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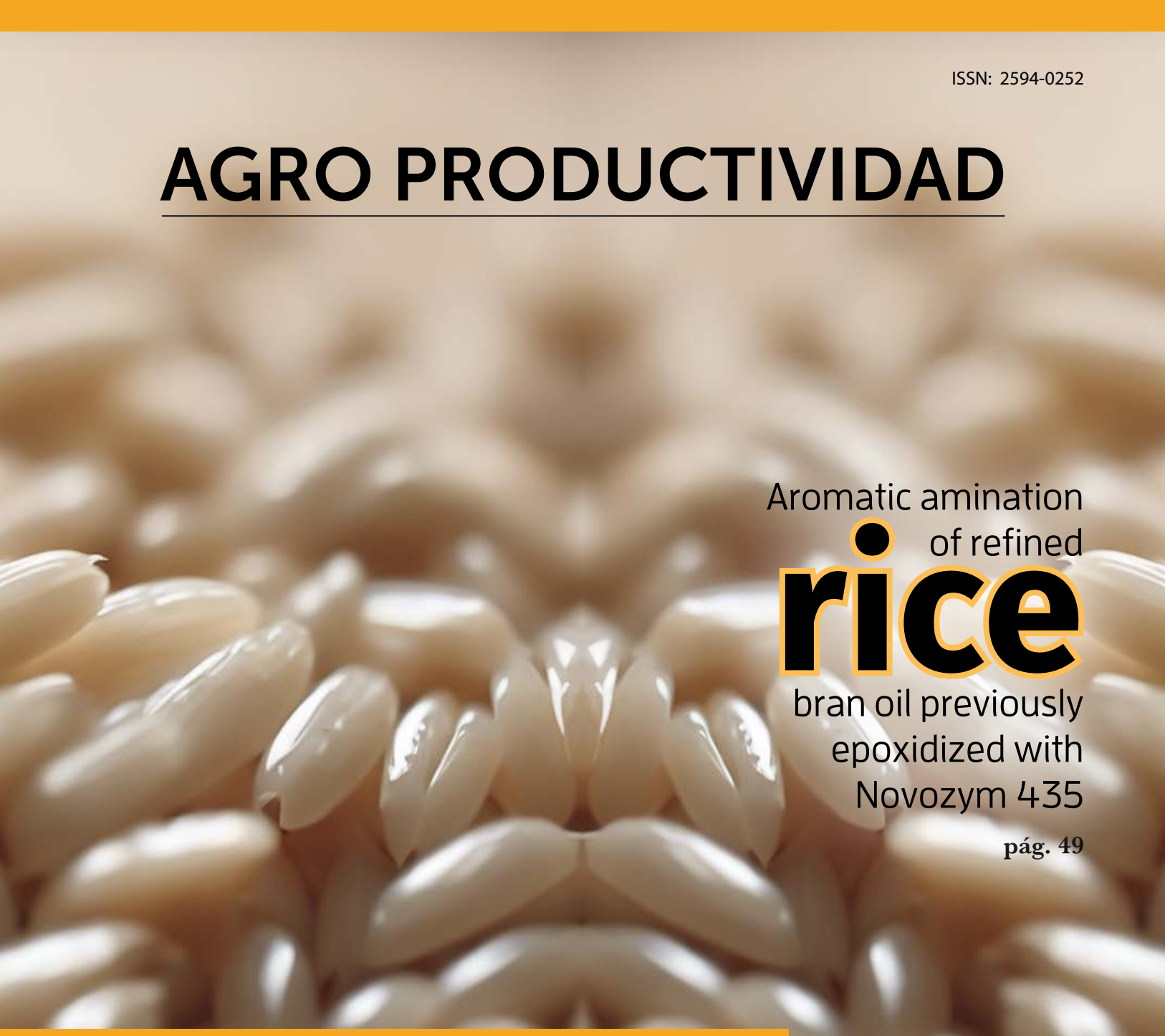

Aromatic amination of refined **rice** bran oil previously epoxidized with Novozym 435

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Colegio de Postgraduados

Contenido

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Disinfestation of *Agave angustifolia* Haw. collected in the field prior to *in vitro* culture

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ABSTRACT

Objective: to determine a disinfestation procedure for *in vitro* establishing of *Agave angustifolia* Haw. explants collected in the field.

Design/Methodology/Approach: seven agave plants per treatment were placed in perlite to contrast six treatments; two agrochemicals (Benomyl 2 g L^{-1} and Actara 0.5 g L^{-1}) and three contact times (10, 20 and 30 days). The stems of the plants were sectioned in 4-6 segments, after treatment with alcohol 80%, chlorine 30% and hydrogen peroxide 20%, to later place them *in vitro*. Usually there is contamination of explants mainly by bacteria, so Pursue® was used at 25 and 50%, placing 20 explants in each concentration for 1 minute. The variables evaluated were percentage of visibly healthy explants, type of contamination. Due to the difference in the number of explants, no statistical tests were performed.

Results: the plants treated with Benomyl presented less contamination by fungi without considering the contact time. Bacteria were present in the different treatments used, however, in the explants with pretreated with Benomyl, 85% was obtained visibly healthy when Pursue® at 50% was used.

Study limitations/Implications: other agrochemicals, such as specific bactericides are desirable to determine through their use, whether they mitigate *in vitro* contamination by pathogenic bacteria.

Findings/Conclusions: pretreatments with agrochemicals for the *in vitro* establishing of plants collected from the field are necessary to increase the number of visually healthy explants. With the Pursue® product at 50%, up to 85% of explants can be obtained without the presence of fungi or bacteria.

Keywords: bacteria, contamination, explant, fungicide, pretreatments.

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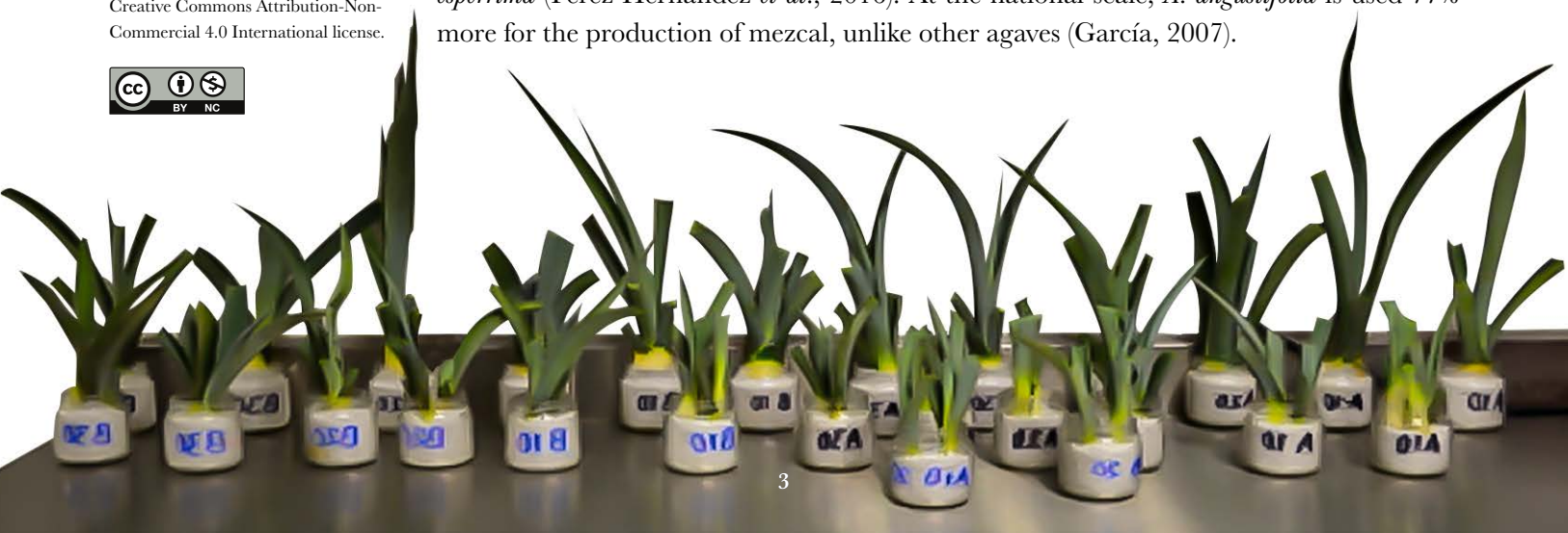
INTRODUCTION

The genus *Agave* is endemic to America; within the 200 existing species, 150 are native to Mexico (Lara-Ávila and Alpuche-Solís, 2016). The uses of these plants are variate: food, beverages, medicinal, fibers (García, 2007). Several species of agave are used for mezcal production, among them are *Agave angustifolia*, *A. cupreata*, *A. potatorum*, *A. salmiana* and *A. esperrima* (Pérez-Hernández *et al.*, 2016). At the national scale, *A. angustifolia* is used 77% more for the production of mezcal, unlike other agaves (García, 2007).



Currently in the municipality of Chilapa, Guerrero, plantations have been made with this species, to obtain mezcal (Barrientos-Rivera *et al.*, 2019). However, it is mentioned that there have been problems for the sexual and asexual multiplication of the species, so in vitro culture is an alternative for obtaining plants (Domínguez *et al.*, 2008). For the genus *Agave*, protocols have been established from the *in vitro* multiplication stage (Aguilar and Rodríguez, 2018). However, these start from plants already established in culture medium. On the other hand, when plants are collected in the field, for subsequent *in vitro* placement, contamination of explants is a difficult problem to solve. Among the most used products for disinfestation are: sodium hypochlorite, ethanol, mercury chloride, tween 20 in different concentrations (Hernández and González, 2010). Even including antibiotics such as sodium ampicillin and chloramphenicol (Alves-Pereira *et al.*, 2014) which have been insufficient options for some species from the field.

Disinfestation protocols are necessary to initiate *in vitro* culture (Bedoya-Pérez *et al.*, 2016), since the rest of the stages will depend on this: multiplication, rooting, and acclimatization, before being stablished in the field. Due to the morphology of agave in addition to the development in the field, the accumulation of dust, presence of fungi and bacteria is greater. Also, by placing fractions of stems in visually aseptic *in vitro* culture medium, the probability of contamination increases.

Therefore, the objective of this study was to determine a disinfestation protocol for the *in vitro* establishing of *Agave angustifolia* explants, from plants collected in the field.

MATERIALS AND METHODS

Plant material

To obtain agave plants *in vitro*, several experiments were conducted without satisfactory results. It was then decided to define an efficient disinfestation protocol for *in vitro* establishing of stem sections.

In April 2017, outstanding (elite) plants of *A. angustifolia* 15-30 cm in length and 3-5 cm in diameter were collected in the field, transported to the *in vitro* culture laboratory of the Genetics building, College of Postgraduates, Campus Montecillo. In the laboratory, the roots were pruned to leave them in approximately 2 cm; then the plants were washed with soap and water, to remove the soil, and they were placed in a cotton cloth to remove the excess of water.

Pretreatments

The pretreatments for disinfestation were six, product of solutions of benomyl 2 g L^{-1} (1-(butylcarbamoyl) benzimidazole-2-ylcarbamate methyl), and actara 0.5 g L^{-1} (3-(2-Chloro-1,3-thiazol-5-ylmethyl)-1,3,5-oxadiaznan-4-ylidene (nitro) amino), plus the contact time of the solutions with the plants of 10, 20 and 30 days. Seven plants of *A. angustifolia* were used per treatment, placed in 100 mL Gerber® bottles with agrolite (Figure 1), 15 mL of the solutions on the substrate and 2 mL on the leaves (depending on the treatment) were added in the first application.

The products added to the substrate were prepared on the same day of application. Solution applications were made on days 1, 10 and 20; with one, two and three applications

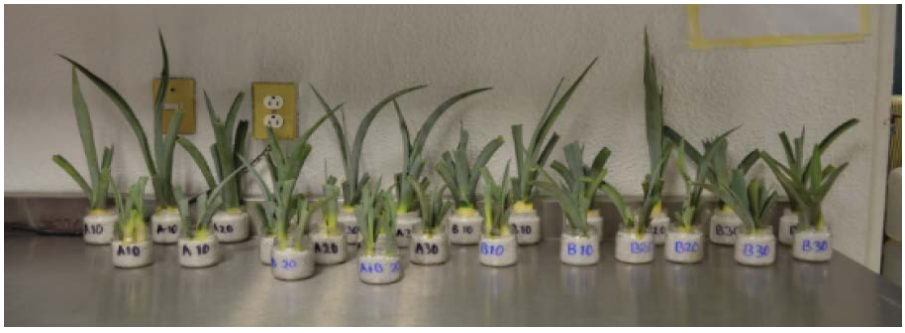


Figure 1. Pretreatments of disinfestation of *A. angustifolia* from the field. A: actara; B, benomyl; 10, 20, 30: days of contact of plants with chemicals.

for days 10, 20 and 30 contact days, respectively. *In vitro* seeding was carried out one day after the aforementioned contact time.

In vitro disinfestation

Plants were extracted from agrolite. The portion corresponding to the stem was washed with soap and water, the roots and leaves were removed.

In a laminar flow hood, ethanol 80%, sodium hypochlorite (commercial) 30%, and hydrogen peroxide (commercial) 20% were used for 3, 2 and 2 minutes respectively. After the disinfestation process, the stems of each pretreatment were cut into a cube shape (Figure 2). Finally, three washes with sterile de-ionized water were applied for 3, 2 and 1 minutes. This process was performed for all nine treatments.

Establishing the *in vitro* culture

After the process of disinfestation, the stems were divided into 4-6 sections. The tissue damaged by the products used was removed and sections of approximately 0.5-0.8 cm were obtained to be placed in test tubes (Figure 2) with 10 mL of culture establishing medium. Which is composed of commercial sugar 20 g L^{-1} and Sigma® agar 6 g L^{-1}, pH 5.7 and sterilized at 20 pounds of pressure in autoclave.

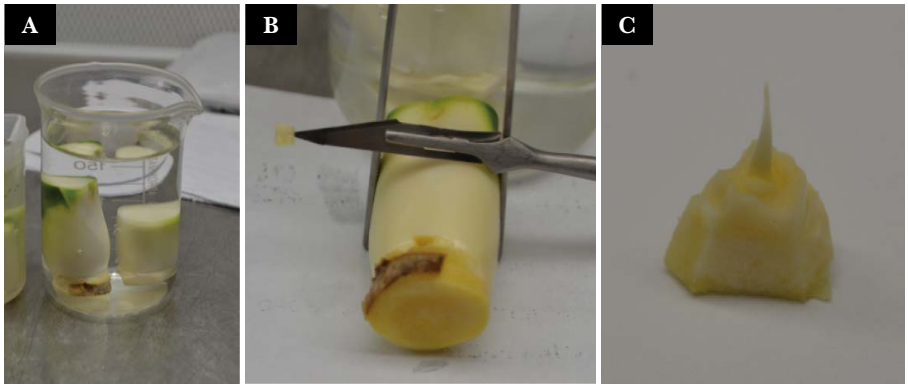


Figure 2. Conditioning of agave stem cuts to be placed in *in vitro* conditions. A: Agave rinse in sterile deionized water; B: Agave stem cutting; C: Stem section for *in vitro* placement.

If the explants were not contaminated, they were sub-cultured two weeks later in test tubes with 10 mL of culture medium with salts and developmental regulators (Table 1), where they remained for four weeks. The subsequent subcultures were made in 200 mL Gerber® bottles with 30 mL of culture medium (Table 1), for the multiplication of agaves.

The number of segments placed per treatment was variable, as those were in function of the stem size of the agave plants. The percentage and type of contamination in the tissues during the first two weeks of the established culture were evaluated to contrast the effectiveness of the disinfestation processes.

Due to the incidence of bacteria in the explants *in vitro*, in November 2017 the product Pursue® (quaternary ammonium salts) was used in concentrations of 25 and 50%; 20 explants were placed in the solutions for 1 minute, then three rinses were made with sterile deionized water of 3, 3 and 1 minutes. These were placed in test tubes with 10 mL of culture medium (Table 1). Only the percentage of contamination was evaluated in these explants.

Experimental design and variables evaluated

The experimental design used was completely randomized with factorial arrangement for treatments; two agrochemical solutions, and three resting times of the explants (Table 2).

Table 1. *In vitro* culture medium used for acclimatization and multiplication of *Agave angustifolia* explants.

Reagent	Concentration
$NH_4\ NO_3$	8 mM
KNO_3	10 mM
$Ca(NO_3)\ 4H_2O$	2.0 mM
$MgSO_4\ 7H_2O$	1.4 mM
$KH_2\ PO_4$	1.3 mM
Chelates$^{\times}$	0.1 mM
Micronutrients†	0.1 mM
Thiamine†††	0.1 mM
Mioinositol††	0.1 mM
BA††	4.43 μM
IBA††	0.42 μM
Sucrose	20 g
Agar	6 g

Chelates$^{\times}$: $FeSO_4\ 7H_2O$ (695 mg) and Na EDTA (931 mg), prepared in 250 mL of deionized water. † Micronutrients: H_3BO_3: 155 mg, $MnSO_4\ H_2O$: 422.5 mg, $ZnSO_4\ 7H_2O$: 215 mg, $Na_2\ MoO\ 2\ H_2O$: 6.25 mg, $CuSO_4\ 5H_2O$: 0.625 mg, prepared in 250 mL of deionized water. †† 10 mg of reactive prepared in 100 mL of deionized water. ††† Thiamine:1 g of reactive in 100 mL of deionized water. All the active chemicals by Sigma®.

Table 2. Treatments used in the process of disinfestation of *A. angustifolia* in *in vitro* culture.

Agrochemical	Exposure days
Benomilo®	10
	20
	30
Actara®	10
	20
	30

Benomyl: 2 g L^{-1}; Actara: 0.5 g L^{-1}; 10, 20 and 30: days of rest in perlite with agrochemicals.

RESULTS AND DISCUSSION

Preliminary results

The first attempts to establish *in vitro* plants of *A. angustifolia* were in November 2016, using seeds and applying alcohol (80%), chloralex (20%) and Timsen® (1 g L^{-1}) for 5, 10 and 15 minutes, respectively. There was 95% of germination, and 66% of the seeds presented contamination, the presence of fungi and bacteria predominated. It is worth mentioning that the explants with fungi were discarded, and treatments were applied to those with bacteria, using different products to try to eliminate pathogens while the plants remained at in vitro conditions.

Puente-Garza *et al.* (2022) performed *in vitro* germination of *A. salmiana* with 93% germination, but did no mention the presence or absence of contamination. Whereas Hernández-Castro *et al.* (2021) obtained 95% germination with seeds of *A. angustifolia* placed in substrate. Therefore, although *in vitro* culture is a novel technique, for *A. angustifolia* its use in seed germination is not justified. It is a more expensive technique, and moreover, germination in substrate is equal to that reported *in vitro*.

Regarding the products used for seed disinfestation, the most used are chlorine and alcohol, or even antibiotics for human use (Hernández and González, 2010). In recent years, protocols have been designed for the *in vitro* disinfestation of explants using hydrogen peroxide, chlorine dioxide and silver nanoparticles (Ramírez *et al.*, 2014; Pastelín-Solano *et al.*, 2020) which are not harmful to people who use them.

Pretreatments with agrochemicals

Although the number of plants placed in agrolite were seven per treatment, the number of explants was different due to the initial size of the plant, which is why no statistical analysis was made.

From the agrochemicals used with Benomilo, 22, 13 and 9 visually healthy explants were obtained with 10, 20 and 30 days of exposure to the products. There was also less contamination by fungi and bacteria; contrary to what occurred when actara was used (Table 3).

The implementation of pretreatments for *in vitro* establishing is little used. This usually happens because many of the agave multiplication protocols are reported on

Table 3. Quantity and condition of explants from *A. angustifolia* placed *in vitro*.

Agrochemical	Exposure days	Explants contidions (%)		
		Fungus	Bacteria	Visually healthy
Actara	10	4	10	9
Benomilo		0	6	22
Actara	20	18	17	7
Benomilo		3	6	13
Actara	30	7	8	4
Benomilo		7	13	9
Total explants		39	60	64

plants already established *in vitro* (Ríos-Ramírez *et al*., 2017) and the disinfestation stage is omitted. Despite the acclimatization, multiplication and rooting *in vitro* are facilitated. This suggests that the methodology is easy for many species in which it is implemented, but there is no mention of methodologies for establishing *in vitro*, those plants collected from the field.

Bedoya-Pérez *et al.* (2016) mentioned a disinfestation protocol similar to the one proposed here, with *Aloysia tryphilla* plants, applying the fungicides Fosetal and Benomil. However, due to the application of other *in vitro* disinfestation treatments, those authors did not report the results corresponding to fungicides.

Of the treatments applied, a lower percentage of fungal contamination was obtained when Benomyl was used, even with 10 days of exposure there was no presence of those pathogens (Figure 3). In a similar way, it can be ratified that it is not necessary for the plants to be maintained for 30 days with the chemicals. In fact, with that time of exposure there were fewer visually healthy explants, 21 and 31 with actara and benomyl, respectively.

With the pretreatments used, a total of 39, 37 and 24% visually healthy explants were obtained, without bacteria or fungi, respectively. But before using the pretreatments, the contamination was 80-100% predominating fungi, even in the explants previously contaminated with bacteria.

Despite the use of agrochemicals, the presence of bacteria in the environment was evident, this could be due to two reasons; that they were endogenous pathogens, or else, the proliferation of those occurred because a specific bactericide was not used in the treatments. Martínez-Rodríguez *et al.* (2014) mentioned that there are endophytic bacteria associated with species of the genus *Agave*, which promote plant growth. The bacteria that proliferated under *in vitro* conditions did not cause damage to the explants, which suggests that they were in symbiosis with the plants.

Seven months after applying the pretreatments, most of the explants had bacteria even when products such as alcohol, chlorine, Timsen®, hydrogen peroxide had been used, so it was decided to use Pursue®. With this procedure applied, an average of 65% of visually healthy explants were obtained (Figure 4). With those we started the process of acclimatization and *in vitro* multiplication of agave.

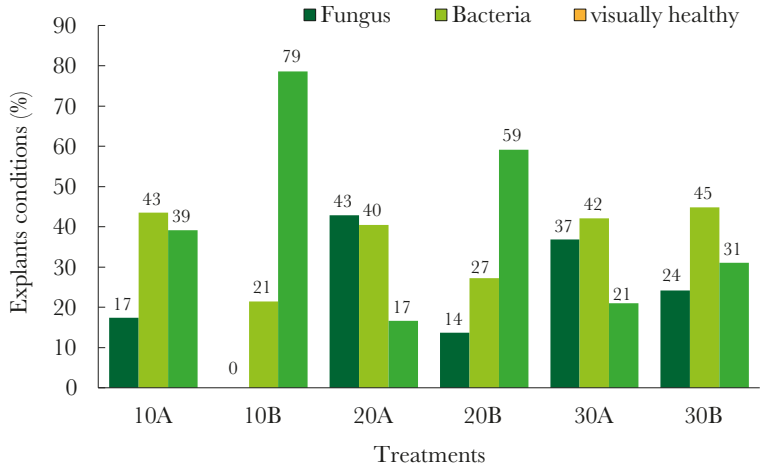


Figure 3. Percentage of condition of *in vitro* explants of *A. angustifolia*, treated with agrochemicals under *ex vitro* conditions. 10, 20, 30 days of exposure to chemicals; A: Actara; B: Benomyl.

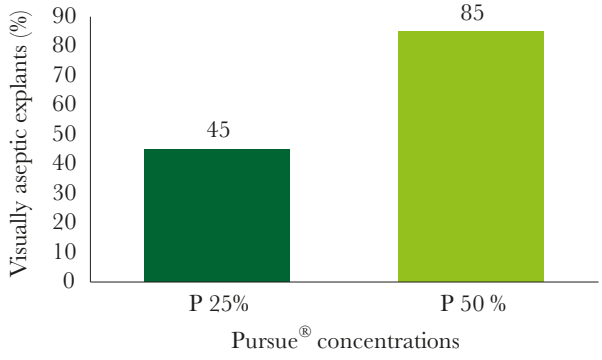


Figure 4. Percentage of visually aseptic explants of *A. angustifolia*, treated with Pursue®. Q: Pursue; n=20.

Alves-Pereira *et al.* (2014) mentioned that contamination by endogenous bacteria is common in *in vitro* culture; moreover, that the application of chlorine or alcohol is not sufficient. This makes it necessary to use antibiotics in the culture medium.

It is worth emphasizing that most *in vitro* studies do not mention how they addressed or solved the problems of contamination of the explants. In this study it was important to obtain visually healthy explants. That is why various products were used, until determining that with Pursue® at 50%, better results are obtained, in regard to the number of explants without visible contamination.

CONCLUSIONS

The use of fungicide and insecticide in agave plants, collected from the field prior to in vitro culture, is an alternative to reduce contamination of explants established in laboratory conditions. The concentration of Pursue® at 50% to disinfect explants with bacteria is satisfactory up to 85% of the treated explants.

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Empowerment processes in management and self-management in two rural communities

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ABSTRACT

Objective: To draw attention to the empowerment in management and self-management that rural women can achieve individually and collectively.

Methodology: Two workshops were conducted with the participation of n=77 people of the communities of Tepexilotla and Carrizal, in order to identify the abilities and capacities that facilitate a better life quality, and greater personal and collective power for rural women.

Results: There is a greater empowerment in the community of Tepexilotla than in Carrizal, because the first had training by academics from educational institutions, as opposed to Carrizal.

Limitations on the study: Machismo as part of the patriarchy limits the participation and attendance of women to the training workshops.

Conclusions: The capacities, strategies and prominence are factors that foster the empowerment process in rural women, driving their participation in management and self-management.

Keywords: Diagnosis, participation, gender, rural development, community organization.

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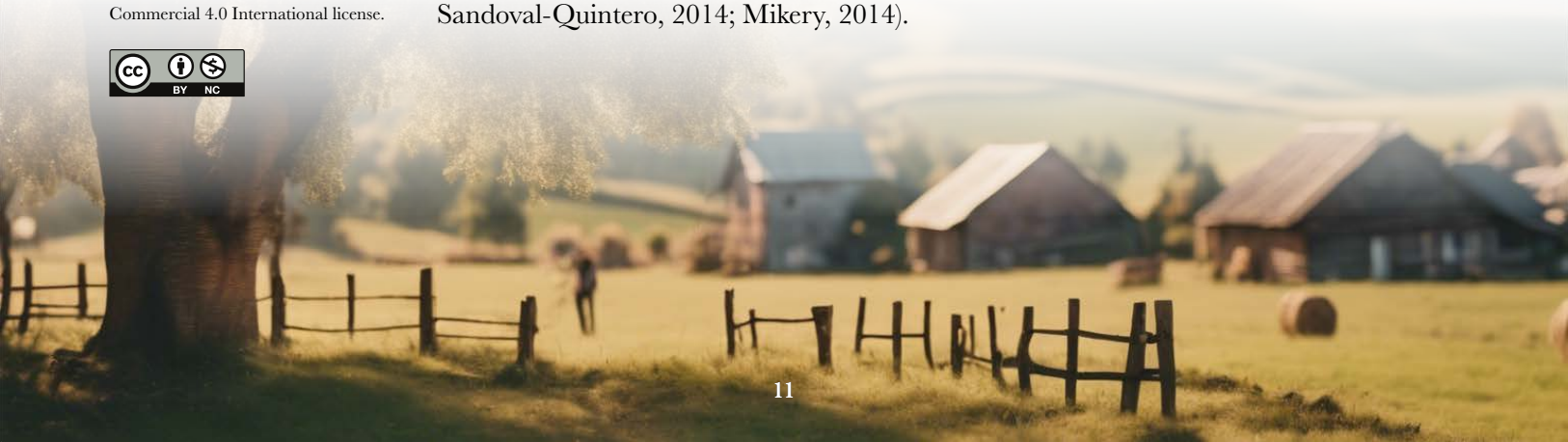
INTRODUCTION

The management and self-management carried out by inhabitants of rural communities allow us to visualize the point of coincidence to reach agreements for participation, inclusion and decision making about common needs. Understanding, recognizing and valuing the contributions of rural women in community resolutions, as well as their contributions to the family both in the cultural and the economic aspects are defining factors in their individual and collective empowerment. The impact of educational institutions (Colegio de Postgraduados, Universidad Autónoma Chapingo) on training in different topics, including technical aspects of production, care for the environment, and human relationships, can contribute to the scientific knowledge of gender and rural women (Pimentel-Aguilar and Sandoval-Quintero, 2014; Mikery, 2014).



Colegio de Postgraduados (COLPOS) and Universidad Autónoma Chapingo (UACh) began training processes in Tepexilotla since 2009 and continued until 2021. From the beginning, the women became involved in training processes, contributing to decrease patriarchal relationships and have generated greater management and self-management of women in Tepexilotla (Pimentel-Aguilar and Sandoval-Quintero, 2014). The participation of women from the community of Tepexilotla in cultural and training workshops has been constant, which has not happened in other communities such as, for example, Carrizal.

This study found that the training process which was offered increased the capacities for communication and interrelation of the participating families. This allowed the inhabitants to suggest new strategies for internal collaboration that motivated greater social and family prominence, both individually and collectively. The participation of women allowed for these internal transformations supported by external agents to become part of the empowerment process of women in the communities of study.

