Journal of Food: Microbiology, Safety & Hygiene

Adjaloo et al., J Food Microbiol Saf Hyg 2017, 2:3
DOI: 10.4172/2476-2059.1000123

Research Article Open Access

Assessment of Honey Quality in Ghana: A Pilot Study of Honeys Produced in Drobo and Berekum

Michael K. Adjaloo[1*], Chris Yaw Asare[2], William Appaw[3] and Owusu Boahene[4]

[1]*Technology Consultancy Centre, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Department of Biochemistry and Biotechnology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[3]*Department of Food Science and Technology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[4]*Kumasi Business School, College of Humanities, KNUST, Kumasi, Ghana*

[*]**Corresponding author:** Michael K. Adjaloo, Technology Consultancy Centre, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, E-mail: mkadjaloo@gmail.com

Received date: June 07, 2017**; Accepted date:** July 01, 2017; **Published date:** July 06, 2017

Abstract

Honey is a natural source of food with countless health benefits. This study was to investigate the quality of honey from well-known honey extraction modes in the Brong Ahafo Region of Ghana. 24 honey samples were taken from bee farms in two districts in the region and subjected to physicochemical and microbiology analysis. Moisture content of the honeys, their pH, water insoluble solids, and sugar were determined. ISO/IEC 17025, ISO 16140-1: 2016 methods of microbial assays were done to determine the presence of aerobes, coliforms, *E.coli* and fungi. Statistical analysis was carried out using the GraphPad Prism 5. The results indicated that moisture content of the honeys ranged from 14.46%-22.31%. The pH values were within the acidity range (4.1-4.3), whiles water insoluble solids ranged from 0.56% to 8.50%; sucrose level ranged from 2.64%-3.12%, but the pressed honey had the highest glucose+fructose (90%). There was significant differences ($p<0.05$) in the microbial quality of the honeys from the two districts, and under the different treatments. Cold extracted honeys recorded the highest bacterial count of 4.614×10^3 cfu/g, honey from pressed method had the least count of 3.30×10^2 cfu/g. Food pathogens were not detected. Aerobes were within safe limits; hence, these honeys were classified as safe for consumption. The results indicated that the best mode of honey extraction was by honey press. All the detected counts fell within the acceptable limits. The study concluded that generally, honey produced locally is safe for consumption.

Keywords: Honey quality; Honey extraction; Beekeeping; Nectar

Introduction

Honey is a sweet substance produced by honey bees using nectar and other additives. It is stored and left to mature in the hive [1], but harvested for human consumption [2]. The precise composition of honey largely depends on the plant species on which bee forages and the regional source [3] however, it is mainly composed of sugars, amino acids, minerals, aromatic substances, pigment waxes and pollen grains [4].

Apart from its consumption as food, honey is known to possess health benefits, such as battling seasonal allergies and infections, wound healing, cough treatment and as an antiseptic [2]. Though not sterile, it is characterised by low moisture and high sugar content which ensures low proliferation of microorganisms [5]. Honey, however, could be associated with some adverse health effects especially when it is contaminated or of low quality. It risks being contaminated with moulds and other microorganisms from the various extraction and filtration methods [6]. According to the Codex Alimentarius [7], honey is a major victim of adulteration and alterations in its composition. It is highly possible for individuals to adulterate honey during the extraction process [3], and thus compromise its quality.

In Ghana apiculture is largely encouraged as a small scale industry because it gives additional income to farmers and rural dwellers. Generally, honey extraction is done using any of four extraction methods viz: hand squeezing/crushing, cold dripping, solar extraction, and, honey press. Fenicia and Anniballi [8] have suggested that these extraction methods have the potential of exposing the honey product to contamination with yeast, wax, pollen, and *Clostridium botulium* endospores which causes botulism in infants, but did not confirm the assertion. Even though honey quality has been a subject of great interest e.g. [9,10], there has not been studies on the effect of extraction methods on the quality of honey produced. This study therefore was carried to evaluate the quality of honeys obtained by the four extraction techniques being employed by beekeepers in Ghana, especially how the individual methods affect the physicochemical composition as well as the micro flora of the honeys vis-a-vis the Codex Standards.

