Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

International Journal of Food Microbiology 105 (2005) 297–304

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Characterization of microorganisms in Argentinean honeys from different sources

Miriam O. Iurlina *, Rosalia Fritz

Departamento de Química, Bromatología, Facultad de Ciencias Exactas y Naturales, UNMDP, Funes 3350, C.P 7600, Mar del Plata, Provincia de Buenos Aires, Argentina

Received 17 November 2003; received in revised form 27 February 2005; accepted 22 March 2005

Abstract

Seventy polyfloral honeys including commercial samples obtained from supermarkets, harvested from apiaries and purchased in bulk were initially examined for total antibacterial activity. From each sample, numbers of aerobic mesophilic bacteria, total coliforms, moulds and yeasts were determined and the presence of *Salmonella* spp., *Shigella* spp., *Clostridium* sulfite-reducers, *Paenibacillus larvae* and *Bacillus* spp. was investigated. Moisture content, pH and total acidity were also determined for all samples. Any honey diluted to concentrations from 75% to 1% (w/v) of full-strength honey showed total antibacterial activity. The numbers of aerobic mesophilic bacteria, moulds and yeasts were less than 10^3 cfu/g for all 70 samples. Faecal coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Clostridium* sulfite-reducers were not detected but *P. larvae* subspp. *larvae*, *Bacillus cereus*, *Bacillus pumilus* and *Bacillus laterosporus* were found among samples. For commercial, apiary and bulk honey the mean values for moisture content, pH and acidity, respectively, were 17.50%, 17.40% and 17.50%; 4.60, 4.10 and 4.20; and 18.30, 20.60 and 21 meq NaOH/kg. *P. larvae* was recovered from 35% of apiaries including hives in which the bees did not display symptoms of American foulbrood disease.

Keywords: Commercial; Bulk and apiary honeys; Microorganisms

1. Introduction

Many authors have reported studies on honeys, some based on physico-chemical and sensorial analysis (Gupta et al., 1992; Singh and Kuar Bath, 1997; Esti et al., 1997), the chemical identification of certain compounds in honeys from different sources and regions (D'Aray et al., 1997; Martos et al., 2000) and, in recent years, studies on the antimicrobial properties of honeys (Allen et al., 1991; Molan, 1992a,b; Willix et al., 1992; Al Somal et al., 1994; Cooper et al., 1999; Taormina et al., 2001). However, except for *Clostridium botulinum* limited quantitative data of bacteria in honeys have been reported in scientific literature (Tysset et al., 1970; Tysset and

* Corresponding author. Tel.: +54 223 4756167; fax: +54 223 4753150.

E-mail address: miurlina@mdp.edu.ar (M.O. Iurlina).

0168-1605/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2005.03.017

Rousseau, 1981; Root, 1983; Nakano et al., 1990; Piana et al., 1991).

According to White et al. (1963) honey is a mixture of fructose (average 38.4%), glucose (average 30.3%), sucrose (average 1.3%) and other carbohydrates (about 12%), minerals (average 0.169%) and proteins (169 mg/100g), with a water content of about 17.2%. The pH of honey ranges from 3.4 to 6.1 with an average of 3.9, while the water activity varies between 0.5 and 0.6. Osmolarity, pH and hydrogen peroxide are the major factors in honey that may be considered for its antimicrobial activity. The principal factor responsible for this activity is hydrogen peroxide produced by the oxidation of glucose by the enzyme glucose-oxidase, which is activated by successive dilutions of honey. In addition, a residual non-peroxide antibacterial activity attributed to phenolic components (phenolic derivatives of benzoic acid, cinnamic acid and flavonoids) was detected in several honeys (Molan and Russell, 1988; Weston et al., 1999). These intrinsic properties of honey affect the growth and survival of microorganisms by bacteriostatic or bactericidal action and, in particular, the low pH and high sugar content of undiluted honeys prevent the growth of many species of microorganisms. In consequence, honey can be expected to contain a small number and a limited variety of microorganisms. Vegetative forms of human disease-causing bacteria have not been found in honey and, as bacteria do not replicate in honey, a high count of vegetative bacteria is indicative of a recent contamination from a secondary source.

Thus, the microorganisms of interest are those that withstand the concentrated sugar, acidity and antimicrobial character of honey. These microorganisms include certain yeasts and spore-forming bacteria; coliforms or yeasts indicative of sanitary or commercial quality, and microorganisms such as *Bacillus cereus*, *Clostridium perfringes* or *C. botulinum*, which under certain conditions (e.g. germination and growth in a non-heated-treated product) could cause illnesses in humans (Snowdon and Cliver, 1996).

