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Jornada de Ingeniería en Alimentos VI

Tuxtepec, Oax
Oct 16-18, 2019
Extended Abstract

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Welcome

The organizers welcome all the participants. We appreciate the support given to the call for Meeting of Food Engineering 2019 by social networks: ResearchGate, Facebook, Twitter, Linked in, WhatsApp, Instagram, Tab UNAM, email and <http://www.bio.edu.mx>

The areas for submitting extender abstract were classified as follows

Num	*Area categories/Division*	*Example:*
I	*Education, Extension, Teaching & Learning*	*Education from elementary school to adulthood. Research opportunities, innovative teaching methods & learning techniques, effective methods for serving your clientele and examples of successful outreach. General teaching and learning strategies, improving students critical thinking, TICs, simulation, WHO, Codex*
II	*Food Chemistry*	*Chemistry and analysis of foods, bulking agents, carbohydrates (cereals, grains, seeds, legumes, pulses)*
III	*Food Engineering*	*Measurement, modeling, optimization and control of food processing systems. (Distillation, fermentation, nanotechnology. drying, transport processes, (bio) chemical reactors, extraction, dehydration, crystallization, food frying, nonlinear systems, cost of production and transportation, instrumentation of processes, techniques of optimization and decision applied to food processes and impact of automation in food engineering).*
IV	*Food Health & Nutrition*	*Diet & Health, Dietary Guidelines, Dietary Supplements, Food Myths & Fads To Address Misconceptions (GMOs, Sugar, etc) Functional Foods, Medical Foods, Microbiome, Omics, Personalized Nutrition, Prebiotics & Probiotics, Sugar & Sweeteners, Vitamins & Minerals*
V	*Food Microbiology*	*Detection and quantification methods, quality control, survival of microorganisms throughout the food contamination and processing environments, preventive controls of pathogens, characterization of emerging pathogens, and microbiology of health and wellness foods.*
VI	*Food Processing & Packaging*	*Improve quality, efficiency, sustainability, lead development new product, processes, packaging material or techniques. Chilling & Freezing, Dehydration, Emulsion Technologies, Extraction, Extrusion, Fermentation, Filtration & Separation, High Pressure Processing, Microencapsulation & Nanoencapsulation, Mixing & Blending, process control & Instrumentation, Processing Equipment, Thermal Processing, Food packaging*

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VII	*Food Safety & Defense*	*Risk Assessment, Management and Communication, Traceability, Quality Systems, Product Testing, Auditing, Crisis Management, Recalls, Laws and Regulations, and Standards, Allergens, Food Fraud, Food Safety Modernization Act, Hazard Assessment (Chemical, Physical & Physical Microbiological), Quality Assurance & Control, Shelf Life, Spoilage Organism.*
VIII	*Food Service*	*Supply preparation, presentation, and delivery of foods*
IX	*Marketing & Management*	*Development of food and beverage products*
X	*Nonthermal Processing*	*Pulsed power engineering, ultra-high pressure, ozone, and reemerging food irradiation*
XI	*Product Development*	*Primary aspects of the development and introduction of new food and beverage product innovation to the global marketplace. This category includes consumer research, product innovation procedures and related business information, as well as the technical and marketing aspects of product development. 3D, Antioxidants & preservatives, Aquatics or Aquaculture, Baby foods, Bakery, Beverages, Botanicals or Bioactive, Colors, Confectionary, Consumer Trends, Dairy Foods & Products, Enzymes, Fats & Oils, Fiber, Fish & Seafood, Flavors, Food Retailing, Food service, Formulation, Fruits & Vegetables, Global Markets & Trade, Meat & Poultry, Mergers & Acquisitions, New Products & Culinary Trends, People & Companies in the News, Pet Food, Proteins R&D, Refrigerated & Frozen Foods, Snacks, Sodium & Salt Replacers, Soups, Sauces & Dressings, Spices & Seasonings, Stabilizers & Emulsifiers, Starches, Supply & Price indexes*
XII	*Public Policy, Food Laws and Regulations*	*Practical, real world implication for food and feed industry of legislative, regulatory, and judicial developments in Mexico and global scale. Non-GMO, Organic, etc.*
XIII	*Quality Assurance*	*Quality assurance, quality control, and food wholesomeness*
XIV	*Refrigerated & Frozen Foods*	*Preservation of foods employing refrigeration or freezing technology*
XV	*Sensory Science*	*Advancements in the science of sensory and consumer research, for product development and marketing research*
XVI	*Sustainability*	*Biotechnology, Food Security, Food Waste, Life Cycle Analysis, Water, Management & Energy Management*
XVII	*Toxicology & Safety Evaluation*	*Science and technology of toxicology and safety evaluation relevant to foods or food components.*

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We invite you to be part of Meeting of Food Engineering 2020 Conference on Tuxtepec Oaxaca next October 16, 2020, at Universidad del Papaloapan to continue its legacy of providing a vital forum for the food research community. It will present the latest advances in food engineering as a multidisciplinary field.

Sincerely

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Oral and Poster Session

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Art by

Olivia Nolasco Hipolito

Conference Session

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Conference Abstract

I. Education, Extension, Teaching & Learning

Title of Speech: Development of Critical Thinking in students, professors and university workers for well-being and performance improvement

***Corresponding Author:** Rubén Hernández Ruiz
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***Area:** Education – Teaching and learning of Critical Thinking
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***Contact number:** 2281211520

*Abstract

Some processes and conclusions of three critical thinking training programs carried out in the last three years are presented: with students and university workers from the Universidad Veracruzana and with professors from the Universidad Juárez Autónoma de Tabasco, both institutions in Mexico.

Programs with students and professors correspond to academic actions for the development of university educational models and the program with employees and managers derives from the need to develop high-performance job skills.

The intention is to show tactics and strategies such as observation, self-observation, being observed, metacognition and mindfulness, both in life and learning experiences, which together make it possible to relink think, feel and express for the awareness of being, being and acting in various areas of management; elements that allow self-regulation to improve well-being and university and professional productivity.

A conclusion within the universities is that training programs in critical thinking should not only be implemented in the first semesters of the careers but should be encouraged throughout the studies. And one in the workplace is that problem solving and decision making must be taught to create heuristics of their own and not just follow instructions.

* Biography:

Ed. D. Rubén Hernández Ruiz is Naval Engineer, dedicated to the design, construction and repair of fishing and work boats. He was Technical Manager and Operations Director of Astilleros Unidos de Mazatlán, in Mexico.

He has been a professor of higher education since 1980. He is currently a multimodal professor in the Universidad Veracruzana where he teaches Critical Thinking for Problem Solving and Reading and Writing Academic Texts.

Among other projects, he is a teacher, employee and manager trainer at the Universidad Veracruzana and teacher trainer in the Universidad Juárez Autónoma de Tabasco.

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Conference Abstract

IX. Marketing & Management. Development of food and beverage products

Title of Speech: Entrepreneurs' communities

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*Abstract

Beginning as an entrepreneur, in my case it was by accident. But when we undertake not everything goes as you planned it. When failure comes, we must do it with awareness, that means to fail with conscience. Failure is not entirely bad; you can learn from it. When this happened I felt discouraged, sad and in a completely uncertain state, now what will I do. There is where this new journey begins, I performed an exercise supported by a couch, called The 7 whys, through this activity I realized that this is what I am passionate about and that is what I would dedicate myself to do from that moment onwards. Finding what we are passionate about, allows us to move on and keep going. If we put persistence, passion and attention to detail, we are moving forward. However, we must learn to make a community. An African proverb says -If you want to go fast, go alone, but if you want to go far, go accompanied-. We will touch 7 types of communities: Family and friends, Other entrepreneurs, Your team, Accelerators or Coworkers, Mentors, Investors and Customers. It takes a whole community to drive a change agent. We must stop being afraid and believe in ourselves. Knowing your market, knowing your numbers, knowing how you differentiate, solving a need and defining your price, all this will lead you to success as an entrepreneur. Oh! Don't be afraid of failure.

Biography:

Biochemical Engineer (1996-2001) and Master in Food Sciences (2007-2009) from the Tuxtepec Institute of Technology and Doctor in Food Sciences (2012-2016) from the Veracruz Institute of Technology and Postdoctoral Stay in the Tuxtepec Institute of Technology (2018-2019). 10 years of experience in the industry and 4 years in the teaching. Research stay in Spain (2008). Co-author of the books of Chemistry with Gafra Editorial in México. Publications in indexed journals and 3 patents applications.

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Conference Abstract

XII. Public Policy, Food Laws and Regulations

***Title of Speech:**
Mexico, maize people ... transgenic?. Food Security and Regulation.

***Corresponding Author:** Alma Xochil Avila Alejandre
Co- Authors: Alejandro Hernández López
***Affiliation:** Universidad del Papaloapan-Tuxtepec; México
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***Contact number:** 2871100664

*Abstract

The maize is Mexico's priority crop, white for human consumption and yellow for industry and fodder. The consumption of products made with maize represents 20% of the total cost of the Mexican diet. In 2017, 28.11 MMt of maize were produced, of both varieties, but, mainly from the US, 14,016 MMt were imported. In the case of white maize, we are almost self-sufficient. However, in yellow maize, there is a deficit. In the USA, 92% of the total corn production corresponds to some transgenic variety. Additionally, in 2017, some authors demonstrated the presence of transgenic maize in 82% of processed corn foods. Although this is not illegal, because according to Mexican legislation what is prohibited is cultivation, it is clear that, beyond the risk to health, what is at risk for the great diversity of Mexican native maize varieties, with the consequent risk to the megadiversity that is recognized to the country, as well as agri-food security. Given the dilemma, in regulatory terms the planting of transgenic seeds is prohibited, so new options must be sought to balance the commercial deficit in this crop.

Biography:

Alma Xochil Avila Alejandre. Doctor in Experimental Biology from the UAM Iztapalapa. Actually, is a Research Professor at the Biotechnology Institute of the Universidad del Papaloapan, Tuxtepec. SNI (Candidate). Shes lines of research include Agricultural Biotechnology and technology transfer with emphasis on the effect of plant growth regulators on the germination of species of agricultural and ecological interest.

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Title of Speech: "Obesity in Mexico"

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Abstract

The Epidemiological Bulletin of Mexico reports the states of Mexico with more obesity were Mexico City and Jalisco. The states of Mexico with more problems as anorexia, bulimia and other disorders were Jalisco, Tamaulipas, Veracruz, Nuevo Leon and Baja California. The states of Mexico with more problems of malnutrition were Gunajuato, Jalisco, Veracruz and Mexico Cd.

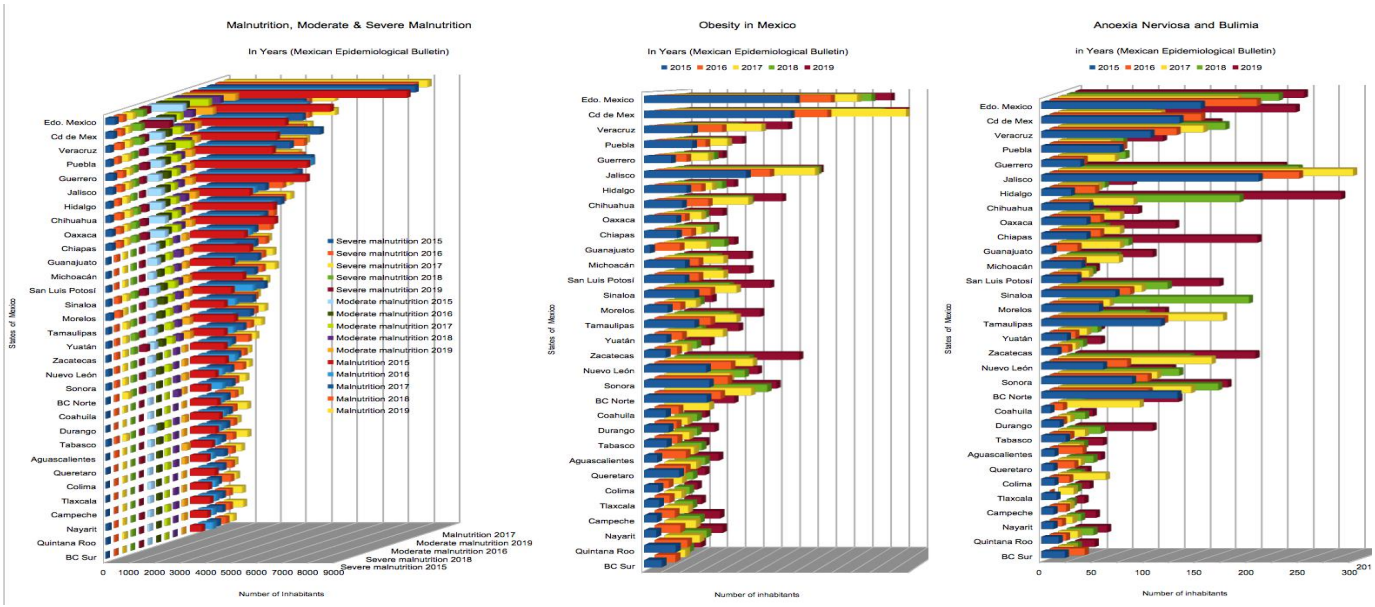

Table 1 Epidemiological Bulletin of Mexico 2016- 2019 week 40

Biography: Susana Lozano-Muñiz, is a Biochemical Engineer graduated at the Technological Institute of Culiacán (ITC), M. Sc. in Microbiology & PhD. on Biotechnology at the Autonomous University of Nuevo Leon at Faculty of Biological Sciences. She has professional experience on physical-chemical & microbiological analysis in quality control laboratories and development. Dr. Lozano created of the engineering career in biotechnology at the University of Papaloapan in October 2007. She is founder and president of the International Biotechnology Foundation. She is editor of the International Biotechnology Color Journal ISSN. She is founder and president of the Mexican Society of Biotechnology and Bioengineering Oaxaca Delegation. She is organizer of the Biotechnology Summit 2012-2020. She is organizer the meeting of food engineering 2017-2019.

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Conference Abstract

***Title of Speech:** The extrusion process in the development of functional foods
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***Abstract:**

Currently, snack foods are being redesigned to increase their nutritional value by adding micro or macronutrients, phytochemical components, vitamins, and antioxidants, among other ingredients, to make the snack foods attractive to consumers by the nutraceutical properties of these compounds. The bioactive compounds typically found in small amounts in foods are currently added as ingredients in the development of new products not only for their bioactive potency but also for their coloring properties, in this sense, some research has been carried out to enrich snacks with antioxidant compounds such as β-carotene. Extrusion cooking is a high-temperature short-time process in which food materials are plasticized and cooked by a combination of temperature, pressure and mechanical shear, resulting in molecular transformation and chemical reactions. This technology uses a continuous process with high productivity, significant retention of nutritional quality, and natural color and favor of food. Therefore, the aim of the study was to optimize the process to develop an extruded snack based on taro flour enriched with mango pulp prepared using a single screw extruder and to evaluate the effect of extrusion temperature, feed moisture content and the proportion of mango pulp in taro flour on some process parameters, physical, functional properties and β-carotene content of the extruded snacks.

Biography:

Biochemical Engineer (2001-2005) and M. Sc. in Food Sciences (2005-2008) from the Tuxtepec Institute of Technology and Doctorate in Science in Biochemical Engineering (2008-2012) from the Durango Institute of Technology. National Researcher Level 1 (SNI 1) (2016-2019). Senior Professor-Researcher C, Profile-Promep (2019-2022), 10 years of experience in the development of extruded products, as well as in the physicochemical and functional characterization of fruits and seeds.

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Title of Speech: Technological preparation of high dietary fiber powders as potential functional ingredients in meat products

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***Area:** III Food Engineering
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*Abstract:

Consumers currently require fast-food meals containing natural ingredients that potentially contribute to their health. In this regard, the preparation of high dietary fiber powders (HDFP) as functional ingredients from underused vegetable raw materials and agroindustrial fruit wastes through technological processing for its easy implementation into the Mexican productive food chain. The aim is to find out a route to contribute to the development of practical and attainable methods to process ingredients that have functional and technological potential. Also, its subsequent use as unconventional ingredients with functional potential in meat products with high consumption in Mexico is an alternative to promote a better use of wastes. This paper describes a practical technological process proposed for the preparation of HDFPs from different fruit and vegetable waste raw materials (Pomace of Carambola, Pineapple or Carrot); as well as the results of its chemical characterization, compositional, techno functional and some physical properties. Likewise, results of the incorporation of HDFPs as ingredients in sausage meat products specifically sausages, as high consumption food products in Mexico, are presented.