Materials and Methods

Source of honey samples

A total of 24 different honey samples (i.e. 12 samples per area) were harvested from honey production farms in Berekum and Drobo in the Brong Ahafo Region of Ghana. The two areas are covered by the wet semi-equatorial climate zone which occurs widely in the tropics. They fall within the vast transition forest of Ghana with a mean annual rainfall ranging between 1,275–1,544 mm during the major rainy season (May to June). The transition forest has been classified as the premier beekeeping zone, as they are rich in bee population and

floristic composition [11]. Berekum and Drobo have renowned honey producing groups which are collaborators of this study.

The samples were categorized on the basis of origin/location, and their extraction methods. They were transported at room temperature in sterile containers. Two sets of analysis were carried out: the first was the physicochemical analyses at the Food Analysis laboratory of the Department of Food Science and Technology; and the second was microbiology analysis at the Microbiology Laboratory of the Department of Biochemistry and Biotechnology all of the Kwame Nkrumah University of Science and Technology.

Physicochemical analysis

Moisture: Moisture in the honey samples was determined using refractometric analysis method as stated by Harmonized Methods for International Honey Commission [12]. All measurements were performed at 20°C after equilibrium. The corresponding percent (%) moisture from the refractive index of the honey sample was calculated by consulting a standard table for this purpose.

pH: The pH was determined based on Association of Official Analytical Chemistry - Official Methods of Analysis [13] methodology. Five (5) g of honey was dissolved in a 15 ml of deionized water and the solution transferred into a 50 ml volumetric flask and topped up to the mark. The pH was then measured using a Mettler Toledo pH meter equipped with a glass combined electrode.

Water insoluble solids: Twenty (20) g of honey was dissolved in 200 ml of warm water (80°C) and the resulting solution filtered into a pre-weighed crucible. The crucible was then dried at 135°C for an hour and cooled in a desiccator and weighed. The crucible was returned into the oven at 30mins interval until a constant weight was obtained. Water Insoluble Solids were then expressed at percentage weight difference.

Sucrose, Glucose and Fructose: Sucrose content was determined by inversion, adding 10 ml of dilute HCl, 50 ml of diluted honey solution and water in a 100 ml volumetric flask. The solution was then heated in a water bath, cooled and diluted to the mark. Determination of reducing sugars and sucrose contents was determined by Layne-Enyon method while fructose was determined by resorcinol reagent method as prescribed in AOAC [13].

Microbiology analysis

Microflora analysis: Fungal isolation was carried out by the standard method ISO 21527-2:2008. Ten grams of each honey sample was separately and serially diluted in 0.1% peptone water. 0.1 ml of each dilution was spread on petri dishes containing sterile Malt Extract Agar (MEA). The plates were incubated for 5-7 days, to observe the development of moulds and yeasts for characterization using the three point method through sub-culturing.

Total aerobic count (TAC): The Total Aerobic Count (TAC) was carried out to determine the presence or otherwise of microbes in the honey samples using the [14] methods. Serial dilutions to the sixth power were prepared using bacteriological peptone as diluent by weighing 10 g of honey into 90 ml of sterile diluent. 0.1 ml of the dilution was inoculated unto sterile plates of Plate Count Agar using the spread plate technique and incubated for 48 h at 37°C. The resulting colonies were recorded. The pure colonies from the TAC were isolated and characterized through biochemical assay: Gram staining, catalase, oxidase, citrate, TSI and motility.