Empowerment is "the process by which women, in a context where they are at a disadvantage due to structural gender barriers, acquire or reinforce their capacities, strategies and prominence, both individually and collectively, to reach an autonomous life in which they can participate in equal terms in the access to resources, recognition and decision making in all the spheres of personal and social life" (CCOO, 2017). Empowerment in praxis manifests in specific processes of management and self-management.

Management is the action of accomplishing and, according to Bozeman (cited in León, 2007), the disciplinary field of public management is associated to the perspective of public policies. The relationship of the community with the outside is understood as management, that is, something that can be accomplished with external agents, such as authorities of different levels (municipal, state or federal), public or private institutions foreign to the community. Considering its etymological definition, self-management is understood as management carried out by oneself (Hudson, 2010). Management implies that the community takes control over how to use a resource of common use, as well as decision making in its form of organization inside the community. Based on this, the objective was to draw attention to the empowerment in management and self-management that rural women can achieve individually and collectively in two rural communities of Veracruz, Mexico.

MATERIALS AND METHODS

Location of the study area

The study was conducted in the communities of Tepexilotla and Carrizal in the municipality of Chocamán, Veracruz, Mexico (Figure 1) (19° 01' N and 97° 02' W), at 1 360 masl. It borders to the north and northwest with Coscomatepec, south with Fortín, and southwest with La Perla.

The climate is temperate-humid with an average temperature of 19 °C. The communities of study have a rich ecosystem where mountain mesophyll forest predominates, made up of cold pine forest and temperate deciduous forest.

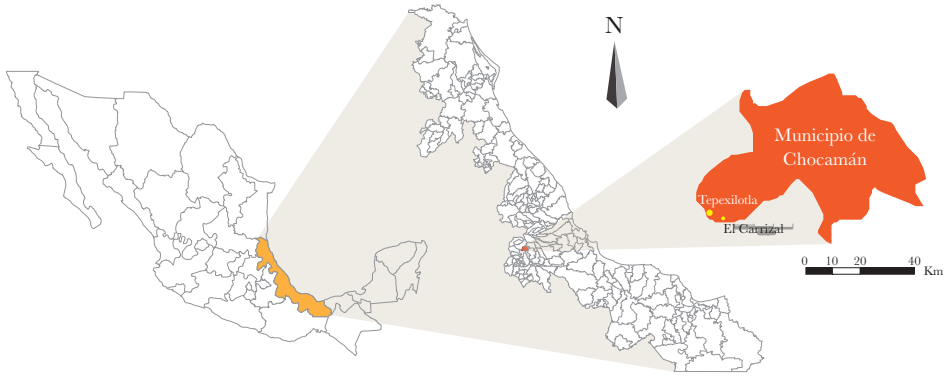


Figure 1. Location map of Tepexilotla and Carrizal. Source: Mikery, 2014

Study methods

The field information was obtained through a participatory diagnosis carried out in February 2019 in both communities with the participation of local inhabitants. This diagnosis with perspective allowed observing the gender relationships inside the community, the forms of organization and the decision making processes that lead to empowerment (León, 1997), as well as the gender relationships between women, between men, and between men and women (Alberti, 2004). When the gender category is incorporated as methodological tool, the social inequality between women and men was evidenced (Núñez, 2000).

Six tools from the methodology proposed by Geilfus (2002) were applied in two workshops with the inhabitants of Tepexilotla and Carrizal. The tools were:

- **Map of the farm with gender perspective**: this allowed identifying the differentiated gender roles in the family about who decides, who is responsible, and who performs the work in the farm.
- **Use of time**: allowed identifying specifically the activities of women in a typical day, and it was a learning dynamic between men and women regarding the real contribution of women in the activities of family exploitation in the farm.
  The use of time is a simple and convincing exercise to dissipate myths about the "limited" role that women have in the family, in the community, and in society.
- **Matrix of priority needs**: this allowed understanding the management activities and processes that the community has carried out and their form of organization, to identify and resolve conflicts.
- **Problem tree and identification of local solutions**: helped to recognize the difficulties and self-management processes that have been carried out by the community.
- **Map of natural resources and land use**: this showed the access points to natural resources and made visible the use of space and resources of the community. The gender perspective used transversally (Lamas, 1996) allowed observing the different levels of participation of women within the decision and action processes that are carried out in the community. This was recorded observationally *in situ*, resulting

in 26 participants in Tepexilotla (8 women, 8 girls, 2 men and 8 boys), and 25 participants in Carrizal (7 women, 1 man, 10 girls and 7 boys) (Table 1).

RESULTS AND DISCUSSION

Tepexilotla and Carrizal are rural communities that have a total population of 139 and 114 non-indigenous inhabitants, respectively. According to the census by IMSS (2019), both communities have a very high degree of marginalization and high social backwardness (INEGI, 2010).

There are clear differences in participation between the two communities that were identified after performing two workshops with attendance of 30% women, 32% boys, 31% girls and 7% men. The women from Tepexilotla showed greater contribution of information than the women from Carrizal. This indicates that the capacities that women in Tepexilotla have developed to communicate their opinions and perceptions about their environment have increased, which is the result of the continuous work that the academic and technical staff from COLPOS and UACh have carried out in issues related to caring for the environment in the community (caring for the river by not throwing out trash or spilling drainage), rescuing the temazcal, promoting the musical culture, and drawing attention to and revaluating the contributions of women in the production unit.

In the community of Carrizal, it was the first time that work was done with participatory workshops as a result of this study, so there was not enough trust and this limited the flow in obtaining information. In this community, more marked patriarchal characteristics were seen that are expressed through "machismo", which limits the women from attending workshops or any other type of activities that help them to develop their capacities and strategies for management and self-management. This was also observed in Tepexilotla at the beginning of the participation-action workshops that institutions in the community promoted, but inasmuch as the courses and workshops were offered, the participation of women increased gradually, resulting in them becoming organized in a savings bank, an agro-ecotourism group, and even small food businesses linked to agro-ecotourism, among others (Figure 2).

The results from the diagnosis explain the capacities, strategies and prominence individually and collectively. The individual capacities are faculties and/or skills that each individual has to perform or develop different tasks (Figure 3).

Table 1. Sociodemographic data of the men and women participants. Source: Prepared by the authors.

Description	Tepexilotla		Carrizal	Total attendance	Percentage
	Day 1	Day 2			
Women	8	8	7	23	30%
Men	2	2	1	5	7%
Girls	8	6	10	24	31%
Boys	8	10	7	25	32%
Total	26	26	25	77	100%

Table 2. Comparative of collective capacities/Map of access to resources.

Location or identified resource	Tepexilotla	Carrizal	Gender informand.			Importance
			Tepexilotla	Carrizal	Total	
Hills	3	2	M2	F1	M2 F1	Medium
Crops	2	---	F1 M1	---	M1 F1	Medium
Houses	10	9	F3 M4	F5	M4 F8	High
Church	2	---	M1	---	M1	Low
Pantheon	1	---	M1	---	M1	Low
Schools	2	---	M1	---	M1	Low
Path	3	1	M1	F1	M1 F1	Medium
River	1	1	M1	F1	M1 F1	Medium
Municipal agency	1	---	M1	---	M1	Low
Other communities	---	2	---	F1	F1	Low
Bridge	1	---	M1	---	M1	Low

*M=male F=female.

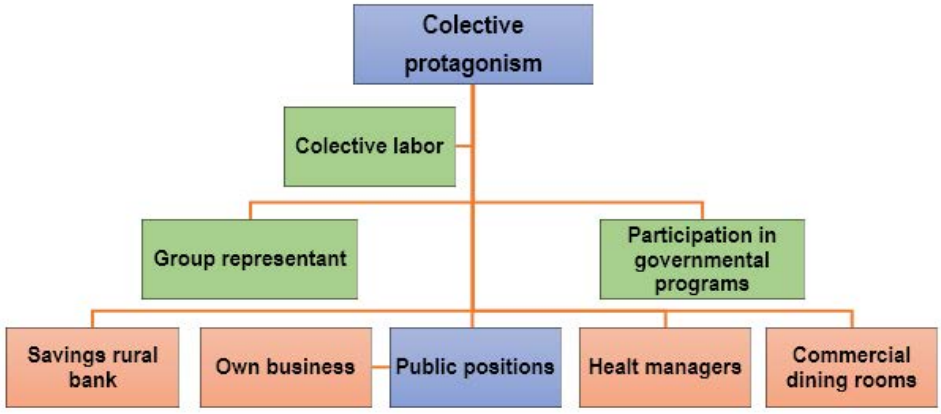


Figure 2. Comparative model-collective prominence. Source: Prepared by the authors.

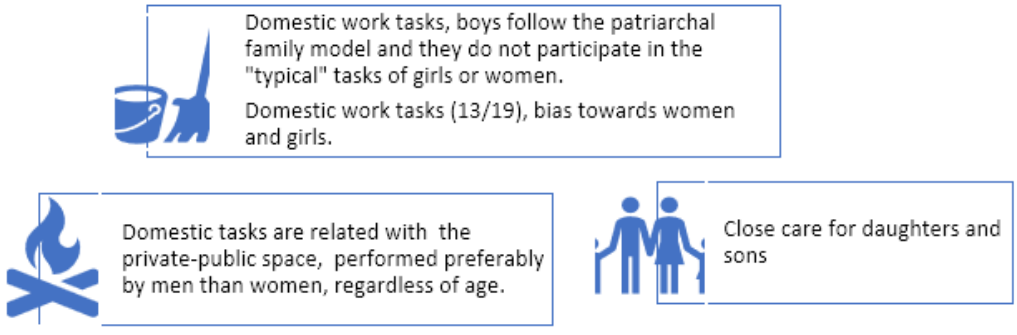


Figure 3. Results of individual capacities. Source: Prepared by the authors.

The three categories (domestic work: interior and exterior and attention to the children) represent the activities of reproductive work that are considered of the domestic scope and exclusive of women. However, the participation of men stands out in domestic work related to the exterior, such as purchasing food. This implies that women who stay at home do not have the opportunity of expressing the needs that could exist in their community to external instances such as the municipal government. Meanwhile, men that are used to being outside the home are related to these instances and with others to manage backing, according to what they consider priority. The priorities of men and women are different, which was seen in an exercise where the resources that they have were identified. For the case of women, the resource that they consider most important was the house, since it is the family's shelter. For their part, men identified the forest, the paths, the crops and the river as important resources because that is where they acquire supplies and sustenance. This indicates that men and women have differentiated valuations of resources (Table 2).

The individual strategies refer to the various ways that women have to acquire the necessary resources for daily use. Both communities agree that water is the primordial need and propose taking care of it and not contaminating it. On the other hand, they also propose the government of the Chocamán municipality should be the one to provide the service of piped drinking water, since the inhabitants must carry water from natural sources of water near the community. They also propose denouncing the people who contaminate this fundamental resource and having communication between neighbors to become organized and thus obtain water from the nearest water sources.

The collective strategies are the way in which men and women identify common problems and agree how to solve them as a group, such as: access to drinking water, adequate management of trash, and electrification. The collective strategies are similar in both communities. The problem of alcoholism and drugs was identified in Tepexilotla, as well as bullying in Carrizal, although there were no adequate proposals to control this situation in both communities, because they do not have clarity as to how to address the causes or recognize the institutions that they can resort to in order to attend to each specific problem; furthermore, they do not have clarity in what they can do themselves to become organized to solve that situation.

Individual prominence implies the salience of one subject over the others, and in this case we are speaking of women, actions and/or decisions that lead them to achieve greater empowerment. The comparative fact with data of women from both communities allows us to note that empowerment is increasing, breaking with the patriarchal structure, although women from Tepexilotla stand out. They seek higher income, which could be interpreted as greater independence in decision making, although this means adding hours to their workday.

A clear case is the women who are health representatives in each community. Something to highlight is that in Tepexilotla women are the ones who attend small businesses of their own that collaborate with the family income, such as: small shops, commercial dining halls, and even rural lodging in their own homes. This is different in Carrizal, where there is only one store that is looked after by a woman.

The collective prominence refers to the groups that stand out or have greater

participation in community activities, whether inside or outside the group. Both sexes participate in collective tasks, although gender division is reflected in the reproductive tasks and of care towards women, and those of load towards men.

The women in Tepexilotla are more prominent in collective activities than women in Carrizal, since they have organized a savings bank and an agro-ecotourism group (mixed), presided by one of them, and participate in programs and projects for community, artistic and cultural support. However, no women from either community are prominent or participate in any public position, even honorary ones.

The women of Carrizal do not participate as group representatives or in government programs. There is knowledge that they do not participate in collaboration of institutions such as COLPOS, UACh or others, and they deny participating in government programs, although it is known that several of them are recipients of the Programas para el Bienestar, and were recipients before of the *Programa Oportunidades*.

Another form of participation are the collective tasks, where both genders participate from both communities, indicating that there is a sense of belonging to the community since they help to improve the conditions of each place. Being a representative of a group promotes community, which is why it is very important for community management and self-management. An example of this is the negotiation they made with the government of Chocamán, as well as with CONAGUA for the defense of the Metlac River to prevent raw sewage from being spilled into it and they attained the protection. This negotiation was made with the initiative of a group of people from Tepexilotla and invited others from Carrizal, where both men and women participated. The way in which they distribute water within the community it is very particular, since it depends on small deposits where they connect and those who own them allow their neighbors to take it. This is an example of self-management, since it is a resource they control and distribute according to certain internal agreements without external intervention. Both communities in the study share territorial proximity, but they are differentiated by small things: 1) empowering actions, 2) self-recognition, and 3) informal training.

CONCLUSIONS

The empowerment process is the formation of awareness, identification of problems, and development of capacities that contribute to the design of strategies that give solution to the problems of each community both individually and collectively. The factors that promote empowerment of rural women are capacities, strategies and prominence. Informal training in gender perspective, inclusion and equity allow strengthening self-trust and self-esteem in the women participants, and with this to achieve projects that give differentiated answers to the needs of each gender that drive their participation in management and self-management.

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Risk factors associated with the use and management of pesticides in the production of nopal (*Opuntia ficus-indica* (L.) Mill)

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ABSTRACT

Objective: To verify the risk factors associated with the use of agrochemicals, through the degree of compliance with Mexican regulations on the Good Use and Management of Pesticides (BUMP).

Design/Methodology/Approach: One-hundred producers, selected from a group of 300 producers who make up the register of the Sistema Producto Nopal in Morelos, Mexico, answered the questionnaire. The questionnaire included plot information, market data, and open questions about the compliance with the checklist for the recognition of areas where Good Use and Management of Pesticides (BUMP) standards are applied in the primary production of vegetables of the National Service of Health, Food Safety and Agro Food Quality (SENASICA)

Results: Out of all the one-hundred producers surveyed, 57 were recognized in BUMP and 43 were not. Whether producers complied or not with these regulations and could have access to the export market depended on the crop area and the economic income it represents. The greatest non-compliance was caused by the non-authorized use of pesticides in the production of nopal, which represents a contamination risk for the worker, the environment, and the consumers.

Study Limitations/Implications: Studies should be carried out about the impact on the health of farm workers and the environment resulting from the inappropriate use of pesticides.

Findings/Conclusions: Although the implementation of BUMP in the nopal cultivation minimized the risks of contamination or poisoning, the non-authorized use of synthetic pesticides for its cultivation poses contamination risks to the worker, consumer, and environment. Therefore, strategies must be generated in the domestic market to guarantee the same food safety than in the international market.

Keywords: BUMP, chemical contamination, pesticides, Opuntia ficus-indica.

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INTRODUCTION

The economic and social importance of nopal (*Opuntia ficus-indica* (L.) Mill) cultivation in Mexico lies in the exportation volume (61,387 t among 15 countries), in the large area occupied by *nopaleras* (both wild and cultivated nopal fields), and in the diversity of nopal-

based products produced and consumed in the country (Badii and Flores, 2001; Maki-Díaz *et al.*, 2015; SIAP, 2022). In the state of Morelos, nopal is grown in a total area of 4,217 hectares, distributed in the municipalities of Tlalnepantla (3,030 hectares), Totolapan (554 hectares), Tlayacapan (512 hectares), and Tepoztlán (61 hectares); consequently, Morelos is the main nopal producer in Mexico (SIAP, 2021).

Nopal plantations, like other agricultural systems, are attacked by different pests and diseases, and the most common management method is chemical control (Pérez *et al.*, 2013). However, improper handling and application of pesticides can entail risks for humans, either as a user or consumer (Fenik *et al.*, 2011). In the last two decades, poisoning incidence from the consumption of vegetables has increased considerably due to the presence of chemical contaminants (Johnston *et al.*, 2006; Tzschoppe *et al.*, 2012). In addition, the environmental impact panorama is critical (Albert, 1996). Therefore, in developing countries such as Mexico, training activities on the safe handling of pesticides have been carried out during the said period, with the aim of making farmers aware of the harmful effects of these products; nevertheless, a significant change in attitude has not been achieved yet and sometimes this awareness cannot be put into practice (Damalas *et al.*, 2006; García, 1999; Isin and Yildirim, 2007). Consequently, government initiatives have been developed and implemented, such as production models based on "Good Agricultural Practices" and "Food Safety" (Pérez *et al.*, 2013). The National Service of Health, Food Safety and Agro Food Quality (SENASICA) promoted, through the Comité Estatal de Sanidad Vegetal del Estado de Morelos, A.C, the implementation of practices for the Good Use and Management of Pesticides (BUMP) among 94 local producers who operate 146 production units in over 185 ha, with the purpose of reducing the risk of chemical contamination in nopal production (SAGARPA, 2020).

Therefore, the objective of this work was to verify the risk factors associated with the use of agrochemicals in the production units that belong to the producers included in the register of the Sistema Producto Nopal in Morelos.

MATERIALS AND METHODS

Study area

The nopal producing region in Morelos is comprised of representative areas of the municipalities of Tlalnepantla, Tlayacapan, and Totolapan. This region is located in the northwest of the state of Morelos (Figure 1); it has a subhumid climate, an average annual rainfall between 913 and 2,341 mm, and an average annual temperature of 17 °C.

Study population

These municipalities have a total population of 40,101 inhabitants, with a similar sex ratio and an average elementary education of 63.7% (INEGI, 2020) (Table 1).

In Tlalnepantla, 90% of the population grows nopal as their primary crop, because it generates a high level of income, mainly in autumn and winter, when it is scarce in other producing states (Rubio, 1997), while, in Totolapan and Tlayacapan, the activity of nopal is shared with other vegetables.

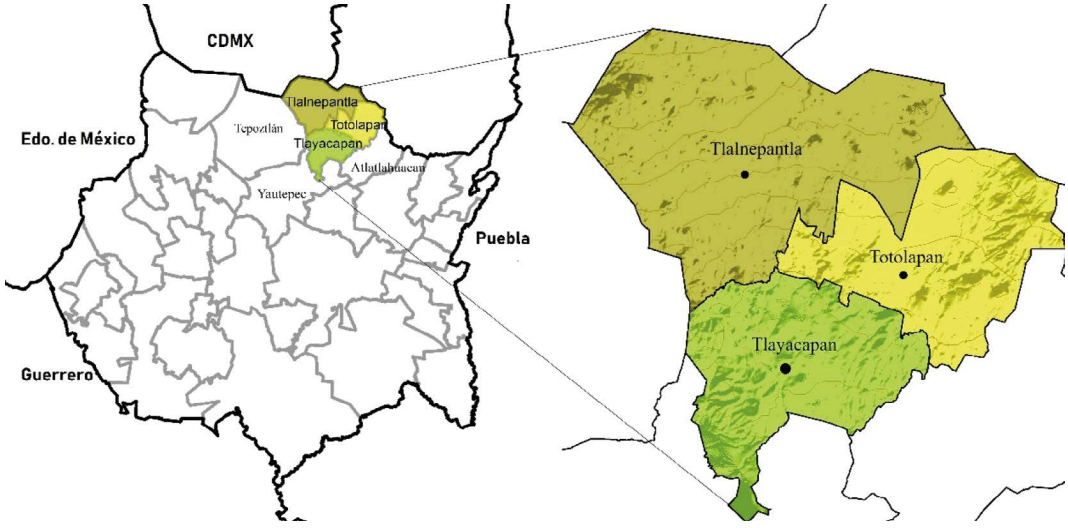

Figure 1. Geographic location of the nopal producing region in Morelos, Mexico.

Table 1. Population characteristics of the nopal producing region in Morelos.

Municipality	Population	Men (%)	Women (%)	Primary education (%)*
Tlayacapan	19,408	49.6	50.4	55.0
Tlalnepantla	7,943	50.1	49.9	69.9
Totolapan	12,750	48.6	51.4	66.3

Source: INEGI, 2020. * People 15 years or older with primary school education.

The register of the Sistema Producto Nopal producers of Morelos was taken as a basis for this study. The simple random sampling method was applied to develop a sample of 100 of the 300 producers that make up the total register.

Questionnaire

The questionnaire was designed as a tool for a descriptive, transversal, and observational process; it involved the voluntary participation and signed consent of the interviewees. The questionnaire included plot information, market data, and open questions about the compliance with the checklist for the recognition of areas where Good Use and Management of Pesticides (BUMP) standards are applied in the primary production of vegetables of the National Service of Health, Food Safety and Agro Food Quality.

Forty-one questions were distributed in eight sections: general information, infrastructure, use and management of pesticides, agrochemical products, personal protective equipment and spraying equipment, preparation of mixtures of agrochemical products, disposal of leftover mixtures, and management of empty containers. The plantations, warehouses, and leftover mixture disposal areas were visited to verify the conditions of use and agrochemical management.

RESULTS AND DISCUSSION

Eighty plots located in Tlalnepantla, fifteen in Tlayacapan, and five in Totolapan were verified. Out of the total producers, 57 were recognized by SENASICA in BUMP and 43 were not. Both groups had received training in BUMP. However, the unrecognized group had not invested in infrastructure or the recognition process. They argued that the income per production area is not enough for the required investment (infrastructure and recognition). All the interviewees (100%) were men between 45 and 60 years old, 99% of whom had attended elementary school and only one had a bachelor's degree. None of the participants mentioned the risk of direct chemical contamination to women and children, since they stated that they themselves were in charge of cultivation work in the field, hiring adult male laborers for the cutting.

Infrastructure

With regard to documentary infrastructure, 100% of the recognized producers had a procedures manual that described the control measures that were applied during the handling of agrochemicals. On the matter of personnel hygiene and safety, all producers complied with the provisions of sections 5.12 and 6.8 of NOM-251-SSA1-2009 (SSA, 2009). Regarding physical infrastructure, 80% of the recognized producers established appropriate storage areas for personal protective equipment, disposal of empty containers, and storage of pesticide application equipment; however, upon verification, 5% of these spaces were in disarray and did not receive maintenance. All the producers (100%) implemented an area for the preparation of pesticide mixtures and the disposal of leftover mixtures, following the corresponding requirements, and 93% provided a maintenance plan signed by the person responsible. In addition, they also shared a record of product doses, which included agrochemicals not recommended for the cultivation of nopal. Thirty-eight percent of the unrecognized producers complied with the physical infrastructure, but not with any of the other requirements.

Signage of basic facilities

One-hundred percent of the recognized producers complied with the provisions of NOM-026-STPS-2008 (STPS, 2008) during verification. When asked what the signs referred to and why they were placed in different areas, they correctly interpreted the images, unlike the unrecognized producers who did not have signs.

Storage of agrochemical products, personal protective equipment, and spraying equipment

Ninety-one percent of the recognized producers had a warehouse, where they established spaces for storage, with separation between pesticides and fertilizers. These spaces were built with non-absorbent and non-flammable material; they were ventilated and well-lit. The door was locked and the key was kept by a trained adult in 86% of the cases. Seventy-four percent of the recognized producers stored their pesticide spraying equipment and personal protective equipment (used during the application activities) in safe areas, consequently preventing health and contamination risks in accordance with section

7.2.1 and 7.2.2 of NOM-003-STPS-1999 (STPS, 1999). On the contrary, unrecognized producers usually stored pesticides and spraying equipment in common areas, putting the health of farm workers at risk. In this regard, several authors have documented that inadequate hygiene and safety measures, lack of training in the handling of agrochemicals, and misuse of personal protective equipment are among the main reasons for poisoning among farm workers (Cárdenas *et al.*, 2010; Gómez-Arroyo *et al.*, 2013; Guzmán-Plazola *et al.*, 2016). Ortiz-Hernández *et al.* (2013) point out that the chronic and acute damages from exposure to pesticides are diverse and indisputable: infertility, psychological damage, chromosomal abnormalities, and increased risk of melanoma, leukemia, and cervical cancer (Thrupp, 1991; Wesseling *et al.*, 1996; Cuenca and Ramírez, 2004).

Agrochemical mixture preparation and leftover mixture disposal

One-hundred percent of the producers with BUMP recognition had areas to prepare agrochemicals and to rinse empty containers. These areas are separated from water sources and have a spill containment pit, with a firm floor that ensures the evaporation of the liquid and a structure that prevents the entry of domestic and wild animals. However, 53% of the unrecognized producers did not have such areas and prepared the agrochemical mixtures in 200-L drums; subsequently, they overspray the leftover mixtures on the edges of the crop and rinse the drums in the area where the mixtures were prepared, potentially polluting soils and water sources through runoff or infiltration. In this regard, Hernández-Antonio and Hansen (2011) studied the pesticide content in water samples from two agricultural areas of Mexico, finding that the concentrations of the atrazine (4.62-15.01 μg L^{-1}) herbicide exceeded the permissible limits for water for human consumption proposed by the World Health Organization (2 μg L^{-1}) and the European Community (0.1 μg L^{-1}), posing a risk to human health.

Use and management of pesticides

All the producers interviewed were trained on the use and management of pesticides. Although they used synthetic pesticides registered before the Federal Commission for the Protection against Sanitary Risks (COFEPRIS, 2017), the use of these pesticides was not authorized for nopal cultivation. However, producers ignored the recommendations on the labels, arguing that no products have been authorized for nopal cultivation. In this sense, although Montoya *et al.* (2013) point out that the agricultural producer must comply with laws, standards, and techniques regarding the handling of agrochemicals, Hernández-González *et al.* (2007) argued that the lack of training on the correct interpretation of the product label encourages its inappropriate use. Lack of knowledge about the safety intervals of the products used could harm the health of both workers and consumers.

Handling of empty containers

One-hundred percent of the producers with BUMP recognition reported having been technically trained to manage empty containers and they had a temporary collection area for triple-washed and perforated containers. In addition, they showed documentary

evidence of having previously sent containers to authorized collection centers. These practices contrasted with the management carried out by unrecognized producers. In this regard, SAGARPA (2012) points out that in Mexico approximately 50 million containers of agrochemicals are generated per year (6,020 tons). Their irresponsible handling means that they end up in irrigation canals, rivers, streams, ditches, and the open field, etc., generating sources of environmental pollution and sometimes poisoning problems. Likewise, García-Gutiérrez and Rodríguez-Meza (2012) point out that the disposal of empty containers is a serious problem resulting from agriculture and represents a high risk to environmental and human health. Overall, producers who did not have recognition by SENASICA failed to comply with these regulations. Their argued that the lack of economic resources was the main limitation for the improvement of their infrastructure.

In this study, the recognized producers had an average surface area of 2.7 ha and their production was destined for the export market, while the unrecognized producers had an average surface area of 1 ha and their production was sold in the domestic market. This situation suggests that the larger the area and the export market, the greater the degree of compliance with national regulations. In this regard, the unrecognized producers indicated that the production area did not cover the expenses required to implement the system and that their product is only sold in the domestic market.

This context poses a risk of chemical contamination for the national consumer, who mainly has access to the nopal produced and distributed by farmers who generally do not implement a proper use and management of pesticides. According to Badii and Valera (2008), pesticides provide great benefits to agricultural production and product quality; however, deficient agricultural practices —*e.g.*, respect for safety intervals, good use and management of pesticides, and the correct use of personal protective equipment— are some of the conditions that result in intoxication or poisoning by agrochemicals, either during their application or the consumption of foods that exceed the maximum allowable residue limit. Therefore, greater surveillance and monitoring of good agricultural practices is necessary, in areas of high agricultural activity, to reduce or prevent damage to both the environment and human health.

During the review of the area for the temporary storage of empty containers, 19 active ingredients that had been used in different agricultural practices were identified (Table 2); several of them had different commercial names and their authorization was indeterminate or in force. In all cases, producers do not use authorized pesticides (mainly insecticides); this is an important indicator of the inappropriate use of these products in the nopal cultivation, consequently posing a potential risk of chemical contamination for both producers and consumers, since the application doses and safety intervals of these products are unknown.

Conventional systems are currently characterized by high consumption and dependence on pesticides; therefore, seeking agroecological strategies that minimize negative impacts on the environment and consumers, through lower consumption of agricultural inputs, is a necessary measure. High concentrations of chlorpyrifos, dimethomorph, malathion, omethoate, imidacloprid, and carbendazim has been observed in nopal samples taken

Table 2. List of pesticide active ingredients found in empty containers stored in temporary warehouses of nopal producers in Morelos, Mexico.