Biography:

She holds a Ph.D. degree, graduated at CINVESTAV, Mexico city. She performed an stance in ICTAN, Madrid, Spain. she is M. Sc. in food science graduated in National School of Biological Science from IPN, Mexico. She holds a B. Sc. on industrial chemistry from University of Veracruz. she worked in the Interdisciplinary Professional Unit from IPN, and from 2005 to current is full-time researcher-professor in the M. Sc. program in food science of the Institute Terminological of Tuxtepec, Oaxaca. she is professor with desirable profile from 2007 to 2024. Actually, is member of National Council of Researchers level 1. Her research interest is focused on the exploitation of underutilized vegetable raw materials, fruit and vegetable residues to obtain functional ingredients with high dietary fiber content to develop food products with functional potential, and preservation of fruits and vegetables by traditional and emerging methods, transformation as nutraceutical ingredients for the development of functional food products such as applied science and technological development.

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Title of Speech: "Vanilla, a crop affected by climate change"

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*Abstract:

"Vanilla" aroma is the most used in the food industry, particularly in the dairy and ice cream industry. However, only 1% of the "vanilla aroma" comes from a natural source, mainly from the fruit of the *Vanilla planifolia* orchid. The aroma of the cured fruits is complex and is due to a mixture of more than 200 different volatile compounds. Quantitatively the most important volatiles are: vanillin, *p*-hydroxybenzaldehyde, vanillinic acid and p-hydroxybenzoic acid; Vanillin being primarily responsible for the aroma of vanilla.

Mexico, country of vanilla domestication was the only vanilla producer in the world for 300 years. Currently, national vanilla production represents less than 1% of world production. In recent years, the increase in extreme temperatures in summer as a result of global climate change has negatively affected vanilla crops, particularly due to the premature fall of the fruit. Therefore, the establishment of strategies and actions to mitigate and adapt vanilla cultivation to climate change is urgently required to make national production of vanilla in Mexico more competitive.

Biography:

Araceli Pérez Silva is a Research Professor at the Tecnológico Nacional de México / Instituto Tecnológico de Tuxtepec. She has 20 years of experience in the research line "Evaluation of the potentiality and aromatic quality of vanilla". She is SNI-1 researcher level. She is currently responsible for the project "Strategies for adaptation and mitigation to climate change necessary for the rescue of vanilla cultivation in Mexico, financed by FORDECYT-CONACYT, project under development by the research group "VaniClim" and in active relationship with the productive sector.

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Art by Olivia Nolasco Hipolito

Oral and Poster session

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Mineral macro and micro content in three bean species *(Phaseolus sp.)*

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Area II

***Abstract:**

Phaseolus vulgaris L., *P. lunatus* L. and *P. coccineus* L. are endemic bean species with greater quantity and frequency of consumption in Mexico, although little is known about the contribution in mineral content. The objective was to determine the mineral content in *Phaseolus vulgaris, P. lunatus* and *P. coccineus,* collected in the Mixteca, Sierra Norte, Sierra Sur and Central Valleys regions of Oaxaca, Mexico. The grain of four populations per species was ground and the mineral content (mg / 100 g dry base) was determined by means of optical emission spectrometry with inductively coupled plasma with three repetitions per sample. The analysis of variance of mineral content showed significant differences ($p \leq 0.05$), between species and populations within each species. *P. lunatus* presented higher Fe and Mn content; *P. coccineus* in Mg, K, Zn and S; and *P. vulgaris* in Ca, Cu, P and Na. The PC-022 population (*P. coccineus*) had a high K content; PL-005 (*P. lunatus*) in Fe; and PV-002 (*P. vulgaris*) in P. The results indicate high variation and differentiation in mineral content and within the species evaluated, considering that their consumption plays a relevant social, economic and food function for the communities.

Keywords: *Phaseolus coccineus, P. lunatus, P. vulgaris,* optical emission spectrometry (ICP-OES).

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Retention of β-glucans in *Lentinula edodes* drying by refractance window

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Area III

Abstract

β-glucans are high molecular weight glucose (polysaccharide) polymers that are found naturally in the cell wall of edible mushrooms, which are highly perishable, so it is necessary to preserve them through some process or technology that guarantees and increases shelf life. Given the above, the objective of this work was to determine the conditions of drying by window refractance for shiitake mushrooms (Lentinula edodes) evaluating its effect on the content of β-glucans. Mushrooms were dried at 70, 80 and 85 °C, using the method of window refractance and β-glucans were determined by megazyme assay kit (K-YBGL), with the objective to compare the β-glucans content in dried and fresh mushrooms. The best conditions for window refractance drying of *Lentinula edodes* corresponds to 85 °C, which obtained an aw of 0.55 which is suitable for storage and β-glucans concentration of 16.33 (g/100g) having a minimum loss of β -glucans.

Keywords: *Lentinula edodes*; shiitake; window refractance; β-glucan

1. Introduction

Lentinula edodes, known commercially as shiitake, is a basidiomycete fungus, native to China, Japan and Korea. Shiitake mushrooms are mainly cultivated in dead trunks and wood of oak trees, characterized by presenting central stalks attached to circular-shaped caps that are light tan to dark brown and 5-25 cm across (Arriaga, 2016).

These favorite mushrooms from the Orient are not only delicious and nutritious food with great flavor and enticing aroma, but they also contain a material well-known for its medicinal benefits, lentinan (þ-1,3 glucan), a water-soluble polysaccharide has proven to be an anti-infective and anti-carcinogenic agent (Xu *et al*., 2015; Zhu *et al*., 2012). In addition, these mushrooms have essential amino acids, vitamins such as B_1, B_2, B_6, B_{12}, riboflavin, niacin, iron and minerals that are activators of the immune system and reduce cholesterol (Guzman *et al.*, 2013; Romero, 2015). Scientific background suggests that, depending on the physicochemical structure and origin, consumption would be associated with beneficial effects on human health such as a decrease in the

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plasma concentration of total cholesterol and a reduction in the glycemic index. Fresh shiitake mushrooms have a short life, so they must be consumed quickly. They are many methods of long-term preservation, being drying one of the process more commonly employed. In addition, some consumers prefer the unique characteristics of dried mushrooms since ancient times (Tian *et al*., 2016)

Refractance window drying is a drying method, where the thermal energy of hot water is transferred to the wet material deposited in a thin film on a plastic conveyor belt. The belt moves while in contact with hot water and this results in very fast drying. This drying method has become an attractive method for the industry due to the quality of the product it offers and its relatively low cost (Jerez *et al.*, 2015).

2. Materials and methods

2.1. Obtaining and classifying shiitake

The shiitake mushrooms (IE-40 strain) were produced in INECOL using oak cheap as substrate (Guzmán *et al*., 2008). An important parameter to determine the commercial quality of the mushroom is the size of the cap (pileus) developed in its mature state, therefore the fresh mushrooms were measured and classified according to the size of cap: G1= < 5 cm, G2= 5 to 15 cm and G3= > 15 cm.

2.2 Humidity content

The humidity content of the shiitake was determined by constant weight technique, under the standard NMX-F-083-1986. The weight loss was evaluated in time at 30 min intervals using an analytical balance (OHAUS brand Pioneer model PA214 25 +1°C).

2.3 Water activity (*aw*)

The water activity (*aw*) of the shiitake was determined in an Aqua Lab 3 series TE model, with a range of 0.03 to 1.0 *aw* 1 g of fresh (or dehydrated) mushroom was placed in a polyethylene tray, closing the equipment chamber and starting the measurement at laboratory temperature 25 + 1 ° C.

2.4 Ash determination

The determination was made under the official standard NMX-F-066-S-1978 using a Thermo Scientific brand flask heated at a temperature of 630 °C.

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2.5 Shiitake refractance window drying

Drying was performed for three sizes of mushrooms, as described previously. All groups were processed at 70, 80 and 85 °C. Mushrooms were dried by the window refractance (RW) method, it was performed in a laboratory prototype, designed to reproduce the infrared radiation (RW) condition, and consisted of a thermal bath (ThermoScientific NESLAB EX 7 Series) with a temperature range of 12 to 200 ° C and a polyester membrane (Mylar type, transparent to infrared radiation with a thickness of 0.5 mm and an area of 23 x 21 cm^2) was placed on the surface of the bath. All experiments were performed in triplicate.

2.6 β-glucans determination

The β-glucan content was determined using Megazyme assay kit (K-YBGL). The extracted samples were hydrolyzed with 37% HCL (V / V) for 45 min at 30 °C, further hydrolyzed for 2 h at 100 °C and neutralized with 2N KOH solution. A mixture of exo-β-(1 → 3) D-glucanase and β-glucosidase in sodium acetate buffer 0.1 M (pH 5) was added which was resting at 40 °C for 1 h. Then, centrifuged at 1500 *g* for 10 min, the glucose content released was determined with GOPOD in a spectrophotometer at 510 nm. In the determination the following correlation was applied:

Where: ΔE = Absorbance of reaction – Absorbance of the blank, BS = β-glucans in % (m/m) in dry sample, F = 100 µg of glucose / absorbance of 100 µg of glucose, W = weight of the dry sample in mg (Bak *et al*., 2014).

In shiitake drying, select a design factor 3^2 where the factors with three levels were temperature (70, 80 and 85 °C) and, mushroom sizes G1, G2 and G3 where the response variables were: β-glucans (g / 100 g of dry mass), humidity (%) and water activity (aw), using Minitab 18 statistic program.

3 Results and discussion

The determination of the optimal drying time by VR was done in triplicate, reporting average data in the table 1.

Table 1. ß-Glucans in fresh/dried shiitake studied

Drying temperature (°C)	Cap size group	A_w	Humidity (%)	ß-Glucan (g/100g of dry mass)
Fresh		0.98	75	17.82a[1]
70	G1	0.71	9.5	12.80c
70	G2	0.73	9.82	13.52bc
70	G3	0.76	10.15	13.96bc
80	G1	0.64	6.65	14.97abc
80	G2	0.68	6.5	15.20abc
80	G3	0.71	6.48	15.78abc
85	G1	0.55	5.27	16.33ab

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85	G2	0.57	5.33	16.57a
85	G3	0.49	5.22	16.9a

[1]Same letter in the column indicate that were no significant differences between the values according to Tukey multiple range test.

The ideal temperature for drying by shiitake refractance window corresponds to 85º C, since a_w of 0.55 was reached in the final product. The a_w of the product makes it suitable for storage as an intermediate moisture food. However, this condition will be preserved by to vacuum packing of the material.

Multivariate analysis (MANOVA) demonstrated that temperature is a significant condition for the retention of β-glucans in the drying by reactance window, with a *P* value of 0.009 and a regression coefficient of 96.78%. Tukey multiple range test applied with a 95% confidence interval showed that the mushrooms of the group 3 dehydrated at 85°C maintained the highest concentration of β-glucans, in comparison to the samples G1 dried at 70°C. In addition, these mushrooms registered a high aw for a dry food. The size of the fungus was not significant for this type of drying.

4 Conclusions

The concentrations of β-glucans varied depending of the size of the mushrooms and dehydration temperature used. The highest concentration difference (5.02%) was observed between the fresh product and G1 mushrooms dehydrated at 70 °C. In contrast, the highest β-glucan concentration was maintained in G3 mushrooms dehydrated to 85 °C. Preliminary results showed that the refractance window drying is an adequate method for obtain a dry product; however, it is necessary to experiment with other parameters (colour, texture, etc.) and to evaluate the storage of dry mushrooms for a_w less than intermediate, with the goal to obtain a dry product with better nutraceutic quality.

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"Retention of phenolic compounds in slices of watermelon dried by refractance window"

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Area III

ABSTRACT

The conservation of phenolic compounds in slices of watermelon pulp (Citrullus lanatus) dried by Refractance Window (RW) was evaluated, at the temperature 70, 80 and 90 °C and slice thickness of 1, 3 and 5 mm. The phenolic content of the fresh sand slices was quantified and after drying using the Folin-Ciocalteu method. The most appropriate treatment was at 70 °C and slice thickness of 3 and 5 mm for 240 min and content of phenolic compounds of 567.8 and 599.8 mg Eq AG / 100 g in dry, basis respectively. The phenolic compounds retention obtained represents a significant preservation of these compounds.

Keywords: *watermelon; refractance window; phenolic compounds.*

1 Introduction

Watermelon (Citrullus lanatus) is a curbitacea native of the tropical Africa. Mexico annually produces 984.3 thousand ton and from these Veracruz State produces about 308,630 ton. Watermelon have pulp (locule), bark (mesocarp) and shell (epicarp). The pulp contains 90% water and 10% in B vitamin, minerals (P, K, Mg, Ca and Fe), amino acids (citrulline and arginine), phenolic compounds and antioxidants (carotenoids and lycopene). Watermelon is highly perishable, with a shelf life of about 12 days at 20 °C according to Martins et al., (2018). Due to the high moisture content in watermelon, it is necessary to find alternatives for its conservation and processing without significant changes in the nutritional qualities. A promising proposal is the drying by Window Refractance (RW) that is a method of drying of 4th generation. RW uses a polyester film transparent to the infrared radiation (Gamboa et al., 2013).

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2 Material and methods

2.1 Watermelon slices obtention

The watermelons used come from the Isla, Ver. Town and were acquired in the Emiliano Zapata market of the City of Orizaba, Ver. The whole watermelon fruit was washed with drinking water and brushed to remove any foreign matter or adhered dust, then the whole fruit was cut its structural components (pulp, bark and shell) were separated. Pulp slices with a diameter of 4 cm and slice thickness of 1, 3 and 5 mm were obtained. Analysis of the content of solids in watermelon juice (8-10 oBrix), moisture and water activity in the slices were carried out to validate the level of ripeness of the fruit, which corresponds to a firm fruit and varies in texture every day after of the cut.

2.2 Moisture content

The moisture content of the watermelon pulp slices was determined by the constant weight technique, under the NMX-F-083-1986 standard. The weight loss was assessed to time intervals each 30 minutes, using an analytical balance (OHAUS brand Pioneer model PA214 series, with a sensitivity of 0.0001 g and with a maximum capacity of 210 g).

2.3 Water Activity

The water activity (aw) of the watermelon pulp slices was determined in the equipment

Aqua Lab 3 series TE, with a water activity range of 0.03 to 1.0. 1 gram of watermelon pulp (fresh or dehydrated) was placed in the tray and starting the measurement at laboratory temperature (25 + 1ºC).

2.4 Color determination

The determination of the color parameters was made by means of a colorimeter HunterLab MiniScan XE Plus The results were captured and processed with the Universal Software version 4.10. The colorimetric parameters L, and of the watermelon pulp slices were obtained with which the color difference ΔE was determined (Eq. 1); ΔE is a unique value which is determined by the differences L (Eq.2), (Eq.3) and (Eq.3):

Eq.1

Eq.2

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Eq.3

Eq.4

2.5 Extraction of phenolic compounds

The phenolic compounds were extracted by means of the maceration method, using 1 gram of fresh and dried watermelon and 10 milliliters of the solvent formed by anhydrous ethanol, water and hydrochloric acid in a 7: 3: 1 (v / v / v) ratio with an extraction time of 24 horas at 4 ° C. Subsequently, the extract was concentrated by means of a magnetic grill with stirring at a temperature of 55 ° C and a speed of 50 rpm, until the volume was reduced by 60 %, once the extract was obtained, 2mM of NaF was added to inactivate the polyphenol oxidase enzyme and prevent the degradation of phenolic compounds during the test.

2.6 Quantification of phenolic compounds

The determination of phenolic compounds was carried out by the Folin-Ciocalteu method, a calibration curve of gallic acid was developed for the quantification of phenolic compounds contained in watermelon pulp slices (before and after drying by WR). 250 µL of each standard solution of gallic acid or supernatant from the extraction of the phenolic compounds in the sample and placed in 25 mL volumetric flasks were taken. They added 15 mL of distilled water and 1.25 mL of Folin-Ciocalteau reagent; subsequently the contents of the flasks were homogenized and let stand 8 min. in the dark, after this time, 3.75 mL of the 7.5% sodium carbonate solution was added to each flask and brought to a volume of 25 mL with distilled water, the flasks were homogenized and kept in dark at At room temperature for 2h, finally, absorbance was measured on a Genesys 20 brand spectrophotometer at a wavelength of 765 nm.

