Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2015, Vol. 65, No. 2, pp. 119–126
DOI: 10.1515/pjfns-2015-0031
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

Effect of Microwave Treatment on Microbial Contamination of Honeys and on Their Physicochemical and Thermal Properties

María de la Paz Moliné[1,2,5], Natalia Jorgelina Fernández[1,4], Sandra Karina Medici[1,6], Diana Fasce[3], Liesel Brenda Gende[1,2,4]*

[1]*Centro de Investigación en Abejas Sociales, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina*
[2]*Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina*
[3]*INTEMA, Instituto de Investigaciones en Ciencia y Tecnología de Materiales, UNMDP-CONICET, Mar del Plata, Argentina*
[4]*CONICET, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina*
[5]*CIC, La Plata, Buenos Aires, Argentina*
[6]*Laboratorio Fares Taie, Mar del Plata, Argentina*

Key words: honeys, microwave, microbial count, physicochemical and thermal properties, *Paenibacillus larvae*

In recent years, microwave heating has become a common method for pasteurization and sterilization of food. Honey is a sweet substance produced by worker honeybees from nectar of flowers. The major microbial contaminants include moulds and yeasts, as well as the spore-forming bacteria, being their counts indicative of honeys' commercial quality and safety. *Paenibacillus larvae* is also of interest since it causes American foulbrood (AFB) in honeybee larvae. The main quality factors that are used in the honey international trade are moisture, hydroxymethylfurfural content (HMF), and enzymatic indices. Moreover, honey exhibits several thermal events, the most important being the glass transition temperature (Tg). The aim of this work was to evaluate microwave effect (800 watts during 45 and 90 seconds) on microbial content in particular over *P. larvae* spores retained in honey, and on physicochemical and thermal properties. Microwave promoted a decrease of microbial count with time of exposure, including *P. larvae*. Moisture content diminished after treatment, while Tg increased linearly, and acidity decremented in the majority of cases. Honeys darkened and HMF exceeded the permissible value. Diastase and glucose-oxidase enzymes were totally inactivated by microwave treatment.

INTRODUCTION

In industrial food processing, microwave energy has been considered a faster process of pasteurization and sterilization of food than conventional methods. Consequently, microwave radiation is regarded as an alternative method for killing bacteria because of its effectiveness, commercial availability, and lower cost compared with other technologies [Celandroni *et al*., 2004]. However, the effect of microwave on bacteria depends on food composition and type of microorganism [Castro *et al*., 1997].

Honey is a sweet product elaborated by worker honeybees from nectar and secretion of flowers. It is collected and transformed by combining with specific substances, then stored in honeycombs until maturation. Chemical composition involves a complex mixture of fructose (average 38.4 g/100 g), glucose (average 30.3 g/100 g), sucrose (average 1.3 g/100 g), other carbohydrates (around 12 g/100 g), minerals (average 0.169 g/100 g) and proteins (enzymes and amino acids) (average 169 mg/100 g), with a moisture content of 17.2 g/100 g [White *et al*., 1963]. It also contains organic acids such as gluconic (0.43 g/100 g), and in a minor amount, acetic, citric, lactic, succinic and formic acids, aromatic substances, pigments, wax and pollen grains [Desalegn, 2013]. Honey has own flavor and aroma, and its color varies from colorless to dark amber. Color has been frequently attributed to presence of polyphenols, tannins, among others [Acquarone, 2004].

Due to its natural properties the number and variety of microorganisms present in honey is expected to be low. Low water activity (aw: 0.56 – 0.62), low pH (3.5 – 4.5), high organic acids content, presence of hydrogen peroxide, phytochemicals and/or antioxidants [Estrada *et al*., 2005; Coll Cardenas *et al*., 2008] inhibit microorganisms growing. Handling and manipulation post harvest are the main sources of contamination, usually by yeasts (*e.g. Saccharomyces*, *Schizosac-charomyces* and *Torula*) and spore-forming bacteria (*e.g.* Aerobic *Bacillus* and anaerobic *Clostridium* spores). Spores and small

* Corresponding Author: Tel: +549223 5468496;
E-mail: molinemdelapaz@gmail.com

fragments of moulds may appear [Frazier & Westhoff, 1978; Kedzia *et al*., 1996]. Among these, microorganisms that cause honeybee diseases are of special interest. *Paenibacillus larvae*, a spore-forming bacterium, is one of the major pathogens of *Apis mellifera* responsible for an infection known as American foulbrood (AFB) [Alippi *et al*., 2002; Lauro *et al*., 2003]. Also, the species *Paenibacillus alvei* and *Melisococcus pluton* in association may cause European foulbrood disease (EFB), which is less important [Coll Cardenas *et al*., 2008]. The Argentine Food Code (AFC) requires total absence of coliforms and *Salmonella-Shigella*, and less of 10 CFU/g of moulds and yeasts. Other countries include a limit of 10^3 CFU/g for aerobic mesophilic bacteria [Peréz *et al*., 2009].