Active ingredient	Tox. Cat.	Type of pesticide	Trade name
Abamectin	III	Insecticide/acaricide	Agrimec® 1.8% CE, Agriver® 1.8 CE
Amitraz	IV	Acaricide	Mitoff, Teracix
Benomyl	IV	Fungicide	Promyl 50 P. H.
Carbendazim	IV	Fungicide	Prozycar 500 F
Captan	IV	Fungicide	Captan 50 plus
Chlorothalonil		Fungicide	
Cypermethrin	IV	Insecticide	Cipermetrina 20 CE
Cymoxanil	IV	Fungicide	Curzate 60 DF
Chlorpyrifos	III	Insecticide	Cyren 480, Carioca
Chlorpyrifos+permethrin	III	Insecticide	Disparo, Ventax
Carbofuran	II	Insecticide	Furadan 5 G
Diazinon	IV	Insecticide	Diazinon-bio 25
Fipronil	IV	Insecticide/acaricide	Regent MG-20 GR
Glyphosate	IV	Herbicide	Faena full
Malathion		Insecticide	
Methidathion	III	Insecticide	Suprathion 40 EC
Monocrotophos	II	Insecticide	Zucron, Bazucron 60*, Dicron
Parathion-methyl	III	Insecticide	Foley 2%*
Permethrin	III	Insecticide	Ambush 34 CE*, Matagus 34*

*Tox. Cat. Toxicological category. * Undetermined authorization, ** Registration in force.

from a collection center supplied by producers who do not implement BUMP. These findings contrasted with the nopal samples from BUMP-recognized producers, where no pesticide residues were detected, highlighting the importance of the proper use of these products and the implementation of the BUMP for the protection of both farmers and consumers (Ramírez-Bustos et al., 2018). In this regard, Ramírez-Bustos et al. (2019) studied the dissipation of chlorothalonil, chlorpyrifos, and malathion in the nopal crop, pointing out that the approximate dissipation time for these pesticides is 10 days and their mean life lasts six days. The final concentrations of the three pesticides were below the reference (0.01 mg/kg) Maximum Residue Limits (MRL), suggesting that these products can be safely applied in commercial nopal production at the concentrations established in the said study.

Finally, the federal and state governments must support small-scale producers, who otherwise will not be able to comply with the infrastructure required to implement the Good Use and Management of Pesticides and reduce the risk of chemical contamination among workers, consumers, and the environment. The results of this study provide information to propose and prioritize actions aimed at addressing the problems of small nopal producers in Morelos, along with strict regulation on the use of pesticides. Likewise, comprehensive strategies should be designed and implemented to make production systems accountable for the health of the population and the environment.

CONCLUSIONS

The implementation of the proper use and management of pesticides, established in national regulations, reduces the risks of chemical contamination in the cultivation of nopal. However, the use of unauthorized pesticides for the cultivation of nopal involves risks of contamination to the worker, the consumer, and the environment. Therefore, strategies must be generated in the domestic market to guarantee the same food safety than in the international market. The competent authorities should authorize molecules that provide producers with options for the use of agrochemicals authorized for the control of the pests and diseases detected during the crop cycle.

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Sustainability Assessment of Two Farming Systems

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ABSTRACT

Objective: To assess the sustainability of two agricultural production systems (native and improved maize) using the MESMIS methodology.

Design/Methodology/Approach: Understanding the social, economic, and environmental factors that make up the production systems, as well as their management methods, in the municipality of Jilotepec, State of Mexico enables the development of sustainable rural development strategies. The study included 30 production systems and compared the level of sustainability between producers of native and improved maize. In September 2022, information was collected in the field, through participatory workshops, semi-structured interviews, and surveys. Additionally, soil sampling was carried out to analyze soil fertility.

Results: The two systems studied have high agricultural production costs and are highly dependent on external inputs, especially those that use improved maize. Most of the systems depend on external economic income. Agriculture is increasingly exposed to drought conditions and changes in rainfall patterns, forcing the population to implement adaptability measures. Owning livestock is an important economic support for production systems. The regional soil is fertile and suitable for growing maize. The perception of happiness among the interviewees is high; they consider their quality of life to be good and therefore do not migrate.

Findings/Conclusions: This study is the result of an integrated analysis of several methodologies used in the sustainability indicators measurement.

Keywords: Rural development, sustainable agriculture, happiness, MESMIS.

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INTRODUCTION

The concept of sustainability has great relevance for the preservation of the diverse lifeforms that exist in our planet; as a social being, man plays a major role, since environmental problems mainly have an anthropogenic origin. These severe environmental problems are awakening a worldwide social conscience to protect and care for the environment.

This is evident in the academic, political, and economic discussions that seek to achieve a harmonious relationship between society and nature (Fonseca-Carreño *et al.* 2020; Javier-Sarandón, 2020).

Sustainability is a relatively new concept. It is a response to the industrial revolution that began to generate negative effects on the environment in the mid-18th century. It began to be openly discussed with the Brundtland report (1987), during the creation of the World Commission on Environment and Development. The discussion focused on satisfying the needs of the current and future human population from three approaches: economic, environmental, and social (González-Esquivel *et al.*, 2006). Noguera *et al.* (2020) point out that "sustainability is a proposal based on environmental rationality, promoting political and social change. On the one hand, from the economic point of view, it seeks the participation of native people and farmers, with the aim of achieving a fair redistribution of wealth and other forms that respect the resilience of ecosystems. On the other hand, from the environmental and cultural point of view, it should include the richness of cultural diversity. It also relates nature with culture, seeking a re-appropriation of nature and life".

Martínez-Castillo *et al.* (2016) mention that "the sustainability of production systems refers to their capacity to maintain their productivity despite major disturbances, both economic and natural, external or internal". This definition implies that the natural, social, economic, and technical characteristics of a production system, along with the pressures or interventions it experiences, will determine its level of sustainability.

Javier-Sarandón (2020) points out that "agricultural production systems that make an intensive use of external inputs have negative consequences, because they are degrading and polluting the environment. This deterioration process is increasingly evident and puts the maintenance of ecosystem balances and human life at risk." Likewise, Borras *et al.* (2012) mention that "agriculture and sustainable rural development have had an increasingly major role in the discussions about potential alternatives to face the challenges posed by the environmental, climate, energy, financial, and food crisis." Linares-Díaz (2019) points out that agriculture plays such a key role in the alleviation of world hunger that the 17 Sustainable Development Goals (SDGs) proposed by the United Nations, in its 2030 Agenda for Sustainable Development, included "end hunger, achieve food security, and improved nutrition and promote sustainable agriculture." Furthermore, to achieve this goal, fundamental changes in production and in current agricultural policies are necessary. These changes must be focused on sustainable agriculture by addressing its 3 pillars: social, economic, and environmental (Wiget *et al.*, 2020; Purvis *et al.*, 2019).

Maize is the most important agricultural product in Mexico, from a food, industrial, political, and social point of view. It is grown practically all year round and in wildly diverse agroclimatic conditions, under rainfed and irrigated systems, and in more than half of the country's agricultural area (7.4 million ha) (SIAP, 2023). Mexico is the center of origin of maize; currently, 59 native breeds have been identified and hundreds of varieties have been adapted to each region or climate (CONABIO, 2008). *Criollo* or native breed sowing contributes significantly to the food security of the poorest rural strata of the country, whose production is focused on self-consumption, either for themselves or for livestock (Turrent-Fernández *et al.*, 2012). In turn, improved maize (hybrids) —whose harvest is destined for

sale and which largely satisfy the needs of Mexican agroindustry— occupies only 20% of the total area sown with maize (SIAP, 2023), basically under artificial irrigation systems and with high production and input costs (Turrent-Fernández *et al.*, 2012).

Given the relevance of the native maize production system in Mexico, Fonseca-Carreño *et al.* (2020), González-Esquivel *et al.* (2006), and other authors have analyzed the sustainability of maize production systems (basically aimed on self-consumption or carried out by small producers), focusing on proposals for food strategies and the analysis of the role of family members in production, as well as on the different social, economic, and political participants involved in the production system.

In agricultural production, establishing some technical concepts is important for an adequate agronomic management of crops. The concept of soil fertility or fertile soil is one of the most widely used in agricultural and forestry contexts. In this sense, Astier-Calderón *et al.* (2002) mention that "soil is a core component of the agroecosystem; therefore, it is necessary to characterize and define its condition, in order to evaluate its sustainability". Regarding soil fertility parameters, Domingo-Santos *et al.* (2006) indicate that water absorption, retention, and supply are fundamental ecological missions of the soil; they are so important that, among the variables used to estimate soil degradation, those related to soil availability are considered even more important than those related to nutrient availability. Soil water is an important ecological factor and, therefore, it is essential to determine the volumes that soil can hold, as well as the proportion of this water that is available to plants.

The economic dimension of sustainability is one of the main challenges faced by agricultural production. Food generation should not only be productive, but also profitable, fair, and sustainable (Uzcanga-Pérez *et al.*, 2020; Bonilla-Bolaños *et al.*, 2019). Masera *et al.* (2008) mention that the sustainable system design should be oriented towards small producers with scarce economic resources; reduce production costs; increase benefits through productivity; conserve traditional agricultural management lore; and offer access to inputs, food, and market.

Agriculture is a social production system where human beings interact with nature. People who participate in this production should feel fully satisfied with their lives. This sense of satisfaction will be called happiness in the rest of this paper. Happiness is a complex concept that has been addressed as the goal of human life, ever since the time of the Greek philosophers. Alarcón (2006) and Beytía *et al.* (2011) defined happiness as the degree to which a person appreciates the totality of their present life in a positive way and experiences pleasurable affections. Therefore, establishing methodologies for measuring happiness in the population is an important step to study the socioeconomic determinants of happiness and to guide public policies towards the improvement of subjective well-being. Fernández-Berrocal *et al.* (2009) mention that, "from a psychological-positive perspective, a happy person would have many positive experiences and few negative ones, perceiving themselves as satisfied with their life as a whole; therefore, happiness is a subjective state of the individual that is a direct result of their self-report".

Assessing the sustainability of 2 production systems (native maize and improved maize) with the MESMIS methodology determines the status of the sustainability components

of production systems and will be the starting point for the identification of those aspects that —if improved (in case of weaknesses) or exploited (in case of strengths)— can balance the social, economic, and environmental aspects of the systems and eventually lead to an integral development of the production systems involved.

MATERIALS AND METHODS

Location: El Saltillo is a community that belongs to the municipality of Jilotepec, State of Mexico, located 90 km northwest of Mexico City (Figure 1). It has an area of 1,384 ha, with a population of approximately 870 inhabitants (INEGI 2021). It is a rural area with a low population density, where agriculture (mainly maize) is carried out under a rainfed regime.

Figure 1 shows the spatial distribution of the 30 production systems under study. The selection methodology used for the assessed systems was first applied to all the systems that use improved maize (7). Subsequently, the best spatial distribution within the community that uses native maize (23) was determined. The participants were contacted and invited to participate in the present study.

The MESMIS Framework integrates the environmental, social, and economic spheres to the concept of sustainability. Seven attributes are measured based on the sustainability characteristics of productive systems: a) productivity, b) stability, c) resilience, d) reliability, e) adaptability, f) self-management, and g) equity. The sustainability indicators to be assessed must be immersed in at least one of the attributes. The six following elements constitute the MESMIS work phases: 1) definition of the systems to be worked on; 2) determination of critical points of the system; 3) selection of strategic indicators; 4) measurement and

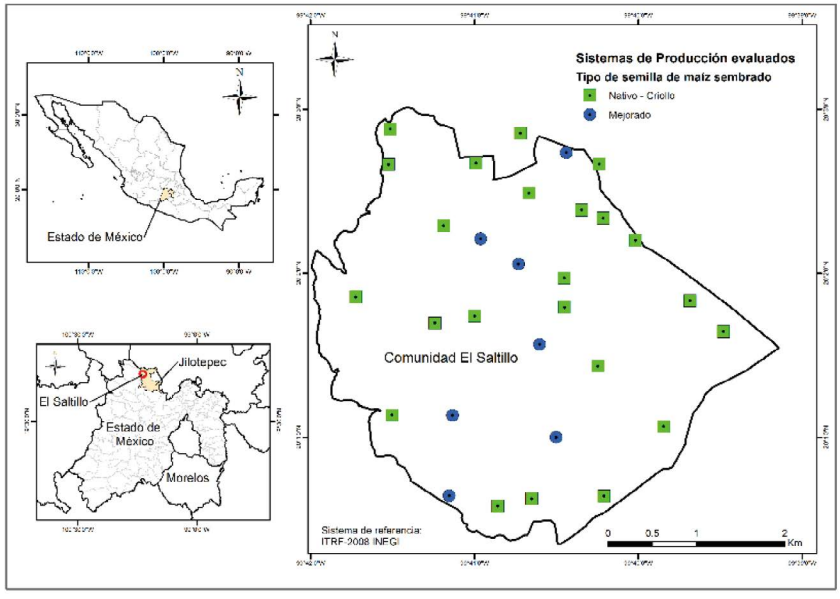

Figure 1. Location of the community of El Saltillo, Jilotepec, State of Mexico and spatial distribution of the assessed production systems.
Source: Figure developed by the authors based on cartographic data from Marco Geoestadístico 2021 (INEGI).

monitoring of indicators; 5) integration of results; and 6) conclusions and recommendations (Masera *et al.*, 1999).

For Phase 1, diagnostic work was carried out on October 2021, enabling the definition of phases 2 and 3. In September 2022, phase 4 was carried out, which included the collection of information in the field. The interview and oral communication technique was the data collection tool chosen for this study (Geilfus, 2002). It consisted of semi-structured questions addressed to members of each family, with topics related to producer data, agricultural production and yields, agricultural and livestock production costs, diversification of economic income, and perception and adaptability to climate change. A survey was applied to different members of each production system in order to measure the perception of happiness. The data used to calculate the Grain Yield Potential (GYP) were collected from the plots of the families interviewed and the collected soil samples were sent to a specialized laboratory to determine their fertility parameters.

Soil water holding capacity was considered as one of the indicators in the environmental field and refers to the difference between field capacity (FC) and wilting point (WP), which are the limits that define the water requirement for the optimal development of a crop —*i.e.*, the water contained between FC and WP is the water that can be absorbed by the crop's root system (Liu *et al.*, 2019). The diversification of livestock species represents a process of sustainability of the production system. González-Flores *et al.* (2020) highlight that species diversity is a strategy that guarantees the sustainability of agroecosystems. In the same sense, Sullivan (2003) indicates that the diversification of livestock species reduces risk or vulnerability, makes the system resilient, and increases sustainability. The Shannon-Weaver index was used to measure the number and diversity of livestock species in production systems.

Within the economic indicators, the Grain Yield Potential (GYP) was calculated, applying the following formula.

$$GYP = \frac{pd \times cp \times gc \times tgw}{Gh} \qquad \text{(LGSEED. 2020)}$$

Where GYP=is the grain potential yield, pd=plant density per hectare, cp=number of cobs per plant, gc=number of grains per cob, tgw=Thousand Grain Weight, and Gh=number of grains per hectare.

The agricultural production cost refers to the outlay or expenses that each system makes when producing maize, with the understanding that a sustainable system should allow for low production costs and high yields. Data refer to input (fertilizers, herbicides, and seeds) and machinery (tractors and harvesters) costs.

Economic income diversification refers to the number of jobs outside the production system held by its members. External labor costs refer to the economic disbursement that the systems make to hire day laborers or agricultural laborers. They are one of the highest economic expenses of the system.

The adaptability actions to climate change refer to the measures that each system has implemented to face the undeniable changes in temperature and precipitation patterns in recent years.

RESULTS AND DISCUSSION

As a result of the diagnostic work carried out on October 2021, the main characteristics of the production systems to be assessed were defined (Table 1), during MESMIS phase 2.

Table 1 describes the main characteristics of the assessed production systems. The first system (S1) is made up of 23 producers who use native and rainfed maize, obtain low agricultural yields, and produce for self-consumption. A second system (S2) is made up of the only 7 producers in the community who use improved maize under a rainfed agriculture system. They produce for self-consumption and to feed livestock and they commercialize live cattle, milk, and derivatives as their main source of income.

Table 2 defines 11 sustainability indicators for phase 3 and describes the indicators to be measured.

In September 2022, phase 4 of the field work was carried out and included the collection of information in the field through various techniques, such as participatory workshops, semi-structured interviews, and surveys. Additionally, soil sampling was carried out to analyze soil fertility.

Initial values were quantitative and qualitative and different units of measurement were used. The said values were standardized on the same scale of values for their jointly analysis (phase 5, integration of results). The Reference Interval methodology was used for this purpose (Galván-Miyoshi 2008). The position of a system is determined in relation

Table 1. Main characteristics of the assessed production systems.

Characteristics	Production systems	
	Native seed (S1)	**Improved seed (S2)**
Production systems evaluated	23	7
Corn seed used	Native seed	Improved seed
Average corn yield (Ton/ha)	1 to 3	8 to 10
Crops in plot	One (monoculture)	
Purpose of production	Self-consumption and for livestock	
Type of plot work	Yoke and tractor	Tractor, harvester, manual
Type of planting	Manual and tractor	Tractor
Type of fertilization	Agrochemicals	
Type of irrigation	Rainfed irrigation	Rainfed irrigation (and drip irrigation with stored rainwater)
Catte management	Grain and stubble fed, grazes on the field after harvesting	Stable, grain and stubble fed, plus complement
Purpose of cattle	Occasional marketing of live cattle	Marketing on dead wight to abattoirs, milk and derivatives
Type of economic income	Government employees, workers and own businesses	Government employees and own businesses
Type of labor	Family and contracted	Contracted

Table 2. Diagnosis criteria and indicators used to assess the sustainability of production systems.

Indicator	Area	Attribute	Diagnostic criteria	Measurement units
Soil organic matter	Environmental	Productivity, Stability	Soil fertility	% (OM)
Soil's ability to retain water	Environmental	Productivity, Stability	Soil fertility	Difference between CC & PM
Cattle diversity	Environmental	Self-sufficiency, Stability	System self-sufficiency	Shannon-Weaver index
Happiness level of local agents	Social	Resilience, Adaptability	Rol of local actor	Happiness survey
Adaptability actions of CC	Social	Resilience, Adaptability	Rol of local actor	Numbers of adaptability actions of CC
Time dedicated to farming activities, women	Social	Self-sufficiency, Equity	Rol of local actor	Time dedicated to farming activities
Time dedicated to farming activities, men	Social	Self-sufficiency, Equity	Rol of local actor	Time dedicated to farming activities
Cost of agricultural production	Economic	Self-sufficiency, Productivity	System self-sufficiency	Investment ($) in agricultural production
Diversification of economic income	Economic	Self-sufficiency, Productivity	System self-sufficiency	Number of external economic income
Investment in external labor	Economic	Self-sufficiency, Productivity	System self-sufficiency	$ Hiring of external labor (pawns)
Potential grain yield (PGY)	Economic	Self-sufficiency, Productivity	Technical efficiency	Kg ha^{-1} of grain

to a maximum and minimum interval, based on an optimal value (maximum or optimal level reachable by the indicator, shown as V_{max} in Table 3) plus a critical threshold (the worst possible value, shown as V_{min} in Table 3). The value of the maximum and minimum intervals ranges from 0 (the worst value) to 100 (the best value). The following formulas were used for that purpose:

Maximization of the indicator's value

$$LS = \left(\frac{(X - V_{min})}{(V_{max} - V_{min})} \right) * 100$$

Minimization of the value

$$NS = \left(\frac{(V_{max} - X)}{(V_{max} - V_{min})} \right) * 100$$

where: LS is the level of sustainability; V_{min} is the critical threshold; V_{max} is the optimum, and X is the indicator value to be standardized.

The reference values of the indicators of agricultural production cost, external labor cost, and GYP were calculated considering a 20% decrease or increase in relation to the indicator value. In other words, regarding the agricultural production cost and the recommendations for the appropriate dosage application, the investment cost can diminish by 20% or, in the worst-case scenario, increase by 20%.

Regarding the environmental indicators, the two production systems have similar OM% and soil water retention capacity conditions (Figure 2). On the one hand, S1 producers have a greater diversity of cattle, sheep, poultry, and others, because animals represent an investment in case of economic need. On the other hand, S2 producers specialize in cattle

Table 3. Indicators, optimal values, and results of the sustainability assessment with previously standardized values.

Indicator	System production		Reference value	
	Seed native (S1)	Improved seed (S2)	V_{min}	V_{max}
Soil organic matter	56.5	61.7	3	4
Soil's ability to retain water	39.1	64.4	9	12
Cattle diversity	72.6	53.8	0	1
Happiness level of local agents	80.2	79.4	0	100
Adaptability actions to Climate change	37.7	70.8	0	4
Time dedicated to farming activities, women	85.4	60.0	0	100
Time dedicated to farming activities, men	42.7	43.8	0	100
Cost of agricultural production	89.8	23.5	16,449	56,580
Diversification of economic income	21.9	66.7	1	4
Investment in external labor	18.2	97.6	840	9,683
Potential grain yield (PYG)	10.3	76.5	2,819	9,656

for the commercialization of carcasses, milk, and by-products (greater number of livestock, less variety of species).

In the social indicators, inhabitants of the area point out that phenomena such as droughts and frosts have increased, both in frequency and intensity, in the last 10-15 years; therefore, some measures have been taken to reduce the negative effects. Almost all the farmers interviewed (28 out of 30) have built ditches to contain rainwater for livestock and also to use it as auxiliary source of irrigation for crops. Additionally, they have built cisterns and/or bought plastic tanks to store water for domestic use. S2 system has taken

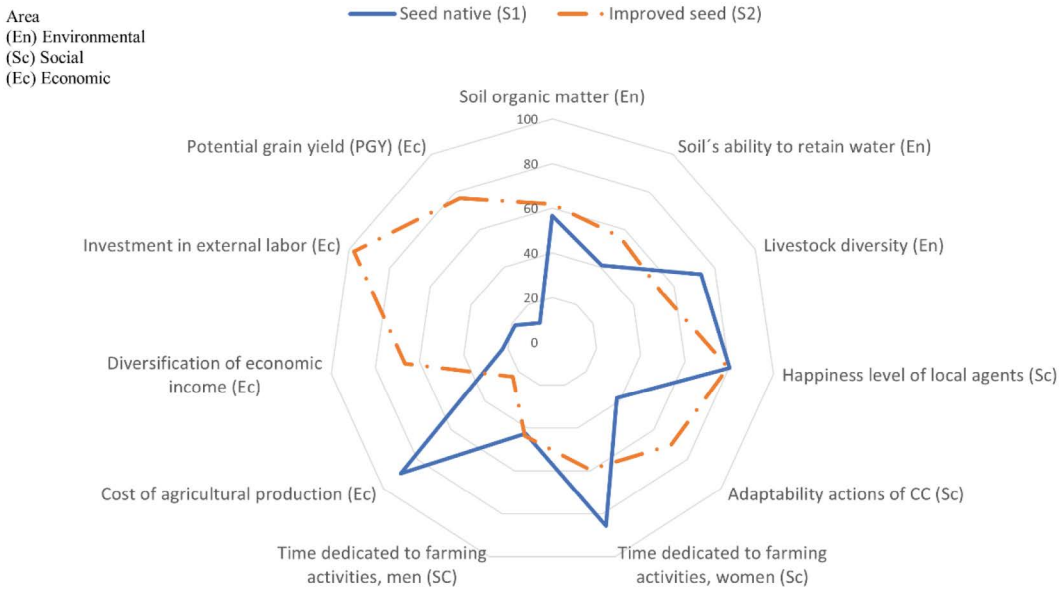

Figure 2. Sustainability evaluation of two agricultural production systems in El Saltillo, Jilotepec, State of Mexico.

more actions to adapt to FC: three producers of this group implemented agricultural drip irrigation systems that take advantage of and make efficient use of the rainwater contained in the watering systems. They have also implemented closed corrals with hermetic roof and floor that allow them to maintain the interior temperature for the benefit of the livestock.

Women play a key role in the operation of the systems. In the S1 system, they take care of the livestock and housework. When they are not studying, young women spend part of their afternoons doing housework and looking after the livestock. In the S2 system, women are hired to help with housework. Men work outside the house and only look after the fields during the weekends, causing a shortage of agricultural laborers, making it more expensive. Overall happiness levels are high for both systems. Aspects such as low noise levels, tranquility, freedom to do things their own way, low levels of violence and crime, and sufficient economic and natural resources, as well as nearby schools and sources of employment, constitute the basis of an overall feeling of a high-quality life, despite the roughness or complications of life in the countryside.

S1 system obtained high levels of sustainability related to agricultural production costs (lower economic investment), while S2 system had high costs in agricultural inputs. There is high dependence on external agricultural inputs. Both systems use agrochemicals such as urea, ammonium sulfate, diammonium phosphate (DAP) and herbicides. Nevertheless, there is evidence that reducing the use of agrochemicals and replacing them with organic fertilizers and sustainable agroecological practices is necessary to produce positive effects on soil conservation and fertility.

The use of technology and agrochemicals results in high maize yields and grain production, but it also increases production costs. Sangermán-Jarquín *et al.* (2009) point out that the use of technologies is an essential factor for greater profitability; however, there is a trend towards the increase of production costs as more developed technology is used and towards a decrease in costs when less developed technologies are used. Consequently, systems of group S2 are not very sustainable in terms of agricultural production costs, given the strong economic investment involved.

In the community, grain is the most important part of the maize crop since it is used for human consumption and also as part of the feed for livestock. Grain is the only form in which farmers commercialize maize; therefore, having high or acceptable maize grain yields guarantees, to a certain point, food self-sufficiency. However, it is also an indicator of soil health or fertility. The grain yield potential indicator was lower than average for all systems, especially for the native maize system. On the one hand, 2022 was a dry year and the delay in rainfall caused five native maize producers to lose their whole harvest and, on the other hand, during the field work stage, the rest of the producers mentioned that the drought "had an impact on maize production, it was a bad year." S2 system has a higher grain yield potential, probably due to the type of maize (improved maize) and the number of agrochemicals used, in addition to the drip irrigation system implemented by some farmers. S1 system turned out to be not very sustainable, as a result of the need to hire more agricultural laborers; although family work is a core part of this system, the work itself is not mechanized enough. On the contrary, S2 system turned out to be more sustainable because they hire fewer laborers and almost all their work is mechanized.

CONCLUSIONS

In the two assessed production systems, aspects such as low sustainability (*e.g.*, high production costs, high dependence on external inputs, high dependence on external labor, and vulnerability to climatic conditions) stand out; although other aspects (*e.g.*, high soil fertility, high perception of happiness and quality of life, and high diversity of livestock) are also emphasized.

Some agricultural practices that involve the use of agrochemical, such as herbicides and fertilizers, clearly degrade the soil, water, and natural capital; therefore, it is necessary to implement agroecological practices that are not only more environmentally friendly, but also encourage increased agricultural yields. As part of the initial agreements with producers, they were given the results of the soil fertility analyses. Two types of recommendations were made regarding the application of fertilizer or herbicide doses. The aim was to establish the necessary doses and to reduce agricultural production costs and the environmental impact.

S1 production systems are highly vulnerable, because the investment in labor, money, and effort is not proportional to the production obtained. Native maize sowing is a cultural issue that can be abandoned or changed for a more profitable activity.

Identifying climate change indicators will allow the creation of future scenarios, the implementation of adaptability actions, and the exploitation of changes in an effective and concrete manner that favors the conservation of native maize.

By assessing different elements present in a production system (such as happiness, soil fertility, grain yields, livestock diversity, perception and adaptability to climate change, time dedicated to farming by women and men), as well as production expenses, this study is the result of the integrated analysis of several methodologies used to measure sustainability indicators.

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Climatic variables that favor the Black Sigatoka (*Mycosphaerella fijiensis* Morelet) [anamorph: *Pseudocercospora fijiensis* (Morelet) Deighton] infestation in a banana-growing zone

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ABSTRACT

Objective: To establish the favorable or unfavorable climatic conditions for the emergence and development of Black Sigatoka in a banana-growing area within the influence zone of the Teapa weather station (27004) in Tabasco, Mexico.

Design/Methodology/Approach: We analyzed temperature data for n=59 years (1961-2019) at the Teapa weather station (27044) in Tabasco, as reported by IMTA (2009) and the Servicio Meteorológico Nacional (until 2019). Relative humidity was calculated using the equation developed by Allen *et al.* (2006). We also established the favorable or unfavorable climatic conditions for the development of Black Sigatoka in Teapa by resorting to the favorability typology posited by Júnior *et al.* (2008).

Results: There are no highly favorable climatic conditions for the incidence and development of this disease. Overall, spring and summer are the less favorable months, while fall and winter offer more favorable conditions.

Study Limitations/Implications: This study should be replicated in other banana-growing areas of Tabasco, since both temperature and relative humidity may differ and, consequently, the frequency of the disease may vary.

Findings/Conclusions: October and March are the most favorable months for Black Sigatoka occurrence. Therefore, comprehensive management and control programs should be designed for this period.

Keywords: Temperature, Relative Humidity, Banana, Yields, Probability, Prediction Models.

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INTRODUCTION

Black Sigatoka (*Mycosphaerella fijiensis* Morelet [anamorph: *Pseudocercospora fijiensis* (Morelet) Deighton]) is an infectious disease that attacks banana and plantain leaves worldwide. The reproduction of this ascomycete fungus can be sexual or asexual (Soares *et al.*, 2021). Black Sigatoka is considered the most harmful and costly of banana diseases, with its control taking up nearly 27% of production costs (Júnior *et al.*, 2008). This disease can cause a 25-100% yield reduction, if no methods and appropriate agronomic practices are in place to control its effects (Nfor *et al.*, 2011).

