bee products, is associated with an image of a natural, healthy and safe product. However, during handling, packaging and storage, microbiological contamination or fraud can occur. In this context, this study aimed to diagnose the physical-chemical and microbiological quality of honey samples produced in family units located in agrarian settlement areas in the district of Cantagalo, Rio das Ostras-RJ. The microbiological analyzes performed were, Counting of Total and Thermotolerant Coliforms, fungi and yeasts, *Staphylococcus* coagulase-positive, *Clostridium* spp. and *Bacillus* spp. and detection of*Salmonella* spp. The physicochemical analyzes performed were moisture, acidity and total soluble solids. Six samples of two different lots of honey were evaluated, and for all these there was no *Salmonella*, of *Clostridium* reducing sulphites and Total and Thermotolerant Coliforms. In the analysis of mesophiles, *Bacillus cereus, Sthaphyloccus* and fungi and yeasts, positive isolation results were obtained, but the counts were lower than the counting confidence criterion, which establishes a minimum number of 25 CFU/mL. Regarding the physicochemical analyses, there was a change in acidity in 50% of the samples, the other physicochemical parameters are in accordance with legislation.
Keywords: Honey, quality, hygienic-sanitary markers and family production.

INTRODUCTION

Honey is considered the easiest apicultural product to be explored, being also the best known and the one with the greatest possibilities of commercialization. It is a product consumed worldwide and highly relevant to human health. In addition to being a food, it is also used in pharmaceutical and cosmetic industries, in the manufacture of animal feed, as animal tissue preservatives and others, due to its known therapeutic actions (FREITAS et al., 2004). The breeding of Apis mellifera bees for economic purposes allows not only the production of honey, but also wax, propolis, pollen, royal jelly and apitoxin, in addition to enabling the provision of pollination services (BEHM et al., 2012).

In Brazil, the importance of beekeeping is consolidated by the multiplicity of floral reserves, allowing tons of honey to be produced, thus meeting the most demanding markets (WIESE, 1993). Our country is ranked in the world ranking among the largest honey producers, with China in first place, followed by Turkey and Argentina (FAO, 2018). Beekeeping is an agricultural production considered free from major health problems, such as drug and pesticide residues (PACHECO, 2006). However, there is still a great unexplored potential (flora and climate), and a great possibility of maximizing production, increasing the beekeeping agribusiness. Therefore, it is necessary that the producer possesses knowledge about bee biology, honey management and harvesting techniques, swarm pests and diseases, economic importance, market and commercialization (EMBRAPA, 2003).

In this context, beekeeping is an activity of great importance, capable of causing positive impacts, both social and economic, as it presents an alternative occupation and income for rural people, in addition to contributing to the maintenance and preservation of existing ecosystems. The beekeeping productive chain provides the generation of countless jobs, jobs and income flow, mainly in the family farming

environment, being, therefore, determinant in the improvement of the quality of life and fixation of the man in the rural environment.

In addition to these facts, it can be characterized by its easy maintenance and low initial cost compared to other agricultural activities (FREITAS et al., 2004). Family farming represents the organization of agricultural, forestry, fisheries, pastoral and aquaculture production. Since the logistics of production depend on family work, linking family and farm, developing and combining economic, environmental, social and cultural functions (FAO, 2013). The existence of family farmers is directly related to the preservation of the historical and cultural heritage of the interior of Brazil and the generation of employment in commerce and services provided in small towns. Family farming brings together important aspects: the family, work, production and cultural traditions, therefore, it can be considered as one that, at the same time as the owner, takes on the work in the establishment. The improvement in this segment's income, through its greater insertion in the market, has an important impact in the interior of Brazil and, consequently, in large cities (ZOCAL et al., 2015). Beekeeping is the creation of honey bees, Apis mellifera L., housed in artificial hives, using methods and equipment developed to better take advantage of the natural productive capacity of these insects (PERUCA et al., 2002). By its nature, beekeeping is a species-conserving economic activity, due to the low environmental impact it causes, enabling the permanent use of natural resources and not destroying the rural environment. Thus, it is one of the few activities that fulfill all the requirements of the sustainability tripod: social, economic and environmental.

The place of collection of honey samples in the present study was the district of Cantagalo in the municipality of Rio das Ostras (RJ), which is mainly constituted by the Cantagalo settlement project, established by the National Institute of Colonization and Agrarian Reform (INCRA), in 1987. Among the various properties in the district of Cantagalo, there are 10 honey collection points, each containing an average of 14 hives, where honey samples were obtained. The district is supplied by the Jundiá river, which rises between the Serra do Pote and the Serra da Careta, runs through the floodplains until it meets the Iriry river. At the meeting of these two rivers, the Rio das Ostras is formed, which gives the city its name. The typical climate is humid tropical, with dry winter and humid summer and temperatures typical of the coastal region, ranging from 15 to 38˚C, with the maximum occurring between the months of November and February and the minimum between the months of June and September. It is worth mentioning that it is part of the Atlantic Forest biome, having a great diversity of ecosystems, with emphasis on the Serra do Mar Corridor, comprising one of the largest forest remnants of the Dense Ombrophilous Forest (ICMBIO, 2020).

