PHYSICOCHEMICAL AND MICROBIOLOGICAL ASPECTS OF HONEY PRODUCED IN MINAS GERAIS STATE, BRAZIL[1]

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ABSTRACT – Samples from ten brands of floral honey produced in different cities of the state of Minas Gerais, Brazil were analyzed for their physicochemical and microbiological characteristics. The studied parameters were humidity, reducing sugars, apparent saccharose, insoluble solids, ashes, total acidity, diastatic activity, content of hydroxymethylfurfural, counting of filamentous fungi and yeasts, sulfite-reducing *Clostridia*, *Staphylococcus aureus*, *Shigella spp*. and *Salmonella spp*. detection, and numeration of total coliforms and *Escherichia coli*. All of these analyses followed official methodology. The physicochemical analyses indicated that a fraction of 60% (6/10) of evaluated brands were in disagreement with Brazilian legislation in, at least, one of analyzed parameters. Respecting to microbiological results, it was verified the presence of filamentous fungi and yeasts in five brands of honey, total coliforms and *E. coli* in two brands, and absence of any other microorganisms in all evaluated brands. Simultaneous presence of filamentous fungi and yeasts with total coliforms and *E. coli* was observed in one sample. The obtained results demonstrate that not all honey samples were in accordance to Brazilian legislation, and the presence of deteriorative and pathogenic microorganisms indicated a possible decrease in the product quality, and a potential risk to consumer.

Keywords: Brazilian legislation, food safety, pathogens, quality parameters.

ASPECTOS FÍSICO-QUÍMICO E MICROBIOLÓGICO DE DIFERENTES MARCAS DE MEL PRODUZIDOS EM MINAS GERAIS

RESUMO – Amostras de dez marcas de mel floral produzidas em diferentes municípios de Minas Gerais, Brasil, foram analisadas quanto suas características físico-químicas e microbiológicas. Os parâmetros estudados foram umidade, açúcares redutores, sacarose aparente, sólidos insolúveis, cinzas, acidez total, atividade diastásica, teor de hidroximetilfurfural, contagem de fungos filamentosos e leveduras, Clostridium sulfito redutores, Staphylococcus aureus, detecção de Shigella spp. e Salmonella spp. e enumeração de coliformes totais e Escherichia coli. Todas as análises seguiram metodologias oficiais. As análises físico-químicas indicaram que 60% (6/10) das marcas avaliadas estavam em desacordo com a legislação brasileira em pelo menos um dos parâmetros analisados. Nos resultados microbiológicos verificou-se presença de fungos filamentosos e leveduras em cinco marcas de mel, coliformes totais e E. coli em duas marcas e ausência para os demais micro-organismos em todas as marcas. A presença simultânea de fungos filamentosos e leveduras e de coliformes totais e E. coli foi observada em uma amostra. Os resultados obtidos demonstram que nem todas as amostras de mel estavam de acordo com a legislação brasileira e que a presença de micro-organismos deteriorantes e patogênicos indicam uma possível diminuição da qualidade do produto e um risco potencial ao consumidor.

Palavras chave: legislação brasileira, parâmetros de qualidade, patógenos, segurança alimentar.

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1. INTRODUCTION

According to Brazilian legislation (BRASIL, 2000), 'honey' is understood as the alimentary product resulting from the nectar of flowers or secretions coming from living parts of plants, or resulting from excretions of plants sucking insects that remain on the living parts of plants, which is collected by bees, transformed and combined by them with their own specific substances, and then stored and left maturating within hive honeycombs. It is a natural product composed mainly by a complex mix of carbohydrates, which presents also, in lower proportions, proteins, organic acids, lipids, vitamins, volatile compounds, phenolic acids, flavonoids, and carotenoids. In all types of honey, fructose, immediately followed by glucose, are the predominant carbohydrates, representing approximately 85-96% of the present total carbohydrates (Blasa *et al*., 2006; Finola *et al*., 2007).

In the last decades, studies have demonstrated a high correlation between the presence of phenolic compounds coming from honeys of several floral origins, and their respective antioxidant and antibacterial activities (Alvarez-Suarez *et al*., 2012; Al-Waily *et al*., 2013). Besides, other factors, such as lower pH and water activity values interfere in the growth and survival of microorganisms. Consequently, this product contains low amount of microorganisms. Their presence is indicative of recent contamination from secondary origin, or cross-contamination. Thus, the microbiological and physicochemical parameters are essential to detect product adulterations during manufacturing, and assure hygienic and adequate production and storage (Iurlina *et al*., 2005; Olaitan *et al*., 2007; Alves, 2013).

