Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil

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Abstract

The development of mold of environmental origin in honey affects its quality and leads to its deterioration, so yeasts and molds counts have been used as an important indicator of hygiene levels during its processing, transportation and storage. The aim of this study was to evaluate the levels of yeasts and molds contamination and their correlation with moisture and acidity levels in *Apis mellifera* L. honey from central Brazil. In 20% of the samples, the yeasts and molds counts exceeded the limit established by legislation for the marketing of honey in the MERCOSUR, while 42.8% and 5.7% presented above-standard acidity and moisture levels, respectively. Although samples showed yeasts and molds counts over 1.0 x 10^2 UFC.g^-1, there was no correlation between moisture content and the number of microorganisms, since, in part of the samples with above-standard counts, the moisture level was below 20%. In some samples the acidity level was higher than that established by legislation, but only one sample presented a yeasts and molds count above the limit established by MERCOSUR, which would suggest the influence of the floral source on this parameter. In general, of the 35 samples analyzed, the quality was considered inadequate in 45.7% of cases.

Key words: honey, *Apis mellifera* L., quality.

Introduction

People are more and more convinced that the quality of their health is directly related to the food they consume. According to the principles of healthy eating, a diet should provide water, carbohydrates, proteins, lipids, fiber, vitamins and minerals, irreplaceable and indispensable components for the proper functioning of the body (Brasil, 2005). In this context, honey is of excellent nutritional value and the move towards contamination-free natural foods, which retain their original nutritional characteristics, and are produced by a system with little impact on the environment has led to an increase in its consumption (Araújo *et al.*, 2006; Arvanitoyannis and Krystalli, 2006).

Another constant concern on the part of consumers and producers is the sanitary quality of food, as it has been estimated that annually there are 1.2 billion cases of diarrhea and some 2.2 million deaths attributed to contaminated food consumption (Organizaçao, 2009). Honey is usually consumed *in natura*, so during harvesting and extraction, hygienic norms must be rigorously applied, because there is no way of reducing or eliminating the pathogenic microorganisms which cause the product to deteriorate. Thus, a lack of proper procedures when handling honey can irreversibly compromise its quality, and make it unfit for sale (Brasil, 1985; European, 2002).

The physical and chemical features of honey mean that it has low susceptibility to the proliferation of microorganisms (low pH, low moisture content, oxidation reduction potential, antimicrobial constituents, etc.). Nevertheless, external factors, such as environmental conditions and handling and storage, can have a negative effect on the final quality (Pereira *et al.*, 2003; Silva, 2007). In general, one can expect to find a reduced number of microorganisms in this substrate, which under normal moisture conditions, would not seem to interfere with the quality of honey. They are not pathogenic and are considered to be just indicator microorganisms (Pereira *et al.*, 2003; Snowdon and Cliver, 1996).

Contamination of honey can occur through primary sources (pollen, floral nectar, dust, soil and the bodies and
digestive tracts of bees) or through secondary sources, during extraction or processing (Almeida-Anacleto, 2007; Alves, 2008; Mendes et al., 2009; Pereira, 2008; Silva, 2007). Certain microorganisms cause honey to ferment, thereby acidifying it and modifying the taste (Pereira et al., 2003; Silva, 2007). Contamination can be avoided by implementing quality control programs, one of which could be the setting up of “Good Manufacturing Practices” by beekeepers, “distribution centers” and warehouses (European, 2002).

Consequently, the aims of this study were to do a yeasts and molds count, to analyze the moisture content and acidity level in Apis mellifera L. honey, produced in a certain region in Goiás State, in order to compare it with the parameters established for products marketed in the MERCOSUR, and then correlate the values found.

Material and Methods

Collection and sample preparation

A total of 35 samples of Apis mellifera L. honey was collected between May and August 2009. They were chosen at random from 29 beekeepers, participating in the “Beekeeping in the Railroad Territory Project”, carried out by the Servico de Apoio às Micro e Pequenas Empresas (SEBRAE) in 10 municipalities in the Pires do Rio, Goiás.