Food pathogens: The TAC results indicated the presence of microbes; therefore tests were carried out to determine the presence of coliforms in the honey samples as they are pathogens commonly found in foods. Violet red bile lactose agar was used to incubate 0.1 ml of dilutions at 37°C for 48 h and observed colonies were reported for Coliforms [14]. Subsequently, the presence of *Escherichia coli* was also tested since it is a pointer to contamination. A volume of 0.1 ml of the prepared serial dilution was inoculated on sterile plates of Brilliance *E. coli* media *via* spread plating and incubated for 48 h at 37°C. The plates were examined for purple colonies which indicate the presence of *E. coli* [15].

Statistical analysis

Data from the various tests were analyzed and presented graphically using the GraphPad® Prism version 5.0. Data were log transformed prior to statistical analysis after which they were untransformed. The Kruskal-Wallis test was done to show the significant difference in the microbial loads recorded for the various modes of extraction. The validity confirmation using a 95% Tukey HSD interval showed acceptable standard errors (0.20) and variability in measurements. All statistical analysis was done at 95% confidence interval.

Results

Physiochemical analysis

Table 1 presents all the results of the physicochemical analysis. The moisture content of the honey ranged from 14.46%-22.31% with solar recording the lowest while press had the highest moisture content. Mode of extraction had significant effect on the moisture content of the honey analyzed ($p<0.05$).

Extraction Technique	Moisture	pH	WSS	Sucrose	Glucose+fructose
Cold	20.24 ± 0.99a	4.1 ± 0.01a	1.74 ± 0.12a	2.64 ± 0.34a	85.5 ± 0.34a
Hand	22.26 ± 0.64b	4.3 ± 0.71a	8.50 ± 0.11b	2.78 ± 0.15a	87.3 ± 0.54a
Press	22.31 ± 0.55b	4.1 ± 0.01a	0.56 ± 0.07c	3.12 ± 0.17a	90.0 ± 0.17a
Solar	14.46 ± 0.78b	4.2 ± 0.02a	1.48 ± 0.04a	2.83 ± 0.24a	86.4 ± 0.12a

Table 1: Physiochemical analysis for honey samples from Berekum and Drobo. WSS=Water Insoluble Solids; Values are mean ± standard deviation; Values with different alphabets in a column are significantly different at $p<0.05$.

The pH values were within the acidity range (4.1-4.3) with no significant difference ($p>0.05$) amongst the different types of extraction. Types of extraction significantly influenced water insoluble solids ($p<0.05$) with values ranging from 0.56% to 8.50%, with press having the least water insoluble solids. Sucrose level ranged from 2.64%-3.12% and were not influenced by the type of extraction. Pressed honey had a higher glucose+fructose (90%) as compared to the other processing techniques ($p<0.05$).

Microbial analysis

The results for the microbial analysis indicated that there were no yeast and moulds; hence no characterization was carried out. Similarly, no food pathogens (*E.coli* and coliforms) were detected (Table 2). However, some aerobic microorganisms were found. These included *Bacillus* spp. and *Streptococcus* spp.

Samples	TAC	TCC	Yeast & Moulds	*E.coli*
1	7.9×10^3	None detected	None detected	None detected
2	<30	None detected	None detected	None detected
3	<30	None detected	None detected	None detected
4	<30	None detected	None detected	None detected
5	3.7×10^3	None detected	None detected	None detected
6	<30	None detected	None detected	None detected
7	<30	None detected	None detected	None detected
8	<30	None detected	None detected	None detected
9	<30	None detected	None detected	None detected
10	6.4×10^3	None detected	None detected	None detected
11	<30	None detected	None detected	None detected
12	<30	None detected	None detected	None detected

Table 2: Microbial analysis on honey samples expressed in colony forming units (cfu/g).