The climatic variables that favor the emergence, incidence, and severity of the Black Sigatoka attack are mainly temperature, relative air humidity, precipitation, and the time that leaves remain wet; the maximum effect has been recorded during the first symptoms (Álvarez *et al.*, 2013; Khan *et al.*, 2015). The optimal temperature range for the development of the disease is 25 °C to 28 °C (Bebber, 2019; Orozco *et al.*, 2008). Relative humidity levels of 92% or higher are a predictor of disease outbreaks (Khan *et al.*, 2015). Jacome and Schuh (1992) report that ≥92% relative humidity favors conidia infection, whether the leaves are wet or not. However, ascospore infections do require wet leaves (Júnior *et al.*, 2008).

To categorize the climatic conditions that favor the incidence and development of Black Sigatoka in any given locality or region, Júnior *et al.* (2008) proposed a classification based on temperature and relative humidity. Based on the abovementioned background, the occurrence of Black Sigatoka in the state of Tabasco must be researched. Consequently, the duration of climatic conditions (*i.e.*, temperature and relative humidity) that favor the occurrence of the Black Sigatoka infection was estimated. The most crucial variables for the disease model are precipitation, relative humidity, and temperature (Bombelli *et al.*, 2013). Improving the quality of weather and climate forecasting can help to estimate the probability of disease occurrence in plants and to predict the emergence or absence of severe epidemics.

MATERIALS AND METHODS

Climatological information and data management

The daily average maximum and minimum temperature data (T_{max} and T_{min}) were retrieved from the ERIC III database developed in 2009 by the Instituto Mexicano de Tecnología del Agua (IMTA). The information covered n=59 years (1961-2019) of data gathered at the Teapa weather station (27044), Tabasco, Mexico. We complemented this information with data up to 2019 recorded by the Servicio Meteorológico Nacional (SMN, 2021). The Teapa weather station is located at 92° 57' 12" W and 17° 32' 56" N. The average annual temperature in the zone ranges from 24 °C to 26 °C, while the total annual precipitation fluctuates between 2,000 and 4,000 mm (Zavala-Cruz *et al.*, 2016). The resulting data were transcribed into an Excel sheet for accessibility. The average daily temperature (T_{med}) was obtained using the following equation (1):

$$T_{med} = \frac{T_{\max} + T_{\min}}{2} \quad (1)$$

The average monthly temperature was subsequently estimated. Consequently, we had 12 average monthly values (T_{med}) for each one of the 59 registered years (708 month data). Finally, an annual average was determined for all 59 years. The same procedure was followed for the relative humidity data.

Estimating relative humidity (HR)

Since neither Eric III nor the Servicio Meteorológico Nacional provide HR data, its percentage was estimated based on the ratio of the actual partial pressure of water vapor

to the partial pressure of water vapor at saturation, using the equation proposed by Allen *et al.* (2006):

$$HR = \left(\frac{e_a}{e_s}\right) * 100 \tag{2}$$

Where *HR* is the relative humidity (%) and "e_a" is the actual partial pressure of water vapor (kPa). We used 348,192 data captured every 10 minutes in 13 automatic weather stations distributed throughout the Tabasco Plain. The following equation (3) was used to carry out these calculations:

$$e_a = \left(\frac{HR}{100}\right) * e_s \tag{3}$$

Where "e_s" is the partial pressure of water vapor at saturation (kPa), obtained from temperature data using the equation proposed by Allen *et al.* (2006):

$$e_s = 0.61078 * \exp\left[\frac{\left(17.269 * T_{med}\right)}{\left(T_{med} + 237.3\right)}\right] \tag{4}$$

A daily average was obtained with the values of "e_a". Subsequently, a regression analysis was carried out between these values and the daily average temperature to find an equation or functional relation to estimate "e_a" based only on temperature data for all existing weather stations in Tabasco. The result was equation (5), which had a coefficient of determination in the validation phase (R^2) of 0.852.

$$e_a = 4.646 - \left(\frac{45.15}{T_{med}}\right) \tag{5}$$

Once "e_a" and "e_s" were established, the daily, monthly, and annual average relative humidity (*HR*) was calculated using equation (2).

Typology of climatic favorability for Black Sigatoka

The different types of climatic favorability for the development of Black Sigatoka were determined for each month of the year using monthly average temperature and relative humidity data. This categorization draws on the climatic favorability types proposed by Júnior *et al.* (2008) (Table 1).

Determining favorability types

Based on the monthly average temperature and relative humidity data recorded each year at the Teapa weather station, a typology of climatic favorability was developed for each

Table 1. Types of favorability for Black Sigatoka development, according to temperature and relative humidity intervals (Júnior *et al.*, 2008).

Favoring class	Description	Temperature ranges (°C)	Relative humidity ranges (%)
1	Highly Favorable	25 a 28	>90
2	Favorable	25 a 28	80 a 90
3	Relatively Favorable	20 a 25 o 28 a 35	>80
4	Poor	20 a 35	70 a 80
5*	Unfavorable	<20 a >35	<70

(*) Favorability type 5 occurs with a <70% relative humidity in any temperature interval.

of the 708 months of the 1961-2019 period, as well as the monthly average (59 data per month) and the total annual average. The typology of favorability (based on temperature and relative humidity) was determined using the limits shown in Table 1. Subsequently, the relative frequency for each month was determined to estimate the probability of each of the five favorability types and their corresponding return period.

Relative frequency analysis

The division of the number of occurrences in a specific period (Table 1) by the historical record was the basis of the analysis of the relative frequencies of temperature and relative humidity (separately or combined). This is how we determined the relative frequency for each of the five types of favorability and each month of the 59 years recorded.

RESULTS AND DISCUSSION

Isolated effect of temperature on the development of Black Sigatoka

None of the 708 monthly average temperature values analyzed met the criteria for the Unfavorable category. In 72.3% of the period under observation, the temperature ranged from Relatively Favorable to Highly Favorable for the development of Black Sigatoka. Temperatures in September and October were Highly Favorable for the manifestation of Black Sigatoka disease, since the values in these months fluctuate between 25 °C and 28 °C. Only 27.7% of the months analyzed were classified as Slightly Unfavorable. In conclusion, the thermal conditions in three out of every four years are conducive to the development of the disease in the influence zone of the Teapa weather station in Tabasco.

Isolated effect of relative humidity on the development of Black Sigatoka

The average relative humidity during the 59 years studied was high (78.8%). No ≥90% HR values were recorded in any month of the analyzed period. Therefore, the Highly Favorable conditions did not occur at the monthly average level. However, 98% of the analyzed months recorded relative humidity values greater than 70%. Consequently, <70% relative humidity occurred only in 2% of the period under observation. Therefore, Unfavorable conditions for the development of Black Sigatoka would take place once every 50 to 51 years. This is a very unlikely phenomenon. Slightly Unfavorable conditions were recorded 58.2% of the time (HR between 70% to 80%). In contrast, Relatively Favorable

to Favorable conditions occurred only 39.8% of the time (HR >80%). If we consider HR alone, Slightly Unfavorable to Unfavorable conditions predominate at the Teapa weather station (27044) in Tabasco, Mexico, most of the time. Hence, these conditions will be present in six out of every ten years.

Combined effect of total average temperature and relative humidity

Using a single average temperature and relative humidity value for the 59 years of recorded data (Table 1), the overall climatic conditions were determined to be Relatively Favorable for the emergence of the Black Sigatoka disease in bananas, in the influence zone of the Teapa weather station (27044).

Combined effect of monthly average temperature and relative humidity on the development of Black Sigatoka for the whole analyzed period

Table 2 shows the relative time of each type of climatic favorability, during each of the 708 months of the 1961-2019 period. This Table shows that there were no Highly Favorable conditions for the development of Black Sigatoka during the whole period. On the one hand, the Table also shows that, most of the time (56.9%), the conditions were Slightly Unfavorable for the development of the disease, with a 2-year return period. On the other hand, Relatively Favorable conditions prevailed during 28% of the time analyzed (*i.e.*, a 4-year return period). Meanwhile, Favorable conditions have an 8-year return period (13.1 %). Finally, Unfavorable conditions had a 50- to 51-year return period (2%).

Table 2 shows the relative frequency and return period for the various climatic conditions recorded in the 708 months under study. It also indicates the frequency with which they can occur in any of the 12 months of the year. In nearly 60% of the period under analysis, the climatic conditions ranged from Slightly Unfavorable to Unfavorable for the development of Black Sigatoka. Based on a monthly analysis, Highly Favorable conditions do not occur, while Unfavorable ones occur once every 51 years.

Figure 1 contains the results of temperature and relative humidity analyses for each of the 708 months; these results determine the type of climatic favorability and its relative frequency. On the one hand, the climatic conditions in January are 100% Relatively Favorable (Type 3, the prevailing type), although they do not reach 100% conditions during November, December, and February. On the other hand, July, August, and September show 100% Slightly Unfavorable climatic conditions (Type 4), with a certain reduction

Table 2. Relative time of occurrence for different types of climatic favorability and their corresponding return periods, from 1961 to 2019, in the influence zone of the Teapa weather station (27044) in Tabasco, Mexico.

Climatic condition	Time analyzed (%) (1961 - 2019)	Return period (years)
Favorable	13.1	8
Relatively Favorable	28.0	4
Poor	56.9	2
Unfavorable	2.0	51

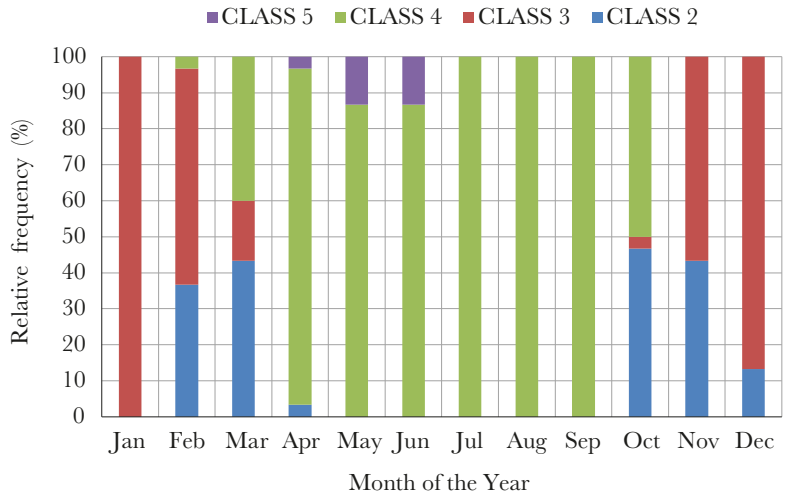

Figure 1. Relative frequency of climatic favorability types for the development of Black Sigatoka for all months of the year in the influence zone of the Teapa weather station (27044) in Tabasco, Mexico.

during April, May, June, and October, while still remaining prevalent. Likewise, Favorable conditions are not fully met (100%) in any month of the year, with >40% values only in October, November, and March.

Figure 2 shows the four types of favorability for Black Sigatoka development in the Teapa banana-growing area, divided into favorable and unfavorable conditions. From April to September (spring-summer), Slightly Unfavorable to Unfavorable conditions for the development of Black Sigatoka prevailed —with values that fluctuate between 96.6% and 100%. Meanwhile, from November to March (autumn-winter), the prevailing conditions range from Relatively Favorable to Favorable (100% in November, December, and January). During these three months, continuous sampling must be conducted to gain a more effective control over Black Sigatoka in the influence zone of the Teapa weather station (27044) in Tabasco, Mexico.

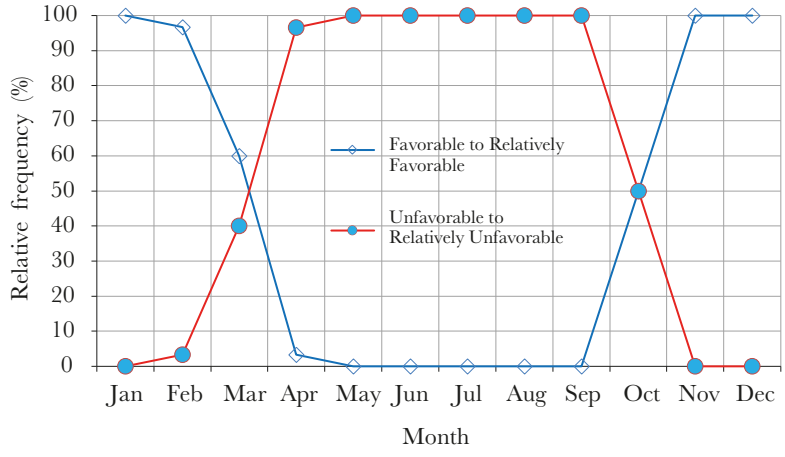

Figure 2. Favorable climatic conditions for the development of Black Sigatoka, for all months of the year, in the influence zone of the Teapa weather station (27044) in Tabasco, Mexico.

Combined effect of monthly average temperature and relative humidity on the development of Black Sigatoka

Table 3 shows the monthly average favorability types for the influence zone of the Teapa weather station (27044) in Tabasco, Mexico.

The monthly average values in Table 3 do not include Highly Favorable and Unfavorable types for the development of Black Sigatoka. From April to September, climatic conditions are Slightly Unfavorable for the development of the disease. These results match those obtained in the analysis of the whole period (708 months). Favorable climatic conditions occur in October, November, and March. Khan *et al.* (2015) reported similar results in four banana-growing areas in Bangladesh, where the highest incidence and severity of Black Sigatoka are recorded in October. These authors found a high correlation between the incidence and severity of the disease and the precipitation and temperature which are influenced by relative humidity.

Table 3. Monthly average temperature and relative humidity values defining the types of climatic favorability for Black Sigatoka infestation in the influence zone of the Teapa weather station (27044) in Tabasco, Mexico, from 1961 to 2019.

Month	T_{max}	T_{min}	T_{med}	*RH*	Favoring class	Description
January	26.6	18.0	22.3	86.9	3	Relatively Favorable
February	28.2	18.5	23.4	85.3	3	Relatively Favorable
March	31.3	20.0	25.7	81.2	2	Favorable
April	33.7	21.8	27.8	76.8	4	Poor
May	34.9	23.0	29.0	74.0	4	Poor
June	34.0	23.0	28.5	75.1	4	Poor
July	33.5	22.4	28.0	76.4	4	Poor
August	33.3	22.5	27.9	76.5	4	Poor
September	32.2	22.6	27.4	77.6	4	Poor
October	30.3	21.7	26.0	80.5	2	Favorable
November	28.8	20.1	24.5	83.4	2	Favorable
December	27.1	18.6	22.9	86.1	3	Relatively Favorable

T_{max}: maximum temperature; T_{min}: minimum temperature; T_{med}: average temperature; *RH*: relative humidity.

CONCLUSIONS

In the analyzed period (1961-2019), the climatic conditions were Relatively Favorable for the manifestation of the Black Sigatoka disease. No Highly Favorable climatic conditions for the emergence of Black Sigatoka occurred in the zone of influence of the Teapa weather station. The methodology and results of this work should be incorporated into a web system, using hourly or daily data to issue an early real-time warning about the risk of Black Sigatoka in the banana-growing region of Teapa, Tabasco.

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Colegio de Postgraduados

Aromatic amination of refined rice bran oil previously epoxidized with Novozym 435

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ABSTRACT

Objective: To aromatically aminate refined rice bran oil (RBO), a by-product of the rice agro-industry, through a chemical-enzymatic epoxidation based on Novozym 435 and p-xylylenediamine insertion.

Design/Methodology/Approach: Refined RBO was epoxidized with H_2O_2/Novozym 435. The resulting epoxidized rice bran oil (eRBO) was functionalized with the p-xylylenediamine aromatic diamine via epoxy ring-opening (X-eRBO), using $ZnCl_2$ as catalyst. Iodine value (IV), saponification value (SV), and oxirane oxygen content (OOC) were determined to evaluate structural changes in oils. The RBO, the eRBO, and the X-eRBO were identified using FTIR, ^{1}H, and ^{13}C NMR.

Results: The IV, the SV, and the OOC suggest that the synthesis of eRBO and X-eRBO were effective. The increase of molecular weight in eRBO point to the formation of ~6 epoxy rings per original triglyceride. The OOC value of X-eRBO was 22% lower than the OOC value of eRBO, implying that an effective aromatic amination was achieved. The FTIR, ^{1}H and ^{13}C NMR spectroscopy analysis confirmed the epoxidation and amination of the RBO.

Study Limitations/Implications: X-eRBO may be a feasible precursor for value-added products, such as crosslinked polymers or corrosion inhibitors.

Findings/Conclusions: Refined rice bran oil was aromatically aminated after two stages under mild thermal conditions. This result was achieved with an epoxidation sequence with H_2O_2/Novozym 435, followed by functionalization with p-xylylenediamine.

Keywords: Epoxidation, amination, use of agro-industrial residues.

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INTRODUCTION

Biorefining is the set of processes that allow chemical products —such as biofuels and high value-added products— to be obtained from biomass. Biorefining raw material includes oilseed crops, domestic or industrial oily residues, and algae, as well as forestry, agricultural, and agro-industrial residues (Ng *et al.*, 2014). Rice (*Oryzae sative* L.) bran is

an agro-industrial by-product, which is extracted from the film that divides the grain and its husk during polishing (Bhosale and Vijayalakshmi, 2015). Its high oleic content (Zúñiga-Diaz *et al.*, 2017) make rice bran a potential source of oil, suitable for human consumption, and a precursor for biodiesel production (Zuñiga-Díaz *et al.*, 2018). To date, the exploitation of rice bran oil (RBO) is limited, particularly in the production of fine chemical products. Raw RBO contains di- and triacylglycerols, phospholipids, waxes, and non-saponifiable lipids, among other components (Garba *et al.*, 2017). Raw RBO that is purified until it reaches a high triglyceride composition is called refined RBO. RBO triglycerides are characterized by C=C unsaturations, which enables the transformation of this oil into value-added chemical products. This transformation can be achieved inserting functional organic groups that gradually provide the physical and chemical properties that are required for products of the polymer, pharmaceutical, and cosmetic industries, among others. To achieve the functionalization of oils, oxirane or epoxy rings must be first formed by inserting an oxygen atom in the unsaturations of the triglycerides —a process known as epoxidation. The subsequent ring-opening enables the functionalization of the oil, forming monomers for subsequent reactions (Pascault and Williams, 2009). Amination is the functionalization that involves the different types of amines; consequently, it is expected to produce precursors which can be tuned to obtain new electrical, rigidity, and adhesiveness properties (Kalita and Karak, 2013). This work reports that rice bran oil can be feasibly functionalized with aromatic amine, using a previous enzymatic epoxidation process based on Novozym 435 lipase. The resulting precursor can be considered for the synthesis of polyurethanes and corrosion inhibitors.

MATERIALS AND METHODS

Reagents. Chemical reagents for analytical grade were obtained from Sigma Aldrich. The refined RBO was obtained from commercial sources.

Physicochemical characterization of the oils

The determinations of IV (Firestone, 1998a), SV (Firestone, 1998b), and OOC (ASTM, 2004) were carried out in triplicate for the various RBO, eRBO, and X-eRBO samples, following international regulations. The IV refers to the amount of iodine that is absorbed per 100 g of the substance under study and it determines the unsaturation level of the C=C bonds of fatty acids. On the other hand, the SV refers to the number of milligrams of KOH required to saponify 1 g of oil and it is a measure of the average molecular weight of a sample. Finally, OOC refers to the amount of oxirane oxygen that is inserted into C=C unsaturations during the epoxidation. Therefore, OOC is a direct measure of the amount of epoxy groups present.

Rice bran oil epoxidation

The epoxidation of RBO was carried out using Novozym 435 lipase as catalyst (Ortiz *et al.*, 2019). In order to synthesize eRBO, 12.0 g RBO, 0.98 g oleic acid, 2.5 g catalyst, and 52.84 g toluene (as a solvent) were mixed for 2 hours at 49 °C. Next,

62.77 g H_2O_2 (30%) were added drop by drop. Subsequently, the system was allowed to react for 24 hours with a gentle mechanical stirring. The catalyst was separated from the resulting mixture using vacuum filtration. During the next stage of the process, the catalyst was repeatedly rinsed with toluene and left to dry at room temperature to be used again. The filtered mixture was then washed with distilled water. An organic layer was extracted and mixed for ~1 hour with 10:1 anhydrous Na_2SO_4 (mixture: Na_2SO_4) to eliminate moisture and unreacted H_2O_2. Finally, the solids were removed by vacuum filtration and the toluene or water was extracted with a rotary evaporator (Petrović *et al.*, 2002).

eRBO aromatic amination

The reaction conditions to achieve amination were temperature-molar ratio of the eRBO to the amount of catalyst ($ZnCl_2$), and a fixed time of 3 hours. The reaction temperatures were 70, 77, and 85 °C, while the eRBO:$ZnCl_2$ molar ratios were 0.84, 0.92, and 1.00, resulting in 9 tests. In each one, 1.02 mmol eRBO was mixed with 2.00 mmol p-xylylenediamine and an amount of $ZnCl_2$ depending on the molar ratio. Once the reaction was completed, the product was cooled at room temperature, dissolved in ethyl acetate, and transferred to a separatory funnel. To remove $ZnCl_2$ and unreacted amine, nine successive washes were immediately carried out with 50 mL of solutions (two washes with a pH=4 acid solution, two washes with a saturated solution of $NaHCO_3$, two washes with a pH=13 basic solution, and three washes with distilled water). Finally, the organic layer was recovered and kept in a vacuum desiccator for 24 hours at 30 °C (Lopez-Tellez *et al.*, 2008).

Infrared spectroscopy and nuclear magnetic resonance

The oil samples were subjected to an infrared spectroscopy (FTIR) analysis using a PerkinElmer® Spectrum™ One FTIR-ATR spectrometer with a 4,000 to 400 cm^{-1} radiation source. Nuclear magnetic resonance (NMR) analysis was achieved with a Bruker Avance™ III HD 400 MHz NMR instrument that uses $CDCl_3$ as solvent. ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively.

RESULTS AND DISCUSSION

Physicochemical characterization: IV, SV, MW, and OOC

Table 1 shows the determinations of IV, SV, and OOC for the RBO and eRBO. The corresponding molecular weights (MW) obtained from the SV are also included.

Table 1. IV, SV, MW, and OOC of the refined RBO and eRBO.

Sample	IV	SV	MW	OOC
	(g I_2/100g)	(mg KOH/g)	(g/gmol)	
RBO	90.40±0.67	191.58±3.92	878.5	0.07±0.02
eRBO	11.61±0.12	171.90±4.62	979.14	5.60±0.65

The average IV for the refined RBO samples was estimated at 90.40±0.67, fully matching previously reported values for RBOs of different varieties and regions: 90 (Gupta *et al.*, 2016), 91.38 (Zúñiga-Diaz *et al.*, 2017), and 95.40 (Hanmoungjai *et al.*, 2000). After epoxidation, the IV was reduced to ∼12% (11.61±0.12). This decrease suggests a significant loss of C=C unsaturations, as a consequence of enzymatic epoxidation based on H_2O_2/Novozym 435. Previously, Gupta *et al.* (2016), using entirely chemical and not enzymatic strategies, reported an IV of raw eRBO of ∼32.2, showing that the process based on Novozym 435 is more efficient. The average SV for the refined RBO of this study was 191.58±3.92, which was also consistent with previous reports: 182.35 (Zúñiga-Diaz *et al.*, 2017) and 178.18 (Hanmoungjai *et al.*, 2000). Based on this SV, the molecular weight of the refined RBO was estimated at 878.50 g/gmol, which is lower than other estimates of raw RBO (923.36 g/gmol) (Zúñiga-Diaz *et al.*, 2017). This effect is possibly associated with the absence of the components removed during the refining of the RBO. The SV of eRBO (171.90±4.62) allowed the calculation of a molecular weight of 979.14 g/gmol. The difference in molecular weights between eRBO and RBO suggests that each triglyceride was epoxidized by the insertion of an average of 6 oxygen atoms and consequently formed epoxy rings; this proof matches the significant decrease of IV in eRBO. Finally, the evolution of the OOC enabled a direct monitoring of the appearance or disappearance of epoxy groups. The OOC in refined RBO was 0.07±0.02, confirming an absence of epoxy or oxirane rings in the initial sample. Gupta *et al.* (2016) have reported a 0.68 OOC of raw RBO, with which the determination of this study can be compared. Once the epoxidation was completed, the OOC in eRBO increased 8.2 times (5.60±0.65) compared to the OOC of the refined RBO. A similar increase (4.7 times) has been reported in raw RBO using chemical epoxidation (Gupta *et al.*, 2016); once more, this result implies that Novozym 435 lipase improves the performance of an epoxidation. The greatest OOC reduction (to 1.28) was recorded after functionalization, with p-xylylenediamine at 70 °C and an eRBO:$ZnCl_2$ molar ratio of 0.92. These were the best conditions for partial aromatic amination, at the fixed reaction time. The OOC reduction is the result of the destruction of epoxy rings by the insertion of the aromatic amino group.

Infrared spectroscopy analysis

Figure 1 shows the FTIR spectra of refined RBO (black line), epoxidized RBO (blue line), and RBO functionalized with p-xylylenediamine (yellow line). The FTIR spectrum of the refined RBO is consistent with previous works (Silverstein and Bassler, 1962; Zúñiga-Diaz *et al.*, 2017). In contrast, the FTIR spectrum of eRBO shows a band in the 842-824 cm^{-1} region, associated with the oxirane group (Petrović *et al.*, 2002). This band can still be observed after functionalization with the aromatic amine. The FTIR spectrum of X-eRBO shows the band of the epoxy group, as well as the appearance of strong bands at 1639 and 1550 cm^{-1}, assigned to secondary and primary amine groups, respectively (Lopez Tellez *et al.*, 2008).

Another proof of the epoxy ring-opening and functionalization is the 3,613-3,149 cm^{-1} band which is related to the vibration of the OH group, which occurs simultaneously when

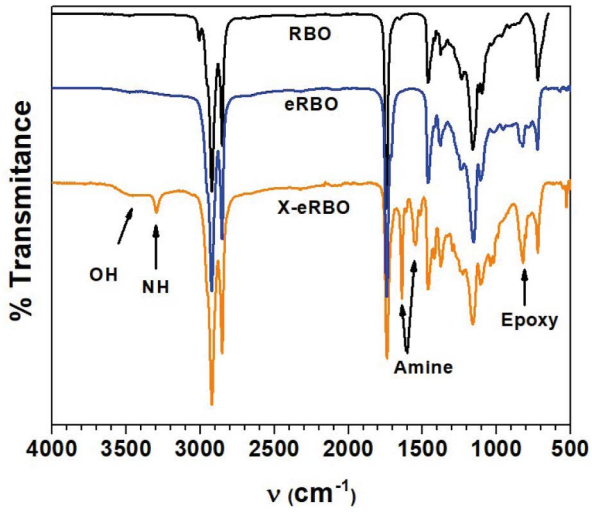

Figure 1. FTIR spectra of refined RBO (black line), eRBO (blue line), and X-eRBO (yellow line).

p-xylylenediamine is inserted into the triglyceride structure. The band centered at 3,294 cm^{-1} proves the primary N-H vibration of unreacted p-xylylenediamine.

^{1}H and ^{13}C NMR analyses of X-eRBO functionalized oil

Figure 2a shows the ^{1}H NMR spectrum for X-eRBO. The observed signals enable the identification and proposal of a generic structure (see box in the figure). The signals found in this work (indicated in parentheses) are consistent with previous reports (Lopez Tellez et al., 2008). The conserved oxirane rings are identified by three signals: protons –CH– of the epoxy group (Ha) at 2.9 ppm, –CH– adjacent to epoxy groups (Hb) at 1.45 ppm, and –CH– adjacent to two epoxy groups (Hc) at 3.1 ppm. The signals that confirm the functionalization of X-eRBO correspond to displacements in $–CH_2–NH–$ and $–CH_2–NH_2Ph$ protons (3.6 ppm) associated with methylene protons (Hd, Hg, at 3.9 ppm), and aromatic protons (He, Hf, at 7.2 ppm). After the oxirane ring-opening, an OH group (Hh, at 2.75 ppm) and a secondary amine (Hi, at 3.65 ppm) were formed.

Figure 2b shows the ^{13}C NMR spectrum of X-eRBO. Considering that the X-eRBO sample remains epoxidized and comparing it with a ^{13}C NMR spectrum of epoxidized canola oil (Madankar *et al.*, 2013), it is easy to see that both are practically identical, which confirms that X-eRBO remains partially epoxidized. The 53-60 ppm signals are associated with the carbon of the epoxy group, while those between 68 and 62 ppm come from the carbon resonance of glycerol in the α and β carbon atoms, respectively. The 128-ppm signal confirms the amination of RBO, since it matches the carbons of p-xylylenediamine (SpectraBase, 2021).

