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Evaluation of the production system and microbiological quality of honeys collected at Sinop, Mato Grosso, Brazil

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Abstract

Honey is a substance of high energy and nutritional value that is part of the diet of many people in the world, and fresh honey consumption is related to various beneficial properties for health. However, during the production system, their microbiological characteristics can be changed by contamination sources, affecting their quality. This study aimed to evaluate the production system and microbiological quality of honeys collected at Sinop, Mato Grosso, Brazil. It was conducted in 10 apiaries, which did not have any stamp for marketing. A checklist with questions on good manufacturing practices was applied in the apiaries. Samples of processed honeys were collected to determine the presence of total coliforms. There were many inadequacies in the apiaries, such as: presence of pets in the vicinity (100%); people allowed to enter the processing area in the honey house (90%); smoke improperly directed when managing the hive (80%); handlers do not wear white uniforms (100%),clean and exclusively (90%) in the apiary; non-availability of water supply system connected to the public network (80%), among others. However, the collected samples of honey did not indicate the presence of total coliforms, a result which does not certify the hygienic and sanitary quality of this product, and does not exempt beekeepers from best conforming to good manufacturing practices Thus, the implementation of quality control programs throughout the production system and the determination of other microorganisms that may be present in honey is needed to ensure the product quality and increase productivity.

Key words: Honey. Beekeeping. Coliforms. Food Hygiene. Identify and Quality Pattern for Products and Services.

Introduction

Beekeeping was introduced in Brazil in 1839, but it was only in 1956 that a significant agricultural, social and technological advance occurred, resulting from the introduction of African bees (*Apis mellifera scutellata*) into the apiaries in the country. The recovery and expansion of this activity began in 1970, having as a milestone the first Brazilian Beekeeping Congress.[1]

Honey, a highly energetic and nutritional substance, is produced by honeybees from the nectar of flowers, and is part of the eating habits of many people worldwide. Natural consumption of honey is associated with various beneficial properties for health, among them antimicrobial, healing, soothing, tissues regeneration and stimulant effects.[2,3]

In Brazil, *per capita* consumption of honey is only 60 grams a year, while in other countries, such as Germany, is 2.4 kilos and in the Central African Republic is 3.4 kilos.[1,4] Currently, state governments and city administrations have created public policies to stimulate the consumption of honey, aiming to improve people's health and promote the development of beekeeping, diversifying the farmers' income without causing damages to the environment.

An example has been observed in Conquista D'Oeste, state of Mato Grosso. Beekeeping was introduced in 2003 with the assistance of the City Administration, and, in 2005, supported by the Brazilian Support Service to Micro and Small Businesses (SEBRAE), the beekeepers received investment funds from the National Program to Strengthen Family Farming (PRONAF). In 2006, they founded the Cooperative of Beekeepers in Mato Grosso (Apisnorte), with members from seven municipalities in the region. After its creation and some technical adjustments, the cooperative obtained the certification stamp from the State Health Inspection Service (SISE/MT), which, together with the stamp awarded by the Federal Health Inspection Service (SIF), it was allowed to market the products throughout the country.[5,6] However, this is not a reality of many apiaries in other cities in the state of Mato Grosso, which do not have the SISE/MT or the SIF certification stamps, preventing them from the selling their product freely. This is mostly due to nonconformities to the good manufacturing practices (GMP) and lack of a warehouse, which are requirements that the Ministry of Agriculture, Livestock and Supply (MAPA) inspects to ensure the hygiene, sanitary and physicochemical quality of honeys, as well as the apiaries productivity.

It is worth noting that, in case of microbiological contamination of honey, this can be due to primary sources (which usually occurs before harvest, and is very difficult to control, such as: pollen, the digestive system of the honey bees, dust, soil and nectar) or secondary (occurring after the harvest period and caused by factors that can be changed, such as handling practices, cross contamination, equipment and facilities).[2,7,8]

Thus, it is necessary to identify and solve the possible risks for honey contamination, from harvest to sale, once the microbiological characteristics will always be associated with the quality of this food. [8-11] Therefore, the present work aimed to assess the production system and the quality of honey samples collected in Sinop, Mato Grosso, Brazil.

Methodology

Cross-sectional study conducted in apiaries located in the municipality of Sinop, Mato Grosso, Brazil, which did not have any kind of certification stamp for marketing the product. The apiaries were selected according to the beekeepers' permission. Thus, initially 14 beekeepers were contacted, out of which only ten authorized the evaluation of their production system and honey, which took place during the harvesting and processing period, from July to September, 2012.