Moreover, microorganisms that cause diseases in honey bees are also of interest. *Paenibacillus larvae*, a spore-forming bacterium, is one of the major pathogens of *Apis mellifera* responsible for an infectious disease known as American foulbrood (Alippi et al., 2002; Lauro et al., 2003). However, *P. larvae* have never been associated with illness in humans. The aim of this work was to determine and characterize microbial populations in Argentinean honeys obtained from commercial sources, apiaries and bulk containers.

2. Materials and methods

2.1. Honey samples

The study was carried out with 70 unpasteurized polyfloral honey samples, which were classified into 3 groups including: 23 commercial honeys from local supermarkets; 37 honeys harvested directly from apiaries; and 10 honeys purchased in bulk for industrial use representing 33%, 53% and 14% of the total of samples, respectively. Apiary honeys were divided into 2 sub-groups: (i) samples from hives with signs of American foulbrood (AFB) disease at the time of sampling (9 samples, 24%), and (ii) samples from hives free of any sign of AFB disease at the time of sampling (28 samples, 76%). AFB disease affects the larval and pupal stages of honeybees (*A. mellifera* L.) The brood combs were evaluated for a patchy appearance of its, sunken capping with a greasy-look and partially open cells. Infected individuals turn brown and then black and the resultant mass becomes a hard scale of material deposited on the side of the cell (Alippi et al., 2002). Commercial honeys came from different regions of Argentina. Apiary and bulk honeys came from apiaries laid in the southeast region of Buenos Aires province. Sampling was performed in triplicate, dividing the samples into 2 parts: one for microbiological and antibacterial activity assays and the other for moisture content, pH and acidity measurements. Commercial samples were analyzed from original containers. For apiary and bulk honeys, representative portions were aseptically collected into sterile containers. All samples were stored in the dark at 10 °C.

2.2. Assay of antibacterial activity

Honeys were screened for total antibacterial activity against *Staphylococcus aureus* (ATCC 25923) according to the agar well diffusion method (Allen et al., 1991). Cultures (100 μL) of *S. aureus* grown in trypticase soy broth (TSB) at 37 °C for 18 h were

added to nutrient agar (150 mL) and immediately poured onto plates. The plates were stored at 4 °C for 24 h before being used, and wells (8 mm diameter) were cut in the agar. Solutions containing 0, 1%, 5%, 10%, 25%, 50% and 75% (w/v) of honey were made in sterile distilled water. A 100-μL aliquot of each sample was added to each well. Cultures were incubated at 37 °C for 18 h. Antibacterial activity was assessed by measuring the size of the zones of inhibition surrounding wells.

2.3. Microbial counts

Ten grams of each sample were homogenized into 90 mL of sterile Butterfield's phosphate-buffered dilution water (0.25 M KH_2PO_4 adjusted to pH 7.2 with NaOH; Butterfield, 1932) with Stomacher 400 Circulator for 3 min. Decimal dilutions were made into the buffer dilution water. Aerobic mesophilic bacteria were counted onto standard plate count agar (PCA) incubated at 30 ± 1 °C for 24–48 h (ICMSF, 1983). Total coliforms were counted onto standard violet red bile (VRB) agar incubated at 35 ± 1 °C for 24–48 h (ICMSF, 1983). Moulds and yeasts were counted onto standard yeast extract–glucose–chloramphenicol (YGC) agar incubated at 22–25 °C for 3–5 days (ICMSF, 1983). Microbial counts were expressed as colony-forming units per gram of honey (cfu/g).

2.4. Bacterial detection

Salmonella spp. was investigated according to a modification of the standard method suggested by the Bacteriological Analytical Manual (BAM, 2001). For the pre-enrichment, 25 g was added to 225 mL of Lactose broth (LB, pH = 7.2 ± 0.1) and cultures were incubated at 35 °C for 24 ± 2 h. The enrichment step was performed onto both tetrathionate (TT, pH = 8.4 ± 0.2) and selenite cystine (SC, pH = 7 ± 0.2) broths incubated at 35 ± 2 °C for 24 ± 2 h. Isolations were examined onto both media Hektoen enteric (HE) and bismuth sulfite (BS) agars, after incubation at 35 ± 2 °C for 24 ± 2 h.