Like other food products, honey must meet numerous quality criteria and certifications before marketing (DEVILLERS et al., 2004). However, with the increased consumption of natural products, honey has been used and marketed more intensively, so that the possibility of fraud, adulteration and inadequate handling also increases (CANO et al., 1992). According to the Ministry of Agriculture, Livestock and Supply (MAPA, 2000), honey is a food product produced by honey bees, from the nectar of flowers or secretions from living parts of plants or excretions of plant-sucking insects. Bees collect, transform, combine with salivary enzymes, store and let them mature in the hive's combs. It is a concentrated solution of sugars with a predominance of glucose

and fructose (MAPA, 2000; CODEX, 2001). It also contains a complex mixture of other carbohydrates, carbohydrates, enzymes, amino acids, organic acids, minerals, aromatic substances, pigments and pollen grains and may contain beeswax from the extraction process (MAPA, 2000; CODEX, 2001). It is considered a food with high energy value for the human body (CRANE, 1987) since 1 gram of honey contains 6.4 kcal (ETTINGER, 2002) (Table 01).

Honey is a complex product, whose composition varies markedly as a result of the original flowering, geographical areas and climatic conditions. In tropical regions, the physical and chemical characteristics of honey are still poorly known, as the bee flora is quite diversified, associated with high rates of humidity and temperature. Thus, the characterization of honeys is fundamental for the knowledge of their physical and chemical properties, taking into account the edaphoclimatic factors (relating to soil and climate) and establishing comparative analysis criteria between different regions (CRANE, 1983). The difference between honeys depends on the variety and amount of plants that flower and produce nectar in the same period (SILVA et al., 2004). The hypothesis that this product has therapeutic properties has contributed to its use as a natural therapy agent due to its antibacterial, antibiotic, anticaries, anti-inflammatory, antimicrobial, biostimulant, depurative, emollient, energetic, immunostimulating and healing actions (BEKERS et al., 2004; WAILI-AL, 2004; AL et al., 2009).

Honey has been widely exploited for its nutritional richness and its composition, which includes micronutrients such as vitamins, minerals. Minerals can be indicators of the geographical origin of honey as well as environmental indicators. Honey bees can be exposed to contaminants present in the apiary, which is why they are considered bioindicators of environmental pollution. The most common mineral found in honey is potassium, followed by calcium, magnesium, sodium, chlorine, sulfur and phosphorus (AZEREDO; AZEREDO; DUTRA, 2003). Traces of iron, copper, zinc and manganese are also found. Vitamins are essential for the growth and repair of tissues, vital for the functioning of organs and the production of specific metabolic reactions in the cellular environment. In honey, complex B vitamins and vitamins A, E and C are found

As a product of natural origin, African bee honey (Apis mellifera) has its own microbiota similar to that which occurs with other food products, but with a characteristic microbiological behavior. This microbiota can be divided into two groups: the micro-organisms inherent in honey, which are introduced by bees into the hive, with nectar, pollen or honeydew, or during the cleaning operation performed by them, when conveying it on or within their body; and microorganisms considered occasional or accidental, introduced by chance due to lack of hygiene in handling or during the honey extraction and processing process (GROSSO et al., 2002).

Although honey is a product that, due to its physicochemical characteristics, does not present high susceptibility to the proliferation of microorganisms, the action of external factors (environmental, handling and storage conditions) can negatively influence its final quality (CAMARGO, 2002). According to decree number 367, of September 4, 1997, by the Ministry of Agriculture, Livestock and Supply (MAPA), the microbiological standards for honey are: absence of Total Coliforms/g in five analyzed samples of a batch, absence of *Salmonella* spp. and *Shigella* spp in 25g in ten samples from the same batch and presence of a maximum of 100 CFU/g of molds and yeasts

in two samples out of five analyzed from the same batch. This ordinance was revoked by normative instruction number: 11 of October 20, 2000 which contains in annex the Technical Regulation of Identity and Quality (RTIQ) of honey, however, this normative instruction does not present microbiological standards for honey. The microbiological characteristics of honey are related to the quality and safety of this food. Important health indicator microorganisms are primarily yeasts, filamentous fungi and spore-forming bacteria. These microorganisms are involved in product deterioration activities, through the production of enzymes, toxins, the metabolic conversion of food, as well as the production of growth factors (vitamins and amino acids) and inhibiting factors of competing microorganisms (GOERZEN, 1991). The main sources of honey contamination are: human beings, equipment, containers, dust, air, insects, animals and water.

Microorganisms can survive in honey, which increases the demand for the adoption of good manufacturing practices as an essential factor in the control of sanitary hygiene for this product. The primary sources of microbial contamination of honey such as pollen, the digestive tract of honey bees, dust, air, earth and nectar are considered difficult to control when compared to secondary sources that can be controlled through the implantation of good manufacturing practices in the warehouse (MARTINS et al., 2003). Secondary sources include: exposure to air during honey extraction; the handlers (skin infections, sneezing or fecal contamination); cross contaminations (animals or animal products) and equipment (including food and water waste). In addition to these, floors, walls and ceilings can also be reservoirs of microorganisms and contaminate food. Another factor little considered is the period of the production cycle. The time of flowering season can interfere with the microbiological quality of honey since, in the low availability of food, bees can forage in fungal colonies (SNOWDOWN, 1999) or even in feces and other sources of organic matter (NOGUEIRA NETO, 1997).

