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Effect of the Tyndallization on the Quality of Colombian Honeys

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Honey is highly appreciated for its functional properties, however its consume is restricted for children under one year old due to the potential presence of *Clostridium botulinum* spores, which could cause botulism. Within this context the thermal treatment of tyndallization could become in an alternative to treat honey with the aim of eliminating *Clostridium botulinum* spores. The objective of this study was to apply thermal treatments (pasteurization and tyndallization) to *Apis mellifera* and *Tetragonisca angustula* honeys, to evaluate the effect on some physicochemical and microbiological parameters associated to honey quality. The honey bottled in amber glass recipients was pasteurized to 65 ºC for 15 and 21 min and was tyndallized to 80 ºC during five and seven minutes. The tyndallized samples were immediately removed from the thermostatic bath and cooled then, were incubated at 37 ºC under anaerobic conditions for 72 h, this cycle was repeated two times more, and after that the samples were analyzed. Harmonized methods of the International Honey Commission were used for physicochemical analyses, while methods from the International Commission on Microbiological Specifications for Foods (ICMSF) were used for microbiological tests. Honey samples heat treated for pasteurization and tyndallization during 15 and 21 min showed statistically significant differences ($p < 0.05$), in terms of moisture and diastase activity, in comparison to untreated honey, although their values were within the limits established by the Colombian regulations, except *T. angustula* moisture. Hydroxymethylfurfural (HMF) raised in *A. mellifera* honey pasteurized and color raised in tyndallized honeys. Mesophilic bacteria, molds and yeasts levels of *T. angustula* honey were not within the limits established by the Colombian regulations. Mesophilic bacteria, molds and yeasts and total coliforms levels decreased in *T. angustula* honey heat treated. While sulfite-reducers anaerobic spores increased in *T. angustula* honey pasteurized and *Clostridium perfringens* increased in *T. angustula* tyndallized honey.

1. Introduction

Although *Apis mellifera* is responsible for almost all of the honey sold in the world, it is not the only producing species; there are species of stingless bees (meliponini) that produce honey and they are mostly originated from America (Michener, 2013). Stingless bees unlike *A. mellifera*, have no stinger and they are not aggressive, which significantly facilitates handling for breeding and maintenance. Stingless bees are mainly a tropical group of more than 500 species (and possibly even 100 more undescribed) (Michener, 2013). These bees are distributed among the northern hemisphere 23.5 ºN (Tropic of Cancer) and temperate regions of the southern hemisphere 35 ºS (Australia and South America) and 28 ºS in Africa (Kwapong et al., 2010). In Colombia there are 101 species of stingless bees, however only 17 of these species, among which may be mentioned *Tetragonisca angustula* and some species of the genus *Melipona* (Nates-Parra, 2005) are used in honey production. The species *T. angustula* (4mm long) known as "angelita" is the stingless bee most widely distributed in Colombia, it lives in all natural regions below 1800 m.a.s.l. (Nates-Parra, 2001). Honey of these species is known for its medicinal value, popularly attributed, and is sold in various local markets (Cepeda et

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al., 2008). *Clostridium botulinum* spores could cause botulism; botulism is a severe neurologic illness induced by the botulinic neurotoxin that affects human beings and several animals (Rall et al., 2003). Approximately one third of cases of infant botulism have been associated with honey consumption (Gilbert et al., 2006). Therefore, honey consume is restricted for children under one year old due to the potential presence of *Clostridium botulinum* spores, this group is susceptible to germination of the spores in the intestinal canal, with subsequent multiplication and toxin formation (Nevas et al., 2006). Thermal pasteurization is a technique widely utilized in the industry to reduce the microbial content of foods and increase their shelf life (D'Addio et al., 2014). However, *C. botulinum* spores are not destroyed with pasteurization, therefore is necessary to search a different heat treatment of sterilization, because this process would inevitably degrade the taste and structure of honey. Tyndallization is a process that can be applied to honey for eliminating *C. botulinum* spores without affecting considerably the quality of honey. The classical tyndallization process, that is intermittent or fractional sterilization, is a means of sterilization by which a food or beverage is heated for successive time periods with intermittent incubation storage for allowing spores germination (Kim et al., 2012). Therefore, the objective of this study was to apply thermal treatments (pasteurization and tyndallization) to *Apis mellifera* and *Tetragonisca angustula* honeys, to evaluate the effect on some physicochemical and microbiological parameters associated to honey quality.