According to AFC [1969], honey must meet the following requirements: maximum moisture of 18 g/100 g, an acidity of 40 meq/kg, a minimum of 8 diastase units (Gothe scale) and a maximum of 40 mg/kg of hydroxymethylfurfural (HMF).

Moisture is the major factor that influences the keeping quality or storability of honey especially concerning the risk of spoilage due to fermentation [White, 1975]. The control of moisture is relevant to prevent microbial growth. Acidity is due to the presence of organic acids that contribute not only to honey flavour, but also to stability against microbial spoilage [Coll Cardenas *et al*., 2008].

Several enzymes are present in honey, such as invertase, diastase, glucose-oxidase, catalase and acid phosphatase. Invertase, glucose-oxidase and catalase are derived mostly from the honeybees. Their activities depend on the intensity of the nectar flow and the amount of nectar processed by the honeybees [Bachmann, 2007]. Enzyme activities are usually measured to evaluate heating or storage effects. The activity of diastase enzyme decreases with time of storage and heating [Kowalski *et al*., 2012]. Glucose-oxidase is more sensitive to temperature than diastase, so that its determination constitutes a good complement in thermally-treated honey's studies [Montenegro *et al*., 2006].

HMF is formed through the acid catalyzed dehydration of hexoses (glucose and fructose) as well as from the decomposition of 3-deoxyosone in Maillard reaction [Belitz *et al*., 2004]. Its content in fresh honey is very low, and it depends on the floral origin and the chemical properties of honey [Singh & Bath, 1998; Fallico *et al*., 2004; Zappala *et al*., 2005]. Its concentration increases during storage in relation to pH and time [Bath & Singh, 1999], but also due to honey heating [Bath & Singh, 1999; Bartakova *et al*., 2011]. The level of HMF in food depends on the equilibrium between formation from precursors and destruction by oxidation [Morales *et al*., 1997].

Honey is a supersaturated solution composed mainly of sugars and water and can be thermally characterized by its glass transition temperature (Tg). This is the temperature at which an amorphous material changes from rubbery to glassy state upon cooling. As Tg is very sensitive to water content as well as to chemical composition [Bhandari & Howes, 1999], it is a very useful tool to detect adulteration [Cordella *et al*., 2003].

The consequences of microwave treatment over honey properties, such as moisture content, acidity [Dranca & Oroian, 2013], HMF and enzyme activities [Kowalski *et al*., 2012], as well as over mould and yeasts [Hebbar *et al*., 2003] and bacteria spores have already been reported. Microwave radiation cannot be thought as simple heating from the inside; the E-field would produce effects on the biological molecules affecting spore structure, different from those attributable only to heat [Celandroni *et al*., 2004]. Gamma radiation method was used to control this disease, in order to inactivate *P. larvae*, although significant effect on chemical parameters and enzyme activities of honey were observed [Baggio *et al*., 2005].

At present, the effect of microwave radiation on *P. larvae* has not been studied yet. So, the aim of this study was to evaluate the microwave impact on microbial content, in particular over *P. larvae* spores, and on physicochemical and thermal properties.

MATERIALS AND METHODS

Samples

Eight honeys were obtained from different geographic sites of Argentina. Five from Corrientes province (27°8′16″ S; 58°50′25″ W), one from Entre Rios province (32°2′51.72″ S; 60°16′51.6″ W) and the other two from Buenos Aires province (37°52′59″ S; 57°36′09″ W). They were kept in hermetic containers and stored at 20–27°C and 50% relative humidity [AOAC, 2000a]. Superficial layer was removed and honey was homogenized with sterile scoop. Microbial content and physicochemical and thermal properties of honeys were determined.

Microwave thermal treatment

Microwave heating studies were conducted in a microconvective oven with turntable attachment (Sharp R-3A55, maximum power of 800 watts; Thailand). Experiments were carried out at high microwave power of 800 watts, and for heating periods of 45 and 90 seconds [Kohn's, 1998]. Microbial content and physicochemical and thermal properties of honeys were anew determined. The samples temperature was measured at the end of the each heating period (Mean temperature at 45 seconds: 83°C; mean temperature at 90 seconds: 108°C).

Analysis of microbial content

Aerobic mesophilic bacteria, moulds and yeast and sulphite-reductive *Clostridium* were counted. Also, *P. larvae* was determined. Untreated honeys were used as a control.

In microbial counts of aerobic mesophilic bacteria and moulds and yeast, ten grams of each sample were homogenized into 90 mL of sterile phosphate-buffered dilution water (0.25 mol/L KH_2PO_4 adjusted to pH 7.2 with NaOH). Decimal dilutions were made into the buffer. Aerobic mesophilic bacteria were counted onto standard plate count agar (PCA) incubated at 37°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).

Sulphite-reductive *Clostridium* count was determined with horizontal method in anaerobes on Agar Sulphite Iron (TSC)

with virgin cape to avoid contact between oxygen and microorganism. Plates were incubated in anaerobe jar at 37°C for 24–48 h [ISO 15213, 2003].

