MICROORGANISMS IN ORGANIC AND NON ORGANIC HONEY SAMPLES OF AFRICANIZED HONEYBEES

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S u m m a r y

This research was carried out to evaluate and compare 11 organic honey samples and six non organic honey samples, respectively, harvested from islands of the triple frontier (Sao Paulo, Parana and Mato Grosso do Sul states) and from the state of Paraná, Brazil. The samples were studied for the presence of coliforms from 35°C, to 45°C and the enumeration of moulds and yeast, a minimum of 1.9×10^2 and a maximum of 1.1×10^3 CFU/g were observed in organic honey and a minimum of 1.8×10^1 and a maximum of 2.5×10^2 CFU/g were in non organic honey. In this studied region, the organic honey presented a microbiological quality inferior to the non organic honey.

Keywords: Africanized honeybees, microbiological analysis, coliforms, moulds and yeast.

INTRODUCTION

Honey is a product with minimum types and levels of microorganisms which are attributed to the natural properties and to industry control. The microorganisms in the honey are initially yeast, moulds, and bacteria spores coming from primary sources when the nectar is being harvested, stored and matured during the procedure. These microorganisms are always present in the air (Snowdon and Cliver, 1996).

Certified organic honey is defined as lacking any chemical contamination, including that related to the migration of honeybees in search of good blossoms which is not directly controlled by beekeepers. Contaminated with chemicals in the final packaging process is possible, though the basic requirement is the ability to control the origin of the product and production process (Buainin and Batalha, 2007).

The primary resources of microorganisms are: pollen, the digestive system of the honey bee, dust, air and flowers (Snowdon and Cliver, 1996). According to Gilliam et al. (1983) the nectar and pollen contain many microorganisms that can be transmitted to the honey. According to Tysset and Rousseau (1981) the secondary sources of microorganisms are humans, equipment, recipients, wind, dust, insects, animals and water.

As a product with a natural origin, the honey of the *Apis mellifera* presents a

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high grade of resistance to proliferation of microorganisms. The action of environmental factors (high relative humidity), manipulation conditions (spores contamination), and high temperature of storage can influence negatively the final quality of the honey (Snowdon and Cliver, 1996).

Although microorganisms (except some yeast and molds) cannot grow in the honey, they can be transmitted when honey is used as an ingredient in the preparation of other foods and multiply until they deteriorate the product (Snowdon, 1999). Microorganisms can be involved in activities such as alterations, enzyme production, antibiotics, toxins, vitamin increasing, amino acids, promoting metabolic conversion and inhibition by microbial competition (Martins et al., 2003).

This research was carried out to evaluate and compare organic and non organic honey samples harvested from the islands of the triple frontier (the states of Parana, Sao Paulo and Mato Grosso do Sul) and from the state of Paraná, Brazil.

MATERIAL AND METHODS

The organic and non organic honey samples were harvested from December 2004 to January 2005 on islands from the triple frontier (the states of Paraná, São Paulo and Mato Grosso do Sul Brazil - organic honey) and (from the state of Paraná - non organic) by beekeepers after the collection was carried out by them from their own apiaries.

The microbiological analysis was made in the Food Laboratory at the Universidade Tecnológica Federal do Paraná, in the town of Campo Mourão, Brazil.

The data were statistically analyzed in a completely randomized design in variance analysis and the F test ($p<0.05$) was used, with three repetitions to each parameter.

The samples were studied for the presence of coliforms to 35°C, and to 45°C. The samples were also studied for the enumeration of moulds and yeasts following the international rules listed in the Compendium of Methods for the Microbiological Examination of Foods (APHA, 1992).

From 25 g of honey, the first dilution was made in 225 ml of 0.1% buffer peptoned water. The preparation of subsequent decimal dilutions were made in tubes containing 9 ml of the same diluents until it was 1/1000. For each dilution, three repetitions were done to test the number of probable coliforms (NMP). The repetitions were done in Lauryl Sulfate Broth (LST Broth) and three repetitions of 1 ml in duplicate of each decimal dilution, were incorporated into Potato Dextrose Agar (PDA) acidified with tartaric acid 10% until the pH 3.5 was reached. The incubation was done in a bacteriological incubator at 25°C for five days for moulds and yeasts, and for the NMP tests with 35°C for two days and regressive counting. The three replications were evaluated by F test at a 5% probability level.

RESULTS

Table 1 presents the F values with the probability (P), coefficient of variation (CV %), averages, and standard deviation of moulds and yeasts from the analyzed samples.