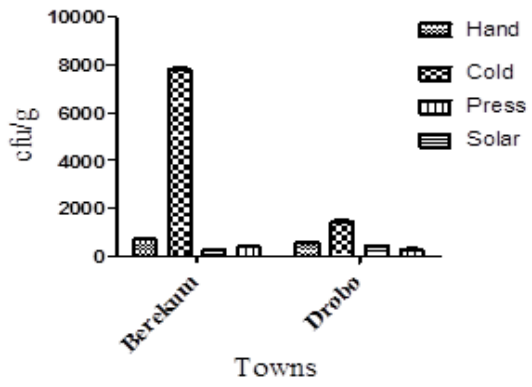

Figure 1: Total aerobic count of honey samples from Berekum and Drobo.

Generally, the samples from Berekum appeared to show higher aerobic counts over those from Drobo (Figure 1). The test for food pathogens (*E. coli* and coliforms) as well as yeast and moulds came out negative as none of these were detected in any of the samples.

The four selected modes of honey extraction showed varying microbial quality. The cold extraction recorded the highest count of 4.614×10^3 cfu/g, this was followed by hand extraction which recorded 6.31×10^2 cfu/g. Solar extraction recorded counts of 3.46×10^2 cfu/g with extraction by press recording the least count of 3.30×10^2 cfu/g (Figure 2).

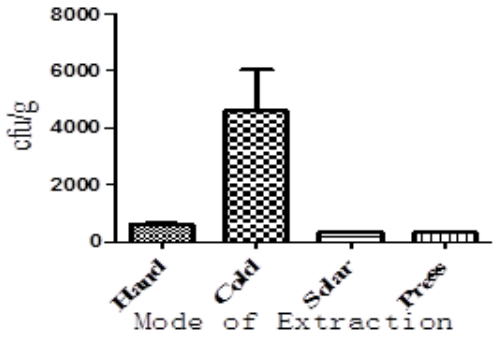

Figure 2: Microbial load of modes of honey extraction.

Again none of the target pathogenic organisms were detected in the products of the four extraction modes Figures 3 and 4. The main sources of variation (town and processing type) as well as interaction between the sources significantly ($p<0.05$) influenced the microbial load of the honey samples studied (Table 3).

Source	*P*-Value
Main Effects	
A: Town	<0.0001*
B: Processing Type	<0.0001*
Interactions: A x B	<0.0001*

Table 3: Effect on honey community and type of extraction on microbial load. *Significant at $p<0.05$.

Discussion

Physicochemical analysis

The results indicate that the moisture content of honeys processed was generally high. Apart from the solar mode of extraction which registered 14.46% all the other processing techniques produced honeys with moisture content exceeding the maximum value of 20.0 g/100 g established by the Codex Alimentarius Commission and EU Commission as the international standard honey moisture content. According to Bogdanov [16], moisture content gives an indication of the shelf-stability of the product. Higher moisture content therefore directly correlates to early fermentation due to yeast. The result for solar based extraction technique, however, was lower than moisture values reported by Deviller et al. [17] and Guler [18]. The result implies

that the honey producers in the two areas need to further training in the post-harvest handling of their honey product.

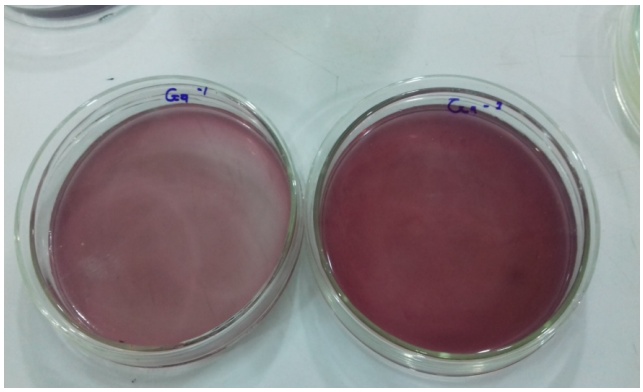

Figure 3: Negative coliform plates on BRBLA after 24 hours.

