



V ISA

International
Symposium on Agave

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Temática 3. Fructanos y otros derivados del Agave

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Temática 4. Aprovechamiento integral y sostenible de los Agaves y subproductos

Dr. Gustavo Viniegra González, Coordinador temática 4

Dra. Lorena Amaya Delgado

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Temática 5. Aspectos socioculturales del Agave y marco normativo

M.C. Juan Gallardo Valdez, Coordinador temática 5

Dra. Vianey del Rio Guerra

Dr. José de Jesús Hernández López

Dr. Ever Sánchez Osorio

PROGRAMA

PROGRAMA

Wednesday October, 12	
8:00-15:00	Registration
ROOM 23A AGAVE	
I Scientific trends on Agave	
Chair: Liberato Portillo Martinez	
8:30-9:30	I-O-01 Keynote Lecture. Dr. Benjamín Rodríguez Garay "Reproducción sexual en el Agave"
9:30-10:30	I-O-02 Keynote Lecture. Dr. Héctor González Hernández "Scyphophorus acupunctatus Gyllenhal plaga de los magueyes en México"
10:30-10:50	I-O-03 Lecture. Fulgencio Alatorre Cobos "Unraveling agave fiber formation by using omics and development biology approaches"
10:50-11:10	I-O-04 Lecture. Sandra Yarenssy Martínez "Putrescine affect in vitro maturation of somatic embryos of two species of Agave"
11:10-11:40	Coffee Break
11:40-12:00	I-O-05 Lecture. Monserrat Hernandez Solis "Effect of osmotic stress on indirect somatic embryogenesis of three species of Agave"
12:00-12:20	I-O-06 Lecture. Laura Acosta Villagran "Regeneration of <i>Agave cupreata</i> plants through direct somatic embryogenesis"
12:20-12:40	I-O-07 Lecture. Jesus Edgardo Gutierrez "Characterization of mayahuelin, a type I Ribosome Inactivating Protein from <i>A. tequilana</i> var. azul and its utilization in Agave phylogeny"
12:40-13:00 VIRTUAL	I-O-08 Lecture. Maria Isabel Hernandez Castillo "Synergism between phytopathogenus cause <i>Agave salmiana</i> diseases in Hidalgo, México"
13:00-13:20	I-O-09 Lecture. Carmen Corona Rodríguez "Control of vascular wilt in <i>Agave cupreata</i> , through biological treatments"
13:20-15:00	Lunch
15:00-16:00	Opening ceremony
Opening lectures	
16:00-17:00	REDBIO. Villalobos Arámbula Víctor Manuel "La biotecnología y la sostenibilidad ante la crisis alimentaria"
17:00-18:00	ISA. Pedro Jesús Herrera Franco. "Materiales compuestos de matriz polimérica con refuerzo celulósico – Una alternativa de alto valor agregado para los agaves"
18:00-20:00 Cocktail	
Thursday October, 13	
8:00-8:30	Registration
ROOM 23A AGAVE	
IV Sustainable and integral exploitation of Agaves and sub products	
Chair: Lorena Amaya Delgado	
8:30-9:30 VIRTUAL	IV-O-01 Keynote Lecture. Dr. Luis Carlos Rosales Rivera "Desarrollo de filamentos para impresión 3D y materiales compuestos usando fibras naturales"
9:30-10:30	IV-O-02 Lecture. Dr. Gustavo Viniegra "Metepante (Agave & corn plantations): analysis and perspectives"
10:30-10:50	IV-O-03 Lecture. Yoselin Avila Lizarraga "Initial assessment of Agro-industrial liquid wastes from <i>Agave fourcroydes</i> Lem. as prebiotic"
10:50-11:10	IV-O-04 Lecture. Jacobo Pérez Barragán "Potential to produce biohydrogen and biogas from <i>Agave tequilana</i> bagasse: effect of tequila production process"
11:10-11:40	Coffee Break
11:40-12:00	IV-O-05 Lecture. Matías Domínguez Laso "LAM project: an alternative for the conservation of regional agaves in Oaxaca"
12:00-12:20	IV-O-06 Lecture. Diego Gallardo Martínez "Biochemical characterization of the liquid residue obtained by mechanical decortication of <i>Agave salmiana</i> leaves"
12:20-12:40	IV-O-07 Lecture. Jose Angel Garcia Bejar "Scale-up of lignocellulosic ethanol production from agave bagasse"
12:40-13:00	IV-O-08 Lecture. Paola Janet Delgado Espitia "Enzymatic hydrolysates of agave bagasse pretreated with ionic liquids: saccharification efficiency and hydrogen production"
13:00-14:30	Lunch

PROGRAMA

Thursday October, 13

ROOM 23A AGAVE

V Industrial Social, normative and ethnobotanic aspects

Chair: Juan Gallardo Valdez

14:30-15:30	V-O-01 Keynote Lecture. Dr. Rogelio Luna Zamora "Bioeconomía y tanatopolítica de los mezcales tradicionales y ancestrales"
15:30-16:30 Virtual/presencial	V-O-02 Keynote Lecture. Dra. Marie-Christine Renard Hubert y Rodolfo Domínguez Arista "La Denominación de Origen Mezcal: inclusiones y exclusiones. El ejemplo del EdoMex"
16:30-16:50	V-O-03 Lecture. Gustavo Viniegra "El aprovechamiento agroforestal minifundista de los agaves"
16:50-17:10 VIRTUAL	V-O-04 Lecture. Doris Adriana Leyva Trinidad "The maguey: the cultural resistance of Ñähñü people in the Alto Mezquital, Hidalgo"
17:10-18:00	Poster Sessions I, II, III, IV, V

Friday October, 14

ROOM 23A AGAVE

II Science and technology of Agave beverages

Chair: Claudia Patricia Larralde Corona

8:30-9:30	II-O-01 Keynote Lecture. Dr. José Adelfo Escalante Lozada "Análisis de la diversidad microbiana y su inferencia funcional durante el proceso de fermentación del pulque para la definición de un microbioma central de esa bebida"
9:30- 10:30	II-O-02 Keynote Lecture. Dr. Sergio Erick García Barrón "Caracterización sensorial del Mezcal: desde los aromas hasta las preferencias"
10:30-10:50	II-O-03 Lecture. Rodrigo Arredondo Fernandez "Determination of the yeast communities' succession and of the physicochemical changes in commercial pulque production from the Hacienda de Xochuca, Tlaxcala, México"
10:50-11:10	II-O-04 Lecture. Fernando Astudillo Melgar "Comparison of the pulque associated microbiome from two different agaves"
11:10-11:40	Coffee Break
11:40-12:00	II-O-05 Lecture. Alma Verdugo Valadez "Comparison of five fermentation processes of Comiteco"
12:00-12:20	II-O-06 Lecture. Rene Quezada "Metagenomic characterization and evaluation of volatile compounds in artisanal mezcal fermentation from Oaxaca state"
12:20-12:40	II-O-07 Lecture. Filiberto A Bautista Moreno "Pasteurization and sterile filtration techniques comparison for aguamiel decontamination. An effort to enhance shelf life of a highly perishable product"
12:40-13:00	II-O-08 Lecture. Jacobo Rodriguez Campos "Discrimination of authentic tequila by some volatile compound markers"
13:00-14:30	Lunch

III Fructans and other agave products

Chair: Lorena Moreno Vilet

14:30-15:30 VIRTUAL	III-O-01 Keynote Lecture. Dra. Georgina Sandoval "Agave fructan bioconjugates: synthesis and biological activity"
15:30-16:3 VIRTUAL	III-O-02 Keynote Lecture. M. en C. Cynthia Fernández Lainez "Efectos benéficos de los fructanos derivados de <i>Agave tequilana</i> : desde la inmunomodulación y protección de la barrera intestinal, hasta su efecto prebiótico en recién nacidos"
16:30 -16:50	III-O-03 Lecture. Eliud de la Cruz García "Fructanase production by yeasts in agave media"
16:50-17:10	III-O-04 Lecture. Noe Luiz Santos "Fouling resistance of agave fructan ultrafiltration process using ceramic membranes"
17:10-17:30	III-O-05 Lecture. Liliana Kelly Vigil Cuate "Effect of the use of agavins and agave syrup in the development of a gummy"
17:30-18:30	Closing Lecture Dra. Anne Christine Gschaeidler Mathis "Aprovechamiento de los agaves en México: panorama, retos y oportunidades"
18:30-19:00	Closing ceremony

CONFERENCIAS

MAGISTRALES

Opening lecture

Pedro Jesús Herrera Franco. "Materiales compuestos de matriz polimérica con refuerzo celulósico – Una alternativa de alto valor agregado para los agaves"

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V Industrial Social, normative and ethnobotanic aspects

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V-O-02 Keynote Lecture. Dra. Marie-Christine Renard Hubert y Rodolfo Domínguez Arista "La Denominación de Origen Mezcal: inclusiones y exclusiones. El ejemplo del EdoMex"

Closing Lecture

Dra. Anne Christine Gschaeidler Mathis "Aprovechamiento de los agaves en México: panorama, retos y oportunidades"

OPENING LECTURE

Materiales compuestos de matriz polimérica con refuerzo celulósico – Una alternativa de alto valor agregado para los agaves –

Pedro Jesús Herrera Franco

Unidad de Materiales, Centro de Investigación Científica de Yucatán, A.C.
Calle 43 No. 130 x 32 y 34, Chuburná de Hidalgo, C.P. 97205,
Mérida, Yucatán, México

Resumen

La naturaleza nos ha el mejor ejemplo del uso de la celulosa como material de refuerzo en la construcción de plantas y árboles. El hombre la ha utilizado desde épocas remotas de la historia de la humanidad para hacer cuerdas, vestido, parte de sus viviendas. En los últimos años, la investigación científica se ha involucrado en la explotación de fibras celulósicas como constituyentes de carga y de refuerzo en materiales compuestos. El uso de estos materiales de origen natural en materiales compuestos se ha incrementado en los últimos años debido a su relativo bajo costo en comparación con materiales convencionales como las fibras de vidrio y de aramida, su capacidad de reciclaje y por el hecho de que compiten bien en términos de resistencia por peso de material. En esta presentación, primeramente, se hace una revisión de las propiedades físicas y mecánicas de las fibras de henequén (*Agave fourcroydes*) y de los conceptos de adherencia con los materiales poliméricos. Posteriormente se muestra la factibilidad del desarrollo de un material compuesto a base de polietileno, un relleno mineral y fibras cortas de henequén. Se muestra que es posible incorporar a la resina termoplástica contenidos de carga de hasta un 50% p/p y optimizar las propiedades de tracción y flexión del compuesto en función del contenido de carga mineral y/o fibra natural y la temperatura de procesamiento. Las fibras de henequén también se han utilizado para mejorar las propiedades del concreto a base de cemento Portland y la posibilidad de utilizar a este concreto fibro-reforzado en la fabricación de vivienda en sectores de la población de bajos recursos. La utilización de microfibras de celulosa para la fabricación de materiales compuestos de baja resistencia y altos volúmenes de producción se ha estudiado para reforzar el concepto de biodegradabilidad. El concepto utilizado es el de mejoramiento de las propiedades interfaciales fibra-matriz que permiten tener un material compuesto con resistencia aceptable y posteriormente después de cumplir con su vida útil, asegurar su degradación para evitar el incremento de los problemas ambientales de contaminación. Finalmente, se mencionan algunos resultados de la utilización de fibras naturales en la fabricación de materiales compuestos utilizando la tecnología de manufactura aditiva.

OPENING LECTURE

Polymeric-matrix composite materials with cellulosic reinforcement – A high added value alternative for agaves –

Pedro Jesús Herrera Franco

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Abstract

Nature has given us the best example of the use of cellulose as a reinforcement material in the construction of plants and trees. Man has used cellulose since ancient times in the history of mankind to make ropes, clothing, part of their homes. In recent years, scientific research has been involved in the exploitation of cellulosic fibers as filler and reinforcement constituents in composite materials. The use of these materials of natural origin in composite materials has increased in recent years due to their relative low cost compared to conventional materials such as glass and aramid fibers, their recyclability and the fact that they compete well in terms of strength per weight of material. In this presentation, first, a review of the physical and mechanical properties of henequen fibers (*Agave fourcroydes*) and the concepts of adherence with polymeric materials is made. Subsequently, the feasibility of developing a composite material based on polyethylene, a mineral filler and short henequen fibers is shown. It is also shown that it is possible to incorporate filler contents of up to 50% w/w into the thermoplastic resin and optimize the tensile and flexural properties of the compound depending on the content of mineral filler and/or natural fiber and the processing temperature. Henequen fibers have also been used to improve the properties of Portland cement-based concrete and the possibility of using this fiber-reinforced concrete in the manufacture of housing for low-income sectors of the population. The use of cellulose microfibers for the manufacture of low-resistance composite materials and high production volumes has been studied to reinforce the concept of biodegradability. The concept used is the improvement of the fiber-matrix interfacial properties that allow having a composite material with acceptable strength and stiffness and later, after fulfilling its useful life, ensure its degradation to avoid the increase of environmental pollution problems. Finally, some results of the use of natural fibers in the manufacture of composite materials using additive manufacturing technology are mentioned.

REPRODUCCIÓN SEXUAL EN EL Agave

Benjamín Rodríguez Garay. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. agavero01@hotmail.com

La familia Asparagaceae es una de decenas de familias de plantas angiospermas monocotiledóneas a la cual pertenece el género *Agave*. Como tal, el género *Agave* posee flores, las cuales son hermafroditas, esto es, que contienen ambos sexos en la misma flor. Las flores son protándricas, esto es que los órganos masculinos (anteras) maduran antes que el órgano femenino (ovario contenido óvulos). Esta particularidad, permite y hace necesaria la polinización cruzada entre individuos, lo cual entre otros es una estrategia de producción de variación genética.

En la actual presentación, se muestran algunos aspectos importantes de la división meiótica “incluyendo accidentes citogenéticos” y sus implicaciones durante el desarrollo del gametofito masculino (polen) en diversas especies de *Agave*. También, se presentan ejemplos del proceso de formación del saco embrionario a partir de la célula madre de la megaspora (2n) en diversas especies de *Agave* y otras de la subfamilia Agavoideae (Asparagaceae) comparadas con especies “no asparagáceas”. Es importante destacar la dificultad técnica que implica la observación y el estudio de estos procesos en las profundidades del ovario de la flor, lo cual ha sido posible con el desarrollo de técnicas de clarificación de tejidos suculentos y gruesos y el uso de técnicas de microscopía confocal.

Una característica sobresaliente de las plantas con flores, es la “doble fertilización”, la cual implica el movimiento de los espermas dentro del saco embrionario para la fertilización de la célula huevo y el núcleo de la célula central. Importantes elementos para estos procesos son la participación del calcio y proteínas motoras como la actina. El tamaño del saco embrionario en los agaves y el tamaño de las células /célula huevo, sinérgidas, antípodas y célula central dentro del gametofito femenino, permiten observaciones y análisis relativamente sencillo. Sin embargo, biológicamente estas características representan un importante reto para el movimiento del esperma que fertiliza al núcleo de la célula central, el cual debe de recorrer grandes distancias para lograr su objetivo. Resultado de esta fertilización es la formación del endospermo, elemento importante para la formación final de la semilla.

También, se discuten métodos de polinización, manejo y conservación de polen y uso del conocimiento de la reproducción sexual para el mantenimiento de variedades de interés comercial y/o mejoramiento genético de agaves por medio de métodos convencionales y biotecnológicos que serán importantes para hacer frente a los efectos del calentamiento global.

***Scyphophorus acupunctatus* Gyllenhal plaga de los magueyes en México**

Héctor González Hernández, Colegio de Postgraduados.

El picudo del agave *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophtoridae) es la principal plaga de los agávaceas cultivadas como el tequilero, mezcaleros, pulqueros, henequén, sisal o nardo; además de atacar agávaceas silvestres de los desiertos o áreas naturales de casi todo el país. El picudo del agave es de origen del norte de México y sur de Estados Unidos de América, aunque actualmente es de distribución cosmopolita, con amplia distribución en países de Centro América, algunos de Sudamérica, con reportes en varios países de Europa, Oriente Medio, África y Oceanía, donde han llevado ejemplares con fines de ornato o para la producción de fibras. En cultivos de agave mezcalero de Oaxaca y tequilero de Jalisco se han registrado daños importantes en la materia prima - cabezas o piñas (mezontle o tallo) - que llega a las fábricas procesadoras, ya que las larvas barrenan esta parte de la planta, pudiendo inclusive consumir el 90 o 100 del tejido vegetal, aunado a que estos daños se pueden asociar con hongos o bacterias fitopatógenas que pueden acelerar la muerte o descomposición de toda la planta. La incidencia del picudo en los agaves cultivados es durante todo el año. En agaves silvestres, el picudo generalmente ataca la planta poco después de producir el escapo floral o quiote, casi al final de su ciclo de vida; mientras que, en agave cultivados como el tequilero, puede atacar desde el estado de hijuelos hasta plantas maduras casi para jimar. Una de las tácticas más sugeridas para el control de esta plaga, aunque correctiva, es el control cultural mediante la remoción de plantas infestadas, que de acuerdo con la madurez de éstas, puede ser por eliminación, jima fitosanitaria o de recuperación. Para el caso de plantaciones de agave pulquero, el control cultural debe incluir la limpieza y eliminación de restos de pencas en el proceso de raspado, para evitar focos de infestación. Como parte de la comunicación química de este grupo de picudos los adultos emiten compuestos feromonales de agregación, donde los machos al llegar a una planta llaman mediante estos compuestos a más individuos de ambos sexos de su población, para comenzar el proceso de colonización y con más individuos poder doblar las defensas de la planta más fácil y rápidamente. Este comportamiento se ha aprovechado para desarrollar sistemas de monitoreo, sintetizando los compuestos feromonales responsables de esta comunicación y usarlos para programa de trámpeo para el monitoreo o para trámpeo masivo con fines de control. De esta forma, para esta plaga ya se cuenta con un diseño de trampa que es efectiva para determinar el comportamiento estacional y establecer períodos críticos para iniciar acciones de manejo como el químico, cultural o biológico. A nivel de plantaciones comerciales de agave, se han aplicado productos de origen natural como distintas cepas de hongos o nemátodos entomopatógenos. Además, en forma natural, se presentan otros agentes de control del picudo del agave, como parasitoides y escarabajos depredadores, que pueden tener un papel importante en la regulación de este picudo.

ANÁLISIS DE LA DIVERSIDAD MICROBIANA Y SU INFERENCIA FUNCIONAL DURANTE EL PROCESO DE FERMENTACIÓN DEL PULQUE PARA LA DEFINICIÓN DE UN MICROBIOMA CENTRAL DE ESA BEBIDA.

Dr. Adelfo Escalante.

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El pulque es una bebida fermentada tradicional alcohólica elaborada a partir de la fermentación de la savia o aguamiel de varias especies de maguey (*Agave*) cultivados para la producción de esta bebida. Es la bebida más ampliamente estudiada en México desde diferentes enfoques: histórico, social, arqueológico, microbiológico, etc. Para su producción, aguamiel fresco o recién colectado es agregado a un tanque en el que se desarrolla la fermentación y que contiene pulque previamente fermentado. La fermentación de esta bebida resulta de la actividad de una microbiota compleja. En este trabajo se presenta el análisis de la diversidad microbiana presente en el pulque: aguamiel, pulque fermentado y durante una fermentación de 6 horas en laboratorio, mediante una aproximación metagenómica para caracterizar su composición microbiana y su diversidad funcional. Se identificaron 6 géneros principales: *Acinetobacter*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Saccharomyces* y *Zymomonas* y 10 especies dominantes: *Acinetobacter boissieri*, *A. nectaris*, *Lactobacillus sanfranciscensis*, *Lactococcus lactis*, *L. piscium*, *L. plantarum*, *Leuconostoc citreum*, *L. gelidum*, *Zymomonas mobilis* y *Saccharomyces cerevisiae*, que están presentes $\geq 1\%$ en al menos una etapa de la fermentación. La abundancia de los géneros y especies cambió durante la fermentación y se asoció a una disminución en la concentración de sacarosa y un incremento en la concentración de ácido láctico, sugiriendo que la competencia por el sustrato principal en el aguamiel, define la diversidad microbiana y metabólica presente durante la fermentación. Se determinó también el perfil funcional de la bebida con base en el contenido de genes para cada etapa analizada y se identificó una abundancia de genes asociada a la bioíntesis de folatos. De forma adicional, se estudió la relación de *S. cerevisiae* y *Z. mobilis*, dos de los microrganismos más abundantes y relevantes por su capacidad de producción de etanol. Los resultados obtenidos sugieren que la levadura *S. cerevisiae* del pulque está relacionada con aislados asiáticos presnetes en el sake y en bioetanol. Por su parte, *Z. mobilis* aislada del pulque representa un linaje distinto a otras *Zymomonas* de otras bebidas fermentadas.

Este proyecto contó con financiamiento de los proyectos PAPIIT UNAM IN207917 e IN211420.

CARACTERIZACIÓN SENSORIAL DEL MEZCAL: DESDE LOS AROMAS HASTA LAS PREFERENCIAS.

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El mezcal es una bebida alcohólica tradicional de México. Posee una importancia tanto cultural como económica para las zonas en donde se produce. La zona de producción abarca una parte importante del territorio nacional, lo que le brinda una identidad sensorial ligada a la región en donde se produce. Es en este sentido que se planteó un proyecto de investigación para entender como la etapa de fermentación de los procesos artesanales de dos zonas productoras influyen en las poblaciones microbianas. Como parte de ese proyecto se derivó otra pregunta, ¿Existen diferencias en el perfil sensorial del producto terminado elaborado bajo diferentes condiciones de fermentación? Para responder esta pregunta, se estableció una estrategia metodológica global que incluía dos etapas: una metodología sensorial y otra instrumental. La metodología mostró que existían diferencias a nivel sensorial entre lotes de un mismo fabricante y que cada mezcal tenía un perfil sensorial diferente. Por otro lado, el análisis instrumental mostró que cada mezcal tuvo un perfil de compuestos volátiles diferente y que el proceso de fermentación también influyó. Al correlacionar las mediciones sensoriales instrumentales mediante herramientas de estadística multivariada, se observó que existe un patrón de compuestos volátiles que explican la formación de las características sensoriales. Por otro lado, como parte de las investigaciones con el mezcal, se planteó la pregunta ¿La preferencia y percepción de los consumidores por el mezcal cambia en función del lugar de residencia? Los resultados muestran que existe una relación entre el lugar de origen de los consumidores y sus preferencias, ya que los consumidores tienden a preferir los productos elaborados en sus lugares de origen, demostrando que la familiaridad guía las preferencias de los consumidores, así mismo, la información tiende a acentuar estas preferencias. Como parte de estos estudios, también se planteó investigar como los consumidores definen el concepto “mezcal”, observando que la familiaridad de los consumidores para con el producto tenía una relación con el tipo y número de palabras empleadas para la definición del “mezcal”. Finalmente, se planteó las preguntas ¿El mezcal desde el punto de vista del consumidor es tradicional? Y si lo es ¿Qué hace que sea tradicional? Para responder estas preguntas, se emplearon constructos psicológicos para explicar el comportamiento del consumidor y su relación con dimensiones que definen a un producto tradicional. Lo que se observó es que el mezcal es altamente tradicional en donde el conocimiento, experiencia con el producto y el consumo tienden a influir en la percepción de la imagen tradicional del mezcal. La información de la caracterización sensorial del mezcal permite caracterizar el efecto de las etapas de elaboración de procesos artesanales. En cuanto a estudio con consumidores, confirman la importancia del estudio de las preferencias, sobre todo para la identificación de los factores que influyen en los patrones de respuesta con el fin de generar estrategias de difusión y posicionamiento de acuerdo a las características del mezcal.

AGAVE FRUCTAN BIOCONJUGATES: SYNTHESIS AND BIOLOGICAL ACTIVITY.

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Plant-derived products have played an important role in maintaining human well-being, for hundreds of years, natural products and their derivatives have been used since ancient times mainly in the development of pharmaceuticals for the treatment of human conditions.

An interesting recently studied source for this purpose are fructans from *Agave* genera, which differs from inulin in the type of linkage. Indeed, inulin is lineal while *Agave* fructans are a complex mixture of fructooligosaccharides containing principally $\beta(2\rightarrow1)$ linkages, and some $\beta(2\rightarrow6)$ and branching moieties, leading to ramified structures.

Functionalization of fructans, such as acylation with fatty acids has also been reported for instance for inulin by chemical esterification, and in our case, by enzymatic bioconjugation of *Agave tequilana* fructans.

This talk describes the synthesis of bioconjugated molecules from agave fructans and its patented production process by biocatalyzed synthesis with lipases. The bioconjugate molecules have been tested *in vitro* and *in vivo*, in applications in foods and nutraceuticals (prebiotic, emulsifier, fat substitute); pharmaceuticals (anti-inflammatory, antitumoral, intestinal vector, and against metabolic syndrome); and cosmetics (prebiotic emollient). Besides, these molecules are non-toxic according to the Ames tests.

Which makes them extremely interesting molecules for different industries. It also contributes to harnessing and diversification of value chains for agave fructans.



EFECTOS BENÉFICOS DE LOS FRUCTANOS DERIVADOS DEL *Agave tequilana*: DESDE LA INMUNOMODULACIÓN Y PROTECCIÓN DE LA BARRERA INTESTINAL, HASTA SU EFECTO PREBIÓTICO EN RECIÉN NACIDOS.

M. en C. Cynthia Fernández Lainez

Los fructanos han sido sujeto de estudio en las últimas décadas debido a sus potenciales beneficios para la salud. Nuestro trabajo aporta conocimientos relevantes acerca de las características químicas y el efecto benéfico de los fructanos extraídos del *Agave tequilana*, una de las plantas endémicas de la región de América Latina. Entre los puntos importantes de nuestra investigación, se encuentra la comparación que realizamos entre el efecto de los fructanos extraídos del Agave mexicano y aquellos extraídos de la planta de achicoria, cuyo uso es amplio en Europa. Utilizamos dos mezclas de fructanos del agave con diferente grado de polimerización y con estructura ramificada. Los enlaces glicosídicos b(2[°]1) y b(2[°]6) son los responsables de la estructura ramificada y se deben a su naturaleza de graminanos, por lo que de aquí en adelante los llamaremos fructanos de tipo graminano (GTFs). Estos GTFs los comparamos con dos mezclas de fructanos de la achicoria, cuya estructura es lineal por su naturaleza de inulinas constituidas únicamente por enlaces b(2[°]1), de aquí en adelante llamados fructanos de tipo inulina (ITFs). Al adicionar estas mezclas de fructanos a cultivos de células de humano que sobre expresan los receptores inmunes tipo "toll" (TLRs), encontramos que los GTFs inhibieron la activación de dichos receptores, lo que se tradujo en un efecto de regulación de la producción de citocinas proinflamatorias en células dendríticas. Al estudiar más a fondo este fenómeno mediante modelaje molecular *in silico*, encontramos que estos GTFs muestran características moleculares que les confieren la capacidad de unirse a residuos de aminoácidos de los TLRs, los cuales fueron previamente descritos como de importancia para la unión de los ligandos naturales de estos receptores, así como para la dimerización, es decir, para la formación de sus unidades funcionales. Más aún, encontramos que dichos efectos son dependientes del tipo de estructura del fructano (lineal o ramificada) y del grado de polimerización. En el contexto del efecto directo que pueden tener los fructanos en el intestino, analizamos su interacción con células del epitelio intestinal para saber si podrían también tener algún efecto benéfico sobre la función de la barrera intestinal, no sólo en estado de homeostasis sino también bajo el efecto de agentes químicos disruptores. Para ello, incubamos líneas celulares intestinales adicionadas con los fructanos, con o sin la posterior adición de los disruptores. Encontramos que ambos tipos de fructanos tienen efecto protector de la barrera intestinal ya que previenen el incremento de la permeabilidad intestinal, determinado mediante resistencia transepitelial. Investigando más a fondo, estudiamos si el efecto protector encontrado estaba influenciado por cambios en la expresión génica de las proteínas que forman parte de la barrera intestinal. Para ello, mediante ensayos de PCR cuantitativo en tiempo real, medimos la expresión de los genes que codifican para claudina 1, claudina 2, claudina 3, E-caderina, ocludina y zonula occludens 1, en células del epitelio intestinal humano. Encontramos que los fructanos tienen una acción protectora, al inducir la expresión de genes relacionados con prevenir la disrupción de la barrera intestinal. También encontramos que uno de los GTFs y uno de los ITFs, ambos de cadena larga, tienen un efecto benéfico sobre la expresión de los genes intestinales estudiados, como es la disminución de la expresión de proteínas formadoras de poros en la barrera intestinal, lo que se traduce en nuestra propuesta del uso de estos carbohidratos como agentes preventivos de desórdenes inflamatorios intestinales. Previamente demostramos la seguridad y eficacia del uso de estos GTFs como prebióticos en niños recién nacidos a término. Si bien

los efectos de los fructanos como prebióticos se atribuyen principalmente a la intervención de la microbiota intestinal, nuestros resultados acerca de su efecto directo con el sistema inmune pueden verse fortalecidos con lo que se encontró en el grupo de niños estudiado. A este respecto, los niños que consumieron formula infantil adicionada con estos GTFs mostraron una estimulación de su sistema inmune a nivel de mucosas, lo cual se corroboró por el aumento en la producción de IgA. De forma similar, se encontró que los niños que consumieron estos GTFs fueron los que presentaron menor número de eventos relacionados con la respuesta inflamatoria, como son los cólicos y la distensión abdominal. Adicionalmente, se demostró que estos GTFs moldearon los perfiles de la microbiota intestinal de los recién nacidos, promoviendo el desarrollo de bifidobacterias e impidiendo la colonización de patógenos como *Clostridium*. Incluso, al hacer un estudio de correlación entre la vía de nacimiento, el consumo de GTFs y los perfiles de microbiota intestinal, encontramos que los phyla bacterianos de los consumidores de GTFs se asociaron más fuertemente con los fenotipos de nacimiento por vía vaginal que a los nacidos por cesárea. En conjunto, nuestros resultados *in vitro* y directamente con los niños, nos impulsan a proponer que los GTFs tienen un efecto inmunomodulador directo que se suma y complementa con el efecto prebiótico en el individuo y que nos permiten vislumbrar el uso más amplio de estos oligosacáridos para beneficio de las poblaciones vulnerables de México y del mundo.



DESARROLLO DE FILAMENTOS PARA IMPRESIÓN 3D Y MATERIALES COMPUESTOS USANDO FIBRAS NATURALES

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El modelado por deposición fundida (MDF) es una de las técnicas empleadas por la manufactura aditiva (también conocida como impresión 3D) con una gran cantidad de ventajas en la fabricación de prototipos como lo son: su alta rapidez, versatilidad y precisión, un bajo coste, una menor generación de residuos y con un sinfín de aplicaciones.

Para proporcionarles una segunda vida económica a las fibras de agave provenientes de residuos de la industria tequilera, se fabricaron filamentos a partir del biopolímero ácido poliláctico (PLA) en diferentes concentraciones (0, 3, 5 y 10% p/p) y se utilizaron para fabricar biocompositos mediante la técnica de MDF, se usaron diferentes ángulos de impresión (0/90° y -45/45°). Las piezas fabricadas fueron sometidas a diferentes estudios como calorimetría diferencial de barrido (DSC), absorción de agua, densidad, morfología y desintegración bajo condiciones simuladas de compostaje. Además, se fabricaron piezas específicas para ser probadas usando las normas internacionales ASTM para tensión, flexión e impacto Charpy. En casi todas las pruebas el ángulo de impresión no tuvo un efecto significativo sobre las propiedades finales.

La inclusión de fibras favorece la formación de estructuras más porosas y con densidades aparentes menores a las piezas de PLA puro (de 1.16 a 0.98 g cm⁻³). La adición de fibras a los filamentos incrementó la cristalinidad de 23 a 44 % (0-10% p/p). En cuestión de densidad las piezas con fibra de agave presentaron estructuras porosas con una mayor cantidad de celdas abiertas en comparación a las piezas fabricadas solo de PLA; incrementando la máxima absorción de agua incrementó de 1.5% para PLA puro hasta un 17% para biocompositos con 10% de fibra.

En cuestión de propiedades mecánicas, las propiedades de tensión y flexión disminuyeron con la inclusión de fibra de agave, mientras que el ángulo de impresión afectó principalmente a la resistencia a la tensión, las propiedades de flexión y la resistencia al impacto, disminuyendo al cambiar el ángulo de -45/45° a 0/90°. En general, un incremento de las concentraciones de fibras de agave tuvo un efecto negativo de todas las pruebas mecánicas. Finalmente, la desintegración usando condiciones simuladas de compostaje de los biocompositos se vio ralentizada al adicionar fibra de agave (de 16.90% para piezas con 10% de fibra hasta 27.54% para el PLA puro).

BIOECONOMÍA Y TANATOPOLÍTICA DE LOS MEZCALES TRADICIONALES Y ANCESTRALES

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Todavía hasta la última década del siglo XX, la imagen que se tenía del mezcal en los círculos de consumo urbano, era la de una bebida barata, rústica y propia de paladares de gusto no refinado, se consideraba como bebida de mal gusto y propia del vulgo y, consecuentemente, con precios irrisorios en el mercado; no obstante, a partir de la primera década de la presente centuria y de manera inesperada, comenzó a gozar de una imagen totalmente opuesta, adquirió el estatus de bebida gourmet, con sabores y aromas complejos, de distinción única y exclusiva, este cambio en el gusto -y en la valoración del mezcal-, ocurrió en sectores de consumo urbano y de altos ingresos, lo que dio como resultado que las grandes corporaciones distribuidoras de vinos y licores en el mundo, integraran presentaciones del mezcal como una de sus bebidas *Premium*.

Pero estas presentaciones de exclusividad y distinción acontecieron con los mezcales elaborados con las tecnologías más rústicas, hoy día clasificadas como tradicionales y artesanales, como se estableció en la NOM-070-SCFI-2016, es decir, con los mezcales elaborados en las comunidades indígenas y campesinas de mayor tradición en su elaboración. En otros términos, su fabricación se inscribe en economías locales con menores niveles de comercialización de sus mezcales cuya manufactura data de tiempos ancestrales, y cuyo sistema de producción está asociado a ceremonias religiosas y fiestas locales, e integrada a la cultura culinaria y de socialización de los miembros de la comunidad.

El éxito comercial y la reciente y pujante penetración de empresas comercializadoras, ha provocado una fuerte e inesperada demanda de estos mezcales, impulsando en muy corto tiempo su precio y provocando en los productores locales fuertes motivaciones para incrementar su producción. En otros términos, la propuesta teórica de este artículo es que la fuerte presión generada por la demanda externa a la localidad, se presenta como un dispositivo desarticulante de los vínculos y arreglos tradicionales de los productores de mezcal y de las dinámicas de interacción y socialización entre los miembros de la comunidad. La propuesta es analizar hasta dónde afectan las consecuencias socioculturales y económicas a partir de la vorágine en la comercialización de este producto.

La metodología empleada hasta el momento ha sido producto de entrevistas telefónicas y presenciales a investigadores, productores y comercializadores de estos mezcales.

LA DENOMINACIÓN DE ORIGEN MEZCAL: INCLUSIONES Y EXCLUSIONES. EL EJEMPLO DEL EDOMEX.

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En el diseño de las Denominaciones de Origen (DO), los criterios respecto de a qué productores y territorios se incluyen o se excluyen son esenciales. En el caso de la DO Mezcal, productores y territorios tradicionales han sido excluidos de una DO apegada a criterios administrativos y políticos más que al anclaje territorial de la bebida y eso, aún en el mismo estado de Oaxaca que ostenta el 90% de la producción; cada demanda de inclusión de nuevos territorios ha estado envuelta en la polémica y ha dado lugar a pleitos políticos y demandas legales. Los criterios que, según el Consejo Regulador, definen la inclusión en la DO, a saber, la existencia histórica de maguey, la ancestralidad de la producción, y su persistencia cultural, son utilizados como argumentos tanto por quienes pretenden ser incluidos como por los que se oponen a ello.

La ponencia intentará, por un lado, dilucidar la relación entre esta polémica y el diseño mismo de la DOM; por otro lado, las ilustrará partir del caso del estado de México.



PRESENTACIONES ORALES

PONENCIAS ORALES

I Scientific trends on Agave

I-O-03 Lecture. Fulgencio Alatorre Cobos "Unraveling agave fiber formation by using omics and development biology approaches"

I-O-04 Lecture. Sandra Yarenssy Martínez Martínez "Putrescine affect in vitro maturation of somatic embryos of two species of Agave"

I-O-05 Lecture. Monserrat Hernandez Solis "Effect of osmotic stress on indirect somatic embryogenesis of three species of Agave"

I-O-06 Lecture. Laura Acosta Villagran "Efecto del ácido indol-acético en la expression de embriones somáticos directos en *Agave cupreata*"

I-O-07 Lecture. Jesus Edgardo Gutierrez "Characterization of mayahuelin, a type I Ribosome Inactivating Protein from *A. tequilana* var. azul and its utilization in Agave phylogeny"

I-O-08 Lecture. Maria Isabel Hernandez Castillo "Synergism between phytopathogenus cause *Agave salmiana* diseases in Hidalgo, México"

I-O-09 Lecture. Carmen Corona Rodríguez "Control of vascular wilt in *Agave cupreata*, through biological treatments"

II Science and technology of Agave beverages

II-O-03 Lecture. Rodrigo Arredondo Fernandez "Determination of the yeast communities' succession and of the physicochemical changes in commercial pulque production from the Hacienda de Xochuca, Tlaxcala, México"

II-O-04 Lecture. Fernando Astudillo Melgar "Comparison of the pulque associated microbiome from two different agaves"

II-O-05 Lecture. Alma Verdugo Valadez "Comparison of five fermentation processes of Comiteco"

II-O-06 Lecture. Rene Quezada "Metagenomic characterization and evaluation of volatile compounds in artisanal mezcal fermentation from Oaxaca state"

II-O-07 Lecture. Filiberto A Bautista Moreno "Pasteurization and sterile filtration techniques comparison for aguamiel decontamination. An effort to enhance shelf life of a highly perishable product"

II-O-08 Lecture. Jacobo Rodriguez Campos "Discrimination of authentic tequila by some volatile compound markers"

III Fructans and other agave products

III-O-03 Lecture. Eliud de la Cruz García “Fructanase production by yeasts in agave media”

III-O-04 Lecture. Noe Luiz Santos “Fouling resistance of agave fructan ultrafiltration process using ceramic membranes”

III-O-05 Lecture. Liliana Kelly Vigil Cuate “Effect of the use of agavins and agave syrup in the development of a gummy”

IV Sustainable and integral exploitation of Agaves and sub products

IV-O-02 Lecture. Dr. Gustavo Viniegra “Metepantle (Agave & corn plantations): analysis and perspectives”

IV-O-03 Lecture. Yoselin Avila Lizarraga “Initial assement of Agro-industrial liquid wastes from *Agave fourcroydes* Lem. as prebiotic”

IV-O-04 Lecture. Jacobo Pérez Barragán “Potential to produce biohydrogen and biogas from *Agave tequilana* bagasse: effect of tequila production process”

IV-O-05 Lecture. Matías Domínguez Laso “LAM project: an alternative for the conservation of regional agaves in Oaxaca”

IV-O-06 Lecture. Diego Gallardo Martínez “Biochemical characterization of the liquid residue obtained by mechanical decortication of *Agave salmiana* leaves”

IV-O-07 Lecture. Jose Angel Garcia Bejar “Scale-up of lignocellulosic ethanol production from agave bagasse”

IV-O-08 Lecture. Paola Janet Delgado Espitia “Enzymatic hydrolysates of agave bagasse pretreated with ionic liquids: saccharification efficiency and hydrogen production”

V Industrial Social, normative and ethnobotanic aspects

V-O-03 Lecture. Gustavo Viniegra “El aprovechamiento agroforestal minifundista de los agaves”

V-O-04 Lecture. Doris Adriana Leyva Trinidad “The maguey: the cultural resistance of Ñähñhu people in the Alto Mezquital, Hidalgo”

UNRAVELING AGAVE FIBER FORMATION BY USING OMICS AND DEVELOPMENT BIOLOGY APPROACHES

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Keywords: Fibers, sclerenchyma, lignification

Introduction. Supported by a world moving toward a green economy, natural fibers are currently classified as high-valorized renewable resources, with wide use in construction, textile, and clothing industry, and biopolymers for high-tech applications^{1,2}. Several Agave species are fiber sources. Although physical-mechanical properties of agave fibers have been determined, the molecular genetic mechanisms underlying their development and ontogeny remain unknown. Our research team has used omics and plant biology approaches to study the fiber formation in *Agave tequilana* and *A. fourcroydes*, two traditional crops of Mexico used for spirits and fiber production, respectively^{3,4}.