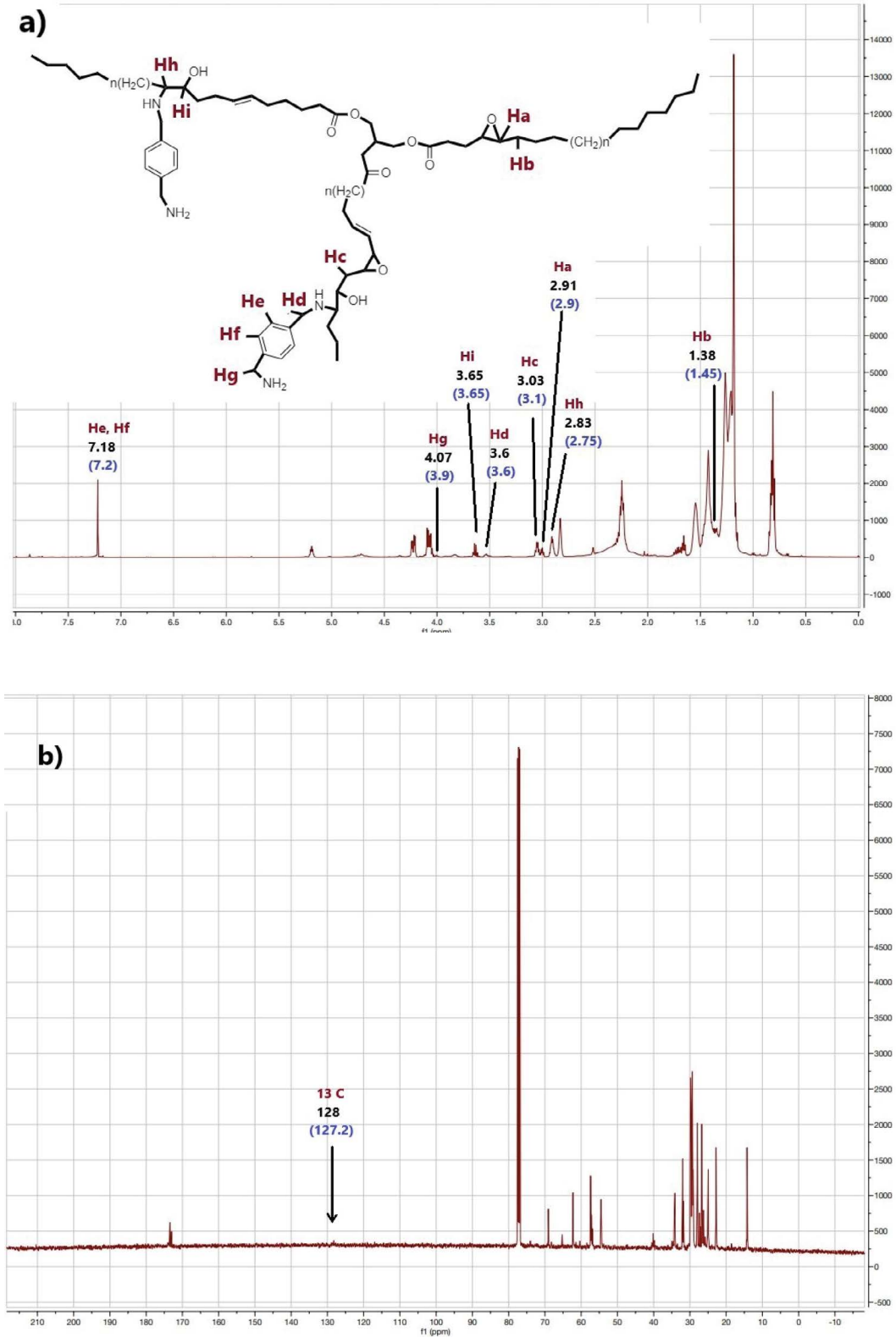

Figure 2. a: ^{1}H and b) ^{13}C NMR analyses of rice bran oil partially aminated with p-xylylenediamine (X-eRBO). b: ^{13}C NMR spectrum of X-eRBO.

CONCLUSIONS

Rice bran oil, a by-product of the rice industry, can be functionalized with the p-xylylenediamine aromatic diamine. The prior epoxidation of this oil using Novozym 435 lipase results in a more efficient strategy for the insertion of oxirane groups than with previously reported procedures. The most efficient aromatic amination occurred under conditions of 70 °C and an eRBO:$XnCl_2$ molar ratio of 0.92. The amination with the p-xylylenediamine resulting from the RBO was partial and confirmed by FTIR, ^{1}H, and ^{13}C NMR analyses.

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Colegio de Postgraduados

Rice bran oil biorefining: functionalization with acrylate

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ABSTRACT

Objective: To obtain acrylated refined rice bran oil (RBO) using a combined functionalization: first, epoxidation with H_2O_2/Novozym 435 lipase, followed by acrylate group insertion.

Design/Methodology/Approach: After being epoxidized with H_2O_2/Novozym 435, the refined rice bran oil was acrylated via epoxy ring-opening, using triethanolamine as catalyst and 4-methoxyphenol as inhibitor. The experimental conditions of temperature (T=100 and 110 °C) and reaction time (t=3 and 4 hours), as well as the ratio of g eRBO (epoxidized oil) to g acrylic acid (1.5 and 2.0) were considered for the functionalization. The functionalizations were monitored using iodine value (IV), saponification value (SV), and oxirane oxygen content (OOC), Fourier transform infrared (FTIR), and nuclear magnetic resonance (^{1}H NMR), which allowed the estimation of the %Acrylation.

Results: The ^{1}H NMR studies indicate that the acrylation of rice bran oil is efficient, which is confirmed with the evolution of IV, SV, and OOC. Using the OOC, the best acrylation condition was identified at T=110 °C, t=3 hours, and ratio of g eRBO to g acrylic acid=1.5, obtaining a %Acrylation of 85.89% via ^{1}H NMR.

Study Limitations/Implications: Partially acrylated rice bran oil may become an intermediate in the biorefining of this oil and be used in the synthesis of crosslinked polymers.

Findings/Conclusions: Refined rice bran oil was efficiently acrylated using two consecutive steps: it was initially epoxidized with H_2O_2/Novozym 435, followed by functionalization with acrylate group.

Keywords: Acrylation, Epoxidation, Biorefining.

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INTRODUCTION

Rice bran oil (RBO) is a by-product of the milling of rice (*Oryzae sative* L.) seeds. This bran has a 10-25 % oil content (Balat, 2011; Saunders, 1985), depending on the variety of rice, the geographical origin, and the extraction method (Most *et al.*, 2005). Once extracted, the oil is dark green and has a high phosphatide content and a large amount of free fatty acids. When the oil is refined, the phosphatides and free fatty acids are eliminated, increasing the triglyceride concentration. These molecules are esters of three fatty acids attached to a glycerol molecule, and may contain C=C unsaturations.

Thanks to these unsaturations, the oil can be transformed into high value-added chemical products, such as those used in the polymer, cosmetic, and food industries. For this purpose, the oils are modified through the addition of functional groups to the unsaturations, a process known as functionalization. Epoxidation is a functionalization in which a C-O-C epoxy group is formed by replacing the C=C double bond and it is usually employed as the initial step for the insertion of subsequent functional groups (Samuelsson *et al.*, 2004). Epoxidation is usually achieved through chemical or chemical-enzymatic processes; in both cases, a catalyst is required in the presence of an atomic oxygen donor (*e.g.*, H_2O_2). Chemical-enzymatic epoxidation has had a better performance than chemical epoxidation; *Candida antarctica* (Novozym 435) is a good option for this process. Meanwhile, acrylation has been studied in different vegetable oils, such as palm (KuShAiri and Choo, 2015; Salih *et al.*, 2015), soybean (Behera and Banthia, 2008; Chu *et al.*, 2014), and linseed or flaxseed (Rana and Evitts, 2015; Wuzella *et al.*, 2012). The typical acrylation strategy consists of the epoxy ring-opening, followed by the insertion of the acrylate group (CH_2=CHCOO).

MATERIALS AND METHODS

IV, SV, and OOC of oils

Using international standards, we determined the IV (Firestone, 1998a), the SV, and the molecular weight (MW) (Firestone, 1998b), as well as the OOC (Standard, 2017) of refined (commercially obtained), epoxidized (eRBO), and acrylated (A-eRBO) RBO. These determinations were carried out in triplicate. The IV measures the amount of C=C double bonds present in the oil under study, while the SV enables the inference of its approximate MW, and the OOC determines the percentage of oxirane oxygen that is added to the C=C bonds in a sample.

Epoxidation of rice bran oil (eRBO)

The epoxidation of RBO was carried out using Novozym 435 as a catalyst (Albarrán-Preza *et al.*, 2016), in the presence of H_2O_2. The optimal epoxidation conditions were: 0.1 mol of refined RBO, 1.1 mol of toluene, and 0.018 mol of oleic acid. Once this mixture was completely incorporated, 2.5 g of Novozym 435 were added; immediately, 56.6 mL of 30% H_2O_2 solution at 44 °C were added drop by drop, every 2-3 seconds. The reaction was maintained under these conditions for 24 hours. Once the process was completed, Novozym 435 was recovered, while the organic phase was extracted with a 5 % $NaHCO_3$ solution to remove the catalyst and free fatty acids. The organic phase was also recovered, washed with anhydrous $MgSO_4$ during one hour, and concentrated using a rotary evaporator (Petrović *et al.*, 2002).

eRBO acrylation

The eRBO acrylation was carried out under the following experimental conditions: temperature (T=100 and 110 °C) and reaction time (t=3 and 4 hours), as well as the ratio of g eRBO to g acrylic acid (1.5 and 2.0). For all tests, 1 g eRBO, 0.0025g 4-methoxyphenol, and 0.0025 g triethanolamine were used. Once these components were mixed, the system

was subjected to the established temperature and the acrylic acid (5 seconds/drop) was immediately incorporated. The mixture was maintained for the above mentioned time (Salih *et al.*, 2015).

Infrared spectroscopy and nuclear magnetic resonance

The samples obtained were analyzed by infrared spectroscopy (FTIR) and ^{1}H nuclear magnetic resonance (NMR). For this purpose, a Perkin Elmer Spectrum™ One FTIR-ATR spectrometer with a 4,000 to 400 cm^{-1} radiation source and a Bruker Avance™ III HD 400 MHz NMR equipment were used.

RESULTS AND DISCUSSION

IV, SV, MW, and OOC monitoring

Table 1 shows the determinations of the IV, SV, MW, and OOC for the RBO and eRBO. The average IV of the refined RBO samples was estimated at 90.63±1.36, matching the findings of previous reports: 90 (Gupta *et al.*, 2016), 91.38 (Zúñiga-Diaz *et al.*, 2017), and 95.40 (Hanmoungjai *et al.*, 2011). Similarly, a SV of 192.10±2.06 was obtained for RBO, based on a MW of 877.19 g/gmol and a OOC of 0.06±0.02; the latter suggests the absence in practice of epoxy groups. The average SV of refined RBO was slightly higher than the previously reported results —187.60 (Hanmoungjai *et al.*, 2001) and 182.35 (Zúñiga-Diaz *et al.*, 2017)— which implies a lower MW of the refined RBO. The IV, SV, MW, and OOC were also determined for the eRBO. The IV was reduced to 0.52±0.16, suggesting an almost complete disappearance of the C=C double bonds after epoxidation. Considering that the RBO is composed of 81.97% unsaturated fatty acids (oleic acid, linoleic acid, and linolenic acid (0.87%) (Most *et al.*, 2005), the RBO could be efficiently functionalized. The eRBO SV was estimated at 170.82±3.48, obtaining an eRBO MW of 985.61 g/gmol. The difference between the MW of the RBO and the eRBO proves an epoxidation with the insertion of 6-7 atomic oxygen atoms into the RBO.

The increase of OOC to 5.12±0.60 in eRBO confirmed that the insertion of the epoxy group was even more efficient than in previous reports. Therefore, the average OOC of the eRBO increased ~85 times with respect to the OOC of the refined RBO. In comparison, the traditional method based on H_2O_2/formic acid (Gupta *et al.*, 2016) only increases OOC by ~4.7 times after epoxidation. The OOC was also determined after the functionalizations with an acrylate group, for all the experimental conditions (temperature, reaction time, and ratio of g eRBO to g acrylic acid). The maximum OOC reduction (to 3.63±0.18) was found under the following conditions: T=110 °C, t=3 hours, and a ratio

Table 1. IV, MW, SV, and OOC of the refined RBO and eRBO.

Oil	IV	MW	SV	OOC
	(g I_2/100g)	(g/gmol)	(mg KOH/g)	
RBO	90.63±1.36	877.19	192.10±2.06	0.06±0.02
eRBO	0.52±0.16	985.61	170.82±3.5	5.12±0.60
A-eRBO	72.33±1.25	1162.79	145.86±3.00	3.63±0.18

of g eRBO to g acrylic acid of 1:1.5. These results imply that the greatest destruction of epoxy rings took place in these conditions due to the insertion of the acrylate group. The resulting sample is now called A-eRBO and its IV, SV, and OOC were determined (Table 1). The dramatic increase in the IV (to 72.33±1.25) in the A-eRBO sample suggests the insertion of the acrylate group (CH_2=CHCOO), given the new increase in the presence of C=C bonds. The SV was estimated at 145.86±2.93 and its MW at 1,162.79 g/gmol, indicating a partial acrylation of the eRBO. A simple analysis suggests that the ~16% increase in the MW of A-eRBO (with respect to the MW of eRBO) implies an insertion of 2-3 acrylate groups of the 6-7 epoxy groups available in eRBO.

Infrared spectroscopy analysis

RBO and eRBO samples were analyzed using infrared spectroscopy. Figure 1 shows the FTIR spectra for these oils. The RBO FTIR spectrum (Figure 1a) matches the findings of Rohman *et al.* (2011) and Zúñiga-Diaz *et al.* (2017); however, there is an absence of the weak signal at ~1711 cm^{-1}, which indicates the presence of free fatty acids. Such absence is attributed to the refinement of the RBO. Finally, the eRBO FTIR spectrum (Figure 1b) shows a strong signal (at 824 cm^{-1}), which is consistent with the presence of the epoxy group (Vlček and Petrović, 2006).

^{1}H NMR analysis of the A-eRBO functionalized oil

Figure 2 shows the ^{1}H NMR spectrum of partially acrylated rice bran oil (A-eRBO). A generic structure of the A-eRBO obtained in this work can be seen in this figure. Resonant protons associated with unreacted epoxy groups are observed in the 2.93 ppm (–CH– of epoxy group), 1.57 ppm (adjacent –CH– protons to epoxy group), and 3.14 ppm (adjacent –CH– protons of two epoxy groups) signals (Lopez Tellez *et al.*, 2008). Patterns of proton signals typical of triglycerides are also observed. Some include the triplet centered at 0.86 ppm (protons of the $–CH_3$ methyl group at the ends of the carbon chains of the triglyceride), the signal at 1.27 ppm ($–CH_2–$ methylene protons of the saturated chains of the $–CH_2–CH_2–CH_2–$ fatty acid), and the signal at 1.65 ppm, resulting from

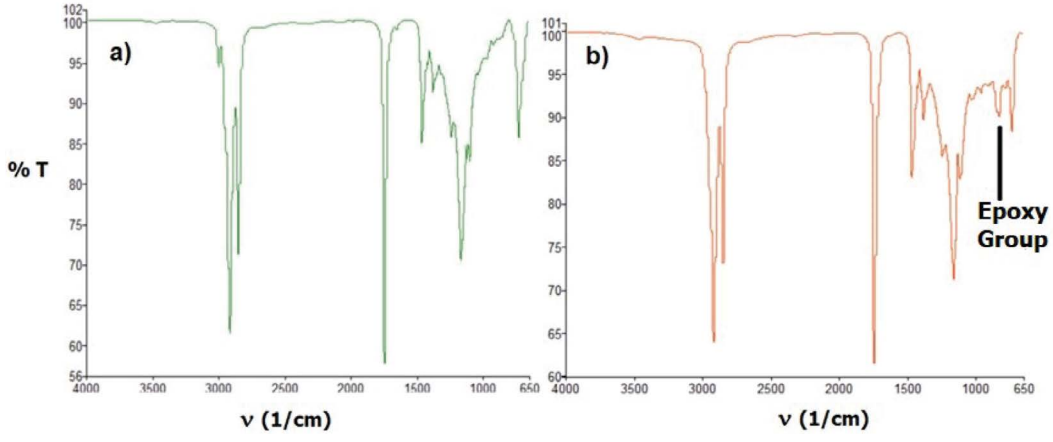

Figure 1. FTIR spectra of refined RBO (green line) and epoxidized eRBO (red line).

the resonances of the β methylene $-CH_2$ protons of the glycerol portion of the triglyceride in $-C{=}OCH_2$-CH_2 (Salih *et al.*, 2015). The insertion of the acrylate group was verified by four signals in the ^{1}H NMR spectrum (Salih *et al.*, 2015). The signals in the 3.58-3.75 ppm (Hk) region are attributed to the protons of the –CH–OH methine group that previously were part of the epoxy group. Likewise, the proton of a neighboring carbon to this methine group is identified in the region at 4.07-4.15 ppm (Hl): the - proton with respect to atomic oxygen of the –CH–CH–O–C=O< acrylate groups, identified as a quartet at 4.07-4.16 ppm. Finally, the signals of the $CH_2{=}CH-$ (vinyl) group in the acrylate group were also identified. In conclusion, the two vinyl protons in $CH_2{=}CH$ (Hm, Hn) and its methine proton (Ho) are observed at 5.80-5.86, 6.40-6.48, and 6.18 ppm, respectively. Nevertheless, the three protons in $CH_2{=}CH$ are not equivalent and therefore have different δ chemical shifts, as a consequence of their orientation (Fu *et al.*, 2010). No signals corresponding to free acrylic acid —which are usually observed at the 10-13 ppm range— were found.

The %Acrylation was determined as an internal reference pattern, based on the areas of the CH_3 methyl group signals. Equation 1 establishes the %Acrylation, following previous reports (Zhang *et al.*, 2011).

$$\%Acrylation = \frac{a_{Acrylate}}{a_{Epoxy} + a_{Acrylate}} \times 100 \quad (1)$$

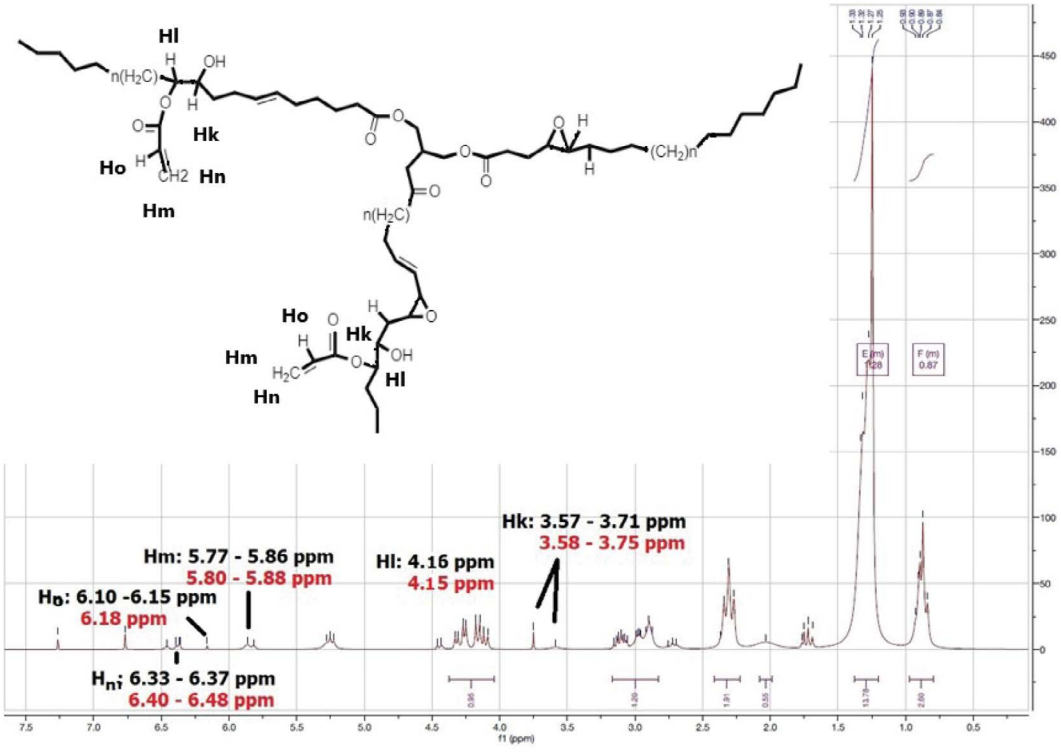

Figure 2. ^{1}H NMR analysis of rice bran oil partially acrylated with acrylic acid (A-eRBO). Values in bold typography are reference values (Fu *et al.*, 2010; Salih *et al.*, 2015).

where a_{Epoxy} is the integrated area of the associated protons of the methine epoxy at 2.76 ppm and $a_{Acrylate}$ is the integrated area of the acrylate protons in the 5.80-6.48 ppm range. The ^{1}H NMR spectrum (Figure 2) indicates that an 85.89% Acrylation was obtained with a_{Epoxy}=296.72 ua and $a_{Acrylate}$=1,806.8 ua. These results are comparable to previous reports of acrylated oils (Scala and Wool, 2002; Sharma *et al.*, 2006).

CONCLUSIONS

Refined rice bran oil can be feasibly functionalized with an acrylate group, through an intermediate epoxidation using H_2O_2, in the presence of Novozym 435 lipase. According to the ^{1}H NMR analysis, the best %Acrylation (85.89%) was estimated for the following conditions: T=110 °C, t=3 hours, and ratio of g eRBO to g acrylic acid of 1:1.5. The evolution of acrylation was confirmed by the of IV, SV, OOC, and MW values recorded.

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Bromatological analysis of annatto (*Bixa orellana* L.) seeds

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ABSTRACT

Objective: to carry out a bromatological and physicochemical analysis of local annatto seeds and a commercial paste.

Materials and Methods: local annatto seeds to which a physicochemical analysis was carried out where ash, humidity (weight difference), dry matter, proteins were determined. ADF and NDF, fat, and in addition, bromatological analysis was carried out on the samples and the commercial pasta.

Results: sample M3 (dark heart-shaped annatto without filaments) presented the highest values. The bixin content was recorded with 4.09% in sample M2 (heart-shaped red annatto without filaments) and the commercial paste was the lowest with 0.56%.

Limitations and Implications of the study: the importance of performing the bromatological and physical-chemical analysis of annatto seeds of local genotypes determined which of the local samples and the commercial paste are the ones that contain the greatest amount of bixin.

Findings and Conclusions: Sample M3 (dark heart-shaped annatto without filaments) presented the highest values. The highest bixin content was found in the smooth heart-red variety and the lowest value in the commercial pasta.

Keywords: Accessions, foods, proximal analysis, bixin.

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INTRODUCTION

Annatto (*Bixa orellana* L. (Bixaceae) is a perennial shrub whose seeds accumulate a high content of the apocarotenoid pigment bixin, a dye used for its pigmenting qualities, from pre-Columbian times to date. Likewise, bixin is economically and culturally important, since it is consumed in large quantities in Mexico and in the world. The commercialization of this pigment is mainly intended for use in the food, pharmaceutical, textile and cosmetic industries (Rivera-Madrid, 2021). Color, in food, plays an important role from the appearance point of view, which is why colorants as food additives are relevant. They are often used to highlight the natural color of food and others to restore the color lost during handling for preservation. he latter is the case, for example, with strawberry and pea preserves, which would be unattractive and unappetizing without

dyes. Food coloring was already practiced in Roman and Egyptian times (La química y la alimentación, 2018).

Natural dyes are generated by microorganisms, plants, animals, or minerals; economically they represent 940 million dollars in sales in the world market of dyes per year, and due to the consumer's caution for the consumption of products that alter or damage their health, it grows around 4% per year. Natural pigments are those obtained from sources present in nature, used to provide color in some products (Rivera-Madrid, 2021). They are subject to the same quality and toxicological safety testing as synthetics, but the FDA and other government agencies do not require them to be certified for chemical purity, and therefore refer to them as non-certified color additives (Camacaro *et al.*, 2018). In Mexico, there are standards for the food industry that regulate the use of bixin in foods (cheeses, yogurts, meats, creams, margarines, etc.); which establish the permitted doses or concentrations depending on the food and mention that this colorant does not contain toxic substances that may cause illness to the consumer and that it is of vegetable origin. These Mexican Standards are NOM-086-SSA1-1994, NOM-120-SSA1-1994, NOM-131-SSA1-1995, NOM-147-SSA1-1996, NOM-185-SSA1-2002, NOM-213-SSA1-2002 (FDA, 2001; Sahaza, 2001). The chemical and biological analysis of food began its operation as a science in the 19th and 20th centuries, with the purpose of making known the characteristics and nutritional value of food (Acero, 2007). The information obtained through bromatology is critical for the assimilation of the factors that condition the properties of foods and, in the same way, for food processing to be safe, nutritious, and pleasant for the consumer; since then, the improvement of the quality, quantity and availability of food supply worldwide has been introduced (Acero, 2007). Its importance lies in the economic, hygienic and legislative aspects, which is why it is not enough on its own, since it is essential to complement its execution with other disciplines, taking into account the assessment of the nutritional properties and composition of natural and processed foods and their possible adulterations; chemical analysis of the quantitative content of lipids, carbohydrates, vitamins, proteins and minerals present in the different foods; also, the technical regulation of the sanitary sale of food; as well as industrial production, seriation and transport.

Likewise, investigate the causes that induce and accelerate food alterations and through this develop preventive measures to avoid food from being a vehicle for microorganisms, toxins or any substance harmful to health (Salazar, 2014).

This research contributes to the knowledge of local annatto samples that have potential bixin content in their seeds and can be cultivated. The specific objective of this work was to carry out the bromatological and physicochemical characterization of annatto seeds from two communities in the municipality of Cunduacán, Tabasco and a commercial paste.

MATERIALS AND METHODS

Annatto seed samples (Figure 1) collected in two communities in the municipality of Cunduacán, Tabasco, were used as plant material: six corresponded to the Yoloxochitl 3rd section community and two samples to Monte Grande. The samples were placed in paper bags with a capacity of 500 g and labeled; each sample weighed approximately 200 g.

Figure 1. Annatto samples and seeds (*Bixa Orellana*) collected in two communities in Cunduacán, Tabasco, Mexico.

These were taken to the Campus Tabasco del Colegio de Postgraduados where they were analyzed in the Animal Science Laboratory and Central Laboratory.

Physicochemical determination and bixin content of annatto seeds

The following parameters were determined for the samples: ashes (Kirk *et al.*, 1996), moisture (weight difference), dry matter (Nielsen, 2019), protein (Kjeldahl, AOAC, 1980), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest and Wine, 1967), fats (Soxhlet, 1990) and bixin extraction (Vázquez, 2001). They were performed on each of the original samples and a repeat for each determination. Of the eight samples collected, all were subjected to the seven determinations mentioned above. Except for dry matter, only two varieties were tested. This was due to the fact that when the collection was made, fresh material was needed, and the harvest date had already passed.

Ashes

The methodology developed by Kirk *et al.* (1996) was used. First, the percentage of organic matter (OM) was calculated with the following formula:

$$\%O.M \equiv \frac{DM - RW}{DM} \times 100$$

(*DM*) Dry Matter; (*RW*) Residual weight.

The percent of ashes was calculated with the following formula:

$$\%C \equiv 100 - \%O.M$$

%*C*: Percent of Ashes; %*O.M.*: Percent organic matter.

Moisture

Moisture determination was only carried out for samples 6 and 8.

$$\%DM = 100 - \%H$$

$P1$: Initial weight; Pf: Final weight.

Dry Matter

The methodology proposed by Nielsen (2019) was used.

$$\%H = \frac{P1 - Pf}{P1} \times 100$$

$\%H$: Percent of moisture; $\%DM$: Percent of Dry Matter.

Protein

The Kjeldahl method was used, and calculations were made using the following formulas:

$$\%P = \frac{(GTHCL)(NHCL)(1.4)}{Pm} \times 6.25$$

$\%P$: Percent of Proteins; $GTHCL$: Total hydrochloric acid consumption; $NHCL$: Normality of hydrochloric acid; Pm: Sample weight.

Two adjustment factors were used: Adjustment factor for nitrogen (0.014 mill equivalents multiplied by 100 divided by final sample weight) and Adjustment factor for protein (0.0625 mill equivalents multiplied by 100).

Neutral Detergent Fiber (NDF)

Yields of recovered neutral detergent fiber were expressed as a percentage. Using the following formula:

$$\%FDN = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Acid Detergent Fiber (ADF)

The yield calculations were made using the following formula:

$$\%FDA = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Fats

The procedure was carried out with the following formula:

$$\%raw\ fat = \frac{M2 - M1}{M} \times 100$$

M: Sample weight; *M*1: flask weight; *M*2: flask weight with fat.

Bixin Extraction

The obtained absorbance was read and the bixin content is calculated using the following formula:

$$\%Bixin = \frac{\left[A_{500} + A_{404} - \left(0.256 \times A_{500}\right)\right]}{286.6 \times 1 \times a} \times 100$$

A=Absorbance of the test solution at the indicated wavelength; 1=Standard cell length (in cm); *a*=Sample concentration (in g/L); 286.6=Absorbance of bixin at 500 nm in chloroform (molar extinction coefficient); 0.256=Factor related to the absorbance of bixin in chloroform at 404 nm and 500 nm.