The Brazilian legislation (BRASIL, 2000) anticipates the physicochemical quality requisites, but does not consider any specific and acceptable microbiological characteristic for the product, orienting only about the hygienic practice for its elaboration. Thus, this present work has as purpose to verify if the physicochemical parameters from a sample of honeys commercialized in the state of Minas Gerais, Brazil, are effectively in accordance with current Brazilian legislation; verifying also, if the microbiological quality of these honeys, produced in the same area, presents some risk to consumer.

2. MATERIAL AND METHODS

Samples

Samples from honey coming from three lots, referring to 10 different brands produced in varied cities of the state of Minas Gerais, Brazil, were analyzed in this study, being obtained from September, 2011 up to July, 2012. These samples were maintained at room temperature, within their original plastic packages of 280 g, up to analysis occurrence. Every honey brand was identified with a sequential letter from 'A' to 'J', according to reception order. The physicochemical and microbiological analyses were developed in the Water and Food Analytical Laboratory (*Laboratório de Análise de Alimentos e Águas* – LAAA) of the *Universidade Federal de Juiz de Fora* (UFJF) Faculty of Pharmaceutics, in the state of Minas Gerais, Brazil.

Physicochemical analyses

The humidity content was determined by refractometry, according to method # 173/IV from *Instituto Adolfo Lutz* (2005). This method is based on the determination of honey refractive index at 20°C. The refractive index was converted into humidity percentage by means of the Chataway table. The determination of reducing sugars was developed according to method # 176/IV from *Instituto Adolfo Lutz* (2005), modified from Lane and Eynon, involving the reduction of Fehling's solution. The apparent saccharose content was determined after the sugar inversion by acid hydrolysis, according to method # 178/IV from *Instituto Adolfo Lutz* (2005). The content of insoluble solids was obtained by gravimetric method # 180/IV from *Instituto Adolfo Lutz* (2005). The content of ashes was determined by honey samples calcination in a muffle, at 600°C, up to constant weight (CAC, 1990). The total acidity was determined according to methodology # 962.19 from AOAC (1998). This method is based on the sum of free and lactonic acidity. The free acidity is determined by titration with 0.05 N NaOH up to achievement of equivalence point, at pH 8.5. The lactonic acidity is obtained by the addition of a given 0.05 N NaOH excess, which is titrated with 0.05 N HCl up to pH coming back to 8.3 value. The diastatic activity was obtained by means of spectrophotometric method at 660 nm, as described in method # 181/IV from *Instituto Adolfo Lutz* (2005), expressed in Gothe units per gram of honey. The hydroxymethylfurfural (HMF) content

was obtained by spectrophotometry at 284 and 336 nm, following the Analytical Norm # 175/IV from *Instituto Adolfo Lutz* (2005). Finally, the obtained results were compared with values established by Normative Instruction # 11, from the Ministry of Agriculture, Livestock, and Supplying (*Ministério da Agricultura, Pecuária e Abastecimento* – MAPA) (BRASIL, 2000).

Microbiological analyses

The microbiological analyses were developed according to methodologies described by Silva *et al*. (2007), for every microorganism group. The analyses developed were counting of filamentous fungi and yeasts, sulfite-reducing *Clostridia*, *Staphylococcus aureus*; *Shigella spp*. and *Salmonella spp*. detection, and numeration of total coliforms and *Escherichia coli*. Aliquots of 25 g of every sample were aseptically weighed and homogenized in 225 mL of 0.1% buffered peptone water (dilution 10^{-1}) with the help of Stomacher equipment. The subsequent dilutions (10^{-2} and 10^{-3}) were developed in tubes containing 9 mL of this same diluent. All analyses were developed in duplicate.