Shigella spp. were tested according to a modification of the method suggested by Pascual Anderson and Calderón García (2000). For the enrichment step, 25 g of honey was mixed into 225 mL of broth for Gram-negative bacteria (GN, pH = 7.0 ± 0.2). The cultures were incubated at 35 ± 2 °C for 16–18 h. Isolations were examined onto Hektoen enteric (HE) agar after incubation at 35 ± 2 °C for 24–48 h.

Clostridium sulfite-reducers were tested as a modification of the Pascual Anderson and Calderón García method (2000). A 5-mL aliquot from each homogenate made for bacterial counts was thermally treated at 80 °C for 5 min. Then, 1-mL aliquots were added at the bottom of tubes containing sulfite–polymixin–sulfadiazine (SPS) agar and the top of each culture was cover with parafin. Cultures incubated at 46 °C and 35 °C under anaerobic conditions for 7 days were checked for growth and gas production.

For *P. larvae*, honeys were processed according to Hornitzky and Clark (1991). Each sample was diluted 1:2 (w/v) with phosphate-buffered saline (PBS, pH 7.2) and centrifuged at 3000×*g* for 45 min. The supernatant was decanted leaving about 3 mL that was mixed with the sediment. An aliquot of this suspension was heated at 80 °C for 15 min and 100 μL spread onto MYPGP agar (Dingman and Stahly, 1983), containing: Muller–Hinton (10 g/L) broth, yeast extract (15 g/L), K_2HPO_4 (3 g/L), sodium pyruvate (1 g/L), agar (20 g/L), glucose (2 g/L). The medium was supplemented, after sterilization, with 6 μg/mL nalidixic acid and 10 μg/mL pipemidic acid to inhibit the growth of other *Bacillus* species. Plates were incubated under microaerophilic conditions (8–10% CO_2) at 35 ± 2 °C for 7 days. *P. larvae* presumptive colonies were initially identified by their appearance on MYPGP agar, catalase test, Gram reaction, and spores staining (Claus and Berkley, 1986).

Honeys were also examined for the presence of other *Bacillus* species. From the suspensions heated at 80 °C for 15 min, a 100-μL aliquot was spread onto J-agar (JA) (Alippi, 1995; Nordström and Fries, 1995), composed of: tryptone (5 g/L), yeast extract (15 g/L), K_2HPO_4 (3 g/L), agar (20 g/L), glucose (2g/L). Plates were incubated at 35 ± 2 °C for 46 h under aerobic conditions. Colonies were identified by their morphological appearance in this medium, Gram reaction, shape and position of spores.

The isolates were maintained both in MYPGP and J-agar and characterized by: the ability to withstand serial transfer in nutrient broth, Voges–Proskauer and indole tests, nitrate reduction, starch hydrolysis and mannitol utilization (Gordon et al., 1973; Priest et al., 1988).

2.5. Moisture content, pH and acidity measurements

Moisture content was determined using undiluted samples. Solid or crystalline honeys were previously heated in a water bath at 40 °C for melting. Refractive index on transparent and translucent liquid samples was determined using an Abbe refractometer at 20 °C and the values recorded were converted to percent moisture using a conversion table (Bianchi, 1984). For pH measuring honey samples were diluted at various concentrations, 1%, 5%, 10%, 25%, 50%, 75% (w/v), in CO_2-free distilled water and the pH value of full-strength honey (100%) was obtained by extrapolation. A HANNA pH-meter (model HI 9321) was used for pH measuring. Acidity content of samples was determined in a solution containing 10 g of honey in 75 mL of CO_2-free distilled water that was tritated with a standard NaOH solution approximately 0.1 N (Bianchi, 1984).

3. Results

3.1. Antibacterial activity

For all honey samples no inhibition growth halo was detected in honeys diluted to concentrations from 75% to 1% (w/v) of its full strength, indicating no bacteriostatic and/or bactericidal effect on *S. aureus* ATCC 25923.

3.2. Microbial counts

Among all the honey samples, aerobic mesophilic bacteria were counted. For commercial and apiary honeys counts were similar, with values that varied between 30–1200 cfu/g and 60–1100 cfu/g, respectively. For commercial honeys the mean count value was 244 cfu/g while for apiary honeys it was about 500 cfu/g. The lowest mean value (223 cfu/g) was obtained for bulk honeys that showed counts ranged between 40 and 500 cfu/g. The largest count estimated mesophilic bacteria (3×10^4 cfu/g) was found in a commercial sample which also contained a total coliform level of 10 cfu/g.