In order to quantify viable spore abundance of *P. larvae*, five grams of honey were homogenized with 5 mL of sterile water [Alippi, 1995]. Serial dilutions (1:2 and 1:10) of the honey solution were prepared and their aliquots (100 μL) were plated on MYPGP agar [Dingman & Stahly, 1983], supplemented with 9 μg/L nalidixic acid to inhibit *Paenibacillus alvei* growth, and incubated at 37°C in microaerophilia (5–10 g/100 g CO_2). The plates were checked after 2 and 4 days to count the number of *P. larvae*-like colony forming units (CFU) for each dilution, which permitted to calculate the number of viable spores per honey gram. Colonies were identified by catalase test [Heyndrickx *et al*., 1996], Gram staining [Beveridge, 2001] and phenotypic characterization [Alippi, 1992].

All microbial tests were performed in triplicate.

Analysis of physicochemical properties

Moisture content, acidity, color, diastase, glucose-oxidase and HMF were determined for all the samples. Untreated honeys were used as a control.

Determination of moisture content was based on the refractometric method [AOAC, 2000b]. The refractive index of samples was measured with a refractometer (Abbe; Science Instrumentation, Argentina) and results were converted with the Chataway Table. Free acidity was determined by titrimetric method with a pH-meter (HANNA Instruments, HI 2211 pH, pH/ORP meter; Rumania, Europe) according to AOAC [2000c] technical, readings were corrected to meq of acid/kg of honey. Measurements of color are based on a visual comparison with a colored glass reference. It was measured in the Pfund colorimeter (HANNA Instruments, C22f digital, The United States) [Tabera *et al*., 2002].

Diastase catalyzes the transformation of starch to maltose. It was determined quantitatively by official method in AOAC [2003] and qualitatively according to Bianchi [1990]; both methods are based in diastase action on starch solution, using iodine-iodized solution to visualize the hydrolysis. In qualitative determination diastase was analysed together with glucose-oxidase. Glucose-oxidase activity determination is based in action of glucose-oxidize on glucose, to produce gluconic acid and hydroxide peroxides [Bianchi, 1990].

The HMF was measured by spectrophotometric method suggested by White [1979] using Carrez solutions. The absorbance of the solutions at 284 and 336 nm was determined using a spectrophotometer (Helios Unicam Gamma, England). The quantitative value of HMF was determined using the proposed formula for the method reported by IHC [1999].

Analysis of thermal properties

Glass transition temperature (Tg) was determined for all the samples. Untreated honeys were used as a control. Tg was analyzed by differential scanning calorimetry (DSC) using a Pyris 1, Perkin Elmer (Massachusetts, USA). Samples were previously homogenized with a mechanical device (IEC CC31R Multispeed Centrifuge Thermo Scientific, United States) for 20 min, then placed in aluminum pans and heated at a rate of 10°C/min from -65°C to 25°C under N_2 atmosphere. Tg was determined as the onset of the step decrease in heat flow plot [Cordella *et al*., 2003].

Statistical analysis

It was performed with RStudio software, and data were considered as binomial distribution and inflated zero. Widespread Linear Model was used for analyzed differences between microbial content and treatment time. Pearson's correlation was used to analyze chemical data. Pearson's linear correlation coefficient (r) was calculated to the relationship between moisture content and Tg, and between HMF and color. For all analyses, $\alpha=0.05$ was the level of statistical significance.

RESULTS AND DISCUSSION

Microbial content

Honey may be expected to contain a small number and a limited variety of microorganisms. Treated honeys presented a lower count of aerobic mesophilic bacteria, moulds and yeast and *P. larvae* than virgin honeys (Table 1). Sulphite-reductive *Clostridium* was not observed.

In this study, aerobic mesophilic bacterial count was lower than those obtained by Iurlina & Fritz [2005]. Microwave reduced significantly ($p<2 \times 10^{-16}$) count in response to the exposure time. Untreated honeys presented higher microbial variety in relation to treated ones. White colonies with radial or spiral forms and yellow colonies were observed. All presented positive-Gram coloration with basilar or coco-basilar structure. Wakita *et al*. [2001] and Iurlina & Fritz [2005] reported colonies of *Bacillus* with concentric ring and irregular border. Mould and yeast counts ranged between 0 to 766 UFC/g in virgin honeys, whereas, Iurlina & Fritz [2005] observed a lower values within a narrower range (164–191 UFC/g) for honeys of southeast region of Buenos Aires province, Argentina. Microwave treatment resulted in a total reduction of these counts after 90 seconds of exposure ($p=0.015$). Similar results were achieved by Hebbar *et al.* [2003].

P. larvae has the ability to generate extremely resilient and long-lived spores [Shimanuki, 1997]. They can be transmitted by beekeepers unwittingly, shifting honeycombs between colonies, or by the honeybees themselves through robbing of colonies weakened by AFB [Fries & Camazine, 2001; de Graaf *et al*., 2001]. It is widely recognized that honey will retain spores of *P. larvae* [Sturtevant, 1936; Hansen & Rasmussen, 1986], and that inter-colony transmission of spores in contaminated honey does occur [Fries & Camazine, 2001; de Graaf *et al*., 2001]. In this study, *P. larvae* count was on average 37 CFU/g, data according to Iurlina & Fritz [2005]. The microwave treatment reduced the count of *P. larvae*, but this decrease was not statistically significant, a different result from gamma radiation method was observed [Baggio *et al*., 2005].