The standard counting of filamentous fungi and yeasts was developed by in-depth plating, with inoculation of 1 mL of every dilution, utilizing the Potato Dextrose Agar (PDA) medium, acidified with 10% tartaric acid up to pH 3.5. Then, the plates were incubated at 25°C during five days. The counting of Sulfite-reducing *Clostridia* was developed by the addition of 10 g of honey sample into 90 mL of Tryptone Soya Broth (TSB), following incubation at 30°C/48 hours. Further, an aliquot of 1 mL of cultivation in TSB was plated with overlay on S*hahidi*-Ferguson Perfringens (SFP) agar, and incubated at 46°C/48 hours. The count of *S. aureus* was developed through inoculation of 0.1 mL of every dilution on plates containing Baird-Parker (BP) agar, with further incubation for 48 hours at 32°C. Colonies considered typical in BP were selected for confirmation of coagulase enzyme production in lyophilized rabbit plasma. For the *Salmonella spp*. and *Shigella spp*. investigation, an aliquot of 25 g of honey samples was added to 225 mL of 2% peptone water for a non selective pre-enrichment with media incubation at 35°C/24 hours. Then, aliquots of 1 mL and 0.1 mL were transferred to the selective enrichment media Tetrationate (TT) and Rappaport (RP), respectively. TT medium was incubated at 35°C/24 hours, and RP medium at 42°C/ 24 hours. Subsequently, differential selective plating was developed in Brilliant Green (BG) and Salmonella Shigella (SS) media, incubating them at 35°C/48 hours. The purification of colonies was done in Tryptone Soya Agar (TSA), with incubation at 35°C/24 hours. The confirmation was developed by biochemical examinations developed in Lysine-Iron Agar (LIA) and Triple Sugar Iron (TSI) agar, both incubated at 35°C/48 hours, with serological analyses being then developed. For numeration of total coliforms and *E. coli*, the fermentation in multiple tubes technique was utilized, developing initially the presumptive test in Lauryl Sulfate Tryptose (LST) broth, with incubation at 35°C/48 hours. For the confirmative tests, the Bile-Brilliant Green (BBG) and EC-mug broths were utilized, which identify, respectively, total coliforms and *Escherichia coli*. The results were expressed in NMP.g^{-1}, according to Hoskins table.

As the current legislation does not establish microbiological standards for bees' honey, recommending only following the Good Agricultural Practice in the honey extraction, the microbiological results were compared with values reference in the literature.

3. RESULTS AND DISCUSSION

Physicochemical analyses

In the samples evaluated in this study, fermentation and granulation were not visually verified before the physicochemical analyses. The results from physicochemical analyses are presented at Tables 1 and 2.

According to results observed, the evaluated samples are within the parameters established by Brazilian legislation for humidity, insoluble solids, total acidity, diastatic activity (*Atividade Diastásica* – AD), and content of hydroxymethylfurfural (HMF). However, it was verified that for remaining parameters, at least one sample, was in disagreement with Brazilian legislation.

It was verified in all evaluated samples that humidity content was within the standards established by Brazilian legislation, which is of, at least, 20% (BRASIL, 2000). The samples presented variation from 16.2% to 17.9%, with a mean value of 17.06%. Similar values were found by Richter *et al*. (2011) and Feas *et al*. (2010), who found mean values of 18.9% and 17.5%, respectively, for the analyzed samples. The small variation observed between the samples could be due to similar handling practices by the apiarists. The humidity content in

Table 1 - Mean values for 'humidity', 'reducing sugars', 'apparent saccharose', and 'insoluble solids', for honey samples produced in the State of Minas Gerais, Brazil

Sample	Humidity (%)	Reducing Sugars (%)	Apparent Saccharose (%)	Insoluble Solids (%)
A	16.30 ± 0.70	64.64 ± 2.34	1.84 ± 0.36	0.02 ± 0.05
B	16.20± 0.28	69.77 ± 1.02	1.96 ± 1.30	0.01 ± 0.00
C	16.80 ± 0.28	68.14 ± 1.28	1.30 ± 0.57	0.01 ± 0.01
D	17.80 ± 0.56	65.99 ± 2.63	3.81 ± 0.26	0.02 ± 0.01
E	17.90 ± 0.71	67.55 ± 1.47	0.88 ± 0.79	0.02 ± 0.00
F	16.20 ± 1.13	63.82 ± 2.86	5.26 ± 4.40	0.05 ± 0.03
G	15.80 ± 0.07	70.15 ± 0.63	2.92 ± 0.98	0.01 ± 0.00
H	17.90 ± 0.14	67.24 ± 0.19	4.22 ± 0.97	0.00 ± 0.00
I	17.80 ± 0.05	69.89 ± 0.46	0.99 ± 0.01	0.01 ± 0.00
J	17.80 ± 0.99	69.56 ± 2.14	1.71 ± 1.70	0.03 ± 0.02
Mean	17.06	67.68	2.49	0.02
Legislation '(BRASIL, 2000)	Maximum 20%	Minimum 65%	Maximum 3%	Maximum 0.1%