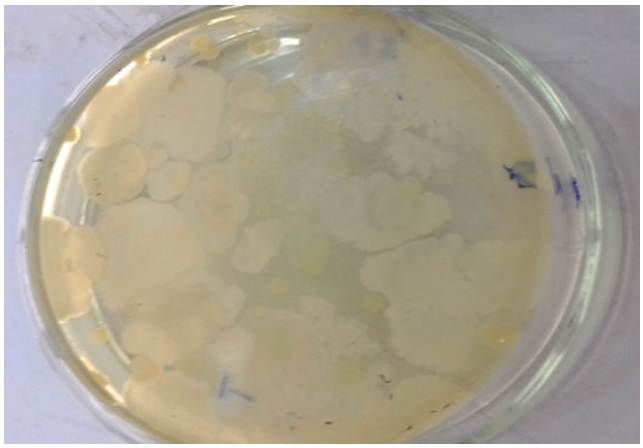

Figure 4: Aerobic count on PCA from Honey samples.
Aerobic microorganisms of on PCA from Honey samples of diverse morphological characteristics.

All the honey samples fell within the acceptable acidity range. The pH is a chemical property and an intrinsic feature of the honey, thus is not influenced by physical methods such as the extraction mode. The acidic nature of the honey is ideal in ensuring a sterile environment as most microorganisms do not do well at low pH. Conversely, the water insoluble solids registered in the processing techniques were above the acceptable limit of 0.1 for solid suspended matter in honey [19]. Extraction by hand recorded the highest insoluble solids (8.5%) which exceeded the acceptable limit by a significant margin, whereas press extraction recorded the least (0.56%) as in Table 1. The solids were mainly pieces of wax. The water insoluble solids are an index of the solid organic contaminants such as insects, wax particles and flower or vegetable debris. The result implies that some attention should be given to the modes of honey extraction to avoid the contamination. Sugar or carbohydrates forms the main constituent of honey and accounts for close to 95% of the dry weight of the honey [16]. The sugar content of honey is useful in indicating the type of honey be it unifloral or multifloral, and it is considered an intrinsic property of the honey. Moreover, the extraction processes are physical and not chemical. Therefore, the sugar content cannot be significantly altered by the mode of extraction [16].

Microbial analysis

The results obtained for all the honeys are acceptable and considered safe as no pathogens were detected. The highest aerobic count recorded (4.614×10^3) falls within the acceptable limit of 1.0×10^4 prescribed by the Food and Drugs Authority of Ghana. Though honey is a relatively poor medium for the growth of microorganisms it is not to be considered sterile [20], therefore the detection of some aerobic organisms is not viewed as strange phenomenon. Some authors have indicated that total aerobic viable count in honey can vary between zero and tens of thousands per gram. For example, Iurlina and Fritz [21] analyzed 70 honey samples from different parts of Argentina and found the total viable count of aerobic bacteria of 1.0×10^3 cfu/g in any of the samples analyzed. Similarly, Kňazovická et al. [22] also reported a mean value of 1.4×10^2 cfu/g of aerobic bacteria isolated from honey. According to Omafuvbe and Akanabi [23], the mean number of aerobic bacteria in honey ranged from 1.0×10^3 to 5.0×10^3 cfu/g. *Escherichia coli* in the honey samples were used as indicator for human and environmental contamination. It is usually the commonest and thus most abundant pathogen in foods. Hence, its absence from the honey samples gives credence to their quality. The detection of *Bacillus* spp. and *Streptococcus* spp. in the honey samples could be due to the ability of these aerobic microorganisms to form spores and thus survive harsh conditions even as persist in honey [24]. This goes to confirm that honey is not absolutely sterile as seen in this study; hence precautions must be taken to ensure that microbial contamination of honey is reduced to the barest minimum.

The absence of food pathogens can be attributed to the physicochemical factors (pH, sugar concentration and moisture) of the honey [20,25]. The physicochemical studies have shown honey to be of acidic pH of mean 4 which is not favorable for most microorganisms to thrive. The low moisture content and viscosity of the honey provides an environment of harsh osmotic conditions that discourages bacteria amplification [26]. The osmotic gradient allows for the net movement of fluids out of the organisms, thus resulting in shrinking of cell membrane and subsequently death of the cells.

