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ORIGINAL PAPER

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Microbiological Qualities of Imported and Locally Produced Honey in Saudi Arabia

Abstract: Forty-eight samples of imported and locally produced honey marketed in Saudi Arabia were collected and subjected to microbiological analysis. Selected parameters adopted were standard plate count (SPC), anaerobic count using cooked meat medium (CMM) and reinforced clostridial medium (RCM), yeast and mould count, lactic acid bacteria count on MRS and M17 media, coliform count and faecal streptococci count. Results were reported as $\log_{10}$ cfu g^{-1} for both types of honey samples studied. Overall results revealed that locally produced honey significantly surpassed ($\alpha = 0.05$) imported honey in most tested parameters. In addition, 100 bacterial isolates were isolated, purified and identified from samples of both locally produced and imported honey. *Bacillus subtilis*, *B. larvae*, *B. licheniformis*; *Micrococcus luteus*, *M. variance*; *Staphylococcus albus*; *Lactobacillus plantarum*, *L. fermentum*, *L. helveticus*; *Enterococcus faecium*; *Lactococcus lactis*; *Streptococcus thermophilus*; *Clostridium perfringenes* and *Cl. difficile* were recognized using the API system.

Keywords: Saudi Arabia, honey, bacteria, microbiological analysis

الجودة الميكروبيولوجية للعسل المستورد و المنتج محلياً بالمملكة العربية السعودية

رشاد رزق الهندي

المستخلص: جمعت ثمان و أربعون عينة من العسل المستورد، و العسل المنتج محليا"، من أسواق المملكة العربية السعودية، و تم إختبارها ميكروبيولوجياً. و كانت المعايير المختارة للدراسة، العد بالأطباق القياسي و عد البكتيريا اللاهوائية، بإستخدام بيئة اللحم المطبوخ، و بيئة الكلوستريديا المغناة، و كذلك عد الخمائر و الفطريات، و عد بكتيريا حمض اللاكتيك، و عد مجموعة بكتيريا القولون و عد بكتيريا ستربتوكوكاي البرازية. قدرت النتائج لوغارتمياً للوحدات المكونة للمستعمرات/ ج عينة في نوعي العسل. و أظهرت النتائج بشكل عام، تفوق العسل المنتج محلياً على العسل المستورد في معظم المعايير المختبرة. و بالاضافة الى ذلك، تم الحصول على 100 عزلة بكتيرية من عينات نوعي العسل. وتم تعريف البكتيريا التالية:

Bacillus subtilis, *Bacillus larvae*, *Bacillus licheniformis*; *Micrococcus luteus*, *M. variance*; *Staphylococcus albus*; *Lactobacillus plantarum*, *L. fermentum*, *L. helveticus*; *Enterococcus faecium*; *Lactococcus lactis*; *Streptococcus thermophilus*; *Clostridium perfringenes* and *Cl. difficile*

كلمات مدخلية: السعودية، عسل محلي، مستورد، بكتيريا، جودة، إختبار ميكروبيولوجي

Introduction

Honey is highly regarded for its health-giving properties rather than merely as a food. Besides, it has been used in folk medicine since ancient times in numerous cultures and continues to be accepted as such by the medical profession up until the present time. Honey has also been reported to have antimicrobial activities (Allen, *et al.* 1991; Brady, *et al.* 1996 and Cooper, *et al.* 2002).

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Clostridium botulinum, the causative agent of infant botulism is relatively uncommon in honey. However, potentially life-threatening neuroparalytic illness caused by toxins secreted by *Cl. botulinum* (Krishna and Puri, 2001) and some other species of this bacterium (Gimenez, *et al.* 1992; Aureli, *et al.* 1986) have been reported. Accordingly, feeding honey to infants less than one year of age seems risky (Arnon, *et al.* 1979; Fenicia, *et al.* 1993; Centorbi, *et al.* 1999). Infant botulism was described as a separate clinical entity for the first time by Midura and Arnon (1976) and has since been reported in many countries (Fenicia, *et al.* 1993; Thomas, 1993; Balslev, *et al.* 1997) and is the most common form of botulism in the United States based on accumulated reports (Spika, *et al.* 1989).

The Kingdom of Saudi Arabia produces honeys of various floral and geographical origins which are

distributed through food stores and groceries all over the kingdom. In addition, enormous quantities of numerous honeys are imported from all over the world, from both developed and developing countries, into the Kingdom. However, it has been observed that there is a lack of published data concerning the microbiological quality and safety of honey marketed in Saudi Arabia.