2. Materials and methods

2.1 Samples

The study was carried out on two selected multifloral honey batches: *Apis mellifera* organic honey and *Tetragonisca angustula* honey. *Apis mellifera* honey batch were provided by beekeepers from "Ecolsierra" network and produced during December 2013 harvest in the province of Santa Marta, Colombia. *Tetragonisca angustula* honey batch were provided by beekeepers from Botanical Garden and produced during June 2014 harvest in the city of Medellín, Colombia. All honey samples were not heated by the producers, and they were taken not more than 4 weeks after the extraction from the hives, batches showed no signs of fermentation or crystallization. The samples were taken directly from the storage containers. They were aseptically transferred to alimentary amber glass recipients. *A. mellifera* honey samples were preserved at room temperature until use, while *T. angustula* honey samples were preserved at 4 ºC until processing.

2.2 Heat treatment

Each honey batch was divided into three parts: one part as a control (without heat treatment), another part for pasteurization at 65 ºC and the last part for tyndallization at 80 ºC. The honey was bottled in amber glass recipients of 50 mL. The honey for pasteurization was heated in a water bath at 65 ºC for 15 during 21 min, immediately removed from the thermostatic bath and cooled in an ice-water bath. Tyndallization was performed to 80 ºC during five and seven minutes, immediately removed from the water bath and cooled in ice-water bath then, were anaerobically incubated at 37 ºC during 72 h using a Gaspak jar. This cycle was repeated two more times, and after that the samples were analyzed.

2.3 Physicochemical analyses

Harmonized methods of the International Honey Commission (Bogdanov et al., 1997) were used for physicochemical analyses: moisture and °Brix by refractometry, diastase activity and HMF using spectrophotometry and colour by spectrocolorimetry.

2.4 Microbiological analysis

International Commission on Microbiological Specifications for Foods (ICMSF, 2002) were used for microbiological tests.

2.5 Statistical analysis

The experimental design was a full factorial analysis of variance with heat treatment time (0, 15 or 21 min), heat treatment type (pasteurization or tyndallization) and bee species (*Apis mellifera* or *Tetragonisca angustula*) as factors was carried out. The means were separated according to the Tukey test ($\alpha = 0.05$). Data are presented as mean values.

3. Results and discussion

3.1 Physicochemical behavior of honeys heat-treated

Tables 1, 2, 3 and 4 present physicochemical analysis for *A. mellifera* and *T. angustula* honeys treated thermally. Moisture increased significantly relative to the control sample, in all heat treatments and for the two species (Tables 1, 2, 3 and 4). There were no significant differences in moisture between 15 and 21 min of treatment, neither there were no differences between pasteurization and tyndallization. However, in neither case *Apis mellifera* honey exceeded the standards set by the regulations for moisture, but moisture level of

Tetragonisca angustula honey exceeded in all cases the permissible levels to *A. mellifera*. It could be possible that this characteristic of high moisture is a natural characteristic for stingless bee honeys. These results moisture coincide with reports made to *T. angustula* honey (Anacleto et al., 2009). The brix degrees had an inverse behaviour to moisture. Moisture was significantly higher in T. *angustula* relative to *A. mellifera*.

Diastase activity decreased significantly in comparison to the control sample, in all heat treatments and for the two species (Tables 1, 2, 3 and 4). Decrease was more noticeable for tyndallization and *A. mellifera* honey. Within the same heat treatment there was no significant difference in diastase activity between 15 and 21 min. Tyndallization reduced diastase activity by 52 % in *A. mellifera* honey and 32 % in *T. angustula* honey; while, pasteurization reduced diastase activity by 5 % in *A. mellifera* honey and 7 % in *T. angustula* honey. Diastase reduction due to heat treatment with similar conditions has been reported for *A. mellifera* honey (Samborska and Czelejewska, 2014).

The HMF is obtained of the threefold dehydration of hexoses, like fructose and glucose (Lanziano et al., 2014). The HMF is a toxic sugar degradation compound (Spigno et al., 2014). HMF increased significantly relative to the control sample, for pasteurization in *A. mellifera* honey (Table 1). Within the same heat treatment, there was no significant difference in HMF between 15 and 21 min. Pasteurization increased HMF by 77 % in *A. mellifera* honey. Samborska and Czelejewska (2014) reported HMF increases in *A. mellifera* honey under heat treatment, although these increases were not as considerable as this study. For *T. angustula* honey, HMF was under the limit of quantification for control and pasteurization. This behavior coincides with results reported by Biluca et al. (2014), when heat treatments were applied to stingless bee honey. HMF levels for *T. angustula* tyndallized honey were lows, although significantly higher compared to control for 21 min. This behavior coincides with Freitas et al. (2010), who reported HMF increase with heat treatment in stingless bee honey.