Forty (57%) of the 70 honeys showed count values equal to or lower than 470 cfu/g for moulds and yeasts population. The lowest numbers were found in commercial samples with counts ranged between 0 and 300 cfu/g, being the mean count about 34 cfu/g. In apiary honeys the counts were found between 0 and 470 cfu/g with a mean value of 164 cfu/g. Bulk honeys had the same range of counts as commercial honeys; however, the mean count was about 100 cfu/g. The incidence of moulds and yeasts for commercial, apiary and bulk honeys were 47%, 59% and 70%, respectively.

3.3. Bacterial detection

Faecal coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Clostridium* sulfite-reducers were not detected in any of the 70 honeys studied.

P. larvae subspp. *larvae* was detected in commercial, apiary and bulk honeys with an incidence of 17%, 60% and 10%, respectively. All apiary samples taken from hives with clinical symptoms of AFB disease were positive for *P. larvae* subspp. *larvae* and of the 28 samples obtained from hives without signs of AFB, 13 (35%) were positive for the bacterium. This microorganism was not detected in 41% of samples.

Sixteen of the seventy samples (23%) contained *Bacillus* spp., being identified *B. cereus*, *Bacillus pumilus* and *Bacillus laterosporus*. Six out of the sixteen positive samples were from commercial honeys, in which *B. cereus*, *B. pumilus* and *B. laterosporus* had an occurrence of 26%, 13% and 26%, respectively. The remaining 9 samples corresponded to apiary honeys, being *B. cereus* the sole species isolated, with an incidence of 24%. From bulk honeys *Bacillus* spp. had an incidence of 10%, being only isolated *B. cereus*.

3.4. Moisture content, pH and acidity determinations

For commercial honeys moisture contents varied from 15.8% to 21.4%, with a mean of 17.50%. pH varied from 3.3 to 5.0 and acidity from 11.5 to 33 meq NaOH/kg, being mean values 4.60 and 18.30 meq NaOH/kg, respectively. Moisture content mean value of apiary honeys was 17.40%, with values ranging from 16.2% to 21.8%. In addition, pH and acidity mean values were 4.10 and 20.60 meq NaOH/kg varying from 3.4 to 4.6 and 12.3 to 32 meq NaOH/kg, respectively. For bulk honey moisture content, pH

and acidity mean values were 17.50%, 4.20 and 21 meq NaOH/kg, respectively.

4. Discussion

4.1. Antibacterial activity

Honeys have long been recognized for their antimicrobial activity against bacteria, moulds and yeasts with unique properties that render it bacteriostatic and bactericidal. The high osmotic pressure, low water activity, low pH, low redox potential of honey, hydrogen peroxide and other phytochemical factors might contribute to the honey antimicrobial nature. Their relative importance depend on the sensitivity of the species and the level of additional factors in any honey (Molan, 1992a,b).

In this study, previous to microbial counts and bacterial detections honeys were diluted into buffer dilution water or broth media. This dilution effect would lead and activation to glucose-oxidase enzyme that generates hydrogen peroxide, which generally is the major antibacterial factor in honey. White et al. (1963) found that the enzyme is practically inactive in full-strength honey, it giving rise to hydrogen peroxide only when the honey is diluted. In addition its activity is suppressed by the unfavourable pH in ripened honey. The enzyme has an optimum pH of 6.1 with a good activity from 5.5 to 8, but the activity drops off sharply below 5.5 (Molan, 1992a). Testing for total antimicrobial activity indicated that none of the honeys had an antimicrobial activity that could affect the recovery of microorganisms after dilution in buffer, or dilution and incubation in pre-enrichment and enrichment broths.