RESULTS AND DISCUSSION

The results obtained from the physicochemical characterization of the samples are presented in Table 1, where variations in the contents between samples are observed.

Regarding ashes content, sample M1 (7.18%) was higher than all other samples. While the lowest value was found in M6. For moisture content the highest value 41.26% was for M6 from the community of Yoloxochitl and the lowest moisture content in the seeds of sample M8 (33.55%) from Monte Grande. In terms of dry matter, the highest value

Table 1. Physicochemical characteristics of annatto seeds.

Samples	Ash	Moisture	Dry matter	Protein	NDF	FDA	Fats
	%						
M1	7.18	-	-	15.00	74.70	87.97	9.42
M2	4.61	-	-	14.28	65.48	81.03	13.80
M3	5.34	-	-	16.03	75.96	91.05	37.16
M4	5.52	-	-	15.58	65.20	80.46	24.41
M5	5.40	-	-	15.99	75.89	88.70	15.04
M6	3.72	41.26	58.73	15.03	58.66	91.10	23.91
M7	4.32	-	-	14.15	53.31	86.65	28.29
M8	3.84	33.55	66.45	15.13	65.46	90.22	28.29

Note: M1, M2, M3, M4, M6, M7=Samples taken from the community Yoloxochilt 3ª. Section. M5, M8=Samples taken from the Monte Grande community (Arias-Pérez and De Dios-Durán, 2013).

66.45% was obtained in M8 and the lowest in sample M6. On the other hand, sample M3 presented the highest value of 16.03% protein, followed by M5 (15.99%) and the sample with the lowest protein content was M7 with 14.15%. For Neutral Detergent Fiber (NDF) the highest value 75.96% was reached in sample M3 and the lowest value 53.31% in sample M7. For Acid Detergent Fiber (FDA) the highest value 91.10% was obtained in sample M6 and in sample M4 the lowest value with 80.46%. For fat content the highest value (37.16%) was obtained in sample M3 and the lowest value of 9.42% in sample M1.

The results reached in this research for the ash variable are similar to those achieved by authors such as Córdoba (1987), Jaramillo and Muñoz (1992), CNP (2001) and SDIC (2001), who, in studies carried out with annatto of the red variety with abundant filaments, found values that varied from 4.50 to 7.97%. For protein content, the results of the study were superior since the range varied from 14 to 16%, while the previously mentioned authors obtained ranges from 13.00 to 14.24%. The same authors found that moisture content ranged from 8 to 13%. Whereas in the study the two samples M6 and M8 presented moisture contents of 41.26 and 33.35%, respectively. Authors such as, Arias-Pérez and De Dios-Durán (2013) in the same variety of hearty red achiote without filaments obtained moisture percentages of 6.43%; this could be since the authors used very small samples of 0.2 g.

Values for NDF ranged from 53.31 to 75.96% and for FDA from 80.46 to 91.10%, which were higher than those obtained by Arias-Pérez and De Dios-Durán (2013), who reported an NDF content of 47.98% and FDA of 39.09%. As the FDA value increases, the digestibility of the seed is reduced as the cell wall is composed of cellulose and lignin (FOSS, 2018). Regarding fat content, the results showed that sample M3 had the highest value of 37.16%, while sample M1 had the lowest content of 9.42%. These results were higher than those achieved by Noguiera-Carvalho *et al.* (2010) who reported only 4.5% of ethereal fat in annatto seeds.

Table 2. Bixin extraction performed on annatto seeds and commercial paste.

Sample	Bixin (%)
Control	0.00
Annatto paste	0.56
M1	3.99
M2	4.09
M3	3.39
M4	3.86
M5	2.00
M6	3.74
M7	1.76
M8	1.08

Note: M1, M2, M3, M4, M6, M7= Samples taken from the community Yoloxochilt 3ª. Section. M5, M8= Samples taken from the community Monte Grande. Paste achiote= Sample taken from the annatto paste.

The results of this study differ from those obtained by Valadez-Villarreal *et al.* (2020), who achieved 8.2% bixin in annatto seeds when using an alkaline method for the extraction of the dye. The results varied according to the samples collected. The bixin content was higher (4.09%) in sample M2, the heartwood red variety without filaments, than in the other samples. In samples M5 (green annatto with abundant filaments), M7 (hearty red with few filaments) and M8 (hearty red with abundant filaments) the values found range between 1.08 and 2%, the lowest value corresponds to the annatto paste with 0.56% of bixin. This could be since this paste is commercial. INTITEC (2013), recommends for technical requirements in processing plants, that the concentration of bixin should not be less than 2.5%.

CONCLUSIONS

To be approved as an additive, a substance must be well characterized chemically and must pass the toxicological controls established by the corresponding health authorities. In addition, its need must be demonstrated in such a way that its use implies technological advantages and benefits for the consumer. Therefore, the annatto grown in rural communities in Tabasco can be an alternative as raw material of high nutritional value for the food industry and the sample with the highest bixin content should be recommended for its cultivation.

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Colegio de Postgraduados

Comarca Lagunera: Between the socioeconomic characterization and the availability of piped water

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ABSTRACT

Objective: This research aims to describe with sociodemographic features the municipalities that make up the Comarca Lagunera. They are located in the states of Coahuila and Durango. In Coahuila, they are: Francisco I. Madero, Matamoros, San Pedro, Torreón and Viesca; and in Durango: Gómez Palacio, Lerdo, Mapimí and Tlahualilo.

Design/methodology/approach: The methodological strategy is quantitative, using descriptive statistics as analysis technique to incorporate synthetic measures (such as indices) and proportions. The information sources come from different instances (CONEVAL, CONAPO and INEGI). In all cases, the level of disaggregation is municipal and the moment of information collection corresponds to 2020. The population volume of the municipality is described, as well as its proportion with regards to the number of inhabitants in the state. Later, the results from the Gini index, marginalization index, proportion of occupied population devoted to primary activities, and proportion of private households without access to piped water are incorporated.

Results: The municipalities that had the highest values for each of the variables are presented. In Mapimí the Gini index is 0.54; in Viesca, 36.2% of the population is dedicated to primary activities; in Tlahualilo, 4.13% of households lack piped water; and the index of marginalization in Torreón is 0.62.

Limitations on study/implications: It was not possible to present the information with a higher level of disaggregation because the chosen variables only have data available up to the municipal level.

Findings/conclusions: The use of indices allows to carry out a very useful characterization and can be strengthened by the inclusion of specific variables that account for the municipal situation.

Keywords: inequality; households without piped water; primary activities.

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INTRODUCTION

The importance of the Comarca Lagunera is fundamental to understand the economic dynamics of northern Mexico. Since it is located between Coahuila and Durango, its proximity to the border links it closely with the cross-border regional economy (Aguilar, 2005). Although Monterrey is identified as the industrial capital in this circuit, Torreón and surroundings are integrated into the intense commercial exchange with Texas. Thus, the Comarca Lagunera is an essential productive enclave to understand the development of northern Mexican economy (Sierra Jiménez, 2008).

Concerning the review of background information that has examined the situation of the region from a socioeconomic perspective, the case of the study by López and Sánchez (2007) stands out; although the study makes a review about the changes in the population volume of the region of interest, the most recent data examined corresponds to the year 2005. In addition, in this case, the number of localities is presented according to their size.

Among the most recent antecedents with socioeconomic information of the region, there is the study by Enríquez, Hernández and Morales (2021). Despite the complexity and the importance of the results, information collection corresponds to the year 2015, since information from the Inter-census Survey is used. In this context, the present study updates the information with gathered in 2020 and presents an integral analysis that incorporates social and economic variables, directed at understanding their relationship with access to water in the households.

In this research, the nine municipalities that make up the Comarca Lagunera were described in sociodemographic terms. In the first place, the population volume of each of these demarcations is stated to present a general outlook, as well as the percentage that it represents with regards to the total of the states. Later, the Gini and marginalization indices are described. In addition, the percentage of the population linked to primary activities and the proportion of households that do not have access to piped water are examined.

Something that must be taken into account is that these results are framed within the metropolitan context integrated by the municipalities of Francisco I. Madero, Matamoros and Torreón —in Coahuila— and Gómez Palacio and Lerdo —which belong to Durango. These five demarcations make up the metropolitan zone (MZ) of the Lagoon, which, according to INEGI (2019), has the ninth place within the most inhabited zones of the country.

MATERIALS AND METHODS

The methodological strategy is quantitative. The type of analysis is descriptive, and the main technique is calculation of proportions and interpretation of synthetic measures (such as indices). The information inputs come from different instances (CONEVAL, CONAPO e INEGI).

First, the four variables were examined: Gini index, marginalization index, proportion of occupied population devoted to primary activities, and percentage of private households without access to piped water. Considering the disaggregation of information at the municipal level and that its date of collection corresponds to the year 2020.

The Gini and marginalization indexes are described and presented in maps, as well as the incorporation of measurements to show a general outlook of the region. Despite of this, the discussion was directed at linking the socioeconomic conditions of the municipalities analyzed through variables related to the consumption and access to water.

Regarding the Gini index, its estimation is in charge of the National Council for Evaluation of Social Development Policy (*Consejo Nacional de Evaluación de la Política de*

Desarrollo Social, CONEVAL). The marginalization index was estimated by the National Population Council (*Consejo Nacional de Población*, CONAPO), as well as the proportion of households without piped water. In the case of the percentage of population occupied in the primary sector, its calculation was made by the authors.

With the microdata from Durango and Coahuila, each set of data were used separately. In every case, the National System for Classification of Occupation (*Nacional de Clasificación de Ocupación*, 2019) from INEGI was used to identify the type of occupation of each individual incorporated in the labor market. With this information, a variable was generated that grouped the occupation into only three categories: primary, secondary and tertiary activities.

RESULTS AND DISCUSSION

In the first place, the volume and the proportion of the population represented with regards to the total of the state are mentioned. Then, the study refers to socioeconomic aspects of the region.

To gain perspective of the size of the population of each municipality, the total inhabitants per municipality is presented according to information from the Population and Household Census 2020. In Coahuila, the ones that stand out are Francisco I. Madero (59,035 inhabitants), Matamoros (118,337 inhabitants), San Pedro (101,041 inhabitants), Torreón (720,848 inhabitants), and Viesca (20,305 inhabitants). From this set of municipalities in Coahuila, Matamoros is the largest one and Viesca is the one with least population. Together, these municipalities have 999,261 inhabitants, which represent 31.75% of the state's population.

In the case of Durango, between the municipalities of Gómez Palacio (372,750 inhabitants), Lerdo (163,313 inhabitants), Mapimí (26,932 inhabitants) and Tlahualilo (21,143 inhabitants), the total population that belongs to these municipalities is 584,138 inhabitants which represent 31.87% of the inhabitants of the state (1,832,650 inhabitants).

In this context, the analysis of sociodemographic variables of this zone is convenient. For this purpose, the main indicators are presented next, taking as reference the 9 municipalities that are closely linked to the Comarca Lagunera.

Gini index

According to Esquivel (2021), the most famous indicator to measure income inequality (or wealth) is the Gini coefficient. This indicator —as others— allows obtaining an estimation of inequality based on the information contained in any distribution. It is constructed from axiomatic principles and tends to have extreme values that serve as reference to know if the inequality is high or low.

In terms of interpretation, the value of this coefficient ranges between 0 and 1, and as gets higher it indicates that there is a higher degree of inequality. Next, a map is presented which includes the nine municipalities selected:

Figure 1. Gini Index, 2020.

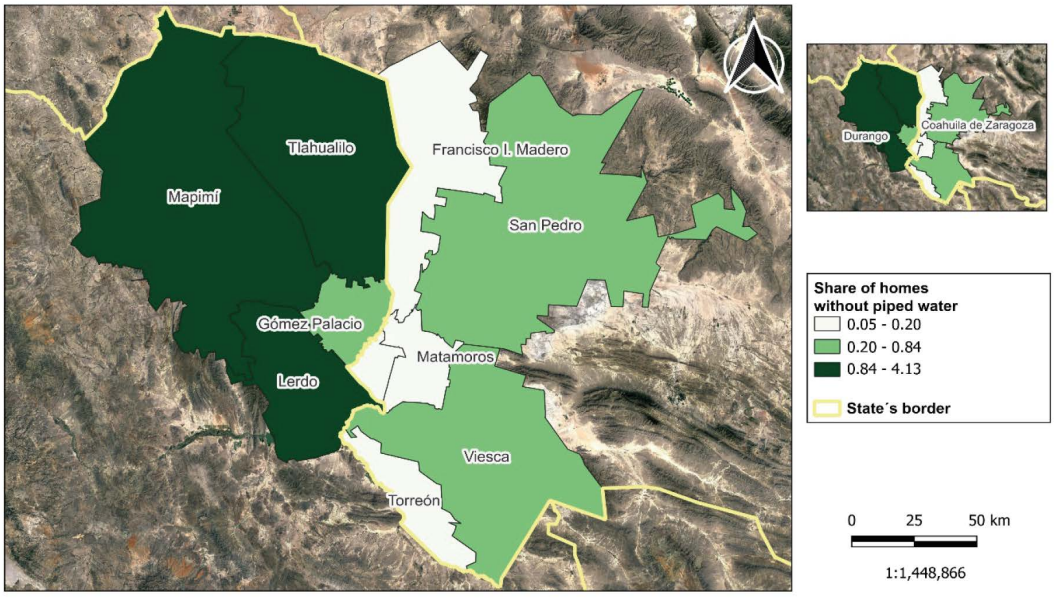

Figure 1. Percentage of private homes without piped water by municipality (2020). Source: Prepared by the authors based on data of CONEVAL, 2020.

The results show that among the municipalities with highest level of inequality there is Mapimí (0.54), followed by Viesca (0.48) and Torreón (0.45). In opposite position, there are San Pedro and Tlahualilo (both with 0.38). Although the range between the highest and the lowest values is close, we must recognize that, given the type of construction of the index, a change of 0.1 is relevant.

In this context, the municipality of Mapimí stands out for having the highest degree of inequality compared to the group of demarcations analyzed. This is important inasmuch as this municipality is not part of a metropolitan zone and the fact that it has a higher degree of inequality can represent that it has more adverse conditions.

Proportion of population devoted to the primary sector

The review of the general context of inequality and the limitations of resources in municipalities leads to examining which of them requires a greater amount of water resources according to its productive structure. To examine this, the proportion of the population devoted to primary activities represented has been calculated with regards to the total occupied population. The inputs used to present this result also correspond to census data.

The municipalities of Viesca (36.4%), Tlahualilo (36.2%) and Mapimí (28.8%) are shown in dark green and they have the largest proportion of occupied population in primary activities. In the opposite situation and in white color, there are the municipalities of Lerdo (6.5%) and Torreón (2%), both characterized for being large cities whose economy is closely linked to tertiary activities. This recalls the importance of the urban structure: the large population centers have a predominantly tertiary economy.

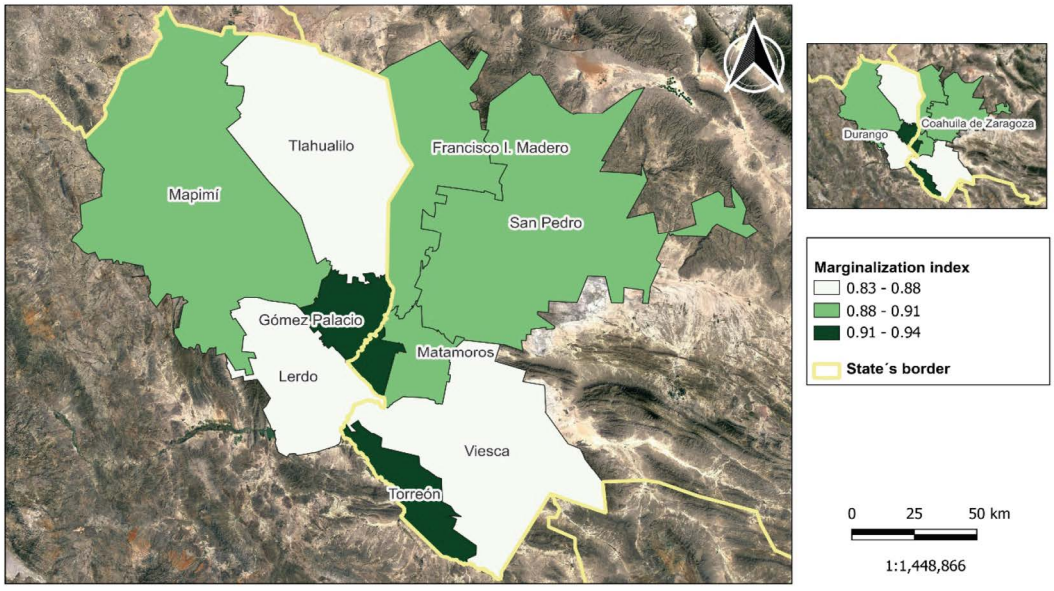

Figure 2. Percentage (%) of economically active population devoted to the primary sector by municipality, 2020. Source: Prepared by authors based on the Mexican census data (INEGI, 2020).

Private households without access to piped water

Finally, a variable related to access to water is presented. This variable was incorporated with the intention of completing the outlook, that is, the proportion of the workforce that performs primary activities accounted for what happens in the public sphere, while understanding the percentage of households without piped water approximates the analysis to the private dimension.

It is important to point out that, according to CONAPO (2021), this variable is part of the construction of a marginalization index. In this regard, it was decided to analyze it separately with the intention of observing differences between the municipalities, since although it is true that they make up the same economic region, the access that households have to water is unequal between municipalities. Next, the results are shown (Figure 3).

Figure 4 shows that Tlahualilo (4.13%), Lerdo (3.2%) and Mapimí (1.15%) have the highest percentages of private households without access to piped water. Although these percentages are apparently 'low', it should be remembered that the impossibility of having piped water calls into question their physical accessibility. This dimension is one of the 6 factors that make up the right to water (Dominguez and Flores, 2016). In the case of the municipalities with lower percentages, this also reminds us that with larger localities there is more urban infrastructure, which -among other things- provides the water service.

This result reminds us of the importance that presenting variables, additional to the indices, allows complementing the analysis. For example, in the case of Tlahualilo, the value of the marginalization index functioned as a first approximation to the situation of the municipality, although reviewing the percentage of households without piped water strengthens the analysis and above all, it allows us to understand that beyond the demand for water being be covered to satisfy the productive needs of the municipality, there is a lack in the urban use of the vital liquid.

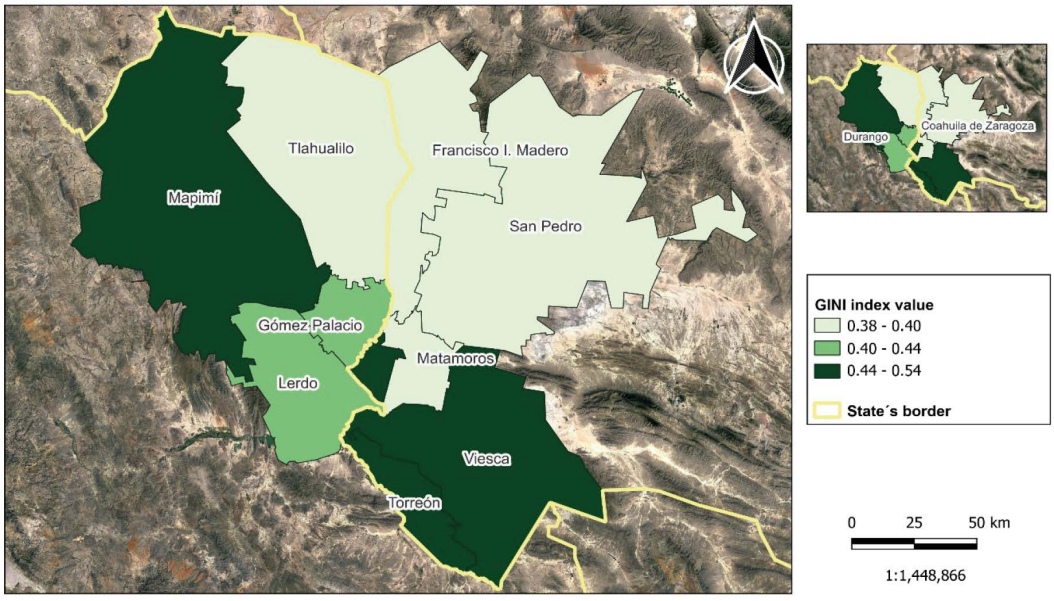

Figure 3. Percentage (%) of households without piped water by municipality, 2020. Source: Prepared by the authors based on data from CONAPO.

The next variable is incorporated with the intention of presenting a general synthesis that allows contrasting the general situation of the municipalities with the characteristics analyzed.

Marginalization index

Measuring the marginalization index informs about the intensity of the deprivation and social exclusion of the population; it is a synthetic measure that serves to understand the situation of the municipalities, despite its utility. Including it is important in order to note the relevance there is in incorporating non-synthetic measures to analyze the situation of the municipalities more meticulously. In this case, the normalized values between 0 and 1 are shown: if the figure is higher, the degree of deprivation is higher. Its estimation is in charge of the National Population Council (*Consejo Nacional de Población*, CONAPO) and is presented in the Figure 4.

The map shows that Torreón (0.94) and Gómez Palacios (0.93) are prominent with high degrees of marginalization. This deserves to be reviewed with greater depth, since it calls into question whether the economies of agglomeration in large metropolis serve as mechanisms to inhibit poverty and marginalization (Sobrino, 2015). Therefore, it would be expected for both cities that are part of the MZ of the Comarca Lagunera to have a lower value in this index.

In addition to this distinction, it is important to note that, jointly, the values of the municipalities that make up the region are high. This leads us to consider that it is a marginalized region even despite the relevance that it has in its productive development. The fact that the region has great economic importance in the metropolitan and national context does not necessarily reflect an improvement in the living conditions of the population.

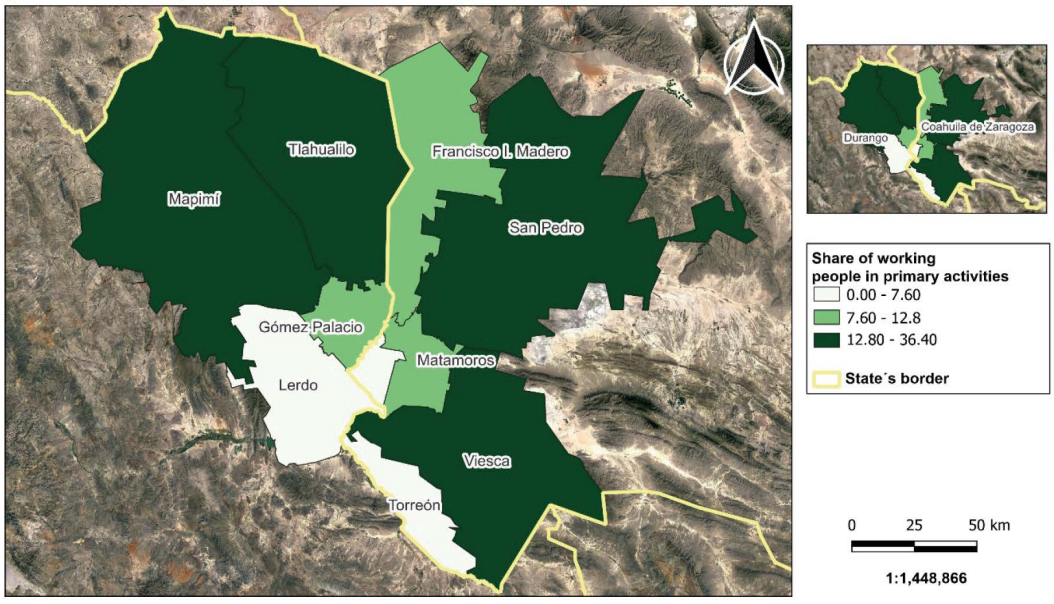

Figure 4. Marginalization index by municipality, 2020. Source: Prepared by the authors based on data from CONAPO.

CONCLUSIONS

In sum, the municipalities that have the highest values in each of the variables analyzed are presented here: Mapimí has a Gini index of 0.56, Viesca is the demarcation where 36.2% of the economically active population is devoted to primary activities. In Tlahualilo, 4.13% of private households do not have access to piped water, while in Torreón the marginalization index is 0.62. It was seen that in the largest localities there is better access to the water service, and this is closely related to the fact that the large urban centers have better infrastructure in services.

This synthesis of results allows us to recognize that although it is true that synthetic measures such as the marginalization or Gini index allow approaching the general situation of the municipalities, reviewing specific variables allows us to make a more rich analysis of the situation in the municipalities. In this case, the fact that the demarcations selected are part of the same economic region points to the fact that between them there is certain homogeneity; however, examining access to piped water allows identifying the existing contrasts between municipalities.

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Social network of producers of dehydrated products with thermosolar technology in Zacatecas, Mexico

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ABSTRACT

Objective: To analyze the social networks and the trust there is among the producers of dehydrated products with thermosolar technology in the agricultural strip of Zacatecas, in order to determine the organizational potential through prevailing levels of trust between producers.

Design/methodology/approach: A survey was applied to 30 producers of dehydrated products who have received training and information about the use of the thermosolar plant, all belonging to the state of Zacatecas. Indicators were used for the social networks analysis.

Results: The results confirmed the importance of direct and indirect links; of all the producers, eighteen had no relationship with any other producer, although the rest showed at least one interaction with another producer. The producers have notable characteristics and acceptable trust relationships.

Limitations on study/implications: The results apply to the selected sample; thermosolar food dehydration technology is still unknown among the producers.

Findings/conclusions: Trust relationships must be strengthened and strategies should be created to disseminate knowledge in a timely and efficient manner with key stakeholders and thus boost their pre-existing interactions.

Keywords: technology, solar energy, food.

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INTRODUCTION

One of the greatest problems that humanity faces currently is the balance between food production and consumption (Murthy, 2009). A way of reducing the loss and waste of foods is through conservation techniques, among which moisture removal from fresh products without processing stands out, minimally processed by drying (using environmental conditions) or by dehydration (using artificial heat) (De Michelis and Ohaco, 2022). Both techniques are used to eliminate water from the enzymes and

microorganisms responsible for the deterioration of products. Dehydration allows conserving and maintaining the quality of the foods for a longer time, favoring the addition of value and their availability (Menchaca and Recio, 2017). Solar dehydration is one of the simplest and most inexpensive techniques, given the availability of electromagnetic radiation from the sun (Guzmán *et al.*, 2020).

The state of Zacatecas has an important tradition using the techniques of drying and dehydrating of horticultural products such as chili peppers, garlic, fruits and vegetables. In 2017, in Morelos, Zacatecas, a thermosolar plant was established to dehydrate horticultural products and, thus, take advantage of the privileged total annual solar irradiance (520 W/m^2) in benefit of the agroindustrial sector of the state (UNAM, 2017). In 2021, the activities of this plant were taken up again to promote their use among farmers with equipment and modernization of this biospace, which provides training and technical assistance to producers in the process of dehydration and value aggregation to various agricultural and livestock products.

The response from farmers has been positive in the elaboration of dehydrated byproducts based on guajillo chili pepper, garlic, peach, apple, nopal, tomato, celery, cilantro and beef. In addition, individually, producers have sought different means of commercialization of their products in stores, fairs, government offices and schools. However, there is the urgent need for strategies that allow the appropriate organization to become integrated as micro-businesses in the generation of agricultural products with added value (Poot *et al.*, 2021). Nevertheless, there is a need to identify the most recommendable legal and organizational figure for these producers, based on the existing trust between them (Luna and Velasco, 2005; Figueroa-Rodríguez *et al.*, 2012). For Cevallos *et al.* (2020) trust constitutes a key component of social capital to favor cooperation and to solve collective action problems, which, at the same time, allow better levels of development. Therefore, the analysis of trust between producers of dehydrated products is fundamental to reinforce the cooperation between them and to strengthen the participation of these in exchange networks for information, knowledge and technology.

This information can be obtained through Social Networks Analysis (SNA). SNA is a quantitative method that studies the social structure from regularities in the pattern of relationships established between social entities (for example, people, groups or organizations) (Kuz *et al.*, 2016). In turn, Aguirre (2011) defines a social structure as a social network composed by a finite group of actors and configured around a series of relationships between them, which can be represented in the form of one or several graphs. The graphs are made up of nodes that are related with other nodes through connections named edges that allow studying the existing relationships between them (Clark, 2006).

SNA has been used to determine the importance in the type of relationship, the levels of trust, and the organization between agricultural producers (Figueroa-Rodríguez *et al.*, 2012) to define the process of information and knowledge exchange for agricultural innovation (Monge and Hartwich, 2008; Aguilar-Gallegos *et al.*, 2016; Villarroel-Molina *et al.*, 2019), and to analyze the commercial relationships between

different links in the value chains (Callejas *et al.*, 2020). However, although this tool contributes to a starting point to develop a better understanding of the direct and indirect interactions between farmers and other stakeholders, something true is that there are no studies about the relationships of trust there are between producers already linked to the market of dehydrated products in the state. Therefore, this study sought to decrease the gap present in the literature. The objective of this study was to analyze social networks and the trust there is between producers of dehydrated products with thermosolar technology in the agricultural strip of Zacatecas, with the aim of defining the potential for organization through the prevalent levels of trust between producers. The hypothesis suggested was that producers of dehydrated products have established networks, although this does not imply that they are stakeholders that have high levels of trust.