The present investigation aims to examine the microbiological qualities of imported and locally produced honey in order to identify their predominant bacterial flora. It is hoped that the results will be beneficial for food safety authorities in the Kingdom and food processors in the food technology sector. Therefore, it was decided to undertake the responsibility to examine the microbiological qualities of honey.

Materials and Methods

Sample collection

A total of 48 samples of imported and locally produced honey were randomly collected from different retail sites in Saudi Arabia. Imported honey samples were of different geographical and floral origins. The locally produced honey samples were mostly from Gizan and Assir regions of Saudi Arabia and of different floral origins.

Microbiological analyses

Standard plate count (SPC) was determined adopting the method reported by Hought *et al.* (1993). An anaerobic count and count of clostridial spores were carried out using cooked meat medium (CMM) and reinforced clostridial medium (RCM). Dilutions of each sample were inoculated separately in tubes of both media after adding 1.5% agar (Horwitz, 1980), counts were reported after incubation at 30°C for 48h. A yeast and mold count was performed adopting the method reported by Frank, *et al.* (1993). Lactic acid bacteria (LAB) counts were monitored using the methods reported by Frank, *et al.* (1993). For selectivity of lactobacilli and lactic streptococci, (MRS; de Mann, Rogosa and Sharpe, Oxoid) and (M17, Oxoid,) broths with added agar were used, respectively. Coliform count was carried out adopting the methods reported by Serra and Escola (1997). One ml of the dilution used in the aerobic count determination was poured into each of two plates, and 8-10ml of violet red bile agar was then added. When solidified, a second layer of culture medium (8-10ml) was added. Plates were then incubated at 37°C for 48h, coliform colonies were then counted and reported. Faecal streptococci count was carried out adopting the methods reported by Serra and Escola (1997). One ml of the dilution used in the aerobic count determination was poured into each of two plates, and 10-15ml of kanamycin agar, was then added. When solidified, plates were incubated and colonies surrounded by a black zone were counted and reported.

Microbial characterization

Some of the resulting colonies on the different selective plating media were randomly chosen and transferred to broths of the same media and incubated as mentioned previously. The procedure of plating, transferring discrete colonies to broths and then incubation was repeated many times in order to obtain pure cultures. Slants of these pure cultures were prepared to carry out characterization and identification of the obtained isolates. Colonies from the selective media were transferred to broth media for propagation and then slant agar was made from which a microscopic examination was performed to define the shape of bacteria after staining by Gram stain. Production of catalase enzyme was also tested.

Microbial Identification

Identification of the isolated micro-organisms was carried out using the available commercial identification kits. Clostridia were identified to species level using an API 20A kit according to the instruction of the manufacturer (Biomerieux, France). Gram positive, catalase negative cocci isolates were identified using the appropriate available identification kit, API 20 Strep kit (Biomerieux, France). An API 50 CHL kit (Biomerieux, France) was used for the identification of lactobacilli isolates. Identification of Gram positive, catalase positive spore-forming isolates was performed according to Buchanan and Gibbons (1986). Faecal streptococci isolates picked from the selective medium used for their counting were identified using an API 20 Strep kit (Biomerieux, France).

Data analysis

The means (μ) for the imported and locally produced honey populations were estimated for each studied item using the obtained results and adopting the method of the equation for estimating

the mean. The means of each of the two honey populations for each item were compared using the test of statistical hypothesis of variance and the difference between two means. Significant differences between the means were then estimated adopting the method of the equation for estimating the difference between two means.

Results and Discussion

Honey is a very popular foodstuff much in demand in Saudi Arabia. Honey in Saudi Arabia is marketed either as imported, mostly from less-developed countries, or as locally produced, mostly by traditional and migratory beekeeping methods. When honey has been the object of research, its antibacterial activities have been the focal research points and little research has been carried out on its microbiological status, quality and safety. Table 1 shows the obtained results of microbiological analysis of imported and locally produced honey. There were no significant differences, estimated at the level of significance of $\alpha = 0.10$, between the means of standard plate count, lactic acid bacteria count on MRS medium and anaerobic count on RCM medium of imported honey and the means of their counterpart items of locally produced honey. This meant that neither of the two types of honey was better than the other in regard to these items of analysis. On the other hand, there were significant differences, estimated at the level of significance of $\alpha = 0.10$, between the means of the yeast and mold count, lactic acid bacteria count on M17 medium and anaerobe count on CMM medium of imported honey and the means of their counterpart items of locally produced honey. This revealed here that imported honey was inferior to locally produced honey regarding these analysis items.