Table 1: Physicochemical analysis for Apis mellifera pasteurized honey

Physicochemical Analysis	Control	Treatment time		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Moisture (%)	16.71 ± 0.55*^{a}	18.52±0.06^{b}	18.19±0.08^{b}	≤ 20 %	≤ 21 %
°Brix	81.30±0.11^{a}	79.38±0.04^{b}	79.65±0.07^{b}		
Diastase activity (DN)	9.25±0.07^{a}	8.75±0.35^{b}	8.8±0.00^{b}	≥ 3	≥ 8
HMF (mg/kg)	7.04±0.99^{a}	12.07±0.58^{b}	12.60±0.16^{b}	≤ 60	≤ 60
Color (mm Pfund)	48.00±0.00^{a}	49.00±0.00^{a}	49.00±0.00^{a}		

* Means ± standard deviation (n = 2). Values in rows with the same letter do not differ significantly at α = 0.05

Table 2: Physicochemical analysis for Apis mellifera tyndallized honey

Physicochemical Analysis	Control	Treatment time		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Moisture (%)	16.71 ± 0.55*^{a}	18.91±0.38^{b}	18.82±0.03^{b}	≤ 20 %	≤ 21 %
°Brix	81.30±0.11^{a}	79.15±0.35^{b}	79.18±0.04^{b}		
Diastase activity (DN)	9.25±0.07^{a}	4.65±0.35^{c}	4.25±0.07^{c}	≥ 3	≥ 8
HMF (mg/kg)	7.04±0.99^{a}	6.22±1.80^{a}	7.18±0.73^{a}	≤ 60	≤ 60
Color (mm Pfund)	48.00±0.00^{a}	57.00±0.00^{b}	58.00±1.41^{b}		

* Means ± standard deviation (n = 2). Values in rows with the same letter do not differ significantly at α = 0.05

Color increased significantly in comparison to the control sample, for tyndallization and for the two species (Tables 1, 2, 3 and 4). Increase was more marked for *T. angustula* honey. Within the same heat treatment, there was no significant difference in colour between 15 and 21 min. Tyndallization increased colour by 20 % in *A. mellifera* honey and 40 % in *T. angustula* honey.

Table 3: Physicochemical analysis for Tetragonisca angustula pasteurized honey

Physicochemical Analysis	Control	Treatment time		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Moisture (%)	22.03 ±1.11*[a]	23.26 ± 0.57[b]	23.45 ± 0.19[b]	≤ 20 %	≤ 21 %
°Brix	75.98 ±0.88[a]	74.70 ± 0.57[b]	74.53 ± 0.18[b]		
Diastase activity (DN)	33.28 ±1.01[a]	30.25 ± 0.92[b]	31.70 ± 3.11[b]	≥ 3	≥ 8
HMF (mg/kg)	ND[a]	ND[a]	ND[a]	≤ 60	≤ 60
Color (mm Pfund)	62.50 ±0.71[a]	62.50 ± 0.71[a]	63.0 ± 0.00[a]		

* Means ± standard deviation (n = 2). ND, non-detectable HMF.
Values in rows with the same letter do not differ significantly at α = 0.05

Table 4: Physicochemical analysis for Tetragonisca angustula tyndallized honey

Physicochemical Analysis	Control	Treatment time		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Moisture (%)	22.03 ±1.11*[a]	23.58 ± 0.08[b]	24.02 ± 0.08[b]	≤ 20 %	≤ 21 %
°Brix	75.98 ±0.88[a]	74.58 ± 0.04[b]	74.15 ± 0.07[b]		
Diastase activity (DN)	33.28 ±1.01[a]	24.08 ± 0.73[c]	21.25 ± 0.21[c]	≥ 3	≥ 8
HMF (mg/kg)	ND[a]	1.61 ± 0.05[a]	4.46 ± 0.21[b]	≤ 60	≤ 60
Color (mm Pfund)	62.50 ±0.71[a]	87.75 ± 0.35[b]	87.75 ± 0.35[b]		

* Means ± standard deviation (n = 2). ND, non-detectable HMF.
Values in rows with the same letter do not differ significantly at α = 0.05

3.2 Microbiological behavior of heat-treated honeys

Tables 5 and 6 show microbiological analysis for *A. mellifera* and *T. angustula* honeys treated thermally. *A. mellifera* honey was within limits established by Colombian regulations for all treatments. Pasteurization for 21 min reduced aerobic mesophilic bacteria to < 1.00 log (CFU/g).