4.2. Microbial counts

According to published data total aerobic viable count values for honeys can range from 0 to several thousand per gram. This variation in bacterial counts may be due to the type of sample (raw, finished or retailed), the freshness of the honey, the time of harvest and the analytical techniques used (Snowdon and Cliver, 1996). Nakano and Sakaguchi (1991) tested 270 honey samples from retail outlets in Japan, and recorded a mean aerobic viable count of 83 cfu/g and, from 35 honey samples of international origin these authors reported viable counts from 0 to 72 cfu/g, with a mean value of 24 cfu/g. From 175 samples of commercial honey from different geographical regions of France, Tysset and Rousseau (1981) found a mean value for viable counts to be 227 cfu/g, with values that varied from 3 to 9500 cfu/g. In our study mean value was closer than those obtained by Tysset and Rousseau (1981), whereas the distribution of our counts was lower (30 to 1200 cfu/g). Tysset et al. (1970) tested 14 samples of freshly harvested French honey and found that viable counts ranged from 25 to 1600 cfu/g, with a mean count below 100 cfu/g. Moreover, from these samples *E. coli*, *Enterococcus*, sulfite-reducers or *Staphylococcus* were not detected. These values were reported within the range of current industry experience where the bacterial levels of finished honeys range from 1 to 5000 cfu/g, although viable counts of finished products reported in informal industry ranges between 10^4 and 10^5 cfu/g and viable counts in raw products can also reach 10^4–10^5 cfu/g (Snowdon and Cliver, 1996). Lower total aerobic counts were reported by Piana et al. (1991) who found numbers varying from 1 to 55 cfu/g. The apiary honeys studied here had similar numbers to those reported by Tysset et al. (1970).

There are few reports that quantify the levels of moulds and yeasts in honey. Among 50 Italian honeys Piana et al. (1991) found moulds at levels ranging from 1 to 43 cfu/g. Moreover, 34 out of 50 samples contained primarily osmophilic yeasts in the range of 1 to 3500 cfu/g, while yeasts ranged from 1 to 200 cfu/g. In 14 French honeys Tysset et al. (1970) found mean counts of yeasts and moulds of 254 cfu/g, with values that varied from 0 to 2500 cfu/g. In 175 honeys, Tysset and Rousseau (1981) found that counts of moulds and yeasts also varied from 0 to 2500 cfu/g, whereas the mean count was 90 cfu/g. Nakano and Sakaguchi (1991) reported, for 35 out of 270 retail honey samples, a yeast mean count of 9 cfu/g, which varied from 0 to 300 cfu/g. In addition, Root (1983) showed among 320 Canadian honeys that yeasts and moulds were present at levels as low as 1 in 10 g and as high as 10^6 spores per gram. Rall et al. (2003) found an incidence of 64% of mould and yeasts in industrial and domestic production honeys with counts ranged from absence to 1.5×10^5 cfu/g. In

our study, moulds and yeasts counts for commercial and bulk honeys were less than 300 cfu/g, whereas for apiary honeys we found a higher incidence for moulds and yeasts. However, for the total of samples analyzed, moulds and yeasts counts varied in a shorter range than those reported by Tysset et al. (1970), and Tysset and Rousseau (1981).

Enumeration of moulds and yeasts provides information on the quality of honeys as well as shelf life and spoilage potential as a high yeast count, increased moisture, moderate temperatures and granulation encourages fermentation (Snowdon and Cliver, 1996). In our study, numbers of yeast and moulds among commercial and bulk samples were similar to freshly harvested honeys indicating that these microorganisms in commercial and bulk honeys have not grown during storage.

4.3. Bacterial detection

Some of the microorganisms reported to be associated with bees include *Bacillus*, *Clostridium*, *E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, etc., and many of these have been found in honey. Studies on survival of some *Salmonella* species or another vegetative pathogenic organisms, which are not normally present in honey, had been reported. Nevertheless, the incidence of these microorganisms is variable, as is the prevalence of species into these genera (Snowdon and Cliver, 1996).

Our results agree with those obtained by Tysset et al. (1970), Aureli et al. (1983), Piana et al. (1991) and Delmas et al. (1994) in that *C. perfringes* or *C. botulinum* was not found in any sample tested. However, Piana et al. (1991) also found unidentified anaerobic spores in 22 out of 50 samples tested at levels between 0.1 and 1 cfu/g. Furthermore *C. botulinum* spores have been found in Argentinean honeys (de Centorbi et al., 1997; Centorbi et al., 1999; Monetto et al., 1999) and some reports have shown a variable incidence of botulinal spores in apiary, drum, retail package samples (Sugiyama et al., 1978; Huhtanen et al., 1981). Rall et al. (2003) reported *C. botulinum* in the 3% of industrialized and domestic honeys, but total coliforms, *Salmonella* spp., *Shigella* spp. were not found in any sample.

In our study, apiary honeys showed a higher incidence for *P. larvae* subspp. *larvae* than those reported by Alippi (1995) who found 8% of the positive samples for the bacterium. On the other hand, Lauro et al. (2003) studied 56 honey samples by both cultural and PCR methods, being *P. larvae* detected in 57% and 91% of samples, respectively. In this study, the occurrence of *P. larvae* subspp. *larvae* in apiary honeys is not directly related to the presence of AFB clinical symptoms. Thus, from those hives without signs of AFB the presence of the microorganism spores was demonstrated at low levels. Spores could be spread into hives by robber bees or inadequate beekeeping practices. The high prevalence (60%) of *P. larvae* subspp. *larvae* in apiary honeys shows widespread distribution of this microorganism among honey combs.