Table 2 - Mean values for 'ashes', 'total acidity', 'diastatic activity', 'hydroxymethylfurfural', for honey samples produced in the State of Minas Gerais, Brazil

Sample	Ashes (%)	Total Acidity (mEq/Kg)	DA (Gothe)	HMF (mg/Kg)
A	0.37 ± 0.18	40.09 ± 16.39	61.37 ± 49.00	32.22 ± 19.31
B	5.35 ± 2.53	35.41 ± 10.13	59.64 ± 11.98	24.09 ± 3.42
C	0.58 ± 0.26	28.45 ± 5.39	23.81 ± 14.51	3.32 ± 3.76
D	0.66 ± 0.53	37.65 ± 19.73	43.10 ± 13.33	12.09 ± 1.66
E	0.39 ± 0.31	38.76 ± 1.82	43.69 ± 20.29	17.56 ± 20.50
F	0.55 ± 0.03	33.01 ± 10.28	24.82 ± 9.12	12.55 ± 2.40
G	0.73 ± 0.29	22.63 ± 10.96	23.60 ± 11.17	7.47 ± 7.32
H	0.37 ± 0.17	25.75 ± 1.83	52.84 ± 43.81	21.76 ± 2.03
I	0.38 ± 0.02	26.49 ± 0.44	40.84 ± 16.03	5.00 ± 0.06
J	0.40 ± 0.03	23.81 ± 0.03	36.44 ± 7.38	10.24 ± 2.94
Mean	0.98	31.21	41.07	14.63
Legislation (BRASIL, 2000)	Maximum 0.6%	Maximum 50 mEq/Kg	Minimum 8	Maximum 60 mg/Kg

DA: Diastatic Activity: HMF: Hydroxymethylfurfural.

the honey could influence the taste, viscosity, and fluidity. Besides, the knowledge of humidity content is important for conservation and storage, being its alteration indicative of fermentation process (Araújo, 2006; Feas *et al*., 2010).

Values for reducing sugars found in both samples (A and F) were below the standards established by current legislation, which is of, at least, 65% (BRASIL, 2000). Between the results obtained by apparent saccharose, samples 'D', 'F', and 'H' presented values above the one established by current Brazilian legislation. Saccharose values above the allowed range could indicate premature collection, due to nectar saccharose being not totally transformed into glucose and fructose. Besides, high contents of saccharose in honey could identify adulteration by addition of partially inverted saccharose syrup. However, some adulterating agents do not possess saccharose, and the adulteration could only be identified with HMF content analysis (Evangelista-Rodrigues *et al*., 2005). It is convenient to emphasize that the addition of sugars or syrups, at any production phase, is considered fraud; and the identification of any type of adulteration is important for economic and public health reasons.

The values found for insoluble solids are within the limits established by Brazilian legislation. Similar results were found by Alves *et al.* (2011), when analyzing honey coming from the several Uruçu apiarian species (*Meliponinae* subfamily). The insoluble solids analysis is an important index for honey purity (Santos *et al.*, 2010).

In this present study, three samples presented values above 0.6% for ashes, possibly indicating honey adulteration with molasses (Mendes *et al.*, 1998). Santos *et al.* (2010) found values for ashes higher than required by legislation, achieving a mean of 1.15%. The ashes value expresses the content of minerals present in the honey, being it influenced by the honey's botanic origin, as well as the technique utilized in the respective determination. Higher ashes values could indicate lack of hygiene and absence of decantation and/or filtration in the final process of honey collection developed by the apiarian; otherwise, it could be even an indicator of honey adulteration with molasses (Mendes *et al.*, 1998; Evangelista–Rodrigues *et al.*, 2005).