During the visits, a GMP checklist[12] was used in the warehouse, based on Ordinance no.6/1985 of MAPA, which approves the hygiene, sanitary and technological standards for honey, bees wax and derivatives.[13] The instrument consisted of 60 questions covering the location of the apiaries, beehives handling, utensils, equipment and materials, honey house, honey, handlers, cleaning, wastes management and water supply. For data analysis, descriptive statistics was used, and the results were expressed in absolute and relative frequency in relation to full conformity (fully meets the requirement), partial conformity (partially meets the requirement) and nonconformity (does not meet the requirement).

Samples of processed honeys (500g) were also collected, bottled in transparent glass jars with threaded caps, previously sterilized in autoclave, kept under room temperature and protected from light.

For the count of total coliforms, aliquots of 25 g of each honey sample were aseptically weighed in Erlenmeyer flasks previously sterilized in autoclave and homogenized with 225 ml of 0.9% saline solution. Dilutions 10^{-1}, 10^{-2} and 10^{-3} were prepared in Durhan tubes containing 10.0 ml of Lactose Broth culture medium (HiMedia Laboratories pvt. Ltd. India). These tubes were incubated under a temperature of 35°C for 48 hours. After the incubation period, from the samples producing gas, a loop of each culture was transferred to the tubes containing 2% brilliant green bile broth, according to the methodology of the most probable number per gram of sample.[14]

Results and discussion

Compliance with the GMP manual is vital not just to ensure increased productivities but also to maintain the honey identity and quality standards. It was found (Table 1) that most of the assessed apiaries had easy access to people, built in shaded yards, with equipment and appliances made of stainless steel and sanitary facilities close to the honey-processing site, in good conditions. The beekeepers fed the hives before the flowering season and used cleaning products registered with the Ministry of Health.

Table 1 – Assessment of conformity regarding the site of the studied apiaries in Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
Apiary site	n	%	n	%	n	%
1. Is the apiary located more than 300 meters from houses, pens/corrals, animal sheds and roads?	6	60	4	40		
2. Does the apiary have easy access for vehicles?	4	40	5	50	1	10
3. Does the apiary have easy access for people?	8	80			2	20
4. Is the apiary located near natural sources of nectar and pollen?	10	100				
5. Is the apiary located near a good quality water source and easy access for the bees, at a distance of 100 to 500 meters?	5	50	5	50		
6. Is the apiary located in a shaded area?	9	90	1	10		
7. Is the apiary located far from contamination sources (sewers, garbage deposits, etc.)?	8	80	1	10	1	10

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity
n = number

Shaded areas for the beehives helps preserve the characteristics of color, moisture, hydroxymethylfurfural content and the invertase and diastase reaction of the honey in the production of propolis, minimizing the heat stress that the bees suffer in tropical regions, where temperatures can be higher than 35°C. They also facilitate the operational flow, the use of appropriate processing equipment and hygiene and sanitary conditions [5,15,16]

The main nonconformities found were difficult access for vehicles and a great distance from water sources for the bees (50%). Difficult access to the apiary increases unnecessarily the time spent with handling, transportation of the products and, sometimes, hives. In addition, the presence of fresh water near the apiary is crucial for maintaining the bee swarms (lower energy expenditure for harvesting), mainly in hot climates,[4,10] as in the region of Sinop, where water is used in thermoregulation and to meet the bees physiological requirements.

It was found (Table 2) that the major conformities relating to appropriate handling of the hives were non-collection of the honey supers in rainy days and the nonuse of smokers that burn plant materials such as sawdust, straw, etc. (90%). These results corroborate study[17] where it was found that 85% of the beekeepers harvested honey in sunny days, thus preventing damages to the honey quality. Regarding nonconformities, we can cite people entering the handling area of the honey house after working directly in the collection of the honey supers in the field (90%) and improper direction of the smoke from the smoker, which was mostly (80%) puffed directly to the combs.

Table 2 – Assessment of proper handling of beehives in the apiaries studied in the city of Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
Beehives handling	n	%	n	%	n	%
1. Does the beekeeper feed the beehives before the flowering season?	7	70	3	30		
2. Do beehives have double screen or queen excluder?	5	50	5	50		
3. Does the beekeeper use previously used and stored supers for production?	10	100				
4. Are honeycombs collected in rainy days?	1	10	9	90		
5. Is there presence of green honey ("decapped") in the frames for extraction?			10	100		
6. Is there absence of bee offspring in any stage of development in the honey frames for extraction?	2	20	7	70	1	10
7. Is the smoke used during handling cold, clean and soot free?	6	60	2	20	2	20
8. Is the smoke blown from smoker directly to the honeycombs?	8	80	1	10	1	10
9. Does the beekeeper use plant materials such as sawdust or straw to fuel the smokers?	9	90	1	10		
10. Does the beekeeper, during handling, wear appropriate clothing (suits, gloves, boots and veils)?	7	70	3	30		
11. Are the people who work directly on the harvest of honeycombs in the field allowed to enter the honey house handling area?	9	90	1	10		