Studies by Tysset et al. (1970) on qualitative examination of the organisms recovered on viable count plates from 12 samples of French honey showed the presence of microorganisms such as *Bacillus*, *Brevibacterium*, *Enterobacter*, *Micrococcus* and *Pseudomonas* with the most common isolates being *B. cereus* and *B. pumilus*. On the other hand, Piana et al. (1991) found *B. cereus* spores in 24 of 50 samples tested. Moreover, Kokubo et al. (1984) found spores in 67 out of 71 honey samples from processing plants and retailers. Most of the spores belonged to the *Bacillus* genus, *B. cereus* being the predominant species. Other species identified included *Bacillus coagulans*, *Bacillus megaterium* and *Bacillus alvei*. On the other hand, in Argentinean commercial honeys, *B. cereus* was detected in 78% of the samples with values lower than 10.000 spores/kg (Monetto et al., 1999). In our study the prevalence of *B. cereus* was lower than that reported by these researches with 16 of 70 samples positive for the microorganism. According to our findings the distribution of *Bacillus* spp. was similar for commercial and apiary honeys. The lowest incidence in bulk honey may be due to the mixing of samples from different beekeepers causing a dilution of honey with uneven distribution of the microorganisms.

4.4. Moisture content, pH and acidity determinations

Anupama et al. (2003) reported moisture content varying from 17% to 22.6%, acidity expressed as formic acid from 0.03 to 0.15 and pH from 3.62 to 5.46 for commercial Indian honeys. da Azeredo et al.

(2003) for monofloral and heterofloral honeys have obtained values below 20% for moisture content. In heterofloral honeys moisture varied from 18.59% to 19.25%. Besides, the range of acidity values varied from 28.2 to 39.5 meq/kg being higher than the range obtained in our study for honeys from both apiaries and commerce. pH values ranged from 3.20 to 3.84.

Yeast and mould contamination of honey is unavoidable, since bees collect them together with the nectar. However, the water activity of ripened honey is too low to support the growth of any species, and no fermentation occurs if the water content is below 17.1% (w/v). For moisture contents between 17.1% and 20% (w/v) the product stability will depend on the microbial content, at more than 20% (w/v) osmophilic yeasts may develop (Molan, 1992a; Tosi et al., 2004). In our study some honeys showed moisture contents higher than 20% (w/v). However, no samples with remaining liquid phase, in which the water content becomes greater than the original honey representing a potential spoilage, were observed. The acidity and pH of honeys would have implications for its microbial ecology. However, studies in which acidity was taken into account found no correlation between antibacterial activity and the pH of honeys studied.

5. Conclusion

The microbiological and some physico-chemical characteristics of Argentinean polyfloral honeys from different sources, commercial, harvested from apiaries and purchased in bulk containers, were determined to provide information on their level and prevalence depending on the source. Numbers of aerobic, mesophilic bacteria, moulds and yeasts identified among the honey samples from different sources were similar, with count values less than 10^3 cfu/g.

Persistent microorganisms recovered were moulds, yeasts and spore-forming bacilli each of which is adapted to survive in the physico-chemical environment of honey with consistently low numbers suggesting that growth is severally restricted. *P. larvae*, the agent of American foulbrood disease, was recovered from 35% of apiary honeys including those from hives not displaying symptoms of the disease. The methods used in this study to detect *P. larvae* are suitable for routine monitoring for this pathogen and the implementation of measures to prevent the disease which usually progress to the extinction of hives.

Acknowledgement

We gratefully acknowledge the financial support of the Universidad Nacional de Mar del Plata (Project No. EXA 233/02).

References

Alippi, M.A., 1995. Detection of *Bacillus larvae* spores in Argentinean honeys by using a semi-selective medium. Microbiología 11, 343–350.

Alippi, M.A., López, C.A., Aguilar, M.O., 2002. Differentiation of *Paenibacillus larvae* subsp. *larvae*, the cause of American foulbrood of honey bees, by using PCR and restriction fragment analysis of genes encoding 16S rRNA. Appl. Environ. Microbiol. 68, 3655–3660.