Methods. *A. fourcroydes* leaves in different development stages, ranked from 3 to 65 cm long, were classified into four categories using an arbitrary classification. Analyzed leaves were divided transversally into three sections (basal, medium, apical), and then each section was sampled and pooled to get a composted sample. These samples were used for histological, carbohydrates (HPAEC-PAD), and lignin (pyrolysis-MBMS) analyses. To know the genetic circuits involved in cell wall metabolism, we carried out a transcriptome mining of RNAseq data of *A. tequilana*, and the orthologous genes retrieved encoding cellulose and lignin enzymes were analyzed for studies of expression levels and phylogenetic analyses.

Results and discussion. Histology data of *A. fourcroydes* showed contrasting differentiation processes between structural and ribbon fibers when spatial-time analyses were carried out for sclerenchyma fibers of leaf. Cellulose, hemicellulose components, total lignin, and monomers showed levels associated with leaf development, especially for the xylose monosaccharide. Transcriptomic analyses in *A. tequilana* have allowed us to dissect the biosynthetic pathways for cellulose and lignin. Orthology analysis revealed most of the orthologs retrieved showed differential expression levels when they were analyzed in different tissues with contrasting cellulose and lignin accumulation. Phylogenetic and structural motif analyses of putative CESA and CAD proteins allowed us to identify those potentially involved with secondary cell wall formation in agave.

RT-qPCR assays revealed enhanced expression levels of *AtqCAD5* and *AtqCESA7* in parenchyma cells associated with extraxillary fibers in *A. tequilana*. Similarly, high expression levels of *CESA7* and *CAD5* in fiber-surrounding cells were also found in *A. fourcroydes* leaves. These data suggest a mechanism of formation of sclerenchyma fibers in agave similar to that reported for xylem cells in model eudicots

Conclusions. Agave leaf's structural and ribbon fibers of agave leaf show different development pattern while chemical composition profiling indicate a close relationship between hemicellulose component levels and fiber development stage. Expression level analysis of key gene for cell wall biosynthesis suggest sclerenchyma fibers formation may involve a cooperative mechanism similar to that reported for xylem.

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Putrescine affect *in vitro* maturation of somatic embryos of two species of *Agave*

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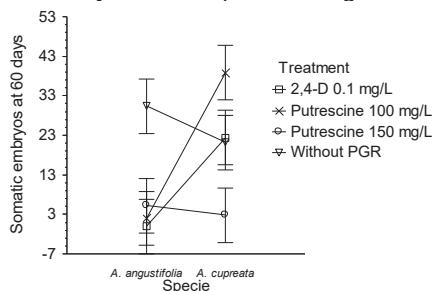
Keywords: propagation, *Agave angustifolia*, *Agave cupreata*.

Introduction. Somatic embryogenesis (SE) has been used as tool for regeneration, propagation, and genetic breeding of several plant species. Indirect somatic embryogenesis comprises three stages: embryogenic callus induction, embryo maturation, and regeneration to plant of somatic embryos. Nowadays, the research carried out on somatic embryogenesis of *Agave* genus has been focused on callus induction, but there have been few reports about maturation stage. The objective of this investigation was to evaluate the effect of exogenous application of putrescine (Put) on somatic embryos maturation of *A. angustifolia* and *A. cupreata*.

Methods. Zygotic embryos of *A. angustifolia* and *A. cupreata* were used as explants, which were placed in medium for embryogenic callus induction (1). After 60 days, embryogenic callus, was transferred to medium for maturation embryos composed of MS salts (2), 30 g/L of sucrose. Was evaluated: two concentrations of putrescine (100 and 150 mg/L), 0.1 mg/L of 2,4-D and without plant growth regulators (PGR) as control. Sixty days later, the percentage of embryo maturation and the number of somatic embryos were evaluated.

Results and discussion. The highest percentage of embryo maturation was obtained in *A. cupreata* (83%) in medium without PGR, whereas in *A. angustifolia* 50% was obtained when 150 mg/L of putrescine was added to medium.

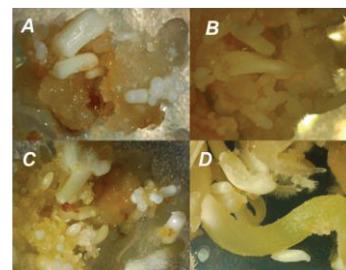
Fig. 1. Effect of putrescine on the number of mature somatic embryos of two species of *Agave*.



Likewise, the highest number of somatic embryos in *A. angustifolia* (30 embryos/explant) was obtained in maturation medium without PGR (Fig. 1) while for *A. cupreata* 40 embryos/explant were obtained with addition of 100 mg/L of putrescine (Fig. 1). It's known that Put addition in SE process can increase the number of somatic embryos through triggers different

activities, namely: increasing SE by regulating 2,4-D synthesis and transport, enhancing cytoskeleton development and the H₂O₂ generated by extracellular Put could underlie tissue differentiation due to the coordinated processes of cell wall maturation and programmed cell death (3).

Fig. 2. Somatic embryos sprouting in maturation medium. A. *angustifolia* without PGR (A and B) and *A. cupreata* with 100 mg/L of putrescine (C and D), at 30 days (A and C) and at 60 days (B and D).



On the other hand, both species showed differences in the maturation embryo time because in *A. angustifolia* somatic embryos in scutellar stage were observed at 30 days (Fig. 2. A) and subsequently the number doubled of embryos at this stage at 60 days (Fig. 2. B) whereas for *A. cupreata*, the presence of somatic embryos in scutellar stage was observed at 30 days (Fig. 2. C) and was higher than *A. angustifolia*, showing regeneration to plant at 60 days (Fig. 2. D). So, this protocol can be reduce the maturation embryos period to 45 days in this species.

Conclusions. The effect of putrescine on the maturation somatic embryos of *Agave* depends on the species.

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EFFECT OF OSMOTIC STRESS ON INDIRECT SOMATIC EMBRYOGENESIS OF THREE SPECIES OF *Agave* spp.

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Keywords: somatic embryogenesis, osmotic stress, Agave.

Introduction. Somatic embryogenesis (SE) is a useful technique to produce several plants from a single explant, commonly used for the propagation of species with commercial importance, such as those from the *Agave* genus, which are used to produce alcoholic beverages and that are endangered for the same reason, since their asexual reproduction is limited. Many factors affect the process of SE, one of these is osmotic stress, which promotes somatic embryo maturation by simulating desiccation in seed formation. The aim of this work was to evaluate the effect of osmotic stress with the exogenous application of ABA and PEG on the maturation of somatic embryos of three *Agave* species: *A. angustifolia*, *A. salmiana* and *A. cupreata*.

Methods. Zygotic embryos were used as primary explant for the callus induction on the media proposed by Alvarez-Aragon *et al.* (2020). After 60 days, calluses were transferred to embryo maturation medium (Alvarez-Aragón *et al.*, 2020), where two concentrations of ABA (3 and 9 mg/L) and PEG 6000 (5 and 7%) were tested for 60 more days in darkness at 25°C. The variables evaluated were percentage of embryogenesis and the number of somatic embryos.

Results and discussion. The first embryos appeared from day 18 in the 9 mg/L ABA treatment for *A. salmiana*, as well as for the 5% PEG treatment for *A. angustifolia* and *A. salmiana*. At 90 days after culture started, all three species showed embryos in the 9 mg/L ABA (Fig. 1A, 1D, 1G) and 5% PEG (Fig. 1B, 1E, 1H) treatments, and *A. angustifolia* showed embryos in the 7% PEG treatment (Fig. 1C). These results agree with those reported for other species (Cruz *et al.*, 2022). Both ABA and PEG have been related to the maturation process of the somatic embryo, since there is a relation between ABA and embryogenic genes as a ABI-3 (Kikuchi *et al.*, 2006) and PEG has been found to promote the meristematic activity in the apical and radicular meristem development in coffee somatic embryos (Valencia Lozano *et al.*, 2021).

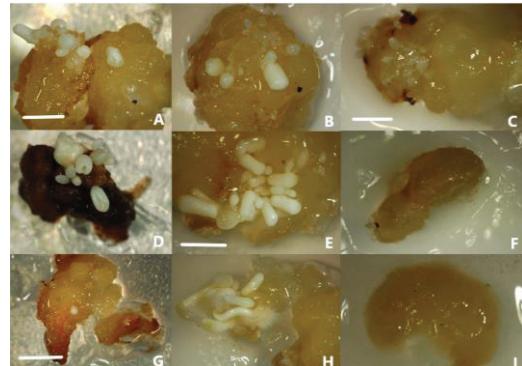


Fig. 1. Expression and maturation of somatic embryos in *Agave* spp. Embryos of *A. angustifolia* (A, B, C), *A. salmiana* (D, E, F) and *A. cupreata* (G, H, I). From left to right: treatment with 9 mg/L ABA, 5% PEG and 7% PEG after 30 days in maturation medium. Bar: 5 mm

Conclusions. Osmotic stress in the form of exogenous ABA and PEG favored the maturation of somatic embryos in the three species evaluated, being the first report of SE in *A. salmiana* and *A. cupreata*.

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EFFECT OF INDOL-ACETIC ACID ON THE EXPRESSION OF DIRECT SOMATIC EMBRYO IN *Agave cupreata*

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Keywords. *Agave cupreata*, embryogenesis, explant

Introduction. *Agave cupreata* is an endemic plant of the state of Guerrero, México with great importance in the production of mezcal. However, its excessive collection for the production of this drink affects the reproduction of the species and decreases populations. An alternative that favors mass multiplication is through of *in vitro* direct somatic embryogenesis (DSE). An advantage of DSE is that its genetic stability is maintained and it can be regenerated in a shorter time, in addition to this a factor has been involved that triggers the induction of DSE with an increase in the concentration of endogenous IAA in plant tissues.

The objective of this study was evaluate IAA at different concentrations on the embryogenic response of *Agave cupreata*

Methods. The explant used was the leaf, obtained from three-month-old *in vitro* *Agave cupreata* seedlings. The explants were cultured in a medium for the induction of direct somatic embryos (IDSE), which consisted of 25% MS salts (Murashige and Skoog, 1962), supplemented with 30 g/L of sucrose and gelled with 8 g/L of agar, vitamins L2 (Phillips and Collins, 1979). With five concentrations of indole acetic acid (IAA): 0.1, 0.5, 1.0, 2.0, 3.0 mgL⁻¹ and combined with the auxin dichlorophenoxyacetic (2,4-D).

Results and discussion.

A good embryogenic response was obtained in foliar explants of *Agave cupreata*. There is evidence that the biosynthesis of IAA is essential for the induction of ES to be carried out and the increase in AIA is the action that triggers the induction of somatic embryogenesis (Pérez-Hernández, 2016), for which five concentrations of IAA were tested of which only the concentration of 0.5 mgL⁻¹ reflected the presence of direct somatic embryogenesis in *Agave cupreata*.

Conclusions. Direct somatic embryogenesis induction in *A. cupreata* is possible. This regeneration pathway can also be used in breeding and/or conservation programs for this species.

Acknowledgements. The first author appreciates the support granted through the CONACYT scholarship (no.1108387), as well as the Autonomous University of the State of Mexico for the facilities granted for the realization of the research.

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Characterization of mayahuelin, a type I Ribosome Inactivating Protein from *A. tequilana* var. *azul* and its utilization in *Agave* phylogeny

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Keywords: *mayahuelin*, *RIPs*, *molecular phylogeny*

Introduction. Ribosome inactivating proteins (RIPs) are a family of proteins that bind to the large subunit of the ribosome, irreversibly blocking protein synthesis during the elongation phase. RIPs are found in fungi and bacteria, but they are more abundant in plants [1]. Plant RIPs are commonly classified into three types: type I, formed by a single chain named A of approximately 30 kDa; type II, are heterodimers formed by a chain A plus a B chain with lectin properties: these heterodimers have a MW from 56 to 65 kDa; type III (pro-RIPs), are inactive precursors that require proteolytic processing to obtain a functional RIP [1]. RIPs are involved in the plant response to abiotic stress, pathogens, and herbivory, but their mechanisms of action are unknown.

The spike leaves of *A. tequilana* var. *azul* contain large amounts of a type I RIP named mayahuelin, to honor Mayahuel, the goddess of agave plants, according to aztec mythology [2]. Mayahuelin has a natural aa substitution (Y76D) in one out of four aa that comprise its active site [2]. This substitution confers to the protein a moderate toxicity, suitable for biotechnological applications. Additionally, *Mayahuelin* nucleotide sequences can be used as molecular markers to resolve the phylogeny of the genus *Agave* [2].

The aims of this work were to evaluate mayahuelin effects on protein translation using an *in vitro* system, to derive molecular phylogenies within the genus *Agave* based on *Mayahuelin* nucleotide sequences, and to carry out bioinformatic data-mining to identify the entire RIP gene family in *A. tequilana* var. *azul*. The *A. tequilana* var. *azul* RIP family may have biotechnological use in the future to combat pathogens and/or increase resistance to stress.

Methods. Mayahuelin was purified from an *A. tequilana* var. *azul* plant by FPLC using a set of chromatographic methods. Its effects on protein synthesis were evaluated using luciferase as a reporter on a cell-free wheat-germ protein synthesis system.

Mayahuelin orthologs from *Agave* species were amplified by PCR. Phylogenetic reconstructions were

obtained using Bayesian inference and Maximum likelihood methods. RIP sequences from *A. tequilana* var. *azul* were mined from a cDNA library and from published transcriptome resources.

Results and discussion. Mayahuelin inhibited luciferase *in vitro* translation in a dose-dependent manner (IC_{50} : 10.53 nM), when tested on a wheat germ cell-free system. We performed a phylogenetic analysis including cultivars of *A. tequilana*, *A. rhodacantha*, and *A. angustifolia* plus wild populations within *Agave* with emphasis in the Rigidae group. Our data showed that *A. tequilana* var. *azul* is closely related to two wild populations from *A. rhodacantha* from Oaxaca and Sonora; this group formed a higher order clade with *A. angustifolia* var. *espadín* and two specimens of *A. rhodacantha* from Jalisco (wild) and Mexico City (ornamental).

We identified at least 35 unigene transcripts encoding RIPs in *A. tequilana* var. *azul*, the largest number reported to date in a single species. Three of these new RIPs also contained active site substitutions at Y76.

Conclusions. Mayahuelin is a translational inhibitor despite the Y76 substitution in the active site. Both *A. rhodacantha* from Sonora and Oaxaca are the closest wild relatives to *A. tequilana* var. *azul*. Some RIPs from *A. tequilana* var. *azul* might be used for defense against pathogens and as molecular markers to derive phylogenies within the Agavoideae subfamily.

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SYNERGISM BETWEEN PHYTOPATHOGENS CAUSE *Agave salmiana* DISEASES IN HIDALGO, MEXICO

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Keywords: Gray spot, Consortium, Synergistic interaction.

Introduction. *Agave salmiana* is economic and cultural importance for the native peoples, who used to obtain ixtle, food and beverage production like “aguamiel” and “pulque” and has a biotechnological potential due to nutritional value for develop functional foods and active components for food industry. Currently, agave plants have bacterial and fungal diseases like anthracnose. The aim of this work is to study the synergistic interaction between phytopathogens isolated from *A. salmiana*.

Methods. *A. salmiana* leaves with necrotic gray spots were collected from agaves in open-field plantations from Cardonal, Hidalgo, Mexico. The phytopathogens were isolated (Li et al. 2009). Molecular identification was done by 16s and ITS Metagenomics. Virulence tests were carried out inoculating in pairwise the phytopathogens in two hosts: apple fruit and healthy agave leaves, which were pricked and inoculated with 20 µl spore fungi suspension (10^6 conidia/ml) and bacterial suspension (10^8 cell/ml). The hosts were incubated at 26 °C; apples for 12 days and agave leaves for 30 days. Lesions were measured with a digital caliper and reported as the surface of an ellipse (Pariaud et al. 2009). The interaction was expressed as the ratio of the lesion-area of the coinoculation of pathogens (Experimental) between the summatory of the area of each one in pure culture (Expected).

Results and discussion. A microbial consortium was selected due that it was frequently isolated from the lesions (Fig.1). The following pairs show a significative positive interaction (synergism) in apple: *Alternaria* sp. (HG) + *Fusarium* sp. (HR), *Penicillium* sp. (HB) + *Fusarium* sp. (HN), *Enterobacter* sp. (BB) + *Alternaria* sp. (HG) and *Enterobacter* sp. (BB) + *Fusarium* sp. (HR) (Fig. 2a). While in agave leaves this effect exists in *Aspergillus* sp. (HN) + *Alternaria* sp. (HG), *Aspergillus* sp. (HN) + *Fusarium* sp. (HB), *Penicillium* sp. (HVE) + *Fusarium* sp. (HB), *Penicillium* sp. (HVE) + *Aspergillus* sp. (HN) and *Enterobacter* sp. (BB) + *Alternaria* sp. (HG) (Fig. 2 b).

On the other hand, *Enterobacter* sp. (BB) inhibited the virulence of *Penicillium* sp. (HVE), *Fusarium* sp. (HB) and *Alternaria* (HAV) in apple (Fig. 2a). However, in agave leaves this *Enterobacter* (BB) strain was no virulent but the combination with *Alternaria* sp. (HG) or *Aspergillus* sp. (HN) it was. The strain *Enterobacter* sp. (BA) in both hosts inhibited the virulence of all the fungi tested (Fig. 2b).

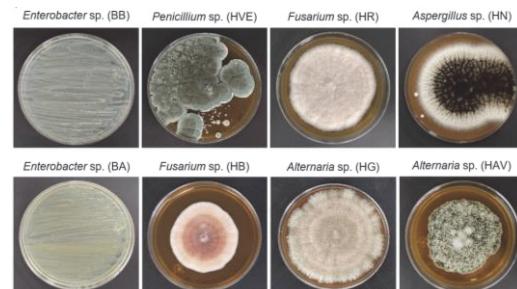


Fig. 1. Microbial consortium isolated from gray spot lesion.

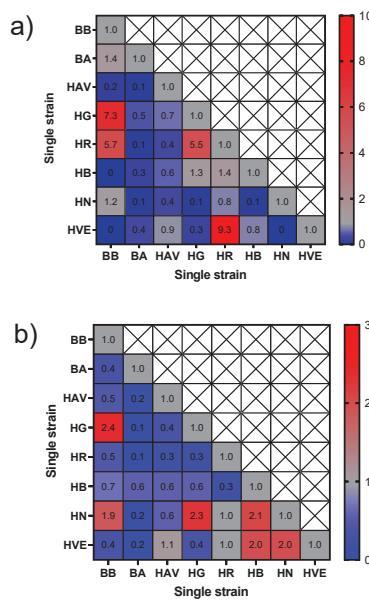


Fig. 2. Heatmap of synergistic interaction between phytopathogens in apple fruits (a) and in agave leaves (b).

Conclusions. Gray spot disease in agaves is the result of interactions within microbial consortium and not as individual and independent agents, indeed these synergic and negative interactions are a challenge as well as an opportunity for biocontrol strategies of diseases in agriculture.

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CONTROL OF VASCULAR WILT IN *Agave cupreata*, THROUGH BIOLOGICAL TREATMENTS

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Key words: Biological treatments, *Fusarium* sp., *Trichoderma* sp.

Introduction. *Agave cupreata*, the raw material for the production of a mezcal with a characteristic flavor, typical of the Balsas River basin in the states of Guerrero and Michoacán. An economically important phytosanitary problem that affects this species is "vascular wilt" attributable to complexes of *Fusarium* species (*F. oxysporum*, *F. solani* and *Fusarium* sp.), which has caused losses of 30 to 100% in the production of raw material for obtaining this drink. Currently, chemical control has been used to reduce the threshold of economic damage of this disease with the consequent environmental and human impact that this implies. Faced with this problem, this paper proposes the alternative use of preventive and sustainable biorational treatments such as biological control.

Objetive. To use preventive and sustainable biological treatments in the control of vascular wilt.

Methods. The dual culture technique was used where two biological treatments were tested, a chemical product as a control with three repetitions, namely: *T. harzianum*, *T. lignorum* and Pentamax on the *Fusarium* sp. (F1MCCR). A completely randomized statistical design with three treatments and three replications was used. After performing the analysis of variance, the DMS Test was used at 0.05 probability.

Results and discussion. In the results of the antagonistic tests, *T. lignorum* showed greater growth on the *Fusarium* sp. (F1MCCR), unlike *T. harzianum*, which grew more than the *Fusarium* sp. strain in 24-48 hours. (F1MCCR), from 48-96h there was no control, however, after 96h it returned to exercise control. In relation to this, Sarro *et al.*, 2011 and Tlapal *et al.*, 2014, agree when mentioning control of the genus *Trichoderma* sp. on different strains of *Fusarium* in *in vitro* tests.

Conclusions

The *Trichoderma harzianum* and *T. lignorum* strains presented effective control over the *Fusarium* sp. (MCCR1).

Acknowledgment. The first author thanks the Autonomous University of the State of Mexico for his training.

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Determination of yeast communities' succession and physicochemical changes in commercial pulque production from the Hacienda de Xochuca, Tlaxcala, Mexico

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Keywords Pulque, yeast succession, chemical analysis

Introduction. Pulque is a traditional Mexican fermented beverage with significant historical, cultural, and nutritional relevance, considered an icon of this country. It is produced by fermentation of sugary sap or aguamiel extracted from several *Agave* species, mainly *A. salmiana* [1]. Pulque production is associated with bacteria and yeast communities that metabolically interact to provide the physicochemical and sensorial features that distinguish pulque [1, 2]. This work aimed to elucidate both the yeast community succession and the main physicochemical changes throughout the production of commercial pulque from the Hacienda de Xochuca, Tlaxcala, Mexico.

Methods. From pulque production process 15 samples were collected. Each sample's physicochemical parameters were recorded *in situ*, and yeast viable cell counts (CFU/mL sample) were carried out by serial decimal dilution. The 85 isolated strains were identified by polyphasic taxonomy [3], and communities' succession was described with Shannon and Sorensen indices. HPLC technique was used to quantify the concentration of sugars, lactic and acetic acids, and ethanol, and GC-MS have been used to identify and quantify volatile compounds.

Results and discussion. By associating the physicochemical results with the yeast diversity indices of each sample, it was possible to characterize each fermentation stage and establish the succession of yeast communities throughout the entire process. Changes in yeast diversity were observed between samples. Non-Saccharomyces (*Candida boidinii*, *Clavispora lusitaniae*, *Kluyveromyces marxianus*, *Meyerozyma guilliermondii*, *Kazachstania gulospora* and *Zygosaccharomyces bailii*) were identified at the beginning, a community that synthesizes aromatic compounds that provide characteristics organoleptic to pulque [4]. In the later stages of fermentation, yeast diversity decreased as sugars were consumed and

ethanol concentration increased. The most persistent species in the whole process were *Saccharomyces cerevisiae*, *S. paradoxus*, and *Starmerella stellata*. These species constitute the main mycobiota of the inoculum used to induce the sap fermentation. Through fermentation process, the physicochemical parameters changes were pH 5 to 3; lactic and acetic acids 0.15 to 0.7% and 0.05 to 0.15%, respectively. In general, most sugars decreased, and ethanol reached 5.5% at final stage

Conclusions. The determination of the succession of the yeast communities in the pulque fermentation process allows us to understand the physicochemical changes associated with the final product, as well as to find and characterize species of biotechnological interest

Acknowledgements. The authors thank CONACYT for the master's scholarship awarded to Rodrigo Arredondo, and the owner of Hacienda de Xochuca, Mr. Guillermo Ramírez, and his workers for the facilities and support to carry out this research.

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COMPARISON OF THE PULQUE ASSOCIATED MICROBIOME FROM TWO DIFFERENT AGAVES.

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Keywords: Pulque, Metagenomic, Microbiome.

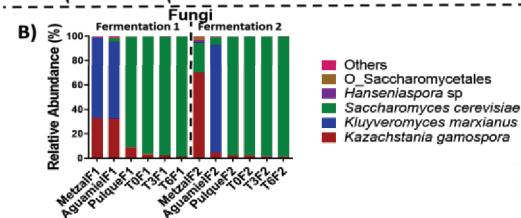
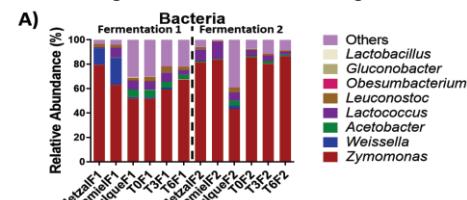
Introduction. Pulque is a traditional Mexican beverage made from the sap extracted from different species of Agaves. Previous studies on the bacterial and fungal diversity associated to this beverage applied different experimental strategies, culture-dependent, and culture-independent methodologies [1]. Recently omics studies have improved the resolution of the relative diversity of species already associated and have found new bacterial and fungal associated to pulque [2,3]. The objective of this work was to determine the core of microorganisms responsible of the fermentation of pulque regardless of the maguey (*Agave*) plant selected for sap (aguamiel) extraction.

Methods. Plant tissue of the cavity or cajete, where the maguey sap accumulates known as metzal and aguamiel were collected from two different maguey plants, as well as pulque (seed) used as starter. The fermentations were carried out for 6 hours at 28-30°C. Next generation sequencing of 16S rDNA gene amplicons from V3-V4 hypervariable regions and ITS1 were performed with Illumina MiSeq 2x250 and they were analyzed with QIIME software for taxonomic assignment using the SILVA database for bacterial and UNITE for fungi, as well as for diversity analyses. The quantification of fermentation metabolites was performed using HPLC with Aminex HPX-87P for carbohydrates and Aminex HPX-87H for organic acids and ethanol.

Results and discussion. The analyzed samples (aguamiel, metzal, and pulque) were on November 7th and 11th of 2019, obtaining 12 samples corresponding to plant tissue, aguamiel, pulque, and fermentation times (0, 3 and 6 hours, duplicated fermentations). The concentration of carbohydrates in sap decreased through fermentation; sucrose was the most abundant carbohydrate in both fermentations (34 g/L and 68 g/L, respectively). We detected an average of 27.5 g/L of ethanol for both fermentations and its concentration increase through fermentation time; the organic acids maintained less than 4 g/L. These results coincide with those reported by Chacón-Vargas [2], highlighting that they found 58 g/L of ethanol more than us. The results of the sequencing showed differences in the relative abundances of the bacteria founded, pulque samples showed more bacteria genus assigned, in the case of fungi, aguamiel and metzal samples presented the greatest diversity, and the dominant fungal species are

the same in both fermentations (Figure 1). The microorganisms founded had already been previously reported in 2 omics works, where their relative abundance was different to the values reported in this work. We found 7 genera of bacteria and 3 of fungi that are preserved throughout the fermentation process, among which are mainly *Zymomonas*, *Lactobacillus* and *Saccharomyces*. This core are genera have already been reported as potential probiotics, which could confer the pulque as a potential probiotic beverage [1].

Fig. 1. Bacterial and Fungal diversity.



Conclusions. We observed that there is a core of microorganisms that are preserved throughout the fermentation process regardless of the agave from which the aguamiel was obtained. It is also proposed to evaluate the core founded as a fermentation starter for the standardization of the pulque production process.

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COMPARISON OF FIVE FERMENTATION PROCESSES OF COMITECO

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Keywords: Comiteco, Agave, Yeast

Introduction. Comiteco is an Agave distillate produced in the Meseta Comiteca Tojolabal in the State of Chiapas, Mexico. It is obtained by the spontaneous fermentation and distillation of a mixture of *A. americana* L. sap (aguamiel), brown sugar (piloncillo), and water.

The objectives of this work were to analyze the association between the artisanal elaboration processes and the yeasts involved in the fermentation microenvironment, to identify and quantify the major volatile compounds and organic acids, and to analyze the principal components.

Method. Five artisanal processes were studied, three in 2017 and two in 2018, from two different producers; by seeding serial dilutions in petri dishes with WL medium added with 0.05% chloramphenicol. Yeast isolates were identified by their phenotypic, biochemical [1, 2], and protein profile by MALDI-TOF/MS. Total reducing sugars, major volatile compounds, and organic acids were identified and quantified by GC and HPLC [3, 4]. A Principal Component Analysis was also carried out.

Results and discussion. All the five comiteco elaboration processes followed the same general recipe and used the same raw materials, but each producer had a particular method to elaborate the beverage that affected the development of the mycobiota during fermentation; since yeast population varied between the processes studied in the two consecutive years. The diversity of yeast species found (Table 1) was low compared to other agave beverages [5]. Low levels of methanol, organic acids, higher alcohols, and high levels of aldehydes and esters were detected, mainly in process 4. Through PCA, PC 1 and 2; explained 63.59 % of the variance and the separation of the processes sampled in the two years was evident.

Table 1. Yeast species identified in the different fermentation processes

Species	Fermentative processes				
	1	2	3	4	5
<i>Kluyveromyces marxianus</i>	X	X	X		X
<i>Zygosaccharomyces bailii</i>	X				
<i>Hanseniaspora uvarum</i>	X	X		X	X
<i>Wickerhamomyces anomalus</i>				X	
<i>Wickerhamiella pararugosa</i>			X		
<i>Saccharomyces cerevisiae</i>				X	X
<i>Trichosporon asahii</i>				X	
<i>Geotrichum silvicola</i>	X	X			

Conclusions. The richness of yeasts species in the processes studied in the first year of sampling was higher than in the second, and only non-*Saccharomyces* species were identified, while in the second year *S. cerevisiae* was the dominant species. These results show that the particular method followed by each producer to elaborate comiteco plays an important role in the yeast diversity and chemical characteristics of the final product.

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METAGENOMIC CHARACTERIZATION AND EVALUATION OF VOLATILE COMPOUNDS IN ARTISANAL MEZCAL FERMENTATION FROM OAXACA STATE

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Keywords. Mezcal, metagenome, fermentation

Introduction. There is a wide range of alcoholic beverages derived from agave where the production processes vary between each region and where the microbiota involved in the fermentation stage is highly diverse⁽¹⁾. During alcoholic fermentations are produced a variety of volatile compounds, but the highest production of ethanol is generated by *S. cerevisiae*; however, the quantity and type of volatile compounds tend to increase by non-Saccharomyces yeasts⁽²⁾.

The objective of this study was to determine the impact of different *Agave* species upon the microbial consortium during the fermentation stage, and the volatile compounds production.

Methods. Samples corresponding to the beginning, half and end of fermentations using *A. americana* (F1) and *A. angustifolia* (F2), were obtained from a mezcal factory. The amplicon Library QC and sequencing were carried out using MiSeq Illumina platform. Subsequently, taxonomic identification was performed through Qiime 2022.2 platform on Linux using the Silva 138 99% OTUs full-length database for bacteria and UNITE (fungal ITS) version 8.3 for yeast. The concentration of ethanol and volatiles compounds were quantified by HS-GC. PCA statistical analysis was carried out using XLSTAT.

Results and discussion. Some differences were observed between the two fermentations, however in both cases the dominant yeast in these fermentations was *Hanseniaspora* followed by *Torulaspora*, this result is unusual because the most predominant yeast in fermentations is commonly *S. cerevisiae* (Figure 1). However, a higher relative abundance of *Torulaspora* was also observed in F1. Figure 2 shows the fermentations progress *Lactobacillus* increased in abundance while *Weisella* decreased.

Among the volatile compounds quantified, those that stand out most in the results was ethyl lactate, reaching 230 g/L in F1 and 300 mg/L F2 at the end of fermentations. Ethyl acetate in F1 the final concentration was 470 mg/L and 550 mg/L in F1. In both cases, these concentrations were higher than those reported in other fermentations. The efficiency of ethanol production during fermentations were between 38 and 47%. The PCA showed that there was a statistically significant difference between F1 and F2.

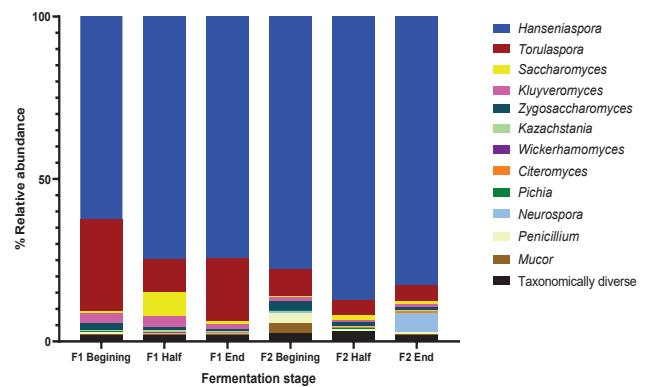


Figure 1 Relative abundance of Fungi

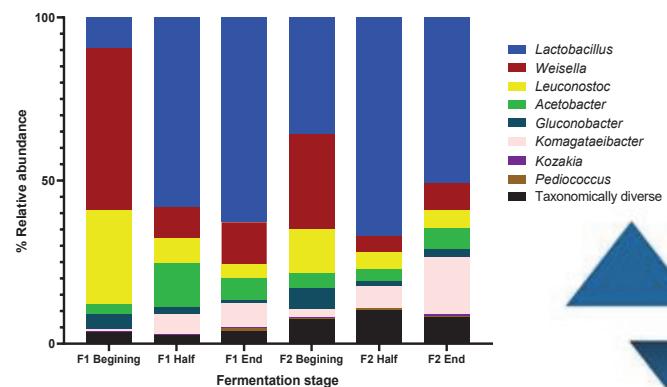


Figure 2. Relative abundance of Bacteria

Conclusions. These results show a statistically significant difference in the metagenomic analysis and the volatile compounds generated in fermentations using different types of *Agave*, but it is necessary to analyze more series to corroborate these results.

Acknowledgements. To CONACYT for the research grant and to the Projects CB 252665 and FORDECYT 296369.

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Pasteurization and sterile filtration techniques comparison for aguamiel decontamination. An effort to enhance shelf life of a highly perishable product.

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Aguamiel, microfiltration, shelf life.

Introduction. *Aguamiel* is the name given to the fresh sap from several *Agave* species when extracted by the scraping method [1]. The *aguamiel* is used mainly as substrate in the production of pulque (a fermented Mexican alcoholic beverage) and it has been associated to several nutritional and prebiotic properties [2]. Because of its high natural microbial load *aguamiel* is subjected to a fast microbial-related fermentation, which makes it to have a very short shelf life (just a few hours from collection) [3]. This issue makes *aguamiel* commercialization to be difficult and restricted to a small geographical area surrounding its point of origin.

In this work we evaluated two experimental approaches (pasteurization and sterile filtration) to reduce the natural microbial load of *aguamiel* and extend its shelf life without compromising its carbohydrates content.

Methods. Sterile filtration (0.45 um) and pasteurization (at 63 and 71°C) techniques were assayed over day freshly collected *aguamiel* from Huitzilac Morelos, México.

Microbial load reduction was evaluated by measuring CFU counts in YPD, MRS and DN media after each treatment. Changes in sugars concentration and FOS profile were measured by HPLC using an Aminex HPX-87 P/H and a CarboPac PA200 columns respectively.

Results and discussion. Both techniques: pasteurization and sterile filtration, were able to reduce the microbial load of *aguamiel* up to seven CFU logarithmic units (fig.1A). However, only sterile filtration was able to get *aguamiel* sterile, which allowed to extend the shelf life of filtrated *aguamiel* for at least 4 weeks, even when stored at 30°C.

Additionally to the microbial load reduction, sterile filtration clarified *aguamiel* until it got a crystalline appeal. This clarification was evaluated as the optical density change after filtration, which changed in three magnitude orders of Optical Density units (1 to 0.002 OD).

None of the evaluated techniques modified significantly either the sugars concentration of *aguamiel* (fig.1B), or its FOS profile (fig.C). Taken together, these results suggest that the prebiotic

character of *aguamiel* remains unaltered after such treatments.

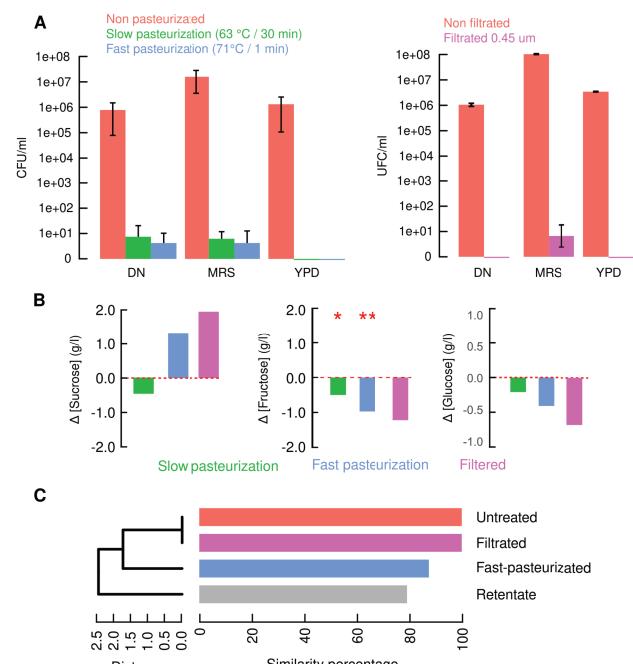


Fig. 1. Effect of pasteurization and sterile filtration over fresh *aguamiel* samples.

Conclusions. Sterile filtration is the technique with more benefits when used to reduce the microbial load of *aguamiel*. Because, besides eliminating the natural microbiota of *aguamiel* leaving its sugar and polysaccharide content unaltered, makes *aguamiel* to have a bright attractive look, which could give an aggregate value to the product.

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Discrimination of authentic tequila by some volatile compound markers

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Keywords: Agave, GC-FID, adulterated beverages

Introduction. According to Mexican law, tequila should be produced with the *Agave tequilana* Weber var. Azul, however, is frequently adulterated, mainly to make it with other sugars or added with alcohol and colorants. Also, the Mexican regulation evaluates only tequila's main compounds (high alcohols, methanol, esters, acetaldehyde, and furfural) to determine its quality. Some volatile compounds could help to discriminate the origin of tequila beverages. Ethyl esters, alcohols, furans, and terpenes have differentiated spirit beverages made with other agaves (Peña-Alvarez, 2006). This work evaluated some volatile compounds that could be markers of the authenticity of tequila.

Methods. The sample was prepared according to Mexican standards with slightly modified chromatographic conditions (NOM-006-SCFI-2012). Thirteen tequila 100% agave samples were analyzed, ten silver (S1-S10), ten rested (R1-R10) and ten aged (A1-A10), and other ten not authentic tequila samples, which were purchased in the local supermarket (N1-N10). The results were analyzed with PCA and cluster analysis.

Results and discussion. Figure 1 shows the PCA and cluster analysis. The PC1 on the positive axis was highly influenced by the following compounds: high alcohols, methanol, 1-propanol ethyl acetate, ethyl octanoate, esters, 2/3methyl-1-butanol, 2-methyl-1-propanol, 2-acetyl furan, 5-methyl furfural, and acetaldehyde. Authentic tequilas had high contents of these compounds. The furfuryl alcohol, perillyl alcohol, ethyl dodecanoate, and furfural were found on the negative axis and related to the unauthentic tequilas. Some of these compounds presented high content in the sample, not authentic tequila (N5, N6, N2, and N7). The scores plot from the two first PCs (Figure 1b) showed two groups: the first one grouped all not authentic tequila samples (negative axis of PC1), and the other one grouped the authentic tequilas. Figure 1c shows the dendrogram performed to discriminate the authentic tequila of the not authentic tequila. We observed the presence of four clusters which are identified as I, II, III, and IV. In clusters I, II, and III were grouped the authentic tequila samples, and in the cluster, IV were grouped all the samples of the unauthentic tequila.