The results obtained for total acidity are within the limits specified by legislation, indicating absence of undesired microbial fermentations responsible for decrease in honey's quality and shelf life. The mean value obtained from the samples for this examination was 31.21 mEq/kg; this value is similar to those observed by Feas *et al.* (2010) and Nanda *et al.* (2003) for multifloral honeys. The total acidity variation (22.63-40.09 mEq/kg) in this present study could be attributed to honey's collection period and respective original botanic species, due to the fact that acidity in honey originates from several organic acids contained in the nectar collected by bees, which thanks to action of glucose-oxidase enzyme, originate the gluconic acid and is also influenced by the amount of minerals present in the nectar (Root, 1985; Silva *et al.*, 2004; Feas *et al.*, 2010).

In this study, the content of hydroxymethylfurfural (HMF) and the diastatic activity (AD) were verified in all samples and showed to be according to legislation, indicating that sampled honeys were not submitted to thermal treatment. Such analyses precisely indicate honey's quality and possible thermal processing; however, they do not evaluate the floral origin of samples. Sereia *et al.* (2011) and Fallico *et al.* (2004) obtained lower values for HMF and DA when analyzing organic honeys and honeys proceeding from orange tree. The content of HMF and AD are worldly recognized parameters to evaluate the honey's freshness. The HMF content results from the degradation of fructose in acid environment and indicates the honey's freshness and conservation status (Terrab *et al.*, 2002; Mogliotti *et al.*, 2011). According to Nozal *et al.* (2001), honey warmed under inadequate conditions after storage, or adulterated with inverted sugar syrup, promotes the formation of HMF, which decreases the product quality (Feas *et al.*, 2010). The AD value indicates the presence of diastase enzyme, which has the function to digest starch molecules and is very sensitive to heat. The Brazilian legislation anticipates HMF values up to 60 mg/kg, and AD values of, at least, 8 in the Gothe scale.

Microbiological analyses

In this present work, the microbiological analyses demonstrated absence of sulfite-reducing *Clostridia*, *S. aureus*, *Shigella spp.*, and *Salmonella spp.* in all honey samples. The presence of filamentous fungi and yeasts, total coliforms, and *E. coli* was respectively confirmed in five, two, and two samples. Out of these samples, one presented as filamentous fungi and yeasts, as total coliforms and *E. coli* (Table 3). The honey is synthesized by chemical transformation of the nectar, pollen, and water collected from flowers by the worker bees. During this process, the bees interact with several environmental factors, including microorganisms and airborne particles, which could be retained on their body surface or inhaled and adhered to their trachea. Besides, the sweeteners used to feed bees could be a microbial source. Such factors are mentioned as the main sources of contamination, and there are few or none control over them (Snowdon & Cliver, 1996; Olaitan *et al.*, 2007).

According to Resolution # 15/94 approving the 'Mercosul Technical Regulation for Honey Identity and Quality', honey brands should present, as a maximum, 10^2 CFU.g^{-1} of filamentous fungi and yeasts. The counting of filamentous fungi and yeasts in this present work varied from 1.0×10^1 to 3.3×10^1 CFU.g^{-1}; indicating that all samples were according to above legislation. In accordance with Alves *et al.* (2011) and Silva *et al.* (2008), honey of floral origin presents its own microbiota. This microbiota could be introduced by bees in the hive, by means of nectar, pollen, or during the cleaning operations they usually develop. Such

Table 3 - Microbiological parameters of honey brands produced in the state of Minas Gerais, Brasil

Sample	SRC	*SA*	*SL*	*SG*	FFY (CFU.g^{-1})	TC (NMP.g^{-1})	*EC*(NMP.g^{-1})
A	<10	<10	Absence	Absence	1.0 x 10^1	3.6	3.6
B	<10	<10	Absence	Absence	<10	<3	<3
C	<10	<10	Absence	Absence	1.0 x 10^1	<3	<3
D	<10	<10	Absence	Absence	<10	9.3	9.3
E	<10	<10	Absence	Absence	<10	<3	<3
F	<10	<10	Absence	Absence	1.0 x 10^1	<3	<3
G	<10	<10	Absence	Absence	3.3 x 10^1	<3	<3
H	<10	<10	Absence	Absence	<10	<3	<3
I	<10	<10	Absence	Absence	3.3 x 10^1	<3	<3
J	<10	<10	Absence	Absence	<10	<3	<3