Physicochemical and thermal properties

The moisture content in honeys depends on several factors, such as harvesting season, degree of maturity and climatic factors. The values determined for virgin honeys are

TABLE 1. Counts of aerobic mesophilic bacteria, moulds and yeasts and *P. larvae* in the analysed honeys.

Sample	Aerobic mesophilic bacteria (CFU / g of honey)			Moulds and yeasts (CFU / g of honey)			*P. larvae* (CFU / g of honey)		
	Unt.	45"	90"	Unt.	45"	90"	Unt.	45"	90"
1	87	23	15	19	ZC	ZC	133	5	ZC
2	45	40	20	766	10	ZC	5	ZC	ZC
3	140	62	25	269	ZC	ZC	ZC	ZC	ZC
4	188	115	63	293	ZC	ZC	59	5	ZC
5	124	59	5	35	10	ZC	ZC	ZC	ZC
6	85	28	10	45	ZC	ZC	50	ZC	ZC
7	24	5	ZC	ZC	ZC	ZC	49	ZC	ZC
8	30	19	4	ZC	ZC	ZC	ZC	ZC	ZC
Mean ± SD	90.3 ± 57.7[a]	43.8 ± 34.7[b]	17.8 ± 20.1[b]	178.4 ± 265.7[a]	2.5 ± 4.6[b]	0[b]	37 ± 46.5[a]	1.3 ± 2.3[a]	0[a]
p		$< 2 \times 10^{-16}$			0.015			0.97	

Unt. (untreated), 45" (treated honey 45 seconds at 800 watts). 90" (treated honey 90 seconds at 800 watts). ZC. Zero Count. *p* significance value (widespread lineal model with binomial distribution and inflated zero). The same letter indicates no significant difference at the 95% confidence level.

TABLE 2. Moisture content, glass transition temperature (Tg) and acidity of the analysed honeys.

Sample	Moisture (g/100 g)			Tg (°C)			Acidity (meq/kg)		
	Unt.	45"	90"	Unt.	45"	90"	Unt.	45"	90"
1	19.6	19	18.8	-52.9	-51.7	-47.8	29	33	44
2	19.6	17.6	16.6	-50.4	-46.3	-44.2	42	35	35
3	21.4	19.8	16.2	-55.3	-53.3	-46.3	46	34	43
4	20.2	18.6	17.6	-53.4	-52.2	-47.3	36	41	30
5	19	17.2	16.6	-52.5	-48.8	-47.9	39	30	35
6	19.4	18.4	17.6	-51.1	-48.5	-44.9	36	31	32
7	21.8	20.8	18.4	-56.9	-56.2	-53.3	36	27	27
8	17.6	17.4	16.8	-47.1	-47.9	-45.3	32	29	32
Mean ± SD	19.8 ± 1.3	18.6 ± 1.2	17.3 ± 0.9	-52.5 ± 3	-50.6 ± 3.3	-47.1 ± 2.8	–	–	–
r	-0.9	-0.9	-0.6	–	–	–	–	–	–

Unt. (untreated), 45" (treated honey 45 seconds at 800 watts). 90" (treated honey 90 seconds at 800 watts). Pearson correlation coefficient (r) between moisture and Tg.

higher than others reported for Argentine honeys [Acquarone, 2004; Iurlina & Fritz, 2005; Silvano *et al*., 2014] and Spanish ones [Bentabol *et al*., 2014]. But, these values were similar to those reported by Karabagias *et al*. [2014] for unifloral Greek honeys. Meanwhile, the values were close to or even higher than those permitted by international food codes as Codex Alimentarius standard for honey 12–1981 (≤20 g/100 g) and the AFC (≤18 g/100 g). After microwave treatment, moisture content diminished (Table 2), as it was expectable because of the sample temperature rise during the exposure to microwave radiation.

Glass transition temperatures (Tg) of honeys from different botanical and geographic origins have already been reported [Cordella *et al*., 2003; Lazaridou *et al*., 2004; Costa *et al*., 2011] but there is no available information about Argentine honeys. From the results it can be seen that Tg shifted to higher values after treatment, which is consistent with the reduction in moisture content. It is well known that water acts as a plasticizer lowering the Tg [Bhandari & Howes, 1999; Ahmed *et al*., 2007]. Moreover, a linear relationship between Tg and moisture content was found (r=-0.94), which is in agreement with other authors [Cordella *et al*., 2003].

Acidity is produced in honey, principally, by the action of the enzyme glucose-oxidase on glucose. The values of most virgin honeys analyzed were below the limit permitted by the Argentine Food Code (40 meq/Kg) and were similar to these described by Aqcuarone [2004]. Bentabol *et al*. [2014] reported similar results for this parameter, these values did

TABLE 3. Color changes and hydroxymethylfurfural content in the analysed honeys.