The samples were collected from individual jars of honey of different sizes (from 0.3 to 1 Litre), ready for sale, or from bulk storage, in which case the honey was transferred to sterilized jars. Each sample was assigned a sequence number according to the order of collection. The samples were stored at room temperature and kept in dry, airy conditions to await analysis.

Analyses were performed in the Physics & Chemistry Laboratories at the School of Agronomy and Food Science at the Federal University of Goiás (UFG); at the Centre for Food Research at the Veterinary Medical School/UFG and at the Food Quality Control Laboratory at the Faculty of Pharmacy/UFG from September 2009 to May 2010.

Yeasts and molds count

Yeasts and molds count was undertaken according to the methodology proposed for animal origin products (Brasil, 2003). A sample of 25 ± 0.2 g of honey was weighed, and 225 mL of 0.1% peptone solution (Apijã, Goiânia, Brazil) was added and serial dilutions were made and inoculated (0.1 mL) on a dry 2% potato glucose agar surface (Merck, São Paulo, SP), acidified to pH 3.5. The Petri dishes were incubated without inverting at 25 ± 1°C, for 5 days, in a BOD incubator. The results were expressed as colony forming units (cfu) of yeasts and molds per gram of honey.

Moisture content

Moisture content in the samples was determined using a refractometer (WYA 2S, Polax) at 20 °C, and the refractive index was then converted for moisture content using the standard table, as recommended by the AOAC (2003). The values were calculated on a wet basis.

Total acidity

Following AOAC (2003), total acidity results were obtained by adding free and lactone acidities. Free acidity was found using the titimetric method, with sodium hydroxide up to the equivalence point. Then, the lactonic acidity was also found using the titimetric method with hydrochloric acid.

Statistical analysis

Analyses were performed in quintuplicate and from the data, the Spearman correlation coefficient between the variables was calculated. For this end, Statistica 7 program was used (StatSoft, 2004).

Results and Discussion

Yeasts and molds counts in the samples ranged from < 1.0 x 10¹ to 5.0 x 10² cfu.g⁻¹ (Table 1). As the maximum level allowed for trading in the MERCOSUR is 10² cfu.g⁻¹, it was considered that 20% of samples showed cell count above this stipulated value. Similar counts of 1.0 x 10¹ to 3.0 x 10² cfu.g⁻¹ were reported by Sodré et al. (2007) in Apis mellifera honeys produced in the State of Piauí, and of < 1.0 x 10¹ to 6.1 x 10² cfu.g⁻¹, reported by Schlabitz et al. (2010) in honeys from the Vale do Taquari region of Rio Grande do Sul State.

The molds which are commonly found in honey may survive, but do not reproduce, so high scores are often related to a recent contamination by the environment or by equipment used during processing (Finola et al., 2007; Pereira, 2008; Snowdon and Cliver, 1996). So, the presence of these microorganisms could indicate the hygiene levels in which the product was processed and are indicators of the practices adopted by the processor (Garcia, 2003; SEBRAE, 2008; SEBRAE, 2009). When evaluating honeys from Western Cameroon, Tchomboue et al. (2007) found that 73.4% of samples had been contaminated by microorganisms, occurring during the post-harvest processing, as there was an absence of microorganisms in honeys collected under ideal hygienic conditions. This hypothesis confirms the need to implement quality control management programs for beekeepers, “honey distribution centers” and warehouses.