SRC: Sulfite-reducing *Clostridia*; SA: *Staphylococcus aureus*; SG: *Shigella spp.*; SL: *Salmonella spp.*; FFY: Filamentous Fungi and Yeast: TC: Total *Coliforms*; EC: *Escherichia coli*.

microbiota includes filamentous fungi and yeasts that, under normal pH and humidity conditions, do not interfere in the honey's quality and are not pathogenic. The exhibited lower counting of filamentous fungi and yeasts could be a result of honeys' natural microbiota, and not simply a result of inadequate practices. Besides, humidity values lower than 20% inhibit the development of filamentous fungi and yeasts in honey samples (Alves *et al*., 2009). As the samples presented humidity value lower than this latter one, such factor could be favored the low counting for these fungi. Values similar to that in this present work were also observed in artisanal honeys produced in the city of Marília, state of São Paulo, Brazil (Pontara *et al*., 2012) or in Portugal (Feas *et al*., 2010). However, in other studies, much higher values were observed in honey samples produced in different Brazilian regions (Silva *et al*., 2008; Alves *et al*., 2009; Sereia *et al*., 2011).

The absence of sulfite-reducing *Clostridia*, *S. aureus*, *Shigella spp*., and *Salmonella spp*. in the honey samples evaluated in this present work corroborate with results found in other studies (Iurlina & Fritz, 2005; Schlabitz *et al*., 2010; Santos *et al*., 2011; Pontara *et al*., 2012; Santos & Oliveira, 2013). Although not finding *Salmonella spp*. in the evaluated honey samples, Feas *et al*. (2010) and Rall *et al*. (2003) verified the presence of sulfite-reducing *Clostridia* in 2 and 3% of samples, respectively. Finola *et al*. (2007) confirmed the presence of sulfite-reducing *Clostridia* in 70% of samples, indicating the possibility of presence of *Clostridium botulinum* spores, being such contamination the main cause of child botulism all over the world.

The presence of coliforms group bacteria indicates failures in the production hygiene practices, reflecting a decrease in the product shelf life and alimentary insecurity by the possible presence of pathogens (Pontara *et al*., 2012). The presence of total coliforms and *E. coli* in 20% of evaluated samples indicates that, although honey possesses properties to embarrass the microbial development, such as antimicrobial substances or lower pH and water activity values, the presence of coliforms group bacteria suggests recent contamination and, therefore, failures in the production procedures that lead to health risk for consumer and product quality loss (Waili *et al*., 2012). Other authors have identified absence of total coliforms and *E. coli* in honey samples of floral origin (Iurlina & Fritz, 2005; Silva *et al*., 2008; Gallez e Fernandez, 2009; Feas *et al*., 2010; Sereia *et al*., 2011; Pontara *et al*., 2012; Santos & Oliveira, 2013).

4. CONCLUSIONS

The conclusion is that not all honey samples produced in the state of Minas Gerais were in accordance with Brazilian legislation; a fact that indicates possible adulteration, product quality decrease, and a public health risk.

5. LITERATURE CITED

ALVAREZ-SUAREZ, J.M.; GIAMPIERI, F.; GONZÁLEZ-PARAMÁS, A.M. et al. Phenolics from monofl oral honeys protect human erythrocyte membranes against oxidative damage. **Food Chemical and Toxicology**, v.50, p.1508-1516, 2012.

ALVES, E.M.; TOLEDO, V.A.A.; MARCHINI, L.C. et al. Presença de coliformes, bolores e leveduras em amostras de mel orgânico de abelhas africanizadas das ilhas do alto rio Paraná. **Ciência Rural**, v.39, n.7, p.2222-2224, 2009.

ALVES, T.T.L.; MENESES, A.R.V.; SILVA, J.N. et al. Caracterização físico-química e avaliação microbiológica de méis de abelhas nativas do nordeste brasileiro. **Rev. Verde**, v.6, n.3, p.91-97, 2011.

AOAC-Association of Official Analytical Chemists. **Official methods of analysis**. 16.ed. rev.4. Washington, 1998. 1170p.

ARAÚJO, D.R.; SILVA, R.H. D.; SOUSA, J.S. Avaliação da qualidade físico-química do mel comercializado na cidade de Crato, CE. **Rev. Biol. Ciênc. Terra**, v.6, n.1, p.51-55, 2006.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 11 de 20 de outubro de 2000. **Regulamento Técnico de identidade e qualidade do mel**. Diário Oficial da República Federativa do Brasil, Brasília, DF, 23 de outubro de 2000. Seção 1, p. 16-17.

