Brazilian Journal of Microbiology 44, 3, 679-683 (2013)
ISSN 1678-4405
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www.sbmicrobiologia.org.br

Research Paper

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

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Submitted: April 14, 2011; Approved: November 13, 2012.

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 1.0×10^2 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 (Organizacão, 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

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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 Goias 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 $< 1.0 \times 10^1$ to 5.0×10^2 cfu.g^{-1} (Table 1). As the maximum level allowed for trading in the MERCOSUR is 10^2 cfu.g^{-1}, it was considered that 20% of samples showed cell count above this stipulated value. Similar counts of 1.0×10^1 to 3.0×10^2 cfu.g^{-1} were reported by Sodré *et al.* (2007) in *Apis mellifera* honeys produced in the State of Piauí, and of $< 1.0 \times 10^1$ to 6.1×10^2 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, Tchoumboue *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

Table 1 - Moisture content, yeasts and molds and acidity values in 35 samples of Apis mellifera L. honey from central Brazil, and the limit established by Brazilian Legislation and MERCOSUR.

Sample	Moisture (%)	Yeasts and Molds (cfu. g^{-1})	Acidity (mEq/kg)
1	15.2	< 1.0 x 10^1	46.79
2	16.4	2.0 x 10^2	32.87
3	17.0	2.0 x 10^2	43.86
4	16.6	1.0 x 10^2	59.85
5	18.0	< 1.0 x 10^1	70.87
6	16.8	< 1.0 x 10^1	39.06
7	18.2	< 1.0 x 10^1	78.13
8	18.4	3.0 x 10^2	65.14
9	18.2	2.0 x 10^2	31.33
10	15.6	< 1.0 x 10^1	20.22
11	18.8	< 1.0 x 10^1	48.61
12	16.6	5.0 x 10^2	39.07
13	15.8	3.0 x 10^2	23.24
14	16.4	< 1.0 x 10^1	26.10
15	16.2	< 1.0 x 10^1	21.04
16	15.6	1.0 x 10^2	32.77
17	18.8	1.0 x 10^2	58.70
18	16.4	< 1.0 x 10^1	21.32
19	16.6	< 1.0 x 10^1	72.30
20	17.2	< 1.0 x 10^1	20.70
21	15.0	1.0 x 10^2	41.06
22	16.6	< 1.0 x 10^1	19.95
23	15.8	1.0 x 10^2	21.76
24	16.2	2.0 x 10^2	25.42
25	17.0	< 1.0 x 10^1	28.77
26	16.4	< 1.0 x 10^1	23.80
27	20.2	< 1.0 x 10^1	40.34
28	17.6	< 1.0 x 10^1	25.25
29	18.6	< 1.0 x 10^1	28.03
30	16.6	1.0 x 10^2	37.87
31	20.6	1.0 x 10^2	73.83
32	17.0	< 1.0 x 10^1	65.39
33	18.0	1.0 x 10^2	63.50
34	15.6	1.0 x 10^2	22.27
35	17.8	1.0 x 10^2	42.62
Limit established by Brazil Legislation	20.0%	-	50 mEq/kg.
Limit established by MERCOSUR	20.0%	1.0 x 10^2 cfu.g^{-1}	40 mEq/kg.

higher activity of water in the liquid phase (Osachlo, 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 ºBrix (Anupama *et al.*, 2003), and as well as that, according to some studies, the higher the moisture level the greater the development of

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	0.5085 (p < 0.01)
Acidity	0.1265 (p = 0.4688)	-	1.0000

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 1.0×10^2 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.

Acknowledgments

The authors wish to thank the Federal University of Goiás for the use of their premises and concession of a scholarship and SEBRAE, Goiás, for its financial support.

References

Abramovic H, Jamnik M, Burkan L, Kac M (2008) Water activity and water content in Slovenian honeys. Food Control 19:1086-1090.

Almeida D (2002) Espécies de abelhas (Hymenoptera, Apoidae) e tipificacão dos méis por elas produzidos em área de cerrado, do município de Pirassununga, Estado de São Paulo. Piracicaba, Brasil, 116p. (M.Sc. Dissertation. Escola Superior de Agricultura "Luiz de Queiroz", USP).