MATERIALS AND METHODS

A survey was applied to 30 producers who participated in the use of the thermosolar plant in Zacatecas. Based on what was presented by Figueroa-Rodríguez *et al.* (2012), the instrument applied was designed to capture information referring to the following points: 1) general information of the producers; and 2) record of the names of other producers that dehydrate agricultural products in Zacatecas. They were asked about the importance according to the type of relationship for family members, friends, neighbors and business acquaintances and the level of trust of each, expressed in an ordinal scale of 1 to 5 (where 1 is not having any trust, 2 has almost no trust, 3 has regular trust, 4 has plenty trust, and 5 has absolute trust); they were also asked if they would lend money, if they would ask to borrow money, and if they would make a society, and the responses to these questions were expressed in a nominal scale of yes or no and considered as a measurement for the level of trust between stakeholders. The surveys were applied during the months of September and October 2022.

Information analysis

For the social networks analysis, the information was codified and registered in databases to construct a mode-one network, in which each node can be related to any other network (Aguilar-Gallegos *et al.*, 2016). SNA indicators were estimated in the symmetrical matrix made up by the producers surveyed, which were the size of the network, the density, and the index of centralization (entry and exit).

The size of the network corresponded to the number of producers who have a relationship with other producers. The stakeholders are linked one to the other through social, technical, management or commercial links; these "links" are represented with lines. Thus, a link is established between two stakeholders when they are related in some sphere (Rendón *et al.*, 2007).

The density of the network is the percentage of existing relationships between the ones possible to carry out (Equation 1) (Rendón *et al.*, 2007); the density (D) was calculated according to the next formula:

$$D = \frac{1l}{n(n-1)} * 100 \quad (1)$$

Where l was the number of existing relationships divided by the number of possible relationships $n(n-1)$. If the value of D was 100%, then the interpretation is that the network is completely articulated and there is an optimal information flow between stakeholders.

The centralization index measures the degree at which a stakeholder is dominant in the network (Aguilar *et al.*, 2017). The values will range between 0 and 1, with 1 being the value for the most centralized graph and there are no links between the other stakeholders and zero when there is no dominating stakeholder and all stakeholders are linked between one another (Cuevas-Reyes *et al.*, 2016; Aguilar *et al.*, 2017). The degree of centralization of entry was estimated to identify the nodes that serve as an important source of information and the degree of centralization of exit to identify the stakeholder who obtains information from different stakeholders.

The Kruskal-Wallis non-parametric test with a significance level of 5% was used to identify the differences between the groups by type of relationship, the importance that they gave to it, and the level of trust. The hypothesis considered was: H_0: the level of trust is equal for family members, friends, neighbors and business acquaintances. The Wilcoxon test with a significance level of 5% was used to establish the differences between the qualitative variables of importance of the relationship and the level of trust. The null hypothesis established for this test was: H_0: the importance given to the relationship is equal to the level of trust in the producers.

With the questions about whether they would lend or ask to borrow money and if they would form a society, 2×2 contingency tables were made, which were used to calculate the coefficient *Phi* (r_{φ}) that determined the correlation between two variables in nominal scale. Finally, three logistic regression models were estimated to calculate the probability of loaning money, borrowing money and making a society (Figueroa-Rodríguez *et al.*, 2012); the Wald entry method was used for the selection of the variables to be included in the model. The empirical model estimated was:

$$P(Y / X) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x)}} + \varepsilon \quad (2)$$

Where β_0 and β_1 were the coefficients of the regression model and the term ε represents the residual error.

The social networks analysis was carried out with the UCINET 6 software for Windows and NetDraw 2.141; while the statistical analysis of the ordinal variables (means and standard deviation), correlation tests and logistic regression models were conducted with the SPSS 27.0 software for Windows (IBM, 2022).

RESULTS AND DISCUSSION

Characteristics of the survey respondents

The producers surveyed were mainly men (93%) and only 7% women, with an average age of 48 years, which ranged between 25 and 75 years, and have on average 20 years of experience with the process of dehydration. Of the survey respondents, 6.7% have primary school studies, 26.7% secondary school, 13.3% high school, 40% undergraduate studies, and 13.3% graduate studies. These results reaffirm what was found by Minjarez *et al.* (2019) in the characterization and classification of producers from the western highlands in San Luis Potosí and what was mentioned by the OECD (2007); the organization stated that the sociocultural and economic traits present in producers from a certain region could also be found throughout the Mexican territory. However, the condition of the farmers surveyed (young and trained adults) will allow generating efficient strategies for training in technological innovations and new management plans, and to improve the commercialization channels.

From the survey respondents, 83.3% had agriculture as the main activity and 16.7% have established a business of their own. However, 58.6% of the total involved mentioned that their income comes from dehydrated products that they trade; 50% have a legally established business where they commercialize their dehydrated products, while the other 50% do not have a legally constituted business and trade informally. The businesses constituted have been functioning for 2 to 50 years, with an average of 14±13 years; annually, they generate 13±21 permanent jobs and 34±35 occasional jobs. In this sense, Acevedo (2017) stated that in most of the world there is an individualistic mentality that makes associations difficult. For their part, González and González (2017) mentioned that the sense of organizing formally has decreased from past experiences that have not had favorable results, in addition to the uses, customs, culture and ideology of the collaborating agent (Tamayo *et al.*, 2019).

Of the producers, 58.8% dehydrate chili pepper and this trend is derived from the productive tradition of the state, since at the national level, Zacatecas has been characterized as being the main producer of dry chili pepper. This agrifood chain is socially and economically strategic for the state and for the generation of jobs in production and commercialization of the product (Aguilar and Esparza, 2010). Other products that are dehydrated in the state are nopal, apple, strawberry, fig, pineapple, celery, parsley, tomato, onion, garlic and dry meat. The diversification of products is something key in Zacatecas, and thus 13.3% of the survey respondents dehydrate more than one product. Although 50% of the survey respondents grow their own raw materials, the remaining 40% acquire it through another producer.

Social networks of producers of dehydrated products

An asymmetrical matrix was constructed with the information gathered, which derives into a total of 85 names mentioned, of which 9 were producers interviewed and 55 were not registered. Figure 1 shows the existence of names referenced most frequently, which means that they have a larger number of networks. However, as mentioned by Figueroa-Rodríguez *et al.* (2012), the fact that a producer has many networks does not imply that he is a stakeholder that has high levels of trust.

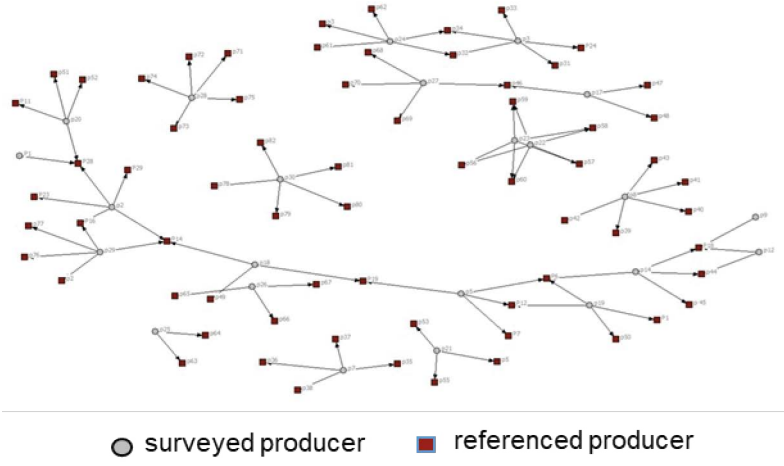

Figure 1. Reference network of the producers of dehydrated products.

The social network of producers of dehydrated products is presented in Figure 2. There were 18 producers who did not have any relationship with any other producer; however, the rest showed having at least one interaction with another producer. The network of dehydrating producers was formed by a total of 12 nodes and 15 relationships (Figure 2). The density of the network was 3.4%, that is, from every 100 relationships, only three materialized. The network was more centralized in degrees of exit (14.27%) than of entry (10.70%); that is, in the network there is one or more modes that are dominant in the establishment of exit links, for this case it was seen that producers 2, 19, 5 and 18 referred between 5 and 3 producers with whom they maintained links. Regarding the stakeholders with more degree of entry, they were producers 5, 18, 14 and 6 with 4 to 3 producers who were their source of information (Cuevas-Reyes *et al.*, 2016; Aguilar *et al.*, 2017).

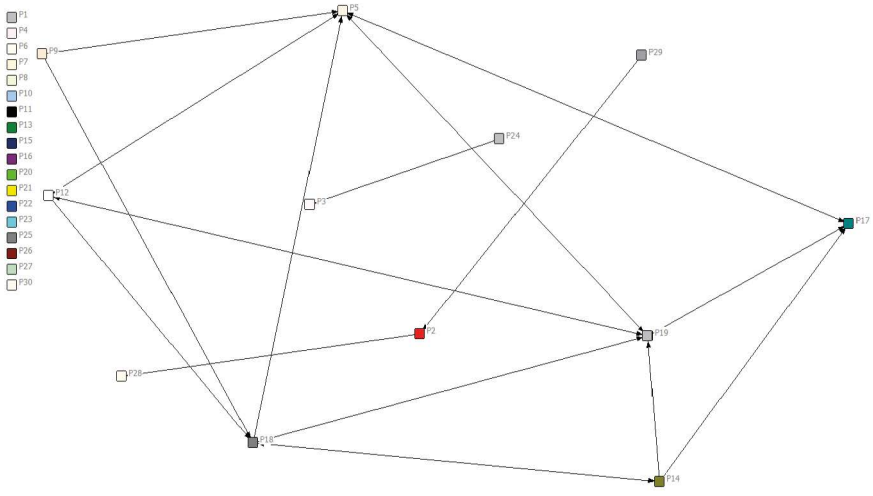

Figure 2. Social network of producers of dehydrated products.

Trust between producers

The trust demonstrated by producers in relation to family members was 75% of absolute trust and 25% of plenty trust. Regarding the relationship with friends, 28% had regular trust, 30% plenty trust, and 42% absolute trust. Of the survey respondents, 44% refer that they maintained a level of plenty trust with their business acquaintances and for 49% of the survey respondents this relationship was of absolute trust. Finally, the producers mentioned having between regular trust (33%), plenty trust (17%) and absolute trust (50%) with their neighbors. These results indicated that there is an important degree of trust; however, as Cevallos *et al.* (2020) points out, trust is integrated by the associative experience, participation and reciprocity to have strengthened social cooperation; this situation must be developed among the producers to be able to become organized. This is how trust plays an important role when committing the members to a deal between peers with responsibility, which allows at the same time generating social relationships and of long-term exchange (Tepox and González, 2021).

From the Kruskal-Wallis test, it was determined that the importance of the relationship that producers maintain between groups ($H(4)=2.106$) was not significant ($p=0.551$), and therefore the null hypothesis is not rejected and there is inference that producers give the same importance to the relationship they maintain with other producers. Statistically, it was shown that the level of trust was equal for family members, friends, neighbors and business acquaintances, since the test statistic was $H(4)=3.408$, with $p=0.333$.

On average, the level of trust was higher (4.27 ± 0.82) than the importance that producers give to the type of relationship between them (4.03 ± 0.76). This was corroborated with the results obtained in the Wilcoxon test $Z=-3.833$ and $p<0.000$, whose contrast was conducted considering 5% of significance. The decision to trust entails a risk, so there is no certainty that the person to whom that trust is given responds in the same way (Acedo and Gomila, 2013). However, trust can be achieved by determining viable behaviors that allow interactions of mutual help to reach the organizational objectives in the best way (Cansino, 2020).

The correlation between independent variables was higher between borrowing money and making a society ($r_{\varphi}=0.5000$, $p\leq0.001$), followed by lending and borrowing money ($r_{\varphi}=0.455$, $p\leq0.001$), and lending money and making a society ($r_{\varphi}=0.417$, $p\leq0.001$). The results obtained indicated that there is a positive and moderate association between variables (Muria and Saura, 1998). Derived from the previous data, it was pertinent to carry out the logistic regression of each dichotomous variable and level of trust.

The results obtained from the estimation of the models are presented in Table 1. Model 1 defined that trust explains loaning money in 72.3% of accuracy in the prediction. The relationship of trust and that of forming a society (model 3) obtained an accuracy of 67.3%, and both explain the relationship between variables in more than 60%; however, trust explains better that a producer is willing to lend money.

In the case of the relationship between trust and asking to borrow money, the estimated model explained 48.5% and the relationship between both variables was not significant. The result obtained was similar to that reported by Figueroa-Rodríguez *et al.* (2012), where they indicated that the scarce relationship between both variables is because the action of

Table 1. Results of the logistic regression model.

Model	Variable	B	E.T.	Wald	gl	Sig.	Exp(B)
Lend money[a]	Trust	1.074	0.304	12.464	1	0.000	2.927
	Constant	−2.410	0.952	6.409	1	0.011	0.090
Ask for money[b]	Trust	0.145	0.252	0.333	1	0.564	1.156
	Constant	−0.295	0.844	0.122	1	0.727	0.745
Form a company[c]	Trust	0.701	0.269	6.803	1	0.009	2.016
	Constant	−1.801	0.886	4.126	1	0.042	0.165

[a] Variable introduced in step 1: Trust. R^2 de Negelkerke=0.188; X^2=14.073, p=0.000.
[b] Variable introduced in step 1: Trust. R^2 de Negelkerke=0.004; X^2=0.564, p=0.564.
[c] Variable introduced in step 1: Trust. R^2 de Negelkerke=0.093; X^2=7.202, p=0.007.

a producer of asking to borrow money from another is attributed not to trust, but rather to the reputation that the producer has within the group.

CONCLUSIONS

Producers of dehydrated products from the state of Zacatecas have characteristics of note and acceptable relationships of trust, which also have important implications to reach a higher number of farmers and to consolidate the existing links. Therefore, it is important to create strategies to disseminate the knowledge about solar dehydration in a timely and efficient manner with key stakeholders, in addition to strengthening actively the pre-existing interactions in producers' networks. It is priority to create awareness of the importance of networks and teamwork. This, as a strategy to promote the cooperation between them and to strengthen the participation in exchange networks for information, knowledge, technology, and commercialization.

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Colegio de Postgraduados

Design of an interpretive trail and conservation of native orchids (Orchidaceae) in an anthropized landscape

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ABSTRACT

Objective: to design an interpretive trail of native orchids in the gardens of Campus Cordoba, Colegio de Postgraduados.

Design/Methodology/Approach: this study is descriptive and field based. First, the feasibility of the trail was validated through an online survey. Afterwards, several field trips were conducted; the script was created, and finally the interpretive script of the trail was validated.

Results: of the respondents, 95% considered the orchid interpretive trail as feasible. The inventory of the trail area allowed to verify that the orchids are accessible at all stations along the route. The design of the interpretive trail has nine thematic stations: 0. Introduction, 1. The *Catasetum* trail, 2. *In vitro* germination, 3. Acclimatization of orchids, 4. Miniature orchids, 5. Establishing orchids in phorophytes, 6. The *Laelias* trail, 7. The "May Flowers" trail, and 8. The "Donkey ears" (*Thricocentrum luridum*). The interpretive trail allows anyone to know and appreciate the diversity of wild orchids, 28 species and their phorophytes, along 700 m of walking trails for a 2.5-hours route.

Limitations of the study/Implications: it is a guided interpretive trail.

Findings/Conclusions: The interpretive trail allows us to appreciate the importance of the Orchidaceae family; which is one of the largest families in Mexico and the world through environmental and ornamental importance.

Keywords: pollinators food, conservation, environmental education, phophytes, visitors.

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INTRODUCTION

Environmental education deals with people's relationship with nature in order to become aware of the accelerated growth of the human population, environmental degradation, the overexploitation of natural resources and the extinction of animal and plant species, in order to achieve informed citizens who know and solve the various environmental situations (Pellegrini, 2009). There are different mechanisms for providing environmental education. The interpretive trail is among them, which aims to communicate about the importance of the conservation of the cultural and natural heritage of a site. For this reason, information is prepared beforehand so that tourists can strengthen their social, historical or environmental culture. As any case as it

may be, through recreation, thus facilitating sensations, emotions and awareness in visitors (SECTUR, 2004; Phillips *et al.*, 2014).

Public parks and green areas of educational institutions can be a good setting for the implementation of interpretive trails, for the awareness of students and the general public. For example, at the Simón Bolívar University, in Venezuela, an environmental education program was designed with several interpretive trails, called Guided Didactic trails, among them, one trail is "The parts of the plant: similarities and differences" (Pellegrini Blanco, Reyes Gil, & Pulido, 2007). At the University of Quintana Roo, Campus Cozumel, Mexico, plant species were classified according to ethnobotanical use, as the interpretation factor for interpretive trails (Chan-Quijano and López-Mejía, 2012). At the Luis A. Martínez Institute, in Riobamba, Ecuador, it was proposed to create an interpretive trail as a pedagogical strategy to promote the use of existing agricultural and natural resources (Calero *et al.*, 2019). For these reasons, public and private gardens with orchids have a good educational potential, through interpretive trails.

Based on the aforementioned, this proposal is intended as a guide for the design of an interpretive trail, where the knowledge of the landscape of anthropized and peri-urban areas is integrated. So that it can be applied in all sites that have biodiversity and culture such as educational centers, cities, municipalities, parks, walking trails, gallery landscapes with *Taxodium* trees, public and private gardens. In a way that, through interpretive trails, the interpretation and learning of what is to be preserved is achieved, composed of elements in the landscape (flora, fauna, bodies of water and culture).

The Orchidaceae family is one of the three most numerous globally (Cartay, 2020), with around 35 000 species (Dressler, 1993); Mexico ranks twelfth, with 1315 species (Solano-Gómez *et al.*, 2019). This family is characterized by having flowers of various sizes, shapes, and colors; they are so attractive that they attract pollinators that facilitate fertilization and seed production (Hágsater *et al.*, 2015). However, human activity has placed this plant family as the second most threatened, after cacti (Alvarado-Martínez, 2012). For example, there are 187 threatened species in Mexico, out of which 74 are endemic (NOM-059-SEMARNAT-2010).

Based on all the above, it was considered pertinent to design an interpretive trail "The Eden of Native Orchids" in the gardens of the Colegio de Postgraduados, Campus Cordoba; with a focus on conservation and interpretation of botanical concepts for the course "Conservation and Sustainable Use of Flora" of the Master's Degree Program in Landscape and Rural Tourism, which can also be used for informative courses on orchids. The objective of this study was to design an interpretive trail of native orchids with a focus on conservation and interpretation of botanical concepts in the gardens of the Colegio de Postgraduados, Campus Cordoba.

MATERIALS AND METHODS

Materials

The site of the intervention is located in Colegio de Postgraduados, Campus Córdoba; which is located at km 348 of the Mexico-Veracruz Federal Highway, in the municipality of Amatlán de los Reyes, Veracruz, Mexico, at the geographic coordinates 18° 51' 21" N,

96° 51' 35" W, at 627 m altitude. The climate is warm and humid, with abundant rainfall in summer (INEGI, 2008), an average annual temperature of 20 °C (INEGI, 2007) and 1900 mm as average total annual rainfall (INEGI, 2006).

The total extension of the Campus is 57 hectares (ha), with 1.38 ha of buildings with administrative offices, classrooms, cubicles, pilot plants, service areas, greenhouses and laboratories. Buildings are surrounded by gardens, paths and experimental and production areas for coffee, lemon and sugar cane crops, among others. In the 2 ha of gardens, some representative specimens of the native vegetation of the Tropical Lowland Forest are preserved, with secondary and introduced vegetation. The population of the Campus is around 150 people, including undergraduate students who are involved in professional stayings or social services, thus occupying the facilities for about 8 hours a day, mainly from Monday to Friday.

Methods

The design of the interpretive trail was based on the concepts proposed by Ham (1992), SECTUR (2004) and Baltazar *et al.* (2014), in four sequential phases: I. Description of the study area, II. Online survey, III. Trail design, and IV. Validation of the interpretive script for the trail, as a tourist activity. Based on the latter, a descriptive and field approach was used, with five routes, as explained below.

Stage I. *Description of the study area*. Explicitly, three exploration visits were made to the gardens of the Campus to identify the characteristics of the feasible space for the interpretive trail: extension, type of vegetation, species of orchids and their hosts, ease of access for people with disabilities, and features with interpretive opportunities of native orchids.

Stage II. *Electronic survey*. With the application of Google Drive Forms, the Campus community was surveyed to find out their interest in the feasibility of an interpretive orchid trail at the Campus.

Stage III. *Design of the interpretive trail*. Based on the concepts proposed by Ham (1992), SECTUR (2004) and Baltazar *et al.* (2014), five tours were made with the following activities:

1 Identification of the trail route. After having inventoried the presence of orchids, the route was defined.
2 Inventory of natural resources. An inventory of 28 species of epiphytic orchids and their phorophytes (hosts) was done in several routes to support the central idea of the trail and to establish the different stations.
3 Determination of interpretation stations. Once the interpretive features were identified (concepts: diversity, biology, botany and reproduction of orchids, pollinators and trail conservation), the interpretation stations on the trail were selected.
4 Map of interpretive resources. The selected area was plotted and the length between stations and the total length of the trail were measured using a GPS device.

5 Definition of the type of trail. According to the interpretive information of the study area, it was determined that this trail is guided.
6 Preparation of interpretive texts. From the compilation of bibliographic information on biology, pollinators and conservation of orchids, on the chosen interpretive features, the corresponding texts are elaborated, which will be deployed with interpretation techniques such as the invitation to participation, the dramatization and the question.

Stage IV. *Validation of the interpretive script of the trail.*

Once the interpretive script of the guided trail was finished: Interpretive Trail "The Eden of the Native Orchids" at Campus Córdoba" of the Colegio de Postgraduados. The validation of thematic content was done by the students of the master's degree in landscape and Rural Tourism to verify the content, the relevance of the texts, and the titles of each station of the trail. The observations were added to the interpretive script at each of the stations on the trail.

RESULTS AND DISCUSSION

Description of the study area

The gardens of the Campus are distributed in an area of 2 ha of native, secondary and introduced vegetation. Tree cover is around 50%, with tree species from Mesophyll Forest and Tropical Lowland Forest such as soursop (*Annona muricata*), ash (*Fraxinus* sp.), native avocado (*Persea* sp.), mamey sapote (*Pouteria sapota*), Mahogany (*Swietenia macrophylla*), Golden rain (*Tecoma stans*) and Mulatto wood (*Bursera simaruba*) and newly established species such as jicaro (*Crescentia cujete*), chicozapote (*Manilkara zapota*), cacao (*Theobroma* spp.) and exotic species such as cashew (*Anacardium occidentale*), neem (*Azadiractha indica*), mango (*Mangifera indica*) and lemon (*Citrus* × lemon) (cf. Baltazar-Bernal *et al.*, 2020). The garden area is home to a collection of 28 native orchids, actively reforested with native tree species.

Survey

Before designing the trail, an online survey was conducted within the Campus community, using Google forms and obtaining 30 responses. The results of the survey showed a general trend around the feasibility of the orchid interpretive trail (95% consider it feasible). Regarding the impact on the knowledge and care of orchids (95% were in favor); the diversity of the flora (90% considered it as a characteristic); the knowledge of orchids (60%) such as the May Flower (*Oncidium sphacelatum*) (45% know it); Donkey's ear (*Trichocentrum luridum*); laelias (*Laelia anceps*) (20% know them); and others (15%).

Interpretive Trail Design

Between 2021 and 2022, five guided tours were carried out by students and two teachers to identify the stations of the trail; to take photographs and notes for description of the orchid species with scientific name, the distance walked, and the time spent during the entire interpretive trail. The interpretive trail has a distance of 700 m and 9 stations that

can be covered in 2 hours and 30 minutes, in a semicircular route that has infrastructure for people with disabilities, and it is feasible to walk throughout the year (Figure 1).

Trail Stations

There are nine stations on the trail, the description of which is set below:

Station 0. Introduction

Welcome to the Interpretive Trail "The Eden of the Native Orchids of Campus Cordoba". During the tour, please pay close attention for you to learn a little more about orchids: a plant family known botanically as Orchidaceae, which is one of the largest in Mexico and the world. Surely, they are very attractive to you due to size and color diversity of their flowers. During the tour, we shall recognize 28 species of orchids (Table 1), some of which are threatened by extinction risk. We will make eight more stops, along 700 m in approximately 2.5 hours on foot. The interpretive trail stations and resources that we are going to use are described in Table 2.

As we move on to the next station; Do any of you want to reflect on the importance of orchids? Which ones do you know? and What do you know about them?

Station 1. Catasetum walk

At this first station, there is an abundance of orchids of the genus *Catasetum* (Figure 2) such as *Catasetum integerrimum* which, as you can see, established naturally in the Robeline palm (*Phoenix roebelenii*), 3 to 4 m height. That medium-sized epiphytic orchid is *C. integerrimum* that blooms here from May to September and lives in the Tropical Lowland Forest on the slopes to the Gulf of Mexico, up to Nicaragua. It is one of the most numerous species here on Campus, tolerating direct sun, shedding its foliage from January to March, and featuring both male and female flowers (Figures 3A and 3B).

You should know that the flower of orchids has three sepals, two petals and a labellum that serves to attract pollinator insects that, when looking for nectar, move the anther that

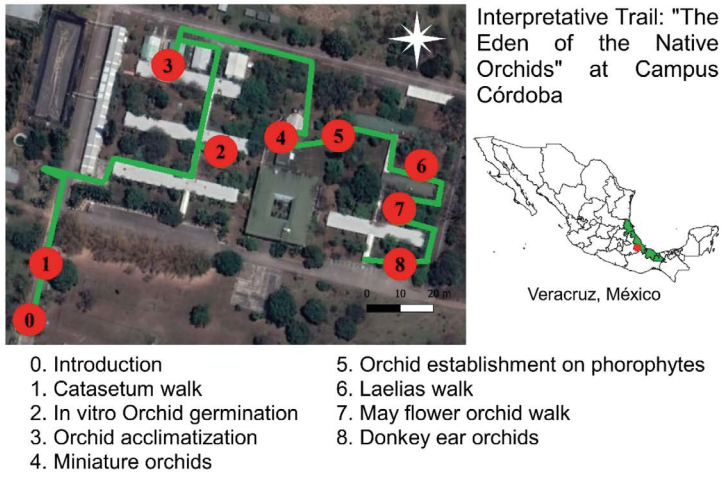

Figure 1. Interpretive trail the Eden of the native orchids at Cordoba Campus. Veracruz, México. Source: elaborated by the author, based on a Google Earth image.

Table 1. Orchids along the interpretive trail "The Eden of Native Orchids of Campus Cordoba".

No.	Species	No.	Species
1	*Brassia verrucosa* Lindl.	15	**Oncidium sphacelatum* Lindl.
2	**Catasetum integerrimum* Hook.	16	**Platystele stenostachya* (Rchb. f.) Garay.
3	*Comparettia falcata* Poepp. Y Endl.	17	*Prosthechea ochracea* (Lindl.) W.E. Higgins
4	*Encyclia parviflora* (Regel) Withner.	18	*Prosthechea radiata* (Lindl.) W.E. Higgins.
5	**Epidendrum cardiophorum* Schltr.	19	*Rhyncholaelia glauca* (Lindl.) Schltr.
6	*Epidendrum ciliare* Jacq.	20	**Specklinia digitale* (Luer) Pridgeon & M.W.Chase
7	*Epidendrum radicans* Pav. ex Lindl.	21	**Specklinia tribuloides* (Sw.) Pridgeon & M.W. Chase.
8	**Laelia anceps* Lindl.	22	*Stanhopea oculata* (G. Lodd.) Lindl.
9	*Lycaste aromatica* (Graham) Lindl.	23	*Stanhopea ruckeri*
10	**Maxillaria densa* Lindl.	24	*Stanhopea tigrina*
11	*Maxillaria elatior* Rchb. f.	25	*Stelis purpurascens* A. Rich. y Galeotti.
12	*Maxillaria variabilis* Bateman ex Lindl.	26	**Trichocentrum lindenii* (Brongn.) M.W. Chase & N.H. Williams
13	*Myrmecophila grandiflora* (Lindl.) Carnevali, Tapia-Muñoz & I. Ramírez	27	**Trichocentrum luridum* (Lindl.) M.W. Chase & N.H. Williams.
14	*Notylia barkeri* Lindl.	28	*Trichosalpinx ciliaris* (Lindl.) Luer

*Naturally pollinated orchids; Source: elaborated by the author.

Figura 2. The Catasetum walk.

covers the polliniums or pollen containers and discharge it into the stigmatic cavity. Thus, pollinating the flower that will later develop a fruit with millions of seeds to reproduce naturally.

Did you know that 80% of orchids need a pollinator to form their fruit? According to some authors such as Ackerman *et al.* (2023), 67% of the 456 orchid species they studied were pollinated by a single pollinator, 14% by two, and 17% by three or more species (Tremblay, 1992). Pollinators are bees such as *Eulaema polychroma*, wasps, flies, butterflies (diurnal and

Table 2. Stations and interpretive resources of the interpretive trail "The Eden of Native Orchids of Campus Córdoba."