ALVES, A.; RAMOS, A.; GONÇALVES, M.A. et al. Antioxidant activity, quality parameters and mineral content of Portuguese monoûoral honeys. **J. of Food Comp. and Anal.**, v.30, p.130-138, 2013. In: http://ac.els-cdn.com/S0889157513000410/1-s2.0-S0889157513000410-main.pdf?_tid=7feb3d6e-2552-11e3-82c6-00000aacb35d&acdnat=1380052381_13ab2c6d9343d8677729a30c1274c77e (acessado em 25 agosto de 2013).

AL-WAILI, N.; AL GHAMDI, A.; ANSARI, M.J.; AL-ATTAL, Y.; AL-MUBARAK, A.; SALOM, K. Differences in Composition of Honey Samples and Their Impact on the Antimicrobial Activities against Drug Multiresistant Bacteria and Pathogenic Fungi. **Arch. of Medical Research**, v.44, p.307-316, 2013.

AL-WAILI, N.; SALOM, K.; AL-GHAMDI, A.; ANSARI, M.J. Antibiotic, Pesticide, and Microbial Contaminants of Honey: Human Health Hazards The Scientific World. **J. Volume**, p.1-9, 2012.

BLASA, M.; CANDIRACCI, M.; ACCORSI, A.; PIACENTINI, M.P.; ALBERTINI, M.C.; PIATTI, E. Raw Millefiori honey is packed full of antioxidants. **Food Chem.**, v.97, p.217–222, 2006.

EVANGELISTA-RODRIGUES, A.; SILVA, E.M.S.; BESERRA, E.M.F.; RODRIGUES, M.L. Análise físico-química dos méis das abelhas *Apis mellifera* e *Melipona scutellaris* produzidos em regiões distintas no Estado da Paraíba. **Ciência Rural**, v.35, n.5, p.1166-1171, 2005.

FALLICO, B.; ZAPPALA, M.; ARENA. E.; VERZERA, A. Effects of conditioning on HMF content in unifloral honeys. **Food Chem.**, v.85, p.305-313, 2004.

FEÁS, X.; PIRES, J.; IGLESIAS, A.; ESTEVINHO, M.L. Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. **Food and Chem. Toxicol.**, v.48, p.3462-3470, 2010.

FINOLA, M.S.; LASAGNO, M.C.; MARIOLI, J.M. Microbiological and chemical characterization of honeys from central Argentina. **Food Chemistry**. v.100, p.1649-1653, 2007. In: http://ac.els-cdn.com/S0308814606000288/1-s2.0-S03088146 06000288-main.pdf?tid=af452078-2635-11e3-b0e9-00000aab0f02&acdnat=13801499 56c225f3546769385 fb4867a0392dcff1c (acessado em 25 setembro 2013).

GALLEZ, L.M.; FERNANDEZ, L.A. Mieles del sistema serrano de Ventania: evaluación de la calidad microbiológica dentro del circuito de la planta de extracción. **Rev. Argent. Microbial.**, v.41, n.3, p.163-167, 2009.

IAL. Normas Analíticas do Instituto Adolfo Lutz. **Métodos químicos e físicos para análise de Alimentos**. 3ª ed. São Paulo, Instituto Adolfo Lutz, 2005. v. 1

IURLINA, M.O; FRITZ, R. Characterization of microorganisms in Argentinean honeys from different sources. **Int. J. of Food Microbiol.**, v.105, p.297-304, 2005.

MENDES, E.; PROENC¸ E.B.; FERREIRA, I.M.P.L.V.O, FERREIRA, M.A. Quality evaluation of Portuguese honey. **Carbohydrate Polymers**, v.37, p.219-223, 1998.

MERCOSUL. Resolução nº 15/94. **Regulamento técnico MERCOSUL de identidade e qualidade do mel**. Buenos Aires: Grupo de Mercado Comum, 1994. In: http:// www.inmetro.gov.br/barreirastecnicas/PdF/ GMC_reS_1994-015.pdf (acessado em 26 jan. 2012).

MOGLIOTTI, P.; GUARALDO, P.; ROSSO, A. et al. Honey quality in Piedmont region. **Italy J. of Food**, v., n., p., 2011.