Honey deterioration is partly a reflection of the action of filamentous fungi and yeasts that can develop in acidic conditions and are not inhibited by the presence of sucrose (SNOWDON; CLIVER, 1996), thus being considered a problem in the honey industry. According to Crane (1979), the increase in humidity and temperature in honey storage favors the development of yeasts, contributing to the fermentation of the product. During fermentation, yeasts attack sugars, producing alcohol and carbon dioxide. In the presence of oxygen, alcohol can be converted to acetic acid. Fermentation usually takes place in micro-environments (such as on top of the package) where there is a higher concentration of water. According to White (1978), another factor to be considered is that, even under adequate sanitary conditions, the natural crystallization process of honey promotes the enrichment of the liquid phase, also contributing to its fermentation. In relation to filamentous fungi, some are associated with the intestinal content of bees, the hive and the foraging environment.*Aspergillus* spp was isolated from bee larvae intestines (HASIG; KAMBUROY, 1996), as well as the genera : *Atichia* spp, *Coniothecium* spp, *Hormiscium* spp, e *Triposporium* spp (SNOWDON; CLIVER, 1996). Rios de Selgrad et al. (1992) found that since honey is contaminated by fungi and yeasts, these microorganisms remain in latent form, without multiplying. The proliferation of fungi can have the following consequences: economic losses due to product deterioration and damage to health due to the possibility that they are mycotoxin-producing fungi, such as aflatoxins (MARTINS et al., 2003).

Bacteria of public health importance that have been isolated from honey are *Bacillus cereus* and *Clostridium botulinum*. *The Bacillus cereus* is a Gram positive, aerobic, spherical spore-forming bacterium, and many strains are capable of producing toxins. This bacteria is commonly found in soils, vegetables, dust, water and in various raw and processed foods such as rice, condiments, milk, vegetables, meat, flour, desserts and cakes (CHRISTIANSSON et al., 1999). Intoxication by *B. cereus* has a generally short incubation period (average of 6 to 12 hours) and the main clinical manifestation is represented by persistent vomiting, resulting from intoxication by the emetic toxin. This toxin is thermostable, resistant to proteases and extremes of pH, in addition to inducing mitochondrial alterations in type 2 "T helper" lymphocytes (ÁGATA et al., 2002). Other types of toxins produced are enterotoxins, responsible for the manifestation of diarrhea and abdominal pain; they can be of three types, hemolytic, non-hemolytic and enterotoxin (FINLAY et al., 2002). The consumption of foods containing a concentration greater than 10^6 UFC de *B. cereus*/g can result in food poisoning (APHA, 2001). This bacterium is currently among the predominant ones in food poisoning outbreaks, causing diarrhea and emesis (FINLAY et al., 2002).

Clostridium botulinum is an anaerobic bacterium, Gram positive, has the ability to sporulate, which gives it resistance to heat and maintains its survival in unprocessed foods. It is the etiological agent of botulism and its natural habitat is the soil (SOLOMON; LILLY, 2001). This microorganism produces the botulinum toxin (neurotoxin) which is the most potent known natural toxin, whose lethal dose is 10-7 mg/k body weight. This small amount in the bloodstream can cause death within minutes through muscle paralysis (SOLOMON; LILLY, 2001). Most cases of botulism are associated with the consumption of homemade foods, especially vegetables, fruits and fish that are inadequately preserved. Intoxication by C. botulinum is a rare disease with an occurrence of 24 cases/ year in the United States. Cases of botulism are rarely identified after consuming processed foods. Canned, smoked or preserved foods, whose thermal treatment did not allow the destruction of the spores, can also be sources of contamination (KÜPLÜLÜ et al., 2006).

Bacteria of the *Staphylococcus* genus are pathogens recognized in Foodborne Diseases from human and animal contamination. They are Gram-positive cocci-shaped bacteria, belonging to the Staphylococcaceae family (FRANCO and LANDGRAF, 2005), immobile, non-sporulated, capsulated or not, facultative anaerobes, and which, when viewed under a microscope, appear mostly in the form of bunches. They have respiratory and fermentative metabolism (BENNETT and MONDAY, 2003) and with greater growth under aerobic conditions, when they then produce catalase (FRANCO and LANDGRAF, 2005), being classified as catalase positive species. In addition, staphylococci utilize a variety of carbohydrates and require nitrogen sources. (BENNETT e MONDAY, 2003). Among the species that are part of the genus *Staphylococcus*, the species: *S. aureus* certainly the one with the highest degree of relevance. The growth and proliferation of *S. aureus* in food represents a risk to the health of the consumer, considering the production of enterotoxins. The primary reason for its search and identification is the attempt to trace whether there was post-processing contamination (BENNETT and MONDAY, 2003), as well as secondary sources of contamination that indicate inadequate hygiene and handling practices. Man is the main reservoir of *S. aureus* and the colonization percentages vary from 60 to 70%

(SANTOS, 2000).