The results indicate clear difference in outcome from the two study areas Figure 1, which could be attributed to environmental factors at the site of honey extraction. The differences in sanitary and hygiene practices at these locations could result in relative levels of contamination of the honeys from Berekum and Drobo. Tysset and Rousseau [6] have observed that contaminants found in honey are usually introduced during extraction and handling rather than in the hive. Honey producers need to be vigilant when processing the honey product as already demonstrated. The cold extraction gave the highest contamination followed by hand extraction. This may be due to the active role of temperature in the survival of microorganisms. Most of the microorganisms within our environment are mesophiles [27] and cannot do well in high temperatures. The room temperature under which both cold and hand extraction are carried out is ideal for these organisms and they therefore thrive well. Again if proper sanitary practices are not observed particularly in hand extraction, there exists the tendency to introduce contaminants by the processor to the product [6] The ultraviolet rays of the sun is known to be lethal to microorganisms as they are unicellular and do not have protective mechanisms against the ionizing rays. This phenomenon is likely to have contributed to the low contamination observed with the honey

extracted by solar means. The major source of contamination of honey is human and equipment [6]. Once these factors are taken care of the risk of contamination is highly reduced. The press extraction requires less human interaction with the product and with the equipment in good sanitary conditions, it is guaranteed to yield highly safe and consumable products. Based on these results it could be concluded that the extraction by honey press is the most hygienic and can be recommended for honey production in the developing economies like Ghana.

The limitation of this study comes from the limited number of samples used for the study, and the microbial biochemical profiling of isolated organisms due to lack of reagents like the API test kits. The number of samples was limited by the unwillingness of honey producers in the study areas to release the samples. Absence of the API test kits made it difficult to identify the microorganism to the species level; hence all identification was done only to their genus. Notwithstanding the above the results have wide application. Most of the honey producers in Ghana and other developing countries employ these methods of extraction. The lessons from the study could serve as basis to re-train them in postharvest handling of the honeys to sustain their quality.

Conclusion

The analysis show significant difference in the moisture content, water insoluble solids (WSS) and microbial quality of the different treatments pertaining to the mode of extraction of the honey samples. The outcome of the analysis from the two locations, Berekum and Drobo show cold extraction to have recorded the highest count of 4.614×10^3 cfu/g with hand extraction recording the next highest of 6.31×10^2 cfu/g. Solar extraction recorded counts of 3.46×10^2 cfu/g with extraction by press recording the least count of 3.30×10^2 cfu/g. None of the detected aerobic counts exceeded the safe limit of 1.0×10^4. No food pathogens (*E. coli*, Coliforms and fungi) were detected in all the samples. Honey produced locally in Ghana by trained beekeepers is thus said to be safe for consumption with infinitesimal health risks on the basis of biological threat. A combination of extraction by honey press and solar is the best mode of extraction to meets Codex and EU standards for moisture, WSS and microbial contamination for honey.

Acknowledgement

The authors are grateful to the Danish Government who supported this study through the Building Stronger Universities Work Package (II). We also wish to express our gratitude to Dr F.C. Mills-Robertson of the Department of Biochemistry and Biotechnology for his assistance and advice during the laboratory analysis.