Table 1. Microbiological analysis of retail honey.

Analysis	Imported honey (n= 27)	Local honey (n= 21)
SPC	4.58 ± 1.00[a]	3.36 ± 1.13
Yeast and mold	1.61 ± 0.64	0.53 ± 0.44
LAB (M17)	3.64 ± 0.91	1.89 ± 0.86
LAB (MRS)	1.04 ± 0.62	0.40 ± 0.40
Anaerobic (RCM)	1.61 ± 0.57	1.41 ± 0.59
Anaerobic (CMM)	2.19 ± 0.62	1.27 ± 0.67

[a], Mean $\log_{10}$ CFU/g ± the standard error.

As evident from the means of the microbiological items studied, honey is generally a product with minimal counts of microorganisms. This is attributable to the natural properties of honey. Although these microorganisms cannot grow in honey, they can persist in it, be carried into a new product in which honey is used as an ingredient and grow in and spoil the new product. Honey could be contaminated by microorganisms from air, food handlers, and cross-contamination. These sources of contamination are controlled by good manufacturing practices. More work will be needed to explain what influences the types and levels of microorganisms in honey have, particularly during harvesting, processing, storage and use as an ingredient (Snowdon, 1999).

Characterization and identification of tested honey microflora

One hundred bacterial isolates were characterized, grouped and further identified in this study as mentioned previously. Table 2 shows the identification of 30 Gram positive, catalase positive isolates to the species level according to Buchanan and Gibbons (1986). A total of 12 *Bacillus* species (*B. subtilis*, *B. larvae*, *B. licheniformis*) were identified; and of the 18 cocci isolates, 15 belonged to *Micrococcus* species (*M. leteus*, *M. variance*), the remaining three belonging to *Staphylococcus albus*.

Table 2. Results of 30 Gram positive, catalase positive bacterial isolates identified to the species level.

Rods	Cocci
Bacillus subtilis (8)	*Micrococcus leteus* (9)
Bacillus larvae (2)	*Micrococcus variance* (6)
Bacillus licheniformis (2)	*Staphylococcus albus* (3)
Total = 12	Total = 18

Table 3 shows the identification of the remaining 70 Gram positive, catalase negative bacterial isolates. Of the 25 Gram positive, catalase negative rod isolates, 22 were identified as *Lactobacillus* species (*Lb. plantarum*, *Lb. Fermentum*, *Lb. helveticus*) and the remaining three rods were unidentified species. On the other hand, the 45 Gram positive, catalase negative cocci isolates were identified as *Enterococcus faecium* (18), *Lactococcus lactis* (12) and *Streptococcus thermophilus* (6), while the remaining nine cocci were unidentified species.

Table 3. Results of 70 Gram positive, catalase negative isolates identified to the species level.

Rods	Cocci
Lactobacillus plantarum (11)	*Enterococcus faecium* (18)
Lactobacillus fermentum (6)	*Lactococcus lactis* (12)
Lactobacillus helveticus (5)	*Streptococcus thermophilus* (6)
Unidentified species (3)	Unidentified species (9)
Total = 25	Total = 45

In summary, identification of the bacterial isolates to the genus level revealed that 45 isolates (45%) belonged to streptococci, 25 (25%) were lactobacilli, 18 (18%) were micrococci and 12 (12%) were *Bacillus* spp.

It was found in the present research that honey contained good bacteria, namely those belonging to the genera lactobacilli and streptococci. These beneficial bacteria can grow in the intestine and alter the balance between the harmful and useful bacteria in favor of the latter. Honey can supply consumers with different types of these beneficial bacteria, or probiotics. Probiotic bacteria are living bacteria which enhance human health, some of which are grouped in the genera lactobacilli and streptococci. During the current work, we obtained many bacterial isolates belonging to the aforementioned genera. Extensive research would be needed to examine them for their characteristics as being probiotics and this was beyond the scope of the present study.