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity
n=number

Proper use of smoke is crucial for a safe handling of the beehives. Its function is to simulate a danger situation (fire), when the bees will be prepared to leave the site, eating as much food as possible and, consequently, gaining weight and greater abdominal distension, which will prevent their moves to sting.[2,18]

It was found (Table 3) that the highest percentage of conformity of equipment, utensils and materials were associated with the purchase of specific processing materials. Eighty percent of the apiaries had stainless steel centrifuges, strainers and filters made of 40 to 80-mesh stainless steel or nylon, and honey packaging materials consisted of nontoxic plastic, glass or other containers, with the SIF certification stamp. However, some nonconformities were found relating to the equipment and proper use, i.e. during honey centrifugation, in 70% of the sites the centrifuges were not properly closed and in half of these sites (n=5) there was no stainless steel table for frames decapping.

Table 3 – Assessment of beekeeping equipment, utensils and materials used in the apiaries studied in the city of Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
Beekeeping equipment, utensils and materials	n	%	n	%	n	%
1. Does the beekeeper use stainless steel knife or chisel?	6	60	4	40		
2. Does the beekeeper use a stainless steel smoker?	7	70	2	20	1	10
3. Does the honey house have stainless steel decapping fork available?	2	20	1	10	7	70
4. Is the table used for decapping made of stainless steel?	5	50	5	50		
5. Are centrifuges made of stainless steel?	8	80	2	20		
6. During centrifugation, is the centrifuge maintained closed?	3	30	7	70		

Beekeeping equipment, utensils and materials	YES		NO		PARTIAL	
	n	%	n	%	n	%
7. Are the settling tanks made of stainless steel or nontoxic plastic material?	6	60	4	40		
8. Are the filters or strainers made of 40-80-mesh stainless steel or nylon?	8	80	2	20		
9. Does the beekeeper use cloth strainer during filtration?	4	40	6	60		
10. Does the beekeeper use female nylon stockings for honey filtration?	6	60	3	30	1	10
11. Is the equipment arrangement suitable to a good operational flow, also regarding the aspects of easy cleaning?	3	30	5	50	2	20
12. Are the honey packaging materials made of nontoxic plastic, glass or other materials approved by SIF?	8	80	2	20		

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity n=number

Improper use of the centrifuge during honey processing can increase the moisture contained in the product, once honey has high hygroscopicity,[19] which favor the multiplication of deteriorating microorganisms. In addition, an inadequate equipment arrangement, preventing a suitable operational flow, mainly for cleaning operations, and non-availability of a stainless steel table for decapping can represent possible sources of contamination during honey processing.[20]

The main nonconformity found (Table 4) in all honey processing sites (100%) was the presence of pets or other farmed animals nearby which may result in food contamination, mainly by fecal material,[20] and non-availability of sanitary facilities with personal hygiene products (70%). Regarding the major conformities found in the honey house, we can cite that 80% had walls at least two meters high and had adequate sanitary facilities close to the processing facility. In 70% of the assessed apiaries, there was no honey house or a place for honey processing. In the apiaries where these facilities were available (90% of the cases), the doors were neither made of metal

nor coated with waterproof material, and not wide enough for the operations and easy flow. In addition, the walls were not made of bricks and covered with tiles, industrial ceramics or similar, in light colors, or other lining material that ensures perfect waterproofing.

Table 4 – Assessment of the honey house or honey processing facility in the apiaries studied in the city of Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
Honey house or processing facility	n	%	n	%	n	%
1. Does the apiary have a honey house?	2	20	7	70	1	10
2. Are there pets or farmed animals in the vicinity?			10	100		
3. Are utensils/equipment used in agricultural practices kept nearby?	5	50	5	50		
4. Are there utensils, clothes, etc. hanging on the inner walls of the site?	2	20	8	80		
5. Are there suitable sanitary facilities at the site?	8	80	1	10	1	10
6. Do the sanitary facilities have personal hygiene products, such as odorless and/or antiseptic liquid soap, non-recycled paper towels for hands available?	2	20	7	70	1	10
7. Does the beekeeper write down the honey output of each hive, as well as the crop output?	2	20	6	60	2	20
8. Is the space enough for equipment installation and honey storage?	3	30	6	60	1	10
9. Do windows have screens and are cleaned at least every two weeks?	2	20	5	50	3	30