References

1. Codex Alimentarius Commission (2001) Codex Standards for sugar (honey). Supplement 2 to Codex Alimentarius. Food and Agriculture Organisation of the United Nations and World Health Organisation, Rome.
2. Nordqvist J (2015) Health benefits and uses in medicine.
3. Ankrah EK (1998) Chemical composition of some Ghanaian honey samples. National science and technology press, Ghana J of Agri Sci 31: 119-121.
4. Agbagwa K, Otokunefor OE, Frank-Peterside TV, Nnenna k (2011) Quality assessment of Nigeria honey and manuka honey. J Microbio and Biotech Res 1: 20-31.
5. Serena MJ, Alves EM, Toledo VDAAD, Marchini LC, Faquinello P, et al. (2011) Microbial flora in organic honey samples of Africanized honeybees from Parana River islands. Food Sci and Tech (Campinas) 31: 462-466.
6. Tysset C, Rousseau M (1981) Problem of microbes and hygiene of commercial honey. Rev Med Vet 132: 591-600.
7. Codex Alimentarius (1994) Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, FAO, Rome.
8. Fenicia L, Anniballi F (2009) Infant botulism. Ann Ist Super Sanita 45: 134-146.
9. Fatimah B, Abubakar G, Aliyu S (2013) Analysis of biochemical composition of honey samples from North-East Nigeria. Biochem Anal Biochem 2: 1000139.
10. Ahmida MH, Elwerfali S, Agha A, Elagori M, Ahmida NH (2013) Physicochemical, heavy metal and phenolic compound analysis of Libyan honey samples collected from Benghazi during 2009-2010. Food Nut Sci 4: 33-40.
11. Adjare SO (1984) The Golden Insect. A handbook on Beekeeping for beginners, Practical Action.
12. Harmonised Methods of the International Honey Commission (2002) IHC responsible for the methods center. Bern: FAM, Liebefeld CH-3003: 20-23.
13. Official Method of Analysis of Association of Official Analytical Chemists (AOAC) International (2005) 18th Edn.
14. ISO 4831 (2006) Horizontal method for the detection and enumeration of coliforms.
15. ISO 16650 (2001) Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Escherichia coli 0157.
16. Bogdanov S, Lullman C, Martin P, Werner V, Russmann, H, et al. (2004) Honey quality, methods of analysis and international regulatory standards: Review of the work of the international honey commission. Swiss bee research centre. Mitt Lebensm Hyg 80: 108-125.
17. Devillers J, M Morlot, MH Pham–Delegue, JC Dore (2004) Classification of monofloral honeys based on their quality control data. Food Chem 86: 305-312.
18. Guler Z (2005) Dogu Karadeniz Bolgesinde uretilen ballarin kimyasal ve duyusal nitelikleri. Gida 30: 379–384.
19. Mohamed M, Sulaiman SA, Sirajudeen KN (2013) Protective effect of honey against cigarette smoke induced impaired sexual behavior and fertility of male rats. Toxicol Ind Health 29: 264-271.
20. Sereia MJ, Alves EM, Toledo VDAA D, Marchini LC, Faquinello P, et al. (2011) Microbial flora in organic honey samples of Africanized honeybees from Parana River islands. Food Science and Technology (Campinas) 31: 462-466.
21. Iurlina MO, Fritz R (2005) Characterization of microorganisms in Argentinean honeys from different sources. Int J Food Microbiol 105: 297-304.
22. Kacaniová M, Melich M, Knazovická V, Hašcik P, Sudzinová J, et al. (2009) The indicator microorganisms value in relation to primary contamination of honey. Lucraristiintifice Zootehniesi Biotehnologii, 42: 159-163.
23. Omafuvbe B, Akanbi OO (2009) Microbiological and physico-chemical properties of some commercial Nigerian honey. Afr J Microbiol Res 3: 891-896.
24. Laaberki MH, Dworkin J (2008) Death and survival of spore forming bacterial in the Caenorhabditiselegans intestine. Symbiosis 46: 95-100.
25. Silva JA (2000) Tópicos da Tecnologia dos Alimentos. 2 edn. São Paulo: VARELA.
26. Marchini LC, Sodre GDS, Moreti ACDC (2004) Mel brasileiro: composição e normas. Ribeirão Preto: A. S. Pinto.
27. Adebiyi FM, Akpan I, Obiajuwa EI, Olaniyi HB (2004) Chemical/Physical Characterization of Nigerian Honey. Pak J of Nut 3: 278-281.