It is of concern to know what microorganisms are present in honey, where they are coming from, and how to control them. Honey can carry spores of yeasts, molds and bacteria and all food, including honey, can be inadvertently inoculated with undesirable microorganisms during processing. Spores are present everywhere, they may even be found in honey in the hive and could come from primary sources such as pollen, the digestive tracts of honey bees, air, earth, and nectar. It is difficult to control the entry of microorganisms from these sources. The vegetative form of microorganisms as opposed to the spore form can be added any time after the honey is harvested. These post-harvest sources of microorganisms can include air, food handlers, cross-contamination, equipment and buildings (Snowdon, 1999).

During the present investigation, representative colonies appearing on RCM and CMM media, which are assumed to enhance the growth of clostridia, were isolated, characterized and identified. Fifty rod-shaped bacterial isolates proved to be Gram positive, catalase negative and sporulated. They were then subjected for identification using an API 20A kit (Biomerieux, France). No *Cl. botulinum* was detected in any of the tested samples. Most of the isolates were *Cl. perfringens* represented by 40 isolates (80% of the identified bacteria) while the remaining 10 isolates (20%) were identified as *Cl. difficile*. The latter species causes a severe and sometimes fatal gastrointestinal tract infection; while the former has been reported to be a causative of food poisoning. This coincided with the results of Quaglio *et al.* (1988) who carried out an epidemiological study on 167 honey samples for detection of *Cl. botulinum* in honey. No *Cl. botulinum* was detected in any of the samples. They isolated *Cl. difficile* and *Cl. perfringens* and concluded that these isolates could be associated with toxigenic intestinal infection in infancy. However, the osmotic pressure and low moisture content, both characteristics of honey, beside some other factors, may be the reason to discourage the proliferation of such bacteria, rendering them inactive (Osato, *et al.* 1999).

Honey is thought to become contaminated as a result of spores of *Cl. botulinum* in the soil sticking to the legs of bees and being transported to the beehive. Commercial as well as rurally produced honey can contain spores of *Cl. botulinum*. No *Cl. botulinum* was detected in the honey consumed by a Dutch case of infant botulism, but honey was the likeliest cause of infection (Wolters, 2001). The results obtained during the current research pointed to the presence of some clostridia other than *Cl. botulinum,* which may stand as an explanation for the findings reported by (Wolters, 2001). In many cultures honey is given to babies to keep them quiet. Parents have been warned not to give honey to infants under the age of one year (Wolters, 2001). Our results coincided also with those reported by Delmas, *et al.* (1994) who examined a total of 116 samples of honey (90 produced in France and 26 from other countries) for the presence of *Cl. botulinum* spores. No *Cl. botulinum* was isolated from any of these samples. However, other *Clostridium* spp. (*Cl. sporogenes* and *Cl. perfringens*) were isolated. They concluded that *Cl. botulinum* spores are unlikely to be detected in French honey. They also added that the presence of other potentially toxigenic *Clostridium* spp. in the honeys is also of concern and should be investigated further.

Conclusions

Honey has a characteristic microflora and microorganisms natively present in honey are of special interest. There is still a risk from feeding honey to infants and susceptible individuals, and more work is needed to follow up the natural bacterial toxins and pathogens which might be present in honey. Emphasis should not be placed only on the incidence of *Cl. botulinum* in honey, but also on other food poisoning clostridia such as *Cl. perfringens*. The beneficial bacteria in honey needs more elucidation and study on the role of honey in stimulating these bacteria (Shamala, *et al.*, 2000) should be given more consideration. Food industry managers should be aware of the potential risk when using honey as an ingredient in other food products. Due to this concern, they should use honey of high quality to avoid any cross-contamination occurring to their end products. It has been observed that Saudi standards give limits and specifications only for physicochemical attributes and count of yeast and mold in honey (SASO, 1993). Based on our findings, we recommend adding other microbiological parameters and standards such as standard plate count and anaerobe count to ensure honey quality and safety for consumption. Adopting the bacteriological quality criteria of Colin *et al.* (1986): absence of coliforms and faecal streptococci and <100 to <1000 yeasts/g, it could be concluded that all tested honey samples were of high bacteriological quality and safe for human consumption in this regard. Finally, the author would like to advise related authorities to establish a local research institution which would take the responsibility of carrying out original research on different aspects of this very popular foodstuff in Saudi Arabia, with the suggested name, Saudi Arabian Board of Honey Research.

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References

Allen KL, Molan PC and **Reid GM** (1991) A survey of the antibacterial activity in some New Zealand honeys. *J. Pharm. Pharmacol.* **43**: 817-822.