Coliforms to 35°C and to 45°C

In all samples analyzed for coliforms enumeration to 35°C and to 45°C, the presented results are <3.0 NMP/g. These data corroborate with Sodré et al. (2007) who analyzed 58 *Apis mellifera* honey samples. The absence of these microorganisms in analyzed honey was expected. These bacteria need water activity > 0.91 to grow (Ribeiro and Seravalli, 2004).

Snowdon and Cliver (1996) already reported the population of coliforms to 45°C in honey; that varied from 10 to 10^2 CFU/g. These authors, however, did not note the moisture content in these samples.

Moulds

The mould and yeast values obtained were: minimum values of 1.9×10^2 and maximum of 1.1×10^3 CFU/g for organic honey, and a minimum of 1.8×10^1 and

Table 1

F value with probability (P), coefficient of variation (CV), averages and standard deviation of moulds and yeasts in microbiological analysis from organic and non organic honey samples

Variation source	Moulds and Yeasts (CFU/g)
F value	9.092 (P = 0.0088)
CV (%)	88.68
Organic honey (n = 11)	5.3×10^2 a (± 318.91)
Non organic honey (n = 6)	1.0×10^2 b (± 92.87)

*Averages followed by different letters, in the same column, are different ($p<0.05$) by F test

a maximum of 2.5×10^2 CFU/g for non organic honey (Tab. 1).

The main microorganisms responsible by honey fermentation are moulds and yeasts which change the sensorial and chemical characteristics of this product.

The presence of yeast in honey occurs due to contamination caused by carelessness and lack of good sanitary practices during management. Such practices include: placing the super on the floor, not washed centrifuges well, extractors made of lath, dark combs and long storage of honey in the super. Through these practices microorganisms can be added to the product (Snowdon, 1999) and depending on the conditions, may change physicochemical, organoleptic and microbiological characteristics (Alves et al., 2005). The presence of osmophilic yeasts represents a risk to food, as they are capable of growing in acid conditions and in high sucrose concentrations. The fermentation process takes place in the sugar hydrolysis with alcohol production and carbonic gas. In the presence of oxygen, the alcohol may be converted in acetic acid (White, 1978).

According to the Resolution MERCOSUL GMC, number 15/94 - technical rules for identity fixation and honey quality, approved by ordinance number 367 from September, 4th 1997, honey may contain a maximum of 100 CFU/g. All samples of organic honey and 27.2% of non organic honey samples in our experiment were higher than 100 CFU/g.

Souza et al. (2006) and Almeida-Anacleto et al. (2009) reported 64.5% and 52.2% of honey samples analyzed, respectively, contained outstanding CFU/g; and thus, inadequate for human consumption. Oliveira et al. (2005), when analysing honey samples harvested by two methods, reported differences in the microbiological quality. In samples harvested from beekeepers and aseptically harvested, 65% and 25%, respectively were more contaminated than the permitted maximum. According to these authors, the installation of an apiary in a wet environment, and climatic conditions may be responsible for the growth of moulds and yeasts above that permitted in aseptically harvested samples.

In the non organic honey, the occurrence of contamination may be associated with secondary sources of contamination, represented mainly by manipulation of conditions, equipment, installations and the environment during the collection, process, packing and storage of the final product. Alves et al. (2009), evaluating two techniques of harvesting - extracting the honey with the use of a disposable syringe; and unseal the honey pot and turning the beehive in a recipient to collect the honey, reported the importance of care during the honey harvest. The mould and yeast for each method demonstrated that an inadequate procedure can increase the microorganism count present in honey, shorting life shelf and it can prove to be inadequate for human consumption.

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Analyzing the results, found a significant increase in the moulds and yeasts observed in organic honey in relation to the non organic. It can be inferred that this contamination is related to the primary sources of contaminations, especially with the botanical, and geographic origin and environmental conditions (Tab. 1).

According to National Institute of Meteorology (INMET, 2005) the precipitation in December/2004 to February/2005 was above average. The period of the honey harvest was followed by intense rain, many times accompanied by strong winds and hail.

In the state of Mato Grosso do Sul, the maximum values of precipitation were 300-420 mm and minimum values were between 120-180 mm, respectively in December 2004 and January 2005. In the state of Sao Paulo, the maximum values of precipitation occurred in December and oscillated from 300-360 mm. In the state of Paraná, in December, the maximum precipitation occurred in the North (240-300 mm) and in January, the maximum varied from 300-360 mm in the East.

Because of this, the data in registers of INMET (2005) in January 2005, revealed that the region had a relative humidity of between 70-95% which possibly contributed to the enrichment of the liquid phase (water activity) of the organic honey. They were elaborated in the same region (islands with forest, with high water saturation in the air).