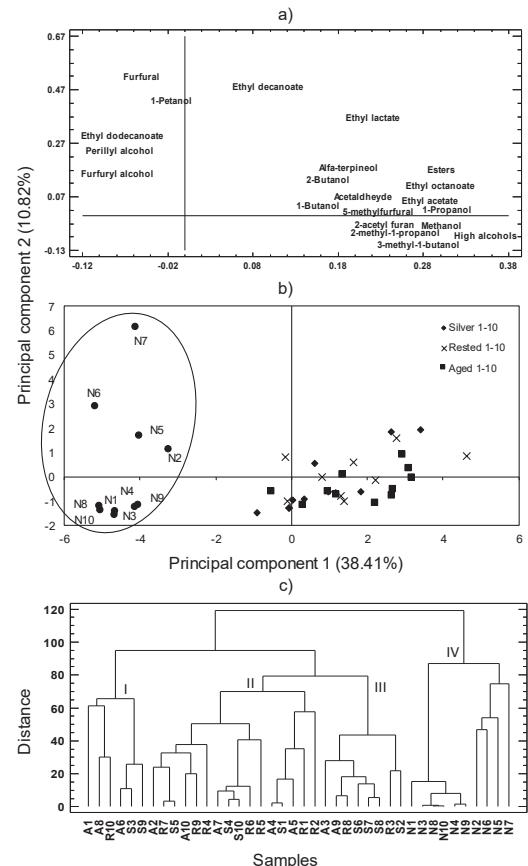


Fig. 1. Principal component analysis and cluster analysis. a) Loading plot for volatile compounds, b) Score plot from authentic tequila and not authentic tequila, c) Dendrogram plot of tequila samples

Conclusions. Compounds such as ethyl octanoate, 2-acetyl furan, perillyl alcohol, and alpha-terpineol are proposed as markers of tequila authenticity.

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FRUCTANASE PRODUCTION BY YEASTS IN AGAVE MEDIA.

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Keywords: Fructanases, Agave, Residues.

Introduction. Agave musts have abundant fructanase-producing microorganisms. Fructanases have high yields and low by-product generation in the production of fructose and fructooligosaccharides [1]. Fungi and yeasts are the most widely used microorganisms in the production of fructanases, being *Kluyveromyces marxianus* the most widely used yeast. Some species of *Pichia* and *Aspergillus*, among others, have also been used in large proportions for fructanase production [2].

In this work, the fructanase activity of different yeasts isolated from agave musts was analyzed, using raw agave processed leaves as substrate.

Methods. From a collection of strains, a pre-selection was made using chicory inulin as substrate. One strain of *K. marxianus* (Km1Y9), one strain of *C. lusitaniae* (Cl1AN4) and one strain of *P. mexicana* (Pm1AN3) were selected and tested on agave flour medium (FM) containing \approx 5.5 g/L sugars and on agave leaf extract medium (EM), containing \approx 5.8 g/L sugars. Cell growth was measured by plate count and fructanase activity by DNS methodology. Protein measurement by Bradford methodology was used to obtain specific fructanase activity.

Results and discussion. The growth kinetics of strains showed that, after 48 h, the strains are in stationary phase in both media.

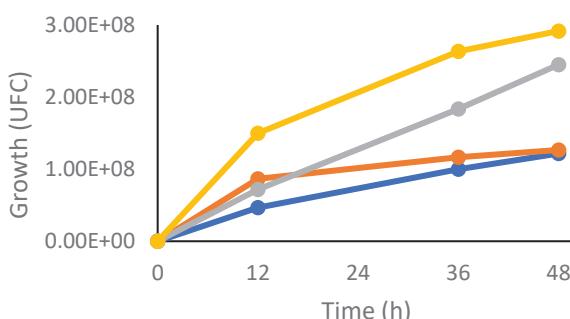


Fig. 1. Cell growth: ●=Pm1AN3 in EM; ●=Cl1AN4 in EM; ●=Km1Y9 in EM; ●=KM1Y9 in FM. EM= Agave leaf extract medium. FM= Agave flour medium.

In both media, Km1Y9 shows its maximum specific fructanase activity at 36 h and Cl1AN4 at 24 h.

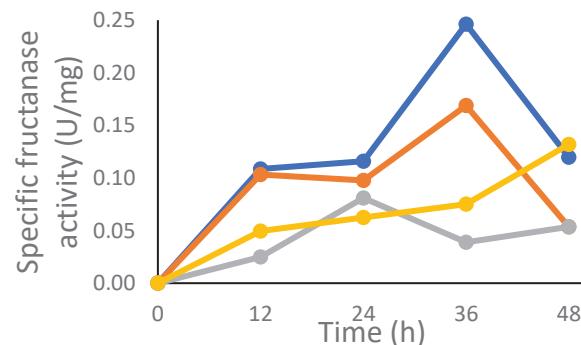


Fig. 2. Specific fructanase activity: ●=Pm1AN3 in EM; ●=Cl1AN4 in EM; ●=Km1Y9 in EM; ●=KM1Y9 in FM. EM= Agave leaf extract medium. FM= Agave flour medium.

Conclusions. The highest cell growth is shown in Pm1AN3, but strain Km1Y9 presented the highest specific fructanase activity in both media, but being higher on the agave flour medium. The results here indicate that the use of agave flour medium is the most convenient for fructanase production, and this also obviates the step of hot water extraction of the fructanes from the leave's tissues, being also more attractive from the economical point of view.

Acknowledgements. The authors acknowledge the CONACYT scholarship granted to EDCG, and the support of IPN projects SIP2022-0653 and SIP2022-1428.

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FOULING RESISTANCE OF AGAVE FRUCTAN ULTRAFILTRATION PROCESS USING CERAMIC MEMBRANES.

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lmoreno@ciatej.mx, **Keywords:** Fouling resistance, ultrafiltration, agave fructans.

Introduction. Membrane technology is a separation process used to fractionate agave fructans to obtain fructooligosaccharides (FOS) and high molecular weight fructans. However, the fouling of the membrane has not been studied, which is usually one of the main limitations of implementing this technology on an industrial scale. Fouling reduces the permeate flow (productivity) concerning time, increasing the cost of the process. Therefore, it is necessary to know the effect of operating factors on fouling. This work aimed to evaluate the temperature, transmembrane pressure (TMP) and fouling concentration during the fractionation process of agave fructans.

Methods. The experiments were performed in a pilot-scale filtration system with a TiO₂ ultrafiltration membrane of 1 kDa (inside-Céram, TAMI Industries, France). The experimental conditions were evaluated in the following ranges: Temperature (30-60°C), TMP (1-5 bar), and concentration (50-150 kg.m⁻³) on the fouling. The fouling resistances of the membrane system were expressed by the resistance-in-series model according to Eq.1 [1-2].

$$J_p = \frac{TMP}{\mu * R_t} \quad (1)$$

Where J_p is the experimental permeate flux with fructan solution, μ_p viscosity in the permeate and R_t is the total resistance of the membrane system. The total resistance of the membrane system (Eq. 2) is the sum of the intrinsic resistance of the membrane (R_m) and the fouling resistance (R_f) represents the reversible and irreversible fouling.

$$R_t = R_m + R_f \quad (2)$$

Results and discussion. The fouling membrane was evaluated in the agave fructan ultrafiltration process. The statistical analysis showed that temperature, TMP, and concentration were significant factors. The analysis revealed that temperature-TMP and TMP² have a significant effect ($p<0.005$) and R^2 of 92.06%. Figure 1 shows the major effects of the studied factors and their interactions in the fouling membrane process. The

lower the TMP and the concentration used, the greater the fouling; this is because, at 30°C, the molecules adopt their extended form, which makes it difficult for them to pass through the membrane, so if the TMP is low, it does not manage to overcome that resistance. However, it is observed that the resistance to fouling decreases with increasing concentration of fructans due to the decrease in the molar volume of fructans [3].

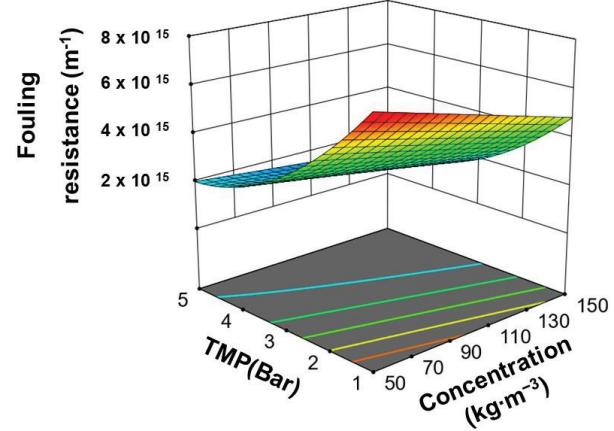


Fig. 1. Response surface plot for fouling as a function of TMP and temperature at 30°C

Conclusions. The feed concentration, temperature and TMP have a negative effect on fouling resistance. Therefore, it is required to operate at high levels to reduce fouling during agave fructans ultrafiltration.

Acknowledgments. The authors are thankful for the financial support of the project SEP-CONACYT 287926, México.

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EFFECT OF THE USE OF AGAVINS AND AGAVE SYRUP IN THE DEVELOPMENT OF A GUMMY

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Keywords: Prebiotics, functional ingredients, optimization.

Introduction. Agave fructans or agavins are branched polymers of fructose units with a sucrose unit. Those have been used like functional ingredients in different foods due to their technological properties and prebiotic effect. The agave syrup is a natural sweetener, used as functional ingredient. It has units of fructose, glucose, and FOS. Thus, those functional ingredients could be used in the formulation of candies like gummies.

The aim of this work was to formulate a gummy using agavins and agave syrup from *Agave angustifolia* Haw using a Central Composite Design.

Methods. It was carried out a Central Composite Design for the development of the gummy using the software STATISTICA. A texture profile analysis was performed using a texture analyzer (TA XT2) to obtain the dependent variables (hardness, cohesiveness, adhesiveness, gumminess, and springiness), the water activity (AquaLab 4TE) also was measured for the gummies. The values of a commercial gummy were established as reference to obtain the desirability specifications on the optimal formulation.

Results and discussion. The agavins (A) and agave syrup(S) showed a significant effect ($p<0.05$) on the dependent variables except for adhesiveness. It was observed that high amounts of A and S provide gummies with high a_w (0.85) and lower hardness (0.20 N) and gumminess (0.21) (Fig 1).

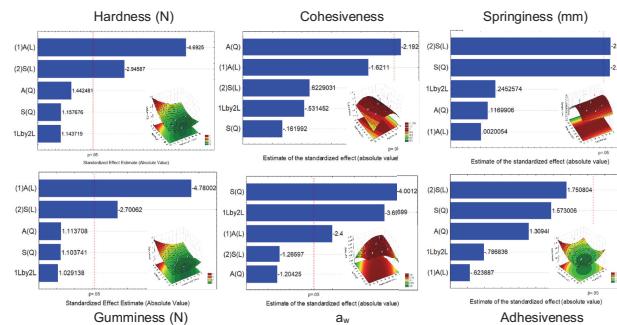


Fig. 1. Pareto charts of standardized effects and surface response of each dependent variable. A (Agavins), S (Agave syrup), L (Linear), Q (Quadratic), 1Lby2L (Interaction agavins and agave syrup in the linear form) $P < 0.05$.

This is because candies with high a_w promote less hardness [1], due to the high content of units of fructose of S and a high amount of OH [2] in A. Those ingredients are very hygroscopic promoting a high a_w and low hardness.

The desirability surface (Fig. 2) for obtain the optimal formulation shows that, the use of A and S give characteristics to the gummies close to the high desirability (1). Other formulations of gummies with different sugar and sweetener have showed that is possible to replace sucrose and glucose syrup without affect the mechanical and optical properties [3].

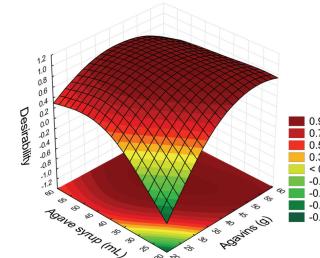


Fig. 2. Desirability surface for the optimal formulation.

Conclusions. The agavins and agave syrup are functional ingredients that can be used to replacing sucrose and glucose syrup without affect the mechanical and physicochemical properties in the formulation of soft sweets such as gummies.

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METEPANTLE (AGAVE & CORN PLANTATIONS): ANALYSIS AND PERSPECTIVES

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Keywords: Metepantle, agave agroforestry, integrated systems

Introduction.

Metepantle, from Náhuatl: *metl* = agave; and *pantli* = wall, is a millenary plantation system of Central Mexico where *Agave salmiana* rows separate lanes of corn plantations. This way a perennial CAM and drought resistant plant (agave) is combined with C3 & C4 annual plants (beans and maize). Agave is used as a cash crop and corn plants are used as subsistence crops. More than 10 thousand hectares of metepantle still subsist as the basis of peasant economies, but climate change and energy transitions may help to provide new perspectives of metepantle in 21st century Mexican economy.

Methods.

Present analysis is supported by a previous bibliographic survey [1] and field work in Valley of Mezquital, Hidalgo State and Nanacamilpa de M. Arista, Tlaxcala State.

Results and discussion.

Figure 1 shows satellite and landscape images of various metepantles in Nanacamilpa (Tlax.).



Figure 1A Satellite image of metepantles. 1B Field view of metepantle. Both, in Nanacamilpa de M. Arista, Tlax.

Measurement showed metepantle areas occupied by 25% *A. salmiana* and 75% corn (*milpa*) often as a mixture of maize and beans [2]. Table 1 shows that agave derivatives (*pulque*, *ixtle* & *lamb*) yield 94% of the estimated farmgate sales.

Table 1 Mass and farmgate sales

Product	Mgha ⁻¹	USD ha ⁻¹
Maize & beans	1.50	375 ¹ (5.6%)
Lamb	0.20	509 ² (7.6%)
Fiber (<i>ixtle</i>)	0.40	800 ³ (12.0%)
Beverage (<i>pulque</i>)	100 hL	5,000 ⁴ (74.8%)

Calculations: 1) Maize, 250 USDMg⁻¹; 2) Lamb, agave and maize roughage = 2.24 Mg, 11/1 conversion and liveweight price 2.5 USDkg⁻¹; 3) Ixtle, 4% in 10 Mg leaves, 2 USD kg⁻¹; 4) Pulque, 50 USDhL⁻¹.

Whereas maize & beans provide to a family of four, 3 kcal and 60 g per capita daily intake, balanced in energy and essential amino acids since maize and beans supplement each other (data not shown).

Present market conditions limit pulque consumption to less than 2 million hL because the market is dominated by barely malt beer (100 million hL). But agave sap can be concentrated into syrups for export and could be used as input to new fermentation industries. In the long run, food industry would need to substitute 250 ktons of polyethylene by bioplastics, such as polylactic acid. Also, there is a deficit of 25 ktons of frozen lamb meat and a growing demand for vegetable fibers in many household items. These results show that metepantle is an ancient agricultural system well adapted to climate change since agave plants are drought resistant and provide a good level of farmgate sales to traditional producers. Such sales are commensurate to the average remittance by Mexican migrants living in USA. Therefore, this is a strategic alternative to alleviate poverty and reduce migration. Maize & beans harvesting may be marginal in the commercial sense but are essential to family subsistence and provide a rationale for metepantle mixed agriculture.

Future demand of bioplastics and other biotechnological derivatives may provide the basis for metepantle expansion in the semiarid regions of Central Mexico.

Conclusions:

It is important to understand and support the present and future of metepantle development because it provides a significant alternative to reduce rural poverty, to adapt to climate change and to support future biotechnological development in the field of food and bioplastic fermentations.

Acknowledgements. This study was partially financed by CONACYT grant FORDECYT-PRONACES /1312404/2020

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INITIAL ASSESSMENT OF AGRO-INDUSTRIAL LIQUID WASTES FROM AGAVE *fourcroydes* LEM. AS PREBIOTIC.

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Keywords: Henequen, prebiotic, agroindustrial liquid waste.

Introduction. In Yucatan, the main use given to henequen (*Agave fourcroydes Lem.*) is to obtain natural fibers. During the artisanal mechanical defiber process the fructo-oligosacharides contained in the henequen leaves are released to the leachate by the action of the added water. These agro-industrial liquid wastes (ALW) contains $4.47 \pm 0.63\%$ of soluble fiber and could be a potential source of prebiotics [1].

The aim of the work is to assess the safety and resistance to conditions of the gastrointestinal tract of liquid wastes from *A. fourcroydes* processing.

Methods. The ALW from *A. fourcroydes* were collected in a defiber factory ubicated in Holactún, Yucatán ($20^{\circ}52'32''N$ $89^{\circ}19'45''O$). The ALW were centrifuged at 4700 rpm, $10^{\circ}C$, for 20'; subsequently, the supernatant was freeze-dried at 0.200 mbar and $-50^{\circ}C$. The lyophilized liquid waste was physicochemically characterized and for saponin quantification the vanillin-sulfuric acid assay was used [2]. The hemolytic activity was evaluated on commercial blood agar plates with 5% of erythrocyte suspension using quillaja bark saponin as control (+) [3]. Finally, the *in vitro* digestibility was evaluated in the simulations corresponding to the stomach (pepsin) and the small intestine (pancreatin, bile salts and pancreatic lipase) [4]

Results and discussion. The results of the characterization of powder from ALW are shown in the table 1.

Table 1. Characterization of lyophilized powder from agroindustrial liquid wastes from *A. fourcroydes*

Color Parameter		Chemical Parameter	
L*	67.22 ± 0.32	Aw	0.84 ± 0.02
a*	-5.21 ± 0.02	Protein (%)	1.02 ± 0.00
b*	37.17 ± 0.26	Total sugars (g/L)	17.09 ± 0.13
C*	37.53 ± 0.26	Saponins (g/L)	15.97 ± 1.21

L* Luminosity (B/W) a*red/green component b*yellow/blue component C* saturation

In the hemolysis test the lyophilized ALW at concentrations below 5g/L did not showed any hemolytic activity. At 20 g/L a hemolytic halo was found (11.87 ± 0.32 mm), while the control of quillaja bark saponin presented a larger hemolysis diameter of 14.57 ± 0.11 mm at the same concentration.

In table 2 the results of the *in vitro* digestibility test are showed.

Table 2. *In vitro* digestibility of FOS contained in the lyophilized ALW of *A. fourcroydes*

Before digestion g/L	Stomach g/L	Small Intestine
1.904 ± 0.050^a	1.905 ± 0.133^a	1.807 ± 0.049^a

Different letters in the same row show a statistically significant difference ($p \leq 0.05$)

Conclusions. The ALW were able to resist degradation by pH changes and the effect of digestive enzymes during *in vitro* simulation, also hemolysis was detected, due to the presence of saponins (15.97 g/L) in ALW solutions at concentrations > 5 g/L.

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POTENTIAL TO PRODUCE BIOHYDROGEN AND BIOGAS FROM AGAVE TEQUILANA BAGASSE: EFFECT OF TEQUILA PRODUCTION PROCESS

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Keywords: Bioenergy, Biofuels, Lignocellulosic biomass, Renewable energy, Sustainability

Introduction. The generation of *Agave tequilana* bagasse (AB) during tequila production process is of economic, social and environmental concern. In 2021, AB waste reached 0.7 million tons, being generated mainly by two tequila production processes.^[1] In the traditional process, AB results from autoclaved pinas (AAB), while in the non-cooked agave process, AB is generated by a diffuser (DAB). The valorization of AB through biofuels production is able to bring positive environmental effects and local and regional development, additionally alleviating problematic disposal problems. The present study investigated for the first time how the use of ABB and DAB might impact on the production potential of biohydrogen and biogas.

Methods. The dried and grinded lignocellulosic materials were pretreated by ozonolysis and then subjected to enzymatic hydrolysis using a binary enzymatic mixture. The hydrolysates obtained were further tested for biochemical hydrogen (BHP) and methane (BMP) potential tests production using the AMPTS II (Automatic Methane Potential Test System, Bioprocess Control), following the methodology described by Holliger et al. [2] for BMP and Carrillo-Reyes et al. [3] for BHP. Anaerobic granular sludge from a full-scale digester treating tequila vinasse was used as the methanogenic inoculum. It was pretreated by heat shock (105° C for 24 h) and then used as the acidogenic biocatalyst. An inoculum-to-substrate ratio of 4:1 (on a volatile solids (VS) basis) was set.

Results and discussion. Observed hydrogen production did not show significant differences between AAB and DAB hydrolysates (average 99.6 ± 8.9 NmL H₂/g VS_{fed}) (Fig. 1A). However, some differences in the kinetics were recorded, as revealed the modified Gompertz model. Particularly a higher H₂ volumetric production rate of 71.6 ± 11.6 NmL/L-h was attained using DAB. On the other hand, AAB supported the highest methane yield of 269.3 ± 8.1 NmL CH₄/g VS_{fed}, which was 10% higher than that of DAB (Fig. 1B). Interestingly, short lag phases were computed in the

BHP and BPM assays regardless of the type of AB tested, pointing out the effectiveness of the integrated pretreatment to release easily fermentable organic compounds.

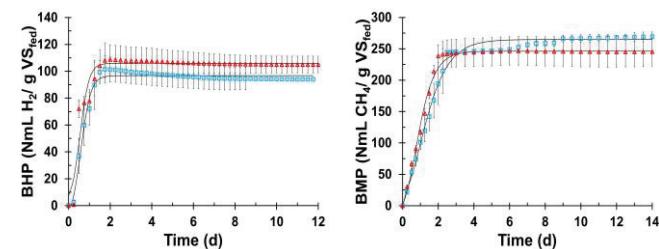


Fig. 1. A) BHP and B) BMP of AAB (■) and DAB (▲). Solid lines represent the predicted data by the Gompertz model.

Conclusions Comparatively, no significant differences were observed in the hydrogen production potential of AAB and DAB, which yielded on average 100 NmL H₂/g VS_{fed}. Regarding biogas production, the use of AAB resulted in a slightly increase (10%) in the methane production potential (270 NmL CH₄/g VS_{fed}) compared with DAB. Therefore, both types of AB tested can be used as biohydrogen and biogas feedstocks, although further process optimization, scaling-up, and techno-economic and environmental assessments are still needed. Overall, the deployment of AB-based biorefinery schemes producing biohydrogen and/or biogas is an opportunity to revalorize such a residue while recovering renewable energy, thus promoting more sustainable tequila industries.

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LAM PROJECT: AN ALTERNATIVE FOR THE CONSERVATION OF REGIONAL AGAVES IN OAXACA

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Keywords: Proyecto LAM, agaves, conservation, sustainable management.

Introduction. Mexico has the greatest diversity of agaves in the world, 210 species are distributed naturally and 176 are endemic to the country. In Santa Catarina Minas Oaxaca, since 2018 the LAM Project has been developed, which is part of the Mezcal de los Angeles Family Cooperative, a cooperative that has a mezcal tradition of more than 100 years in the production of maguey and mezcal. Given the lack of information to counteract the effects of pests and diseases on our crops, as well as information for the propagation of our agaves, we took on the task of documenting their biological and reproduction strategies, thus giving rise to the LAM Project, becoming in an arm of the family project where we set ourselves the goal of rescuing, conserving and propagating agaves through seeds. At the same time we document the processes of pollination, flowering and reproduction of the agaves that make up its bank of mother plants. This project is proposed as a proposal for sustainable and friendly management with agrobiodiversity, in addition to promoting the care and conservation of biodiversity, as well as the provision of ecosystem services, promoting polyculture and the use of organic fertilizers. LAM Project is proposed as a regional development model for the conservation, management and sustainable use of regional agaves through the systematization of their biological processes and friendly interactions with biodiversity, sharing their findings with other small producers in the region.

Methods. The two spaces where we house the mother plants are our on-site laboratory, from which we systematically document the process of propagation and reproduction of the agaves in the collection. Each year the activities to be documented are determined for three main topics: 1.-reproduction, 2.- germination and propagation, and 3.- pests and diseases. For 2021, the work focused on documenting the floral phenology of the agaves in the collection and the permanent monitoring of the incidence of pests and diseases to establish the dynamics of reproduction of pests, as well as their change in habits and the incidence of climate change.

Results and discussion. LAM Project has managed to form the first agave seed bank in Oaxaca. After the

documentation of the floral phenology, it was possible to determine one of the reproductive strategies of the plant consisting of the refraction of light during the night. It was found that, during the night, the flowers are more fertile and show changes in their structure in order to catch the pollen that arrives. Regarding the incidence of pests, it was confirmed that the weevil continues to be the main pest for agave crops, even in those that are managed in polyculture, in addition to the fact that the insect has changed its attack system on the plant, doing so now from the base, which decreases the possibility of being detected in time to avoid the death of the plant and the increase in the population.

Conclusions. Documentation of floral phonology confirms that agave flowers are most fertile at night, which means that pollinators of this species are nocturnal. Trapping for a consecutive year shows that there is a presence of weevil throughout the year, which shows an important change in the behavior of this pest, which is worrying as it is the pest that most affects agave crops throughout the country.

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BIOCHEMICAL CHARACTERIZATION OF THE LIQUID RESIDUE OBTAINED BY MECHANICAL DECORTICATION OF *Agave salmiana* LEAVES.

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Keywords: *Agave juice, decortication, and saponins.*

Introduction. *Agave salmiana* is currently used to produce *pulque*, a beverage made of fermented agave sap (*aguamiel*). Remaining *A. salmiana* leaves are thicker (15 cm) than *A. sisalana* leaves (2 cm). Hence decortication of the former, requires mechanical flattening with 30% yield of juice that has not been characterized yet. Here we confirm the presence of saponins and sapogenins in such liquid residue.

Methods. Leaves of *Agave salmiana* were decorticated, using a small machine specially designed for such a purpose. The residual liquid (agave juice) was collected and stored frozen. Protein [1], total carbohydrate [2], reducing sugars [3] and pH were measured. Saponins extraction was done using the following solvents: hexane, methanol, chloroform, butanol, and ethyl acetate, by the incubation of equal volumes of juice and each solvent with constant stirring at room temperature by half a day. Saponin and sapogenin presence were confirmed using TLC with mobile phase made of ethyl acetate: ethanol: water: ammonia (65: 25::9::1). The plates were developed by spraying 1% anisaldehyde and 10% sulfuric acid in methanol; followed by steady heating at 100 °C.

Results and discussion. The pH was 5.5 ± 0.2 . Analysis showed (g/L) sugar concentration 189.93 ± 3.38 ; and reducing sugars, 50.72 ± 2.5 ; showing that nearly 75% of CHO are in the form of complex molecules that could correspond to a variety of fructans.[4] Protein content, was $105.9 \text{ g/L} \pm 13.7$ with an estimated C/N ratio of 7.0, higher than reported values for whole *A. salmiana* leaves [5]. TLC analysis showed two major spots with R_f values, 0.1 and 0.5, compatible with steroidal saponins and sapogenins, similar to previous reports [6, 7]. Best extraction solvent was butanol because it yielded a sharper TLC spot resolution (Fig. 1 B). Future structural identification and dosage will be done using reference saponin and sapogenin samples and GC mass spectrographic analysis. Extrapolation of this experience requires development of large-scale decortinating machinery adapted to *A. salmiana* leaves, which is currently under way.

Conclusions. It is shown that agave juice obtained as a by-product of mechanical decortication of *Agave salmiana* leaves can be used as a source of steroidal

saponins and sapogenins. Therefore, industrial decortication of *Agave salmiana* leaves can be considered as a potential source of such biopharmaceuticals for a variety of future applications.

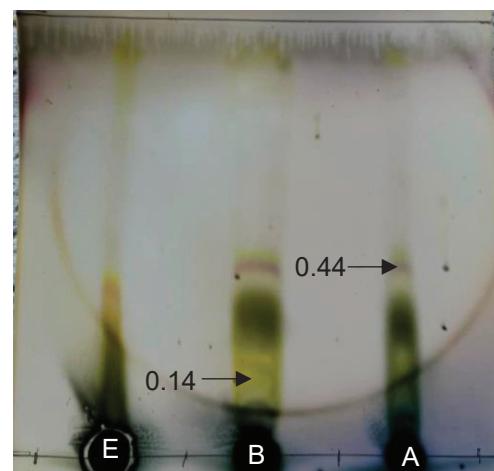


Fig 1. TLC of three extraction of agave juice using different solvents, E: Ethanol, B: Butanol and A: Ethyl acetate. Number indicates the R_f

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SCALE-UP OF LIGNOCELLULOSIC ETHANOL PRODUCTION FROM AGAVE BAGASSE

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Keywords: *Lignocellulosic ethanol, agave bagasse, pilot production*

Introduction. Lignocellulosic ethanol has been of special interest in the last years due to it does not compromise food safety and has a low environmental impact. Agro-industrial wastes can be used for ethanol production. One of the most important agro-industrial wastes in Mexico is *Agave tequilana* weber var. azul bagasse (ATB), which is generated as a by-product of the tequila beverage production process. ATB is produced in large quantities, and its current disposal is a pollution problem, therefore, is necessary its revalorization [1].

The objective of this work was to evaluate the production of lignocellulosic ethanol from ATB at a pilot scale of 35 and 200 L.

Methods. ATB was pretreated by autohydrolysis in a continuous tubular reactor [2]. Enzymatic hydrolysis experiments were carried out in stirred tank reactors of 35 and 200 L with 10% of solids loading at 50 °C, pH 5.0, and constant stirring. Fermentations were carried out in the same reactors by lowering the temperature of the hydrolyzed material to 30 °C and adjusting the pH to 4.5. *Saccharomyces cerevisiae* Ethanol RED was inoculated to a final concentration of 20×10^6 cells/mL. Carbohydrates, organic acids, furans, and phenols were quantified by HPLC, as well as ethanol and volatile compounds by gas chromatography [3].

Results and discussion. Figure 1 shows the kinetic profiles of sugar release during enzymatic hydrolysis of ATB (A) and sugar consumption in the fermentation process (B) for the two reactors (35 and 200 L).

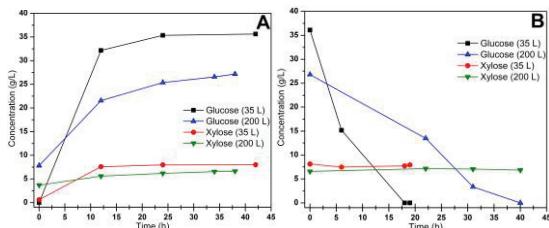


Fig. 1. Release and consumption patterns of glucose and xylose in the enzymatic hydrolysis (A) and fermentation process (B).

As shown in Fig. 1, the maximum glucose concentration reached in the 35 L reactor was 35.61 g/L. On the other hand, in the 200 L reactor, the maximum glucose

concentration was 27.16 g/L, reaching a steady state at 24 h of operation. Regarding the fermentation process, glucose depletion in the 35 L reactor was obtained at 18 h, however, in the 200 L reactor, it was detected between 30 and 41 h.

Table 1 contains the kinetic parameters of the enzymatic hydrolysis and fermentation process at the two production levels.

Table 1. Kinetic parameters of ATB's lignocellulosic ethanol production in 35 and 200 L reactors.

Reactor	Hs (g/Lh)	Qs (g/Lh)	Qp (g/Lh)	Ethanol (g/L)
35 L	2.680	1.677	0.971	18.462
200 L	1.795	0.587	0.352	15.142

Hs: Volumetric rate of glucose production in enzymatic hydrolysis.

Qs: Volumetric rate of glucose consumption in fermentation.

Qp: volumetric rate of ethanol production in fermentation.

According to table 1, the highest ethanol productivity was achieved in the 35 L reactor with 0.971 g/Lh, however, for the 200 L reactor, only productivity of 0.352 g/Lh was obtained. The differences in the release of sugars are attributed to poor transfer processes due to the rheological properties of the system at 200 L. Similarly, the ethanol yields could be because of the presence of inhibitory compounds in the fermentation broth.

Conclusions. ATB is a promising alternative for bioethanol production; nevertheless, multiple research and development efforts are still required to achieve techno-economically feasible productivities.

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ENZYMATIC HYDROLYSATES OF AGAVE BAGASSE PRETREATED WITH IONIC LIQUIDS: SACCHARIFICATION EFFICIENCY AND HYDROGEN PRODUCTION

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Keywords: Biohydrogen, ionic liquids, enzymatic hydrolysis.

Introduction. In recent years, the use of enzymatic hydrolysates of agave bagasse for the hydrogen (H_2) production has been studied. However, the use of pretreatments that allow biomass delignification has been implemented as a strategy with the aim of increasing the efficiency of enzymatic hydrolysis. There is a great variety of pretreatments that have been studied for the disintegration of lignocellulosic biomass. However, in recent years, the use of ionic liquids has been highlighted since they allow breaking and eliminating the crosslinked matrix of lignin and hemicellulose that embeds the cellulose fibers, resulting in an enzymatically hydrolysable material, rich in cellulose and hemicellulose (1).

In this way, the present study aims to evaluate at different scales the pretreatment of agave bagasse with [EOA][OAc] to subsequently use the enzymatic hydrolysates in a dark fermentation process for H_2 production.

Methods. The pretreatment of agave bagasse with [EOA][OAc] was evaluated by the methodology previously established (2). The biomass lignin percentage was calculated based on the NREL/TP-510-42618 procedure of the NREL. Enzymatic hydrolysis was carried out following the parameters previously established (2). The evaluation of H_2 production was carried out according to the specific H_2 production protocol issued by the Latin American Biohydrogen Network (3).

Results and discussion. The agave bagasse has a delignification greater than 20% ($28.64 \pm 1.73\%$). It has previously been shown that it is possible to obtain efficiencies greater than 90% in enzymatic saccharification with these delignification percentages (4). In this way, it was determined that regardless of the scale at which it is carried out, has a delignification that allows cellulose and hemicellulose to be more viable.

Table 1 shows the results of enzymatic hydrolysis using different reactor scales. When comparing the results of the enzymatic hydrolysis, it was determined that there is no significant difference between the final concentration of TC obtained with the different configurations used. Therefore, it is considered that the hydrolysis system with an operating volume of 25 mL is comparable to the system in which a volume of 6 L was used.

Table 1. Yields of saccharification of agave bagasse pretreated with ionic liquids

Biomass	Hydrolysis reactor	Final concentration TC (g/L)	Saccharification specific rate _{max} (g TC/L·h)	Saccharification efficiency (g TC/g bagasse)
Untreated bagasse	Serological bottles 125 mL (Operating volume 25 mL)	21.26 ± 1.23	1.14 ± 0.13	0.185 ± 0.011
	Helical reactor 10 L (Operating volume: 6 L)	26.12 ± 0.01	8.81 ± 1.03	0.227 ± 0.001
Bagasse pretreated-B	Serological bottles 125 mL (Operating volume 25 mL)	51.58 ± 0.49	3.79 ± 0.20	0.448 ± 0.004
	Helical reactor 10 L (Operating volume: 6 L)	51.38 ± 1.38	2.38 ± 0.25	0.447 ± 0.003

When evaluating the H_2 production from the enzymatic hydrolysates, there is no significant difference between the volumetric H_2 production rate and the H_2 maximum cumulative volume using the hydrolysate with untreated bagasse hydrolysate and pretreated bagasse (4.85 ± 0.28 L H_2 /L·d and 3.62 ± 0.86 L H_2 /L·d, 0.93 ± 0.03 L H_2 /L and 0.76 ± 0.04 L H_2 /L, respectively). However, the hydrolysate obtained with the pretreated bagasse, having a higher concentration of TC, requires a smaller volume of hydrolysate in dark fermentation systems.

Conclusions. Using the enzymatic hydrolysates of the agave bagasse pretreated with ionic liquids as a substrate allows to increase the yield of H_2 per kilogram of bagasse, this fact brings with it operational advantages, since, now of proposing a scaling of the H_2 production, it would be required lower volumes of hydrolysate for the operation of the fermentation system.

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EL APROVECHAMIENTO AGROFORESTAL MINIFUNDISTA DE LOS AGAVES.

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Palabras clave: pulque, bacanora, minifundios.

Introducción.

Los agaves han sido, por milenios, un complemento de la agricultura de subsistencia porque: a) Permitió la supervivencia humana durante las sequías extremas [1], b) Generó servicios ambientales [2] y c) Enriqueció la oferta de productos para el consumo local [2].

Para muchos pequeños productores, los derivados de los agaves son la única opción para sobrepasar el umbral de 100 mil pesos (5 KUSD) que establece un costo de oportunidad atractivo para evitar la migración campesina [3]. Su limitación principal es la forma, inequitativa de su inserción en el mercado [3]. Aquí se identifican algunas líneas de investigación y desarrollo que puedan mejorar los ingresos para los campesinos interesados en el aprovechamiento minifundista de los agaves.

Método.

Se recurrió a revisiones hemerográficas y trabajo de campo en los estados de Hidalgo, Sonora y Tlaxcala con entrevistas de productores, agentes comerciales y funcionarios.

Resultados y discusión.

1. **Producción de pulque (*A. salmiana*)**. Las plantaciones comerciales son muy lucrativas con ventas superiores a \$500,000/ha. Pero, desde 1960 el consumo se estancó en menos de 2 millones de hL. A diferencia de la cerveza la cual creció al 7% anual, hasta alcanzar 100 millones de hL. Esto explica el crecimiento del área con cebada para la cerveza y la disminución del área con *A. salmiana*, a pesar de que el pulque podría ser 10 veces más lucrativo que la cebada. Sin embargo, hay tres mercados emergentes: los jarabes de agave para exportación; las fibras duras, para sustituir bosas de plásticos, y, la carne de ovinos importada, para producir barbacoa. Lamentablemente, no hay oferta tecnológica para maquinaria, procesos y venta de esos productos en pequeña escala. Sobre todo, se carece de un servicio o financiamiento públicos para organizar cooperativas y empresas rentables de interés social.
2. **Producción de bacanora (*A. angustifolia*)** En los 35 municipios serranos de Sonora trabajan mil pequeños productores que en promedio venden 300 L de bacanora, licor artesanal parecido al mezcal, protegido por su

denominación de origen. Su precio, (descontando impuestos), varía de \$80/L hasta \$700/L, pero muy pocos productores han logrado registrar y certificar su bebida y son estos pocos los que controlan el comercio para su venta regional o de exportación, pues se carece de servicios públicos de bajo costo para ese fin, y los pequeños productores no pueden pagar los elevados costos de certificación ofrecidos por los laboratorios privados de ese ramo. Tampoco hay oferta de servicios públicos de crédito y organización para los pequeños productores de bacanora que representan el 90% de la oferta.

Conclusiones

El análisis de las cadenas del pulque y del bacanora, indica una deficiencia nacional de servicios públicos de asistencia técnica, de investigación y desarrollo de alternativas para la pequeña industria de los agaves. Esto dificulta la adaptación y aprovechamiento agroforestal minifundista de los agaves que sería sostenible, siempre y cuando se desarrollasen programas adecuados y participativos para ese fin. Ello permitiría crear una plataforma productiva, adaptada al cambio climático y generadora de empleo para mitigar la pobreza rural en las regiones semidesérticas, las cuales abarcan más del 40% de nuestro territorio y afectan al 80% de la población rural que viven en la pobreza extrema.

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THE MAGUEY: THE CULTURAL RESISTANCE OF ÑHÄÑHU PEOPLE IN THE ALTO MEZQUITAL, HIDALGO

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Introduction. The maguey has been important in the life of Mesoamerican people, and the cultural history of the Mexican plateau (1). The Maguey has been used in many ways as feed, home, clothes, fodder, fiber, and medicine. In addition, ecological benefits such as carbon capture and groundwater recharge (2). However, despite the maguey's cultural, social, environmental, and economic importance in the Ñhäñhu culture, its cultivation has decreased. The objective is to offer a vision of the devaluation of the maguey, to try to understand why the peasants continue to cultivate it in the Alto Mezquital.

Methods. The work was developed in the municipalities of Ixmiquilpan, Cardonal and Santiago de Anaya, belonging to the Alto Mezquital. A qualitative study was carried out, using data collection techniques such as semi-structured interviews ($n=100$) and in-depth interviews with key informants ($n=10$), as well as participant observation. The information was analyzed qualitatively.

Results and discussion. The permanence of the maguey is still maintained on a small and medium scale (2-35 hectares), because it is part of the peasant economy at the family and communal level. At the family level, temporary agriculture is practiced, through the establishment of maguey, and within its rows, the intercropping of corn, beans, squash, and other species associated with metepantle (milpa between rows of maguey trees), such as quelites. This strategy is part of the Ñhäñhu culture and its ways of life, highlighting the production and marketing of sheep barbecue, whose main ingredient is the maguey leaves. Pulque and maguey flowers are two products that are consumed frequently. However, pulque has been replaced by beer, coupled with the fact that there are few tlachiqueros and young people do not get involved in this activity. Other uses of the maguey are the penca for feeding sheep and for obtaining fiber (ixtle), as well as obtaining aguamiel to produce maguey honey, which has promoted its conservation, although a program is necessary for reforestation of the magueyeras to avoid the extinction of the species. Despite the commercial

pressure of this crop, its establishment prevails in an area of more than 1,500 hectares. In the region, there are efforts by the Government, state institutions, research centers and farmers, who have carried out collective actions around the Maguey, to promote and conserve this species. Some farmers have created cooperatives to find a local market. Despite fluctuations in sales prices, farmers persist in growing maguey varieties to meet their food and housing needs, as well as the balance of the agroecosystems in which they live and reproduce. In addition to the intensive use of the species, the problems observed in its different stages of development, such as the incidence of various insect pests, mainly the maguey weevil, put the permanence of the maguey at risk. Other typical pests of the crop are the "Chinicuil", the "White Worm" and the "Escamoles", which represent an economic gain. There are diseases such as gray spot, wilt, marginal spot, ring spot, and smallpox or bold, whose control is unknown.