Another group of microorganisms of health relevance is the group of total and faecal coliforms. The term "fecal coliforms" was used for many years to describe coliforms that fermented lactose with gas production. 44,5ºC. *Escherichia coli* and some strains of *Klebsiella* spp. and *Enterobacter* spp. have this characteristic of thermotolerance, however, only *E. coli*, its primary habitat is the human and animal intestines. *Klebsiella* spp. and *Enterobacter* spp., they can be found in other environments, such as vegetables and soil, where they persist longer than that of pathogenic bacteria of intestinal origin (SILVA; CAVALLI, OLIVEIRA, 2006).

In general, the coliform group is used to assess the health status of foods. However, they can be used to assess general aspects of quality, since they are routinely used to assess the quality of the final product and the hygiene used in its processing (SANT'ANA et al., 2003). Among the groups of indicator microorganisms stands out as the best indicator of fecal contamination to *Escherichia coli* (JAY, 2005), for being easily isolated in conventional culture media and more resistant for a longer period of time (SOUZA, 2006). This bacterium has a tendency to change from a commensal organism to an opportunistic pathogen and to an extremely specialized bacterium (HART; WINSTANLEY, 2001), causing diseases in healthy hosts; therefore, it is desirable to determine its incidence in a coliform population. The human gastrointestinal tract is susceptible to foodborne infections, and its main causative agents are represented by members of the Enterobacteriacea family. Among the bacteria in this family, the diarrheagenic categories of *E. coli*. Their infections are limited to colonization of mucosal surfaces or can spread through the body, having been implicated, in addition to infection processes, also in cases of meningitis (NATARO; KAPER, 1998).

Another group of microorganisms of health relevance is represented by the genus Salmonella spp. which is widely distributed in nature, the intestinal tract of man and animals being the main natural reservoir. Due to its ability to spread in the environment, this bacterium can be isolated from different places, and consequently, from different food raw materials. It can also be transmitted by the man himself, without clinical symptoms, in which case the condition of asymptomatic carrier is characterized (CARDOSO, 2006).

Food safety is an issue of growing importance in public health, and governments around the world have intensified their efforts to improve (WHO, 2007). Therefore, it is necessary to prevent these foodborne diseases, through the institution of effective preventive measures and training, together with the implementation of good hygiene practices, from the field to the final consumer, which will contribute to the minimization of contamination and /or unwanted bacterial growth in food products, including honey (SOUZA, 2006).

MATERIAL AND METHODS

SAMPLING

In this study, a total of six samples of honey obtained during the period of October 2019 from family farms in the district of Cantagalo-Rio das Ostras located in areas of agricultural settlement with remnants of the Atlantic Forest were evaluated. Samples were obtained in 250 ml vials or in one liter glass bottles. The samples were sent to the Food Microbiology laboratory at Polo Ajuda UFRJ Campus Macaé for microbiological analyzes added at room temperature. The processing of honey samples was carried out in a biological safety cabinet, with the surface of the honey package disinfected with cotton soaked in 70% alcohol and opened aseptically. Then, 25g of the

honey sample were weighed and transferred to an Erlenmeyer flask containing 225mL of 0.1% peptone water, and this mixture was homogenized for three minutes. From this dilution (1/10), the suspensions were seeded in media suitable for the type of microorganism to be isolated. Microbiological analyzes were determined using the methodology recommended by the APHA (American Public Health Association).

MICROBIOLOGICAL ANALYSIS OF HYGIENIC AND SANITARY MARKERS OF HONEY

The microbiological analyzes included the Counting tests of mesophilic bacteria, enumeration of Total and Fecal Coliforms at 45 0C, filamentous fungi and yeasts, *Staphylococcus* coagulase-positive, *Clostridium* spp. reducing sulphite, *Bacillus cereus* and research of *Salmonella* sp.

- Standard count of viable strict and facultative aerobic mesophilic bacteria

The samples were diluted in 0.1% peptone water, homogenized, submitted to serial decimal dilutions and plated, using the Pour Plate technique, in standard bacterial count agar (PCA). Plates were incubated at 36 ± 1 oC for 48 hours (ISO 4833, 2003). The results were expressed in Colony Forming Units (CFU) per volume expressed in mL according to APHA (2001).