2.7 Drying watermelon pulp slices by Refractance Window (RW)

Drying by RW was performed in a laboratory prototype, designed to reproduce the infrared radiation (IR) condition, consisted of a Thermo Electron Neslab EX 17 Digital One stainless steel thermal bath with a temperatures range from 12 to 200 °C, and a polyester membrane (Mylar type, transparent to infrared radiation (IR) with a thickness of 0.5 mm and an area of 23 x 21 cm^2) was placed on the surface of the bath.

The factorial design 3^2, two factors and three levels, temperature (70, 80 and 90 °C) and slice thickness (1, 3 and 5 mm); response variable was the phenolic compound (mg Eq gallic acid / 100 g dry mass). The humidity (%), water activity (), color (L, , and ΔE) are reported.

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3 Results and discussion

In the bromatological analysis of fresh watermelon pulp (*Citrullus lanatus*) a humidity of 90.13%, ashes of 0.411%, water activity of 0.9825 was obtained. The determination of total solids and pH was performed in pulp juice; obtaining results of 8.80 °Bx and a 5.42 pH, according to Perkins *et al*., 2007; Cenobio *et al*., 2004 a watermelon is considered ripe with a total solids content in pulp between 8.5-11.5%, likewise the content of phenolic compounds is quantified, obtaining as a result 639.8 mg Eq GA / 100 g in wet base, which represents a significant contribution of antioxidants to the daily diet; However, watermelon, having 0.982 water activity, confirms the condition of perishable food.

The treatments were carried out in triplicate, which shows the means of the results obtained from the slices of watermelon pulp, which are found in table 1, which shows that the slices dehydrated at 90 ° C, drying time of 150 minutes, and thicknesses of 1.3 and 5 mm, have a respective content of 123.8, 169.3 and 321.8 GA / 100 g of dry mass, these slices having the lowest retention of phenols after drying by RW, the slices dried at 80 ° C, drying time of 210 minutes and slice thicknesses of 1, 3 and 5 mm, they had a respective retention of 413.8, 461.8 and 457.8 GA / 100 g of dry mass; which is considerably higher compared to dried slices at 90 °C. However, dehydrated slices at 70 °C and thickness of 5 mm with a drying time of 240 minutes they have a greater retention of phenols with a content of 599.8 GA / 100 g of dry mass.

Whereas, the color analysis for samples dried at 70, 80, 90 °C, with 1 mm of slice thickness was found ΔE (from the natural slice - dried slice), are 9.52, 8.86, and 7.69 respectively , which results in a significant color change with a tendency to darken (from red to black), this phenomenon is due to being a thin slice allows for a better thermal transfer by infrared radiation caused by water vapor in contact with the Mylar film, however this directly favors the oxidation of the polyphenolic compounds causing the color change (darkening).

According to the multivariate analysis (ANOVA) carried out in the Minitab 2018 software, it showed that temperature is a significant factor for the retention of phenolic compounds in the drying by RW, with a P value of 0.002 and a regression coefficient of 96.02 %. Through a comparison of Tukey averages with a 95% confidence interval, he showed that a temperature of 90 °C is a significantly different drying condition, given that there is less retention of polyphenols in the slices.

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Table 1 Results of the analysis of humidity, color, water activity and total phenolic content in slices of watermelon pulp before and after the refractance window removal process.

Treatment	Time (min)	Humidity (%)		L			ΔE	Phenolic compounds (mg Eq GA/ 100 g of dry mass)
Fresh	0	90.13	0.982	13.72	14.22	6.17	--	639.8
P91	150	8.33	0.376	22.42	10.58	7.37	9.52	123.8
P93	150	7.54	0.376	21.73	15.84	8.36	8.48	169.3
P95	150	8.34	0.379	18.48	11.9	6.39	5.32	321.8
P81	210	8.7	0.376	16.86	6.11	4.46	8.86	413.8
P83	210	8.75	0.407	17.82	8.17	5.21	7.38	461.8
P85	210	8.6	0.393	17.29	10.9	5.91	4.89	457.8
P71	240	7.51	0.365	17.47	7.61	5.13	7.67	531.8
P73	240	7.79	0.421	16.11	10.49	5.23	4.54	567.8
P75	240	8.53	0.535	16.72	11.2	5.23	4.4	599.8

All the treatments used reduce the moisture content <8 +0.5% in slices of dehydrated pulp. The water activity achieved in watermelon slices in all treatments reflects a value below an intermediate moisture food.

4 Conclusions

The control of the process conditions such as: temperature and slice thickness were significant parameters in RW drying, which directly impact the retention of phenolic compounds (mg Eq GA / 100 gr dry mass), Water activity and ΔE (color change).

The RW processes is suitable for drying watermelon slices, with the best treatment at 70 °C, a slice thickness 5 mm and a drying time of 240 minutes when achieving a retention of phenolic compounds of 599.8 mg Eq GA/100 g of dry mass, which represents a significant preservation of these compounds.

In the future it is proposed to perform a calorimetric analysis of watermelon slices pre and after dying treatment, this to optimize the drying times and retention of the compounds of interest.

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Effect of a broccoli-based paste in an animal model of menopause

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Area IV

Abstract

Menopause is characterized by hypoestrogenism, vaginal dryness, hypertension, osteoporosis, obesity, hyperglycemia, and dyslipidemia. The dietary properties of broccoli (anti-inflammatory, antioxidant, effects like estradiol), may contributes to counteracting the menopause effects. In this study we evaluate a food functional base on broccoli. Methodology: In a gonadectomized rat was evaluated the effect of fed with Broccoli fettuccini. Values of glucose, col-HDL, total cholesterol, triglycerides, body weight and quantity of food consumed, phase of sterol cycle, were studied, over three months post ovariectomy. Results: With the functional food we observe, minor glycemia, HDL and cholesterol did not change; higher levels of triglycerides in the last month. The rats were in diestrum. The body weight was lower, and the composition was reduced. Discussion: The low glucose level, is an advantage for menopausal patients with hyperglycemia or prediabetes. The elevated triglyceride levels can be treated with more consumption of fiber. On the other hand, upon do not modify the sterol phase, may suggest that do not have a carcinogenic effect. Conclusions: Broccoli-based pasta had no negative effect on weight, total cholesterol, blood glucose, or the estrous cycle, but it did increase the concentration of lipids, making it a potential functional food during menopause.

Keywords: *Menopause, Brassica oleracea, broccoli functional food*

1. Introduction

Menopause is the permanent cessation of menses, after significant decrease of ovarian estradiol production. At the onset of menopause, there is a decrease in inhibin levels, hormone that regulates the synthesis of FSH, with normal or slightly low levels of estradiol. This give rise to a shortening of the estrogen-dependent follicular phase and, therefore, to shorter menstrual cycles. Serum levels of FSH begin to increase due to atresia of ovarian follicles, decreasing estrogen production (Torres Jiméneza, 2018).

The alterations that can occur in menopause are genitourinary (dry vaginal mucosa, alkalization of pH, poor lubrication and structural changes in the urethra; cardiovascular (hypertension and risk of ischemic disease); bone (osteopenia and osteoporosis); nervous system central (depression and mild cognitive impairment); in addition to hot flashes, overweight, obesity, insulin resistance and dyslipidemia, among others (Mauvais-Jarvis, et al., 2017). Broccoli (*Brassica Oleracea* L. var. *Cymosa*) is a food with a high content of macronutrients such as water, proteins, lipids, carbohydrates, micronutrients such as vitamins (C, A, E, K, B-12, B-6, thiamine, riboflavin, niacin, pantothenic acid, folates);

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plant pigments, as well as phytonutrients such as glycosylates and indoles, both antioxidants (Mengpei-Liu, 2018).

Phenolic compounds found in plant tissues have antioxidant, hepatoprotective, renal function protective, analgesic, and anti-inflammatory effects, onco-protective, therefore, its phytoestrogens, can contribute to counteract the signs and symptoms of menopause. Thus, the potential functional benefits of broccoli products represent an opportunity to be included in human diet. This work is the beginning of the study of the effects of a broccoli-based paste, in an animal model of menopause, to assess its impact on the profile of lipids, glucose, and body weight. Subsequently, oxidant levels, hormonal profile, inflammation markers and blood pressure regulators among others will be evaluated.

2. Methodology

Female rats of the Wistar strain, weighing approximately 300 g, were used and underwent ovariectomy. They were subsequently divided into two groups; the first was fed commercial food (LabDie. trat 5012); the second group was fed with broccoli-based pasta, each group had 6 rats, both groups were fed exclusively with the food assigned and followed for three months. Both groups were fed ad libitum and was recorded the daily consumption. In all animals, body weight, amount of food consumed, lipid profile, blood glucose, and the estrous cycle only were determined in the first and second month. The lipid profile was determined in peripheral blood obtained from the tip of the tail with the device Mission cholesterol meter, 3 in 1 lipid test; while to determine blood glucose, was determined with Accu-Chek® Active device.

Prior to ovariectomy, the rat was anesthetized with 60 mg/kg pentobarbital, intraperitoneally. The rat was placed in prone position; after performing asepsis and antisepsis of the lumbar region, a longitudinal middle incision was made, then the subcutaneous, muscular and fatty tissue was dissected, to allow the uterine tuba to bind and cut the ovary. This maneuver was done on the contralateral side. Finally, it was closed by plans and left in recovery for fifteen days. Vaginal lavage: the rat was placed in prone position to locate the vaginal intraoocyte, with a Pasteur pipette the vaginal wash was performed with approximately 0.5 ml of distilled water, to then make a smear on a slide; which was allowed to air dry, to subsequently perform histology staining.

Hematoxylin-eosin staining developed as follows, initially the samples were hydrated by the sequence pass in alcoholic solutions of concentration decreases. They were then incubated with hematoxylin and eosin, and then dehydrated by sequential passage in alcoholic solutions of increasing concentration and finally with xylene. Finally, they were mounted and observed a 40x magnification.

For the description of variables, the average, deviation standard, median and interquartile range were used, the ANOVA test of repeated range measurements and the Tukey post hoc test were applied. p less than 0.05 was as significative.

A fettuccine type pasta was elaborated, as follows: the broccoli flour was obtained using a convection oven at 75 °C for 2 h, then the grinding and sieving was carried out, to then mix it with wheat semolina (50:50). The formula was: 100 g of wheat flour/100 g of semolina /1 egg/300 mL of water. With the obtained mass the paste was formed in the form of

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fettuccini, finally it was dried 3 hours in a freezer. Then, commercial or functional food was given for different periods of time (1,2 and 3 months), to Wistar ovariectomized rats (menopause model) (Suliga, et al., 2016).

3. Results

The composition of the paste obtained was better than commercial food, in terms of concentration of lipids, proteins and carbohydrates, but not with respect to the amount of fiber (Table 1).

Table 1 Composition of the different preparations of the broccoli-based pasta

Parameter evaluated (%)	Broccoli/spaghetti	Labdiet rat 5012
Humidity	35.68 ± 0.35	12
Ashes	2.05 ± 0.2	8
Lipids	1.25 ± 0.03	4
Proteins	45.64 ± 0.001	22
Carbohydrates	11.7± 0.5	59.8
Fiber	2.45±0.25	5
Phenolic compounds		
Anthocyanins (mg)	24.17 ± 1.21	-----

The commercial food increased the concentration of glucose, at first month, the concentration was minor with functional food; similar condition was observed at third month (108 vs 140 mg/dL), p 0.012. The levels of total cholesterol and col-HDL were similar always of observation (100 mg/dL, p 1; 50 mg/dL p 0.52, respectively). The triglycerides concentration was always similar, except at third month, which was higher in the group treated with broccoli paste (71 vs 57 mg/dL, p 0.011) (Table 2).

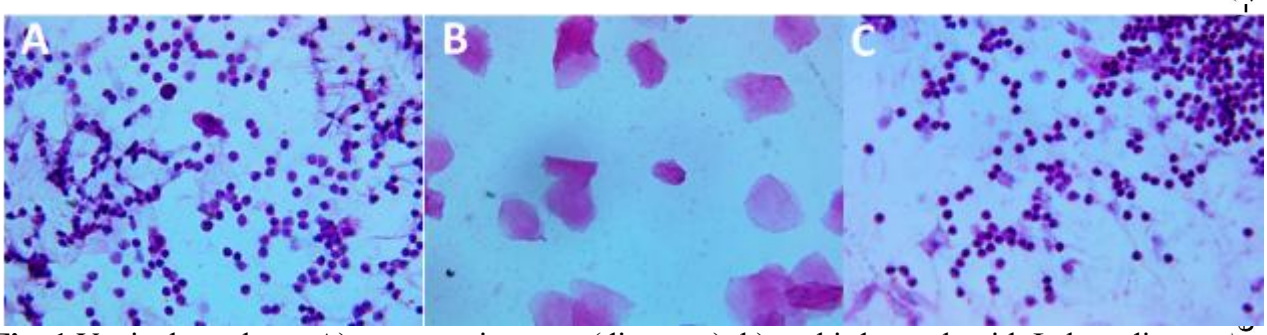

Fig. 1 Vaginal cytology. A) post-ovariectomy (diestrum), b) at third month with Labrat diet 5012 (estrum), C) at third month with broccoli spaghetti (diestrum). Hematoxylin-eosin staining; 40X magnification.

In relation to body weight, it was observed that both groups had a similar increase (94 ± 33 g), compared to those fed with broccoli paste (77 ± 33 g) (p 0.1). Finally, the group that

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significantly increased its intake was that of commercial food, compared to the one that consumed broccoli pasta, p 0.04 (Table 3).

Table 2 Glucose, and lipid profile

	CF	***FF***
	Glucose (md/dL)	
	Med (IR)	Mean ± SD
15 d post Ov	120.5 (118-129)	108 ± 10.6
1st month	116.5 (113-130)	105 ± 16.5
3rd month	140.5 (133-145)[a]	108.7 ±7.9
	p= 0.012[a]	p = 0.672
	Cholesterol total (mg/dL)	
	Med (IR)	Med (IR)
15 d post Ov	100 (100-100)	100 (100-100)
1st month	100 (100-100)	100 (100-100)
3rd month	102 (100-113)[b]	100 (100-100)
	p=0.003[b]	p = 1
	Triglycerides (mg/dL)	
	Mean (SD)	Mean (SD)
15 d post Ov	47 ± 3.1	57.7 ± 25.7
1st month	63.3 ±15.5	60.7 ± 24.9
3rd month	57.8 ± 13.1[c]	71 ± 17.1[d]
	P=0.018[c]	p = 0.011[d]
	HDL (mg/dL)	
	Mean (SD)	Mean (SD)
15 d post Ov	45.9 ± 7.8	40.1 ± 5.9
1st month	49.7 ± 8.9	47 ± 8.8
3rd month	55.8 ± 5.8	38.8 ± 5
	p=0.15	p=0.52

The cytological analysis indicated that after the ovariectomy they were in diestrum, and the evaluation at the first and third month with labdiet rat 5012, were in estrus, while those fed with the paste, remained in diestrum (**Fig**. 1).

Table 3 Food consumed

	Labdiet rat 5012	*Broccoli´s paste*	p
15 post surg	20.8 ± 1.5 gr	21.8 ± 1.9 gr	
1st month	21.7 ± 2.9 gr	19.3 ± 2.4 gr	
3rd month	19.5 ± 2.7 gr	15.3 ± 5.7 gr	0.04

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4. Discussion

Most of the signs and symptoms of menopause are due to estradiol depletion, among them is vaginal dryness, emotional fragility, vasomotor symptoms, insulin resistance, dyslipidemia; as well as overweight and obesity, among many others (Kim et al., 2013).