Conclusions. The maguey continues to be part of everyday life and means of subsistence (partial or total) of the families of the Alto Mezquital. In this way, its cultivation does represent an economic alternative and can contribute to triggering the sustainable development of rural communities. However, the cultivation of maguey is uncertain, because it is still maintained on a small scale, but there are factors such as lack of interest, abandonment of the crop, looting of the leaves, pests, and diseases, migration, and aging of the field. endanger the permanence of this crop.

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POSTERS

POSTERS

I Scientific trends on Agave

IP1P	"IN VITRO CONSERVATION OF GERMPLASM OF MEXICAN AGAVES" <i>Eugenio Pérez Molphe Balch, Lucía Isabel Chávez Ortiz, Alberto Isaac Reyes Silva, Ma. de Lourdes de la Rosa Carrillo & Adilene Dávila Galván.</i>
IP2P	"IMPORTANCE OF IN VITRO HARDENING FOR A SUCCESFUL EX VITRO ACLIMATIZATION IN <i>Agave</i> spp" <i>Luisa Gutiérrez Quintana & Liberato Portillo.</i>
IP3P	"BENEFICIAL INSECTS AND PESTS OF THE AGAVE CROP, PERSPECTIVES AND NEW RESEARCH" <i>William David Rodríguez.</i>
IP4P	"IN VITRO PROPAGATION OF <i>Agave Potatorum</i> (TOBALÁ) BY DIRECT ORGANOGENESIS AND CALLOGENESIS" <i>Enrique Rodríguez de la Garza, Fátima Gutiérrez Tenorio, Mauro Alejandro Salazar Muñoz, Alejandro González Rodríguez, Daniela Magallanes Enríquez & César Armando Puente Garza.</i>
IP5P	"MICROPROPAGATION OF AXILLARY BUDS IN <i>Agave</i> spp.BY TEMPORARY INMERSION SYSTEMS" <i>Marlene Ortiz, Antonia Gutiérrez, Karla Vega, Manuel Rodríguez & Ernesto Tapia.</i>
IP6P	"EFFECT OF SALICYLIC ACID ON THE INCREASE OF BIOMASS AND SUGARS IN <i>Agave cupreata</i> TREL. & BERGER AND <i>Agave salmiana</i> GENTRY" <i>Ana María Roque Otero, Amaury Martín Arzate Fernández, Aurelio Domínguez López & Rubí Martín Arriaga.</i>
IP7P	"EFFECT OF PGPR BACTERIA ON THE DEVELOPMENT AND ACCLIMATIZATION OF VITROPLANTS OF <i>Agave cupreata</i> " <i>Eréndira Tututi Guillén.</i>
IP8P	"USO DE BIOESTIMULANTES A BASE DE ALGAS MARINAS SOBRE EL CRECIMIENTO DE PLANTAS MICROPROPAGADAS DE <i>Agave maximiliana</i> ". <i>Marco Valdés, Fernando Santacruz, Mireya Hernández, José Castañeda, Patricia Zarazúa & Lino de la Cruz.</i>
IP9P	"MORPHOLOGICAL DIVERSITY OF VARIETIES OF AGAVE IN RANCHO SAN ISIDRO, THE FIRST PULQUE PRODUCER IN THE STATE OF TLAXCALA, MEXICO" <i>Areli Flores Morales, D. Verónica Aquino Juárez, Eric Ocaranza Sánchez & Ángeles Sánchez Contreras.</i>
IP1V	"PROGRESS IN CRYOBIO TECHNOLOGY OF <i>Agave</i> spp" <i>Lourdes Delgado Aceves, Liberato Portillo & María Teresa González Arnao</i>
IP2V	"TEST FOR BIOCONTROL OF PHYTOPATHOGENS OF <i>Agave salmiana</i> IN TEMPORARY IMMERSION SYSTEMS" <i>Maria de Lourdes Pliego, Isabel Hernández, Ana Laura López Escamilla & Mayra de la Torre.</i>

POSTERS

I Scientific trends on Agave

IP3V	"GEO-LOCALIZATION OF DISEASES IN AGAVE" <i>Yaxk'in U Kan Coronado & Mayra de la Torre.</i>
IP4V	"GROWTH OF <i>Agave potatorum</i> UNDER BIOFERTILIZATION IN LOW FERTILITY SOILS" <i>Verónica Martínez Gallegos, Angélica Bautista Cruz, Jair Sanjuan Martínez & María Gabriela Ramirez Valadez.</i>
IP5V	"LEAF ANATOMY IN AGAVE SALMIANA SUBSP. SALMIANA (ASPARAGACEAE) FROM THE EDO. OF MEXICO, INTRASPECIFIC VARIATION" <i>Lorena E. Chávez Güitrón, Estela Sandoval Zapotitla, Florencia del C. Salinas Pérez, Ulises Rosas & Alejandro Vallejo Zamora.</i>
IP6V	"USE OF INMERSION TEMPORAL SYSTEM FOR MASSIVE PRODUCTION OF AGAVES" <i>Ana Collí Rodríguez, Gabriel Ojeda, L. Felipe Sánchez-Teyer & Itzamná Salas.</i>
IP7V	"MICROPROPAGATION OF AGAVE CUPREATA" <i>Gabriel Ojeda, Antonia Chan May, Emmanuel Maldonado Puc, Abril Chan May, Rocio Ake May, Rafael Chan Balam, Erik González Balam, Javier González Xool & L. Felipe Sánchez Teyer.</i>
IP8V	"STUDIES OF ORYZALIN ON MERISTEMATIC TISSUE OF AGAVE H11648" <i>Miguel Ángel Herrera Alamillo, María Teresa Álvarez Padilla, Gabriel Ojeda & L. Felipe Sánchez Teyer.</i>
IP9V	"POTENTIAL DISTRIBUTION OF THE WEEVIL AND THE AGAVES OF MEXICO" <i>René Bolom Huet, Gabriela I. Salazar Rivera, Anne Christine Gschaeidler Mathis & Jhony Navat Enríquez Vara.</i>

II Science and technology of Agave beverages

IIP1P	"CHARACTERIZATION OF THE MEZCAL FERMENTATION PROCESS IN FOUR FACTORIES AND TWO REGIONS WITH DENOMINATION OF ORIGIN IN MEXICO" <i>Patricia Alejandra Becerra Lucio, Hugo Ruiz González, Natalia Labrín Sotomayor & Yuri Jorge Peña Ramírez.</i>
IIP2P	"SPECTRAL FINGERPRINTING OF DISTILLED AGAVE BEVERAGES BY FTIR – ATR" <i>Diana Nefertiti Regla Corona, Julisa Edith Lopez Rodriguez & Pedro Martin Mondragon Cortez.</i>
IIP3P	"PHYSICOCHEMICAL EVALUATION OF TWENTY MEZCALS -CERTIFIED AND ARTESANAL- PRODUCED IN DURANGO" <i>Martha Rosales Castro, Sandra J. Alvarado Aguilar & Ma. Guadalupe Reyes Navarrete</i>
IIP4P	"ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOSITION OF PULQUE, A TRADITIONAL MEXICAN FERMENTED BEVERAGE FROM AGAVE" <i>Elizabeth Flores Rodriguez, Manuela del Rosario González González & Rita Miranda López.</i>

POSTERS

II Science and technology of Agave beverages

IIP5P	"SHELF LIFE AND ELABORATION OF A SYMBIOTIC BEVERAGE" <i>David Blanco & Beatriz Perez Armendariz.</i>
IIP6P	"CAPACIDADES FERMENTATIVAS DE LEVADURAS NO SACCHAROMYCES AISLADAS DEL PROCESO DE FERMENTACIÓN ALCOHÓLICA DEL BACANORA" <i>Luis Alberto Cira Chávez, María Isabel Estrada Alvarado, Laura Elisa Gassós Ortega, Sergio de los Santos Villalobos & Octavio Dublan García.</i>
IIP1V	"MINORITY BACTERIAL COMMUNITIES DURING THE FERMENTATION PROCESS OF MEZCAL FROM GUERRERO, MEXICO" <i>Yadira Evaristo Priego, Eneas Aguirre Von Wobeser, María Mayra de la Torre Martínez & Pavel Sierra Martinez.</i>
IIP2V	"METHANOL ANALYSIS OF ARTISANAL AND INDUSTRIALLY PRODUCED AGAVE WHITE SPIRITS" <i>César Iván Godínez Hernández, Joshimar Castro Aguilar & Juan Rogelio Aguirre Rivera.</i>

III Fructans and other agave products

IIIP1P	"AGAVE FRUCTAN METABOLIC PROFILES IN PLANTS OF Agave angustifolia HAW. UNDER TWO DIFFERENT CROP MANAGEMENT STRATEGIES" <i>Ruth E. Márquez López, Patricia Araceli Santiago García & Mercedes G. López.</i>
IIIP2P	"BIOFILM MADE FROM FRUCTANS BY AGAVE LACTIC ACID BACTERIA" <i>Nayeli A. Martha Lucero, Gustavo Viniegra González & Alma Cruz Guerrero.</i>
IIIP3P	"PHYSICOCHEMICAL AND SENSORY CHARACTERIZATION OF MAGUEY SYRUP FROM SINGUILUCAN AND CARDONAL REGIONS IN HIDALGO STATE, MEXICO" <i>L. Moreno Vilet, M.F. Aguilar Aguilar, S.E. García Barrón, J. Jaimez Ordaz & A. E. Cruz Guerrero.</i>
IIIP4P	"ANALYSIS AND CHEMICAL CHARACTERIZATION OF AN Agave angustifolia Haw EXTRACT RICH IN FRUCTANS" <i>Y.E. Camacho Rodríguez, S. V. Avila Reyes, B.H. Camacho Díaz, N. Monterrosas Brisson, M. L. Arenas Ocampo & A. R. Jiménez Aparicio.</i>
IIIP5P	"MAGUEY SYRUP: OPTIMIZATION OF THE ARTISANAL PRODUCTION PROCESS" <i>L.G. González-Olivares, L. Moreno Vilet, E. Contreras López & J. Añorve Morga.</i>
IIIP6P	"CHARACTERIZATION OF ACETYLATED AGAVINS OF Agave Angustifolia Haw" <i>Carolina Buitrago Arias, Brenda H. Camacho Díaz, Rita Martínez Velarde, Liliana Alamilla Beltrán & Antonio R. Jiménez Aparicio.</i>

POSTERS

III Fructans and other agave products

IIIP7P	"AGAVE FRUCTANS AS SUGAR SUBSTITUTES IN SOFT GEL FORMULATIONS" <i>Hugo Espinosa Andrews & Rogelio Rodríguez Rodríguez.</i>
IIIP8P	"VALORIZATION OF AGUAMIEL PRODUCED IN COMMUNITIES OF THE HIDALGO STATE: SUSTAINABLE PRODUCTION OF AGUAMIEL SYRUP RICH IN OLIGOFRUCTANS DESTINED FOR MEDIUM AND MEDIUM-HIGH ECONOMIC SECTORS" <i>C.J. Figueredo Urbina, S.E. García Barrón, A. Castañeda Ovando & L. Moreno Vilet.</i>
IIIP1V	"EFFECT OF CHEMICAL MODIFICATIONS ON THE ANTIBACTERIAL ACTIVITY OF AGAVE FRUCTANS FRACTIONS" <i>Dafne I. Díaz Ramos, Maribel Jiménez Fernández, Oscar García Barradas & Rosa I. Ortiz Basurto</i>
IIIP2V	"DESIGN OF A DOUBLE EMULSION WITH HIGH DEGREE OF POLYMERIZATION AGAVE FRUCTANS (AFHDP)/WPC FOR THE PROTECTION OF PROBIOTICS" <i>Naida Juárez Trujillo, Maribel Jiménez Fernández & Rosa I. Ortiz Basurto.</i>
IIIP3V	"IN VITRO EVALUATION OF AGAVE SALMIANA AND CHICORY INULIN FRUCTAN MIXTURES AS ANTICANCER POTENTIAL IN COLON CELLS" <i>Pa. Alvarez García, L.E. Alcantara Quintana, M.A. Ruiz Cabrera, Ra. González García & A. Grajales-Lagunes.</i>
IIIP4V	"MORPHOSTRUCTURAL CHARACTERIZATION OF <i>Saccharomyces boulardii</i> AGAVINS/WHEY PROTEIN BEADS OBTAINED BY IONIC GELATION" <i>María Sady Chávez-Falcón, Carolina Buitrago-Arias, Sandra Victoria Avila-Reyes, Roberto Campos-Mendiola, Brenda Hideliza Camacho-Díaz, Antonio Ruperto Jiménez-Aparicio</i>

IV Sustainable and integral exploitation of Agaves and sub products

IVP1P	"FOAMING CAPACITY OF Agave lechuguilla GUISHE" <i>Lorena Vargas Rodríguez, Jesús Vargas Medrano & Luis Fernando Medrano Fujarte.</i>
IVP2P	"LIMITATIONS AND PERSPECTIVES OF IXTLE ADDED VALUE CHAINS" <i>Evelyn Cázares Jiménez & Gustavo Viniegra González.</i>
IVP3P	"PRODUCTION OF FORAGE FROM LEAVES OF THREE AGAVE SPECIES IN A SEMIARID REGION OF NORTHERN GUANAJUATO" <i>Juan Teodomiro Frías Hernández, Ronnie Cummins & José Ignacio del Real Laborde.</i>
IVP4P	"PROTOTIPO COMERCIAL DE MEMBRANA BIOPOLIMÉRICA DE RESIDUOS DE LA AGROINDUSTRIA MEZCALERA PARA USO DÉRMICO" <i>Tania Indira Portillo Ayala, María del Pilar Buera, Argelia López Bonilla, Luz Arcelia García Serrano & Brenda Hideliza Camacho Díaz.</i>
IVP5P	"AGAVE SALMIANA SILAGE METAGENOMICS CHALLENGES EXTRACTING HIGH-QUALITY DNA FROM A COMPLEX MATRIX" <i>Fred E. Hernández Perea, Eneas Aguirre Von Wobeser & Mayra de la Torre.</i>

POSTERS

IV Sustainable and integral exploitation of Agaves and sub products

IVP1V	"EXTRACTION AND CHARACTERIZATION OF CELLULOSE FROM AGAVE BAGASSE" <i>Miguel Angel Lorenzo Santiago, Jacobo Rodríguez Campos, Silvia Maribel & Contreras Ramos.</i>
IVP2V	"DETERMINATION OF THE WATERPROOFING CAPACITY OF THE RESIDUE VINASSE OF THE MEZCAL INDUSTRY" <i>Luis Alberto Ordaz Díaz, Lizbeth Aguire Ramirez, David Enrique Zazueta Álvarez, Mónica Yazmin Flores Villegas, Maribel Madrid del Palacio & Ana María Bailón Salas.</i>
IVP3V	"INTRODUCTION OF <i>Agave salmiana</i> INTERCROPPED WITH FOREST AND FRUIT TREE SPECIES IN LERMA, MÉXICO, MEXICO" <i>Omar Franco Mora, Francisco J. Sandoval Figueroa, Susana Sánchez Nava, Jesús R. Sánchez Pale & Álvaro Castañeda Vildózola.</i>
IVP4V	"CHARACTERIZATION OF THE PRODUCTS OBTAINED FROM THE PIROLYSIS OF AGAVE CUPREATA BAGASSE" <i>Gonzalo Canche Escamilla, Natalia Uc León & Santiago Duarte Aranda.</i>
IVP5V	"DEVELOPMENT OF A MANUAL PROTOTYPE FOR MEAD EXTRACTION" <i>Patricia Luna Florencia Salinas, Rafael Hernández Socorro Ruiz & Lorena Chávez.</i>
IVP6V	"LIMITATIONS AND PERSPECTIVES OF IXTLE ADDED VALUE CHAINS" <i>Evelyn Cázares Jiménez & Gustavo Viniegra González.</i>
IVP7V	"COMPARATIVE LANDSCAPE ANALYSIS OF A TRADITIONAL AGAVE SYSTEM (METEPANTLE) vs. MONOCULTURE AGAVE SYSTEM" <i>Gilberto Hernández Cárdenas, Daniela Aguiña Islas & Gustavo Viniegra González.</i>

V Industrial Social, normative and ethnobotanic aspects

VP1P	"SOCIAL COMMONS OF MAGUEY PULQUERO IN HIDALGO MEXICO" <i>Jozelin María Soto Alarcón, Diana Xóchitl González Gómez, Sergio Erick García Barrón & Eduardo Rodríguez Juárez.</i>
VP2P	"MEZCAL FROM NUEVO LEÓN, WHOSE MEZCAL IS IT?" <i>Cuauhtémoc Jacques Hernández, Juan Alonso Ramírez Fernández & Eduardo Pérez Tijerina.</i>
VP3P	"THE EXCLUDING CHARACTER OF THE BACANORA APPELLATION OF ORIGIN CERTIFICATION AND ITS IMPACT ON THE COMMERCIAL NETWORK TOPOLOGY" <i>Vianey del Rio Guerra, Gustavo Viniegra González, Luis Alberto Cira Chávez, Sergio de los Santos Villalobos & María Isabel Estrada Alvarado.</i>
VP1V	"STANDARDS AND CERTIFICATIONS FOR CLASSIFYING AGAVE HONEY AS ORGANIC" <i>Mariana Calixto López, Luis Fernando Gómez Ceballos & Paola A. Fuentes Pérez.</i>

POSTERS TEMATICA I

Scientific trends on Agave

IN VITRO CONSERVATION OF GERMPLASM OF MEXICAN AGAVES.

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Keywords: *Agave*, *germplasm bank*, *in vitro propagation*.

Introduction. Agaves are a source of textile fibers, alcoholic and non-alcoholic beverages, sugars, and useful chemical compounds, as well as their great ornamental value. These plants are currently one of the most attractive options for substituting crops with higher water requirements. Many of the agaves that are exploited are not cultivated, so overexploitation of wild populations has placed many species under threat. This is aggravated by the low efficiency of their natural propagation systems. Plant Biotechnology has proven to be an important tool, both for massive multiplication and *in vitro* conservation of germplasm of *Agave* species.

The objective of this work was the establishment and maintenance of an *in vitro* germplasm bank that preserves 41 Mexican species and varieties of *Agave*.

Methods. *In vitro* cultures of the selected species were established, this through the disinfection of seeds or suckers, and their subsequent inoculation in culture medium. Subsequently, protocols for the *in vitro* propagation of these species were developed. This through the sprouting of lateral meristems in media added with cytokinins (1). Finally, protocols for the *in vitro* conservation of viable tissues through slow growth conditions were developed, this by adding osmotic agents such as mannitol and sorbitol to the culture medium (2).

Results and discussion. Protocols for *in vitro* propagation of species of interest were developed. Depending on the species, average multiplication rates from 8.6 to 35 shoots per explant were obtained, per 60-d culture cycle (Fig. 1a). At longer incubation times, such as 120 d, shoot production was greater than 100 per explant (Fig. 1b). This response was generated in medium added with low concentrations of cytokinins (1–2 mgL⁻¹ of BA, 2iP or metatopolin). No higher concentrations were used, nor were auxins added, to avoid somaclonal variation. The rooting of the generated shoots was carried out in basal medium with efficiencies higher than 90%. In the transfer to soil, an average survival of 84% was achieved. Finally, an *in vitro* germplasm bank was established and maintained, in which shoots of all species of interest are conserved. In this bank slow growth conditions are used to reduce its maintenance costs. For this, basal medium added with 50 gL⁻¹ of mannitol or sorbitol is used.



Figure 1. Shoot production from lateral meristems in explants of *Agave* stem. a) *A. horrida* var. *perotensis* after 60 d of incubation; b) *A. guinegola* after 120 d of incubation. In both cases, the stem segment with the shoots was cut in half for a better visualization of the response.

The species that are currently conserved in the germplasm bank are: *Agave americana* var. *comiteco* and var. *expansa*, *A. angustifolia*, *A. asperrima*, *A. attenuata* var. *dentata*, *A. bovicornuta*, *A. bracteosa*, *A. celsii*, *A. cerulata*, *A. chiapensis*, *A. colimana*, *A. cupreata*, *A. difformis*, *A. duranguensis*, *A. funkiana*, *A. gigantensis*, *A. guadalajarana*, *A. guinegola*, *A. horrida* var. *perotensis*, *A. karwinskii*, *A. kerchovei*, *A. lechuguilla*, *A. marmorata*, *A. nizandensis*, *A. obscura*, *A. ornithobroma*, *A. palmeri*, *A. parrasana*, *A. parryi* var. *huachucensis*, *A. peacockii*, *A. potatorum*, *A. salmiana*, *A. shawii*, *A. sobria*, *A. sobria* var. *roseana*, *A. stricta* var. *nana*, *A. tequilana*, *A. titanota*, *A. victoria-reginae*, *A. vizcainoensis* and *A. wocomahi*. It should be mentioned that the first species were incorporated into this bank in 2006, and their tissues are still viable and capable of multiplying *in vitro*.

Conclusions. It was shown that *in vitro* culture is a viable tool for the conservation of germplasm of the agave species included in this work. The material preserved under retarded growth can be propagated, also in *in vitro* conditions, when required.

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IMPORTANCE OF *IN VITRO* HARDENING FOR A SUCCESSFUL *EX VITRO* ACCLIMATIZATION IN *Agave* spp

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Keywords: agar, *ex vitro* establishment, maguey

Introduction. Due to the new increasing global demand of *Agave* (maguey) propagules, improving its micropropagation has become an urgent subject to science and with it proper acclimatization methods that ensure their *ex vitro* survival. It is well known that *in vitro* plants differ from field ones physiologically and anatomically increasing their vulnerability to field conditions [1]. The arid environment, where these plants are usually found, is harmful to the not acclimatized micropropagated plants and leads them to high mortality rates. *In vitro* hardening has been implemented as a technique that favors the acclimatization process of micropropagated plants [2]. The aim of this study was to demonstrate the benefits of implementing hardening cultures as part of the micropropagation process and to compare the effect of different types of *in vitro* hardening culture medias in the growth of three *Agave* species.

Methods. Two different experiments were carried out. First experiment. *A. tequilana* somatic embryos were obtained and grown using two strategies; one in liquid MS culture media (temporary immersion system) and the other using MS solid media. Later, the plantlets from both methods were transferred to greenhouse for *ex vitro* acclimatization and after 120 d the plants were observed to determine the most proper method with the lowest mortality rate and best desirable anatomic characteristics. Second experiment. Five types of solid LOG culture media with different concentrations of sucrose (30, 45, and 60 g/L) and agar (8, 10, and 12 g/L) were tested to grow plants of *A. tequilana*, *A.victoria-reginae*, and *A. rzedowskiana* during one month; the plant size (PS) and the root length (RL) were compared among treatments and later statistically analyzed by PERMANOVA test in order to determine which treatment conferred better characteristics to the plant.

Results and discussion. First experiment. As well as in other studies [3], the modification of the culture media as a hardening step before *ex vitro* acclimatization has proven to improve the survival rate. In this study the highest survival rate (97%) was obtained in the plantlets that were hardened in solid MS media in comparison to those grown in liquid MS media without any hardening (84%). Also, the plants from solid hardening showed a high significant difference ($P=0000$) in the length and

width (10.22 and 1.12 cm) in comparison to not hardened ones (7.59 and 0.56 cm) (Fig. 1A).

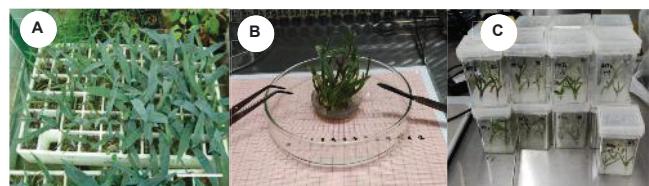


Fig. 1. *Agave* plants from two experiments. A) *Ex vitro* *A. tequilana* plantlets (left grown in liquid MS media, right grown in solid MS media), B) and C) Micropropagation of *Agave* spp for the second experiment.

Second experiment (Figs. 1B and 1C). Neither the PS nor the RL showed significant differences for the agar factor. Depending on the species, the addition of sucrose in culture media is thought to increase the nutrient function of persistent leaves and favor the rooting [1]. The results obtained showed that RL had an interaction with sucrose at 45 and 60 g/L with *A. tequilana* and *A. victoria-reginae*.

Conclusions. Implementing a hardening phase during the micropropagation process of *Agave* spp is beneficial to their *ex vitro* acclimatization because it improves its survival rate as well as their anatomical characteristics.

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BENEFICIAL INSECTS AND PESTS OF THE AGAVE CROP, PERSPECTIVES AND NEW RESEARCH.

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Keywords: *Insects, Appellation of Origin, perspectives.*

We present a synthesis of the insects reported associated with the crop of the genus *Agave* L. (Asparagaceae) with appellation of origin for spirits in Mexico, through a detailed review of published records. We found 89 species of insects collected in different *Agave* species. The natural enemies of insects collected in agaves were 71 species. The need for research on these plants and their associated insects is highlighted.

Introduction. The genus *Agave* L. is endemic to the American Continent and is one of the largest genera of the Mexican flora -200 species, 148 of them endemic (Trejo- Salazar et al. 2016) [1]. The *Agave*' species have been considered keystone species, offering food resources to wildlife and economic resources for humans (Lindsay et al. 2011) [2]. The objective is to provide a checklist of insects recorded as associated to *Agave* plants in Mexico and their natural enemies.

Methods. We constructed two checklists of insect species grouped by genus, subfamily, and family. Moreover, the checklists presented the geographical distribution, host name and biological notes and references. The first checklist includes the insects collected on plants of the genus *Agave* with appellation of origin for spirits in Mexico (Tequila, Mezcal, Raicilla and Bacanora), and the second checklist includes their natural enemies.

Results and discussion. In Mexico, there are 89 species of insects collected in *Agave* with appellation of origin. These species belong to 25 families and 5 orders (Table 1). The diversity of natural enemies of insects recorded in agave is 71 species (Table 2). Insects were found in 10 species of the genus *Agave*.

Table 1. *Insects collected in crop of the genus Agave with Appellation of Origin for spirits in Mexico.*

Orden	Families	Species
Coleoptera	11	59
Hemiptera	5	18
Hymenoptera	4	5
Lepidoptera	3	3
Orthoptera	2	4

Table 2. *Natural enemies of insects collected in crop of the genus Agave with Appellation of Origin for spirits in Mexico.*

Orden	Families	Species
Coleoptera	4	10
Diptera	1	1
Hymenoptera	7	56
Neuroptera	1	2
Thysanoptera	1	2

The species with the most recorded in agaves is *Scyphophorus acupunctatus* Gyllenhal, the species with the most recorded natural enemies is *Aonidiella aurantii* (Maskell). The natural enemy that attacks the most insects in the agaves with Appellation of Origin for spirits is *Scutellista caerulea* (Fonscolombe). Research on the natural enemies of insects in these crops is highly relevant to improve decisions about agricultural management, habitat conservation, and generate value to products and production chains.

Conclusions. Knowing the insect diversity and their natural enemies in agaves allows us to understand the effects of habitat transformation and provides information to understand the interspecific and intraspecific interactions that insects have with agaves in the different phenological phases and their environment. Many studies are missing to know the diversity of insects present in agaves.

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IN VITRO PROPAGATION OF *Agave potatorum* (TOBALÁ) BY DIRECT ORGANOGENESIS AND CALLOGENESIS

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Keywords: *Agave Tobalá, micropropagation, organogenesis, callogenesis*

Introduction

Agave potatorum, commonly known as Tobalá, is a wild species endemic to the state of Oaxaca. *A. potatorum* takes 8 to 12 years to flower, and does not reproduce asexually, rather by seed at the end of its gametophyte phase [1]. The use of *A. potatorum* for mezcal production is a threat to wild populations because most plants are foraged and used before floriation, which reduces the number of reproducers and the production of seeds, hindering the natural regeneration of the species [2].

Therefore, the aim of this project is to establish new and efficient *in vitro* methods for the reproduction of *A. potatorum* by direct and indirect organogenesis, in order to satisfy the demand of the mezcal market, which is expected to have a value of nearly 29 million USD and a growth rate of 7.99% by 2030 [3], while preserving the species by avoiding its predation.

Methods

One hundred plants of *A. potatorum* were obtained from *in vitro* germination. For germination, 300 seeds obtained from Loma Noble Agaves (Oaxaca, Mexico) were disinfected, subjected to mechanical scarification consisting of two cuts, one on each side of the seed, and then placed in germination medium (0.44 g/L Murashige Skoog, 12 g/L agar, pH 5.75-5.85) under a 12:12 photoperiod. After 2 weeks, the seedlings were transferred to propagation medium (4.4 g/L Murashige Skoog, 30 g/L sucrose, 4 g/L Phytagel, pH 5.75-5.85).

One month old plants, were transferred to propagation media containing 3 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D, Treatment 1) and 3 mg/L Naphthaleneacetic acid (NAA, Treatment 2) for callogenesis and 10 mg/L Benzylaminopurine (BA, Treatment 3) for direct organogenesis. The plants were cultured under 12:12 photoperiod at 30°C for 6 weeks.

Results and discussion

BA treatment resulted in the development of axillary shoots in 63% of the plants, averaging 11.05 ± 8.10 shoots per plant with no presence of calli. Individual shoots were turgent and elongated, with an average longitude of 1.69 ± 1.12 cm (figure 1, A). NAA and 2,4-D treatments resulted in the formation of callous tissue at the base of the corresponding plants (Fig. 1, B and C) varying mainly in appearance and texture (Table 1).

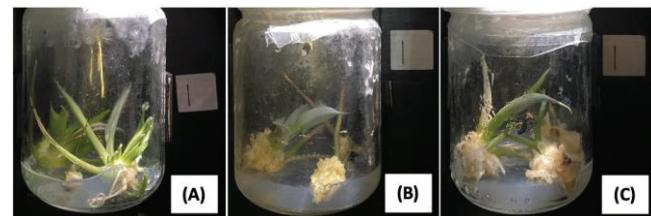


Fig. 1. Plant material obtained from treatment with BA (A), 2,4-D (B) and NAA (C).

Table 1. Callogenesis results for NAA and 2,4-D treatments. Values represent mean \pm stdv after 6 weeks. Different letters denote significant statistical differences ($p < 0.05$)

Treatment	% Calli induction	Callous mass (g)	Appearance
NAA	40.00	0.81 ± 0.56^a	Friable, white shade
2,4-D	56.67	0.99 ± 0.77^a	Compact, yellow shade

Conclusions

A reliable, profitable, and sustainable reproduction method is essential to satisfy the growing market of mezcal without endangering the different *Agave* species, such as *potatorum*. Throughout this research, it was proved that *in vitro* propagation methods can eventually fulfill this demand. The subsequent stage is to obtain full plants by the generation of embryos from calli, and the rooting of axillary shoots, that can then be adapted and used in the field.

Acknowledgements

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MICROPROPAGATION OF AXILLARY BUDS IN *Agave* spp. BY TEMPORARY IMMERSION SYSTEMS.

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Keywords: Bioreactor, TIS's, Biotechnology

Introduction. Agave propagation represents an important contribution to culture in Mexico, its production provides many jobs to the Mexican population due to its multiple applications in different industries: from obtaining fibers, food, and medicines, to its main use, which is the production of spirits. Conventional Agave cultivation methods present difficulties such as low development, heterogeneity in plantation and low or null availability of new shoots for new plantations [1]. An alternative for Agave sourcing is the micropropagation by Temporary Immersion Systems (TIS's). This semi-automated method, unlike conventional micropropagation, allows obtaining a larger number of plants, with characteristics of interest and better adaptability to *ex vitro* conditions [2].

Methods. Shoots of *Agave* spp. in proliferative stage of multiplication were cultivated in MS culture according to the methodology described by Robert *et al.*, [3]. Four different immersion frequencies (T1-T4) were evaluated in comparison with the semisolid system (T5). At 30 days, the Multiplication Rate (MR), and the quality of explants obtained were measured by height and diameter of shoots (cm) and number of leaves. Percentages of hyperhydric shoots and plants with quality compliance to send to rooting were also measured. The immersion frequency with the best results obtained was then evaluated on a larger scale at 30, 60 and 90 days, to validate that the treatment in TIS's is achievable for large scale production. The results were analyzed by variance analysis and Tukey's test in the Statgraphics software.

Results and discussion. Better results were obtained by Treatment 4 rather than Semisolid System and the other treatments in bioreactors in MR at 30 days (Table 1). These results had statistically significant differences (*p* value: 0.0000), therefore T4 was selected to be evaluated on a larger scale at 30, 60 and 90 days compared to the Semisolid System (T5).

Table 1. Multiple Range Test for MR by Treatment

Treatment	Cases	Method: 95.0 percentage Tukey HSD	
		Media	Homogeneous Groups
T5	5	1 X	X
T2	5	2.17 X	X
T1	5	2.38 X	X
T3	5	2.5 X	X
T4	5	3.7 X	X

Figure 1 shows that the number of shoots obtained at 30, 60 and 90 days was larger with the use of TIS's.

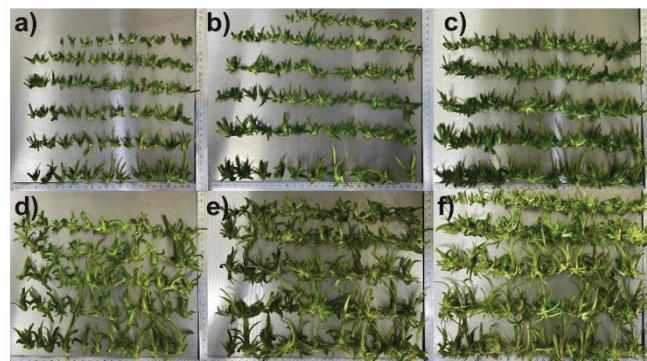


Fig. 1. Shoots at 30 (a), 60 (b) and 90 (c) days in treatment 5 (control) vs. shoots from Treatment 4 (Bioreactor) at 30 (d), 60 (e) and 90 (f) days.

Conclusions. The MR as well as the quality parameters of shoot diameter, height and number of leaves were higher in the temporary immersion systems compared to the conventional method. The renewal of the atmosphere inside the bioreactors prevents the accumulation of harmful gases such as ethylene, which improves tissue oxygenation [4]. Moreover, the intermittent contact with the culture media allows a greater use of the nutrients, and so the development of the Agave shoots in the Temporary Immersion System was highly favored.

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EFFECT OF SALICYLIC ACID ON THE INCREASE OF BIOMASS AND SUGARS IN *Agave cupreata* TREL. & BERGER AND *Agave salmiana* GENTRY

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Keywords: maguey, development, carbohydrates

Introduction. Agave has great economic, agroecological and cultural importance due to its multiple uses in which the production of distilled and fermented beverages stands out (García-Mendoza, 2011). The type and quantity of sugars contained in the central stem determines the quantity and quality of alcohol produced, however, the period to produce agave is so long (Bautista *et al.*, 2001). For this reason, alternatives focused on increasing the sugar content and shortening the agave cycle have been sought. On the other hand, salicylic acid is a plant growth regulator, and several studies have been reported that the productivity can be increased for it (Hayat *et al.*, 2005).

The objective is to carry out an investigation driven to increase the size and content of sugars in this genus using applications of salicylic acid (SA).

Material and Methods. In a semi-hydroponic system, *A. cupreata* and *Agave. salmiana* plants were established. Concentrations of (1.8, 5.4 and 10.8 µM) of salicylic acid were evaluated. Three months later, the height and width of the leaves and the diameter of the stem were measured, plants were sectioned into three parts (leaves, central stem and root), the fresh and dry weight of the biomass was determined, and the quantification of sugars was carried out according to the DNS Miller Method.

Results and discussion

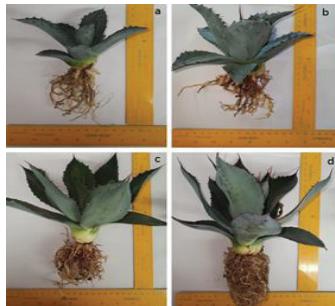


Fig. 1. Effect of salicylic acid on agave plants; a) Control *A. salmiana*, b) T2 (3.6 µM) *A. salmiana*, c) Control *A. cupreata* and d) T2 (3.6 µM) *A. cupreata*.

As shown in Figure 1, depending on SA level an increase of biomass in both species was observed, with the concentration of 5.4 µM was the best, in contrast, when the SA level was incremented the biomass decreased, which coincides with those reported in *Triticum aestivum* L. by Hayat *et al.* (2010) who pointed out that by soaking the seeds in a 10.8 µM solution of AS the activity of the nitrate reductase enzyme is stimulated, increasing the dry and fresh weight of the plants.

Conclusions. The effect of salicylic acid in agave plants growing in a semi-hydroponic system might the size of the leaves, the diameter of the stem and the fresh and dry biomass of the plant, which indicates that this type of plant with CAM metabolism responds positively to this growth regulator even applied in low concentrations and when increasing the concentration, but in high levels can produce an inhibitory effect.

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EFFECT OF PGPR BACTERIA ON THE DEVELOPMENT AND ACCLIMATIZATION OF VITROPLANTS OF *Agave cupreata* Trel. & A. Berger

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Keywords: *Micropropagation, bioinoculants, growth promoting*

Introduction. *Agave cupreata* is a species of great economic importance in Michoacán state due to its use in the elaboration of mezcal, nevertheless, the excessive extraction has been causing the decrease in its natural populations (Pérez et al., 2016), for this reason is important to find a sustainable way to obtain plants, the micropropagation is one of them. However, one of the main challenges of this technique is the acclimatization, since it is required to increase the survival of the plantlets and its vigor, in the moment of transferring them from an *in vitro* to an *ex vitro* medium, besides this, the use of growth-promoting rhizobacteria proliferate the good development and vigor of these plants (De la Torres-Ruiz et al., 2016), therefore, the objective of this work was to evaluate the effect of bacterial bioinoculants on the growth and acclimatization of *A. cupreata* plantlets.

Methods. A total of 140 vitroplants were obtained through micropropagation, which were transplanted to peatmoss substrate, where humidity and temperature were decreased. A collection of rhizobacteria was characterized for its properties as growth promoter and the outstanding ones were selected in the tests to subsequently formulate individual and consortium inoculants, which, were implemented to the plantlets in 14 treatments. Then, morphological measurements were made monthly.

Results and discussion. Strains AesM9-8 and AcuM11-4 were selected for their ability to solubilize phosphates and calcium; AtoM6-4 for having effect in all essays as PGPR, ACJ-14, Lad-7 and C1-13, as nitrogen fixers y G50-78 for its ability to synthesize auxins. In the essays, it was obtained that all rhizobacteria had a positive effect on plantlets (fig 1.), thus standing out AcuM11-4 with AesM9.8 and G50-78 in fresh weight (increasing a 29.68%), AcuM11-4 with AesM9-8 in number of leaves (13.63%), and the individual inoculation ACJ-14 in height (125%) this compared to the control without inoculum and 50% fertilization (19.45, -9.52 and 91.45% respectively) and the control without inoculum and 100% fertilization (8.24, -11.81 and 86.75% respectively).

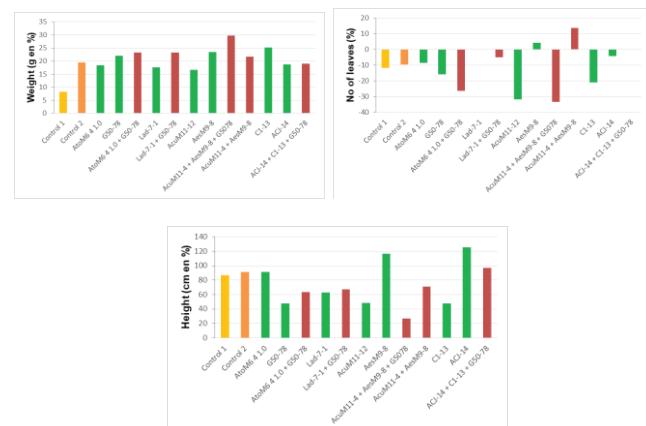


Fig 1. Effect of rhizobacteria on morphological measurements of *Agave cupreata*

Conclusions. The results show that the strains ACJ-14, AcuM11-4, AesM9-8 y G50-78 have potential as growth promoter in the acclimatization of agave plants due to its characteristics like promoter.

Acknowledgements. In gratitude to conacyt, for the economic support provided.