Allen, K.L., Molan, P.C., Reid, G.M., 1991. A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol. 43 (12), 817–822.

Al Somal, N., Coley, K.E., Molan, P.C., Hancock, B.M., 1994. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. J. R. Soc. Med. 87 (1), 9–12.

Anupama, D., Bhat, K.K., Sapna, V.K., 2003. Sensory and physico-chemical properties of commercial samples of honey. Food Res. Int. 36, 183–191.

Aureli, P., Ferrini, A.M., Negri, S., 1983. Ricerca delle spore di *Cl. botulinum* nel miele. Riv. Soc. Ital. Sci. Aliment. 12 (6), 457–460.

BAM. Bacteriologycal Analytical Manual, 2001. In: FDA Bacteriologycal Analytical Manual online. Association of Official Analytical Chemists International, online.

Bianchi, E.M., 1984. Determinación del contenido de humedad (Cap III): pp. 17–22, Determinación de acidez (Cap IV): pp. 23–25. In: Determinación de la Calidad de Miel. Sgo del Estero, Rep. Argenitna: Ed. CEDIA-Univ. Nacional de Sgo del Estero.

Butterfield, C.T., 1932. A selection of dilution water for bacteriological examinations. J. Appl. Bacteriol. 23, 355–367.

Centorbi, J.H., Aliendro, O.E., Demo, N.O., Dutto, R., Fernandez, R., Puig de Centorbi, O.N., 1999. First case of infant botulism associated with honey feeding in Argentina. Anaerobe 5, 181–183.

Claus, D., Berkley, R.C., 1986. Genus *Bacillus* Cohn 1872, 147^{AL}. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2. Ed. Williams and Wilkins, Baltimore, USA, pp. 1104–1138.

Cooper, R.A., Molan, P.C., Harding, K.G., 1999. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J. R. Soc. Med. 92 (6), 283–285.

da Azeredo, C.L., Azeredo, M.A.A., de Souza, S.R., Dutra, V.M.L., 2003. Protein contents and physicochemical properties in honey

samples of *Apis mellifera* of different floral origins. Food Chem. 80, 249–254.

D'Aray, B.R., Rintoul, G.B., Rowland, C.Y., Blackman, A.J., 1997. Composition of Australian honey extractives: 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. J. Agric. Food Chem. 45, 1834–1843.

de Centorbi, P.O., Satorres, E.E., Alcaraz, L.E., Centorbi, H.J., Fernandez, R., 1997. Detection of *Clostridium botulinum* spores in honey. Rev. Argent. Microbiol. 29.

Delmas, C., Vidon, D.J.-M., Sebald, M., 1994. Survey of honey for *Clostridium botulinum* spores in eastern France. Food Microbiol. 11, 515–518.

Dingman, D.W., Stahly, D.P., 1983. Medium promoting sporulation of *Bacillus larvae* and metabolism of medium components. Appl. Environ. Microbiol. 46, 860–869.

Esti, M., Panfili, G., Marconi, E., Trivisonno, M.C., 1997. Valorization of the honeys from the Molise region through physicochemical, organoleptic and nutritional assessment. Food Chem. 58 (1–2), 125–128.

Gordon, R.E., Haynes, W.C., Pang, C.H., 1973. The genus *Bacillus*. Agric. Handb. 427 (ARS-USDA, Washington, 283 pp.).

Gupta, J.K., Kaushik, R., Joshi, V.K., 1992. Influence of different treatments, storage temperature and period on some physicochemical characterisation and sensory qualities of Indian honey. J. Food Sci. Technol. India 29 (2), 84–87.

Hornitzky, M.A.Z., Clark, S., 1991. Culture of *Bacillus larvae* from bulk honey samples for the detection of American foulbrood. J. Apic. Res. 30 (1), 13–16.

Huhtanen, C.N., Knox, D., Shimanuki, H., 1981. Incidence and origin of *Clostridium botulinum* spores in honey. J. Food Prot. 44, 812–814.

ICMSF, 1983. Métodos recomendados para el análisis microbiológico en alimentos. Microorganismos de los Alimentos I. Técnicas de Análisis Microbiológicos, (2da. ed.)Acribia, Zaragoza, España, pp. 105–280.

Kokubo, Y., Jinbo, K., Kaneko, S., Matsumoto, M., 1984. Prevalence of spore-forming bacteria in commercial honey. Ann. Rep. Tokyo Metr. Res. Lab. Pub. Health 35, 192–196.