Sample	Color (mm pfund)			HMF (mg / kg)		
	Unt.	45”	90”	Unt.	45”	90”
1	55	73	73	25	55	101
2	66	89	86	0	42	51
3	68	125	90	0	8	49
4	71	117	90	5	13	80
5	70	90	92	4	21	67
6	51	68	66	0	37	84
7	53	68	63	30	19	50
8	73	81	107	108	87	110
Mean ± SD	63.4 ± 9	88.9 ± 21.6	83.4 ± 14.9	22 ± 36.9	35.3 ± 26.2	74 ± 23.7
r	0.25	0.1	0.26	–	–	–

Unt. (untreated), 45” (treated honey 45 seconds at 800 watts). 90” (treated honey 90 seconds at 800 watts). Pearson correlation coefficient (r) between color and HMF.

TABLE 4. Diastase index, diastase activity and glucose-oxidase activity in the analysed honeys.

Samples	Diastase index (DU)			Diastase activity			Glucose-oxidase activity		
	Unt.	45”	90”	Unt.	45”	90”	Unt.	45”	90”
1	22	0	UM*	+	–	–	+	–	–
2	11	0	UM*	+	–	–	+	–	–
3	31	0	UM*	+	–	–	+	–	–
4	18	2	UM*	+	–	–	+	–	–
5	15	2	UM*	+	–	–	+	–	–
6	5	0	UM*	+	–	–	+	–	–
7	9	0	UM*	+	–	–	+	–	–
8	7	0	UM*	+	–	–	+	–	–

Unt. (untreated), 45” (treated honey 45 seconds at 800 watts), 90” (treated honey 90 seconds at 800 watts), UM*: unmeasured, + (activity present), – (activity absent).

not exceed the maximum established in the European Legislation for free acidity (50 meq/Kg). Microwave caused, in most cases, reduced acidity probably due to volatile acids evaporation. This was in disagreeing with Dranca & Oroian [2013].

The color of honey has not been legislated, but can be considered as another quality criterion. In this study a little range of color was observed. It is recognized that the color varied according to the melliferous areas, our results agreed with data previously obtained by Aloise [2010] for the same geographic zone. Similar range was observed in Greek unifloral honey [Karabagias *et al*., 2014].

One of the effects of thermal treatments of honey is the acceleration of Maillard reaction [Ibarz *et al*., 2000; Wong & Stanton, 1989]. These reactions would be associated to the non-enzymatic chemical changes of browning, leading to the formation of a variety of brown pigments, and formation of intermediate products as the HMF. In honey, Maillard reactions are the sugars condense with free amino acids producing a variety of brown pigments. The difference in browning rate of honey could be explained by differences in its amino acid and reducing sugar contents. Other factors that would influence the kinetics of Maillard browning could be the type and thermal stability of amino acids and reducing sugars which participate in the reaction [Turkmen *et al*., 2006].

In this study, a darkening of honeys was observed and HMF amount incremented rapidly (r=0.3) (Table 3). The trends in the variation of HMF content of the samples clearly depicted the sensitivity of honey to the period of heating and temperature. The same was observed by Kowalski *et al.* [2012], the increase caused by microwaves of the HMF is not gradual and its formation did not take place in the individual honeys in the same way, depending on floral origin. On the other hand, sample eight showed high concentration of HMF before treatment, associated to, probably, adultera-

tion or previous overheating. Excessive amount of HMF has been observed as evidence of overheating. Also, HMF is toxic and cancerogenic [Michail *et al*., 2007].

Diastase index values of virgin honeys were in agreement with reported values of Argentine honeys [Aquarone, 2004]. In Greek unifloral honeys, the range of diastase index values was a little more wide [Karabagias *et al*., 2014]. It this thought that the amount of enzyme activity is influenced by nectar thickness and sugar content and by the fact how many times it is transferred from honeybee to honeybee before nectar is turned into honey [Bonvehi *et al*., 2000].

The parameter of diastase activity is regulated by international codes, while there are no requirements for the activity of glucose-oxidase. Diastase index dropped below the acceptable value (Table 4), meanwhile, Hebbar *et al*. [2003] obtained similar results. Kowalski *et al.* [2012] showed that a short microwave treatment (0–2 min) with a low power level (63 watts) did not influence the honey quality estimated by means of diastase number.

In qualitative determination of both enzymes it was not observed activity neither of diastase (blue color) nor glucose--oxidase (colorless) in treated honeys (Table 4). Glucose-oxidase data were in agreement with the study by Kretavictus *et al.* [2010], where the authors observed a decrease of its activity after exposure to a conventional thermal treatment.

CONCLUSIONS

Microwave treatment was found very effective in reducing aerobic mesophilic bacteria, moulds, yeasts and *P. larvae* too. This was the first register about the effect of microwave on *P. larvae*.