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USO DE BIOESTIMULANTES A BASE DE ALGAS MARINAS SOBRE EL CRECIMIENTO DE PLANTAS MICROPROPAGADAS DE *Agave maximiliana*

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Palabras clave: Fertilización, desarrollo vegetal, extractos de algas

Introducción

Agave maximiliana Baker es una especie perenne frecuente en bosques de pino-encino entre los 2000 y 3000 msnm. Habitualmente carece de reproducción asexual. Esta especie es utilizada en la elaboración del mezcal, llamado raicilla, producido en el estado de Jalisco principalmente (1). En algunas zonas, su población se ha visto disminuida por la sobreexplotación (2).

El objetivo del estudio fue evaluar el uso de bioestimulantes a base de algas marinas en plantas de *Agave maximiliana* obtenidas por cultivo *in vitro*.

Métodos

Se emplearon extractos líquidos (EL) (ácido, neutro y alcalino) obtenidos de polvo de alga de *Padina gymnospora* (2 g/L), además del producto STYMULUS® MAXX (1.5 mL/L) sobre brotes de *A. maximiliana* obtenidos por micropropagación y establecidos en invernadero. Los extractos se aplicaron en combinación con el fertilizante PETERS PROFESSIONAL® 20-10-20 (5 g/L). La aplicación de los bioestimulantes (20 mL/planta) y del fertilizante (25 mL/planta) se realizó semanalmente sobre el suelo durante 120 d hasta su evaluación.

Resultados y discusión

Cuadro 1. Crecimiento en plantas de *Agave maximiliana* después de 120 d después de su establecimiento en invernadero.

T	IPF (g)	CD (mm)	NH	NR
T1	7.4 ± 8.1c	6.8 ± 1.7b	4.5 ± 0.6d	10.0 ± 0.9bc
T2	51.1 ± 7.2b	15.4 ± 1.5a	8.7 ± 0.5bc	13.8 ± 0.9a
T3	68.3 ± 7.4ab	18.8 ± 1.6a	11.2 ± 0.5a	11.4 ± 1.0ab
T4	77.4 ± 7.0a	18.3 ± 1.5a	9.6 ± 0.5ab	10.6 ± 0.9b
T5	22.4 ± 8.6c	6.8 ± 1.5b	7.1 ± 0.6c	7.2 ± 1.3c
T6	5.3 ± 8.4c	4.3 ± 1.7b	4.3 ± 0.6d	9.3 ± 1.0bc

Valores indican promedios ± error estándar. Letras diferentes en la misma fila indican diferencias significativas entre los tratamientos de cada evaluación. ($P < 0.05$). Prueba de Diferencia Mínima Significativa (DMS). IPF = Incremento en T= Tratamiento, T1= Control (agua), T2= Fertilizante (F), T3=

F + EL-Ácido, T4= F + EL-Alcalino, T5= F + EL-Neutro, T6= STYMULUS® MAXX. IPF = Incremento en Peso Fresco. CD = Crecimiento en Diámetro. NH = Número de Hojas. NR = Número de Raíces. EL = Extracto Líquido.

La aplicación de los productos nutritivos proporcionó un incremento significativo en todas las variables de crecimiento evaluadas comparado con los agaves que sólo recibieron agua (Cuadro 1), resultados similares fueron reportados en *A. angustifolia* (3). Por otro lado, la aplicación de bioestimulantes proporcionó resultados heterogéneos, en la variable IPF, el promedio más alto se obtuvo con la aplicación de EL-Alcalino, por otro lado, con la aplicación de EL-Ácido se obtuvo el mayor CD y NH, la aplicación de STYMULUS® MAXX no proporcionó resultados positivos. Respecto al uso de extractos de alga pueden ser variables por múltiples motivos, como los compuestos bioactivos presentes, la especie o variedad cultivada (4) entre otros.

Conclusiones

La aplicación de fertilizantes en combinación con extractos líquidos de *Padina gymnospora* promueve el crecimiento en invernadero de plantas de *Agave maximiliana* obtenidas por cultivo *in vitro*.

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MORPHOLOGICAL DIVERSITY OF VARIETIES OF AGAVE IN RANCHO SAN ISIDRO, THE FIRST PULQUE PRODUCER IN THE STATE OF TLAXCALA, MEXICO

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Keywords: Agaves, Morphology, Aguamiel

Introduction. The maguey pulquero (*Agave salmiana*), grows in the semi-dry and cold highlands (García, 2011). In the State of Tlaxcala, one of the leading producers of the maguey is the Rancho San Isidro, located northeast in the municipality of Nanacamilpa; it has more than 40 hectares of extension, where four varieties of maguey are grown (Chalqueño, Púa Larga, Ayoteco and Manso). Each type has unique morphological characteristics regarding crop exploitation and productivity. The present work contributes to the knowledge of these varieties, for the conserved cultural traditions through better sustainable use.

Methods. The selection of plants was carried out during 2019-2020 at Rancho San Isidro, located in Nanacamilpa, in the semi-dry season. Counting on the support of the owners of the plant material, especially Mr. Rodolfo del Razo. The selection of plants was made based on the plant's phenotype: height, color, the diameter of the rosette, number of sheets or 'pencas', including its diameter, length, weight, and number of spines were measured. In addition to registering the production of 'aguamiel' for each variety.

Results and discussion. The maguey varieties commonly known as Manso, Chalqueño, Ayoteco and Púa Larga, belong to the Agavaceae family, *Agave* genus and *salmiana* species (García, 2011).



Fig. 1. *Agave salmiana*, variedades: A) Manso, B) Chalqueño C) Ayoteco y D) Púa Larga.

These varieties have been reported as the main producers of 'aguamiel' in the area by Madrigal et al., (2013) and Álvarez-Duarte et al., (2018). The morphological characteristics of these 4 varieties of *Agave salmiana* are presented in tables 1 and 2.

Table 1. Characteristics of 4 varieties of *Agave salmiana*

Variety	height (m)	Diameter (m)	No. Pencas	Aguamiel/day (L)	°Brix
Chalqueño	2.44±0.26	4.23±0.22	27.7±2.5	1.85 ± 0.11	11.5 ± 0.39
Púa larga	2.29±0.11	3.24±0.29	28.3±2.1	1.72 ± 0.27	12.1 ± 0.26
Manso	2.10±0.14	2.65±0.12	29.0±2.0	1.87 ± 0.44	11.5 ± 0.35
Ayoteco	2.28±0.21	2.9±0.22	26.7±1.5	1.98 ± 0.40	11.2 ± 0.56

Table 2. Morphological characteristics of pencas of varieties of *agave salmiana* (6 years)

Variety	Longitude (cm)	Diameter (cm)	Weight (Kg)	Spines / penca
Chalqueño	119.5±6.3	28.6±1.7	7.61±1.0	202.3±0.6
Púa larga	102.7±8.8	29.2±0.8	6.92±0.1	138.3±7.4
Manso	103.2±7.7	29.0±1.8	7.33±1.0	115.3±10.0
Ayoteco	155.5±6.6	30.9±4.7	7.82±0.7	95.7±5.5

Conclusions. *Agave salmiana* is the most domesticated species, with the most remarkable diversity among the traditional varieties cultivated in Tlaxcala. Agronomic management has managed to increase the productivity and quality of the sap in individuals with larger rosettes and less spiny leaves, as in the Ayoteco variety. To safeguard diversity, it is essential to keep those that are culturally valued.

Acknowledgements. Mr. Rodolfo del Razo for the donation and identification of the specimens collected.

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PROGRESS IN CRYOBIOTECHNOLOGY OF *Agave* spp.

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Keywords: *in vitro culture, cryopreservation, vitrification-based protocols*

Introduction. In recent years, various methods have been adapted for the long-term conservation of the *Agave* genus using cryopreservation techniques (Delgado-Aceves et al. 2021; 2022). The application of these procedures has been possible thanks to advances in tissue culture techniques and the definition of suitable conditions for the *in vitro* regeneration of plants. The selection of the biological material to cryopreserve is based on the origin, availability, size of the explant and peculiarities of each species (González-Arnao and Engelmann, 2013).

The objective of this work is to present the advances achieved in the application of cryopreservation to different forms of *in vitro* culture of various *Agave* species.

Methods. For the development of cryopreservation experiments, somatic embryos of *Agave tequilana* cv. 'Chato' species and shoot-tips of *A. peacockii* species isolated from *in vitro*-grown plants were used. The illustration of the tissue culture techniques used to generate the biological material subjected to cryopreservation is presented in Figure 1. Two vitrification-based techniques: Droplet-vitrification and the V-cryoplate method were applied. The cryoprotective conditions that allowed obtaining the highest levels of recovery and *in vitro* regeneration (%) of plants from the cryopreserved tissues were defined.



Fig. 1. Biotechnological methods applied for the *in vitro* culture of *Agave* genus.

Results and discussion. Cryopreservation protocols adapted for each *in vitro* form studied of agave are described in Table 1.

Table 1. Technical description of cryopreservation protocols for *Agave* spp.

Biological material	Cryopreservation procedure
Shoot tips <i>Agave peacockii</i>	Droplet-vitrification: shoot-tips of 1 mm in length×1 mm wide were precultured on MS semisolid medium with 0.3 M sucrose for 1d, loaded in solution with 0.4 M sucrose and 1.6 M glycerol for 20 min, exposed to vitrification solution PVS2 for 15 min, and immersed in liquid nitrogen in droplets of PVS2 placed on aluminium foil strips. Post-cryopreservation regrowth 96% (Delgado-Aceves et al. 2022).
Somatic embryos (SEs) <i>A. tequilana</i> cv. 'Chato'	V-cryoplate: SEs (1–2 mm length) at the coleoptilar stage were precultured on MS semisolid medium with 0.3 M sucrose for 1d. Encapsulated SEs on cryoplates were exposed to loading solution 1 M sucrose and 2 M glycerol for 15 min, followed by the dehydration with a PVS solution and immersed in liquid nitrogen. Post-cryopreservation regrowth 83% (Delgado-Aceves et al. 2021).

Conclusions. The research developed represents different available biotechnological alternatives to conserve efficiently various biological materials and agave genotypes for the long-term.

Acknowledgements. This work was supported by The Rutherford Foundation (Grant 31255-2) and Consejo Nacional de Ciencia y Tecnología (CONACYT).

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TEST FOR BIOCONTROL OF PHYTOPATHOGENS OF *Agave salmiana* IN TEMPORARY IMMERSION SYSTEMS

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Key words *Agave salmiana*, phytopathogens, temporary immersion systems.

Introduction. *Agave salmiana* is essential for rural economy in Hidalgo, Mexico. Nevertheless, there are different plagues and phytopathogens that seriously affect plantations and wild magueys. Two phytopathogens were isolated and identified; *Colletotrichum* sp. and *Ceratocystis* sp., the first causes anthracnose (*marginal spot of leaves*) and the second was isolated from a leave-lesion similar to marginal spot. Temporary immersion systems (TIS) provide the most natural environment for *in vitro* culture of plant shoots and seedlings. TIS have different biotechnological application, for example plant micropropagation and production of plant-derived secondary metabolites (1,2).

Our aim was to use TIS, as an aseptic and controlled bioreactor-system, to infect maguey shoots with phytopathogens for developing the illness and test biocontrol agents.

Methods. Agave plants and microorganisms. *Agave salmiana* seeds were collected from DESCTI, disinfected, and seeded according to the protocol used in the Laboratorio Regional de Biodiversidad y Cultivo de Tejidos Vegetales del Instituto de Biología, UNAM. Agaves of 36 weeks were used in the experiments. *Colletotrichum* sp. and *Ceratocystis* sp. were isolated from leaves of *A. salmiana* with typical fungal lesions, purified, characterized by ITS Metagenomics and conserved. *Trichoderma* sp. was isolated from soil from La Costa de Hemosillo, Sonora and *Bacillus subtilis* AcX from *Agave angustifolia*, spores of both were conserved in filter paper disks at 4 °C.

TIS. Consists of two bottles 500 ml with wide mouth and polypropylene cover connected by silicone tube (Fig. 1). Each container is connected to its own pressurized-air line, controlled by two independent timer clocks, coupled with three-way solenoid valves (2).

TIS experiments. Murashige and Skoog's medium with sucrose 30 g/L and supplemented with 5 mg/L 6-benzylaminopurine (BAP) was used. 1 µL of a 5 x 10⁷ spores/mL suspension of *Colletotrichum* sp. or *Ceratocystis* sp. were inoculated in an agave leaf with a syringe. When the lesion appears 1 µL of a 5 x 10⁷ spores/ml suspension of *Trichoderma* sp. or of a 5 x 10⁷ cell/ml suspension of *Bacillus subtilis* AcX were injected close to the lesion.

Results and Discussion. TIS experiments. *Trichoderma* sp. as well as *B. subtilis* Acx were able to control *Ceratocystis* sp. and all the agave plants survived. *Colletotrichum* sp. was

more virulent, however, 67% of the plants survived when either *Trichoderma* or *B. subtilis* were injected to the leave (Fig. 2). In previous experiments with agave leaves from agaves about 5 years old, *Colletotrichum* sp. was highly virulent, hydrolyzed the site of inoculation and dry the leave, while *Ceratocystis* sp. was less virulent although cause a lesion. Thus, the experiments in TIS seems to reproduce what happen in nature.



Fig 1. TIS used for infected maguey shoots.

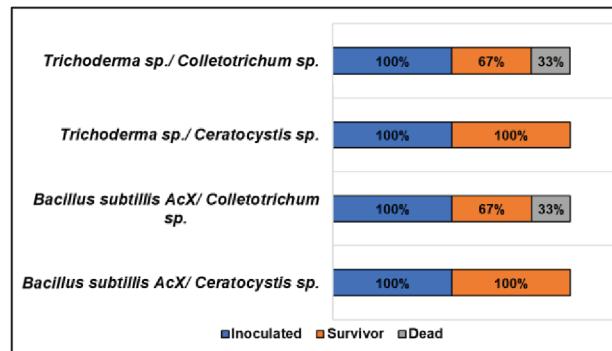


Fig. 2. Percent of survival in different treatments.

Conclusions. *Trichoderma* sp. and *B. subtilis* can control the phytopathogens *Ceratocystis* sp. and *Colletotrichum* sp. in agave plants. TIS seems to be appropriate for studying biocontrol antagonist of phytopathogens in agave plants.

Acknowledgements. CONACyT project 1312404. Jorge Peralta for providing the strain *B. subtilis* AcX.

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GEO-LOCALIZATION OF DISEASES IN AGAVE.

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Keywords: Agave disease, geolocation technology, artificial Intelligence.

The diseases in agave plant are extensively studied in lab conditions, but in the field numerous pathogens and plagues affect the agaves. Agave farmers attribute all the problems to the sisal weevil, but in Hidalgo other plagues and mainly phytopathogens causes important damages and even the dead of plants. Indeed, the problem is extended across the state, so we propose a monitoring system of the diseases in the agave, evaluating the propagation of phytopathogens via a geo-localization techniques and artificial intelligence to identify the site of infection and collaborate to eradicate the diseases and plagues in the plantations. via an integral plan.

Introduction. The state of Hidalgo has a tradition of using agave plants in gastronomy, for agave syrup and “pulque” production, as forage, to obtain fibers, building houses, etc. In recent years plagues and diseases, become a serious problem for the production, ecological equilibrium and social-economical activities that depend on the agave plant.

The aim of this work is to localize the disease in the agave plants and propose a strategy to control the propagation of plagues and phytopathogens, via a low cost techniques of geo-localization [1] and a smartphone without internet connection.

Methods. The project is developed inside the District of Education, Health, Science, Technology and Innovation (DESCTI) at Hidalgo. We implemented a survey in the application Kobo-collect [2] to obtain the data of geo-localization of agave plants, degree of disease, photographs of infection, lesions. and condition of the plant. The geo-data were analyzed with the Geographical Information System (GIS) program QGIS and python scripts to locate the sites of infection.

Results and discussion. The disease in agave plants is expanded in the DESCRI, 92 percent of the plants present diseases in their leaves or/and the base of the plant (Table 1). According to the localization most of the plants are in the south-west part of the district, and the second area with a mayor population density is the north of the main access (upper right corner, Figure 1). Therefore, we analyzed the dispersion of the illness locating a main infection point at the south-west hill, and at the north road of the District.

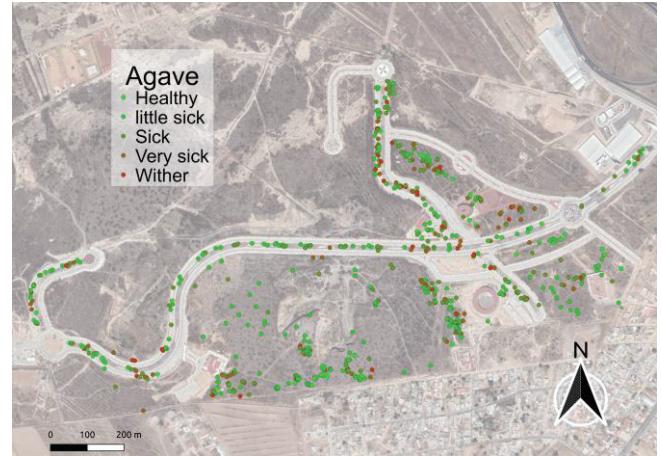


Fig. 1. Map of the Agave distribution in the DESCRI.

Illness site	Number of Agaves	Percentage
Leaves	378	55
Base	43	6
Base and leaves	215	31

Table 1. Location of phytopathogens lesions in agaves-

Conclusions. The geolocalization technology used in this work is a low-cost tool to evaluate the health status of the agave plants, to identify the plagues and phytopathogens, to follow the dynamic propagation of pest and diseases, as well as the dynamic results of integrated pest management strategies. The District is the first site where this technology will be applied to remove the main infection and infestation points and restoring the agrobiodiversity of the zone.

Acknowledgements. We thank all the personnel, researchers, and students at CIAD Hidalgo that help with this project.

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GROWTH OF *Agave potatorum* UNDER BIOFERTILIZATION IN LOW FERTILITY SOILS

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Keywords: maguey, Mixteca alta, plant growth-promoting bacteria

Introduction. *Agave potatorum* (*A. potatorum*) is one of the most important wild maguey species for mezcal production. In the MAO, these agaves are in high demand, which has led to overexploitation of natural populations, a situation that makes the species highly vulnerable due to the steep slopes, precipitation and easily eroded sandy soils where they are grown. Plant growth-promoting rhizobacteria (PGPR) facilitate the availability of some soil nutrients through direct and indirect mechanisms, such as the production of enzymes, organic acids, and phytohormones (2,3). Unfortunately, studies of biofertilizer application on *A. potatorum* under field conditions are relatively scarce. The objective of this study was to evaluate the effect of five biofertilizers on the growth of *A. potatorum* under field conditions in clay soils of the MAO.

Methods. The study site is located in Santiago Tilantongo, Mixteca Alta, Oaxaca, Mexico, (17°03'N, 97°17'W and 2900 masl). Before establishing the experiment, some physical and chemical properties of the soil described in NOM-021-RECNAT-2000 were determined: pH (8.2), electrical conductivity (0.350 dS m⁻¹), phosphorus-Olsen (2.5 mg kg⁻¹), bulk density (1.45 g cm⁻³), organic matter (1.5%), and proportion of sand, silt and clay (65, 20 and 15%). Under a completely randomized design, the effect of three bacterial consortia based on PGPR1: *Enterobacter cloacae* + *Azotobacter* sp., PGPR2: *Pseudomonas* sp. + *Azospirillum* sp., PGPR3: *Azospirillum* sp. + *Azotobacter* sp. and a control (without bacteria) on the growth of *A. potatorum* was evaluated. Each treatment had 30 replicates. The variables evaluated after 12 months were: plant height (AP), rosette diameter (DRO), stem diameter (DT), number of unfolded leaves (NHD), root volume (VR), root density (DR), stem dry biomass (BST), aerial dry biomass (BSA), total biomass (BT), leaf area (AF), total stem soluble solids (°Brix), nitrate (NO₃), chlorophyll content (SPAD) and stomatal density (SD). Soil available phosphorus (DF) and total nitrogen (TN) contents were determined.

Results and discussion. Analysis of variance and multiple comparison of means test (Tukey, P≤ 0.05) revealed that the three biofertilizers positively

influenced the vegetative development of *A. potatorum* and the availability of NT and FD in soil with respect to the uninoculated control. Normally, FD is produced as a consequence of the production of organic acids by various soil bacteria (4). RPCV3 increased 67% PA, 66% BSA, 53% BT, 13% DT, 108% AF, 17% NO₃ content, 11% SPAD units, 72% and 20% stomatal density, compared with the control. The increase in the growth variables of *A. potatorum* can be attributed to several direct and indirect mechanisms carried out by RPCV: these bacteria influence the fertility of agricultural soils, specifically in the availability and absorption of nutrients such as phosphorus and nitrogen, the production of phytohormones (auxins and gibberellins), production of siderophores and synthesis of enzymes such as 1-aminocyclopropane-1-carboxylate deaminase, phosphatases and nitrogenase (5).

Conclusions. The PGPR3 consortium demonstrated the potential to increase soil phosphorus and nitrogen availability, positively stimulate *A. potatorum* development under low fertility conditions and may be a practical and efficient option to promote *A. potatorum* plant growth in the field. However, more research is needed to gain a deeper understanding of the relationships between different agave-associated PGPR species and their synergistic interactions involved in promoting plant growth.

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LEAF ANATOMY IN *AGAVE SALMIANA* SUBSP. *SALMIANA* (ASPARAGACEAE) FROM THE EDO. OF MEXICO, INTRASPECIFIC VARIATION

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Palabras clave: anatomic description, leaf, variation.

Introduction.

Species present variation in structural characteristics that are linked to processes of response to the climatic conditions of the environment or to different types and levels of disturbance (1). The leaf is one of those that present greater variation among species and within the same species (2); however, there is no information about the variation of leaf anatomical characters in agaves, so the study of its anatomical variation at intra-specific level could provide knowledge about the level of its diversification and would allow to know its better utilization.

The present research aims to know the variation of 38 quantitative leaf characters of *Agave salmiana* subsp. *salmiana* through comparative analysis at two levels: among three leaf regions and among three localities in the State of Mexico.

Methods.

Five individuals from 3 localities (San Martín de las Pirámides, Tecámac and Teotihuacán) of *Agave salmiana* subsp. *salmiana* were used. Three regions of the stalk were taken: marginal zone, intermediate zone and middle vein, to obtain transverse sections, epidermis and dissociated. Twenty data per character were obtained. Linear and area measurements were made with the ImageJ v.1.48 program. In order to know the level of variation of these parameters, two levels of comparison were analyzed: between three regions across the leaf and between the three locations. Traits were evaluated by one-way ANOVA and Tukey's test ($P \leq 0.05$). To identify the patterns of variation of anatomical characters among the three localities and the contribution of these in the separation of groups, a linear discriminant analysis was performed.

Results and discussion.

A general anatomical description of the leaf is presented. Comparisons between the three leaf regions indicate that 18 characters (47.38%) have significant differences in at least one of the three regions, of which 5 were different for each of the regions: width of abaxial cuticle, total length of parenchyma at both ends of the leaf, length of fibers and length of fibrotracheids. From the comparison among the three localities it was found that 32 characters (84.21%) show significant variation in at least one of the localities, 17 characters were variable among the three localities, some of these are: length of occluding cells in both epidermis, surface area of adaxial epidermal cells, width of parenchyma cells in abaxial

palisade and length of fibers. The clustering of the data for the three localities and the loading value of the characters in the linear discriminant analysis are shown (Fig. 1 and Table 1).

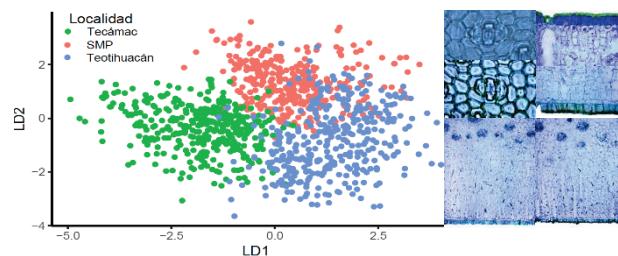


Fig. 1. Linear discriminant of anatomical data; and examples of characters showing variation

Table 1. Two first linear discriminants (97.22% accuracy) with the characteristics with the highest loadings

	LD1(0.7228)	LD2 (0.2772)	
LCOADA	0.4363028	ASCEADA	-0.3546828
LCOABA	0.51148458	ACABA	-0.3517833
ACADA	-0.31626032	ACPABA	-0.3307679

Conclusions.

The internal variation in the leaf is less than 50%, there is anatomical variation in more than 80% of the characters analyzed among the populations of *Agave salmiana* subsp. *salmiana*, which adds to the knowledge of the diversity of this resource. The level of local variability and the possible adaptive value of some of these characteristics allow us to consider each population as a genetic resource of great value for its exploitation. Of the variables that distinguish the localities, it was observed that six are part of the dermal tissue, six of the fundamental and three of the vascular.

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USE OF IMMERSION TEMPORAL SYSTEM FOR MASSIVE PRODUCTION OF AGAVES

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Keywords: *Henequen, Scaleup, Biofactory.*

Abstract.

In this work, various growing conditions were evaluated in Temporary Immersion Systems (TIS), using agave as a source of explant, to optimize a productive flow for the scaled production of plants. The results showed that using irrigation every 30 hours, for 120 seconds starting from “microcuts”, increased the multiplication rate up to 5 times. This technology was useful in the multiplication phase, however, was not useful for growth phase. The results obtained will be used in productive flows for the scaled production of different species of agaves, to support growers.

Introduction. The propagation systems in agaves by tissue culture could be a complementary strategy to guarantee the adequate availability of plants that allows sufficient inventories to maintain active and continuous production of beverages such as mezcals, tequila, bacanora, sotol, pulque, among others. Semisolid culture, somatic embryogenesis or TSI, has been reported previously for several agave species [1,2].

Methods.

In all the treatments were used explants from the multiplication phase in a semi-solid medium and with 30 days of inoculation. Different volumes of medium, number of structures, immersion frequencies and immersion time were tested (table 1). All treatments includes 10 replicates.

Table 1. Conditions evaluated.

Media (ml)	Explants per container	Aeration frequency (Hr)	Immersion time (seconds)
500, 800	50,80,100	8, 10, 12, 24, 30	50, 60, 120 and 180
1000			

Results and discussion. Several tests were carried out, among them: volume of the media to be used, immersion times for each crop, aeration frequency and the number of structures in the container. Based on all these tests and trials carried out with this SETI type system, the results obtained were as following: once the configuration of the system with the requirements of the crop was carried out, it was determined that the optimal medium volume is 1 L, since the structures grew uniformly without any indication of stress. By placing 100 structures per bioreactor and with the mesh inside the same system, the volume of the medium was in contact with all tissues during immersion time up to grew. The immersion frequency comparison showed

that every 30 hours is the more adequate time, during short periods of 120 seconds of immersion, in which the multiplication coefficients increased up to 5, without vitrification, formed seedlings were observed and collected for the growth phase. When testing this system in the growth phase, it was observed that the *in vitro* plantlets were deformed and in this way the quality was not appropriate for acclimatization, suggesting that the bioreactors have greater utility in the multiplication phase for this crop.



Fig. 1. Process of the use of TSI in Agave. A) Explants at initial time B). Immersion phase. C-D) multiplication after 21 days.

Conclusions. The use of irrigation every 30 hours for 120 seconds and a 100 explants per container increased the multiplication rate up to 5.

Acknowledgements. Authors thanks CONACYT for financing by FORDECYT: 292474 and 296369.

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MICROPROPAGATION OF AGAVE CUPREATA

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Keywords: Micropropagation, Agave Cupreata, Guerrero.

Abstract: Micropropagation is the technique of vegetative multiplication of plants through the *in vitro* culture of plant cells, tissues or organs, and could be used from massive propagation as a complementary tool to guarantee plant material for planting. In this work we established a successfully process from the selection of mother plant up to produce plants for nursery derived from two clonal lines of *A. cupreata*.

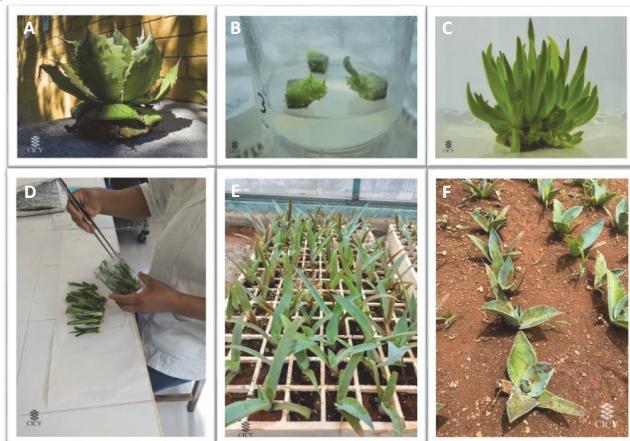
Introduction. Micropropagation is the technique of vegetative multiplication of plants through the *in vitro* culture of plant cells, tissues or organs. In addition to the rapid and efficient multiplication of valuable cultivars, this methodology also produces disease-free plants and sometimes rejuvenated, more vigorous and fast-growing individuals without involving genetic engineering. The main objective of micropropagation is the rapid and efficient multiplication of plants to producing individuals large scale that are morphologically and functionally identical to the mother plant (faithful phenotype).

Methods.

The selected plants of *A. cupreata* were used to extract the meristematic zone in a laminar flow hood, the explants obtained were placed on 25 ml of propagation medium with 10 g / L of agar in "Gerber" type bottles of 120 ml, at the rate of four explants per bottle. After 4 weeks the explants were incubated in a multiplication medium MSB-10 that contains 2-4,D (1mg/ml), 6BAP (2mg/ml), subculturing every 21 days. Depending on the size of the obtained material (microcuts, C1, C2 and C3) the individualized plants (C2 and C3) were incubated in an MSB-1 growth culture medium, whereas microcuts and C1 were subculture in multiplication medium. The plants with 5 cm in size are taken out for adaptation *ex vitro*. The time from microcuts to *ex vitro* could take 2 to 3 subcultures depending on each clonal line.

Results and discussion. Currently we have 2 clonal lines (cup9 and cup22) of *A. cupreata*. The multiplication rate of the cup 9 is 4, but for its growth it requires 5 or 6 subcultures, on the other hand, the cup 22 line showed a multiplication rate of 2.5 but for its

growth it requires 2 or 3 subcultures. For microcuts in a multiplication medium we use 20 plants per container, in phase C1, C2 and C3 a growing culture medium was used 25 plants per container. In all cases the cycle duration is 21 days. All plants above 5 cm are taken out *ex vitro*, the plants were impregnated at the base with a commercial rooter (Radix 1500®), later they were sown in the tray to which the opening of the pools has previously been made with the help of a pointed wood, The tray is placed in wet chambers added with 1gl-1 of commercial fertilizer 13-40-13 (Hakaphos violet®) and 1 ml⁻¹ of the fungicide propamocarb-hydrochloride 64% (Previcur® Bayer®) for 5 days. After this period, they were removed from the wet chambers and incubated in the greenhouse for up to a period of 4 to 5 months until they are ready for planting in the nursery. Weekly waterings are carried out during this phase. We have more than 5000 plants produced from two mother plants.



*Fig. 1. Process for the production of *A. cupreata*. A) Mother plant B) Induction C) Micropagation D) Ex vitro E) Greenhouse, F) Nursery.*

Conclusions. We have established a successfully process from the selection of mother plant up to produce plants for nursery derived from two clonal lines of *A. cupreata*.

Acknowledgements. Authors thanks CONACYT for financing by FORDECYT: 292474 and 296369.

Studies of Oryzalin on meristematic tissue of Agave H11648

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Keywords: *H11648, Oryzalin, Agave.*

Abstract. The induction of artificial polyploidy is a strategy for plant breeding. There are few studies in the genus *Agave* on this procedure. In the present work, an experimental design was used to two groups (Control and experimental); in the first the explants were induced in Murashige and Skoog medium (1962) without oryzalin compared with the experimental group induced at 2.5 μ M and 5 μ M of oryzalin at 15, 30 and 60 minutes of exposure, their response was monitored every 7 days until day 28. So far, a response has been obtained from the formation of outbreaks in both concentrations of oryzalin at 15 and 60 minutes of exposure. The best conditions will be discussed.

Introduction. Polyploidy is a natural trait that occurs more frequently in plants than in animals and is considered a fundamental mechanism in the evolution of new species. Despite the applications that the technique of induction of polyploidy has had in the improvement of plants of agricultural and medicinal interest, few species of the *Agave* genus have been subjected to the action of these chemicals to improve them genetically. The aim of this work is to compare several concentrations of oryzalin to generate polyploidy in *Agave H11648*.

Methods. A total of 30 plants of agave H11648 collected from the CICY nursery of 3 months old were used. Each one was characterized morphometric and disinfest using 70% alcohol and then washed with a 40% sodium hypochlorite solution to finally wash them with sterile distilled water. The treatments with oryzalin were carried out by adding 4 ml of solution to the meristematic cube in an eppendorf tube of 5 ml and then washed with sterile distilled water 3 times. After that, the meristematic cube was incubated in semisolid MS medium, with ABA 44.4 μ M mg/ml and 2,4-D 0.11325 μ M in a culture room in continuous light, at 25 °C.

Results and discussion. The preliminary results obtained so far are as follows: A partial response to the induction of shoots was obtained since the plants were of bulbils adapted in greenhouse manifested a different size in each of them. We obtain shoots after 45-60 days before treatments in both 2.5 and 5 μ M. At this moment we are waiting for the development of the shoots to determine the ploidy level of the generated plants using Flow Cytometry.

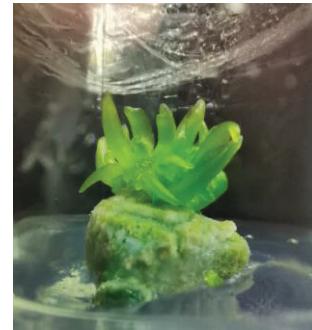


Fig. 1. Shoot development after 60 days of treatment in MS with 2.5 mM of oryzalin after 15 minutes of exposure.



Fig. 2. Shoot development after 60 days of treatment in MS with 5 mM of oryzalin after 60 minutes of exposure.

Conclusions. The preliminary results obtained indicate that 60 minutes are necessary to obtain a better response to induction treatment with oryzalin at both 2.5 and 5 μ M. However, although a meristem with outbreaks was obtained at 15 minutes of exposure. It is necessary to have a plant material with very similar morphometric characteristics to draw precise conclusions from this study.

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Potencial distribution of the weevil and the agaves of Mexico

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Keywords: picudo, plaga, distribución

Introducción.

Scyphophorus acupunctatus Gyllenhal (Coleoptera: Dryiphthoridae) conocido comúnmente como picudo del Agave, es originario de América, con amplia distribución mundial (1). En México, este picudo es considerado una plaga y se ha registrado en 12 estados (Gto., BCS, Pue., Qro., Tlax., Yuc., Mor., Jal., Gro., Tam., Oax. y Hgo.), principalmente donde se cultiva *Agave tequilana*, *A. salmiana*, *A. cupreata*, *A. angustifolia*, *A. potatorum*, *A. karwinski* y *A. americana* (2). Actualmente, con el cambio de uso de suelo y las constantes variaciones climatológicas es necesario contar con modelos que nos permitan conocer las áreas de distribución potencial de esta plaga y sus especies hospederas.

Métodos.

Utilizamos modelos de distribución de especies (SDMs) para obtener las áreas de distribución y la idoneidad climática potencial para *S. acupunctatus* y especies hospederas distribuidas en México (*A. tequilana*, *A. angustifolia*, *A. cupreata*, *A. karwinskii*, *A. potatorum* y *A. salmiana*), por medio de análisis de máxima entropía en Maxent. Con datos de presencia de las especies analizadas, y colectas evaluamos la contribución de variables bioclimáticas (3) en la distribución potencial.

Resultados y discusión. La distribución potencial para el picudo del agave ocurre principalmente en las zonas cálidas del centro y sur del país. Las zonas de idoneidad climática con potencial de presencia son el norte (BC) y el sur (Chis) del país. Observamos potencial de distribución del picudo en zonas con *A. potatorum* y *A. karwinskii* en Oaxaca. La distribución de *S. acupunctatus* está explicada principalmente por la temperatura media del trimestre más húmedo del año (31%) al igual que para *A. salmiana* (51.3%), mientras que la estacionalidad de las precipitaciones contribuye en la distribución de *A. angustifolia* (28.4%), *A. potatorum* (26.3%) y *A. tequilana* (31%). Por su parte las distribuciones de *A. cupreata* y *A. karwinskii* están explicadas por la precipitación del trimestre más húmedo (30.6%) y la precipitación del trimestre más frío (41.6%) respectivamente.

Tabla 1. Contribución de las variables climatológicas a la distribución potencial del picudo y sus hospederos

Variable climatológica	<i>Scyphophorus acupunctatus</i>	A	B	C	D	E	F
Media anual diurna	20.18	6.44	11.53	0.82	10.91	8.26	3.76
Temperatura media del trimestre más húmedo	31	14.19	20.09	0.27	23.24	51.29	0.26
Temperatura media del trimestre más seco	1.77	24.08	3.52	10.22	1.77	9.87	16.81
Precipitación del mes más húmedo	3.14	13.4	30.61	13.87	11.2	0.7	27.87
Precipitación del mes más seco	2.8	0.43	0.1	0.52	0.02	7.52	6.41
Estacionalidad de la precipitación	15.69	28.37	15.94	32.66	26.3	2.85	31.03
Precipitación del trimestre más húmedo	3.1	12.53	5.05	0.16	3.87	0.65	1.66
Precipitación del trimestre más frío	22.32	0.55	13.15	41.59	22.7	18.85	10.2

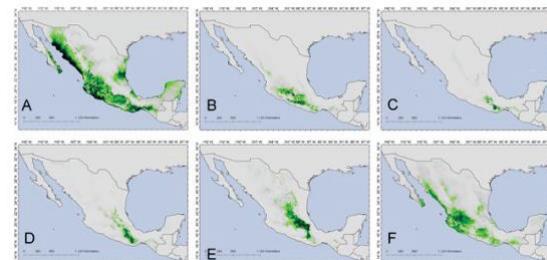
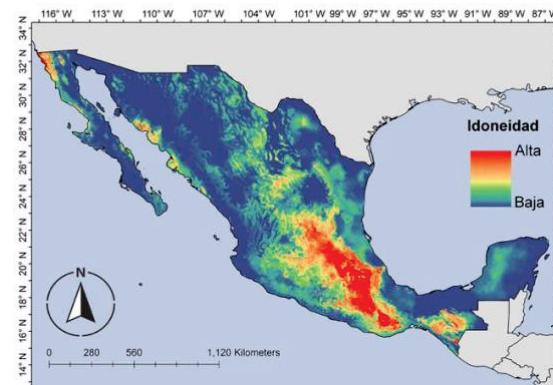


Fig. 1. Distribución potencial de *Scyphophorus acupunctatus* en México A) *A. angustifolia* B) *A. cupreata* C) *A. karwinskii* D) *A. potatorum* E) *A. salmiana* F) *A. tequilana*

Conclusiones.

Los resultados obtenidos muestran la distribución conocida de las especies en estudio y nos permite conocer las zonas con potencial para la presencia de *S. acupunctatus* así como zonas de idoneidad climática para el cultivo de agaves de interés comercial y su conservación.

Agradecimientos. A la beca posdoctoral del autor Salazar-Rivera, a los productores de agave por las facilidades prestadas y datos proporcionados para este estudio. Al proyecto FORDECYT 292474.

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POSTERS TEMATICA II

**Science and technology
of Agave beverages**

CHARACTERIZATION OF THE MEZCAL FERMENTATION PROCESS IN FOUR FACTORIES AND TWO REGIONS WITH DENOMINATION OF ORIGIN IN MEXICO

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Keywords: distilled beverage, ferments, dissolved solids

Introduction. Agaves are the raw material used to make Mezcal; they contain fructans with particular structural characteristics¹. They are sugars that are difficult to ferment with yeasts, commonly used in ethanol production from grains. In Mexico, the production of Mezcal overcomes this problem through spontaneous fermentation with indigenous "domesticated" microorganisms that could be different in every region, even in every factory, resulting not only in an efficient ethanogenic process but also in the production of metabolites, which give it particular organoleptic properties².

This characterization is part of a broader study that aims to understand the origin, diversity, changes, and contribution of the microbiota in the Mezcal process.