-Counting of total and thermotolerant coliforms by the multiple tube technique

The presumptive test for the honey samples was carried out using the Lauryl Sulfate Tryptose Broth (LST). In this test, three aliquots of three sample dilutions were inoculated into a series of three LST tubes per dilution. The samples were incubated at 35 ºC for 24 to 48 hours in a bacteriological incubator. Dilutions that present a presumptive positive reaction, evidenced by the change in color of the medium and gas production, were submitted to the confirmatory test of total coliforms in tubes containing 10 mL of 2% Lactose Verde Brilliant Bile Broth 2% (VBBL) and incubation at 35 ºC by 24/48 hours. For Coliform Confirmation Thermotolerant aliquots of positive dilutions were placed in test tubes containing 10 mL of EC Broth and incubated in a water bath at 44.50 ± 0.2 °C for 24/48 hours, considering positive those with turbidity and gas retention in the Durham tube (BRASIL, 1997; BRAZIL, 2018). Positive samples for Thermotolerant Coliforms were isolated in Eosin Methylene Blue Agar (EMB) and submitted to biochemical characterization tests to confirm *Escherichia coli* (KONEMAM et al., 2008). The number of positive tubes in both VBBL Broth and EC Broth were checked in the NMP tables, and the results expressed in NMP/g.

-Detection of molds and yeasts

The samples were diluted in 0.1% alkaline peptone water (APA), homogenized and subjected to serial decimal dilutions and plated, using the technique : *Spread Plate*, in 2% glucose potato agar acidified to pH 3.5 (BDA). Plates were incubated at 25 ± 1°C for 5-7 days. Results were expressed in CFU /mL (ISO 21527-1, 2008).

- Counting and Identification of *Staphylococcus* spp.

The samples were diluted in 0.1% alkaline peptone water (APA), homogenized and subjected to serial decimal dilutions and plated, by técnica *Spread Plate* in Baird Parker Agar for enumeration of *Staphylococcus* spp. The typical black colonies with double halo of lipolysis were seeded in Mannitol Red Agar of phenol at 7.5% of sodium chloride (NaCl), for observation of the morpho-dying aspects by the Gram stain technique, coagulase test and other tests phenotypics for characterization of genus and species. The identification was carried out using the following standardized procedures: catalase and potassium hydroxide

(KOH) test, nitrate reduction test, Voges-Proskauer, urease production, DNAse and sugar fermentation (ISO 6888-1, 2003).

-Counting of *Bacillus cereus*

A 0.1 ml aliquot of the dilution 10^{-1} was sown in duplicate, according to the method of *Spread Plate* ((Figure 2), on the surface of sterile disposable Petri dishes containing selective medium for *Bacillus cereus* (MYP / microMed). Plates were incubated at 30°C for 24-48 hours. Subsequently, the presumptive identification of the colonies was carried out according to the growth characteristics of the colonies, observing the formation of precipitation zones around the colony, indicating the production of lecithinase and the occurrence or not of mannitol fermentation. The suspicious colonies were transferred to tubes containing Nutritive Agar (Merk®) slanted and incubated at 30 ºC for 24 hours, later tests for identification were performed (APHA, 2001; KONEMAN et al., 2008).

-Counting of *Clostridium* spp. sulfito-redutor

Two enrichment media were used, cooked meat broth (CMM/BBL) and trypticase broth - peptone - glucose - yeast extract (TPGY) and in Sulfite Polymyxin-Sulfadiazine Agar (SPS - Vetec) plus 5% yolk emulsion of egg being incubated in an aerobic environment and the other in anaerobiosis, both at 35 ºC for 48-72 hours. Colonies that showed growth only in anaerobiosis were considered positive (KÜPLÜLÜ et al., 2006; SOLOMON; LILLY, 2001).

-Detection of *Salmonella* sp.

To detect Salmonella, samples were homogenized in 0.1% alkaline peptone water (APA) and after incubation for 16-20 hours at 36 ± 1 ºC, 1 and 0.1 mL aliquots were transferred to Selenite cystine broth, Rappaport Vassiliadis and Sodium Tetrathionate, respectively. After incubation for 24-30 hours at 41 ± 0.5 oC in a water bath, isolation was performed on selective media: XLD and SS agar with incubation for 18-24 hours at 36 ± 1 ºC (ISO 6579.2002), to observe the typical characteristics of Salmonella spp.

PHYSICAL-CHEMICAL ANALYSIS OF THE QUALITY OF HONEY:

-Determination of moisture

Honey moisture was determined according to the methodology of AOAC (1997). The principle of this method is to determine the refractive index of honey at 20 °C, which is converted to moisture using the Chataway reference table. Initially, 3 to 4 drops of the sample were transferred to the prism of the refractometer. Then, the refractive index was read at 20 °C.

-Total soluble solids

It was determined by direct reading of the samples in a bench-top Abbe refractometer, according to the technique established by Instituto Adolfo Lutz (2008). Total soluble solids are directly related to all substances that are dissolved in a given solvent. They are expressed in ºBrix and have a tendency to increase with maturation.

-Determination of acidity

The acidity content of honey was obtained by titration of the filtrate with 0.1N NaOH, according to the technique established by Instituto Adolfo Lutz (2008), with the results expressed in meq.kg-1. Initially, 10g of each sample was weighed into 250 ml Erlenmeyer flasks where 50 ml of distillate was added. Then, 2 to 4 drops of phenolphthalein were added and titration with a sodium hydroxide solution, at a concentration of 0.1062 mol/l, until reaching a pink color. The volume spent is noted and the calculation made according to the equation below:

Acidity in meq/kg = V x f x 10, where f is the factor of the 0.1 mol/L NaOH solution and V is the volume used in the titration.