There are currently many supplements that try to contribute to improve some of the different alterations observed at this stage; however, it is difficult to find a food or preparation that meets properly (Suresh, et al., 2017). Knowing the above, a functional food based on broccoli was developed, since it is known that it has various components that can contribute to improving menopausal disorders (Jiménez-Zamarripa, 2018). The consumption of both types of food was similar in the first two months (approx 20 g), however, in the third month the consumption of broccoli pasta (15 g) was reduced. The nutritional contribution provided by the technical sheet of commercial food is based on a daily contribution in a rat that consumes between 15 at 30 g per day as follows: proteins: 27,020%, Fats (ether extract): 13,103%, Carbs: 59,877%. Although the nutritional contribution of the functional food has not been calculated, we can suggest that by having more proteins, in addition to moisture and low levels of lipids, carbohydrates and fiber, broccoli pasta is a good candidate to be a functional food, in addition, the presentation in the form of fettuccine type pasta, which can be accepted by the general population Ovariectomy is a model that simulates the pathophysiological changes of menopause, so it was used in this work. Cytology showed that the functional food does not modify the estrous cycle achieved with ovariectomy (diestrum), which is adequate, since it has no potent estrogen effects to modify it; therefore, it would not have the adverse effects of a hormone replacement therapy. It was also observed that blood glucose did not increase, a situation that is beneficial, since hyperglycemia has been observed in menopausal women (Zambrana, et al., 2014). The lipid profile was only altered in triglycerides, a situation that could be corrected by increasing fiber in the human diet (Armah, et al., 2015). or in the Broccoli spaghetti. On the other hand, the lower intake of broccoli pasta suggests that it may have a satiety effect. All of the above suggests broccoli pasta as a potential functional food.

5 Conclusions

Broccoli-based pasta had no negative effect on weight, total cholesterol, blood glucose, or the estrous cycle, but it did increase the concentration of lipids; making it a potential functional food during menopause.

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Ayahuasca healing and chemical properties: a mini-review

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Abstract
Ayahuasca, is a psychoactive psychotropic beverage made from the *Bannisteriosis caapi* vine and the *Psychotria viridis* (chacruna) leaf, for many purposes since time inmemorial. *B. caapi* samples had detectable amounts of harmine, harmaline and tetrahydroharmine (THH), while some samples of *P. viridis* had little or no detectable levels of N,N-dimethyltryptamine (DMT). The common feature in the beverage is the presence of harmala alkaloids from *B. caapi*. Although these harmala alkaloids are not particularly psychoactive on their own, they can facilitate the activity of N,N-dimethyltryptamine (DMT) from *P. viridis* by the inhibition of the enzyme monoamine oxidase (MAO) in the liver and central nervous system. The aim of this work is present a mini-review of the use of Ayahuasca on how it works, its chemical composition and its neurobiological effect on the consumers. The consumption of this beverage produces Psychoneuroimmunology effects which are related to the interactions among behavioral, neural and endocrine, and immune processes.

Keywords: *Psychoneuroimmunology; Stem cell proliferation; Epigenetic*

1. Background

1.1. Preparation of Ayahuasca

Ayahuasca word refers both to the liana *Banisteriopsis caapi*, and to the brew prepared from it. In the Quechua languages, aya means "spirit, soul", "corpse, dead body", and waska means "rope" and "woody vine", "liana". This traditional brew made out of *Banisteriopsis caapi* which contains harmine and tetrahydroharmine (THH), alkaloids of the class β-carboline, which act as inhibitors of monoamine oxidase (MAOI) and which allow the primary psychoactive component dimethyltriptamine (DMT) from *Psychotria viridis*, also known as chacruna, to enter activity (Morales-García, 2017).

At the beginning of the last century, syncretic religions combining Amerindian shamanism, African religiosity, European esotericism, and Christianity began to use ayahuasca. In the 1980s, these churches expanded from the Amazon into Brazilian urban centers (Labate

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2004) and, since the 1990s, globally (Labate & Feeney, 2012). One of first description of ayahuasca concerned only with the identification of the sources-species was performed by Schultes, in 1957.

All start with the method to prepare the ayahuasca for cultural uses and related to religious or divine purposes (MacRae, 1999) . In the Amazon, they do not talk about hallucinogens, but about tools to communicate with other species. Ayahuasca is above all, a way of transcending the barrier that separates humans other species and, in our visions, to communicate with plants and animals (Narby, Revalec, & Kounen, 2008).

Rivier & Lindgren (1972), reported the preparation of ayahuasca by natives as follow: fifteen stems of *Banisteriopsis Caapi* (approx. 60 cm long and I to 4 cm in diam.) are crushed with a short thick pole and cut into pieces 10 cm long. In a 15 L metal vessel reserved for this purpose, layers of vine are packed alternating with leaves of *Psychotria sp*., until the vessel is full. Then, 10 L of water are added, and the mixture is boiled for one hour. The vegetable sediments are eliminated by filtering through a strainer. As soon as it is cold, the decoction is ready for consumption.

1.2. Chemical Composition of Ayahuasca

Due to the facts stated above and the healing aim, ayahuasca has attracted the attention of many researchers to elucidate its chemical composition since long time ago. However, it is difficult to uniformize the chemical composition, because is depending on the origin of the plants, the person who prepared it and the methods used for the preparation, thus the composition has a wide range of concentrations. The concentration of psychoactive compound in tea can vary considerably among plants and, consequently, the ayahuasca prepared with them (Wang, et al., 2010, McIlhenny, et al., 2009, McKenna et al., 1984, Rivier & Lindgren ,1972).

The alkaloidal constituents of a number of ayahuasca brews, cultivars of *B. caapi* and a variety of admixture plants were qualitatively and quantitatively investigated using two-dimensional TLC and HPLC as the primary analytical tools.

Santos, Navickiene, & Gaujac, (2017) collected twenty samples from an ayahuasca preparation process from a religious group of the municipality of Fortaleza, Brazil, and analyzed by the developed SPE (Solid-phase extraction) method combined with HPLC–UV/DAD. It has been observed a considerable variation in the composition as compared with other results of analysis of ayahuasca. Their results showed that harmine, harmaline, tetrahydroharmine, harmalol, and DMT concentrations in the samples ranged from 0.3 to 36.7 g/L. Savoldi, et al., (2017), studied the behavioral changes over time following ayahuasca exposure in zebrafish. The ayahuasca used in this study contained (mean ± SD) 0.36 ± 0.01 mg/mL of DMT, 1.86 ± 0.11 mg/mL of harmine, 0.24 ± 0.03 mg/mL of harmaline, and 1.20 ± 0.05 mg/mL of tetrahydroharmine. Again, a clear difference with other reports are observed.

McKenna and coworkers reported the composition of different ayahuasca samples prepared from different persons. Similarly, the results showed differences among the the samples and containing between 3.5 mg/ml and 4.8 mg/ml total alkaloid for two samples analyzed

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(McKenna et al., 1984). In one experiment was investigated if Ayahuasca had any effect on binocular rivalry and explored if these eventual actions of Ayahuasca could shed some light into the concept of interhemispheric fusion. One of the inclusion criteria for experimental subjects was to ingest at least 50 mL of Ayahuasca having concentrations of alkaloids as follow: Harmine 1.36 mg/L, THH 1-05 mg/L, and DMT 0.73 mg/L.

After the decoction, Ayahuasca from sundry area only DMT, Harmine, Harmaline and Tetrahydroharmine were detected. Table 1 shows these fractions of total alkaloids, its percentage of each component in samples of Ayahuasca from different origins.

1.3. The Neurobiological Background of Ayahuasca

A growing number of studies indicate that the psychotherapeutic potential of ayahuasca is based mostly on the strong serotonergic effects, whereas the sigma-1 receptor (Sig-1R) agonist effect of its active ingredient dimethyltryptamine raises the possibility that the ethnomedical observations on the diversity of treated conditions can be scientifically verified (Frecska, Bokor & Winkelman, 2016). Preclinical, observational, and experimental studies suggest that ayahuasca and its alkaloids have anxiolytic, antidepressive, and antiaddictive effects (Santos, et al., 2018). From a pharmacological perspective its active ingredients are the reversible monoamine-oxidase inhibitor harmine, the serotonin reuptake inhibitor tetrahydroharmine which make the serotonin receptor (5-HT2) agonist component N,N-dimethyltryptamine (DMT) bioavailable for oral use, relatively potent and long-acting (Callaway et al., 1999,). Employing a combination of electroencephalogram (EEG) recordings and quantification of ayahuasca's compounds and their metabolites in the systemic circulation it has been reported that ayahuasca to induce a biphasic effect in the brain (Schenberg, et al., 2015)

The benefits of the consumption of Ayahuasca can be explained from the point of view of the people that use the beverage. The interpretation of these benefits is reported by the called gurus or persons that have the skills to prepare the brew (Polizzi, 2018). Therefore, there are reports on these facts and the gurus are who explained these effects, but without scientific arguments. Moreover, some skilled psychologists also have the knowledge to explain these effects as well. Nevertheless, serious research on ayahuasca effects are trying to elucidate the chemical or biochemical effect of the active compounds contained in ayahuasca (Frecska, Bokor, & Winkelman , 2016, Schenberg, et al., 2015, Frecska et al., 2013, Frecska, et al., 2003,). The next point below are related to the benefits of the ayahuasca and some are supported by incipient scientific evidences (Horgan, 2018, Lawn, et a;., 2017, Bradford, 2016, Frecska, Bokor, & Winkelman , 2016, Frood, 2015, Osório et al., 2015, Barbosa, et al., 2012, Santos, et al., 2007).

Physical and Psychological Cleaning Ayahuasca, is traditionally known - for millennia - as a purgative for body and soul. In that sense, there is an organic-inner and psychological-spiritual cleansing.

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Table 1. The results of the analysis of Ayahuasca (*Banisteriopsis sp*.) and Chacruna (*Psychotria sp*.) and of the drug.

Local name and Origin	Plants	Total Alkaloids (%(w/v)	Alkaloids	%	mg/100 mL
Tsipu makumi (white Hayahuasca), Culina Indians Zapote, Peru, 22,7,68	*Banisteriopsis Caapi* *Psycotria viridis*	0.064	Harmine Tetrahydroharmine DMT	26 11 21	17.0 13.2 13.0
Tsipu sueni "desati" (Crude black Ayahuasca) Culina Indians Zapote, Peru, 9, 10, 68	*Banisteriopsis Caapi* *Psycotria spp* *Lyngodium venustum*	0.013	Harmine Tetrahydroharmine Harmaline 232	62 18 4 6	Not drunk
Tsipu sueni "desati" Boiled black Ayahuasca Culina Indians Zapote, Peru, 13, 10, 68	*Banisteriopsis Caapi* *Psycotria spp* *Lyngodium venustum*	0.038	Harmine Tetrahydroharmine Harmaline DMT 232	47 6 4 31 6	18 2.3 1.5 12 2.3
Tsipu sueni "desati" (Crude black Ayahuasca) Culina Indians Zapote, Peru, 13, 10, 68	*Banisteriopsis sp.* *Psycotria sp.*	0.005	Harmine Tetrahydroharmine Harmaline 232	56 11 Trace 10	Not drunk
Tsipu sueni "desati" (Boiled black Ayahuasca) Culina Indians Zapote, Peru, 13, 10, 68	*Banisteriopsis sp.* *Psycotria sp.*	0.015	Harmine Tetrahydroharmine Harmaline DMT 232	43 10 Trace 36 6	6.6 1.5 5.4 0.9
Shuri fisopa (Tukondi) (Black Ayahuasca) Sharanahua Indians Marcos, Peru, 7.10.68	*Banisteriopsis Caapi* *Psycotria viridis* *Lyngodium venustum*	0.049	Harmine Tetrahydroharmine Harmaline DMT 232	37 20 2 20 20	18 9.8 1.1 9.8 9.8
Shuri fisopa Oshinipa (Red Ayahuasca) Sharanahua Indians Marcos, Peru, 7.10.68	*Banisteriopsis Caapi (4)* *Psycotria viridis (5)* *Lyngodium venustum*	0.052	Harmine Tetrahydroharmine Harmaline DMT 232	37 14 3 30 16	19 7.2 1.6 16 8.2
Shuri (Ayahuasca) Sharanahua Indians Marcos, Peru, 1.8.68. Received from J. Siskind (61)		0.034	Harmine Tetrahydroharmine Harmaline DMT 232	22 9 1 41 16	7.1 2.9 0.3 14 5.2
Shuri (Ayahuasca) Sharanahua Indians Piro Indians Rio Urubamba, Peru Received from G. Baer (5)	*Banisteriopsis sp.* *Horowa leaves = Chacruna*	0.038	Harmine Tetrahydroharmine Harmaline 232	21 40 4 6	

Adapted from Mckenna et al., 1984

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Self-assessment or self-analysis. "Ayahuasca serves to activate compensatory mechanisms of behavior, applied to self-analysis and the search for resolutions to present conflicts, both emotional and general adaptive"

Adaptogen function One of the purposes that induce human beings to consume ayahuasca, is related to some cognitive processes that allow an improvement in adaptive efficacy.

Ayahuasca is a dehallucinating "These "master plants"are badly called drugs or hallucinogens, when they are really dehallucinating, because they allow us to perceive reality as it is, and to get out of cultural and social hallucination. Visionary plants provide you with a direct experience of other realities, of ultimate reality, of reality with capital letters: the great mystery. Visionary plants are living beings with whom it is possible to communicate by integrating them into our metabolism. "

Spiritual realization. An exploration of modified states of consciousness is carried out, in order to work in a path of spiritual openness and self-discovery. It is a path to spiritual search.

Acute sensitivity, empathy and interior visualization. "These plants can improve memory, they can make colors brighter, they can make you hear more acutely, and they can make you experience extraordinary empathy, a form of direct communication with nature. They also allow us to see within ourselves, you they allow you to communicate with yourself, they allow you to express yourself "

Knowledge and recognition of our psychic evolution through regression. Regression therapy unlocks traumas, fears, phobias, emotional blockages implanted in our subconscious during our childhood, intrauterine life or even possible previous lives, which represent our current trends and limitations. Ayahuasca allows us to develop our own learning inherent in the evolutionary line itself and discover or rediscover what our personal objective is, what are the right decisions in everyday life.

Consciousness of our sub-conscience. The subconscious is the hidden of our being, it is the memory of all the experiences that our conscious does not remember, it is where the traumas, fears and memories that unconsciously affect us without knowing where they come from are established. Ayahuasca's ability to open the unconscious makes it a substance of great potential in self-psychotherapies.

Remedies for addictions and toxicomania. Ayahuasca is not addictive, which allows rehabilitating drug addicts or subjects of compulsive behavior. It is an excellent remedy to treat dependencies (Tófoli and Arauju, 2016).

2. Effects for drinking Ayahuasca:

Here there is a description of the feeling of people that drink ayahuasca. It is worthy to explain that this is an information collected from diverse sources such as newspapers and blogs coming in some cases from the shamans because this the only route to find out experiences revelated from the users. Moreover, the people that try to use the ayahuasca could have different purposes and then the results could be very different experience from person to person.

Perception, spatiotemporal orientation, beliefs about reality and the self, cognitive and emotional processes can all alter significantly during the experience. Visions of beautiful

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visual scenery are commonly reported together with some typical elements of the "ayahuasca world": ayahuasca beings, power animals, spirit guides, tropical motifs, vibrant, and varying geometric patterns known from the literature of the cultural anthropology of shamanism (Frecska, Bokor and Winkelman, et al., 2016). In other experiences for drinking ayahuasca changes in the behavior such as sensory-perceptive changes in the thinking process and increased mood/affection without loss of contact with reality (Pinto, 2010)
If the plants talk to the patient (grandmother or ego) about something like forgiveness, acceptance or resignation of something (about do some spiritual work) and the patient doesn't would like to do/work on it, so that the patient could experience vomit and/or diarrhea. The majority of users vomit (Levy, 2016, Jung, 2009)... For example: If the sensation of vomiting starts but the person accepts work on plant demands (grandmother/ego demands) that he/she should forgive and cries, the vomiting stops. Other example, if the patient needs to clean the body for some kind of problems (toxin, Cancer, myomatosis) the diarrhea starts for clean the body

A study, led by Jaime Hallak, a neuroscientist at the University of São Paulo, Brazil, gave one mild dose of ayahuasca to six volunteers who had been diagnosed with mild to severe depression that was unresponsive to at least one conventional antidepressant drug (Santos et al., 2017). None had drunk ayahuasca before. After drink Ayahuasca, the participants sat in a quiet, dimly lit room. Physicians used standard clinical questionnaires to track their depression symptoms. Improvements were seen in two or three hours (though the psychedelic effects of an oral dose take around five hours to wear off) — a rapid effect, as conventional antidepressants can take weeks to work. The benefits, which were statistically significant, continued to hold up in assessments over the next three weeks. Three of the participants vomited, a common side effect of ayahuasca, but otherwise the procedure was well tolerated

3. Discussion:

The effectiveness of the brew varies between different batches of trees in the sample. Psychoneuroimmunology can be defined as the study of interactions between behavior, neural and endocrine function, and immune processes (Ader et al, 1995). Ayahuasca can be used as in psychoneuroimmunology process. Frecska *et al* in 2016 find out (1) the therapeutic effects of ayahuasca are best understood from a bio-psycho-socio-spiritual model, and (2) on the biological level ayahuasca may act against chronic low grade inflammation and oxidative stress via the Sig-1R which can explain its widespread therapeutic indications. The indigenous and mestizo communities regularly use Ayahuasca to treat physical ailments, mental problems and frequently handle their social issues, spiritual crises with the help of the brew.