Methods. A sampling was carried out in four Mezcal factories in two regions with Denomination of Origin in Mexico: La Soledad (LS) and El Marillero (EM) from Oaxaca, and La Vinata (LV) and Piedras de Lumbre (PL) from Michoacán. Dissolved solids (DS), pH, and temperature were determined in the cooked agave juice (time 0) and fermented juices at three fermentation times (initial-1, middle-2, and final-3) and three depths of the fermentation vat (surficial, middle, and bottom). InfoStat v. 2020³ was used for T Test between the time of ferments from each factory. During the sampling, it was possible to identify variants in the production process, specifically in fermentation.

Results and discussion. In each factory, a variation was observed pH and temperature throughout the fermentation time and depth of the vat; this modifies the conditions of the substrate and causes a succession in the community of microorganisms, creating suitable conditions for the production of alcohol, varying between 12 °Brix and 28 °C, conditions reported to obtain higher levels of ethanol⁴. According to the T-test, only pH and temperature show significant differences between all fermentation times in terms of vats depths, unlike DS contents that were similar (Table 1). Each batch of production was different in species of agave, amount of production, type of fermentation vat, type of distiller, and production practices, which make each process unique.

Table 1. Soluble solids (DS), pH, and temperature (Temp.) in cooked juice and ferments at three depths of the fermentation vats from four Mezcal factories.

Factory	Depth	n	DS Mean (°Bx)	pH Mean	Temp. Mean (°C)
LS	Bottom	4	19.5*	4.1*	24.0*
	Mid	4	19.1	4.1*	23.7*
	Superficial	4	18.5	4.1*	21.6*
EM	Bottom	4	18.1*	4.0*	24.2*
	Mid	4	15.9*	4.0*	24.9*
	Superficial	4	13.4	4.0*	26.0*
PL	Bottom	4	13.5	3.6*	26.3*
	Mid	4	13.3	3.6*	25.8*
	Superficial	4	13.0	3.6*	25.3*
LV	Bottom	4	15.2	3.6*	26.1*
	Mid	4	15.2	3.8*	27.0*
	Superficial	4	14.6	3.8*	25.5*

Marginal notes: La Soledad (LS) and El Marillero (EM), La Vinata (LV) and Piedras de Lumbre (PL). Means with * show significant difference ($p<0.05$).

Conclusions. The data obtained during the sampling open the door to works where the entire Mezcal production process is involved in understanding the microbiota's origin, its diversity, what changes occur, and how it contributes to the organoleptic profile and yield of alcohol.

Acknowledgments. PABL acknowledges support from a doctoral fellowship (786763) awarded by CONACyT.

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Spectral fingerprinting of distilled agave beverages by FTIR - ATR

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Keywords: Distillates, markers, FTIR - ATR.

Introduction

In Mexico, several traditional distilled beverages are produced. Different species of Agave are exploited for their production; according to the official Mexican standards, Tequila (*A. tequilana* Weber), Bacanora (*A. Angustifolia* Haw) and Sotol (*Dasyliurion spp*), while Mezcal allows several species. Each production processes differ according to the production area and, therefore, generates different compositional profiles. The objective of the work is to obtain spectral profiles by implementing the FTIR - ATR technique for the search of possible markers in Mexican distilled beverages (Tequila, Mezcal, Bacanora and Sotol).

Methods

Different commercial brands were used (47) of Tequila, Mezcal, Bacanora and Sotol of the silver, gold, aged, extra aged 'and crystalline classes. The Fourier transform infrared spectrometer (FTIR) used was the Agilent model CARY 360. In addition, an all-reflection interaction (ATR) attachment was used. Samples were placed in this fixture and measured before and after evaporation in the ATR fixture.

Results and discussion

Figure 1, shows the infrared spectra of Tequila, Mezcal and Bacanora samples (all aged) measured before and after evaporation.

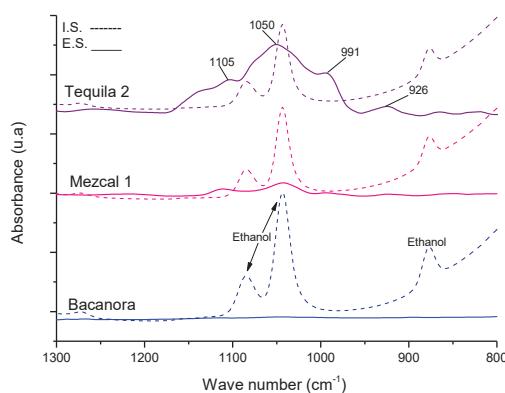


Fig. 1. FTIR - ATR spectra (1300 to 800 cm⁻¹) of Tequila, Mezcal and Bacanora, in the initial sample (I.S.) represented with a dotted line and in the evaporated sample (E.S.) represented with a continuous line

Table 1 shows the physicochemical properties of the distillates that showed differences.

Table 1. Physicochemical properties

Sample	%Alc. Vol.	Color			pH	Brix	Acidity
		L*	a*	b*			
Tequila 1	36.95	93.03	61.41	14.15	4.76	15	40.63
Tequila 2	34.5	93.58	61.73	13.34	4.4	15.25	60.92
Mezcal 1	35.17	94	62.01	10.24	4.2	15.15	119.50
Mezcal 2	37.02	80.8	54.73	42.13	4	16.07	154.07
Bacanora	47.37	96.66	64.32	1.27	4.93	18.45	57.04
Sotol	38.15	95.63	63.42	5.23	4.2	15.77	39.35

In the spectra obtained from the initial samples, the bands corresponding to ethanol were identified, and the different heights are related to the concentration, which can be associated with the % alcohol volume (table 1). On the other hand, in the spectra of the evaporated samples, signals were observed in the interval between 1200 – 900 cm⁻¹ where bands attributed to the stretching and flexion of the C-O bond, stretching of the C-O-H and C-C bond, glycosidic bond of the C-O-C bond, among others, appear. These bands have different heights due to different concentrations, which could be associated with organic molecules corresponding to carbohydrates, acids, esters, furans and terpenes, resulting from the respective barrel maturation or finishing process.

Conclusions

In this work, the fingerprinting of Mexican distilled beverages was carried out using the FTIR - ATR technique, with which the identification of possible markers in the matured distillates of the various beverages analyzed was achieved.

Acknowledgements

Thanks to CIATEJ for providing the facilities to carry out the project.

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PHYSICOCHEMICAL EVALUATION OF TWENTY MEZCALS -CERTIFIED AND ARTESANAL- PRODUCED IN DURANGO, MEXICO

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Keywords: Quality, mezcal, NOM-070-SCFI-2016

Introduction. Mezcal is a Mexican alcoholic beverage obtained from the distillation of fermented agave juice. Durango is one of the States of the Mexican Republic that produce mezcal since it has the origin denomination. It is manufactured both in artesanal (rustic) way, by communal lands holder and small producers, as certified mezcal, although few producers have promoted the certification of their production [1]. The certified companies produce mezcal according to established in the Official Standard NORMA OFICIAL MEXICANA NOM-070-SCFI-2016, BEBIDAS ALCOHÓLICAS-MEZCAL-ESPECIFICACIONES. This Standard establishes the characteristics and specifications that the mezcal must meet for its production, packaging, and marketing. Small producers do not follow the Norm, they store and sell the product in any type of bottle, both glass and plastic [2]. On the other hand, there is EL CONSEJO MEXICANO REGULADOR DE LA CALIDAD DEL MEZCAL (MEXICAN MEZCAL QUALITY REGULATORY COUNCIL) monitors compliance with the Standard and aims to be the mezcal certification body throughout the Mexican Republic.

The aim of this work was to evaluate the mezcal quality of ten certified brands and ten mezcal produced in artisanal vinatas in Durango State and define whether to comply with the established regulatory parameters.

Methods. Ten certified mezcals were purchased in commercial establishments in Durango Mexico (numbered from 1 to 10), and ten mezcal were collected in artisanal vinatas (numbered from 11 to 20) in Nombre de Dios and Mezquital Durango. The quality was evaluated in terms of NOM-070-SCFI-2016. The parameters analyzed were alcoholic content (% Alc.vol NMX-V-013-NORMEX-2019); dry extract (NMX-V-017-NORMEX-2018, in g/L); furfural (NMX-V-004-NORMEX-2018, in mg/100 mL of anhydrous alcohol); methanol, higher alcohols and aldehyde (NMX-V-005-NORMEX-2018, in mg/100 mL of anhydrous alcohol). Samples were analyzed in triplicate.

Results and discussion.

Table 1 depicts the values of physicochemical values of analyzed samples. In **bold** are the minimum and maximum values found in each parameter. In red are highlighted values outside the range established, corresponding to furfural content in mezcals 16 and 20.

Table 1. Physicochemical parameters in certified (1-10) and artisanal (11-20) mezcal.

Nº	% ALC	D.E	FUR	MEOH	HI ALC	ALD
1	40.2	0.09	5.0	190.6	178.6	9.1
2	39.3	0.08	0.7	48.0	63.6	8.8
3	40.5	0.03	4.6	158.8	172.5	10.9
4	48.0	0.09	0.6	144.0	354.5	17.3
5	46.0	0.08	0.2	229.9	235.1	18.0
6	44.9	0.08	2.6	251.7	445.8	28.8
7	47.0	0.06	0.2	123.1	351.9	10.8
8	35.3	0.9	0.2	66.2	136.4	9.7
9	40.8	0.17	0.1	224.1	230.6	6.4
10	38.2	0.03	3.1	280.5	321.2	6.4
11	45.2	0.05	0.2	267.3	310.3	10.3
12	52.0	0.04	0.2	132.7	262.1	14.2
13	43.0	0.07	0.3	245.0	294.2	8.9
14	46.1	0.16	0.2	178.9	264.6	14.9
15	39.7	8.01	0.6	201.4	268.0	1.5
16	39.8	0.04	16.5	224.9	39.2	12.9
17	42.0	5.11	0.2	154.5	187.4	4.1
18	47.6	0.12	0.4	86.6	135.8	3.4
19	43.2	3.62	0.8	73.9	118.9	1.4
20	46.7	0.05	18.4	225.9	168.5	14.7

No.= Sample number; % ALC= alcohol content, D.E= Dry extract, FUR= furfural, MEOH= methanol, HI ALC= high alcohols, ALD= aldehydes.

Accepted ranges for mezcal (NOM-070-SCFI-2016): % Alc (35-55%); Dry extract (0-10 g/L); Furfural (0-5 mg/100 mL AA); Methanol (30-300 mg/100 mL AA); High alcohols (100-500 mg/100 mL AA); Aldehydes (0-40 mg/100 mL AA).

Conclusions. the certified mezcals comply with the quality parameters established by the regulations. The artisanal mezcals comply with the maximum permissible values, except in two samples exceed the furfural concentration.

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Antioxidant activity and phenolic composition of Pulque, a traditional Mexican fermented beverage from Agave

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Keywords: pulque, bioactive compounds, agave

Pulque is a traditional Mexican alcoholic beverage produced from the fermentation of the mead extracted from the stems of many species of the Agave family, such as *A. salmiana*, *A. mapisaga* and *A. atrovirens*; it is a liquid of whitish color, viscous consistency, slightly acidic, and low alcohol content (1,2).

Introduction. Antioxidant compounds have the ability to inhibit or delay the oxidation of other molecules in biological systems, they protect against oxidative stress across different mechanisms of action, as the stabilization of the reactive oxygen species (ROS) (3,4). Phenolic compounds are secondary metabolites widely distributed in the nature, especially in plants; all of them have an aromatic ring with different grade of hydroxylation, which have shown antioxidant capacity against free radicals or ROS (3).

The objective of this work was to determinate the phenolic content and antioxidant capacity from the pulque's methanolic extract produced in two municipalities located in southern Guanajuato.

Methods. The pulque's samples were obtained from the municipalities of Tarimoro (TAR) and Valle de Santiago (VDS), located in the southern of Guanajuato; an methanolic extract was prepared (30:70 v/v, pulque: methanol). Total phenolic content was measured by the Folin-Ciocalteu method, and the results were expressed as milligrams of gallic acid per liter (mg GAE/L). The antioxidant capacity was determined by DPPH (2,2-diphenyl-picrilhydrazil) radical, ABTS⁺ (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical, and the FRAP assay (ferric reducing ability of plasma by TPTZ, tripyridyltriazine). The results were expressed as micromoles of Trolox equivalents antioxidant capacity per mL (TEAC, μ M/mL) for ABTS and FRAP, and as percent of inhibition for DPPH. An analysis of variance (ANOVA) was applied, and the results express the average and standard deviation, a Tukey's test was used for comparison of the mean values at a significance level of 0.05. Each analysis was performed three times.

Results and discussion. The TAR's extract showed more abundance 153.75 mg EAG/L of phenolic content, and the lowest value was shown for VDS's extract, 86.17 mg EAG /L (Table 1).

Table 1. Bioactive compounds in methanolic extracts from pulque.

Location	Total Phenols (EAG mg/extract)	Antioxidant Capacity		
		DPPH (% Inhibition)	ABTS (TEAC, μ M/extract)	FRAP
TAR	153.75±10.57 ^a	31.18±2.72 ^a	195.44±12.99 ^a	230.84±21.11 ^a
VDS	86.17±17.61 ^b	9.66±2.12 ^b	67.04±19.97 ^b	40.63±11.62 ^b

The different letter indicate statistical difference at a significance at level $\alpha = 0.05$.

Antioxidant capacity was highest in the TAR's samples in comparison with VDS's extracts, in which the inhibition percent 31.18% y 9.66% for DPPH radical were found; 195.44 y 67.04 μ M TEAC/mL for the ABTS⁺ radical; 230.21 y 40.63 μ M TEAC/mL for the FRAP assay, respectively (Table 1). The highest standard deviation could be affected because of the variability that pulque presents in the process that it is produced, according with the pulque is an artisanal beverage. In addition, the difference between the averages could be influenced by the variety from the Agave, the production conditions, and the geographic location where the plant is cultivated.

Conclusions: The TAR's methanolic extracts reported more abundance of phenolic compounds, and the highest antioxidant capacity, they showed a statistically significant difference ($\alpha = 0.05$) in comparison with VDS's extracts.

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SHELF LIFE AND ELABORATION OF A SYMBIOTIC BEVERAGE T2

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Keywords: Aguamiel, shelf life, probiotic strains.

Introduction. A symbiotic drink is a way to improve gastrointestinal function. In this work, the fermentation conditions for the elaboration of a symbiotic aguamiel-based beverage were established. In the working group, strains with probiotic potential have been identified and characterized, which were used to make the symbiotic drink. Aguamiel is rich in fermentable and prebiotic sugars (Pedraza & Esquivel, 2018). The purpose of developing the symbiotic beverage with health benefits aimed at various sectors of the population, offering the benefits that probiotics and natural products such as aguamiel can offer through biotechnological processes.

Methods.

The probiotic strains were inoculated in selective medium and we obtained inoculum strains for the bioprocess conditions. The probiotic strains used in this research were *Lactobacillus sp* and *Bacillus sp*. Aguamiel was used as a culture medium, it was pasteurized at 80 ° C for 30 minutes (Márquez Morales et al., 2021). After the pasteurized process aguamiel was inoculated to 2% v/v preinoculum were generated with probiotic strains and incubated for 37°C for 48 h. A suitability and preference test was carried out, where 4 varieties of symbiotic beverage were tested. Shelf life was analyzed under conditions of 4°C, 20°C and 42°C, to measure its viability, UFC count and Brix degrees were used in triplicate (Mercado et al., 2016). Subsequently, sensory evaluation was carried out to identify the degree of acceptability and suitability test of the product obtained.

Results and discussion. The symbiotic drink was obtained using the corresponding probiotic strains and the established fermentation conditions. The beverage was studied for food safety, NOM-092-SSA1-1994, NOM-111-SSA1-1994 and NOM-113-SSA1-1994 standards of Mexico. The growth kinetics in aguamiel was determined (Figure 1), the result showed than exponential phase to 48 hours at 37 °C. In this it's possible to obtain cells in the range of 11-12 log CFU/ml in the fermentation medium. The sensory evaluation indicated the preference of the beverage with *Lactobacillus sp*. with blueberry as flavoring with 22% (Figure 2). Comparing the beverage with a commercial one which leaves it in a second place of preference.

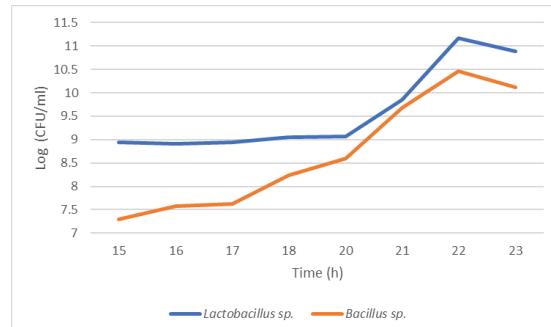


Figure 1. Growth kinetics in aguamiel medium

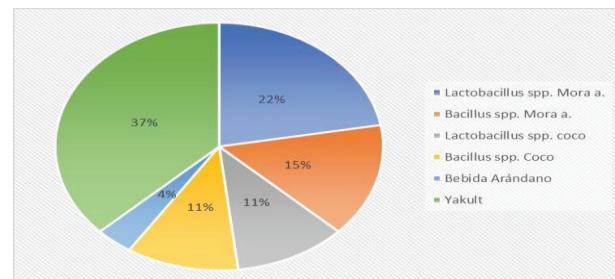


Fig. 2. Symbiotic beverage suitability test

In the suitability test, it obtained a 76% general acceptability in parameters such as aroma, flavor, texture. In the shelf-life study three temperature treatments of 4°C, 20°C and 42°C were applied. The optimal condition for the beverage was refrigerated, however, the sensory evaluation of shelf life shows a difference in its flavor after 15 days. Li et al (2021) reported that probiotic strains like *Lactobacillus sp* in milk has differences. In this research, we found that probiotics strains inoculated at aguamiel had significative effect on the growth with 4.55×10^{10} CFU/ml than Li et al obtained (1×10^{10} CFU/ml).

Conclusions. It was possible to obtain a symbiotic drink using mead. The cooling treatment was widely accepted by the study group. Regarding the shelf life of the drink, the first 15 days presents organoleptic characteristics acceptable to the consumer and achieving sufficient CFU/ml.

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CAPACIDADES FERMENTATIVAS DE LEVADURAS NO SACCHAROMYCES AISLADAS DEL PROCESO DE FERMENTACIÓN ALCOHÓLICA DEL BACANORA.

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Keywords: Bacanora, Non-Saccharomyces yeasts, alcohol fermentation

Introduction.

The bacanora is the exclusive distilled beverage of the state of Sonora, colorless and with a high alcohol content, it is made from the natural fermentation of the *Agave angustifolia* Haw, produced until now in an artisanal way (1). The purpose of the present work was to evaluate two non-*Saccharomyces* yeasts that participate in the alcoholic fermentation of the bacanora elaboration process, evaluating their fermentative capacities and determining their aromatic influence on the final product.

Methods. Alcoholic fermentation was carried out using two non-*Saccharomyces* yeasts (*Kluyveromyces* sp. and *Issatchenka* sp.) previously isolated from the fermented musts of *A. angustifolia* Haw. The fermentation media consisted of agave juice adjusted to 100 g/L (12 °Bx) of reducing sugars and added with 1 g/L of ammonium sulfate. Fermentation kinetics were carried out for 72 hours at 30 °C, 200 rpm and 5 x 10⁶ cells/mL of each yeast as initial inoculum. Biomass, reducing sugars and ethanol were determined. In addition, the main volatile compounds were quantified by gas chromatography (2).

Results and discussion. The results of sugar consumption and ethanol production by the different yeasts evaluated are shown in figure 1. The two yeasts presented a similar behavior, however, *Kluyveromyces* sp. was able to produce a greater amount of ethanol with a value of 42.6 g/L compared to the 36.3 g/L produced by *Issatchenka* sp. Regarding the main volatile compounds produced during alcoholic fermentation, it was possible to quantify Acetaldehyde, Ethyl Acetate, Methanol 1-Propanol, Isobutanol, Isoamyl Acetate, 1-Butanol, Isoamyl Alcohol, Ethyl hexanoate, Ethyl Octanoate, Ethyl decanoate, Phenethyl, Phenethyl Alcohol, being *Kluyveromyces*

the yeast that produced the highest amount of higher alcohols and esters, mainly isobutanol, isoamyl alcohol, ethyl acetate and acetaldehyde.

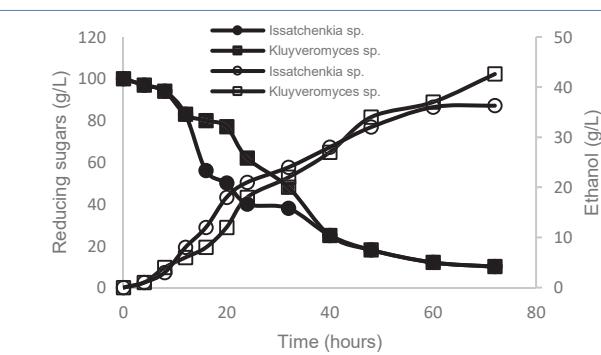


Fig. 1. Substrate consumption and ethanol production by *Issatchenka* sp. and *Kluyveromyces* sp.

Conclusions. The yeasts *Issatchenka* sp. and *Kluyveromyces* sp. isolated from the fermented musts of the bacanora production process, are suitable to be used as starter cultures in the production of bacanora due to their fermentative capacities and to be producers of important aromas and flavors in the drink.

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MINORITY BACTERIAL COMMUNITIES DURING THE FERMENTATION PROCESS OF MEZCAL FROM GUERRERO, MEXICO

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Keywords: *bacteria, fermentation, mezcal, metagenomic*

Introduction. Mezcal is a product obtained from the distillation of fermented agave. Factors such as the species of agave, the local climate, the production tools and the experience of the mezcalero producer favor the growth of certain microbial populations, which adapt to the nutrients in the must and synthesize volatile compounds, which confers a distinctive character of the species, region and production plant (1).

The objective of this project was to identify the bacterial consortium present in the fermentation process of mezcal produced in Guerrero.

Methods. The bacterial diversity of 7 samples of mezcal must collected at a factory in Plan de Guerrero, Guerrero, Mexico, was analyzed every 24 h, since the onset of the fermentation from was analyzed, carrying out a manual protocol for the extraction of Metagenomic DNA, which was sent for sequencing to University Medical Center (Boston, Massachusetts, USA), using the Earth Microbiome Project protocol. Here, we focused the analysis on minority groups (<1% abundance).

Results and discussion. In the fermentation of the agave for the elaboration of mezcal from Guerrero, the minority groups of bacteria included Enterobacteriaceae, Rhodanobacteraceae and *Bacillus* and *Lysinibacillus* with an increasing trend after 96 hours, which was present in the final stage. In addition, genera such as *Kozakia*, *Halotalea*, *Salinicola*, *Bavaricoccus*, *Kosakonia*, *Aerococcus*, *Clostridium*, *Enterococcus* and *Gluconoacetobacter* were found, which are mostly considered sources of contamination, likely from human contact, and the air (2). These contaminants were present in the early stages of fermentation and with frequencies <1%, it is likely that factors such as pH, water activity and sugar content could suppress their growth (3).

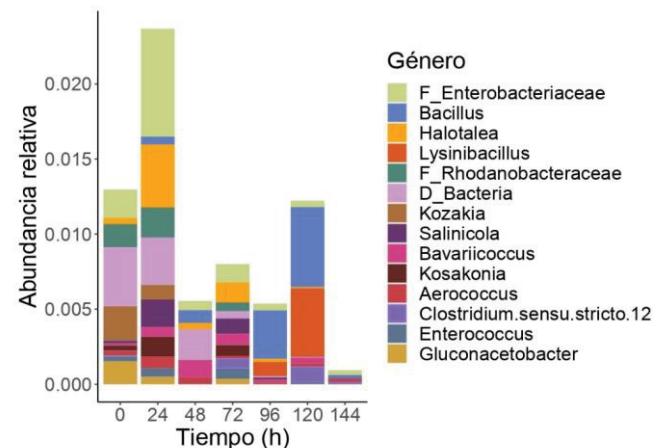


Fig.1. Minority bacterial communities in mezcal from Guerrero.

Conclusions. The minority microbial communities of must fermentation in mezcal production are represented by bacteria such as Enterobacteriaceae, Rhodanobacteraceae, *Bacillus*, *Lysinibacillus*, *Kozakia*, *Halotalea*, *Salinicola*, *Bavaricoccus*, *Kosakonia*, *Aerococcus*, *Clostridium*, *Enterococcus* and *Gluconoacetobacter*.

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METHANOL ANALYSIS OF ARTISANAL AND INDUSTRIALLY PRODUCED AGAVE WHITE SPIRITS

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Keywords: Methanol, agave spirits, Mexican regulations.

Introduction. Methanol is a highly toxic compound for human consumption, so its content is used as a quality indicator in many countries for fermented and distilled alcoholic beverages, because it represents a risk to the consumer's health. The Mexican government has established 300 mg of methanol / 100 mL of anhydrous alcohol as the maximum content permitted. In the production process of agave spirits, methanol is produced particularly during the cooking and fermentation phases. Thus, this compound is produced through the chemical reaction of pectins under high temperatures in an acid medium during the agave heads cooking, but also by demethylation of pectins, due to enzymes produced by certain microorganisms during alcoholic fermentation [1,2,3], and probably also from cellulose during fermentation of juices with bagasse. Therefore, this work aimed to evaluate the methanol content in diverse commercial samples of agave spirits.

Methods. Fifty-four samples of artisanal and industrial agave spirits without any type of maturation in barrels, that is, white schnapps, acquired directly from producers or in stores [37 of artisanal mezcal (**AM**); three tequila (**T**); four industrial mezcal (**IM**); seven named agave liqueurs (**AL**); and three pulque spirits (**PS**)]. The ethanol and methanol analyses were carried out by HPLC and GC, respectively.

Results and discussion. According to compliance with the methanol content specification, three groups were established, over and under specification and non-detectable groups (Figure 1). **AM** samples being the largest subgroup in all three groups, in part because they are more than a half of the samples. However, the proportion between those that exceed and those that are within the specification was similar, perhaps because fermentation with bagasse and deficient distillations still persists in the **AM**, but also there exist producer controlling certain factors of their process that definitely keep their mezcal within the methanol specification. Regarding the non-detectable group, in eight **AM** samples methanol wasn't detected, probably because they are not made from agave, but from sugar cane alcohol with a little mezcal or agave distillation tails, a common practice in **AL** production.

Following this group, the **PS** lacked methanol due to the nature of the wine and because pectins or cellulose are not involved during fermentation, so their distillate is free of methanol. On the other hand, in the group that exceeds the methanol specification, two **IM** were very close to the specification, probably because were fermented with bagasse like the artisanal ones. In addition, two **AL**, which it is most likely due to the use of poorly distilled cheap spirits or distillation by-products such as heads and tails mixed with cane alcohol, which, due to their high concentrations, is insufficient for dilution. Lastly, two small subgroups of the group under specification were **T** and **IM**, because they are not probably fermented with bagasse and probably do apply greater control of the distillation stage.

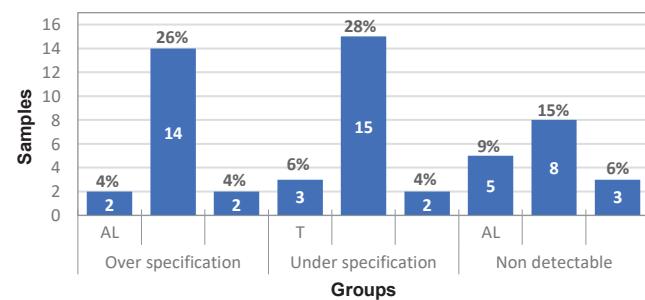


Fig. 1. Distribution of samples according to the reference value of methanol content of Mexican regulation (300 mg of methanol /100 mL of anhydrous alcohol).

Conclusions. There were differences in the content of methanol in agave spirits produced by different ways, as a reflex of differences in their process control.

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POSTERS TEMATICA III

**Fructans and other agave
products**

AGAVE FRUCTAN METABOLIC PROFILES IN PLANTS OF *Agave angustifolia* HAW. UNDER TWO DIFFERENT CROP MANAGEMENT STRATEGIES

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Keywords: Fructans, Agave, HPAEC-PAD

Introduction. In the past years, with the rising global consumption of mezcal, the cultivation of *Agave angustifolia* Haw. has grown intensively, prompting to develop of new management strategies using chemical inputs to increase crop yield. Besides, *A. angustifolia* Haw. has the potential to meet the increasing demand of fructans since, it can also be used as raw material for the production of prebiotics [1]. Although the plant size is apparently advantageous for farmers in terms of fructans amount because larger plants might produce higher usable matter, though, a large sized plant producing lower fructans amount is not necessarily a good resource. Therefore, it is necessary to know more about the type and concentration of fructans to give better alternatives.

The objective of this study was to investigate fructans fluctuation in plants of *A. angustifolia* Haw. (1 to 3 years-old) using two different crop management strategies in the field: a traditional management without any agrochemical reagents (TM) and an agricultural system with fertilized management (FM).

Methods. Fructan extracts were analyzed through a series of analytic techniques such as Thin Layer Chromatography (TLC) and High-Performance Anion-Exchange Chromatography (HPAEC–PAD) to identify simple carbohydrates and fructans [2].

Results and discussion. Analyses showed that the concentration of simple carbohydrates and fructans changed during plant development, young plants of both crop managements mainly stored simple sugars as glucose, fructose, sucrose, and FOS while old plants mainly stored high DP fructans (Fig 1). However, concentrations of glucose and fructose did not show significant differences between the two-crop managements, while sucrose and fructans were more abundant in fertilized plants (FM) (Fig 2). Moreover, plants under traditional management showed least amount of fructooligosaccharides (FOS: short DP fructans) and high DP fructans.

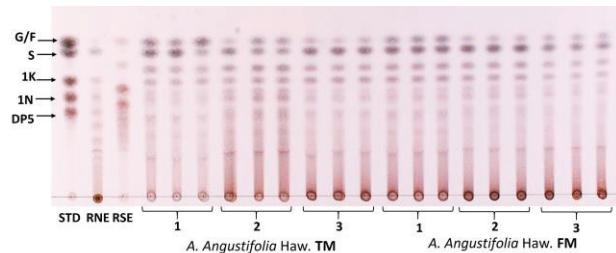


Fig. 1. Thin layer chromatography (TLC) from *A. angustifolia* Haw. with traditional management (TM) and fertilized management (FM).

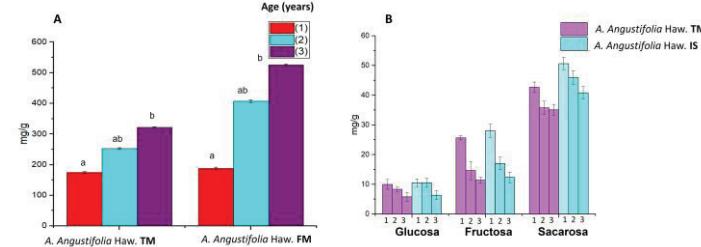


Fig. 2. Fructans and simple carbohydrates contents of *A. angustifolia* Haw. plants at different crop management strategies.

Conclusions. FM plants presented larger amounts of sucrose, fructooligosaccharides (FOS: short DP fructans), and high DP fructans. And plants under traditional management (TM) showed the least amount of sucrose and fructans.

Acknowledgements. The authors thank “Maestro Naxuhal” and “Mil queridas” for providing vegetal material.

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BIOFILM MADE FROM FRUCTANS BY AGAVE LACTIC ACID BACTERIA

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Keywords: Biofilm, lactic acid bacteria, fructans

Introduction.

Lactic Acid Bacteria (LAB) may produce biofilms made of exo-polysaccharides (EPS) which are important in sugar refineries. For example, *Leuconostoc* sp. biofilms from sucrose, fouling pipelines. Such biofilms are resistant to adverse environmental factors and require low levels of complex nutrients. LAB biofilm reactors have been reported to have high conversions with high C/N ratios [1]. To the best of our knowledge, there are no reports of biofilms made from fructans by epiphytic LAB isolated from *Agave salmiana*. Here we report biofilm formation by such kind of organisms.

Methods.

Wild LAB strains were isolated from *Agave salmiana*. Strains, 1 to 8, were *Lacticaseibacillus* sp., Strain 9 was *Enterococcus* sp. [2]. Strain 10 was *Lactobacillus delbrueckii subspecies bulgaricus* isolated from dairy products. Culture media were MRS with glucose, fructose or fructans as the main carbon source. All experiments were done in triplicate. Cell aggregation (%) and hydrophobicity were tested [3]. Biofilm formation was assayed using crystal violet [4].

Results and discussion.

All tested LAB had a strong self-aggregation phenotype (> 86%). Figure 1 shows that all strains had strong hydrophobicity with chloroform (> 60%) but rather low with ethyl acetate (< 30%).

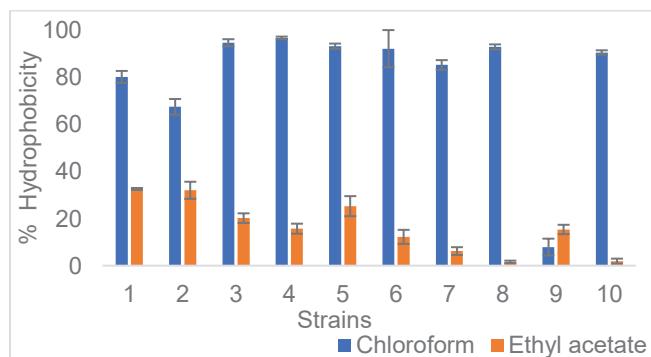


Figure 1. Solvent hydrophobicity test of LAB strains

Figure 2 shows a low level of biofilm formation with fructose and higher levels with glucose or fructans. The crystal violet test was significantly higher with fructans for most of the *Lacticaseibacillus* strains (2, 4, 5 and 8) as compared to those of *Enterococcus* sp (strain 9) and *Lactobacillus delbrueckii subspecies bulgaricus* (strain

10). Additionally, the cell-free fructans control was negative.

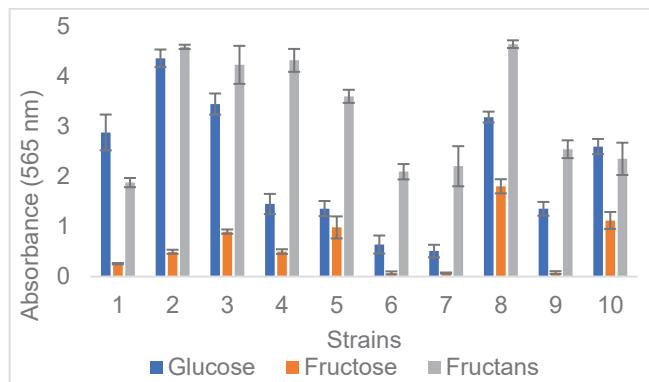


Figure 2. Biofilm formation with different strains and carbohydrate substrates.

Conclusions. Present results show that epiphytic *Lacticaseibacillus* sp isolated from *Agave salmiana*, have the ability to form biofilms when the carbon source is made of fructans but have low biofilm formation with fructose. These results support the use of fructan solutions in biofilm reactors for continuous production of lactic acid production instead of using fructan hydrolysates rich in fructose.

Acknowledgements. N.A. Martha-Lucero had a postgraduate scholarship (853186) from CONACYT.

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PHYSICOCHEMICAL AND SENSORY CHARACTERIZATION OF MAGUEY SYRUP FROM SINGUILUCAN AND CARDONAL REGIONS IN HIDALGO STATE, MEXICO.

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Introduction. In Mexico, the Hidalgo state is the major producer of agave sap (called *aguamiel*) and *pulque*. In the Singuilucan and Cardonal regions, organized communities of producers are engaged in producing maguey syrups from agave sap. This kind of syrup is a traditional product used as a sweetener. It is handcrafted from the heat concentration of sap of different agave species until it reaches around 70°Brix. In order to promote its consumption and commercialization in different markets, there is an interest in characterizing the functional components, such as the Fructooligosaccharides (FOS), as well as the sensory attributes, that in turn depend on the agave sap composition. Previous work [1] showed large variations in the carbohydrate profile and pH of agave sap obtained from different cultivars in the same region. This work aims to evaluate the physicochemical and sensory parameters of maguey syrup from two regions (North: Cardonal and Southeast: Singuilucan) in the Hidalgo state.

Methods. Two maguey syrup samples (ML and MR) were obtained from the Cardonal region (Thafi's) and three samples of different periods from Singuilucan (Rancho La Gaspareña: RLG). Physicochemical parameters such as color, pH and Brix degrees were analyzed for each sample. The carbohydrate profile was determined using HPLC methods to quantify the amount of FOS, glucose, fructose and sucrose. Additionally, a sorting task was carried out to determine if there were perceptible sensory differences and a free description of the samples was included [2].

Results and discussion. The Cardonal syrups presented acid pH (3.6-3.8) and dark color ($L^*<1$, $h^*<28$), while the Singuilucan syrups are pH between 4.4 - 6.3, with yellow and amber colors ($L^*=10-19$, $h^*=50-65$). Fig 1, shows the great differences in carbohydrate profile. The sorting task profile showed sensory differences, which refer to a pattern of specific sensory descriptors for each sample, according to the correspondence factor analysis.

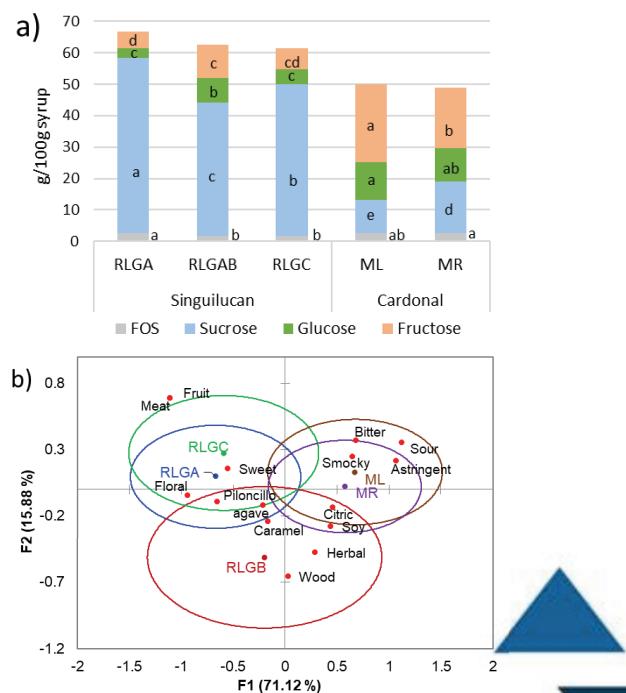


Fig. 1. a) Carbohydrate profile b) Correspondence factorial analysis on the citation frequency of sensory attributes.

Conclusions. The maguey syrups from different regions present differences in physicochemical and sensory attributes. The amount of FOS has been found between 1.7-2.6g/100g syrup, which is a low value to be considered a product with a potential prebiotic effect.

Acknowledgments. The authors thank the project CONACYT 317510, for financial support.

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ANALYSIS AND CHEMICAL CHARACTERIZATION OF AN *Agave angustifolia* Haw EXTRACT RICH IN FRUCTANS

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Keywords: Extraction, chemical profile, Agavins

Introduction. Fructans are water-soluble fructose polymers that offer nutritional and technological advantages [1,2]. The extract obtained from the stem of *Agave angustifolia* Haw is rich in fructans and its addition to different matrices has shown to have beneficial effects at the gastrointestinal level [3].

The aim of this work was to compare the chemical and physicochemical composition of an extract of *Agave angustifolia* Haw obtained in CEPROBI-IPN and commercial fructans of *Agave tequilana* and *Chicorium intybus* L. (Chicory).

Methods. The physicochemical profile (degrees Brix, % humidity, water activity, % protein and total dietary fiber), particle size distribution, morphological analysis and mapping elements by Environmental Scan Electronic Microscopy (ESEM), besides, the sugars profile by HPTLC and determination of total phenols and flavonoids was also performed.

Results and discussion. *A. angustifolia* showed the highest content of protein and total dietary fiber (2.19% and 79.73% respectively), compared to the two commercial sources. Regarding water activity and particle size distribution, no significant differences were found. In the profile of compounds by HPTLC were found mainly carbohydrates (glucose, fructose, ketose), and a lesser extent phenols and flavonoids. *A. tequilana* presented the highest percentage of humidity (4.07%) among the three species.