Concerning to physicochemical and thermal properties variation, the treatment led to several undesirable consequences such as darkening, increase of HMF content and enzymes inactivation. Despite this, from this study it can be concluded that microwave treatment could be successfully applied by properly selecting power and exposure time to control benefits and disadvantages.

ACKNOWLEDGEMENTS

To Laboratorios Fares Taie (Mar del Plata). This work was financed for El Concejo Interuniversitario Nacional (CIN) and for PICT 1625 for LB.

REFERENCES

1. Acquarone C.A., Parámetros fisicoquímicos de mieles, relación entre los mismos y su aplicación potencial para la determinación del origen botánico y/o geográfico de mieles argentinas. 2004, *in*: Las Tesinas de Belgrano, Tesinas de Ciencias Exactas y Naturales, n° 19 (ed. Universidad de Belgrano). Publicaciones, Departamento de Investigación, Facultad de Ciencias Exactas y Naturales, Universidad de Belgrano, Buenos Aires, Argentina, pp. 1–56 (in Spanish).
2. AFC. 1969, Código Alimentario Argentino, Alimentos azucarados, Capitulo X, Artículo 782. Ministerio de Agricultura, Ganadería y Pesca, Ciudad de Buenos Aires. Argentina (in Spanish).
3. Ahmed J., Prabhu S.T., Raghavan G.S.V., Ngadi M., Physico-chemical, rheological, calorimetric and dielectric behaviour of selected Indian honeys. J. Food Eng., 2007, 79, 1207–1213.
4. Alippi A.M., Characterization of *Bacillus larvae* White, the causative agent of American foulbrood of honeybees. First record of its occurrence in Argentina. Rev. Argent. Microbiol., 1992, 24, 67–72.
5. Alippi A.M., Detection of *Bacillus larvae* spores in Argentinian honeys by using a semi-selective medium. Microbiology, 1995, 11, 343–350.
6. Alippi A.M., López C.A., Aguilar M.O., 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., 2002, 68, 3655– 3660.
7. Aloise V., Determination of quality chemical parameters of honey from Chubut (Argentinean Patagonia). Chil. J. Agr. Res., 2010, 70, 640–645.
8. AOAC. 2000a, Official method 920.180 Honey (Liquid, Strained or Comb) preparation of test samples, Official Methods of Analysis of AOAC International, 17th ed. Washington, DC.
9. AOAC. 2000b, Official method 969.38 Moisture in honey, Official Methods of Analysis of AOAC International, 17th ed. Washington, DC.
10. AOAC. 2000c, Official method 962.19 Acidity (Free, Lactone, and Total) of Honey, Official Methods of Analysis of AOAC International, 17th ed. Washington, DC.
11. AOAC. 2003, Official method 958.09, Diastatic Activity of Honey, Official Methods of Analysis of AOAC International, 17th ed. Washington, DC.
12. Bachmann L.H.E., Estudios preliminares de caracterización miel de abeja: determinación de carbohidratos por GC/MS y análisis enzimático. 2007, *in*: Tesis de grado, n° 4, 6, 8 (ed. Sistema de Bibliotecas UACh). Facultad de Ciencias, Universidad Austral de Chile., Valdivia, Chile, pp. 23–27 (in Spanish).
13. Baggio A., Gallina A., Dainese N., Manzinello C., Mutinelli F., Serra G., Colombo R., Carpana E., Sabatini A.G., Wallner K., Piro R., Sangiorgi E., Gamma radiation: A sanitating treatment of AFB-contaminated beekeeping equipment gamma radiation sanitation in beekeeping management. Apiacta, 2005, 40, 22–27.
14. Bartakova K., Drackova M., Borcovcova I., Vorlova L., Impact of microwave heating on hydroximethylfurfural content in Czech honeys. Czech J. Food Sci., 2011, 29, 328–336.
15. Bath P.K., Singh N., A comparison between *Helianthus annuus* & *Eucalyptus lanceolatus* honey. Food Chem., 1999, 67, 389–397.
16. Belitz H.D., Grosch W., Schieberle P., Food Chemistry. 2004, 3rded., Springer Verlag, Germany, pp. 260–263.
17. Bentabol Manzanares A., Hernández García Z., Rodríguez Galdón B., Rodríguez Rodríguez E., Díaz Romero C., Physico-chemical characteristics of minor monofloral honeys from Tenerife, Spain. Food Sc. Tech., 2013, 5, 572–578.
18. Beveridge T.J., Use of the Gram stain in microbiology. Biotech. Histochem., 2001, 76, 111–118.
19. Bhandari T.R., Howes T., Implication of glass transition for the drying and stability of dried foods. J. Food Eng., 1999, 40, 71–79.
20. Bianchi E.M., Control de calidad de la miel y la cera. 1990, *in*: Boletín de los Servicios Agrícolas, n° 68/3 (ed. FAO), (in Spanish).