Lauro, F.M., Favaretto, M., Covolo, L., Rassu, M., Bertoloni, G., 2003. Rapid detection of *Paenibacillus larvae* from honey and hive samples with a novel nested PCR protocol. Int. J. Food Microbiol. 81, 195–201.

Martos, I., Ferreres, F., Tomás-Barberán, F.A., 2000. Identification of flavonoid markers for the botanical origin of *Eucalyptus* honey. J. Agric. Food Chem. 48, 1498–1502.

Molan, P.C., 1992a. The antibacterial activity of honey: 1. The nature of the anibacterial activity. Bee World 73, 5–28.

Molan, P.C., 1992b. The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. Bee World 73, 59–76.

Molan, P.C., Russell, K.M., 1988. Non-peroxide antibacterial activity in some New Zealand honeys. J. Apic. Res. 27, 62–67.

Monetto, A.M., Francavilla, A., Rondini, A., Manca, L., Siravegna, M., Fernandez, R., 1999. A study of botulinum spores in honey. Anaerobe 5, 185–186.

Nakano, H., Sakaguchi, G., 1991. An unusually heavy contamination of honey products by *Clostridium botulinum* type F and *Bacillus alvei*. FEMS Microbiol. Lett. 79, 171–178.

Nakano, H., Okabe, T., Hashimoto, H., Sakaguchi, G., 1990. Incidence of *Clostridium botulinum* in honey of various origins. Jpn. J. Med. Sci. Biol. 43, 183–195.

Nordström, S., Fries, I., 1995. A comparision of media and cultural conditions for identification of *Bacillus larvae* in honey. J. Apic. Res. 34, 97–103.

Pascual Anderson, M.R., Calderón García, V., 2000. Investigación de Shigella (Cap. 7): pp. 63–71, Investigación y recuento de *Clostridium* sulfito-reductores (Cap. 8): pp. 73–75. In: Microbiología Alimentaria. Metodología analítica para alimentos y bebidas. 2da Edición. Madrid, España: Ed. Díaz de Santos, S.A.

Piana, M.L., Poda, G., Cesaroni, D., Cuetti, L., Bucci, M.A., Gotti, P., 1991. Research on microbial characteristics of honey samples of Udine province. Riv. Soc. Ital. Sci. Aliment. 20, 293–301.

Priest, F.G., Goodfellow, M., Todd, C., 1988. A numerical classification of the genus *Bacillus*. J. Gen. Microbiol. 134, 1847–1882.

Rall, V.L.M., Bombo, A.J., Lopes, T.F., Carvalho, L.R., Silva, M.G., 2003. Honey consumption in the state of Sâo Paulo: a risk to human health? Anaerobe 9, 299–303.

Root, A.I. (Ed.), 1983. The ABC and XYZ of Bee Culture. The A.I. Root Co., Medina, OH.

Singh, N., Kuar Bath, P., 1997. Quality evaluation of different types of Indian honey. Food Chem. 58 (1–2), 129–133.

Snowdon, J.A., Cliver, D.O., 1996. Review article. Microorganisms in honey. Int. J. Food Microbiol. 31, 1–26.

Sugiyama, H., Mills, D.C., Kuo, L.-J.C., 1978. Number of *Clostridium botulinum* spores in honey. J. Food Prot. 41, 848–850.

Taormina, P.J., Niemira, B.A., Beuchat, L.R., 2001. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int. J. Food Microbiol. 69 (3), 217–225.

Tosi, E.A., Ré, E., Lucero, H., Bulacio, L., 2004. Effect of honey high-temperature short-time heating on parameters related to quality, crystallisation phenomena and fungal inhibition. Lebensm.-Wiss. u.-Technol. 37 (6), 669–678.

Tysset, C., Rousseau, M., 1981. Problem of microbes and hygiene of commercial honey. Rev. Med. Vet. 132, 591–600.

Tysset, C., Durand, C., Taliergio, Y.P., 1970. Contribution to the study of the microbial contamination and the hygiene of commercial honey. Rev. Med. Vet. 146, 1471–1492.

Weston, R.J., Mitchell, K.R., Allen, K.L., 1999. Antibacterial phenolic components of New Zealand manuka honey. Food Chem. 64, 295–301.

White, J.W., Subers, M.H., Schepartz, A.I., 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim. Biophys. Acta 73, 57–70.

Willix, D.J., Molan, P.C., Harfoot, C.G., 1992. A comparision of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J. Appl. Bacteriol. 73 (5), 388–394.