RESULTS AND DISCUSSION

MICROBIOLOGICAL ANALYSIS

A total of six samples from two different lots of honey were analyzed, and for all these samples there was a total absence of Clostridium sulfite reducing agents, *Salmonella spp* and group of total and faecal coliforms. For the other analyses, positive isolation results were obtained, but the counts were lower than the confidence criterion, which establishes a minimum number of 25 CFUs. Table 01 presents the results of the microbiological analyzes to assess the health and hygiene profile of the honey in question and the discussions will be presented below (Table 02)

-Total count of strict and facultative aerobic mesophilic heterotrophic bacteria

Results obtained in the counts of mesophilic bacteria were less than or equal to 101 CFU/g in the six samples analyzed. It is known that the count of mesophilic bacteria is important because it includes most of the contaminants in food of animal origin, and it can reach high counts when the food is kept at room temperature, in disagreement with the norms for food preservation (SILVA, 2002).

The results obtained in the present work were similar to those of some samples analyzed by Schlabitz et al. (2010), when evaluating the microbiological quality of honey sold in the region of Vale do Taquari/RS, where they obtained results ranging from $1x10^1$ to 2.7×10^2 CFU/mL for 12 samples analyzed. According to the ICMS (2002), the number of mesophilic aerobic microorganisms detected in foods has been one of the most commonly used microbiological indicators of quality as indicators of adequacy of cleaning, disinfection and time/temperature control techniques during processing, transport and storage. This importance is justified by the vast majority of foodborne pathogenic bacteria are part of this group (FRANCO and LANDGRAF, 2005). This microbial indicator also allows obtaining information about deteriorating changes and commercial validity. Therefore, the low count of these indicators reflects adequate and unfavorable hygienic conditions for the multiplication of potentially pathogenic microorganisms for human consumption (LIRA, PEREIRA, 2001; SILVA, 2002).

-Counting of *Bacilus cereus*

The results obtained in the present study detected that in only two samples there was no typical growth of Bacilus cereus, while in the other honey samples the count was less than or equal to 10^1. UFC/g.

In a study carried out in Argentina by Iurlina et al. (2006), the prevalence of *Bacillus* genus bacteria in different products was detected and it was found that in the universe of 70 analyzed honey samples, 27 (38.6%) had such contamination and 23% of all honey samples had specific contamination by *Bacillus cereus.* López and Alippi (2007) found an incidence of contamination by B. cereus of 27% in Argentine honey samples. Despite the discrepancy of such results that demonstrate the presence of this bacterium in an intense way, in the present study we obtained a very low count of colonies of *Bacillus cereus*. Therefore, the samples were considered safe for consumption and it can be inferred that the hygienic-sanitary standards recommended by law were complied with.

- Counting and identification of *Staphylococcus aureus*

A total of 66.67% (4/6) of the honey samples had counts less than or equal to a 10^1 of typical Staphylococcus colonies that were later submitted to biochemical characterization, with no detection of the S. aureus species. In approximately 33.33% (2/6) there was no growth of typical bacterial colonies of coagulase-positive Staphylococcus. Similarly to the present study, Santos (2013) detected

the absence of Staphylococcus aureus in the analyzed honey samples. Contamination by Staphylococcus aureus in honey mainly comes from secondary sources. Post-processing contamination of food is common, due to human contact with already-processed food or exposure to inadequately sanitized surfaces.

- Clostridium *spp. sulphite* reducers

In the analysis for detection and counting of Clostridium spp. sulfite-reducing agents there was no typical growth in selective culture media and in the techniques recommended in the literature. Several studies report the prevalence of Clostridium spp in honey samples and disagree with the results of the present study that detected the absence of this group. Schocken-Iturrino et al. (1999) analyzed 80 samples of Brazilian honey, six (7.50%) of which were contaminated with *C. botulinum*.

- Filamentous fungi and yeast counts

In the six honey samples analyzed, a count greater than or equal to 10^1, therefore, it is within the limits recommended by Ordinance Number: 367, of September 4, 1997, of the Ministry of Agriculture, Livestock and Supply (MAPA), which establishes the maximum limit of 1.0×10^2 UFC/g. This ordinance was revoked by normative instruction number: 11/2000, which includes the RTIQ of honey as an annex, however, this normative instruction does not present microbiological standards for honey. The counts for these microorganisms in the present study were lower than those reported in the literature. Sodré et al. (2007) detected a count of 1.7 x 104 and Schlabitz et al. (2010) who, evaluating the microbiological quality of Apis mellifera honeys, reported a value of 2.7 x 102 CFU/g. The presence of filamentous fungi in the final product may be related to its ability to support high concentrations of sugar, acidity and the antimicrobial properties of honey. The most common molds found in honey are those of the genus: *Penicillium*, *Mucor* and *Aspergillus*, which can produce toxic metabolites. Yeasts can grow under conditions of low pH and are not inhibited by sucrose. The presence of osmophilic yeasts in honey is a problem, as their growth is only limited by the amount of water available. Some conditions, such as increased humidity, moderate temperature, granulation, and high yeast count favor honey fermentation.