21. Bonvehi J.S., Torrento M.S., Raich J.M., Invertase, activity in fresh and processed honeys. J. Sci. Food. Agric., 2000, 80, 507–512.
22. Castro V., Arias M.L., Antillon F., Jimenez M., Efectos de las microondas sobre la sobrevivencia de algunas bacterias patógenas en comidas populares costarricenses. Rev. Costarric. Cienc. Méd., 1997, 18, 19–27 (in Spanish).
23. Celandroni F., Longo I., Tosoratti N., Giannessi F., Ghelardi E., Salvetti S., Baggiani A., Senesi S., Effect of microwave radiation on *Bacillus subtilis* spores. J. Appl. Microbiol., 2004, 97, 1220–1227.
24. Coll Cardenas F., Villat C., Laporte G., Noia M., Mestorino M., Características microbiológicas de la miel. Revisión bibliográfica. J. Veterinaria Cuyana., 2008, n° 3, 1 y 2, 29–34. (in Spanish).
25. Cordella C., Faucon J. P., Cabrol Bass D., Sbirrazuolli N., Application of DSC as a tool for honey floral species characterization and adulteration detection. J. Therm. Anal. Cal., 2003, 71, 279–290.
26. Costa P.A., Moraes I.C.F., Bittante A.M.Q.B., Sobral P.J.A., Gomide C.A., Carrer C.C., Thermal and Rheological Properties of Brazilian Honeys. 2011, *in*: International Congress on Engineering and Food 11th, Engineering Properties of Foods, vol. 2 (eds. S.P. Taoukis, N.G. Stoforos, V.T. Karathanos, G.D. Saravacos). School of Chemical Engineering, National Technical University of Athens, Athens, Greece, pp. 1329–1330.
27. de Graaf D.C.d., Vandekerchove D., Dobbelaere W., Peeters J.E., Jacobs F.J., Influence of the proximity of American foulbrood cases and apicultural management on the prevalence of *Paenibacillus larvae* spores in Belgian honey. Apidologie, 2001, 32, 587–599.
28. Desalegn A., The antibacterial activity of honey. Int. J. Curr. Res. Aca. Rev., 2013, 1, 102–116.
29. Dingman D.W., Stahly D.P., Medium promoting sporulation of *Bacillus larvae* and metabolism of médium components. Appl. Environ. Microbiol., 1983, 46, 860–869.
30. Dranca F., Oroian M., Impact of microwave heating on chemical properties of Romanian honeys. J. Agroaliment. Proc. Technol., 2013, 19, 464–469.
31. Estrada H., Gamboa M.M., Chavez C., Arias M.L., Evaluación de la actividad antimicrobiana de la miel de abeja contra *Staphylococcus aeureus, Staphylococcus epidermis, Pseudomona aeuroginosa, Escherichia coli, Salomnella enteritidis, Listeria monocytogenes y Aspergillus niger*. Evaluación de su carga microbiológica. 2005, *in:* Archivos Latinoamericanos de Nutrición, Vol. 55 (ed. Capítulo Venezolano). Publicación Oficial de la Sociedad Latinoamericana de Nutrición, pp. 167–171 (in Spanish).
32. Fallico B., Zappala M., Arena E., Verzera A., Effects of conditioning on HMF content in unifloral honeys. Food Chem., 2004, 85, 305–313.
33. Frazier W.C., Westhoff D.C., Food Microbiology. 1978, 3rded., Tata McGraw-Hill Publishing Company, NewYork, pp. 185–193.
34. Fries I., Camazine S., Implications of horizontal and vertical pathogen transmissions for honey bee epidemiology. Apidologie, 2001, 32, 199–214.
35. Hansen H., Rasmussen B., The investigation of honey from bee colonies for *Bacillus larvae.* Dan. J. Plant Soil Sci., 1986, 90, 81–86.
36. Hebbar H.U., Nandini K.E., Lakshmi M.C., Subramanian R., Microwave and infrared heat processing of honey and its quality. Food. Sci. Technol. Res., 2003, 9, 49–53.
37. Heyndrickx M., Vandemeulebroecke K., Hoste B., Janssen P., Kersters K., De Vos P., Logan N.A., Ali N., Berkeley R., Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvifaciens* (Nakamura 1984) Ash et al. 1994, a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *larvae* (White 1906) Ash et al. 1994, as a subspecies of *P. larvae*, with emended descriptions of *P. larvae* as *P. larvae* subsp. larvae and *P. larvae* subsp. *pulvifaciens*. Int. J. Syst. Bacteriol., 1996, 46, 270- 279.
38. Ibarz A., Pagán J., Garza S., Kinetic models of non- enzymatic browning in apple puree. J. Sci. Food Agric., 2000, 80, 1162––1168.
39. International Commission on Microbiological Specifications for Foods (ICMSF). Microorganismos de los alimentos. Su significado y métodos de enumeración. 2000., vol 1 (ed. Acribia S.A.). Zaragoza, pp. 165–179.
40. IHC. 1999, Harmonised methods of the international honey commission. International Honey Commission, pp. 1–54.
41. ISO. 15213. 2003, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. International Organization for Standarization.
42. Iurlina M.O., Fritz R., Characterization of microorganisms in Argentinian honeys from different sources. Int. J. Food Microbiol., 2005, 105, 297–304.
43. Karabagias I.K., Badeka A.V., Kontakos S., Karabournioti S., Kontominas M.G., Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. Food Chem., 2014, 165, 181–190.
44. Kedzia B., Kedzia A., Hołderna-Kedzia E., Studies on the occurrence of clostridia spores in honey. *Bacillus* and *Clostridium*. Wyd. Pszczel., 1996, 37–39 (in Polish).
45. Kohn´s B.J., Técnicas de quirófano. 1998, 8thed., Harcourt Brace, Madrid, pp. 207–224 (in Spanish).
46. Kowalski S., Lukasiewicz M., Bednarz S., Panus M., Diastase number changes during thermal and microwave processing of honey. Czech J. Food Sci., 2012, 30, 21–26.
47. Kretavictus J., Kurtinaitiene B., Racys J., Ceksteryte V., Inactivation of glucose oxidase during heat-treatment de-crhistallization of honey. Agriculture., 2010, 97, 115–122.
48. Lauro F.M., Favaretto M., Covolo L., Rassu M., Bertoloni G., Rapid detection of *Paenibacillus larvae* from honey and hive samples with a novel nested PCR protocol. Int. J. Food Microbiol., 2003, 81, 195–201.
49. Lazaridou A., Biliaderis C.G., Bacabdritsos N., Sabattini A.G., Composition, thermal and rehological behaviour of selected Greek honeys. J. Food Eng., 2004, 64, 9–21.
50. Michail K., Matzi V., Maier A., Herwig R., Greilberger J., Juan H., Kunert O., Wintersteiger R., Hydroxymethylfurfural: an enemy or a friendly xenobiotic? A bioanalytical approach. Anal. Bioanal. Chem., 2007, 387, 2801–2814.
51. Montenegro S.B., Avallone C.M., Aztarbe M., Osuna M., Actividad enzimática en miel de *Apis mellifera*. Comunicaciones científicas y tecnológicas., 2006, T095, 1–3.
52. Morales F.J., Romero C., Jimenez-Perez S., A kinetic study on 5-hydroxymethylfurfural formation in Spanish UHT milk stored at different temperatures. J. Food Sci. Tech. Mys., 1997, 34, 28–32.
53. Peréz A., Alexander H., Matos Chamorro A., Análisis comparativo de la calidad fisicoquímica, microbiológica y organoléptica