According to the theory of Paul Maclean triune brain the human brain, is composed of three brains (Maclean, 1990). The reptilian / reptilian brain is mainly composed of the basal ganglia, the brainstem, the cerebellum and the medulla, also called the primitive brain, it controls instinctive behaviors and focuses on the most basic survival needs including

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aggressiveness, domination, territoriality and rituals. The limbic system is the second brain also known as the mammalian brain, this is responsible for emotions, a system based on evasion (unpleasant sensations such as pain) and attraction (pleasant sensations such as pleasure). This is made up of the tonsil, the hippocampus, the thalamus, the hypothalamus, the pineal gland, among others. It is responsible for motivation, the emotion we feel when feeding, reproducing and parental behavior as well as advanced thinking, reason, speech, planning, abstraction. The Neo cortex or Isocortex is the most evolved area of the cerebral cortex, is in charge of higher functions, generates motor orders, spatial control, sensory perception, conscious thinking among others (MaLean, 1990)

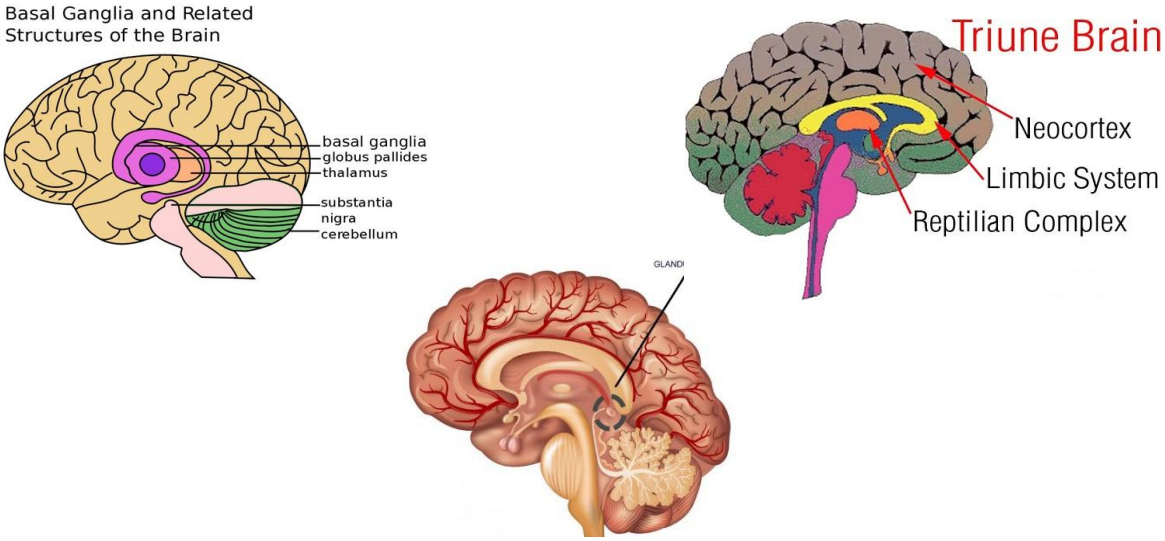

Fig. 2 Tripartite brain

It is important to highlight this part of the CNS (Central Nervous System) and its functions since when Ayahuasca is consumed in a ceremony, it generates neuronal movement in each one, making the segregation of neurotransmitters more active according to the situation that the patient presents (Alonso, et al., 2015, Estrella-Parra, et al., 2019)

The memories released by Ayahuasca are not eliminated from brain storage, they are cleared of dense, negative emotions, which involve pain, suffering, blockage, injury, etc. From this the individual can be more aware of their behaviors and improve decision making so as not to incur illnesses, emotional vices, depressions and more. The physical body is benefited by healing along with emotions. The pineal gland is a key piece in this process since in the CNS it is responsible for regulating the biorhythms of the organism, it is considered “the portal of the soul” this is a small gland that is found in the limbic brain, produces melatonin, has Pineapple shape, hence its name (Drăgoi, et al., 2016). Proper stimulation of this gland greatly improves consciousness, mood and vital energy (Neyra-Luzuriaga, 2018). René Descartes in his books "Treaties of man and soul" mentions that the pineal gland is the "main seat of the soul." He considered the human being as divided into two substances, the mechanical body (extensive substance) and the soul (thinking

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substance), and it is through the pineal gland that the soul communicates with the body. It is located at the height of the eyebrow halfway to the forehead and neck, that is to say exactly in the middle of the brain, many ancestral cultures and philosophies relate it to the third eye or sixth chakra (López-Muñoz, et al., 2011).
When activated it brings new experiences, beyond human nature related even to deep spiritual search. This is why the pineal gland is very important physically and spiritually. The activation of the pineal gland can be achieved with the continuous practice of meditation and in this case with the consumption of Ayahuasca, resulting in the development of perception and extrasensory faculties, potentializing creativity, feeling happiness, reducing stress, regulating free radicals, expansion of consciousness (Hamill, et a; 2019, Barbosa, et al., 2012, Halpern, et al 2008). Very often the human being has saturated systems due to poor diet, high consumption of flours and fats, lack of physical activation, current rhythm of life, stress, lack of contact with nature, which requires detoxification of the gland for a remarkable improvement of the functioning of the physical and mental body itself that is received with the intake of ayahuasca duly monitored by the facilitator, guide or shaman, as it is known in the different areas where it is provided.

The creation of a habit or pattern of behavior requires a continuous repetition of twenty-one days, that is, to keep the information in our brain-mind, this period makes the information or activity permanently present in our system, the issue is that it protects information that hurts as already mentioned above, the valuable thing is that it can also be done with information that allows the growth, healing or transformation of the person, so the importance of waiting twenty one days after the intake of ayahuasca to be able to return to ingest it if necessary. In such a way that resetting the systems is possible through ayahuasca since it favors the detoxification of cells and neurons allowing the release of toxins secreted by stagnant emotions and frequently activated, affecting the human being.

Psychologically the intake of ayahuasca will be recommended by the therapist according to the previous analysis of the situations by which the individual approaches therapy, it is important that they be three continuous intakes waiting for the aforementioned time for the assimilation of the process and the rearrangement of the information worked, this by the same sequence that ayahuasca provides in the brain and physical, mental, psychological and spiritual systems. Being the same individual who decides the frequency or not of the subsequent intake, and there is a follow-up for the handling of the doubts that the patient could have and / or due registration of the behavioral and emotional changes presented (Estrella-Parra, et al., 2019).

Conclusions: The psychoneuroimmunology performed by the intake of ayahuasca can help improve health in different types of diseases, depending on the patient's health needs, physical, mental, spiritual, by epigenetic means, activating the production of stem cells to solve the necessary problem in each case. However, the consumption of ayahuasca still is under study looking to elucidate the acute and chronic psychological and physiological effects of the ayahuasca, as well as to fully identified its active compounds. The religious

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and traditional usage is recognized and because this reason many people is using this brew and the results could be very personal and consequence of the ritual and place where it is consumed.

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Autochthonous lactic acid bacteria isolated, characterized, and identified from fermented habanero pepper puree (*Capsicum chinense* jacq)

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Abstract

Spontaneous fermentation of habanero pepper puree was carried out for three months at room temperature into containers designed for this process. Samples were taken every week for subsequent analysis. The microbial content in the food matrix inside the tank was analyzed, and strains of presumptive mesophilic lactic acid bacteria (LAB) were isolated and then purified. Selected bacteria isolates were characterized biochemical and physiologically. LAB was identified by 16S rDNA analysis and by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The microbial content had a typical behavior of a microorganism growth curve. Three species of lactic acid bacteria, one belonging to the genus *Bacillus* and two to *Enteroccocus* were identified in the present study. Further studies will be necessary to know the fermentative potential of these LAB.

Keywords: *Lactic acid bacteria; fermentation; habanero pepper puree.*

1. Introduction

In recent years, there has been an increased interest in the consumption of novel fermented food products with new flavors and aromas, in which a wide variety of raw materials, microorganisms and manufacturing techniques are used. Fermented chili peppers are a popular food globally, whose market gradually increases. Food fermentation can be caused spontaneously by the native microbiota of the raw materials or by starter cultures that contain functional microorganisms, which modify the substrates biochemically and organoleptically (Tamang *et al*., 2016). The use of starter cultures is an alternative to control and optimize fermentation processes, especially when they have been selected from the native microbiota of the raw material. Recently, successful fermentations of vegetables and fruits have been reported using native lactic acid bacteria, such as in eggplant, carrot, pineapple, tomato juice, kimchi and chili peppers of the *Capsicum annum* species (Di Cagno *et al*., 2016). However, there are no scientific reports of the isolation of lactic acid bacteria from fermented habanero pepper fruit (*Capsicum chinense* Jacq.). Otherwise, fermentation of habanero pepper inoculated with exogenous starter cultures of *Leuconostoc citreum* from jalapeño pepper fermentation has been reported (Peredo-Lovillo *et al*., 2017). Therefore, the main objective of the present study was to isolate, characterize and identify the lactic acid bacteria present in the spontaneous fermentation of habanero pepper puree (*Capsicum chinense* Jacq.).

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2. Materials and methods

2.1. Spontaneous fermentation

Mashed habanero pepper was prepared accordingly to the procedures established by Indamaya SA de CV company, using var. Jaguar as raw material. One-hundred and fifty kilograms were deposited into a 200 L plastic drum (tank fermentation), sealed and maintained at ambient temperature (35 - 37 °C), with a special design for keeping the anaerobic condition generated during a three-month fermentation. Samples obtained weekly from each drum were put in sterilized plastic bags and preserved at 4 °C until processed within 24 h.

2.2. Isolation of lactic acid bacteria (LAB)

Culture agar media deMan-Rogosa-Sharpe (MRS) with 0.05 % (w/v) L-cysteine-HCl adjusted to pH 6.5, and supplemented with 0.002 % (w/v) of Bromophenol blue dye. Blue colonies were selected as LAB members (Lee and Lee, 2008). Incubation was carried out at 35 °C for 24 h. Repeated streaking onto fresh MRS cysteine-HCl Petri plates produced pure bacterial strains.

2.3. Characterization of LAB isolates

Selected bacteria isolates were characterized by Gram tinction, assimilation of carbon source by API 50CH and enzyme activity on API ZYM system (BioMérieux) and susceptibility to antibiotics (Multidisk Bio Rad). These assays were implemented according to the manufacturer's instructions. *Lactobacillus plantarum* was used as control.

2.4. Molecular identification of selected isolates

The identity of the selected strain was determined based on partial length 16S rRNA gene sequence analysis. The genomic DNA was prepared using the Puregene Yeast/Bact Kit B (QIAGEN). The complete 16S rRNA fragment was prepared by PCR amplification using Platinum Taq DNA polymerase (Invitrogen) and oligonucleotides fD1 (50-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-30) and rD1 (50-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-30) (Weisburg *et al*. 1991). PCR conditions consisted of an initial denaturation step at 94 °C for 5 min followed by 35 amplification cycles of 94 °C/1 min, 55 °C/45 s, and 72 °C/1 min. The amplified fragment was verified directly by nucleotide sequence determination of both strands. A 1200 bp fragment of sequences were analyzed for homology using the BLASTN program and 16S rDNA gene sequences of type strains were retrieved from the nonredundant GeneBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version X (Kumar *et al*., 2018).

2.5. Identification of isolates by MALDI-TOF mass spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex LT mass spectrometer using Biotyper 3.1 software (Bruker Daltonics) was used to identify the selected strains isolated, according to De la Torre *et al*., 2018.

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3. Results and discussion

An increase in the microbial content was observed the first four weeks of fermentation, decrease for the fifth week and remains constant until the final of the fermentation (**Fig. 1A**). This behavior is consistent with the microorganism growth curve, and similar results are reported in several vegetables fermentation processes like peppers (Alberto *et al*., 2013), carrots, beets and cabbage (Garder *et al*., 2001).

During the spontaneous fermentation of habanero pepper puree fifty strains were isolated along the fermentation process. Three were selected for identification and characterization. These isolates were differentiated by morphology and physiological traits. The strains were named LAC-5, LAC-6 and LAC-7, all isolated in the first week of fermentation. Phylogenetic analysis of rDNA 16S sequence revealed that LAC-5 is clustered with *Bacillus* species group, while LAC-6 and LAC-7 are clustered to the *Enterococcus* group (**Fig. 1B).**

Table 1 shows the results of the identification and characterization of the strains isolated in the fermentation process. The molecular identification revealed that the strain identified as LAC-5 correspond to *Bacillus sp (B. pumilus; B. safensis)* with 98% of identity; LAC-6 to *Enteroccocus (E. mundtii)* with 100% of identity; and LAC-7 to *Enterococcus (E. faecalis)* with 100% of identity. Both genera identified are related to food fermentation processes.
There are scientific reports of the benefits of the *Enteroccocus* genus in the development of aromas and in the quality of food products such as cheeses, vegetables and sausages (Moreno *et al*., 2006; Franz *et al*., 1999; Giraffa, 2002); the genus *Bacillus* is widely used in fermented foods based on soybeans and rice in Asian and African countries, such as Natto (Japan), Chongkukjang (Korea), Knema (India, Nepal, Bhutan), Thua nao (Thailand), Pepok (Myanmar) and Sieng (Cambodia, Laos) (Shin & Jeong, 2015); the conservation and characteristic aroma of these fermentations are derived, in part, by the release of amino acids, ammonium and other volatile compounds. The MALDI-TOF method only could identify the strain LAC-5 as *Bacillus pumilus,* same result that the obtained by molecular identification. However, was unable to identify the strains LAC-6 y LAC-7, both belonging to the *Enteroccocus* genus. This result could be attributed to any limitation of the method, it is know that the limitation of this technology is that identification of new isolates is possible only if the spectral database contains peptide mass fingerprints of the type strains of specific genera/species/subspecies/strains (Singhal*et al*., 2015). The percent of identity greater than 97% obtained in the three strains, provides reliability in the molecular identification results (Reller *et al*., 2007; del Rosario and Mendoza, 2004). The two phylogenetic groups and the proximity of the sequences can be observed in **Fig. 1B.** The three strains show differences in the assimilation of carbon source (API 50CH), enzyme activity (API ZYM system) and antibiotic susceptibility, which would allow to conclude that they correspond to three different species (**Table 1**).