10. Are walls made of brick, covered with tiles, industrial ceramics or similar material, in light colors or other lining material to ensure waterproofing?			9	90	1	10
11. Is the floor made of waterproof material, resistant and easy to clean?	7	70	3	30		
12. Are the walls of the honey house at least two (2) meters high?	8	80	2	20		
13. Is the ceiling or other upper surfaces in perfect conditions (free of cracks, leaks, moisture and mold)?	8	80	1	10	1	10
14. Are doors made of metal or lined with waterproof material, wide enough for the operations, and easy transit?			9	90	1	10

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity
n=number

A similar study[21] conducted in the micro-region of Pau dos Ferros, Rio Grande do Norte state, found few honey houses available, and many beekeepers extracted honey in improvised places without the required hygienic conditions. This is a national reality, resulting from the fact that the profits from the sale of honey are often not enough to build an appropriate facility, provided with areas for receiving, extracting, and storing honey, as well as suitable sanitary facilities for handlers.[12]

It was found that the honey produced in 70% of the apiaries was not transported from the production site to the warehouses in appropriate packages, specific for the purpose, closed and protected from light, rain or dust. Nearly 60% of the packages containing honey were not placed on appropriate wood pallets or other material to prevent direct contact with the floor, and 60% of the honey frames, after collected, were not packaged with appropriate material for transportation to prevent their contact with the ground (Table 5).

Table 5 – Assessment of the honeys produced and handlers in the apiaries studied in the city of Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
	n	%	n	%	n	%
Honey						
1. Are the honey frames, after harvest, placed in appropriate materials for transportation, preventing contact with the ground?	4	40	3	30	3	30
2. Are the honey supers, when arriving at the honey house, placed on properly cleaned pallets, preventing their contact with the ground?	4	40	4	40	2	20
3. Is honey stored in such a way not to receive direct light?	5	50	3	30	2	20
4. Are the packages containing honey placed on wood pallets or other material to avoid direct contact with the floor?	5	50	4	40	1	10
5. Is honey transported from the production site to the warehouses in appropriate packages, specific for the purpose, closed and protected from sun, rain and dust?					2	20

	YES		NO		PARTIAL	
	n	%	n	%	n	%
Handlers						
1. Does the honey house staff wear uniforms consisting of pants, apron or overalls, cap, hat or hairnet, boots or waterproof shoes, all in white color?			10	100		
2. Are the uniforms always clean and of exclusive use of the apiary, i.e. are the employees prohibited to leave the site wearing their work clothes?			9	90	1	10
3. Do handlers usually take a shower before starting work?	6	60	4	40		
4. Do handlers have their nails usually cut and free from nail polishes?	9	90			1	10
5. Do handlers wear earrings, watches, bracelets, amulets and other jewelry items within the honey house?	2	20	8	80		

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity
n=number

These results point to the risk of biological contamination during the honey production system by micro-organisms present in the ground, which can trigger and speed up the food fermentation process when the food already has an increased moisture content,[20] caused by processing failures, such as maintaining the centrifuges open. It is known that the containers of honey products must protect against the action of physical, chemical and biological agents to prevent alterations in the characteristics desired by the consumer market. The containers must be of non-toxic plastic, glass or other acceptable material by a competent authority.[22] Storage of the products must be made in places equipped with dehumidifiers,[5] protected against light and with temperature control (25 to 30ºC).

In a food production system, personal hygiene and operational flow are key factors in preventing foods contamination and for the quality assurance of the end- product. Handlers interfere directly on the food quality, either as a vector of microorganisms and/or improper production, compromising the honey hygiene and sanitary safety.[23]

It was found that 60% of handlers had the habit of showering before work and 90% had their fingernails always cut and free of polishes. However, during the product handling inside the honey house, 100% of the staff did not use complete, white uniforms (pants and apron or overalls, hat, cap or hairnet, waterproof boots or shoes), 90% did not use uniforms exclusively in the apiary, and the employees were allowed to leave work wearing the uniforms.

In another study,[17] it was found that in only 8% of the sites visited, handlers took a shower before entering the honey processing facility; 15% used to wash hands before processing; 46% had fingernails cut and polish-free; 23% wore uniforms, comprised of pants, apron, cap and mask, all of white color and always clean and used exclusively inside the processing house. It was found that in 39% of the honey extraction facilities, handlers wore watches, amulets and jewelry. Another study[24] also found a low percentage of satisfactory hygiene conditions of the food handlers working in honey production in the city of Pires do Rio, Goiás state. In contrast, a survey,[21] which assessed good practices in honey production in the city of Pau dos Ferros, Rio Grande do Norte state, found that the beekeeper used proper clothing (overalls, mask, glove and boot) and those who worked in the harvest of the beehives in the field did not enter the handling area of the honey house, as well as the supers or frames, which remained outside the processing area.