- Research of *Salmonella* spp.

In all six samples, the result was the absence of Salmonella spp., being in compliance with the Brazilian legislation (BRASIL, 2000) which only foresees the absence of Salmonella spp. in 25 g of the product. The results obtained in this study are in line with other studies available in the literature that demonstrate the absence of Salmonella spp. in honey samples, and compliance with legal microbiological standards. The microbiological quality of a given food including honey is directly related to the hygienic conditions of production and handling of the samples and the form of storage, thus involving a more social than a botanical context and, as it is a pathogenic microorganism, it requires attention and refers to cross contamination of the product through its handling (FRANCO 2008). The species of the genus Salmonella have the ability to spread in the environment, and this bacterium is isolated from different places, and consequently, from different food raw materials, and can also be transmitted by humans without clinical symptoms.

- Count of Total Coliforms and Thermotolerant Coliforms

In the six samples analyzed, a count of less than <3.0 NPM/g of total coliforms and thermotolerant coliforms was obtained, meeting the standards established by Normative Instruction Number: 11, of October 20, 2000, from MAPA, which establishes absence (<3.0 NPM/g) for total coliforms. Similar results to

the present study were found by Pires (2011), who verified the microbiological quality of honey from Apis mellifera bees produced in Piauí. In all honeys evaluated, the presence of microorganisms from the coliform group was not detected. Souza et al. (2012), when evaluating the microbiological characteristics of 21 honeys produced in the Northeast Region of the State of Bahia, they found values < 3.0 NMP/g. Microorganisms belonging to the coliform group can be used to reflect the microbiological quality of products in relation to shelf life or safety. The presence of this microbiota in honey can be attributed to inadequate handling, observed at the time of collection of samples and during filling, or by inappropriate temperature conditions during production or conservation of the product, in addition to the fact that unsterilized bottles are used.

PHYSICOCHEMICAL ANALYSIS

A total of six honey samples from two different batches were evaluated and the results obtained from these physicochemical analyzes are shown in Table 03.

-Moisture

Moisture analysis of honey samples detected an average content of 18.3%, and ranged from 18% to 18.8% (Table 03). These values are below the maximum limit allowed by current legislation, which establishes a criterion of 20% as established by the Ministry of Agriculture, Livestock and Supply (MAPA, 2000). Similarly, the analysis of honeys from Paraíba obtained results between 17.59 to 20.3% (RODRIGUES et al, 2005). Silva et al (2004), analyzing honeys from Piauí, found moisture contents ranging from 17.6 to 19.7%. Weather conditions on the day of harvesting and extracting the honey influence its water content, as it is a hygroscopic product, that is, it absorbs water. Water contents above 20% indicate that the honey was harvested before it became "ripe" (unsealed) or had water added due to improper processing (LEAL, SILVA and JESUS, 2001). The higher the honey's water content, the more it becomes prone to unwanted fermentation (BERTOLDI, GONZAGA and REIS, 2004). The water content is an important quality parameter, as it allows to say the duration of the product, conservation, palatability, stability of the honey and its fermentation. The higher the water content, the more likely the honey is to ferment during storage. As a hygroscopic product, honey can absorb and retain moisture during extraction on wet days and with inadequate storage and in poorly closed containers.

-Acidity

The acidity values for the six honey samples analyzed ranged from 27.35 to 82,19 $meq.kg^{-1}$, averaging 51,83 $meq.kg^{-1}$, only 50% of the samples comply with the parameters recommended by the Codex Alimentarius (2001), which establishes a maximum acidity of 50 meq.kg. Brazilian legislation establishes a maximum limit of 60 $meq.kg^{-1}$ of acidity for bee honey (Brasil, 2000). The results of this present work according to the two literatures are similar, 50% of the samples would comply with the recommended standards. The acidity of honey is mainly related to gluconic acid, which is produced by the glucose oxidase enzyme on glucose. The action of this enzyme remains even after processing, thus remaining in activity during honey storage (VENTURINI, 2007). Other factors that can be attributed to honey acidity would be the action of bacteria during maturation and inorganic ions present in the composition of this bee product, such as phosphate and chloride. The degree of acidity detected in the samples of the present study may have a relationship with the detection of yeasts obtained in microbiological analyses. The determination of acidity can provide valuable

information in assessing the conservation status of a food product (IAL, 2008), since yeasts are microorganisms that can grow in honey by tolerating acidic conditions and high levels of sucrose, while yeasts osmophilics grow when osmotic pressure is high. These can even grow in mature honey, fermenting it easily (SNOWDON, 1999). Honey fermentation results in yeast growth converting sugar into alcohol, carbon dioxide, organic acids and other combinations with undesirable flavors and odors. Yeasts found in honey with predominance are: Saccharomyces, Schizosaccharomyces and Torula (SNOWDON, 1999).