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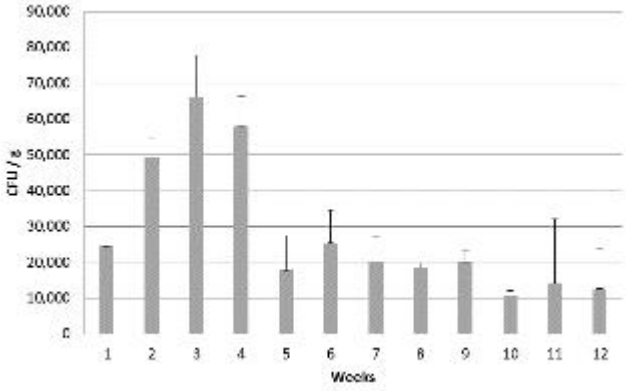
A



B

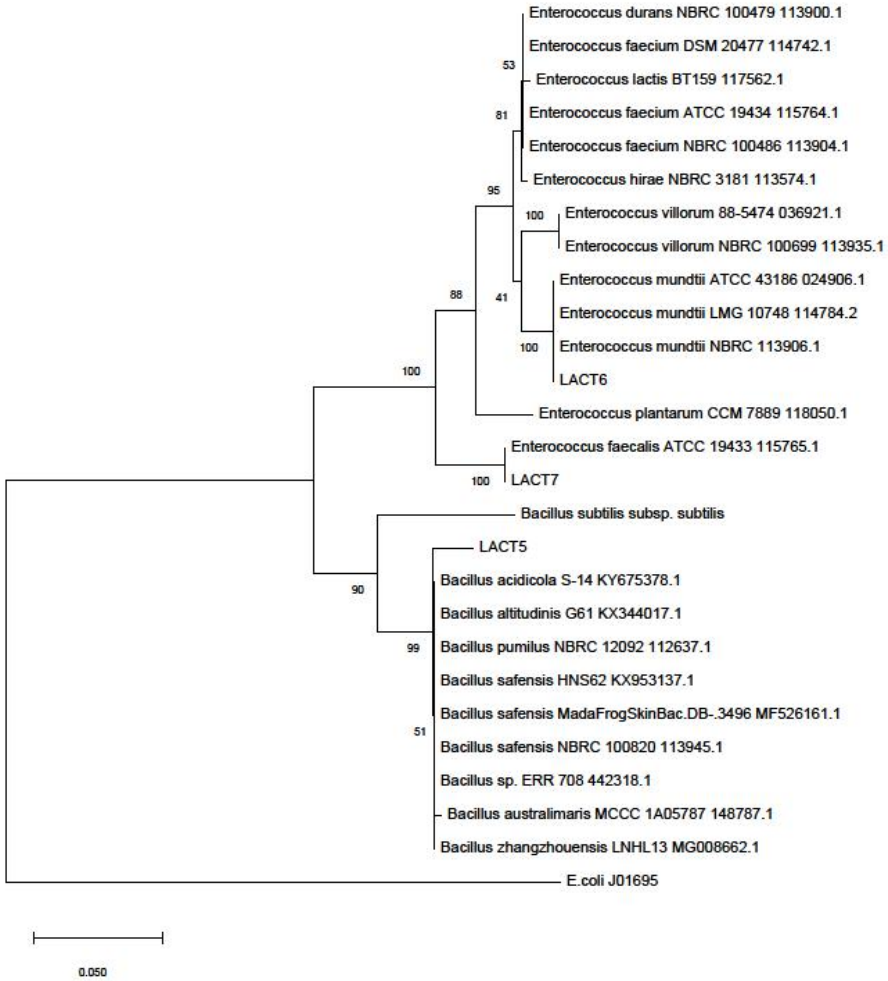


Fig. 1. A) Microbial behavior during the twelve weeks of the spontaneous fermentation of habanero pepper puree; **B)** Phylogenetic tree constructed with homologous sequences to LAC5, LAC6 and LAC7, isolated from the spontaneous fermentation of habanero chilli puree.

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Table 1. Identification and characterization of the strains isolated from spontaneous fermentation of habanero chilli puree.

	LAC 5	LAC 6	LAC 7	*Lactobacillus plantarum*[&]
Molecular identification	*Bacillus* sp *(B. pumilus; B. safensis)* 98% of identity	*Enteroccocus (E. mundtii)* 100% of identity	*Enterococcus (E. faecalis)* 100% of identity	-
MALDI-TOF	*Bacillus pumilus*	No peak found	No peak found	-
Gram	+	+	+	+
API CH50[¥]	GAL, INO, MAL, XLT, LYX	GAL, ADO, INO, ARB, ESC, MAL, SUC, XLT, LYX	GLY, RIB, GAL, GLU, FRU, MNE, MAN, N-AcG, ARB, ESC, SAL, CEL, MAL, MLZ, GEN, TAG, 2-Keto-GNT	L-ARA, RIB, GAL, GLU, FRU, MNE, RHA, MAN, SOR, 1-MDM, N-AcG, AMY, ARB, ESC, SAL, CEL, MAL, LAC, MEL, SUC, TRE, MLZ, RAF, GEN, TUR, D-AR, GNT
API ZYM*	ALP, E C4, E C8, LIP, C14, LAA, VAA, CAA, TR, A-CT, AP, PHO	ALP, E C4, E C8, LIP C14, LAA, A-CT, AP, PHO, AGA, BGA, BGL, NAG	ALP, E C4, E C8, LIP C14, LAA, A-CT, AP, PHO, BGA	E C4, E C8, VAA, AP, PHO
Antibiotic[+] susceptibility	Resistance (AM, CF, PE, FEP, CFM, CTX, SXT, DC, GE) Sensible (E,TE,LVX)	Resistance (AM, CF, PE, FEP, CFM, SXT, DC, GE) Indeterminate (E, CTX, LVX) Sensible (TE)	Resistance (AM, CF, PE, FEP, CFM, SXT, DC, GE). Indeterminate (E, CTX, LVX) Sensible (TE)	Resistance (PE, FEP, CFM, DC, GE) Sensible (AM, E, CF, CTX, SXT, TE, LVX)

[¥] Carbon source: GLY: Glycerol; ARA: L-arabinose; RIB: D-ribose; ADO: D-adonitol; GAL: D-galactose; GLU: D-glucose; FRU: D-fructose; MNE: D-mannose; RHA: L-rhamnose; INO: Inositol; MAN: D-mannitol; SOR: D-sorbitol; 1-MDM: 1-Methyl-D-mannoside; N-AcG: N-Acetyl-D-glucosamine; AMY: Amygdalin; ARB: Arbutin; ESC: Esculin; SAL: Salicin; CEL: D-cellobiose; MAL: D-maltose; LAC: D-lactose; MEL: D-melibiose; SUC: D-sucrose; TRE: D-trehalose; MLZ: D-melezitose; RAF: D-raffinose; XLT: Xylitol; GEN: Gentiobiose; TUR: D-turanose; LYX: D-lixosa; TAG: D-tagatose; D-AR: D-arabitol; GNT: Gluconate; 2-keto-GNT: 2-keto-gluconate.

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*Enzyme abbreviation: ALP, Alkaline phosphatase; E, Esterase; LIP, Lipase; LAA, Leucine arylamidase; VAA, Valaine arylamidase; CAA, Cystine arylamidase; TR, Trypsin; A-CT, a-chimotrypsin; AP, Acid phosphatase; PHO, Naphthol-AS-BI-phosphohydrolase, AGA, a-galactosidase; BGA, b-galactosidase; BGL, b-glucosidase; NAG, N-acetil-b-glucosaminidase. +Antibiotics: AM, Ampicillin 10 mg; CF, Cephalotin 30 mg; CTX, Cefotaxime 30 mg; CFM, Cefuroxime 30 mg; DC, Dicloxacillin 1 mg; E, Erythromycine 15 mg; FEP, Cefepime 30 mg; PE, Penicillin 10 U; TE, Tetracycline 30 mg; LVX, Levofloxacin 5 mg; GE, Gentamicin 10 mg; SXT, Trimethoprim-sulfamethoxazole 25 mg.
& *L. plantarum* was used as control for physiological evaluation

4. Conclusions

In the present study, three species of lactic acid bacteria, one belonging to the genus *Bacillus* and two to *Enterococcus* were isolated, identified and characterized. Various scientific studies have demonstrated the contribution of both genera in the sensory properties of various fermented foods. Further studies will be necessary to know the fermentative potential of these LAB. This information could contribute to the development of starter culture that can be used in the commercial production of habanero pepper puree, with a stable and consistent quality.

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Study of the effect of deep eutectic solvents (DES) in pineapple waste applying experimental design

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AREA VI

ABSTRACT

This study describes the process of pretreatment of lignocellulosic material with Deep Eutectic Solvents (DES) in order to obtain greater access to the available cellulose through the removal of lignin present in pineapple waste (crown, peel and core). Pretreatment is a crucial preliminary stage in the saccharification of cellulose to obtain fermentable sugars for the production of second-generation bioethanol. Four different DES are used based on choline chloride (ChCl), ChCl:Glycerol, ChCl:Formic Acid, ChCl:Lactic Acid and ChCl:Ethylene Glycol. A design of a block of Greco-Latin Square (GLS) is proposed, four factors are analyzed (Temperature, time, LSR and DES), the response variable is the percent of cellulose available. The GLS study allowed the selection of the two most influential DES, subsequently; it was proposed a Composite Central Design (CCD) to explore the best operating conditions which were the independent variables LSR and time, as well as the response variable the percent of cellulose available. From the CCD it is concluded that the DES ChCl:Formic Acid is the most influential with middle LSR (1:22 w/w) and low times (12 h). This study demonstrates that DES have promising characteristics in the pretreatment of lignocellulosic material.

Keywords: *Pineapple Waste, Deep Eutectic Solvents, Experimental Design*

1. Introduction

Different methods of pretreatment for organic waste used as biomass in obtaining second-generation bioethanol exist. Amongst the most employed are: acid hydrolysis, alkaline hydrolysis or a combination of both, which, manage to break the structure of the lignocellulosic material that composes the cell wall of plants. Main advantage of acid

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pretreatment is the high solubility of hemicellulose and lignin with a high glucose yield; however, acid recovery and corrosion resistant equipment are expensive. Further, fermentation inhibitors, such as hydroxylmethylfurfural and furfural are produced in high concentrations, which reduces the effectiveness of this method. On the other hand, NaOH treatment causes swelling of the lignocellulosic material by increasing the surface access, decreasing the degree of polymerization and breaking the lignin-carbohydrate bonds. However, it requires long residence times and the formation of unrecoverable salts exist which are also incorporated into biomass. Further, high peroxide costs make it a non-competitive method on a large scale (Badiei *et al*., 2014).

An alternative to this type of problem is the pretreatment with "green" solvents that have emerged based on the application of Deep Eutectic Solvents. They are liquid eutectic mixtures that are usually formed by simply heating two components (at least one as a solid), which act as hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). The main advantages of the DES are the high solubility of lignin (Francisco *et al*., 2014), its easy preparation through economic procedures, and there is no energy consumption of purification and waste disposal of the solvent. Also, the recovery and reuse of the solvent is possible (Kumar *et al*., 2015), low toxicity or non-toxic compounds can be used to prepare them, many DES and their components have superior biocompatibility and biodegradability; therefore, enzymes are stable and active in the presence of a DES (Tang *et al*., 2017). The discovery and recent application of DES provide a new vision of its potential application for lignin removal and cellulose exposure, thanks to its low ecological footprint and its attractive price, DES are now increasingly interesting both academically and industrially (Álvarez-Vasco *et al*., 2016).

The use of pineapple agro-industrial waste as a raw material for the production of bioethanol, is due to the fact that the state of Veracruz has the first place in the production of pineapple consumed nationwide (SAGARPA, 2017; SIAP, 2018). In 2017 Veracruz planted 34 thousand 926 hectares of pineapple obtaining 604 thousand 929 Tons of pineapple. It is estimated that the pineapple waste that derived from agroindustrial and food processes were was approximately 338,760.24 - 393,203.85 Tons for the state of Veracruz in 2017 (Sánchez *et al*., 2015). In this study, the most efficient type of DES through an experimental design of Greco-Latin Square (GLS) was selected; while taking into consideration the interrelation between the liquid solid ratio (LSR), temperature (T), time (t) and type of DES. Being the response variable the percent cellulose available, subsequently, a composite central design (CCD) was used to explore the best operating conditions.

2. Materials and methods

Choline chloride (ChCl) ≥ 98% pharmaceutical grade as hydrogen bond acceptor (HBA), lactic acid 85%, formic acid 88%, anhydrous glycerol 99.9% and ethylene glycol 99.9% were used as hydrogen bond donors (HBD), all of them of the brand J.T. Baker. Pineapple

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waste (crown, peel and core) was obtained from a pineapple purchased in the immediate vicinity of the Zapata market in the city of Orizaba, Veracruz.

2.1 Pineapple waste preparation

The crown, peel and core wastes of a pineapple were dehydrated in a Lab-line instrument Inc. Model 3471 drying oven at 80°C for 24 h. Subsequently, the dehydrated waste was grounded and dried again at 80°C for 4 h. The humidity was quantified in an ADAM AMB-50 thermobalance.

2.2 DES preparation

The DES were prepared in molar ratios, ChCl:Lactic acid (1:2), ChCl:Formic acid (1:4), ChCl:Glycerol (1:2) and ChCl:Ethylene glycol (1:3), according to the Table 1

Table 1. Relationships to form the DES in molecular weights (g/mol)

ChCl:Glycerol	ChCl:Formic acid	ChCl:Lactic acid	ChCl:Ethylene glycol
140 : 184	140 : 184	140 : 180	140 : 186

Each HBD was kept in an electric grill at a constant temperature of 90°C and magnetic stirring of 250 rpm while the ChCl was added; these conditions were maintained for 60 min or until a uniform transparent liquid was reached without subsequent purification steps. Subsequently, the DES obtained was allowed to cool in a desiccator.

2.3 Chemical pretreatment of lignocellulosic material with DES

The study of the effect of the four DES on pineapple waste was carried out by using an experimental design of Greco-Latin Square (GLS). The variables taken into consideration to analyze were type of DES (ChCl:Glycerol, ChCl:Formic acid, ChCl:Lactic acid and ChCl:Ethylene glycol), temperature (70, 80, 90 and 100°C), time (10, 14, 18 and 22 h) and LSR (1:15, 1:18, 1:21 and 1:24 w/w), at 250 rpm using electric grills. For the LSR the solid sample was 4 g and for each DES it was 60, 72, 84 and 96 g respectively. The response variable being the percent cellulose available. With this design the two DES with the greatest influence on the response variable with their respective operating conditions were identified.

The resulting solid residue was filtered, washed with water to reach a pH between 4 and 6, and dried 24 h at 80 °C using a LAB-LINE INSTRUMENTS 3471 electric oven and reach a humidity of less than 10%. The determination of lignocellulosic composition was carried out by using the Determination of Structural Carbohydrates and Lignin in Biomass technique, established by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.*, 2008).

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2.4 Study of pretreatment with DES

Based on the analysis on the information produced by the experimental design of GLS, can be defined a response surface design. For this study a composite central design (CCD) was proposed, and operated at a constant temperature. The design is summarized in the Table 2.

Table 2. Factors and levels of operation of the CCD

Level	Time (h)	LSR (w/w)	Fixed factors	
			DES	Temperature
-1	12	1:22	ChCl:Formic acid	
0	17	1:24		90°C
1	22	1:26	ChCl:Lactic acid	

3. Results and discussion

3.1. GLS factorial experimental design of pretreatment with DES

The results of the GLS are shown in the Table 3, being the DES; ChCl:Formic acid and ChCl:Lactic acid, those that presented the greatest influence on the response variable, percent of available cellulose (20.57 and 19.91% respectively).

Table 3. GLS results with different types of DES

DES	LSR	Temperature (°C)	Time (h)	% Cellulose
ChCl:Glycerol	1:15	70	10	12.127
ChCl:Formic acid	1:24	90	22	20.572
ChCl:Lactic acid	1:15	90	18	19.914
ChCl:Ethylene glycol	1:15	100	22	15.932

The statistical analysis was carried out with the Minitab® 18 software. Figure 1 shows the analysis of the average of the main effects, it is observed that the highest percent of cellulose available in the pineapple waste was obtained by using: LSR 1 (1:15 w/w), DES 2 (ChCl:Formic Acid), temperature 3 (90°C) and time 1 (10 h), standard deviation of each factor appears in the Table 4. With the objective of studying and procuring a greater quantity of available cellulose, a CCD was proposed and established as fixed factors: temperature (90°C), and two mixtures of DES (ChCl:Formic acid and ChCl: Lactic acid).

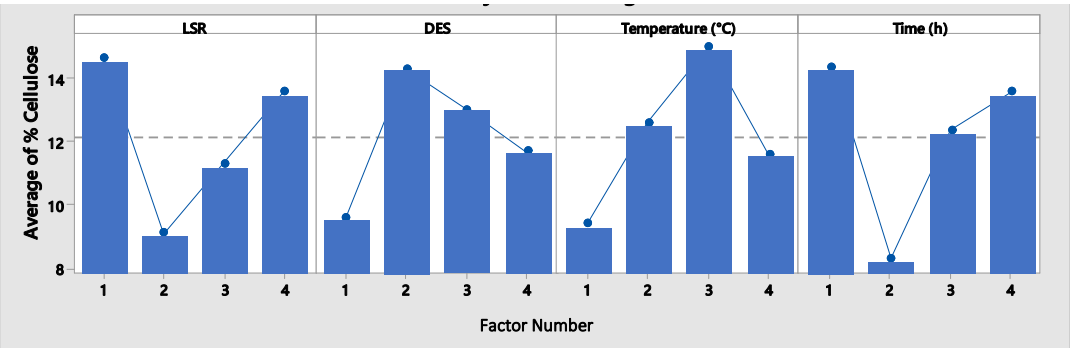

Fig. 1. Effects for response variable percent of cellulose

Table 4. Standard deviation for of each factor

Standard Deviation															
LSR				DES				Temperature (°C)				Time (h)			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
4.198	2.059	3.889	6.689	1.876	5.156	6.964	3.335	1.989	3.736	6.136	5.378	3.412	1.820	5.326	5.790

3.2. CCD study of pretreatment with DES

The best results in the exploration of the operating conditions set out in the CCD are shown in the Table 5 for the ChCl:Formic acid and ChCl:Lactic acid respectively.