Finola et al. (2007) explain that the high microorganism count can increase the acidity in honeys. Osmophilic yeasts ferment honey by acting on the glucose and fructose, forming alcohol and carbon dioxide. Alcohol, in the presence of oxygen, can be broken down into acetic acid and water, which makes the fermented honey more acidic (Pereira et al., 2003; Silva, 2007). Yeasts can grow in low pH conditions and high levels of sucrose, and its development is promoted by the formation of glucose crystals, due to a
higher activity of water in the liquid phase (Oschalo, 2004). But some studies have shown that a high level of acidity is not always an indicator of fermentation by microorganisms (Balanza et al., 2004; Evangelista-Rodrigues et al., 2005; Welke et al., 2008). This study found no correlation between the yeasts and molds counts and the acidity of the honeys (Table 2). From an analysis of the data it can be seen that in part of the samples with high acidity values, the yeasts and molds count was below the standard established by MERCOSUR (MERCOSUL, 1999). So these results corroborated what has been said earlier, that an increase in acidity in honey is not related to one single parameter, which in this case, was the yeasts and molds count.

Total acidity ranged from 19.9 to 78.1 mEq/kg, so 25.7% of the samples exceeded the limit permitted for marketing in the Brazil (Brasil, 2000) and MERCOSUR (MERCOSUL, 1999) (Table 1). These values are higher than those observed by Terrab et al. (2004) in thyme honeys, whose maximum value was 48.6 mEq/kg. Acidity in honey is associated with same factors, such as floral sources, amount of minerals, time of harvesting, and also the amount of gluconic acid resulting from enzymatic action on glucose (De-Rodriguez et al., 2004; Finola et al., 2007; Kücük et al., 2007; Mendes et al., 1998, Mendes et al., 2009; Olaitan et al., 2007; White Jr., 1989).

A maximum limit for acidity in honey is also set out in legislation adopted by the European Union, where the limit is even lower, 40 mEq/g (União, 2001), but this parameter alone should not disqualify the product (Balanza et al., 2004; Evangelista-Rodrigues et al., 2005; Welke et al., 2008). These regulations may be reviewed so that honeys with acidity levels above presently accepted standards, but which show no signs of fermentation, may be accepted and this level may be considered as a feature not related to the loss of quality, but to the origin of the product (bee species, flowering, climate or region).

Moisture content levels ranged from 15% to 20.6% and only two samples exceeded the 20% moisture limit established by Brazil (Brasil, 2000) and MERCOSUR (MERCOSUL, 1999) (Table 1). This result may be related to the relatively low moisture levels of the air in this region and to the beekeeper’s concern in collecting only combs with at least 90% operculation. According to Evangelista-Rodrigues et al. (2005), Apis bees have a habit of operculating the combs only when the honey is already at the point of collection, that is, with moisture content levels between 17% and 18%.

The moisture content influences the flavor, preservation, viscosity, specific weight, crystallization and palatability, and it also contributes to the development of fermenting microorganisms (Abramovic et al., 2008; Almeida, 2002; Araújo et al., 2006; Silva, 2007). The values found were close to those of 16.6 to 20.8%, reported by Almeida (2002), and to those of 18.6 to 21%, found by Salgado et al. (2008), both in honeys produced in upstate São Paulo. The moisture content influences important characteristics of honey, such as viscosity and °Brix (Anupama et al., 2003), and as well as that, according to some studies, the higher the moisture level the greater the development of