Arnon SS, Midura TF, Damus K, Thompson B, Wood RM and **Chin J** (1979) Honey and other environmental risk factors for infant botulism. *J. Pediatr.* **94**: 331-336.

Aureli P, Fenicia L, Pasolini B, Gianfranceschi, M, McCroskey LM and **Hatheway CL** (1986) Two cases of type E infant botulism caused by neurotoxigenic *Cl. butyricum* in Italy. *J. Infect. Dis.* **154**: 207-211.

Balslev T, Ostergaard E, Madsen IK and **Wandall DA** (1997) Infant botulism. The first culture-confirmed Danish case. *Neuropediatrics* **28**: 287-288.

Brady NF, Molan PC and **Harfoot CG** (1996) The sensitivity of dermatophytes to the antimicrobial activity of Manuka honey and other honeys. *Pharmaceutical Sci.* **2**: 471-473.

Buchanan RE and **Gibbons NE** (1986) *Bergey's Manual of Determinative Bacteriology,* 10th Ed. Williams and Wilkins Co., Baltimore.

Centorbi HJ, Aliendro OE, Demo NO, Dutto R, Fernandez R and **Puig-de-Centorbi ON** (1999) First case of infant botulism associated with honey feeding in Argentina. *Anaerobe* **5**: 181-183.

Colin ME, Flamini C, Malaussene J and **Pourtallier J** (1986). Quality of commercial honeys. *Cah. Nutr. Dietetique* **21**: 219-222.

Cooper RA, Molan PC and **Harding KG** (2002). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J Appl. Microbiol.* **93**: 857.

Delmas C, Vidon DJM and **Sebald M** (1994) Survey of honey for *Cl. botulinum* spores in eastern France. *Food Microbiol.* **11**: 515-518.

Fenicia L, Ferrini AM, Aureli P and **Pocecco M** (1993) A case of infant botulism associated with honey feeding in Italy. *Eur. J. Epidemiol.* **9**: 671- 673.

Frank JF, Christen GL and **Bullerman LB** (1993) Tests for groups of microorganisms. *In:* **Marshall, RT (ed.)** *Standard methods for the examination of dairy products.*

Gimenez JA, Gimenez MA and **Das Gupta BR** (1992) Characterization of the neurotoxin isolated from a *Cl. baratii* strain implicated in infant botulism. *Infect. Immun.* **60**: 518-522.

Horwitz W (1980) *Official methods of analysis of the association of official analytical chemists,* 13th Ed. Association of Analytical Chemists, Washington, D.C.

Hought GA, Maturin LJ and **Koenig EK** (1993) Microbiological methods. *In:* **Marshall RT (ed.)** *Standard methods for the examination of dairy products.*

Krishna S and **Puri V** (2001) Infant botulism: case reports and review. *J. Ky. Med. Assoc.* **99**: 143-146.

Midura TF and **Arnon SS** (1976) Infant botulism. Identification of *Cl. botulinum* and its toxins in faeces. *Lancet* **2**: 934-6.

Osato MS, Reddy SG and **Graham DY** (1999) Osmotic effect of honey on growth and viability of *Helicobacter pylori*. *Dig. Dis. Sci.* **44**: 462-464.

Quaglio P, Messi P and **Fabio A** (1988) Bacterial isolates of the genus *Clostridium* in honey samples. *Igiene-Moderna* **90**: 486-496.

SASO (1993) *Honey,* Saudi Standard No. 101. Saudi Arabian Standards Organization, Riyadh, Saudi Arabia.

Serra BJ and **Escola JR** (1997) Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. *J. Agricult. Food Chem.* **45**: 725-732.

Shamala TR, Shri JY and **Saibaba P** (2000) Stimulatory effect of honey on multiplication of lactic acid bacteria under in vitro and in vivo conditions. *Lett. Appl. Microbiol.* **30**: 453-455.

Snowdon JA (1999) The microbiology of honey—meeting your buyers' specifications (why they do what they do). *Am. Bee J.* **139**: 51-60.

Spika JS, Shaffer N, Hargrett-Bean N, Collin S, MacDonald KL and **Blake PA** (1989) Risk factors for infant botulism in the United States. *Am. J. Dis. Child.* **143**: 828-832.

Thomas DG (1993) Infant botulism: a review in South Australia (1980-1989). *J. Paediatr. Child Health* **29**: 24-26.

Wolters B (2001) First case of infant botulism in the Netherlands. *WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe Newsletter* **67**.

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