Table 5. DCC results at different operating conditions

DES	Conditions (Temperature 90°C)		% Cellulose
	LSR	Time (h)	
ChCl:Formic acid	1:22	12	20.689
ChCl:Lactic acid	1:24	17	19.800

4. Conclusions

The design of the GLS allowed to determine that DES ChCl:Formic acid and ChCl:Lactic acid were the most influential in the response variable of percent cellulose after pretreatment. When comparing the behavior of the two DES, the mixture ChCl:Formic acid presented a greater efficiency with middle LSR conditions and low times. This study demonstrates that DES have promising characteristics in the pretreatment of lignocellulosic material and allows the definition of new exploration areas to maximize cellulose conversion.

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Comparative quality index study of a kéfir type drink prepared with starter culture or kéfir grains

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AREA VI

Abstract
Kefir is a fermented milk whose consume is increasing because its positive effects on health. This dairy product is prepared with a starter culture at industrial level. However, the demand by consumers for a Kefir produced by traditional way using kefir grains is increasing because of their sensorial properties, both contain numerous species of bacteria and yeasts. The aim of this research was to produce a Kefir drinks using kefir grains or starter culture and evaluate the properties of quality index for 21 days at 4 °C. In the preparation, commercial ultrapasteurized (UHT) whole milk was used, 2 batches of 1L were obtained. One sample was inoculated with 8 g of kefir grains and incubated at 25 °C/24 h, while another 0.075 g of starter culture "GENESIS®" brand was inoculated and incubated at 29 °C/24 h, until pH 4.6 was reached in both samples. Kefir prepared with grains reached a pH 4.30 ± 0.01, acidity of 0.85 ± 0.01% lactic acid, 62.66 ± 0.57% syneresis, 5.26 ± 0.05 °Brix, 1.0331 ± 0.05 g/mL density, 2.93% protein and 2.76 % fat at 21 days of storage. On the other side Kefir drink prepared with starter culture was obtained at pH 4.43 ± 0.01, acidity 0.75 ± 0.01% Lactic acid, syneresis 55.33 ± 0.57%, 6.63 ± 0.05 °Brix, density 1.0335 ± 0.05 g/mL, 3.03% protein and 2.93% fat in the same storage time. This study demonstrated that both kefir are within the parameters established by NOM-185-SSA1-2002 and Codex STAN 243-2003. Kefir made with kefir grains was the most feasible in terms of price.

Keywords: *Kefir; kefir grains; Starter culture.*

1. Introduction

Kefir is a fermented milk originated in the Caucasus. This fermented milk is acidic, slightly carbonated and has small amounts of alcohol, which distinguishes kefir from traditional fermented milks (yogurt) (García *et al*., 2006; Grønnevik *et al*., 2011). Kefir can be made industrially using starter culture or in a traditional way using kefir grains (Otles and Cagindi, 2003). However, the demand of kefir produced with grains is increasing because it has positive effects on health, such as antimicrobial effect, antihypertensive, hypocholesterolemic, anti-inflammatory, immune system and plasma glucose level. These types of beverages have the ability to modulate disturbances in immune function after

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exercise aiding in muscle synthesis and regeneration after physical activity (O'Brien *et al*., 2016). These health benefits are due to the great diversity of bioactive compounds that are formed during fermentation such as: amino acids (serine, lysine, alanine, threonine, tryptophan, valine, methionine, phenylalanine and isoleucine), vitamins (A, C, K), minerals (Mg, Ca, P, Fe, Co and Mn), lactic acid, CO_2 and ethanol (Rosa *et al*., 2017). The objective of this research was to produce Kefir from kefir grains or starter culture and evaluate the properties of quality indices for 21 days at 4 °C.

2. Materials and methods.

Kefir grains were provided by Engineer Jaime Castillo Ransom. Kefir starter culture (Genesis®) brand was imported from Bulgaria to prepare Kefir that was used to produce the kefir beverages. The kefir starter culture was composed of: *Lactococcus lactis sp.lactis, Lactococcus lactis sp.lactis biovar diacetylactis, Lactococcus lactis sp. cremoris, Leuconostoc mesenteroides sp. cremoris, Lactobacillus kefir, Saccharomyces unisporus* , commercial UHT whole milk that was used was from a known trademark. The microbiological composition of the kefir grains was not known.

2.1. Elaboration of Kefir drinks

The method described by Stewart *et al*. (2019) was followed with some modifications where 8 g of kefir grains were inoculated in 1 L of UHT brand milk, was fermented for 24 h at 25 °C in a Zeigen brand stove, Model: DFA-700, until a pH of 4.6 was obtained, once the pH was reached, the kefir grains were separated from the beverage with the help of a mesh n° 60; The drink was stored at 4 °C for later analysis. According to the conditions established by the "Genesis" brand, 0.075 g of starter culture was inoculated in 1 L of UHT whole milk at 30 ° C, the inoculated milk was fermented at 29 °C for 24 h in a Zeigen brand stove, Model: DFA-700 until obtaining a pH of 4.6, once the pH was reached, the beverage was stored at a temperature of 4 °C for later analysis.

2.2. Quality index analysis

The pH was measured according to Gul *et al*. (2015), using a Denver Brand calibrated potentiometer, Model UB-10, the electrode was immersed in 10 mL previously homogenized the Kefir sample. The acidity was determined according to NOM-155-SCF1-2012. Soluble solids were determined by the method described by the 932.12 (AOAC, 1996). The syneresis was determined by centrifugation at 4000 rpm for 20 min at 10 °C in a Hettich Brand centrifuge, Model: 380 R, 10 mL of sample in conical tubes were added according to the method described by Rojas *et al*. (2007). Density was determined by the use of a pycnometer as described by Santillán *et al*. (2015).

2.3. Proximal chemical analysis

The moisture content was determined by the gravimetric method according to the 16.032 (AOAC, 2000). The ash content was determined following the method 942.05.90 (AOAC, 1990), previously drying the samples at 60 °C for 8 h under vacuum and subsequently calcining at a temperature of 550 °C. Protein determination was carried out by the Kjeldalh

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method according to the method 12.1.07 (AOAC, 2005). The fat content was determined following the Gerber method. Carbohydrate content was determined by weight difference. Total solids (TS) and non-fat solids were determined according to NOM-155-SCF1-2012.

2.4. Direct Cost Analysis

For the analysis and decision making of the direct cost of production of the kefir produced with starter culture and with kefir grains, the raw material costs and an estimate of the labor force for the preparation of 1 L and 100 L.

3. Results and discussion

3.1. Comparative pH of kefir made from kefir grains or starter culture.

The initial pH of the samples after 24 h of fermentation was 4.60 ± 0.01, without differences between them (Figure 1). During the storage time from day 7 to 14 it can be observed that for both samples the pH was stabilized, the kefir starter culture 4.52 ± 0.01 and 4.37 ± 0.01 for the kefir made with grains. The pH obtained from the kefir made with grains was lower compared to the starter culture. This was possibly due to the multiplication of lactic acid bacteria in the presence of yeasts, therefore they produced lactic acid and acetic acid during the storage period (Kök *et al.*, 2013). According to NOM-185-SSA1-2002 the maximum pH for fermented milk drinks is 4.4, obtaining in this investigation a pH of 4.43 ± 0.01 in 21 days for kefir with starter culture, presented a decrease of 0.17 with respect to the initial pH , being within the limits established by the standard, however the kefir made with grains reached a pH of 4.30 ± 0.01 in 21 days having a decrease of 0.37 in relation to the initial pH and decrease of 0.10 in relation to the pH established by the standard, not however, it must be considered the diverse composition of the grain in terms of bacteria and yeasts.

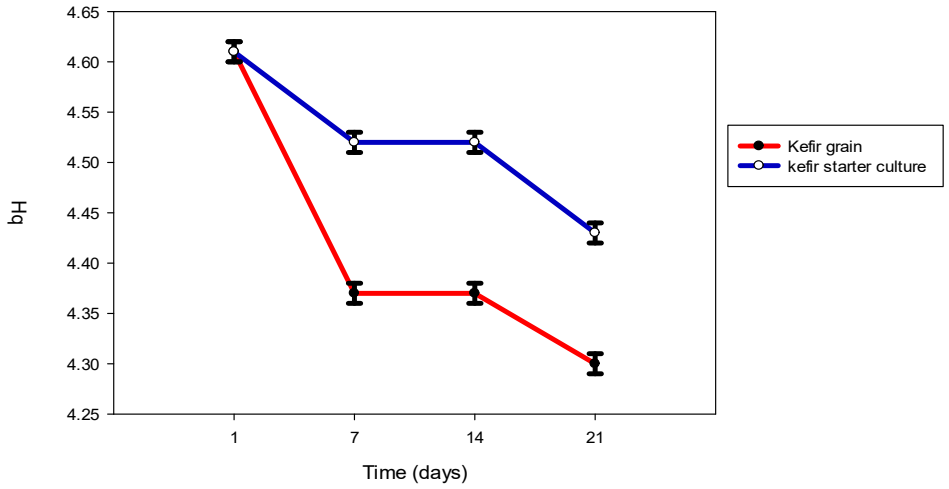

Fig. 1. Comparative pH of kefir made from grains or starter culture during 21 days of storage at 4 °C

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3.2. Comparative study of the acidity of kefir made from kefir grains or starter culture.

Considering that kefir is a fermented milk beverage, it must be kept within the quality parameter established by FAO/WHO in Codex STAN 243-2003, which stipulates that kefir must have a minimum titratable acidity of 0.6% lactic acid. The results showed that both the kefir produced with grains or starter culture started with 0.64 ± 0.01% lactic acid after 24 h of fermentation, finding that after 21 days of storage at 4 °C the analyzed samples were significantly different, 0.75 ± 0.01% kefir starter culture and 0.85 ± 0.01% kefir grains. (Figure 2). Kefir made with grains exhibited higher titratable acidity content as it generates greater degradation of lactose to lactic acid due to the microbial activity of some lactic acid bacteria such as *Lactobacillus Kefiri, Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillius parakefirsp., Lactobacillus delbrueckii subsp.bulgaricus, Bifidobacteria spp, Lactobacillus acidophilus* (Kök *et al*., 2013). Syneresis of kefir drink made from kefir grains or starter culture.

The percentage of syneresis in the kefir grain samples was 42.33 ± 0.57-62.66 ± 0.57% for 21 days, on the other hand, the kefir starter culture had 40 ± 0.05-55.33 ± 0.57% at the same storage time, presenting differences between the samples analyzed (Figure 3). The higher percentage of syneresis in the kefir grain samples may be due to the high acidity content, since this is a factor that influences this parameter, therefore, the syneresis increases as the acidity increases. Parra (2014) reported a similar behavior in a yogurt when obtaining 51% of syneresis, which concluded that the increase in acidity conducive to casein micelles is contracted, promoting the expulsion of serum.

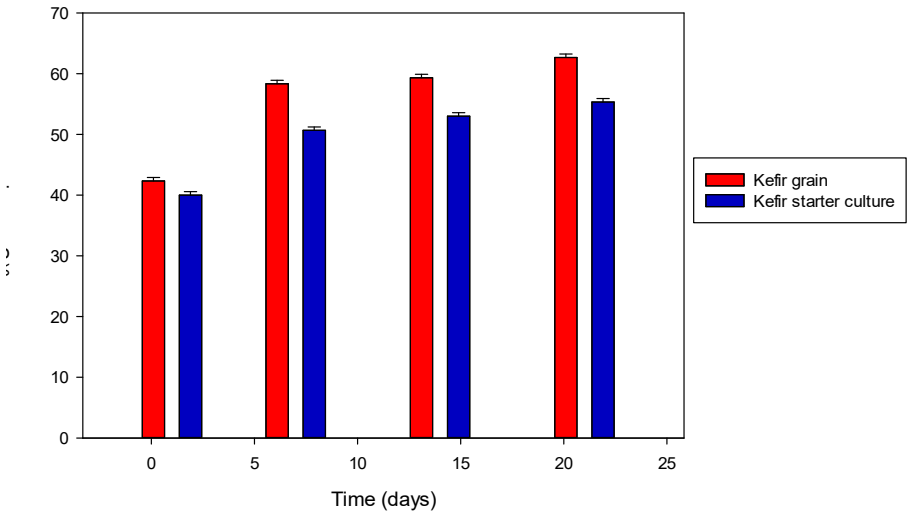

Fig. 2. Percentage of syneresis of kefir made from grains or starter culture during 21 days of storage at 4 °C

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3.3. Soluble solids content of kefir drinks prepared using kefir grains and starter culture.

The content of soluble solids in both samples decreased (Table 1) during the same storage time, having significant difference between the samples analyzed, this can be attributed to the content lactose as the main source of substrate which was metabolized and transformed into lactic acid and ethanol during fermentation, consequently favoring the reduction of soluble solids in the beverage.

Table. 1. Soluble solids content in kefir grains and starter culture

TREATMENTS	DAY 1	DAY 7	DAY 14	DAY 21
KEFIR GRAIN	8.23±0.20A	6.56±0.01A	6.63±0.05A	5.26±0.05A
STARTER CULTURE	9.50±0.20B	8.83±0.11B	8.96±0.05B	6.63±0.05B

Uppercase letters mean significant difference (P <0.05) between treatments during 21 days of storage at 4 ° C

3.4. Density of kefir drinks prepared using kefir grains and starter culture.

As reported by Harper and Hall (1981), the experimental density of dairy products ranges between 1,032 and 1,036 g/mL, the density in these analyzed samples is within the established parameter (Table 2), however the density of Kefir made with grains is smaller, having significant difference between the samples analyzed. According to Moradi and Kalanpour. (2019) established that kefir's own microorganisms produce an extracellular exopolysaccharide called water-soluble branched glucogalactan, which has a large number of OH groups. Based on this, it could be said that the density in the kefir obtained from the grains decreases due possibly to the fact that during sieving certain kefiran residues pass to the kefir, having an influence on the decrease in the density of said beverage, due to the presence of Kefiran's own OH groups.

Table. 2. Density of kefir drinks prepared using kefir grains and starter culture.

TREATMENTS	DAY 1	DAY 7	DAY 14	DAY 21
KEFIR GRAINS	1.026±0.01A	1.032±0.01A	1.032±0.01A	1.033±0.05A
STARTER CULTURE	1.030±0.01B	1.033±0.05B	1.033±0.01B	1.033±0.05B

Uppercase letters mean significant difference (P <0.05) between treatments during 21 days of storage at 4 ° C

3.5. Proximal chemical composition of kefir drinks prepared using kefir grains and starter culture.

Codex STAN 243-2003, establishes that the minimum protein content for a kefir must be 2.7% and less than 10% fat, therefore both samples analyzed in this investigation are within

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the established ranges, where the samples analyzed showed significant differences (Table 3). Rosa *et al* (2017), reported that the moisture content for a kefir is 90%, 6% carbohydrates and 0.7% ashes, which differ slightly from those obtained, since it is necessary to consider the composition of the grain, animal origin and type of milk.

Table. 3. Proximal chemical analysis of kefir drinks prepared using kefir grains and starter culture.

TREATMENTS	Moisture (B.H)	Ashes (B.H)	Fat (B.H)	Carbohydrates (B.H)	Protein (B.H)	%Total solids	% Non-fatty solids
KEFIR GRAIN	88.71±0.01A	0.66±0.02A	2.76±0.05A	4.94±0.02A	2.93±0.05A	11.28±0.01A	8.52±0.05A
STARTER CULTURE	88.45±0.04B	0.62±0.02B	2.93±0.05B	4.97±0.03B	3.03±0.05B	11.55±0.04B	8.61±0.05B

Uppercase letters mean significant difference (P <0.05) between treatments during 21 days of storage at 4 ° C

3.6. Analysis of direct costs for the production of Kefir with lyophilized or grains

In the Table 4 presents the analysis of direct costs for the production of kefir made with lyophilized culture or with grains.