According to Abu-Jdayil et al. (2002) the water content presented in the samples is inversely related to the viscosity being linked to the relative density of the honey. Physical (high osmolarity, high viscosity and acidity) and chemical factors (flavonoids, hydrogen peroxide and fenolic compounds) give honey antiseptic and antibacterial properties (Dustmann, 1993).

In previous studies, using the same samples, it was verified that the organic honey had a clearer coloration (extra light amber and light amber). In non organic honey the amber and dark amber coloration are predominant. According to Frankel et al. (1998) dark honey has high antioxidant potential, the main ones are: pinocembrin, pinobanksin, cuisine, galangine, ascorbic acid (C vitamin), catalase and selenium.

Comparing *Apis mellifera* honey from other social native Brazilian bees, with Meliponinae honey, showed a difference in relation to the bacteria. In general, Meliponinae honey has higher bacteriostatic and bactericide properties than the *Apis mellifera* honey (Cortopassi-Laurino and Gelli, 1991).

The low viscosity and osmolarity in honey samples make it possible to infer that the smallest internal attrite of the molecules determines the higher fluid grade. This higher fluid grade with small opposition to the transmission currents and oxygen dissolution and small anti bacterial action, favors the growth of aerobic and anaerobic osmophilic yeast.

CONCLUSION

In the studied region, the organic honey presented a microbiological quality inferior to the non organic honey. The high moisture observed in organic honey samples was the main factor that determined the microbial flora growth.

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MIKROORGANIZMY W PRÓBKACH MIODU EKOLOGICZNEGO I KONWENCJONALNEGO UZYSKANYCH OD ZAFRYKANIZOWANYCH PSZCZÓŁ MIODNYCH

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S t r e s z c z e n i e

Badania przeprowadzono w celu dokonania oceny i porównania próbek miodu ekologicznego i konwencjonalnego zebranego z pogranicza trzech stanów: Sao Paulo, Paraná i Mato Grosso do Sul oraz stanu Paraná w Brazylii. Dane opracowano statystycznie w układzie kompletnie zrandomizowanym z zastosowaniem analizy wariancji i testu F ($p<0,05$), z trzema powtórzeniami dla każdego parametru. Próbki zostały zbadane na obecność bakterii z grupy coli w temperaturze 35°C i 45°C oraz określono liczebność pleśni i drożdży, zgodnie z międzynarodowymi zasadami Kompendium Metod Badań Mikrobiologicznych Żywności. We wszystkich analizowanych próbkach liczba bakterii z grupy coli zarówno w temperaturze 35°C jak i 45°C była niższa od 3,0 NMP/g. Oznaczając liczebność pleśni i drożdży uzyskano wartości od $1,9 \times 10^2$ do $1,1 \times 10^3$ CFU/g dla miodów ekologicznych oraz od $1,8 \times 10^1$ do $2,5 \times 10^2$ CFU/g dla miodów konwencjonalnych. W przypadku wszystkich próbek miodu ekologicznego i w 27,2% próbek miodu konwencjonalnego uzyskano wyniki przewyższające 100 CFU/g. Zanieczyszczenie przewyższające dozwolone maksymalne wartości wykryto w 65% próbek pozyskanych od pszczelarzy i w 25% próbek pozyskanych metodą sterylną. W przypadku miodów konwencjonalnych zjawisko to może być związane z wtórnymi źródłami zanieczyszczenia, przede wszystkim z warunkami przeprowadzanych zabiegów, stanem sprzętu, instalacji oraz warunkami środowiska podczas zbierania, przetwarzania, pakowania i przechowywania ostatecznego produktu. Obecność pleśni i drożdży zarówno w miodach od pszczelarzy jak i uzyskanych w sterylnych warunkach wskazuje, że nieodpowiednie postępowanie z miodem może zwiększyć w nim liczbę mikroorganizmów, skracając przez to okres jego trwałości lub sprawić, że będzie on nieprzydatny do spożycia. Istotnie wyższa zawartość pleśni i drożdży obserwowana w miodzie ekologicznym w porównaniu do miodu konwencjonalnego może świadczyć o tym, że zanieczyszczenie to jest powiązane z pierwotnymi źródłami, szczególnie z botanicznym i geograficznym pochodzeniem oraz warunkami otoczenia. W analizowanym regionie miody ekologiczne charakteryzowały się gorszą jakością pod względem mikrobiologicznym niż miody konwencjonalne. Wysoka zawartość wody w próbkach miodów ekologicznych była głównym powodem rozwoju flory mikrobiologicznej.

Słowa kluczowe: zafrykanizowane pszczoły miodne, miód ekologiczny, miód konwencjonalny, analiza mikrobiologiczna, bakterie z grupy coli, pleśnie, drożdże.