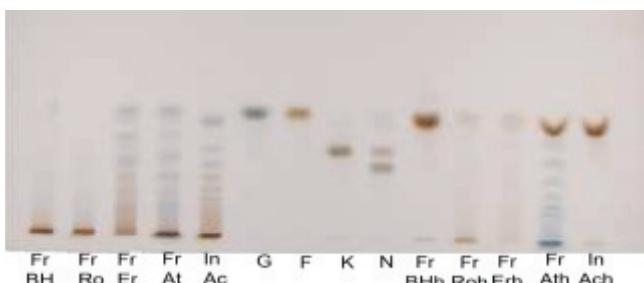


Fig. 1. HPTLC analysis of fructans samples. Ac: Achicoria, G: Glucose, F: Fructose, K: Ketose, N: Nystose

Element mapping showed that commercial fructans of *A. tequilana* presented higher percentage of Si (10.73 %) with respect to the other species, while highest percentage (1.05%) of Ca were found in *A. angustifolia*.

Table 1. Elements maps in fructans samples. Fr: fructans, In: Inulin, BH: Barranca Honda, Ro: El Rodeo, Er: La Era, At: *Agave tequilana* (comerciales), Ac: Achicoria.

Sample	C	O	Na	Si	Ca	Fe	S	P	K	Mg
Fr-BH	61.2 5 ± 5.49	33.9 9 ± 0.61	2.85 ±0.0 9	5.90 ±0.19	1.05 ±0.04	0.22 0.07	0.00 1	0.75 ±0.0 2	0.04 ±0.0 2	0.03 ±0.0 1
Fr-Er	70.8 8 ± 5.15	28.3 1 ± 2.71	0.08 ±0.09	-	1.56 ±0.04	-	0.19 ±0.13	0.14 ±0.10	1.07 ±0.82	0.44 ±0.28
Fr-At	51.7 8 ± 0.88	36.7 8 ± 0.61	1.10 ±0.09	10.7 3 ± 0.19	0.14 ±0.04	0.36 ±0.07	0.01 1	0.01 ±0.0 2	0.05 ±0.0 2	0.06 ±0.0 1
In-Ac	56.1 9 ± 2.58	35.6 0 ± 1.22	0.95 ±0.04	9.20 ±1.24	0.08 ±0.02	0.29 ±0.06	0.01 1	0.01 ±0.0 2	0.03 ±0.0 2	0.02 ±0.0 2

Conclusions. This information reveals that there are significant differences between the samples of the extract obtained in CEPROBI-IPN and between the commercial samples. This may indicate that the obtained results could be attributed to the different extraction process (Industrial and pilot plant).

Acknowledgements. CONACyT and Instituto Politécnico Nacional.

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MAGUEY SYRUP: OPTIMIZATION OF THE ARTISANAL PRODUCTION PROCESS

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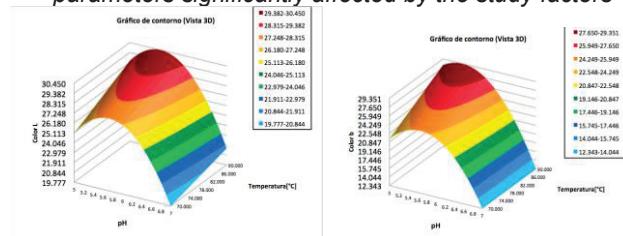
Keywords: maguey syrup, optimization, response surface

Introduction. Maguey is an endemic natural resource of America with approximately 200 species, of which 150 are found in Mexico [1]. Aguamiel is a product derived from these species, which has only been used to produce traditional drinks such as pulque [2]. However, aguamiel and its derivatives can offer health benefits by preventing cardiovascular, bone and respiratory problems [3,4]. That is why the objective of this research is to standardize the artisanal manufacturing process of agave syrup through the physicochemical characterization of aguamiel and through a factorial design to determine the optimal production factors.

Methods. The sampling was carried out in the town of San Gabriel Azteca, municipality of Zempoala, Hgo. Twenty aguamiel samples were collected in 125 mL sterile flasks. The aguamiel was pasteurized at 90 °C for 10 minutes in an autoclave and pH, color, °Brix, total sugars, reducing sugars and viscosity were analyzed. After these measurements, the sample was stored at -20 °C. A Plackett-Burman design was carried out with two factors (pH and process temperature) and three levels. The fixed output parameter was the total solids (70 °Bx). An ANOVA was carried out using the Addinsoft 2020 software. In the end, physicochemical analyzes of the maguey syrup and sensory analysis were carried out with a panel of expert producers of artisanal syrup.

Results and discussion.

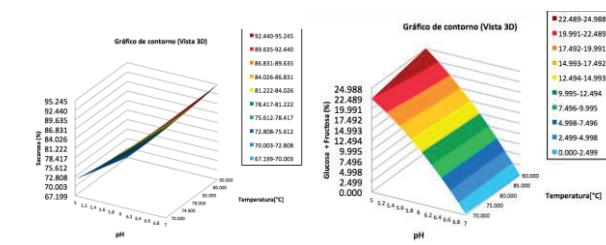
Fig. 1. Response surface for the parameters L (a) and b* (b), parameters significantly affected by the study factors



Aguamiel presented a pH that ranged between 4.20 and 7.68, °Brix from 10.1 to 17.4, viscosity from 12.8 to 18.8 cP and color parameters L* a* b* from 33.53 to 48.68, -0.2 to 8.41 and 2.49 to 29.88 respectively. Regarding reducing sugars, the analysis showed a concentration of 0.03 to 1.75 g/L and total sugar

concentrations of 6.81 to 39.45 g/L. These results indicate that the samples are different between varieties and within the same variety, derived from the place of growth of each maguey, with no correlation between the parameters.

Figure 2. Response surface graphs of the effect of pH and process temperature on the concentration of sucrose (a) and glucose + fructose (b).



Producers selected two syrups. However, crystal formation was observed during storage in the syrups made at 90 °C. So the best syrup was made at a pH of 6 and at 80°C and was selected as a standardized treatment for the production of syrup.

Conclusions. The physicochemical characterization of aguamiel is not dependent on the maguey variety. pH mainly affects color and carbohydrate composition. However, the temperature has a direct effect on the crystallization. It is possible to achieve standardization of the agave syrup production process through temperature and pH control.

Acknowledgments. The authors thank the project CONACYT 317510 for financial support.

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CHARACTERIZATION OF ACETYLATED AGAVINS OF *Agave Angustifolia* Haw.

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Keywords: Modification, optimization, polysaccharides

The chemical modifications of native polysaccharides are used to extend their applications due to their physicochemical properties change providing new functionalities and thus give them different uses in the food industry, tissue engineering, the supply and controlled release of drugs, among others. This functionalization occurs by taking advantage of the free carbonyl and hydroxyl groups that are distributed along the skeleton of these, creating derivatives with properties determined and adapted to the needs. The properties that have generally been modified are physicochemical and biological. This is achieved through oxidation, esterification, amidation, among others, resulting in molecules with new functional groups [1]. Acetylation modification is mostly used when you have branched polysaccharides which have more sites for possible substitution. The main mechanism is that acetyl groups can cause the branches of polysaccharides to stretch and change their spatial orientation [2]. Therefore, the aim of this work was to optimize the acetylation of *Agave angustifolia* Haw.

Methods. Acetylation modification of agavins was performed using dimethylformamide as a reaction medium, sodium acetate and acetic anhydride. Subsequently, the optimal concentration of the reactants of the reaction was identified. Acetylated agavins were characterized by NMR, RAMAN, and DRX.

Results and discussion. Through NMR spectroscopy, the degree of substitution of the modified agavins was determined, obtaining a 2.3 with an acetylation percentage of 76%, which corresponds to the amount of acetylated hydroxyl groups. The modification by acetylation could be observed by the Raman spectrum (Fig.1), in the region from 1245 to 1550 cm⁻¹, where the vibrational response of the modified agavins is decreased. This substitution of acetyl groups can occur in two ways, with bonds through groups O or groups N depending on the polysaccharide, in the case of agavins it occurred through O bonds belonging to the different OH groups that it has in its structure [3]. The samples of native and modified agavins are presented in Fig. 2, it is observed that both agavins have a characteristic wide band of amorphous materials, however, acetylation gives rise to crystallographic planes characteristic of a structure with

a higher degree of crystallinity. The modified agavinas have a rearrangement in the chains, which are represented in the appearance of new peaks in the diffractogram of the MA.

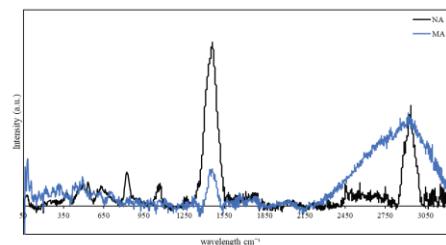


Fig 1. Raman spectrum of native (AN) and modified (AM) agavins

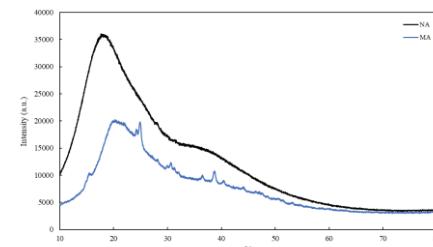


Fig. 2. Diffractogram of native and acetylated agavins

Conclusions. It was determined that DMF affected the performance of the acetylation reaction; by means of a response surface design, the optimal ratio of the reaction compounds (Agavins 11.7%, AA 65.6%, AcNa 0.25% and DMF 22.36% w/w) could be determined, obtaining a maximum yield of 52%.

Acknowledgements. Consejo Nacional de Ciencia y Tecnología (CONACyT) and Secretaría de Investigación y Posgrado (SIP).

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AGAVE FRUCTANS AS SUGAR SUBSTITUTES IN SOFT GEL FORMULATIONS.

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Keywords: Functional foods, Texture, *A. Tequilana Weber*

Introduction. Gelatin gels are treats widely consumed by people of different ages. Their texture and appearance can be varied by changing the concentration of solids, type, and concentration of protein or water content. Their formulation requires high amounts of carbohydrates such as sucrose and glucose, which contributes to their flavor, texture, and consistency. Their high sugar concentration makes them creditors to nutritional warning seals, including sugar excess and high-calories. These products typically possess high glycemic index values; for example, a portion of 30 g can raise the GI values close to 80. Their inappropriate consumption can contribute to overweight and obesity. Agave fructans offer several technological properties, including low-calorie sweeteners, texture, and viscosity modifiers.

This work explored agave fructans as a substitute for sugar in soft gel formulations through physicochemical and textural properties.

Methods. Three gel formulations were prepared, substituting the mass of sucrose/glucose with agave fructans from *A. Tequilana Weber* (0, 50, 100%) following a procedure proposed by Rodriguez-Rodriguez et al. [1]. Physical properties were compared, including pH, water content, water activity, color, and texture.

Results and discussion. The formulation of gels with different concentrations of agave fructans was successfully produced (Fig. 1). The agave fructans reduce the crystallization of sucrose during manufacturing. The pH values and water content of the samples showed no significant differences ($p < 0.05$) between the samples. The water activity value of the F50 sample was significantly lower than the F0 and F100 samples ($p < 0.05$).



Fig. 1. Gel formulations 0%, 50% and 100% sucrose substitution (from left to right).

Color impacts the acceptability of food products. The CIElab color parameters showed significant differences

between samples ($\Delta E, p < 0.05$). The results exhibited that the ΔL^* and Δa^* values decreased with the agave fructans substitution, enhancing the greener color in the F100 formulation.

On the other hand, the texture of soft candies is influenced by the production process, water content/water activity, gelling agent, and sugar content, principally. Cohesiveness, elasticity, and resilience did not show significant differences between the samples ($p < 0.05$).

Table 1. TPA results of gel formulations

	Hardness (gr)	Gumminess (gr)	Chewability (gr)
F0	858±29 ^a	822±24 ^a	798±32 ^a
F50	1813±144 ^b	1696±134 ^b	1646±138 ^b
F100	1169±64 ^c	1110±54 ^c	1053±60 ^c

The F50 sample showed the highest hardness, gumminess, and chewiness values compared to the F100 and F0 samples. Typical hardness values of soft candies must range between 400 and 1600 gr[2], which agrees with those obtained in our study. Agave fructans and sucrose competed for water molecules in the sample, favoring the structure of gels.

Conclusions. The reduction of sucrose with agave fructans is possible in gelatin soft gels, displaying appropriate textural and appearance products. The results suggested that Maillard reactions between gelatin and agave fructans are responsible for the color difference of the samples.

Acknowledgments. This research was funded by FODECIJAL, grant number 2019-8052.

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VALORIZATION OF AGUAMIEL PRODUCED IN COMMUNITIES OF THE HIDALGO STATE: SUSTAINABLE PRODUCTION OF AGUAMIEL SYRUP RICH IN OLIGOFRACTANS DESTINED FOR MEDIUM AND MEDIUM-HIGH ECONOMIC SECTORS

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Introduction. According to data from the Agrifood and Fisheries Information Service (SIAP), in 2018 Hidalgo State contributed 78.14% of the total production of pulque agave, generating 194 million 579 thousand liters of aguamiel or agave sap [1]. In search of diversify the use of aguamiel, producers have undertaken the task of manufacturing aguamiel syrup. However, they have faced several challenges, being one of the most important the lack of standardization in their production process. This situation has prevented the product (aguamiel syrup) is marketed at a high cost, despite having beneficial properties for human health. Therefore, the aim of this work is to optimize the semi-industrial manufacture of aguamiel through the characterization of agave, aguamiel (raw material) and food product (aguamiel syrup), which can show beneficial properties for human health (hypoglycemic and prebiotic activities) with standardized quality and ready for sale.

Methods. The project is divided into four stages. In the first stage, a census is carried out in aguamiel-producing municipalities in the state of Hidalgo, to generate a database. For this purpose, an instrument was designed, which has been applied to the producers during fieldwork. After the application of the survey, the systematization and analysis of data is carried out. At the same time, the morphological characterization and tissue collection for genetic analysis were carried out. Analysis of data will allow to identify the main aguamiel producers and the best agave varieties. In consequence, in-situ sampling will be done to perform the physicochemical characterization of raw material (aguamiel). The second stage will begin with a genetic study of the aguamiel-producing agave varieties. In the same way, the design and optimization of the agave syrup production process will be carried out. In the third stage, the in-vitro and in-vivo studies will be tested to determine the bioactivities of the aguamiel syrup. Once the process has been optimized and the agave syrup with the best physicochemical and sensory profiles has been produced, prebiotic and hypoglycemic activity

tests will be done. Fourth stage will consist of doing a pre-feasibility study, using the results of the previous stages and with the support of aguamiel producers. Likewise, with the pre-feasibility study and once the production process has been optimized, the technology transfer will be carried out to the producers so that they can start with the production of aguamiel syrup with standardized quality. During this period, talks will be organized with the producers, in which they will be trained on the standardization of the production process. Finally, regional discussion forums will be designed to promote association between producers, and at the same time to identify the main challenges facing the sector and design strategies collaboratively, in a synergy between researchers, producers and linking with government agents that support this management.

Results and discussion. Five municipalities in the Hidalgo State have been studied, in which a high agrobiodiversity of pulque agave has been registered, which are maintained in traditional production systems. In Mezquital Valley, they call this production system *Ñu'ta* in the Hñähñu language, while the Llanos de Apan they call *Metepantles*, or simply *Magueyeras* in other localities. Maguey varieties have been characterized ethnobotanically and morphologically. The size of the plant, the shape, number, and size of the leaves, together with the shape and number of lateral teeth and color, are the main characteristics that allow them to be distinguished. In total, samples have been collected from 159 individuals, obtaining DNA concentrations greater than 50 ng/ μ L of DNA with acceptable DO, which indicates pure DNA with few extraction contaminants.

Conclusions. In Mezquital Valley, the preferred traditional variety is *Xamini*, while in the Llanos de Apan it is the *Manso*. Even when there are large traditional varieties that produce high volumes of agave sap, producers prefer those varieties with sweeter, since they seem to give a higher yield in syrup.

EFFECTS OF CHEMICAL MODIFICATIONS ON THE ANTIBACTERIAL ACTIVITIES OF AGAVE FRUCTANS FRACTIONS.

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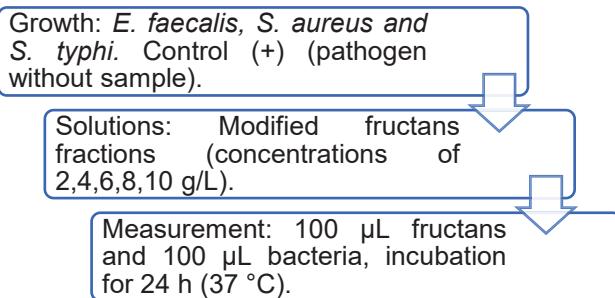
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Keywords: Fructans, chemical modification, antibacterial activity.

Introduction. Modified biopolymers have been studied for their biological characteristics,⁽¹⁾ but little is known about the effect of these modifications on antibacterial potential. Several investigations have been conducted to study the structural modifications and improvements of polysaccharides.⁽²⁾ In this regard, high-performance agave fructans fractions (HPAF) and high degree of polymerization (HDPAF) possess a complex and branched structure that when modified could lead to the development of new antibacterial agents with improved characteristics. The objective of the present investigation is to evaluate the effect of acylation, acetylation and succinylation in agave fructan fractions on their antibacterial activity.

Methods. Antibacterial Activity: Microdilution Method (3)



Results and discussion

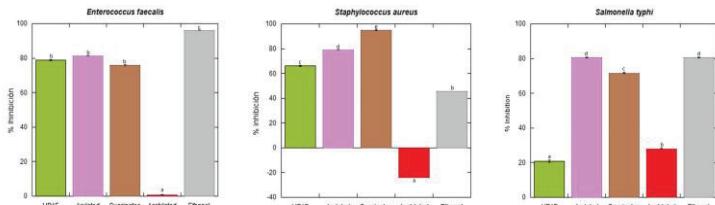


Fig. 1 Percentage inhibition of HPAF and its derivatives against pathogenic bacteria. Different letters indicate differences ($p < 0.05$) between the fraction and its derivatives.

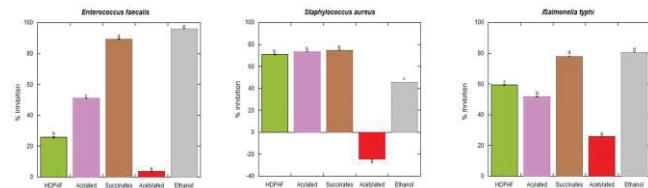


Fig. 2 Percentage inhibition of HDPAF and its derivatives against pathogenic bacteria. Different letters indicate differences ($p < 0.05$) between the fraction and its derivatives.

Figures 1 and 2 show that the acylated and succinate fructan fractions presented a greater inhibition against pathogenic bacteria in relation to the unmodified ones, while the acetylated ones did not present such activity, the differences ($p < 0.05$) observed could be related to the characteristics and chain length of the incorporated groups, as well as the defense mechanisms of each bacterium.⁽⁴⁾

Conclusions. It was shown that the antibacterial activity of the modified fructans was dependent on the structure of the functional group, degree of polymerization and degree of pathogenicity of the bacteria. These findings suggest that the modifications are an alternative for the development of potential natural antibacterials.

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DESIGN OF A DOUBLE EMULSION WITH HIGH DEGREE OF POLYMERIZATION AGAVE FRUCTANS (AFHDP)/WPC FOR THE PROTECTION OF PROBIOTICS

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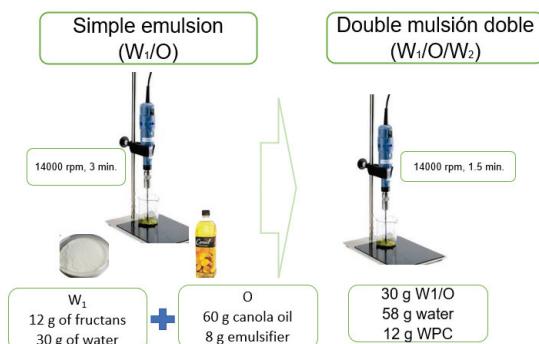
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Keywords: Probiotic, double emulsion, AFHDP.

Introduction. Currently, the use of microencapsulation in the development of functional foods with high added value ingredients has increased in the food industry. Such is the case of microcapsules added with probiotics and prebiotics. Double emulsions are complex polydisperse multiphase systems consisting of an emulsion in which the dispersed droplets are themselves an emulsion. Their compartmentalized structure gives them the potential to separate, encapsulate, protect and facilitate the sustained release and transport of bioactives (Juárez-Trujillo *et al.*, 2020). In this type of systems, biopolymers are used to stabilize them. An example of these biopolymers are agave fructans with a high degree of polymerization and Whey Protein Concentrate. Therefore, in this work the effect of a combination of FAGP/WPC on the viability of *B. longum* and *L. acidophilus* was evaluated.

Methods.



Results and discussion.

Well-defined double emulsions were formed with a structure that allows the encapsulation of probiotics (Figure 1). The particle size of the double emulsions was 16.49 µm, a common particle size for double emulsions due to the two-step manufacturing process. Similar results have been reported by other authors (Juárez-Trujillo *et al.*, 2021). The viability of the double emulsions was greater than 9 log CFU/g solid. The viability could be due to the fact that the protein-fructan mixture protects the probiotics from stress during the formation process double emulsion. Various authors have reported that the minimum viability to obtain adequate benefits for health must exceed 6 log CFU/g (FAO/WHO, 2002).

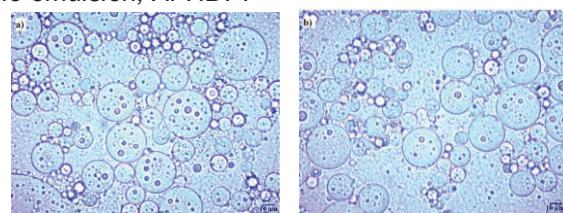


Fig. 1. Micrographs of double emulsions with a) *B. longum* and b) *L. acidophilus* and FAGP.

So the double emulsions developed in this work may have a beneficial effect when consumed since have higher viability. Likewise, in double emulsions, the stability of the system is of utmost importance for its commercial application (Ding *et al.*, 2022), so the stability of the emulsions was determined by the creaming index, finding the absence of phase separation. of double emulsions.

Table 1. Physicochemical properties of double emulsion made with *B. longum* or *L. acidophilus* and FAGP

Property	Value
Tensión superficial (mN/m)	28.67 ± 0.03
Average diameter size (µm)	16.49 ± 7.43
Creaming index (%)	ND
Viability double emulsion <i>B. longum</i> (log CFU/g solid)	9.23 ± 0.09
Viability double emulsion <i>L. acidophilus</i> (log CFU/g solid)	9.32 ± 0.03

Conclusions. A stable compartmentalized protection system was developed for the protection of encapsulated probiotics with potential for commercial application.

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IN VITRO EVALUATION OF AGAVE SALMIANA AND CHICORY INULIN FRUCTAN MIXTURES AS ANTICANCER POTENTIAL IN COLON CELLS T3

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Keywords: *fructans, cancer cell, Agave salmiana.*

Introduction. Colon and rectum cancer, occupies the second place in causes of death (935 000) and third place on new cases (1.93 million). Beneficial modification of microbiota can be carried out by prebiotics which, induce the production of short chain fatty acid (SCFA) by the proliferation of probiotic in gut microbiota producing great benefits such as cancer colon prevention. Some studies have reported the benefits of the individual use of inulin (lineal fructans) or Agave (branched fructans) [1] on gut microbiota. However, the mixtures of both fructans on colon cancer cells is scarce. The aim of this study was to evaluate the effect of lineal and branched fructan mixtures on the apoptosis of three type on cancer colon cells.

Methods. Inulin and *A. salmiana* fructans mixtures were prepared in proportions of 1:0, 0:1, 1:1, 3:1, 1:3 respectively at concentration of 2, 3.5 and 5mg/mL. Each colon cell line CRL1831 (ATCC CRL-1831) (health cell), HT29 (ATCC HTB-38) (cancer cell grade 1-2) and SW480 (ATCC CCL-228) (cancer cell grade 3-4) was supplemented with fructan mixtures (200 µL), and apoptosis assay were carried out.

Results and discussion. Health cells (CRL1831) gave the lowest percentages of apoptotic cells in comparison to cancer cells, the best results were agave-inulin (3:1) at 120h and the concentration was indistinct. As an example figure 1 shows the results for cancer cells HT29 at 72 h, the percentages of apoptotic cells were approximately 100% with agave-inulin (3:1) at a concentration of 5mg/mL. These results suggest that there is a synergistic effect between both type of fructans on the apoptosis of these cells. For cancer cell SW480 the 100% of apoptosis was obtained by agave-inulin (1:1) with a concentration of 5mg/mL. These results indicate that these cells need less concentration of branched fructans than HT29 cells. In general, SW480 cells showed a stronger apoptosis than HT29; this agrees with other studies where apoptosis

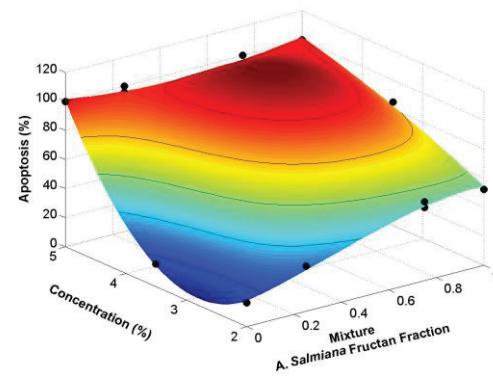


Figure 1 Apoptosis percentages of HT29 cell at 72h

resistance was higher in HT29 cells, possibly due to its morphology (most similar to small intestine enterocytes) [2]. Normally, cancer cells tend to avoid apoptosis signaling pathways by suppressing the p53 protein and overexpressing BCL-2, preventing mitochondrial membrane permeabilization and thus inhibiting apoptosis.

Conclusions. Fructan mixtures do have a synergic effect on the apoptosis process, can prevent the apoptosis of healthy colon cells and can induce the apoptosis of colon cancer cells.

Acknowledgements. CONACYT for scholarship.

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Morphostructural characterization of *Saccharomyces boulardii* encapsulates obtained by ionic gelation.

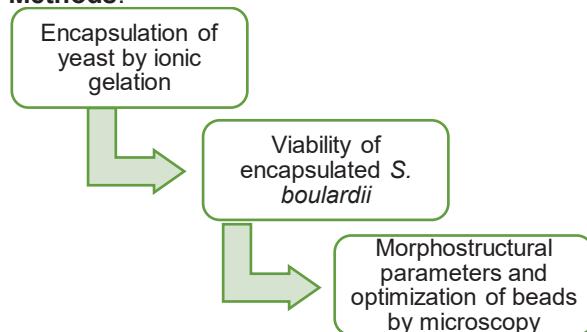
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Keywords: prebiotics, encapsulation, image analysis

Introduction. Encapsulation is a process where bioactive compounds and/or cells are encapsulated with wall materials or their mixtures in order to protect them from adverse conditions [1]. Different techniques have been developed, such as ionic gelation, which has advantages compared to other processes due to its low cost and availability of equipment. Agavins and whey protein with prebiotic effect, in mixture, have been shown to be favorable materials for the encapsulation of probiotic microorganisms [2, 3]. Encapsulation by ionic gelation of *Saccharomyces boulardii*, a probiotic yeast commonly used in the treatment of gastrointestinal disorders, using agavins and whey protein as wall materials, could increase its survival against environmental and gastrointestinal conditions. Therefore, the objective of this work was to evaluate the morphostructural characteristics of *S. boulardii* encapsulates obtained by ionic gelation and the encapsulation percentage of the different wall material formulations.

Methods.



Results and discussion. The results showed that agavins had a significant influence on the protection of *S. boulardii* compared to whey protein, since beads with intermediate and high percentages (3.75 and 5%, respectively) of agavins had the highest encapsulation efficiency percentages. Micrographs of the different types of beads were obtained by stereo microscopy (Figure 1) and their morphostructural parameters (area, perimeter, circularity and solidity) were analyzed. The results showed that whey protein determined the size of the encapsulates compared to agavins. In addition, the whey protein affected the morphology of the encapsulates, producing more spherical beads with a

smoother surface which, in combination with the polysaccharides, modified the properties of the wall material as well as the particle size [3]. Furthermore, agavins and whey protein had a similar influence on the circularity and solidity behavior.

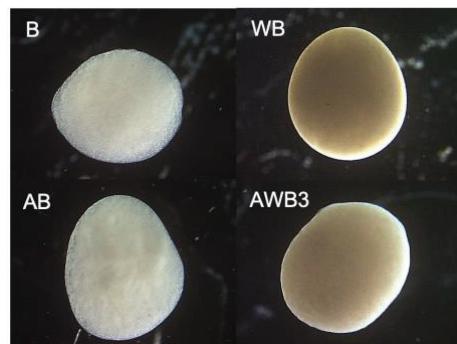


Fig. 1. Micrographs obtained by stereo microscopy (3X) of beads with *S. boulardii*. B: Control alginate, AB: Control agavins, WB: Control whey protein, AWB3: agavins/whey protein

Conclusions. The combination of agavins and whey protein with alginate had a significant influence in obtaining higher encapsulation efficiency percentages compared to the controls, using the ionic gelation technique. Currently, it is expected to know the behavior of the encapsulates through morphostructural characterization and mathematical modeling to correlate size, shape, surface area, bioavailability of the biomaterial, encapsulation efficiency, etc., for the design and manufacture of large-scale encapsulates.

Acknowledgements. Centro de Desarrollo de Productos Bióticos, and Secretaría de Investigación y posgrado (SIP).

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exploitation of Agaves
and sub products**

FOAMING CAPACITY OF *Agave lechuguilla* GUISHE.

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Keywords: saponins, *Agave lechuguilla*, guishe.

Introduction. *A. lechuguilla* is the agavaceae second most abundant and distributed in Mexico, which is mainly used for obtaining the fiber or ixtle through the carving of its leaves. The residue "guishe" (approx. 75%/weight of the leaf) has been minimally revalued. It is known to contain saponins with antihemolytic activity. Group of Otomi in Ixmiquilpan Hgo. has a cultural feature the use of guishe as a domestic soap.

The objective of this work was to evaluate yields and foaming capacity of saponins in aqueous (AG) and ethanolic (EG) extracts of the leaf guishe of *A. lechuguilla*.

Methods. The saponins were extracted by maceration of the guishe and tested according to the methodology presented in Figure 1. Sigma label saponin was used as a standard.

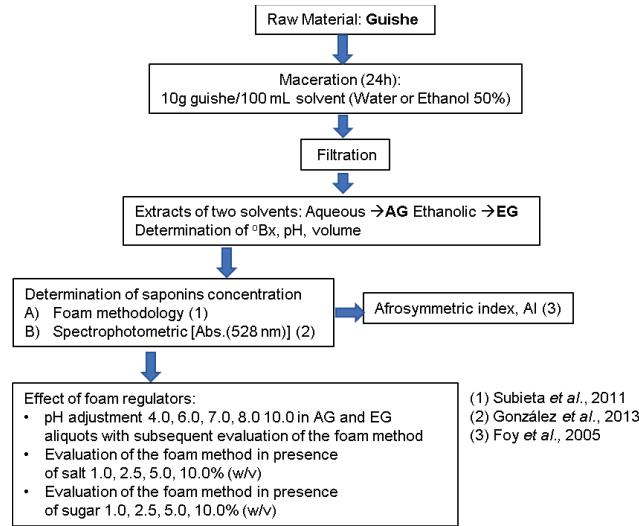


Fig. 1. Diagram of experimental methodology

Results and discussion. The results are presented in Table 1. According to °Bx, ethanol 50% extracts 1.73 times more substances than deionized water; but without exclusivity of saponins, the best qualitative evidence of soapy substances or saponins is foaming. According to the results, the foam method demonstrated reliability for the determination of saponins concentration in both crude extracts. AG reached a value of 21.5 mg of saponins for AI (Afrosimetric index), value that places it with higher saponinic potential than Sigma standard saponin (136.0 mg to generate 1 cm of foam). AI is a useful

indicator or analytical basis to demonstrate the efficiency of the product purification stages.

Table 1. Results of the test guishe extracts

Determination	AG (Aqueous GUISHE)	EG (Ethanolic GUISHE)
pH (day 0 → day 4); °Bx (day 0)	4.5-4.2; 5.35 ± 0.4949	4.9-4.8; 14.6 ± 0.7707
Foam method $mg = (mm - 5.394) \div 6.2958$, $r^2 = 0.9211$ -Concentration (saponin/extract) -Yielding (saponin/guishe)	-3.6462 mg/mL -22.2410mg/g (2.22 %, w/w)	-5.8248 mg/mL -32.0364 mg/g (3.20 %, w/w)
Spectrophotometric method (528 nm) $mg = (Abs - 0.0791), r^2 = 0.9975$ -Yielding (saponin/guishe)	111.8205 mg/mL (11.18 %, w/w)	154.4880 mg/mL (15.44 %, w/w)
Afrosimetric index AI Using 3.3462 mg/mL, [mm = (0.41 mg + 1.1609), $r^2 = 0.9688$]	21.5588 mg saponins to form 1 cm of foam	Interference of ethanol in foam forming, made measurement impossible
pH effect (4.0, 6.0, 7.0, 8.0, 10.0) in foam forming	Minimal variation between all foam values of the two extracts at different pH. The greatest variability occurred for AG with the maximum value 37.8 mm (pH 7.0) vs at minimum 30.01 mm (pH 4.0) (corresponds to 2x the highest standard deviation, 9.89 % of all trials)	
Sugar (sucrose) 1.0, 2.5, 5.0, 10.0 % in foam forming	Negligible effect, in both extracts. The maximum effect was obtained by reducing ≤ 21.71% the foaming for AG (sugar 10 %)	
Salt (NaCl) 1.0, 2.5, 5.0, 10% in foam forming	The foam formation in both extracts was reduced. The maximum effect was obtained for EG by reducing ≤ 53.42 % (salt 10 %)	

*Standard saponin [mm=0.1557 mg+11.189, $r^2=0.954$], led to 136.0886 mg for IA

The regulators of foaming tested, pH and sugar concentrations were basically unchanged; however, the NaCl salt caused the foam reduction (directly proportional to the salt concentration), reaching up to 53.42% reduction for EG with 10% salt. Possible ethanol-salt synergy is suspected for foam reduction.

Conclusions. The yields of saponins in the guishe of *lechuguilla* invite to deepen in investigations; the AI, shows the attractive foaming capacity that it potentiates for a diversity of foaming products, for now of the food and beverage industry (dairy, coffees, desserts) and even wines and beers among others. The above with the timely management of official regulations for its risk-free use.

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LIMITATIONS AND PERSPECTIVES OF IXTLE ADDED VALUE CHAINS

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Keywords: *Ixtle, added-value chains, diagnosis.*

Introduction. Sales of artisanal textile products made of agave fiber (ixtle) provide a supplementary income to many peasant families, but they seem to work with low productivity and meager sales value, despite the new ecological perspective for natural fibers as substitutes for plastics [1]. Hence the need to identify the main limitations and perspectives for thousands of this kind of artisanal cottage shops.

Methods. This work involved interviews in three kinds of enterprises having very different educational, technical and commercial skills: a) A traditional hñahñu (otomi) family together with a survey of half a dozen of similar artisans in El Alto Mezquital, Hidalgo, b) An artisanal and well known textile shop in Contla de Juan Camatzi, Tlaxcala [2] and c) A medium size enterprise called Celutex in Matehuala, SLP, with modern textile machinery and advanced marketing skills [3]. This way it was possible to contrast situations and identify the missing links to develop ixtle added value chains working within fair market conditions.

Results and discussion. Interviews and surveys showed that hñahñu artisans were most interested in acquiring cheap and effective decorticating machines and better marketing conditions to increase their revenues, usually lower than 10% of the final sales value (Fig. 1). Interviews with the Tlaxcala weaving shop showed their concern with the lack of legal support to protect their traditional designs (Fig. 2) from the encroaching and plagiarism by international corporations. They were interested to weave ixtle fibers from other regions such as El Mezquital. The interview with the CEO of Celutex indicated the need to increase ixtle supply for their present commercial operations. A dry run to produce hundred grocery bags with the ixtle fabrics supplied by Celutex (Fig. 3), showed the feasibility of creating new added value chains with better opportunities for ixtle artisans.

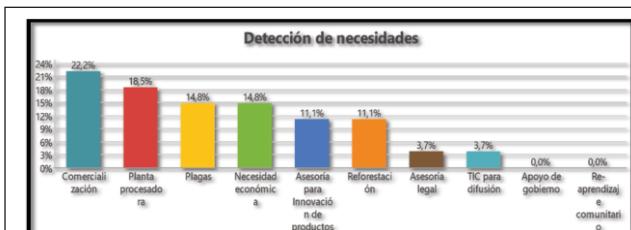


Figure 1. Detection of needs identified by artisans (Source. Cázares, 2020).



Figure 2. a) Materials for wool and ixtle texture (Source. Cázares, 2020), b) Textile workshop (source Netzahualcóyotl workshop, 2022) <https://www.netzahualcoyotl.org/>.



Figure 3. Biodegradable ixtle bag designed by artisans from the State of Mexico. (Source. Cázares, 2020).

Conclusions. It is feasible to create new ixtle added value chains with increased revenues for the small artisanal shops together with an increase on the supply of ixtle fabrics woven either, in artisanal or industrial enterprises. Special attention should be given to the new Mexican Law protecting intellectual communal rights of artisanal designs [4].

Acknowledgements. You can include here the funding of your investigation.

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PRODUCTION OF FORAGE FROM LEAVES OF THREE AGAVE SPECIES IN A SEMIARID REGION OF NORTHERN GUANAJUATO.

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Keywords: Agave leaves; Silage; Forage Production in arid zones.

Introduction. Agave species thrive in semi-desertic areas and only their stems, containing storage sugars, are used for spirits. Leaves, which are more than 50% of the biomass, are seldom used. They provide a dependable source of animal feed produced with scarce rainfall.

This paper compares forage production with harvested leaves from six-year-old agaves of three different species in the semiarid ecosystem of Northern Guanajuato against normal forage crops in the area.

Methods. Three agave species, *Agave salmiana* var. *salmiana*; *A. salmiana* var. *crassispina* and *A. americana*, were planted in El Arenal, Municipality of San José Iturbide, Guanajuato in a 2-hectare field at a density of 2,000 plants per hectare. Field was initially subsoiled and plow forming 50 cm. high furrows following the slope. At planting time, pups were planted below the furrow to protect the plant and provide humidity. Plants have received no irrigation nor any agricultural chemicals during the whole trial.

In June 2021, leaves were counted and pruned from 4 randomly selected plants per each species. They were weighted immediately. To determine dry weight, three subsamples of two leaves per plant (approx.100g/subsample) were oven dried at 50°C and weighted daily until constant dryness was reached.

The experimental site receives an average annual precipitation of 450 mm, between June and September, with Freezing temperatures and hailstorms from November to February. Under these conditions the productivity of crops under dryland production is poor and only irrigated crops succeed consistently.

A full literature review and field survey was conducted on forage production to record the Dry Weight of corn, sorghum, oats, alfalfa, and native grasses in the region and on production of dry stubble from dryland grown corn to compare against agave biomass production.

Results and discussion. Table 1 shows biomass production of leaves of Agave species obtained in this study. Table 2 shows the comparison of annual yields for the common forage sources in Mexico and Agave foliar biomass all compared as Dry Biomass.

Table 1. Foliar biomass of six-year-old plants of three Agave species grown in Northern Guanajuato, Mexico.

Agave Species	Leaves/plant	Ave. Leaf weight (kg)	Ave. Weight leaves per plant (kg)	Foliar Biomass /ha.* (tons)	Foliar biomass /ha. * (yr/ton)	% DW
salimiana v.s	25	12.5	312.5 (65.6)	625.0 (131.2)	104.2 (21.8)	21
americana	57	4.5	456.5 (61.5)	513.0 (123.1)	85.5 (20.5)	24
salimiana v.c	27	3.4	91.8 (21.1)	183.6 (42.2)	30.6 (7.0)	23

*2,000/ha. Number in brackets is Dry Weight.

Table 2. Forage production of different crops grown in Central Mexico (Ton Dry Biomass / ha/ yr) under irrigation and dryland farming vs. three Agave species.

	Irrigation plus agrochemicals			Dryland farming without agrochemicals		
	A. salmiana v.salmiana	A. americana	v. crassispina	A. salmiana	A. americana	v. crassispina
Alfalfa	Sorghum	Oats	Corn silage	Native grasses	21.8	7.0
			42.5	1.6	20.5	
			9.5	0.8		
			27.5			

Conclusions. Agaves grown under these dryland conditions produce higher volumes of forage biomass without irrigation or agrochemicals. They are an alternative to common forage crops. Harvesting agave foliar biomass permits the use of the stem for other purposes creating a double return for the grower. Pups from the same field are also a source of income during the years of growth.