The assessment of the cleaning practices, wastes management and water supply (Table 6) showed that 70% of the apiaries used cleaning products registered with the Ministry of Health. On the other hand, 80% of the processing facilities did not have a water supply system connected to the public main; 70% did not perform frequent removal of the wastes from the processing area to eliminate sources of contamination and do not have cleaning products identified and stored in proper places.

Table 6 – Assessment of cleaning practices, wastes management and water supply of the apiaries studied in the city of Sinop, Mato Grosso, Brazil, 2012.

	YES		NO		PARTIAL	
	n	%	n	%	n	%
Cleaning						
1. Is the cleaning frequency of the installations appropriate?	4	40	1	10	5	50
2. Are the cleaning products registered with the Ministry of Health?	7	70			3	30
3. Are the cleaning products identified and stored appropriately?	1	10	7	70	2	20
Wastes management						
1. Are the containers for collection of wastes inside the apiary easy to clean and transport, properly identified and constantly cleaned; use appropriate trash bags?	1	10	6	60	3	30
2. Is there frequent removal of wastes from the processing area to avoid contamination sources?	3	30	6	60	1	10
Water supply						
1. Is the water supply system connected to the public main?	2	20	8	80		

YES: full conformity; NO: Nonconformity; PARTIAL: Partial conformity
n=number

Because the beekeepers did not have public water supply available, they decided for digging semi-artesian wells in their properties, due to their low cost and easiness. As a result, the water collected from free aquifers is more vulnerable to contamination.[25] Treated water is one of the key aspects for the food hygiene and sanitary assurance, preventing the circulation of microorganisms during cleaning operations of the rooms, equipment, appliances, transport vehicles and handlers.[26]

It is worth noting that, despite several nonconformities observed in the assessed apiaries, none of the collected honey samples indicated the presence of total coliforms, and it was not necessary to conduct the test for *Escherichia coli*. The negative result for fungi and yeasts in the same samples[27] brings additional information on the microbiological quality of the honeys produced in the city of Sinop.

A similar result was found in a study in the Taquari Valley, Rio Grande do Sul state, where none of the commonest contaminants of this food, such as *Staphylococcus aureus, Salmonella* spp., *Clostridium botulinum* and total and thermotolerant coliforms were detected in the assessed honey samples, besides low counts of aerobic and mesophilic microorganisms, a result that the authors considered were due to the antibacterial characteristic of the product.[28] Similar studies[9,29,30] also found total coliforms in samples of honey collected in the states of Minas Gerais, Rio de Janeiro and Paraná, respectively. However, in another study[7] the presence of total and thermotolerant coliforms, molds, fungi and yeasts were detected in honey samples collected in the city of Parintins, state of Amazonas. In the study, the authors did not investigate the production chain and suggested that the results were linked to the early stages of the crop, when flowers are still scarce and the honeybees have to find food in other locations close to animals-raising farms, thus contaminating the honeys.

Despite such analyses are all-important to ensure the honey quality and the consumer health, they are not included in the technical regulation proposed by Ordinance no. n°67/1997 of MAPA[31] and by the normative instruction no. 11/2000,[32] which should be reconsidered.[33]

The microbiota of honey does not affect the honey quality because it is not pathogenic.[34] However, the presence of coliforms under a temperature of 35°C and 45°C, as well as molds and yeasts, are hygiene and sanitary indicators associated with handling and the infrastructure of the apiaries, and may cause diseases. Therefore, further studies should be carried out to evaluate the honey production chain, once the use of the best honey-producing practices is closely associated with an adequate production infrastructure, especially the availability of a Honey Products Extraction Unit (honey houses).[35,36]

Conclusion

The implementation of quality control programs throughout the production system and determination of other microorganisms that may be present in honey, such as yeasts, molds, *Staphylococcus aureus, Salmonella* spp. and *Clostridium botulinum* are needed to ensure the product end quality and increase yields and profits. The negative results for contamination by total coliforms found in the present study do not exempt the beekeepers from compliance with GMP and do not certify the hygiene and sanitary quality of this product.

Moreover, beekeeping is of key importance for family farms, once it contributes to the pollination of the crops, ensures foods production for the families, prevents families from leaving the rural areas, ensures social inclusion, income and jobs generation. There should be local government efforts to stimulate beekeepers to conform to the good manufacturing practices and, consequently, to a better development of the business operations.

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