UNIVERSIDAD AUTÓNOMA DE COAHUILA

FACULTAD DE CIENCIAS QUIMICAS

DEPARTAMENTO DE INVESTIGACIÓN EN ALIMENTOS

TESIS

"Cambios Bioquímicos y Microbiológicos Durante el Procesamiento y Almacenamiento del Aguamiel"

Que presenta

MC. Sandra Luz Villarreal Morales

Para obtener el grado de

Doctor en Ciencia y Tecnología de los Alimentos

Saltillo. Coahuila Junio de 2018.

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La Facultad de Ciencias Químicas a través del jurado examinador hace constar que la tesis titulada

"Cambios Bioquímicos y Microbiológicos Durante el Procesamiento y Almacenamiento del Aguamiel"

Presentada por

MC. Sandra Luz Villarreal Morales

Ha sido aceptada como requisito parcial para obtener el grado de

Doctor en Ciencia y Tecnología de los Alimentos

El trabajo presentado ha sido dirigido por el siguiente comité

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ABSTRACT

Aguamiel is a beverage obtained from the maguey pulquero in adult stage; it is considered a beverage with functional properties due to its nutritional composition, which includes prebiotics such as fructooligosaccharides and probiotics such as *Lactobacillus* sp. However, the shelf life of the beverage is short and the native microbiota in the aguamiel ferments the beverage to pulque, changing the organoleptic characteristics of the aguamiel.

The biochemical changes and microbial diversity of cooked aguamiel during storage at room temperature (25°C) and refrigeration temperature (4°C) were characterized in this study, for a period of 5 days and 10 days respectively, using aguamiel collected from *Agave salmiana* and *Agave atrovirens* during the winter and summer seasons. With the purpose of increasing the shelf life of the beverage with functional properties, for a longer period of time and ensuring its food safety by applying controlled treatments such as pasteurization and ohmic heating.

In the first stage, the microbial characterization of cooked aguamiel was performed using culture-dependent methods, by isolating and molecular identifying the cultivable microorganisms present in the beverage during aguamiel storage. This microbial characterization allowed to verify that the traditional thermal treatment applied to aguamiel by the collector, not inhibit the growth of microorganisms characteristic of the beverage such as *Lactobacillu plantarum, L. paracasei* identified during summer; *Leuconostoc mesenteroides* and *Kluyveromyces marxianus,* identified during winter; *Acetobacter* sp., *Gluconobacter* sp, *Saccharomyces paradoxus* and *Kazachstania gamaspora*, identified only in *A. atrovirens* aguamiel; *Exigobacterium* sp. identified only in aguamiel of *A. salmiana* and opportunistic microorganisms as *Erwinia chrysanthemi, Enterobacter* sp., *Klebsiella sp.* and *Serratia* sp. The microbial diversity (abundance of microorganisms) was greatest in *A. atrovirens* aguamiel for both seasons.

In the second stage, the chemical changes of the cooked aguamiel during storage were evaluated, where it was verified that *Agave* specie and harvest season affect the aguamiel chemical composition, as well as, the uncontrolled heat treatment applied by the collector. These changes compromise the beverage functional properties and its effects on consumer health. In particular, the storage of aguamiel at room temperature, aguamiel collected during the summer and *A. atrovirens* aguamiel were greater affected by a decrease in pH, °Brix and density and an increase in sugar and protein content; these changes are related to the development of microbiota during the aguamiel storage, agave specie and season of aguamiel collection.

In the third stage a controlled treatment (pasteurization) and a treatment of ohmic heating were applied to aguamiel with the purpose of changing to a minimum, the quality parameters of the beverage and conserving the aguamiel for a longer time, ensuring the food safety of the same one and avoiding the change of its chemical and organoleptic characteristics. The best pasteurization treatment in aguamiel was at 80 °C/ 10 min, where the microbial load was inhibited, but the residual activity of the enzyme peroxidase (POD) was high (21%). The aguamiel treated by ohmic heating to 80 °C/ 25 V in a time of 4 min allowed maximum reduction of POD activity (5-10%) and inhibition of microorganisms in a shorter time without effects on browning and sedimentation index, so this treatment was the best.

In the last stage, the metagenomic characterization of the microbiota in fresh and cooked aguamiel was realized, using independent cultivation methods such as DGGE. In this stage, the non-cultivable microorganisms present in the fresh beverage and during its storage were identified. The microbial diversity analyzed in fresh aguamel allowed to establish that during summer, there is a greater diversity for both Agave species. Other species of microorganisms that were not present in cooked aguamiel were identified as *Leuconostoc gelidum, Pediococcus* sp., Uncultured *Trichococcus* sp., Uncultured *Leuconostoc,* Uncultured *Lactococcus,*

Saccharomyces cerevisiae and the yeast *Kazachstania zonata* (not previously reported in aguamiel).

The microbial diversity analyzed during the storage of cooked aguamiel allowed identification of other species of microorganisms not previously identified. Aguamiel from the summer season had the highest number of non-cultivable yeasts and strains such as *L. garlicum, Solibacillus silvestris, Uncultured Streptococcus* sp. and *Kluyveromyces lactis;* winter season included the following strains *Leuconostoc citreum, L. rapi, Candida stellata* and *Clavispora lusitanae;* the *A. salmiana* aguamiel included a greater number of different species of *Leuconostoc* sp. and *A. atrovirens* the biggest number of non-cultivable yeasts. While most of the microorganisms appeared in agauamiel at room temperature.

These changes in the microbiota can be used in the food industry to make bread, elaborate beverages and syrups from aguamiel, as well as to control the fermentation processes in alcoholic beverages such as pulque, selecting the microorganisms that could provide specific organoleptic characteristics to the beverage. In addition to the treatments proposed to ensure the safety of aguamiel, may be scaled to industrial level, to control the production process of aguamiel as a functional beverage, avoiding undesirable characteristics in it and taking advantage of the nutritional properties of this beverage.