Agave has beneficial effects on soil retention, CO₂ sequestration and produces forage with no water footprint.

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ANALYSIS OF THE AGAVE LANDSCAPE IN THE PRODUCTION OF MEZCAL FROM A CIRCULAR ECONOMY PERSPECTIVE

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Keywords: Agave, mezcal, bagasse.

Introduction. During the last decades there has been an exponential growth in the consumption of distilled agave beverages, which has generated reconfigurations in the industry's production chain, starting with the planting and obtaining of agave until the final stage with bagasse as waste. The study of the production chain and the implementation of new technologies as an alternative to ecosystem damage is important in view of the unstoppable growing demand for agave products. Most agave residues degrade slowly due to their solid and organic nature, which corresponds to their lignocellulosic biomass, cellulose, hemicellulose and lignin. Moreover, if they are not subjected to an adequate utilization process, they result in a deficient waste disposal

Methods. Bagasse samples were obtained from three different mezcaleras and the content of total phenolic compounds, total flavonoids, and antioxidant activity were determined using the cation radical of 2,2-diphenyl-1-picrylhydrazyl acid (DPPH-) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS-+) [1].

Results and discussion.

Biopolymeric membranes made from fibrous material obtained from the mezcal industry in the state of Morelos (Fig.1), with *Opuntia ficus-indica* mucilage, *Aloe Barbadensis Miller* mucilage, and agave fructans. The membranes were subjected to the process of antioxidant capacity and the content of phenolic compounds (Fig. 2). The physicochemical analysis of agave bagasse from the mezcal industry yields enough information to implement some new technology and innovation that producers can appropriate. It has been observed that due to the solid state fermentation, there is presence of secondary metabolites: phenolic compounds, flavonoids [2]. The highest concentration of compounds of biological interest was presented in bagasse in the sonication process, which presented the highest extraction of the different phytochemical compounds. This behavior was reflected in the

antioxidant activity of the extracts during the same fermentation time.

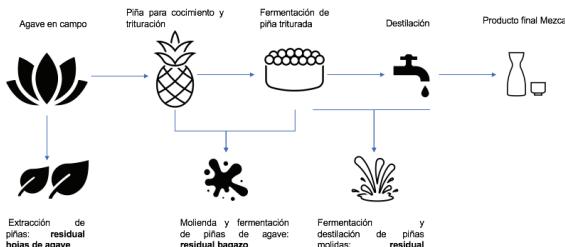


Fig. 1. Mezcal production chain in Morelos



Fig. 2. Bagasse membrane

Conclusions. The physicochemical analysis of agave bagasse contribute to the utilization of these residues, they can be used in functional processes, food formulation or, due to their properties, in the cosmetic industry.

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Agave *salmiana* silage metagenomics challenges extracting high-quality DNA from a complex matrix.

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Keywords: Silage, Agave, microbiome

Introduction. Silage is one of the most used methods to conserve forage; it is based on a spontaneous fermentation process led by the epiphytic assemblage of microorganisms in the fodder (1). However, silage is not only based on fodder and can be used to keep artisanal or industrial by-products, like sugar cane bagasse, or food vegetables. *Agave salmiana* silages have been used as forage but due to their high fiber content, their digestibility is not ideal (2). Guishe is the by-product of agave leaves decortication, after the long fibers are recovered either manually or using mechanical decorticating machines. This residue can be ensiled and used as roughage. However, the success of silage relies on the interactions between the microorganisms present before/during, and after the fermentation. Metagenomics is a feasible way to analyze the dynamics of agave silages which in turn will help to understand the basic principles of this process and help to develop new starter cultures or strains for future applications. For this purpose, it is important to screen out contaminating DNA including from chloroplasts and mitochondria to focus the analysis of microbial DNA.

Hence, the aim of this work was to assess different DNA extraction procedures using *Agave salmiana* guishe as raw material.

Methods. We tested 4 DNA extraction protocols: Power soil Kit Quiagen (control), SDS method (3), phosphate buffer (4), and a CTAB-based protocol. The extracted DNA was quantified and passed through quantity and quality assays. After that, samples were sent to 16S rRNA seq. SDS-modified extraction samples were sent to the facilities of BGI company, Beijing, China for metagenomic shot-gun seq. The result files were analyzed in the Kbase server.

Results and discussion. The manual extraction methods yielded higher DNA amounts than the commercial kit methods. The phenol extraction method was not acceptable because it yielded too many mineral salts, carbohydrates, and phenol itself in the final product. Instead, the SDS protocol using sorbitol and PVP without phenol yielded better results. Furthermore, 16s RNA seq reveals that the CTAB protocol selected Chloroplast DNA over microbial DNA. Shot-gun metagenomics using the SDS extraction protocol showed 90% of agave DNA and only 10% microbial DNA. This could be the result of agave cell breakdown during the process of fiber

mechanical extraction. A preliminary sample of the microbial taxa found by DNA metagenomics is shown in Fig. 1.

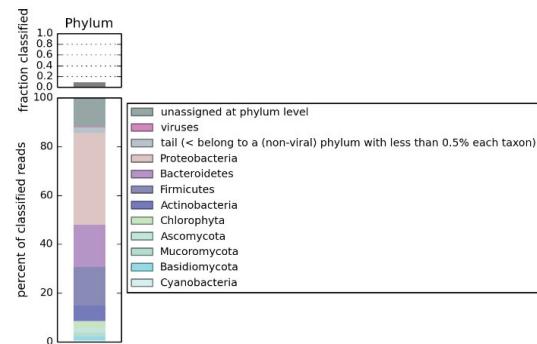


Figure 1.- Classify Taxonomy of Metagenomic Reads with Kaiju, percent of classified reads at Phylum level.

Conclusions. *A salmiana* guishe is a complex matrix which implies that the select DNA extraction metagenomic method must include a purification and host DNA depletion process that guarantees the most accurate microbiome abundance and taxonomical identification.

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EXTRACTION AND CHARACTERIZATION OF CELLULOSE FROM AGAVE BAGASSE

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Keywords: agave bagasse, agroindustrial wastes, biopolymers

Introduction.

Cellulose is the most abundant renewable resource in nature, its diverse properties and industrial applications have sparked greater interest in this biopolymer. The tequila-producing industry produces large amounts of waste after the production of tequila, being the vinasses and bagasse the largest generation. The residual material that remains after harvesting and cooking the "piña" after sugar extraction is commonly referred to as agave bagasse. It consists of long fibers with about 5-10 cm [1]. Currently, bagasse is used, among other things, as feed for ruminants and as organic material for compost. The aim of this work is to obtain cellulose from the fibrous residues of the tequila industry, which can be used as a natural reinforcement in bioplastics, in the pharmaceutical and automotive industries.

Methods.

The agave bagasse was obtained from tequila companies in Arenal, municipality of Jalisco, Mexico. A pretreatment of 10 g bagasse was carried out, using distilled water and stirring for two hours. The alkaline treatment was carried out using 2% (p/v) NaOH and stirring for two hours at 80 °C [2]. Bleaching was carried out with a solution composed of 0.5% H₂O₂ and 4% (p/v) NaOH, in a 1:20 ratio (fiber: solution), with stirring for two hours at 50 °C [3]. The fibers were subjected to drying at 40 °C for 24 h. FTIRs were carried out using a Perkin-Elmer infrared spectrometer (Spectrum 100/100 N FT-IR) and micrographs were obtained using a Phillips XL30 scanning electron microscope.

Results and discussion.

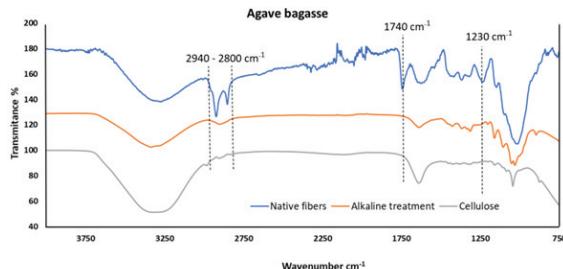


Fig. 1. Infrared spectra for native fiber, fibers after of alkaline treatment and cellulose

The native fiber showed a peak at 1230 cm⁻¹. This is attributed to the stretching of C-O-C bonds of the alkyl aryl ether, a compound present in the structure of lignin

and a peak of 1740 cm⁻¹ is associated with the stretching of C = O groups linked to aliphatic carboxylic. The peaks in 2940 and 2800 cm⁻¹, in the native cellulose, are assigned to the asymmetrical and symmetrical stretching vibrations of alkyl, aliphatic and aromatic rings (H-C-H) [2].

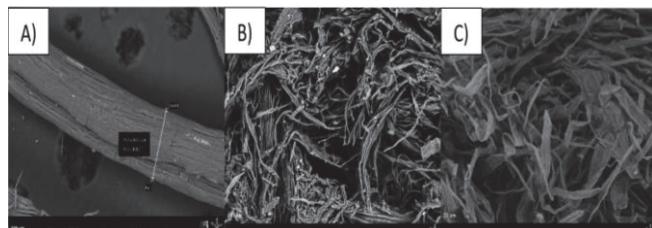


Fig. 2. Micrographs of (A) native fibers, (B) fibers after alkaline treatment (b) and agave bagasse cellulose

In Figure 2A, the fiber without treatments can be seen. After the alkaline modification, individual fibers that have separated from the cellulosic filaments can be seen, however, traces of lignocellulosic compounds can be observed (Figure 2B). Cellulose fibrils are more available due to the removal of lignin and hemicellulose (Figure 2C).

Conclusions.

The alkaline treatment contributed to the separation of the fibers and intervened in the decomposition of lignin and hemicellulose. The use of agricultural residues as a natural source of polymers has become an excellent option for innovation and the development of new products and biodegradable materials.

Acknowledgements.

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DETERMINATION OF THE WATERPROOFING CAPACITY OF THE RESIDUE VINASSE OF THE MEZCAL INDUSTRY

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Keywords: vinasse, waterproofing, Agave

Introduction. The production of mezcal in the state of Durango follows a traditional process that currently seeks to technify it by promoting its development. However, the problem they face is the growth of the mezcal industry in recent years, which has caused an environmental crisis due to the waste by-products that are generated, such as vinasse and bagasse². The vinasse represents an environmental problem causing a persistent modification in the environment where it is dumped, so it must be treated before being discharged.
¹Waterproofing agents are chemical substances or compounds that have the objective of stopping water by preventing its passage and are widely used in the coating of parts and objects that must be kept dry³. The main objective of this project is the manufacture of a vinasse-based waterproofing agent (IMPERVIAL) for the reuse of this residue.

Methods Mixtures of bar soap, alum and vinasse, they were carried out in duplicate, properties such as: appearance, color, yield (mL/cm^2), solar reflectance (%), drying (h), etc. were measured. Were made to create the waterproofing using the experimental design (Table 1).

Table 1. Experimental design

Treatment	Vinasse (%)	Alum (%)	Soap (bar) (%)
1	100	0	0
2	0	100	0
3	0	0	100
4	70	30	0
5	70	0	30
6	70	15	15
7	50	50	0
8	50	0	50
9	50	25	25
10	34	33	33
11	60	40	0
12	60	0	40
13	60	20	20

Results and discussion. The IMPERVIAL 2, 4, 7, 9, 11 and 12 samples showed no filtration, however, in the Cold Flexibility Test the IMPERVIAL 9 and 12 samples showed cracks.

Table 2. Comparison between Impervial 4 and 11 vs commercial.

Type	Vinasse and alum based waterproofing		commercial waterproofing
	IMPERVIAL (4)	IMPERVIAL (11)	
Property	IMPERVIAL (4)	IMPERVIAL (11)	COMEX®
Color	Brown	white	Red/white
Density (g/mL)	1.5	1.9	1.3
touch dry (min.)	39	48	60
full dry (h)	1.3	1.26	24
Filtration	without filtration	without filtration	without filtration
Yield (mL/cm^2)	10	9.7	-----
solar reflectance (%)	100	98	83.6

Conclusions. IMPERVIAL 4 and 11 treatments demonstrated excellent quality properties when compared to commercial waterproofing⁴ on concrete surfaces. It is a great alternative that fulfills the function of a commercial waterproofing.

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INTRODUCTION OF *Agave salmiana* INTERCROPPED WITH FOREST AND FRUIT TREE SPECIES IN LERMA, MÉXICO, MEXICO.

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Keywords: corn, milpa, *Paulownia*.

Introduction. Corn has been the main crop in the mountains and plane lands of the municipality of Lerma, México, México. Importance of corn in diet, daily life, and culture for otomí region is clearly appreciated. Nevertheless, low prices for corn yield and the increase of its production cost reduce interest in continuing traditional corn production. Within Lerma municipality there are different reactions to high labor and cost of corn production; 1) continue producing corn investing money, 2) producing with low investment, 3) look for alternative crops, 4) stop any cropping and try to sell the land, etc.

Present work aims the introduction, or possible reintroduction, of *Agave salmiana* in a plane land located in "Pueblo Nuevo" Tlalmimilolpan, in the municipality of Lerma, México, Central México (1). First objective is to evaluate the *A. salmiana* development when growing alone or intercropped with forest, fruit tree species or corn. Final destination of *A. salmiana* plants is production of gourmet agave syrup.

Methods. *Agave salmiana* plants were obtained from PROBOSQUE in 2021 and allowed to grow one year under greenhouse conditions. In 2022, they were planted in "Pueblo Nuevo", Lerma, México.

*Fig. 1. View of plantation of *Agave salmiana* and *Paulownia tomentosa* in "Pueblo Nuevo", Tlalmimilolpan, Lerma, México, Mexico, 2022.*



Treatments or plantation interval of agave plants and forest or fruit species or corn are show in next table

*Table 1. Plantation system for *Agave salmiana* in "Pueblo Nuevo", Tlalmimilolpan, Lerma, México, Mexico, 2022.*

<i>Agave salmiana</i>	Objetive of system
Alone at 1.5 m	Syrup production (SP)
Alone at 3.0 m	SP
<i>Paulownia tomentosa</i> intercropping (I)	SP; fodder source, ornamental and attraction of bees (2)
<i>Pinus ayacahuite</i> I	SP; Christmas tree
<i>Cupressus lusitanica</i> I	SP; windbreak, ornamental
<i>Senna mexicana</i> I	SP; fodder source, ornamental (3)
<i>Juglans regia</i> I	SP; nut production
<i>Prunus persica</i> I	SP; fruit production
<i>Prunus serotina</i> I	SP; fruit production
<i>Ficus carica</i> I	SP; fruit production
<i>Zea mays</i> I	SP; corn production

Some ecophysiological and grow data from agave will be obtained i.e. grow rate and relationship with intercropped species will be discussed.

Results and discussion. Development of agave and the intercropped species will be scientifically reported and locally shared to interested people.

Acknowledgements. M. Sc Sara Aguirre, Vanesa and Ximena hard work at planting is lovely appreciated.

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CHARACTERIZATION OF THE PRODUCTS OBTAINED FROM THE PYROLYSIS OF AGAVE CUPREATA BAGASSE

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Keywords: Biooil, Biochar, Bagasse agave.

Introduction.

The biomass plays a very important role as renewable energy sources, since it can be used to satisfy the requirements of biofuels [1]. There are many ways to supply these biofuels, including thermal and biological conversion [2]. Pyrolysis is a thermo-chemical process that consists of the thermal degradation of biomass in the absence of oxygen, resulting in the formation of solid (biochar), liquid (biooil) and gaseous products (syngas). The biooil and syngas can be used as boiler fuel or upgraded to renewable transportation. The biochar can be used on the farm as un soil amender that can sequester carbon. In Mexico, the agave industry focuses on the production of alcoholic beverages such as Tequila, Mezcal or Bacanora [3]. The agave bagasse is the residue that remains after cooking, grinding and extracting the fermentable juice of the agave stem. Mezcal production generated in 1017, 254,889 tons of stem bagasse, which can result in a great pollutant for the environment if they are not treated and/or used efficiently [4].

In this work, the feasibility of using *Agave cupreata* bagasse as a source of lignocellulosic biomass for the production of bio-oil, char and syngas through a pyrolysis process was evaluated.

Methods.

The bagasse of *A. cupreata* was characterized according to the TAPPI methods. Extractable, Klason lignin and holocellulose were determined. A Thermo Fisher Scientific tubular furnace with a temperature controller, a cooling system and a bio-oil and gas collection system was used. One quartz tube 1 inch in diameter placed inside the oven was used as reactor. The pyrolysis was carried out at maximum temperatures of 450 y 550 °C, using a flow of N₂ of 10 mL/s and heating rate of 10 or 30 °C/min. The biooil and biochar were characterized by FTIR spectroscopy and elemental analysis.

Results and discussion.

The extractable content was 16.7%, while that of lignin (17.8%) was higher than that reported for other agaves, and had a Holocellulose content of 65.5%. In the pyrolysis of *A. cupreata* bagasse, at the temperatures used in this work, a carbonaceous material, a liquid or bio-oil is produced consisting of volatile compounds that condensed and a non-condensable gas. The

highest bio-oil yield was obtained at 550 °C, 30 °C/min (Table 1). The biochar obtained shows a high content of carbon and oxygen, as well as a high calorific value in the range of 20 to 23 MJ/Kg in comparison with the carbon obtained from other lignocellulosic residues. The FTIR analysis of bio-oil shows the presence of hydroxyls, ester groups, as well as hydrocarbon chains and aromatic groups.

Table 1. Performance of agave bagasse pyrolysis products

Maxima temperature (°C)	Heating rate (°C/min)	Yield (% in weight)		
		Biooil	Biochar	Gas
450	10	35.4	30.1	34.4
550	10	37.1	27.1	35.8
450	30	34.3	30.5	35.2
550	30	45.2	24.6	30.1

Conclusions.

The composition and thermal properties of *A. cupreata* bagasse as well as the products of pyrolysis were determined. The results obtained show the potential of bagasse of *Agave cupreata*, as a source of lignocellulosic biomass for the production of bio-oil, biochar and synthesis gas, that can be used for the production of energy

Acknowledgements.

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DEVELOPMENT OF A MANUAL PROTOTYPE FOR MEAD EXTRACTION

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Keywords: prototype, acocote, mead.

Introduction. Plants of the Agave genus are native to the Americas, with the highest concentration of species native to Mexico, and are called magueys. The agaves most commonly used for the extraction of mead are: *A. salmiana* and *A. atrovirens*. A plant produces from 3 to 6 liters of mead and depending on its robustness from 500 to 1,000 liters in its productive life (1). The extraction is buccal and is done with different instruments (2): guaje (acocote), acocote of wood and plastic bottle of 3L adapted with hose. When this liquid is obtained by mouth suction, the mead is contaminated with microorganisms that are not typical of the beverage (3), so this project aims to develop an instrument that prevents the extraction from being done with the mouth of the tlachiquero, in order to reduce the bacterial load. The objective of this study is to develop a prototype designed with a guaje (*Lagenaria siceraria*) for manual extraction of mead.

Methods. For the design of the prototype, the physicochemical characterization of the mead was carried out, determining: pH, viscosity, density, Brix degrees and refractive index, according to **NMX-V-022-1972 "Aguamiel"**. To develop the prototype, an acocote, a vacuum pump of 4 watts of electrical-mechanical power and a backup system based on lithium batteries were used; food grade hose was used to connect the guaje and the pump.

Prototype battery efficiency test: The number of times and the time it sucks a fixed volume (1.8 L) of mead was determined. The microbiological quality of mead obtained from 8-year-old *Agave salmiana* plants was evaluated using the designed prototype (**Fig. 1A**) and an extraction instrument currently used by plastic bottle tlachiqueros (**Fig. 1B**). The collected samples were sown in triplicate on Mac Conkey, PDA and AST. For bacterial counts, serial 10^6 , 10^8 and 10^{10} dilutions were performed in AST and the reading was taken at the 10^6 dilution.

Results and discussion. The results of the physicochemical characteristics of the mead are as follows: pH (4.98), viscosity at 20 ° C (5.91 cp). The results of the physicochemical characteristics of the mead are as follows: pH (4.98), viscosity at 20 ° C (5.91 cp), density (4.6 g/mL), Brix (9.2) and refractive index (1.3466). These are in accordance with NMX-V-022-1972. In the efficiency test of the prototype battery, it was demonstrated that it sucks a volume of 1.8 L during 200 times in a time of 120 min.



Fig. 1. A) prototype and B) bottle plastic instrument

In the bacterial count in the prototype the isolated microorganisms presented loads over 1.92×10^9 CFU/ml with the prototype (Fig. 2A) and with the bottle mead extraction instrument 4.02×10^9 CFU/ml (Fig. 2B), so that with the extraction performed with the designed prototype the CFU/ml are reduced.



Fig. 2. Bacterial count. A prototype and B) bottle plastic instrument.

Conclusions. A manual mead extraction prototype was designed. In the efficiency test of the prototype battery, it was demonstrated that it sucks (1,800 ml) for 200 times in a time of 120 min. In the bacterial count of mesophiles in the prototype, although it exceeds the CFU/ml, the value decreases considerably with respect to the extraction of mead with the bottle instrument.

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LIMITATIONS AND PERSPECTIVES OF IXTLE ADDED VALUE CHAINS

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Keywords: *Ixtle, added-value chains, diagnosis.*

Introduction. Sales of artisanal textile products made of agave fiber (ixtle) provide a supplementary income to many peasant families, but they seem to work with low productivity and meager sales value, despite the new ecological perspective for natural fibers as substitutes for plastics [1]. Hence the need to identify the main limitations and perspectives for thousands of this kind of artisanal cottage shops.

Methods. This work involved interviews in three kinds of enterprises having very different educational, technical and commercial skills: a) A traditional hñahñu (otomi) family together with a survey of half a dozen of similar artisans in El Alto Mezquital, Hidalgo, b) An artisanal and well known textile shop in Contla de Juan Camatzi, Tlaxcala [2] and c) A medium size enterprise called Celutex in Matehuala, SLP, with modern textile machinery and advanced marketing skills [3]. This way it was possible to contrast situations and identify the missing links to develop ixtle added value chains working within fair market conditions.

Results and discussion. Interviews and surveys showed that hñahñu artisans were most interested in acquiring cheap and effective decorticating machines and better marketing conditions to increase their revenues, usually lower than 10% of the final sales value (Fig. 1). Interviews with the Tlaxcala weaving shop showed their concern with the lack of legal support to protect their traditional designs (Fig. 2) from the encroaching and plagiarism by international corporations. They were interested to weave ixtle fibers from other regions such as El Mezquital. The interview with the CEO of Celutex indicated the need to increase ixtle supply for their present commercial operations. A dry run to produce hundred grocery bags with the ixtle fabrics supplied by Celutex (Fig. 3), showed the feasibility of creating new added value chains with better opportunities for ixtle artisans.

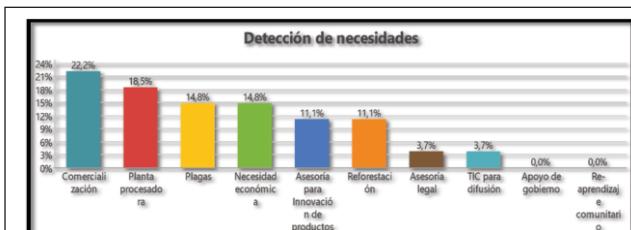


Figure 1. Detection of needs identified by artisans (Source. Cazares, 2020).



Figure 2. a) Materials for wool and ixtle texture (Source. Cazares, 2020), b) Textile workshop (source Netzahualcóyotl workshop, 2022) <https://www.netzahualcoyotl.org/>.



Figure 3. Biodegradable ixtle bag designed by artisans from the State of Mexico. (Source. Cazares, 2020).

Conclusions. It is feasible to create new ixtle added value chains with increased revenues for the small artisanal shops together with an increase on the supply of ixtle fabrics woven either, in artisanal or industrial enterprises. Special attention should be given to the new Mexican Law protecting intellectual communal rights of artisanal designs [4].

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COMPARATIVE LANDSCAPE ANALYSIS OF A TRADITIONAL AGAVE SYSTEM (METEPANTLE) vs. MONOCULTURE AGAVE SYSTEM T4

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Keywords: Metepantle, UAV image, productive analysis

Introduction. Metepantle (*metl*, agave and *pantli*, walls) is the millenary way of cultivating agaves rows interspersed with corn and bean fields. It is adapted to climate change and is an important source of cash products such as a fermented beverage (*pulque*) textile fibers (*ixtle*) and roughage for lamb meat (*barbacoa*). Here we show landscape features to be considered in future economic and mass balance analysis to help compare agave traditional system (polyculture) to agave intensive system (monoculture)

Methods. Landscape analysis was done on the first week of September 2022, using satellite and UAV (Unmanned Aerial Vehicle) imagery in two localities, a) Cardonal, Hidalgo State (Fig. 1A), and b) Nanacamilpa, Tlaxcala State (Fig. 1B). Allometric estimates were obtained from published work [1] and public data bases. To interpret the landscape of each parcel the distances between rows and the agave individuals in each mosaic were digitized. Additionally, the diameters of the agaves were measured in the field as a basis for future estimates of their biomass [2 & 3] to be published elsewhere.



Fig. 1. Landscape comparison between plots a) Metepantle in Santa Teresa Dabotha, Hidalgo, b) Monoculture in Nanacamilpa, Tlaxcala, in Mexico

Results and discussion.

Six digital orthomosaics (smooth image composites) were made between the two study areas from almost 600 photos taken with the UAV. The total surface of the 6 land parcels analyzed was 13ha. The length of agave rows (m) in Dabotha ranges from an average of 93 ± 10 to 152 ± 43 with average distances between rows of 6 ± 4

to 19 ± 4 . The total number of rows was 57. The monoculture parcel was studied in Nanacamilpa. The total number of rows was 84 with average length of 69 ± 24 . In the polyculture system of Nanacamilpa (images not shown), the length of the rows was 93m and a total of 9 rows. The average agave diameter (m) of the agaves was 1.9 ± 0.8 for Dabotha and 1.5 ± 0.5 for Nanacamilpa. The statistics allowed us to define the planting structure and arrangement of the agaves in the plots in traditional and intensive systems. Good correlation ($n=50$; $r=0.85$) was found between agave diameters and heights with data obtained in field work. Correlation between field and orthomosaic diameter measurements was good ($n=50$; $r=0.87$). In Dabotha the land strips were void from corn plants because of severe local drought. In Nanacamilpa with better rainfall, metepantle showed significant maize production.

Conclusions. It is feasible to use aerial image composites (orthomosaics) to estimate the number, spacing, diameter and height of *A. salmiana* plants both in traditional polyculture system (agave, corn and beans) as well as intensive monoculture system. Such data will help to estimate the evolution of agave productivity in both systems using aerial photography.

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POSTERS TEMATICA V

**Industrial Social,
normative and
ethnobotanic aspects**

SOCIAL COMMONS OF MAGUEY PULQUERO IN HIDALGO MEXICO. [T5]

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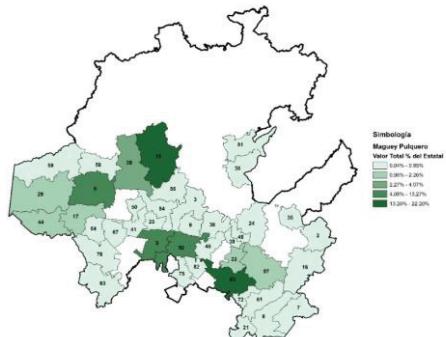
Keywords: Social commons, Maguey pulquero, Hidalgo México

Introduction. The commons refer to the collective process of organization and negotiation to preserve the commons and generate well-being. [1] The social dimension of the commons analyzes the collective organization to structure rules and protocols to care for the commons. It involves political, educational, and organizational systems defined by a community. The research aims to analyze the social dimension of the commons of the maguey pulquero in Hidalgo, Mexico.

Methods. SIAP (2022) data were initially used to identify the municipalities that planted maguey pulquero in Hidalgo. [2]. The analysis of the social common focuses on three levels: Public Policy, the Network composed of producers, researchers, and peasants (Red MagNop); and the experience of the cooperative Alegría del Maguey, an organization composed of rural women Hñähñus. Participant observation in the Alegría del Maguey since 2018 to 2020 were employed to collect data. The first two authors attended the forums convened by the Red MagNop in 2021 to discuss the Maguey Sustainable Management Act in Hidalgo. The information was classified according to the social commons criteria proposed by Gibson-Graham, Cameron, and Healy [1].

Results and discussion. The Mezquital Valley concentrated the most extensive area that planted and harvested the maguey (Fig. 1). Cardonal municipality presented the highest yields of this crop and stands out for not having irrigation. The peasant practices to preserve the maguey are associated with the Hñähñu knowledge, which over the centuries has improved to preserve maguey biodiversity through the reforestation of different varieties of maguey. Some scholars identified that the agave agrobiodiversity is related to pulque production [3].

Fig. 1. Agave pulquero planted in Hidalgo State.



Source: Elaborated by the authors based on SIAP (2022).

The Law for the Sustainable Management of Maguey [4] establish the protocols to access, use, care, responsibility and benefits of the maguey and its derivatives in Hidalgo. It defines the protocols for researchers who employ maguey pulquero for research purposes. The Law demands the recognition of the property rights of indigenous communities, knowledge and the use of local varieties in Article 33.

Red MagNop brings together the interests of producers, researchers and farmers who, since 2017, have undertaken collective actions regarding the cultivation of maguey and nopal crops in the state of Hidalgo.

Since 1998, the Alegría del Maguey has organized for planting and processing of maguey in the community of San Andrés Dabochta, Cardonal. The twenty-one partners, mainly women, have developed organizational, productive and environmental protocols to conserve maguey and take advantage of mead. Collective actions include agave reforestation. They employed peasant knowledge to manage the maguey pulquero.

Conclusions. Three levels of collective organization involve the maguey pulquero social commons: public policy, the Red MagNop that incorporates producers, peasants and researchers and the Alegría del Maguey cooperative that processes nectar.

Acknowledgements. The authors thank the project CONACYT 317510 for financial support.

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MEZCAL FROM NUEVO LEÓN, WHOSE MEZCAL IS IT?

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Keywords: Mezcal, Appellation of Origin, Nuevo León.

Introduction. In the Scientific Tourism Campus of the University Astronomical Observatory of UANL (on Cerro El Picacho to 2,400 masl at Iturbide) where hiking, astronomical observation, scientific geotourism, etc. are carried out. The idea of producing mezcal arose given the abundance of wild maguey (*Agave americana*, *A. asperrima*, *A. gentryi* and *A. montana* magueyes mezcaleros that it shares with Tamaulipas¹) and that in Nuevo León this activity survives with local rustic methods such as the “Choneño” mezcal (name taken from La Ascensión or “La Chona”, municipality of Aramberri). There was a “rancho de vino” on campus, which motivated us to give mezcal historical and tourist value, and although Nuevo León does not belong to the territory of the DOM, we took on the task to research the historical background and current situation of the mezcal production in the entity. The results found excited the governor who publicly expressed his interest in requesting the AOM expansion to include NL (as recently happened with the State of Mexico, Morelos and Sinaloa), which raised expressions against mainly the producers of Oaxaca, which invites us to reflection and discussion Whose is the mezcal? What is the DOM?

Methods. Consult from historical archives, bibliographic and hemerographic sources to find documents that demonstrate the historicity of mezcal production. Field trips in the municipalities where this activity historically existed and still survives. Documentation of the status of the mezcal agaves and the mezcal production process.

Results and discussion. Wild maguey populations are abundant. The tradition of making Mezcal is still alive in Aramberri, Bustamante and Santiago. Abundant documents were found that demonstrate the importance that mezcal had in Nuevo León, dating back to 1828 when Jean-Louis Berlandier toured the state and in his diary he narrates, referring to Monterrey, “In the remote towns of the capital, where the Agave is born, there are many factories of mescal”, expanding later that mezcal was manufactured in Salinas Victoria and Villaldama, including the original production statistics², information generously documented in other sources that begin in 1883 until the first decade of the 20th century, this is how we can tell that Aramberri, Bustamante, Doctor Arroyo, Galeana, Higueras, Lampazos, Mina, Monterrey, Iturbide, Sabinas Hidalgo, Santiago, Salinas Victoria, Vallecillo, Villaldama and Zaragoza, were the mezcal producing municipalities

when Nuevo León was an important producer at the national level, surpassing Tamaulipas and below Oaxaca, both with AOM and the mezcal was producing in 24 states (Fig. 2). In other sources it has been possible to document the international awards obtained, the names of places and producers of mezcal, as well as the names of mezcal brands at that time before the decline of that activity due to the advent of beer.



Fig. 1. Scientific Tourism Campus of UANL. Pine and oak forest with agaves (*A. gentryi* y *A. asperrima*)

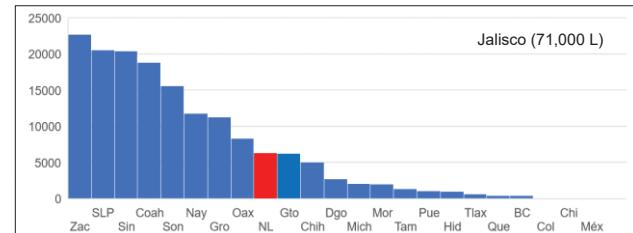


Fig. 2. Average production of mezcal from 1889 to 1907 in the Anuario Estadístico de la República Mexicana.

Conclusions. There are sufficient sources that demonstrate the existence of natural and human factors as support to the technical justification study to request for modification from the AOM to include part of Nuevo León state and thus question the exclusion of territories by AOM or its very existence.

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THE EXCLUDING CHARACTER OF THE BACANORA APPELLATION OF ORIGIN CERTIFICATION AND ITS IMPACT ON THE COMMERCIAL NETWORK TOPOLOGY

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Keywords: bacanora, appellation of origin, certification, topology network.

Introduction. Bacanora is a traditional agave distillate with appellation of origin (AO) and *sui generis* organoleptic characteristics, limited to 35 municipalities in the state of Sonora. The vast majority of producers don't certify their drink due to the complexity and cost of the procedures, generating an asymmetric network with many suppliers and few buyers. [1].

AOs should protect the interests of the producers, their local environmental resources, their ancestral know-how, and their cultural practices [2, 3], since they are assets closely linked to the typical characteristics of the region [4]. Here we present the current schematization of the bacanora's commercial topology and the impact it has on this network, the greater affordability of its certification.

Methodology. Field work was used in the municipalities of the bacanora's AO in the state of Sonora (2017-2020), interviews with producers, commercial houses, and officials of the Sonoran Bacanora Regulatory Council.

Results and discussion

	Code	Producers	Trademarks	Sold in:	Price/liter* (USD)
Bacanora with trademark and AO certification	B1	7	12	•Liquor stores •Online •Exporting	37.50 - 75.00
Bacanora without trademark or AO certification	B2	≈1000	0	•Vintas •Relatives in the USA •Intermediaries (wholesale)	7.50 - 15.00
Bacanora with trademark and without AO certification	B3	38	40	•Liquor stores •Online •Exporting	37.50 - 75.00

Table 1. Bacanora's economic sales scheme

Source: Own elaboration based on producer interviews, point of sale observation, and online sales

*Currency exchange of an average of September, 2021 from BANXICO

Table 1 shows that there are about a thousand producers (B2) and only 7 are certified (B1). This limits the access of B2 to the formal trade that offers better sales prices to 38 commercial agents without AO certification (B3). Fig. 1a shows the topology of the few companies with vertical integration from production to sale, with low transaction costs (B1). Fig. 1b shows many small producers (B2) with few buyers (B3) who control the final sale and generate high transaction costs for the producers (B2). Fig. 1c would be the topology of B2 with greater diversified access to the formal market.

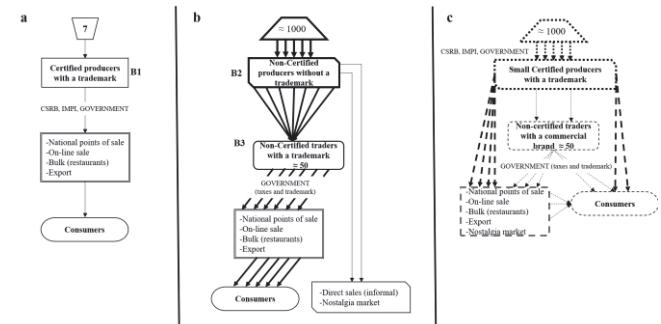


Figure 1. Bacanora network topology: (a) vertical chain with a very narrow production base and low internal transaction costs; (b) vertical chain with a very broad production base, high transaction costs, and asymmetric distribution of wealth; (c) a hypothetical small world network where AO certification is accessible to small producers. Thinner lines represent smaller connections. Source: Own elaboration.

Conclusions. The current certification procedure for the bacanora's AO excludes small producers who do not have access to the best-paid market and therefore their ancestral know-how is in danger. So, it is necessary to promote certification and technical assistance so that these small producers can access the market with better prices, lower their transaction costs and preserve the *sui generis* characteristics of the bacanora.

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STANDARDS AND CERTIFICATIONS FOR CLASSIFYING AGAVE HONEY AS ORGANIC.

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Keywords: Agave honey, certifications, products, organics.

Introduction. In recent years, people have become even more interested in their food for health care. In the study "Who Cares, Who Does?" conducted by Kantar in 2019, shows that in the case of Mexico, 59% stated that they purchase products that take care of the environment and 75% prefer natural products (Ávalos, 2019) (1).

The importance of organic food consumption is that they do not have chemicals, synthetics, or additives, managing to produce food naturally with the highest possible quality, and obtaining multiple benefits for health, the field, and the environment (García Naranjo Loayza, s.f.) (2).

Mexico is the world's fourth largest producer of organic food, growing more than 45 products organically exporting to countries such as Japan, the USA, and the European Union (Secretaría de Agricultura y Desarrollo Rural, 2017) (3).

There are no standards and certifications specifically for the production, marketing, and export of mead, so they were analyzed on wild plants since the final product is obtained from maguey.

The objective of this article is to analyze the standards and certifications at national and international level to be able to export agave honey, which is a product extracted from maguey pulqueiros (*Agave salmiana*) that is produced mainly in the states of Hidalgo, Tlaxcala, Estado de México and Puebla.

Methods. The standards and certifications of organic products in Mexico, the United States of America, the European Union, and Japan were analyzed to determine the similarities and differences in the requirements of each country.

In the same way, parameters of mead in different species of maguey were analyzed, data obtained from the article "Aguamiel y su fermentación: Ciencia más allá de la tradición" published in the Mexican Journal of Biotechnology (Guzmán Pedraza & Contreras Esquivel, 2018) (4).

Results and discussion. With the information of the standards of the different countries, summarized in **Table 1** the labeling in the United States, the European Union and Japan is only for 100% organic products. They also have equivalent agreements with different countries, meaning that the authorities of both nations

recognize their certification processes as equal and can export their products among themselves.

Table 1 Comparative table on standards of different countries

Standars	Ley de Productos Orgánicos	NOP	Reg. (UE) 2018/848	JAS
Country	Mexico	United States of America	European Union	Japan
Logo				
Regulatory agencies	Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA)	U.S. Department of Agriculture (USDA)	European Commission	Ministry of Agriculture, Forestry and Fisheries (MAFF)
Labeling	•100% organic. •Organic. •Made with organic ingredients. •Some organic ingredients.	•100% organic. •Organic.	•Organic	•Organic
Countries with equivalent agreements	United States of America (In progress)	Canada, European Union, Japan, Taiwan, Korea, Switzerland, and the United Kingdom.	Argentina, Chile, Japan, Switzerland, United States of America, etc.	United States of America, Australia, Canada, Switzerland, Argentina, United Kingdom, etc.

Table 2 shows the content of sugars in the soluble solids of the sap obtained from various species of Agave, where it can be observed that the carbohydrate content varies according to the species of Agave.

Table 2 Chemical composition and physicochemical characteristics of mead of different agave species, Source: (Guzmán Pedraza & Contreras Esquivel, 2018)

Parámetro	Agave mapizaga	Agave atrovirens	Agave salmiana
pH	4.50	6.29	4.63
°Brix	ND	11.10	75.53
Fructuosa (%)	3.73	3.63	5.22
Sacarosa (%)	1.01	1.43	4.69
Glucosa (%)	3.05	3.18	3.48
FOS (%)	1.17	1.72	ND
Proteínas (%)	0.35	0.39	0.97

Conclusions. It is important that farmers who want to be certified have information on the following points: Organic plan, land requirements, seeds, harvesting, transportation, storage, and labeling.

Finally, verifying that the products consumed are certified organic, through the seals of nationally and internationally accredited certification bodies generate added value. In this way, it will not only bring health

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