Table 5: Microbiological analysis for Apis mellifera honey treated thermally

Microbiological Analysis	Control log(CFU/g)	Pasteurization log(CFU/g)		Tyndallization log(CFU/g)		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Aerobic mesophilic bacteria	1.84 ± 0.09*	2.18 ± 0.10	< 1.00	1.93 ± 0.04	2.13 ± 0.02	≤ 2.48	
Molds and yeasts	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	≤ 2.00	≤ 2.00
Total coliforms log(MPN/g)	< 0.48	< 0.48	< 0.48	< 0.48	< 0.48	≤ 1.00	
Faecal coliforms log(MPN/g)	< 0.48	< 0.48	< 0.48	< 0.48	< 0.48		
Escherichia coli	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	
Sulfite-reducers anaerobic spores	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00		≤ 2.00
Clostridium sp.	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00		
Salmonella /25g	Absent	Absent	Absent	Absent	Absent	Absent	

* Means ± standard deviation (n = 2).

In contrast with *A. mellifera* honey, *T. angustula* honey control was not within the limits established by Colombian regulations for mesophilic bacteria, molds and yeasts levels. This behavior agrees with the results reported by Ferrufino and Vit (2013) for honey of *Melipona grandis* and *Tetragonisca fiebrigi*. With heat treatment mesophilic bacteria decrease in *T. angustula* honey, but levels are still high.

Molds and yeasts levels decreased, relative to control, in all heat treatments for *T. angustula* honey (Tables 5 and 6), decrease was more noticeable for pasteurization. This trend coincides with Tosi et al. (2004) that reported reduction in molds and yeasts in *A. mellifera* honeys heat-treated.

Total coliforms decrease as well in all *T. angustula* honeys heat treated. Sulfite-reducers anaerobic spores raised in *T. angustula* pasteurized honeys; in samples pasteurized for 15 min sulfite-reducers anaerobic spores exceeded the levels allowed by regulations. *Clostridium perfringens* increased in *T. angustula* tyndallized honeys.

Table 6: Microbiological analysis for Tetragonisca angustula honey treated thermally

Microbiological Analysis	Control log(CFU/g)	Pasteurization log(CFU/g)		Tyndallization log(CFU/g)		*Apis mellifera* honey requirements log(CFU/g)	
		15 min	21 min	15 min	21 min	NTC 1273 (2007)	Res. 1057 (2010)
Aerobic mesophilic bacteria	2.74 ± 0.14*	2.06 ± 0.40	2.22 ± 0.15	2.49 ± 0.08	2.31 ± 0.15	≤ 2.48	
Molds and yeasts	2.96 ± 0.12	1.30 ± 0.43	1.30 ± 0.43	2.36 ± 0.08	2.42 ± 0.03	≤ 2.00	≤ 2.00
Total coliforms log(MPN/g)	0.78 ± 0.25	< 0.48	< 0.48	< 0.48	< 0.48	≤ 1.00	
Faecal coliforms log(MPN/g)	< 0.48	< 0.48	< 0.48	< 0.48	< 0.48		
Escherichia coli	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	
Sulfite-reducers anaerobic spores	< 1.00	2.39 ± 0.12	1.85 ± 0.21	< 1.00	< 1.00		≤ 2.00
Clostridium perfringens	< 1.00	< 1.00	< 1.00	2.59 ± 0.16	2.45 ± 0.21		
Salmonella /25g	Absent	Absent	Absent	Absent	Absent	Absent	

* Means ± standard deviation (n = 2).

4. Conclusions

Tyndallization affected significantly properties of *A. mellifera* honey, as diastase was reduced by 52 % and colour was increased by 20 %. Pasteurization increased HMF by 77 % in *A. mellifera* honey. Considering that *A. mellifera* honey has very good microbiological quality would not be necessary to apply these heat treatments. In contrast, tyndallization reduced diastase activity by 32 % in *T. angustula* honey and colour was increased by 40 %. Despite this reduction, diastase levels are sufficiently high and *T. angustula* honey tyndallized accomplish with the limits set by regulations. In addition, tyndallization reduced sulfite-reducers anaerobic spores in *T. angustula* honey, indicating that this heat treatment works to remove spores. However, tyndallization raised *Clostridium perfringens* in *T. angustula* honey. This result indicates that for *T. angustula* honey is necessary to combine tyndalization with the application of natural antimicrobials to help eliminate microorganisms that this heat treatment does not eliminate.

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References

Anacleto D., Souza B., Marchini L., Moreti A., 2009, Composition of the honey of samples originated from Jataí bees (*Tetragonisca angustula* Latreille, 1811), Ciênc. Tecnol. Aliment. 29, 535-541 (In Portuguese).