Almeida-Anacleto D (2007) Recursos alimentares, desenvolvimento das colônias e características físico químicas, microbiológicas e polínicas de mel e cargas de pólen de meliponíneos, do município de Piracicaba, Estado de São Paulo. Piracicaba, Brasil, 134p. (Tese de Doutorado, Escola Superior de Agricultura "Luiz de Queiroz").

Alves EM (2008) Identificacão da flora e caracterizacão do mel orgânico de abelhas africanizadas das ilhas floresta e laranjeira, do alto do Rio Paraná. Maringá, Brasil, 77 pp. (Tese de Doutorado, Centro de Ciências Agrárias, UEM).

Anupama D, Bhat KK, Sapna VK (2003) Sensory and physico-chemical properties of commercial samples of honey. Food Res Int 36:183-191.

AOAC - Association of Official Analytical Chemists. (2003). *Official methods of analysis*. 17. ed. 2nd revision. AOAC. Gaithersburg.

Araújo DR, Silva RHD, Sousa JS (2006) Avaliacão da qualidade físico-química do mel comercializado na cidade de Crato, CE. Rev Biol Cienc Terra 6:51-55.

Arvanitoyannis I, Krystalli A (2006) An empirical examination of the determinants of honey consumption in Romania. Int J Food Sci Technol 41:1164-1176.

Balanza ME, Ordóñez A, Barrera M (2004) Acidez total y lactónica de la miel de abejas: correlación com otros parâmetros. Seminário Internacional de calidad de miel de Rafaela, Rafaela, SF. http://www.alimentosargentinos.gov.ar/foros/apicola/biblio/11-diciembre/Acidez%20total%20y%20lact%F3nica%20de%20la%20miel_%20Balanza-2.pdf.

Brasil (1985) Portaria nº 06, de 25 de julho de 1985. Aprova as normas higiênico-sanitárias e tecnológicas para mel, cera de abelhas e derivados. Brasília, DF, 1985.

Brazil (2000) Instrucão Normativa nº 11, de 20 de outubro de 2000. Aprova o Regulamento Técnico de Identidade e Qualidade do Mel. Brasília, DF, 2000.

Brazil (2003) Instrucão Normativa nº 62, de 26 de agosto de 2003. Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Ministério da Agricultura, Pecuária e Abastecimento, Brasília, DF, 2003.

Brazil, Ministério da Saúde (2005) Guia alimentar para a populacão brasileira: Promovendo a alimentacão saudável. Editora MS, Brasília, DF.

De Rodríguez GO, Ferrer BS, Ferrer A, Rodríguez B (2004) Characterization of honey produced in Venezuela. Food Chem 84:499-502.

European Commission (2002) Opinion of the scientific committee on veterinary measures relating to public health on honey and microbiological hazards. http://ec.europa.eu/food/fs/sc/scv/out53_en.pdf.

Evangelista-Rodrigues A, Silva SEM, Beserra EMF, Rodrigues ML (2005) Análise físico-química dos méis das abelhas *Apis mellifera* e *Melipona scutellaris* produzidos em duas regiões no Estado da Paraíba. Cienc Rural 35:1166-1171.

Finola MS, Lasagno MC, Marioli JM (2007) Microbiological and chemical characterization of honeys from central Argentina. Food Chem 100:1649-1653.

Garcia W (2003) Guia de Buenas Prácticas de Apícolas y de Manufactura. Secretaría de Agricultura, Ganadería, Pesca y Alimentos, Buenos Aires.

Kücük M, Kolail S, Karaoglu S, Ulusoy E, Baltac C, Candan F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem 100:526-534.

Mendes CG, Silva JBA, Mesquita LX, Maracajá PB (2009) As análises de mel: Revisão. Rev Caatinga 22:7-14.

Mendes E, Brojo Proenca E, Ferreira IMPLVO, Ferreira MA (1998) Quality evaluation of Portuguese honey. Carbohydr Polym 37:219-223.