-Total soluble solids

The honey samples analyzed showed an average value of 79.73%, with values between 79.6 and 80%. In honey, this result represents, quite accurately, the amount, in percentage of total sugars. Current legislation does not require the Brix level analysis to determine the quality of honey, but this measure was performed to compose yet another variable for comparing the results. In an analysis of 15 samples from different cities in the state of Goiás, the average found was 81.04 ºBx, with the highest and lowest result found being 85 and 78.3 ºBx, respectively (SILVA et al, 2003). Carvalho et al. (1998) and Marchini & Moreti (2001), when performing analyzes reported values from 76.0 to 80.1%, thus having results similar to those of the present study.

CONCLUSION

According to the parameters of current legislation, it is highlighted that the present analysis detected the most significant presence, from a microbiological point of view, by filamentous fungi and yeasts, mesophilic bacteria, *Bacillus cereus* and coagulase-positive *Staphylococcus*, despite the Positive isolations from honey samples are in accordance with the standards established by Decree Number: 367/97 (Brazil, 1997A), Decree Number: 451/97 (Brazil, 1997) and RDC Number: 12/2001. The reduced amount of sanitary marker microorganisms may be associated with the presence of antimicrobial factors in honey, the adoption of good hygiene practices during handling, care in the storage process and the location of hive breeding areas far from polluting sources. The honey samples were obtained from a family beekeeping unit located near the sources of the Jundiá and Iriri rivers, settlers of the agrarian reform, in remnants of the Atlantic forest in the district of Cantagalo, Rio das Ostras-RJ, preserved from human action, deforestation, urbanization and real estate speculation.

The physicochemical analyzes of total soluble solids and moisture of the six honey samples are within the recommended limits. And only two samples showed acidity values above the limit established by current legislation (BRASIL, 2000), which may have been influenced by the botanical origin of the flowering, by climatic and geographic conditions or by the harvest of honey before its complete maturity and by the presence of osmophilic yeasts which may also increase the risk for fermentation to occur.

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Component	Approximate quantity
Moisture (%)	15,8
Energy (kcal)	309
Protein (g)	0,0
Carbohydrate (g)	84
Lipid (g)	0,0
Dietary fiber (g)	NA
Cholesterol (g)	NA
Ashes(g)	0,1
Calcium(g)	10
Magnesium(g)	6
Phosphor (g)	0,38
Iron (g)	4
Sodium(g)	0,3
Copper (g)	6
Retinol (g)	Tr
Zinc (g)	NA
Thiamine (g)	0,1
Riboflavin (g)	Tr
Niacin (g)	Tr
Pyridoxine (g)	Tr
Vitamin C (g)	0,1

Table 01. Physicochemical composition of honey:

Source: TACO, 2011; NA: Not analyzed; Tr: Trace.

Samples	Temperature	°Brix	Moisture	Acidity
1	23,5° C	79,60	18,6	64,79
2	22° C	79,90	18,4	60,01
3	24° C	80,00	18	27,35
4	23,5° C	80,00	18	82,19
5	26° C	79,90	18	29,83
6	27° C	79,00	18,8	45,36

Table 02. Physicochemical analysis of six honey samples from family farming in Cantagalo-Rio das Ostras.

Figure 1:Three initial honey samples analyzed.

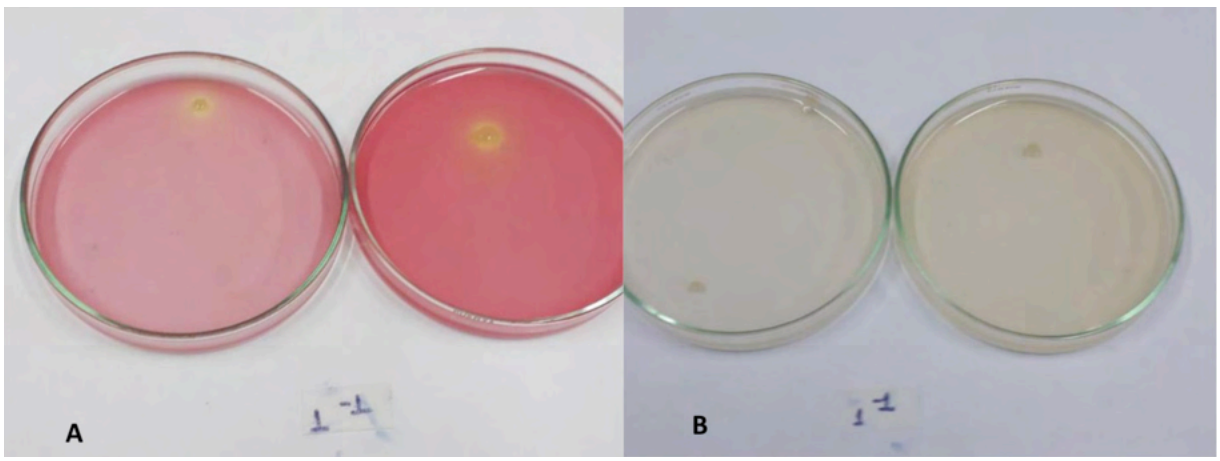

Figure 2: Bacillus cereus count on MYP Agar after incubation at 37°C/24-48h; B: Mesophilic Bacteria Count on PCA Agar after incubation at 37°C/24-48h; copyright collection.