Biluca F.C., Della Betta F., De Oliveira G.P., Pereira L.M., Morilla L., Gonzaga L.V., Costa A.C., Fett R., 2014, 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment, Food chemistry 159, 244–249.

Bogdanov S., Martin P., Lüllmann C., 1997, Harmonized Methods of the European Honey Commission, Apidologie Extra Issue, 1-62.

Cepeda M., Nates-Parra G., Téllez G., 2008, Acta Biológica Colombiana 14, 181. Trade of meliponiculture products in Colombia. Proceedings of IV Colombian Meeting of Wild Bees, LABUN, Bogotá, Colombia (In Spanish).

D'Addio L., Di Natale F., Budelli A., Nigro, R., 2014, CFD Simulation for the Pasteurization of Fruit Puree with Pieces, Chemical Engineering Transactions, 39, 1699-1704.

Ferrufino U., Vit P., 2013, Pot-Honey of Six Meliponines from Amboró National Park, Bolivia, 409. Pot-Honey: A legacy of stingless bees, Eds. Vit P., Pedro S.R.M., Roubik D.W., Springer Science+Business Media, New York, USA.

Freitas W.E.S., Aroucha E.M.M., Soares K.M.P., Mendes F.I.B., Oliveira V.R., Lucas C.R., dos Santos M.C.A., 2010, Physical-chemical parameters of honey stingless bee (*Melipona subnitida*) after heat treatment, Acta Veterinaria Brasilica 4, 153-157 (In Portuguese).

Gilbert S., Lake R., Hudson A., 2006, Risk Profile: *Clostridium botulinum* in Honey. Institute of Environmental Science & Research Limited, Christchurch, New Zealand.

ICMSF (International Commission on Microbiological Specifications for Foods), 2002, Microorganisms in Foods, vol 7: Microbiological Testing in Food Safety Management, Eds. ICMSF, Kluwer Academic/Plenum publishers, New York, USA.

Icontec (Colombian Institute of Technical Standards and Certification), 2007, Colombian Technical Standard. Honey. NTC 1273. Icontec Institute, Bogotá, Colombia (In Spanish).

Kim H., Kim H., Bang J., Kim Y., Beuchat L.R., Ryu J.H., 2012, Reduction of *Bacillus cereus* spores in sikhye, a traditional Korean rice beverage, by modified tyndallization processes with and without carbon dioxide injection, Letters in applied microbiology 55, 218–223.

Kwapong P., Aidoo K., Combey R., Karikari A., 2010, Stingless Bees. Importance, Management and Utilisation. Unimax Macmillan Ltd., Accra North, Ghana.

Lanziano C.S., Rodrigues F., Rabelo S., Guirardello R., Da Silva V.T., Rodella C.B., 2014, Catalytic conversion of glucose using TiO_2 catalysts, Chemical Engineering Transactions 37, 589-594.

Michener C.D., 2013, The Meliponini, 3. Pot-Honey: A legacy of stingless bees, Eds. Vit P., Pedro S.R.M., Roubik D.W., Springer Science+Business Media, New York, USA.

Ministry of Social Protection, 2010, Whereby it is set up the technical regulation on sanitary requirements that must comply bee honey for human consumption. Resolution 1057. Ministry of Social Protection, Bogotá, Colombia (In Spanish).

Nates-Parra G., 2001, Guide for breeding and management of bee "angelita" or "virginita" (*Tetragonisca angustula* Illiger). Andrés Bello Agreement, Science and Technology Series 84, Bogotá, Colombia (In Spanish).

Nates-Parra G., 2005, Corbiculated bees from Colombia: Hymenoptera: Apidae. National University of Colombia, Bogotá, Colombia (In Spanish).

Nevas M., Lindström M., Hörman A., Keto-Timonen R., Korkeala H., 2006, Contamination routes of *Clostridium botulinum* in the honey production environment, Environmental Microbiology 8, 1085–1094.

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

Samborska K., Czelejewska M., 2014, The influence of thermal treatment and spray drying on the physicochemical properties of polish honey, Journal of Food Processing and Preservation 38, 413–419.

Spigno G., Moncalvo A., De Faveri D.M., Silva A., 2014, Valorisation of stalks from different grape cultivars for sugars recovery, Chemical Engineering Transactions 37, 745-750.

Tosi E.A., Ré E., Lucero H., Bulacio L., 2004, Effect of honey high-temperature short-time heating on parameters related to quality, crystallization phenomena and fungal inhibition, LWT - Food Science and Technology 37, 669–678.