MERCOSUL - Mercado Comum do Sul. - Resolucão nº 56, de 29 de setembro de 1999. Aprova o Regulamento técnico "Identidade e Qualidade do Mel. Montevidéu, 1999. http://www.mercosur.int/msweb/portal%20intermediario/Norma/Normas_web/Resoluciones/PT/Res_056_099_RTM%20Identidade%20Qualidade%20_Ata%203_99.PDF.

Olaitan PB, Adeleke OE, Ola IO (2007) Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci 7:159-165.

Organizacão Pan-Americana da Saúde (2009) Guias para o gerenciamento de riscos sanitários em alimentos. OPAS/OMS, Rio de Janeiro.

Osachlo L (2004) Aplicacão do sistema de análise de perigos e pontos críticos de controle no processamento industrial de mel de abelhas (*Apis mellifera*). Brasília, Brasil, 67 pp (Esp. Monografia. Centro de Excelência do Turismo, UNB).

Özcan M, Arslan D, Ceylan DA (2006) Effect of inverted saccharose on some properties of honey. Food Chem 99:24-29.

Pereira APR (2008) Caracterizacão de mel com vista a producão de hidromel. Braganca, Brasil, 85 pp (M.Sc. Dissertation, Escola Superior Agrária de Braganca, Instituto Politécnico de Braganca.

Pereira FM, Lopes MTR, Camargo RCR, Vilela SLO (2003) Producão de Mel. EMBRAPA, Teresina.

Salgado TB, Orsi RO, Funari SRC, Martins AO (2008) Análise físico-química de méis de abelhas *Apis mellifera* L. comercializados na região de Botucatu. PUBVET 2:art. 175.

Schlabi0tz C, Silva SAF, Souza CFV (2010) Avaliacão de parâmetros físico-químicos e microbiológicos em mel. Rev Bras Tecnol Agroind 4:80-90.

SEBRAE – Servico Brasileiro de Apoio às Micro e Pequenas Empresas (2009) PAS Mel: Manual de Boas Práticas Apícolas. SEBRAE/NA. Brasília, DF.

SEBRAE – Servico Brasileiro de Apoio às Micro e Pequenas Empresas (2008) PAS Indústria: Manual de Seguranca e Qualidade para Apicultura. SEBRAE/NA Brasília, DF.

Silva MBL (2007) Diagnóstico do sistema de producão e qualidade do mel de *Apis Mellifera*. Vicosa, Brasil, 97 pp (M.Sc. Dissertation, Departamento de Tecnologia de Alimentos. Universidade Federal de Vicosa).

Snowdon JA, Cliver DO (1996) Microorganisms in honey. Int J Food Microbiol 31:1-26.

Sodré GS, Marchini LC, Moreti ACCC, Otsuk IP, Carvalho CAL (2007) Caracterizacão físico-química de amostras de méis de *Apis mellifera* L. (Hymenoptera: Apidae) do Estado do Ceará. Cienc Rural 37:1139-1144.

Souza BA, Marchini LC, Dias CTS, Oda-Souza M, Carvalho CAL, Alves RMO (2009) Avaliacão microbiológica de amostras de mel de trigonídeos (Apidae: Trigonini) do Estado da Bahia. Cienc Tecnol Aliment 29:798-802.

StatSoft Inc (2004) Statistica: Data Analysis Software System (version 7). (1 CD-ROM).

Tchoumboue J, Awah-Ndukum J, Fonteh FA, Dongock ND, Pinta J, Mvondo ZA (2007) Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon. Afr J Biotechnol 6:908-913.

Terrab A, Recamales AF, Hernanz D, Heredia FJ (2004) Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chem 88:537-542.

União Européia. Comissão do Conselho Europeu - Diretiva CE nº 110, de 20 de dezembro de 2001. Define as normas comuns para o mel adaptando-as a legislacão geral aplicada a produtos alimentícios. Bruxelas, EU, 2001.

Welke JE, Reginatto S, Ferreira D, Vicenzi R, Soares JM (2008) Caracterizacão físico-química de méis de *Apis mellifera* L. da região noroeste do Estado do Rio Grande do Sul. Cienc Rural 38:1737-1741.

White Jr, JW La miel, In: Dadant H (1989) La colmena y la abeja mellifera. Hemisfério Sul, Montevideo.