Table. 4. Analysis of direct costs for the production of kefir drinks prepared using Kefir grains and starter culture.

Kefir	With starter culture Production quantity		With kefir grains Production quantity	
Raw material	1L	100 L	1L	100 L
Whole milk UHT commercial brand	$ 20.50	$2,050	$ 20.50	$2050
Starter culture (1 capsule)	$ 42.72	$4,276	$ 0.00	$0.00
Kefir grains (8g/L)	$ 0.00	$0.00	$ 24.00	$2,400
Workforce	$ 5.45	$545	$ 5.45	$545
Cost	$ 68.67	$ 6,871	$ 49.95	$ 4,995

4. Conclusions

The results obtained in this investigation showed differences between the quality index evaluated in beverages, because the kefir made with grains has lower values in terms of pH, density and ° Brix; higher values for acidity and syneresis after 21 days at 4 ° C compared to kefir made with starter culture. However, both results of the kefir quality index were within the parameter established by NOM-185-SSA1-2002 and Codex STAN 243-2003.

This research aims to continue studying the quality index of kefir made with grains or starter cultures using cow's milk.

According to the production feasibility of the direct cost about making a liter of kefir with starter culture is equivalent to $ 68.67 and the cost of kefir with grains is equivalent to $ 49.95. It is worth mentioning that the cost of grain kefir is initial since these grains have the

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ability to spread, reducing long-term costs. Therefore, from the economic point of view, the production of kefir from grains would be more feasible due to the difference in production.

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Development and Validated Method QuEChERS for Pesticides Residues in Beeswax by GC-MS/MS

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AREA XVII

Abstract

Beeswax has been appreciated as a pharmaceutical, cosmetic commodity and food additive. Thus, pesticides monitoring has become a public health issue in view of the growth of the levels agrochemicals in the hive products. This study was developed with modification QuEChERS EN 15662 and validated according to the European Union SANTE/2015/11945 guidelines a multi-residue method for the quantification 28 pesticides from different chemical group (acylalanine, benzilate, carbamate, dicarboximide, juvenile hormone mimic, neonicotinoid, organochlorine, organophosphorous, oxadiazine, oxime carbamate, pyrethroid, pyridinecarboxamide and triazole) in beeswax. Beeswax samples were extracted by modified QuEChERS method and analyzed by GC-MS/MS. Acceptable values were obtained for the following parameters: linearity, limit of detection (0.010-0.014 µg g^{-1}) and limit of quantification (0.014-0.029 µg g^{-1}), recoveries at twelve spike levels of 0.010 and 0.120 µg g^{-1} were within the 70-120% range with and associated precision RSD <20% and measurement uncertainty test (<40%).

Keywords: *Beeswax; QuEChERS; GC-MS/MS, pesticides.*

1. Introduction

Beeswax has been appreciated as a pharmaceutical, cosmetic commodity and food additive (Niell et al., 2013). However, various studies documented the exposure of agrochemicals and how bees store these products in the hive that leads to exposure to offspring, wax and honey (Mullin et al., 2010; Goulson et al., 2015; Amulen et al., 2017). Thus, pesticides monitoring has become a public health issue in view of the growth of the agrochemical levels in the hive products (Ravoet et al., 2015). The analytical methods must be available for routine analysis for determination of pesticides residues, that requires prior steps of sample preparation due to the low concentrations of the analytes and the complexity of the matrices (Tette et al., 2016). QuEChERS method was development by Anastassiades et al., (2003), that has the advantages simplification the steps, to detect analytes in very lower concentration for different and complex matrices, also reduces time, reagents, materials, and contaminated residues (Ewa et al., 2011). Several studies on multiresidue development by GC-MS/MS that determined pesticides in bee products have been reported in the literature (Niell et al., 2014; Orso et al., 2014; Malhat et al., 2015; Ravoet et al., 2015; Amulen et al., 2017). This study describes multiresidue method developed using a modified QuEChERS EN 15662 method (CEN, 2008) and validated according to the European Union SANTE/2015/11945 for beeswax by gas chromatography with mass mass (GC-MS/MS).

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2. Materials and methods

2.1. Chemicals and reagents

Toluene and acetonitrile absolv grade 99.9%, water Milli-Q quality and HPLC grade, acetic acid glacial HPLC grade 99.9% were used (Tedia). For extraction QuEChERS were employed prepackged bags (DisQuE ™ Pounch for 50 ml CEN), with 1 g sodium Citrate (NaCitrate), 1 g sodium chloride (NaCL), 4 g of magnesium sulphate ($MgSO_4$), and 0.5 g of Sodium hydrogencitrate sesquihydrate (NaSesquihydrate) (Waters). Finally, for cleanup was a used dispersive tube (15 ml), containing 150 mg of primary and secondary amine (PSA) sorbent, 900 mg of anhydrous $MgSO_4$, C_{18}, and 50 mg graphitized carbon black (GCB) (Thermo). All pesticide reference standards were of high purity grade (≥97.1% Chem service, ≥95.5% AccuStandard). All pesticide standards were stored at -18°C. Individual stock solutions were prepared at 2000 mg L^{-1} in toluene or methanol according to the solubility of the standard and stored in a freezer at 18° C. The working solutions were prepared through appropriate dilutions of the stock solutions in acidified acetonitrile 1% with acetic acid glacial.

2.2. Sample extraction and cleanup

The samples provided by Mexican beekeepers from Milpa Alta, Mexico City in 2017, placed in sterile bags and stored at -18°C until analysis. First, beeswax samples were analyzed and those that were free of pesticides were used as blank. The employed procedure was a modified QuEChERS method EN 15662 (CEN, 2008). Beeswax was crushed to decrease the particle size; it was carried out by exerting pressure on the sample with the help of a mortar. Beeswax sample blank was homogenized and 5 ± 0.001 g was weighed into 50 ml centrifuge tube, 5 ml of water Milli-Q quality or HPLC grade, 500 µl fortified with internal standard Diethyl-d10-amine hydrochloride, 10 ml of acetonitrile were added. Sample was shaken vigorously in QuEChERS shaker for 2 min at 500 oscillations min^{-1}. The samples were heated in a water bath at 80 °C (to melt the beeswax) for 10 min, allowed to cool at room temperature for 15 min. Prepackged QuEChERS extraction, 1 g sodium chloride (NaCl), 4 g of magnesium sulphate ($MgSO_4$), and 0.5 g of Sodium hydrogencitrate sesquihydrate (NaSesquihydrate) was added and the sample was shaken for 2 min at 500 oscillations min^{-1}, ultrasonic bath for 5 min and centrifuged for 2 min at 3500 rpm, 5 ml of the supernatant transferred to a one dispersive cleanup tubes containing 150 mg of primary and secondary amine (PSA) sorbent, 900 mg of anhydrous $MgSO_4$, C_{18}, and 50 mg graphitized carbon black (GCB), to remove any organic acids, polar pigments and other compounds. The tube was shaken vigorously in a vortex for 1 min and centrifuged for 2 min at 3500 rpm; an aliquot of supernatant (2 ml) of extract of the sample was filtering through a membrane 0.20 µm Acrodisc PTFE (Waters). The extracted samples were stored in glass screw caps bottles by GC-MS/MS analyses.

2.3. Gas chromatography with mass mass spectrometric detection (GC MS/MS)

For gas chromatography analysis, we used Agilent 7890 B GC, with Multi-Mode Inlet (MMI) injector and under the following temperature conditions: 60 °C for 0.2 min, then increased at 600 °C min^{-1} to 330 °C at 0 min, ultra-inert inlet liner 2 mm dimpled, splitless, two capillary columns were used; the first was Agilent HP-SMS UI(Agilent HP-SMS UI 15 m x 0.25 mm, 0.25 µm) at a flow 1.07 ml min^{-1}, the second column Agilent HP-SMS UI (15m x 0.25mm, 0.25µm set from Aux EPC 4 He to SMD(Selective Mass Detector)) at a flow 1.4 ml min^{-1}, with flow modulation and backwash in the middle of the analytical column, Helium 99.999% was used as gas carrier injection. Column oven temperature program was: 60 °C (1 min), heating at 40 °C min^{-1} to 170 °C

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(0 min), heating at 10 °C min^{-1} to 310 °C (3 min), remaining at this temperature until 25 min and injection volume 2 µl. The mass spectrometer Agilent 7010C Triple Quadrupole GC-MS/MS Detector was operated set to collect data in the multiple reactions monitoring (MRM) using electronic impact (EI) to 70eV.

2.4. Method validation

Following attributes of the extraction method were validated: limit of detection (LOD), limit of quantification (LOQ), accuracy (percentage recovery), precision, linearity and uncertainty. The validation process involved three replicates of spiked samples prepared with known concentration of pesticides (0.005, 0.007, 0.010, 0.015, 0.020, 0.030, 0.040, 0.050 and 0.060 µg mL^{-1}). The parameters that were measured were according to SANTE/2015/11945 (SANTE, 2016).

3. Results and discussion

This study presents a QuEChERS method optimized for beeswax, a complex matrix, for the quantification pesticides from different chemical group (acylalanine, benzilate, carbamate, dicarboximide, juvenile hormon mimic, neonicotinoid, organochlorine, organophosphorous, oxadiazine, oxime carbamate, pyrethroid, pyridinecarboxamide and triazole) analyses by GC-MS/MS (28 pesticides). QuEChERS method in beeswax samples was validated under the requirements of the SANTE guideline (SANTE, 2016). MRM transitions for quantification and confirmation, and optimized parameters for GC-MS/MS operation mode are present in Table 1. Linear range was from 0.005 to 0.060 µg ml^{-1}, LOD and LOQ were evaluated from 0.005, 0.007, 0.010, 0.015 and 0.020 µg ml^{-1} (corresponding at 0.010, 0.014, 0.020, 0.030 and 0.040 µg g^{-1}), except for isomer compositions of cyfluthrin and propiconazole that were concentration of 0.0011-0.0124 mg l^{-1} and 0.0015-0.0177 mg ml^{-1} respectively. LOQs were compared with MRLs (Maximum residue limits) established in the European Union (European Union, 2015). The value LOQs obtained for boscalid, chlorpyrifos, chlorpyrifos methyl, cyfluthrin I, cyfluthrin II, cyfluthrin III, lambda-cyhalothrin, diethofencarb, esfenvalerato, fenvalerate, metalaxyl, propiconazole I and II, pyridaben, tebuconazole and vinclozoline were between 0.014-0.022 µg g^{-1}. These values were lower that MRLs, which are established of 0.050 µg g^{-1}, except pyridaben that is 0.020 µg g^{-1} (CODEX, 2016). For mevinphos, parathion, permethrin MRLs are not specified, so that Comission European established in general default 0.01 µg g^{-1}, for these cases (European Union, 2015). LOQs for alpha-BHC, bromopropylate, chlorfenvinphos, endosulfan sulfate, ethion, fenitrothion, fenthion, heptachlor epoxide (Isomer B), mevinphos, parathion, parathion methyl, permethrin I and II and phorate, were above the value MRLs that is 0.01 µg g^{-1}. For a squared correlation coefficients determination (R^2) were higher than 0.98. The recoveries percentage ranges between 91.4-105.2%, were considered acceptable. The precision was reliable as most % RSD was lower than 20% and uncertainty was lower is than 40% (3-30%) at the levels analyzed. Pesticides that had a LOQs greater than the MRL were rejected for validation, although they met the other parameters indicated in SANTE guideline (SANTE, 2016).

The importance of validating the pesticides listed is because they have been reported in different studies. In honey, Orso et al., (2014) found chlorpyrifos (0.03 mg kg^{-1}), endosulfan sulfate (0.9 mg kg^{-1}); Eissa et al., (2014) detected γ-BCH (0.002-0.039 mg kg^{-1}), chlorpyrifos ethyl (0.09-0.011 µg g^{-1}), fenitrothion (0.016-0.021 mg kg^{-1}), bromopropylate (0.028-0.13 mg kg^{-1}). Malhat et al., (2015) reported heptachlor (0.0054 mg kg^{-1}), heptachlor epoxide (0.0082 mg kg^{-1}), cyhalothrin (0.0035 mg

kg^{-1}), permethrin (0.0019 mg kg^{-1}) and fenvalerate (0.0099 mg kg^{-1}). Al Naggar et al., (2015) analyzed honey, pollen and bees samples and detected chlorpyrifos (0.0033, 0.0264, 0.031 mg kg^{-1}), chlorpyrifos methyl (0, 0.0175, 0 mg kg^{-1}), fenthion (0, 0.0057, 0 mg kg^{-1}). Concentrations below 10 µg g^{-1} are indicated in the reports, so it is necessary to optimize the QuEChERS extraction method that was developed in this study to reduce LOQs.

4. Conclusions

For the QuEChERS method developed a validated to detect pesticides residues in beeswax samples by GC-MS/MS was suitable only for 16 pesticides. All parameter validated satisfied the guide of validation SANTE/2015/11945. Nevertheless, QuEChERS method for beeswax samples is necessary to optimize method for down pesticides LOQs, that were higher the MLR. The validated method showed to be fast, efficient and reliable; it can be used in the monitoring of pesticides in bee products from meet the needs of beekeepers, who request the service for detection pesticides to National References Center of Pesticides and Contaminants (CNRPyC).

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Table 1. MRM transitions of quantification and confirmation (MRM_1 and MRM_2). Operational parameters of QuEChERS method for pesticides in beeswax by GC-MS/MS.

Compound	TR (min)	MRM_1 Quantification	MRM_2 Confirmation	CE (eV) MRM_1	CE (eV) MRM_2	MRM Ratio
Alpha-BHC	7.61	180.9>145.0	218.9>183.0	15	5	86.95
Boscalid	16.68	140.0>76.0	140.0>112.0	25	10	104.92
Bromopropylate	13.97	338.8>182.9	340.8>182.9	20	20	97.49
Chlorfenvinphos	10.75	266.9>159.1	268.9>161.0	15	15	67.1
Chlorpyrifos	9.91	313.8>257.8	313.8>285.8	5	15	40.71
Chlorpyrifos methyl	9.10	285.9>92.9	287.9>92.9	20	20	29.45
Cyfluthrin I	16.22	162.9>127.0	198.9>170.1	5	25	60.18
Cyfluthrin II	16.30	162.9>127.0	198.9>170.1	5	25	52.32
Cyfluthrin III	16.44	162.9>27.0	198.9>170.1	5	25	42.95
lambda-Cyhalothrin	14.97	208.0>181.0	181.1>152.0	5	25	179.54
Diethofencarb	9.80	225.0>96.0	225.0>168.0	30	10	61.75
Endosulfan sulfate	13.08	271.9>237.0	273.8>236.9	15	15	13.09
Esfenvalerato	17.63	419.1>166.8	225.0>147.1	10	10	331.15
Ethion	12.42	230.9>129.0	230.9>175.0	20	10	79.94
Fenitrothion	9.57	277.0>109.0	260.0>109	15	15	35.73
Fenthion	9.89	278.0>109.0	124.9>47.0	15	10	38.64
Fenvalerate	17.45	419.1>166.8	225.0>147.1	10	10	304.74
Heptachlor epoxide (Isomer B)	10.58	352.8>262.9	354.8>264.9	15	15	62.99
Metalaxyl	9.34	234.0>146.1	234.0>174.1	20	10	57.76
Mevinphos	5.62	192.0>127.0	192.0>65.9	10	20	25.88
Parathion	9.96	290.9>109.0	138.9>109.0	10	5	125.3
Parathion methyl	9.13	262.9>109.0	262.9>79.0	10	30	21.12
Permethrin I	15.64	183.1> 65.1	183.1>153.1	10	15	142.13
Permethrin II	15.76	183.1>165.1	183.1>153.1	10	15	107.87
Phorate	7.46	128.9>65.0	121.0>47.0	15	30	128.41
Propiconazole I	13.06	172.9>109.0	172.9>74.0	30	45	145.48
Propiconazole II	13.19	172.9>109.0	172.9>74.0	30	45	116.83
Pyridaben	15.84	147.2>117.1	147.2>132.2	20	10	48.07
Tebuconazole	13.48	125.0>89.0	125.0>99.0	15	20	62.68
Vinclozoline	9.09	187.0>124.0	197.9>145.0	20	15	81.81
ISTD Diethyl-d10-amine hydrochloride	9.86	325.9>294.0	327.9>296.0	5	5	-

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