de la miel de abeja (*Apis mellifera*) producida en diferentes regiones de Perú. Rev. Invest. Univ., 2009, 1, 5–11 (in Spanish).
54. Shimanuki H., Bacteria. 1997, *in*: Honey Bee Pests, Predators, and Diseases (eds. R.A. Morse, K. Flottum). A.I. Root Company., Medina, Ohio, pp. 35–54.
55. Silvano M.F., Varela M.S., Palacio M.A., Ruffinengo S., Yamul D.K., Physicochemical parameters and sensory properties of honeys from Buenos Aires region. Food Chem., 2014, 152, 500–507.
56. Singh N., Bath P.K., Relationship between heating and hydroxymethylfurfural formation in different honey types. J. Food Sci. Tech., 1998, 35, 154–156.
57. Sturtevant A.P., Quantitative demonstration of the presence of spores of *Bacillus larvae* in honey contaminated by contact with American foulbrood. J. Agricult. Res., 1936, 52, 697–704.
58. Tabera A.E., Libonatti C.C., Díaz M.D., Relevamiento de muestras de mieles procedentes de la zona de Tandil. 2002, *in:* Boletín Apícola n° 20 (ed. Dirección de Industria Alimentaria). SAGPyA, Argentina, pp. 13–17 (in Spanish).
59. Turkmen N., Sari F., Poyrazoglu E.S., Velioglu Y.S., Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem., 2006, 95, 653–657.
60. Wakita J., Shimada H., Itoh H., Matsuyama T., Matsushita M., Periodic colony formation by bacterial species *Bacillus subtilis.* J. Phys. Soc. Japan., 2001, 70, 911–919.
61. White J.W., Subers M.H., Schepartz A.I., The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim. Biophys., 1963, 73, 57– 70.
62. White Jr., Physical characteristics of honey. 1975, *in:* Honey, a comprehensive survey (ed. E. Crane). Heinemann, London, pp. 509–514.
63. White J.W., Spectrophotometric method for hydroxymethylfurfural in honey. J. Assoc. Off. Anal. Chem., 1979, 62, 509.
64. Wong M., Stanton D.W., Nonenzymic browning in kiwifruit juice concentrate systems during storage. J. Food Sci., 1989, 54, 669–673.
65. Zappala M., Fallico B., Arena E., Verzera A., Methods for the determination of HMF in honey: a comparison. Food Contr., 2005, 16, 273–277.

Submitted: 30 October 2014. Revised: 12 March 2015. Accepted: 13 March 2015. Published on-line: 15 April 2015.