| Sample | Moisture (%) | Yeasts and Molds (cfu. g⁻¹) | Acidity (mEq/kg) |
|--------|--------------|-----------------------------|-----------------|
| 1      | 15.2         | <1.0 x 10⁴                   | 46.79           |
| 2      | 16.4         | 2.0 x 10⁵                    | 32.87           |
| 3      | 17.0         | 2.0 x 10⁵                    | 43.86           |
| 4      | 16.6         | 1.0 x 10⁵                    | 59.85           |
| 5      | 18.0         | <1.0 x 10⁶                   | 70.87           |
| 6      | 16.8         | <1.0 x 10⁶                   | 39.06           |
| 7      | 18.2         | <1.0 x 10⁴                   | 78.13           |
| 8      | 18.4         | 3.0 x 10⁶                    | 65.14           |
| 9      | 18.2         | 2.0 x 10⁷                    | 31.33           |
| 10     | 15.6         | <1.0 x 10⁴                   | 20.22           |
| 11     | 18.8         | <1.0 x 10⁴                   | 48.61           |
| 12     | 16.6         | 5.0 x 10⁶                    | 39.07           |
| 13     | 15.8         | 3.0 x 10⁶                    | 23.24           |
| 14     | 16.4         | <1.0 x 10⁴                   | 26.10           |
| 15     | 16.2         | <1.0 x 10⁴                   | 21.04           |
| 16     | 15.6         | 1.0 x 10⁷                    | 32.77           |
| 17     | 18.8         | 1.0 x 10⁷                    | 58.70           |
| 18     | 16.4         | <1.0 x 10⁴                   | 21.32           |
| 19     | 16.6         | <1.0 x 10⁴                   | 72.30           |
| 20     | 17.2         | <1.0 x 10⁴                   | 20.70           |
| 21     | 15.0         | 1.0 x 10⁷                    | 41.06           |
| 22     | 16.6         | <1.0 x 10⁴                   | 19.95           |
| 23     | 15.8         | 1.0 x 10⁷                    | 21.76           |
| 24     | 16.2         | 2.0 x 10⁷                    | 25.42           |
| 25     | 17.0         | <1.0 x 10⁴                   | 28.77           |
| 26     | 16.4         | <1.0 x 10⁴                   | 23.80           |
| 27     | 20.2         | <1.0 x 10⁴                   | 40.34           |
| 28     | 17.6         | <1.0 x 10⁴                   | 25.25           |
| 29     | 18.6         | <1.0 x 10⁴                   | 28.03           |
| 30     | 16.6         | 1.0 x 10⁷                    | 37.87           |
| 31     | 20.6         | 1.0 x 10⁷                    | 73.83           |
| 32     | 17.0         | <1.0 x 10⁴                   | 65.39           |
| 33     | 18.0         | 1.0 x 10⁷                    | 63.50           |
| 34     | 15.6         | 1.0 x 10⁷                    | 22.27           |
| 35     | 17.8         | 1.0 x 10⁷                    | 42.62           |

Limit established by Brazil Legislation: 20.0% - 50 mEq/kg.
Limit established by MERCOSUR: 20.0% 1.0 x 10⁷ cfu.g⁻¹ - 40 mEq/kg.
microorganisms, which culminates in increased total acidity due to fermentation (Özcan et al., 2006).

This study found no correlation between moisture content and the yeasts and molds counts of the honeys (Table 2), possibly because in the majority of samples (94.3%), moisture levels were below 20%, and according to Snowdon and Cliverb (1996) a minimum of 20% moisture is needed for the development of yeasts and molds. Moreover, according to Souza et al. (2009), in order to get precise information on the relationship between moisture and the growth of microorganisms, determinations over a certain time interval would be necessary to find the mean growth of microorganisms.

A positive correlation was found between the moisture and acidity levels of the samples to the extent that 50% of the variations in acidity were shown to be related to variations in moisture. These results may be related to the glucose oxidase enzyme activity, which is promoted by higher levels of moisture in honey. The activity of this enzyme gives rise to gluconic acid, which increases honey acidity (Mendes et al., 2009).

Conclusion

The results of the analyses carried out on honeys from a region within Goiás State indicated that 20% of the samples had yeasts and molds counts above 1.0 x 10^2 cfu.g^-1. However, there was no direct relationship between moisture content levels and the presence of these microorganisms. The acidity index was above the permitted standard, but contamination by microorganisms was identified in only one of these samples, which would suggest the influence of other factors in the increase of total acidity in honey of this region.

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Table 2 - Spearman correlation coefficient between the yeasts and molds counts, moisture content and acidity in Apis mellifera L. honey, produced in central Brazil.

| Yeasts and molds | Moisture | Acidity |
|------------------|----------|---------|
| Moisture         | -0.0860 (p = 0.6233) | 1.0000 |
| Acidity          | 0.1265 (p = 0.4688) | -1.0000 |

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