PONTARA, L.P.M.; CLEMENTE, E.; OLIVEIRA, D.M. *et al*. Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees. **Safety**, v.1, n.0, p., 2011.

NANDA, V. SARKAR, B.C.; SHARMA, H.K. et al. Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. **J. of Food Comp. and Anal.**, v.16, p.613-619, 2003.

NOZAL, M.J.; BERNAL, J.L.; TORIBIO, L.; JIMENEZ, J.J.; MARTIN, M.T. High performance liquid chromatography determination of methylanthranilate, hydroxymethylfurfural and related compounds in honey. **J. of Chromatography**, v.917, n.1-2, p.95-103, 2001.

OLAITAN, P.B.; ADELEKE, O.E.; OLA, Y.O. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. **African Health Sciences**, v.7, n.3, 2007.

PONTARA, L.P.M.; CLEMENTE, E.; OLIVEIRA, D.M. et al. Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees. **Ciênc. Tecnol. Aliment.**, v.32., n.3, p.547-552, 2012

RALL, V.L.M.; BOMBO, A.J.; LOPES, T.F. et al. Honey consumption in the state of São Paulo: a risk to human? Anaerobe**, v.9, n.6, p.299-303, 2003.**

RICHTER, W.; JANSEN, J.; VENZKE, T.S.L. et al. Avaliação da qualidade físico-química do mel produzido na cidade de Pelotas/RS. **Alim. Nutr.**, v.22, n.4, p. 547-553, 2011.

ROOT, A. I. **ABC y xyz de la apicultura: encyclopedia de la cria cientifica y prática de las abejas**. Buenos Aires: Editorial Hemisfério Sur, 1985. 723p.

SANTOS, D.C.; MARTINS, J.N.; SILVA, K.F.N.L. Aspectos físico-químicos e microbiológicos do mel comercializado na cidade de Tabuleiro do norte do Ceará. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v.5, n.1, p.79-85, 2010.

SANTOS, D.C.; OLIVEIRA, E.N.A.; MARTINS, J.N. Caracterização físico-quimica de méis comercializados no Município de Aracati- CE. **Acta Vet. Brasilica**, v.5, n.2, p.158-162, 2011.

SANTOS, D.C.; OLIVEIRA, E.N.A. Características físico-químicas e microbiológicas de méis de *Apis mellifera* L. provenientes de diferentes entrepostos. **Comunicata Scientiae**, v.4, n.1, p.67-74, 2013.

SCHLABITZ, C.; SILVA, S.A.F.; SOUZA, C.F.V. Avaliação de parâmetros físico-químicos e microbiológicos em mel. **Rev. Bras. de Tecnol. Agroind.**, v.4, n.1, p.80 – 90, 2010.

SEREIA, M.J.; ALVES, E.M.; TOLEDO, V.A.A. et al. Microbial flora in organic honey samples of Africanized honeybees from Parana River islands. **Ciênc. e Tecnol. de Alim.**, v.31, n.2, p.462-466, 2011.

SILVA, C.L. QUEIROZ, A.J.M.; FIGUEIREDO, R.M.F. Caracterização físico-química de méis produzidos no Estado do Piauí para diferentes floradas. **Revista Brasileira de Eng. Agric. e Amb.**, v.8, n.2-3, p.260-265, 2004.

SILVA, N.; JUNQUEIRA, V.C.A.; SILVEIRA, N.F.A. et al. **Manual de métodos de análise microbiológica de alimentos**. Livraria Varela. 3° edição, São Paulo, 2007.

SILVA, M. B. L. CHAVES, J.B.P.; MESSAGE, D. et al. Qualidade microbiológica de méis produzidos por pequenos apicultores e de méis de entrepostos registrados no Serviço de Inspeção Federal no Estado de Minas Gerais. **Alim. e Nutr.**, v.19, n.4, p.417-420, 2008.

SNOWDON, J. A., CLIVER, D. O. Microorganisms in honey. **Int. J. of Food Microbiol.**, v.31, p.1-26, 1996.

TERRAB, A.; DIEZ, M.J.; HEREDIA, F.J. Characterisation of Moroccan unifloral honeys by their physico-chemical characteristics. **Food Chemistry**, v.79, p.373-379, 2002.

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