Station/distance	Orchid*/Host	Resources	Message/Expected learnings
0.Introduction	2	Trail brochure Orchid flowers and fruits	Trail presentation Orchids importance
1. *Catasetum* walk	2,7 *Phoenix roebelenii*	Orchid flower Female flower Male flower Catasetum fruit	Orchid flower parts Ochid pollinators Flower manual pollination Flower and orchid fruit development
2. *In vitro* orchid germination 240 m	2	Catasetum fruit cut in half Culture medium Orchid seds	*In vitro* germination Threatened orchids
3. Orchid acclimatization 110 m	1,20,21	Vitroplants Tray with lid and substrate	Importance of orchid acclimatizing
4. Miniature orchids 130 m	20,21,27,11 *Pouteria sapota*	Seedlings on the bark on mamey tree *Specklinia digitale* and *S. tribuloides* in flowering	Naturally established miniature orchids Mamey tree as a phorophyte Threatened orchids
5. Orchid establishment on phorophytes 65 m	5,8,15	Plant with pseudobulbs Plant produced *in vitro* and acclimatized	Pseudobulbs importance Orchid plant division Establishing orchids
6. *Laelias* walk 65 m	1,5,7,8,9,10,11,12, 13,22,23,24 *Fraxinus* sp. *Persea* sp	Avocado and ash trees Established *Laelias*	*Laelia anceps*, *Lycaste aromatica*, *Maxillaria elatior*, *M. densa*, *Stanhopea oculata* and *S. trigrina*. Threatened orchids
7. May flower orchid walk 50 m	2,4,13,15,17,18,19 *Azadiractha indica* *Cedrela odorata* *Persea* sp.; *Yucca* sp.	Neem and cedar trees *Oncidium sphacelatum* *Myrmecophila gradiflora*	Largest orchid species from the trail and Mexico
8. Donkey ears orchids 50 m	14,26,27 *Thuja occidentalis*	Thuja trees *Trichocentrum lindenii* *T. luridum*	Phorophyte *Trichocentrum lindenii* *T. luridum*

Source: elaborated by the author; *Orchids references included in Table 1.

Figure 3. *Catasetum integerrimum*: (A) Female flower, (B) Male flower, and (C) Fruits.

nocturnal) and birds such as hummingbirds, according to Hills *et al.* (1972) and Rodriguez *et al.* (1992). Please notice that, according to Ackerman *et al.* (2023) and Ackerman (1996), a large majority of orchids deceive their pollinators, as they do not provide any reward. The deception is alimentary, or orchids imitate oviposition and mating sites; also, they provoke pseudo-antagonism and even sexual deception.

This is the orchid *Epidendrum radicans* that grows on stones, that is, it is lithophytic and reaches up to 80 cm in height and is distributed from Mexico to Central America and Colombia. Here on the Campus *E. radicans* blooms in full sun from February to April, and is pollinated by the butterflies *Anartia fatima* and *Danaus* spp., according to Wolfe (1987); also, this orchid has formed fruits naturally.

You can now appreciate the importance of pollination for the reproduction of these beautiful plants, some of which take up to 11 months to fruit, according to Baltazar *et al.* (2023). On the Campus, it has been identified that two orchids are self-pollinating, seven have pollinators, and 19 lack pollinators. What to do in the latter case? Well, artificial pollination can be performed. Do you want to learn how to do it? Let's go!

Station 2. *In vitro* orchid germination

We let you know that, as a result of pollination, one to four capsules can be formed per plant (Figure 3C). Then, amazingly, when the fruit ripens and opens, it releases about three million "dust-like seeds" that are dispersed by the wind. Only a few of them will give rise to a new plant in association with mycorrhizal fungi because they are so tiny that they do not have enough nutrient reserves and because they have a very tiny and undifferentiated embryo, according to Hágsater *et al.* (2015).

Please note know that the rates of symbiotic germination and survival of orchid plants in natural environments are less than 1%, according to Teixeira da Silva *et al.* (2015) and Chen *et al.* (2020), thanks to its association with a mycorrhizal fungus; but in anthropized environments they are much lower. The alternative is *in vitro* germination of those dust-like seeds, in the laboratory of plant tissue culture. Come and see how it is done.

First, the ripe fruit is washed and disinfected in that laminar flow hood. It is then manipulated with those tweezers and cut with the scalpel to extract the seeds (Figure 4A).

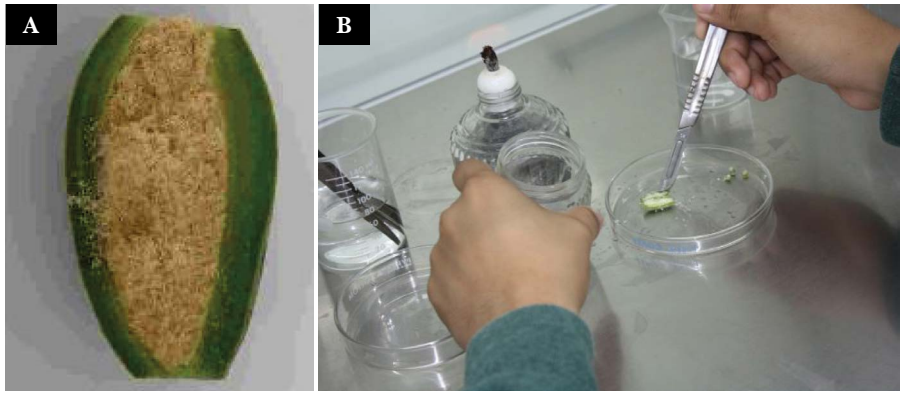

Figure 4. *In vitro* reproduction of orchids. (A) Fruit of *Catasetum integerrimum* cut in half, (B) Handling of orchid fruit in the laminar flow hood.

The seeds are then placed in the Murashige and Skoog (1962) 50% culture medium with 15 grams of sugar using a scalpel (Figure 4B). It will take 45 days for the seeds to germinate in a controlled environment. In this laboratory we have achieved 95% germination of seeds of *C. integerrimum*, *Myrmecophila grandiflora* and *Specklinia digitale*, and 80% with *Lycaste aromatica* and *Oncidium sphacelatum*.

Have you already learned the scientific and common names of the orchids we have seen? Do you remember what percentage of germination is achieved in natural environments? Do you remember Why? Does any of you want to comment something on *in vitro* germination of orchids?

Station 3. Orchid acclimatization

At three and six months after germination, subcultures are made in the laboratory. Afterwards, to acclimatize the seedlings they are moved to the greenhouse to gradually adapt them to the natural conditions of the field, for 12 months. Vigorous *in vitro* developed seedlings are selected, with four well-developed leaves and 5-10 cm tall. They are extracted from containers and placed in tap water. They are washed until the culture medium is completely removed to prevent the development of pathogens (Figure 5A).

The seedlings are then immersed in a Captan solution at 1 g L^{-1} and planted in a plastic tray with sterilized substrate of Chilean Sphagnum moss or Peat moss Premier®, in an autoclave at 120 °C, for 60 minutes and watered to field capacity (Figure 5B). Immediately, the tray is hermetically covered for the first two weeks in 70% shade and at a temperature of 24 °C. Then, trays are opened gradually. Plants are watered manually with a spray bottle and they are covered again. In the fourth week, they are completely uncovered and watered daily by sprinklers and put in 50% shade for six to 10 months (Figure 5C).

Finally, in the last three months, the plants are placed in stress with a 50% reduction in watering, and exposure to 70% sunlight to stimulate the hardening of the leaves and the development of vigorous roots, covered by the velamen, that white tissue that covers the root of epiphytic orchids. Survival rate can be between 40 and 100%.

Why do you consider the acclimatization process of seedlings produced *in vitro* to be important? If the germination rate is 95% and the acclimatization rate is 80%, what is the actual survival rate of orchids produced *in vitro*?

Figure 5. Acclimatization of orchids. (A) Seedling washing, (B) Trays with substrate and (C) Acclimatized *Lycaste aromatica* seedlings.

Station 4. Miniature orchids

This is a perennial Mamey fruit tree (*Pouteria sapota*), native to the Mexican tropics and Central America, up to 50 m tall and 80 cm in diameter at breast height (dbh), with a broad, pyramidal and irregular crown, with simple leaves of 15 to 30 cm (Figure 6A). The bark of the adult Mamey is fissured, which allows mosses and lichens to grow and creates a perfect moistured micro environment for mycorrhizal fungi.

Do you remember that mycorrhizae are essential for the symbiotic germination of orchid seeds? This makes the Mamey tree an excellent natural host (or phorophyte) for orchids.

Seedlings of *Specklinia digitale* and *S. tribuloides*, miniature orchids, have been observed here in mamey bark (Figure 6B). Both species bloom most of the year, as does *S. digitale* (Figure 6C), a threatened orchid, according to SEMARNAT's Official Mexican Standard 059 [2010/2019] (NOM-059-SEMARNAT-2010). It has been observed here at the Campus, that this Mamey fruit tree is the only phorophyte of the miniature orchids. The fruit fly *Drosophila* sp. is identified as pollinator of *S. tribuloides* (Karremans *et al.*, 2015) and possibly also of *S. digitale*, as both orchids form fruit.

Other orchids, hosted by the Mamey tree, are *Platystele stenostachya*, a miniature orchid with light yellow flowers and an orange labellum; *Maxillaria elatior*, a medium-sized orchid with solitary red-orange flowers smaller than 5 cm; and *Trichocentrum luridum*.

Do your remember why the mamey is a good phorophyte for miniature orchids? Why are mycorrhizal fungi important for orchids?

Station 5. Orchid establishment on phorophytes

Orchid plants are divided and established during the rainy season. Plants with four pseudobulbs are used, since it has been seen in *Myrmecophila grandiflora* (Figure 7A) that with a single pseudobulb the plant dies. Also, orchid plants propagated *in vitro* and already acclimatized can be used. In order to promote the anchoring of orchid plants, native

Figure 6. Miniature orchid host: (A) The Mamey fruit tree (*Pouteria sapota*), (B) *Specklinia* sp. seedlings, and (C) *Specklinia digitale* in bloom on the bark of the Mamey tree.

Figure 7. Establishing orchids: (A) Plants of *Myrmecophila grandiflora*, (B) Acclimatized plants of *Lycaste aromatica* and (C) Establishing orchids in the phorophyte.

phorophytes with cracked bark are chosen, and placed at the junctions of the branches that form a V or an L, fixed with thick cotton or henequen thread, with a minimum duration of 6 months, so that the roots of the plant are anchored to the bark of the tree.

During this period, watering should be supplied on days when it does not rain. The plants will reach a height of 15 cm from the base of the pseudobulb to the tip of the largest leaf and their roots will reach 10 cm in length on average, as in *Lycaste aromatica* (Figure 7B and 7C), as indicators of survival. Hernández-García *et al.* (2021) reported they successfully established 30 specimens of *Laelia anceps* in ash (*Fraxinus* sp.) and Creole avocado (*Persea* sp.) with this technique in November 2020 (Figure 8).

Figure 8. Establishing *Laelia anceps* on *Persea* sp.

Let's set up an orchid plant! Does anyone remember the suggestions for the most convenient orchid establishing? What characteristics must a phorophyte have to be considered ideal? What care should be taken in order to establish the plants?

Station 6. Laelias walk

This orchid is popularly known as "Lily of All Saints"; *Laelia anceps* is a medium-sized epiphytic orchid, with floral stalks reaching 80 cm and inflorescences of two to five lilac flowers, highly prized for its beauty. Did you know that *L. anceps* was one of the most widely used orchids in the first hybridization programs in the world? This is an account published by Halbinger and Soto (1997).

At Campus Cordoba, the *Laelias* trail has 40 specimens; *L. anceps* blooms from late October to early December (Figure 9) and occasionally in April. As *L. anceps* is traded in markets in the region, mass harvesting has decreased populations in their natural habitat. According to Baltazar-Bernal *et al.* (2020) *L. anceps* is found in trees and pots in backyards and is pollinated by *Bombus medius*.

This plant you see here is known in Mexico as "little spider"; it is *Brassia verrucosa*, a medium-sized orchid, with inflorescences of more than 12 fragrant flowers, with a bright white labellum and green polka dots, which blooms from June to August. These you see here are *Epidendrum cardiophorum* with small, creamy-green flowers and *Maxillaria densa* that blooms in February. *Maxillaria variabilis* is a small orchid, with yellow or burgundy flowers smaller than 1.5 cm that bloom in autumn, winter and summer. Here you can also see *Stanhopea oculata* and *Stanhopea tigrina* which are threatened orchids according to NOM-059-SEMARNAT-2010. Both species bloom in July, but there is no fruiting. *S. tigrina* is an endemic species that grows in a region of Mexico. This is *Lycaste aromatica* that blooms in April and gives off an exquisite cinnamon fragrance.

Can anyone of you explain what an endemic orchid is?

Figure 9. Specimens of *Laelia anceps* at *Laelias* walk station.

Station 7. May flower orchid walk

This majestic orchid is our "May Flower" also known in México as "Golden rain", *Oncidium sphacelatum*. It is an orchid that develops abundant floral stalks that can reach 1.5 meters in length, with more than 150 yellow flowers with brown spots, which blooms in April and May. Because it is one of the most abundant and most photographed orchids at Colegio de Postgraduados, Campus Cordoba, it has become a main attraction (Figure 10). It is a representative species in the central area of the state of Veracruz, where it is used in religious traditions and as an ornamental plant.

The vast majority of the *O. sphacelatum* specimens on Campus, reproduced after being established in 2017 following the procedure already described, based on the division of specimens. They were located on neem (*Azadiractha indica*), cedar (*Cedrela odorata*), avocado (*Persea* sp.) and Izote [Ee-zo-teh] (*Yucca* sp.) plants (Figure 11).

Figure 10. The Mexican "May Flower" – Lluvia de oro (lit. Golden rain; *Oncidium sphacelatum*).

Figure 11. May Flower walk in one of the gardens at Colegio de Postgraduados, Campus Córdoba.

Damon and Cruz (2006) reported that pollination of *O. sphacelatum* is natural by the pollinator *Centris mexicana*; but this occurs in one flower out of every 5000 on Campus. This orchid has been germinated *in vitro*. As an attractive orchid, *O. sphacelatum* is used in landscape projects, also as *Encyclia parviflora* and *Myrmecophila grandiflora*, which bloom from April to June and March and April, respectively.

Myrmecophila grandiflora is the largest orchid in Mexico and, surprisingly, once the flower is pollinated, the fruit develops and matures in as little as 30 days. So, in one floral stalk you can found flowers and fruits developing at the same time (Figure 12). Those other orchids are *Prosthechea ochracea* and *Prosthechea radiata* which bloom in May-July and July-September, respectively. Does anyone remember which is the largest orchid in Mexico?

Station 8. Donkey ear orchids (*Trichocentrum luridum*)

The thuja (*Thuja occidentalis*) is a small, introduced evergreen tree that reaches 4 m in height, with a trunk up to 40 cm in dbh, with narrow longitudinal striations on its bark, and flattened branches that form a globose crown of dense foliage (Figure 13).

The thuja retains its leaves, which is critical for hosting *Notylia barkeri*, an orchid that blooms from March to April; *Trichocentrum lindenii* which has purplish foliage and 30 cm flower stalks, that blooms in July; and *Trichocentrum luridum* (Figure 14) which has green foliage and flower stalks 1 to 1.5 m in length, that blooms in March and April. Both *Trichocentrum* species are pollinated by bees of the genus *Centris*, according to Carmona and García (2009), to form fruit and reproduce by symbiotic germination.

Can anyone share their thoughts on the characteristics of an ideal phorophyte for orchids? You may begin with those trees we observed, Mamey, Avocado and Thuja, three of the most visited native hosts on the trail.

Figure 12. *Myrmecophila grandiflora* with flowers and fruits.

Figure 13. Thuja (*T. occidentalis*) is host for *Trichocentrum lindenii* and *Trichocentrum luridum*.

Figure 14. *Trichocentrum lindenii* with purplish leaves and *T. luridum* with green leaves.

CONCLUSIONS

With nine stations involving 28 species, 700 m length and 2.5 hours to cover the routes on foot, the orchid interpretive trail is a good alternative to inform the public on the importance, problems of extinction risk, and the reproduction of orchids; since it is possible to achieve with it an active and participatory learning. It is essential to plan, manage, and regulate education tourism activities to reduce their impact on anthropized landscape as it were ecosystem.

The objective was accomplished, to show this design of the Interpretive Trail "The Eden of the Native Orchids at Campus Cordoba" as both, an environmental and ornamental theme. Something similar might be done in public parks in order to raise awareness among students and citizens; thus, going beyond the merely informative to an active social participation, for example with a permanent campaign to repopulate orchids.

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Applied interpolation methodology with GIS used for artisanal fishing zoning in Bahía Magdalena, Mexico

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ABSTRACT

Objective: To limit the artisanal fishing zones in the mirror-still water of Bahía Magdalena, by means of interpolation.

Design/methodology/approach: Social, economic and biological production data were collected through surveys from a sample of 56 artisanal fishing cooperatives. The data are integrated into a relational database with geospatial reference and geostatistically processed with interpolation methods using a geographic information system.

Results: Four zoning maps were established based on the interpolation of the variables target species, fishing effort, extraction volume and sale value.

Limitations on study/implications: The remoteness and difficult access to the study area.

Findings/conclusions: The methodology can be used at the national level to generate a delimitation of the priority zones for artisanal fishing in Mexico, contributing to decision making and management plans that can consider variables of the fisherman's social life.

Keywords: Artisanal fisheries, Krigging, Bahía Magdalena, Zoning.

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INTRODUCTION

Presently, artisanal fisheries represent between 30% and 40% of the global fishing production (Villegas, 2012), while for Mexico they represent 40% of the total fishing production in the country (Ramírez, 2011; Ojeda, 2012). Although the riverbank fishing activity has a fundamental role in the economic production of the country, there are no management plans, structured methodologies and proposals that integrate the factors that intervene in fishing and the analysis of the social subject into a systemic study (Beltrán & Magadán, 2010; Díaz *et al.*, 2013; Ojeda, 2012), in order to recognize the importance of action and transformation exerted on the fishing socio-environment.

The use of Geographic Information Systems (GIS) in the analysis of coastal environments is an area of opportunity in the organization and planning of the productive activity of artisanal fishing. Storing information databases with different variables of importance, the ease of spatial visualization, and the capacity to analyze the results statistically (Meaden & Do Chi, 1996) make GIS a tool of great use for the study of the marine environment in interrelation with the livelihood and production mode of coastal zones and, with this, to generate integral studies.

The development of methodologies for the evaluation of marine resources is necessary, integrating the use of remote sensors based on the potentialities of GIS and promoting the possibility for management and collection of necessary data (Mumbyab *et al.*, 1995). Technological resources must be priority, although it is necessary to recognize, select and incorporate the data that interact in the management of marine resources to the GIS (Pan, 2005), such as the existing relationship between the coast and the marine ecosystem, the interaction between different organizations, communities and social subjects, the establishment of a line of communication that provides understanding between scientific contributions and the livelihood of coastal communities, or the knowledge of the strengths and limitations of the natural medium. The description of a methodological protocol that incorporates local knowledge from the fishing communities (Close & Hall, 2006; De Freitas & Tagliani, 2009), and the economic, productive, biological and geographic factors to a GIS is nowadays a priority need that must be present in fishing management plans in Mexico.

The storage and analysis of the data under the different processes provided by the use of GIS allow obtaining specific and multivariate results that may be presented under different modalities, both cartographic (Silva *et al.*, 2010) and regionalization (Erisman *et al.*, 2011), according to the intention and need of the researcher or study at issue.

For the case of studies conducted in Mexico, there are approaches quite close to the development of integral proposals of data incorporation, as is the case of the Collaborative Science Program (*Programa de Ciencia Colaborativa*, PCC), which integrates local, scientific and common perception knowledge to elucidate the socioenvironmental context in which fisheries develop (Jiménez *et al.*, 2018), and others that make use of the multi-functionality of GIS as a tool for working with and analyzing relational databases.

The objective of this study is geographically zoning, through a methodology of geostatistical interpolation with the use of a GIS, the mirror-still waters known as the Bahía Magdalena lagoon complex (BMLC) using different management variables and handling of socioeconomic resources. The variables related are the impact of cooperative action on the environment, the target species, the geographic limits of the zone, and the economic aspects of sale value and extraction volume of marine resources.

MATERIALS AND METHODS

The working units are the riverbank or artisanal fisheries of the BMLC, which are characterized by making use of small vessels and implementing artisanal fishing arts at a distance from the coast no greater than 12 nautical miles; these fisheries generate 2,502 direct jobs in the surrounding area (Ojeda & Ramírez, 2012). The main legal form in

which the fishermen are associated to perform this activity is the Cooperative Fishing Production Society (*Sociedad Cooperativa de Producción Pesquera*, SCPP) which includes more than 90% of the population that has the legal possibility of gaining access to the extraction of the fishing resource (CONAPESCA, 2012; Villegas, 2012).

A database was created as a census of a representative sample of the artisanal fishing cooperatives. The sample size was obtained through the application of a formula to estimate the proportion of a known population universe of cooperative organizations (Rojas, 1995; Hernández *et al.*, 2010).

$$n = \frac{N * Z^2 * p * q}{d^2 * (N-1) + Z^2 * p * q}$$

From a population of 132 SCPP a random sampling with a total of 56 registered organizations was applied. The data that were collected are of social, organizational character, of impact on work, means of production and commercialization.

The data are integrated into a relational database with geographic references. The database used the ArcView 3.2 software of ESRI and a series of fields are established with specific characteristics to store the information gathered from each segment that makes up the research.

The structure of metadata with which the base study was constructed follows the technical norm recommended by the National Institute of Statistics and Geography based on ISO 19115:2003, where the minimal dispositions for the elaboration of metadata from the geographic data of national interest are established (INEGI, 2015); it is composed not only of the base structure of organization and conformation, but rather uses the resource of geographic location and characterization (Barbosa, 2013).

To generate the results expressed in this study, three vector layers were interrelated: 1) fishing zones, 2) cooperatives, and 3) fishing permits, which were approached under the structure of relational table in a *Dbase* (.dbf) format and linked directly to a geospatial visualizer in *shapefile* (.shp) format, having as a basis a raster *Rapideye* image of the geographic zone with *Enhanced Compression Wavelet* (ECW) format, a resolution of 2.5 meters pixel and color composition of three bands. Each vector layer has the following specific characteristics of information and composition:

1) Fishing zones: The layer has a polygon structure with a trace scale of 1:25,000 and a projection of the geoid WGS84. This vector layer delimits the fishing areas because of their geographic condition and marine characteristics; the polygon area represents 8,030.09 Km^2 of the surface and is divided into three differentiated zones: Pacific coast with 7,203.49 Km^2, estuary and Canals with 151.96 Km^2, and coastal lagoon with 674.64 Km^2.
2) Cooperatives: Layer made up by geographic points on land obtained through GPS geo-referencing with an error range plus/less 3 m. The layer offers the information of identity with regards to the organization of each SCPP, shows 56 fiscal addresses

of SCPPs, and incorporates quantitative and qualitative data of each organization gathering socioeconomic data, environments and livelihood of riverside fishermen.

3) Fishing permits: The layer is composed by vector points in the sea proposed by the fishermen themselves through the use of a participatory methodology where the fisherman, through observation of the satellite photograph and the use of GIS, pointed out and recorded the main fishing points that he approaches to perform his task; it also records verification points collected by joining fishing trips for each of the species registered (shrimp, crab, squid, marine scale, pen shell; *generosa*, *roñosa* and *chocolata* clams; octopus and shark), and data such as the volume permitted and quota granted for each SCPP registered as well as official data present in the permit.

The three vector layers serve as a basis of information so that cartographic results can be presented through the interpolation method, to delimit the fishing area according to four criteria of interest: 1) target species, 2) extraction in kilograms, 3) sale value, and 4) fishing effort.

Kriging interpolation method

The kriging interpolation method is a geostatistical technique (Bosque 1997) (Bosque 1997) that serves to interpolate data and/or create maps, which allows predicting the values of a series of cells (Villatoro *et al.*, 2008; Londoño *et al.*, 2010) from focalized values, assuming that the spatial distribution of the points follows a correlation (García *et al.*, 2010; Murillo *et al.*, 2012; Paredes *et al.*, 2013), "*the advantage they have against other interpolation models lies in including the behavior of the variable in space" (Quiroz 2011: 20) "it is based on the hypothesis that the spatial variations of the variable are statistically homogeneous throughout a surface*" (Ruiz *et al.* 2010: 111).

For the case of coastal zones and mirror-still waters, the cartography of fishing resources is a priority and is part of the objectives of management, emphasizing the spatial aspect of the information based on the capacities of the interpolation models (Ruiz *et al.* 2010).

The base vector delimitation was taken from the "fishing zone" layer, highlighting the differences in areas, and the quantitative information that the layers of cooperatives and fishing permits offer was interpolated on it, to obtain as a result the specialized cartography that shows: 1) the spatiality of the target species, 2) the extraction volume, 3) the value obtained from the sale, and 4) the number of fishing efforts. The layers were interrelated by the closest neighboring process that links the site to be estimated and the data used in the valuation (Emery, 2008); thus, the model offers as a result the projective parameters where the resulting zoning delimits the areas of interest.

The mapping, analysis of geo-referencing data, and interpolation model used a projection system in format of degrees, minutes and seconds with the geoid and WGS84 datum, maximum 25° 19' 12" and minimum 24° 18' 36" latitude North and maximum 112° 47' 24" and minimum 111° 50' 60" longitude West.

The values obtained from the interpolation were reclassified in function of their specificity or quantified range in order to obtain the cartography with differentiated polygons that promote their zoning.

RESULTS AND DISCUSSION

Zoning by target species permitted

The resulting data offer as result the great diversity found in the riverside zone, where three extractive zones can be differentiated punctually with perfectly represented characteristics and impact of target species:

1) The adjacent estuary zone shows a clear impact of the target species crab and mullet.
2) The bay zone shows a diversity of fisheries devoted to the extraction of clams such as *roñosa*, *catarina*, *chocolata* and *generosa*, as well as extraction of shrimp, pen shell, and different species of marine scale, we can affirm that the greatest diversity of target species is extracted mainly from bay waters.
3) The Pacific coast zone provides the habitat for fisheries of shark, marine scale, squid and octopus.

Figure 1 shows that the zoning can be represented based on the target species, where the estuary area is habitat for populations of crustaceans and "minor" marine scale species, and on which the action of trappers has a much higher influence.

The bay area is habitat of bivalve species (species that settle in the seabed) and which thanks to the conditions of bathymetry, salinity and organic matter find the place ideal for their reproduction; this is the place of action of harvesting divers.

In addition to this, the Pacific coast area has target species that carry out migratory processes, so their fishing is seasonal; this zone is the place of action of a much reduced group of fishermen.

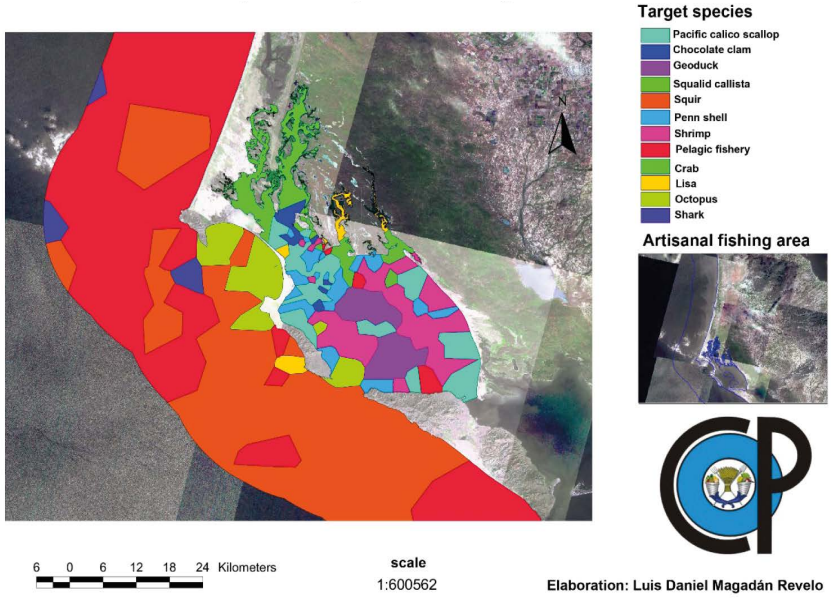

Figure 1. Target species.

Zoning by extraction in kilograms

In this interpolation exercise, the amount of extraction in kilograms found in the study area can be observed (Figure 2), which shows that in estuary zones and part of the Pacific coast is where most of the extraction takes place, which represents an area mostly dominated by a range of extraction between 8,001 and 30,000 kg, presenting small zones that denote areas that reach extraction volumes of up to 99,000 kg of product obtained by cooperative.

Similarly, we can observe that within the bay zone, the average extraction of kilograms of product by SCPP is 1,000 to 8,000 Kg, which represents the smallest value range in terms of extraction.

In the northeastern area of the bay, a strip can be seen that denotes an average extraction of 8,001-30,000 Kg which, compared to the areas with the distribution map per target species, shows that in the zone described there is extraction of estuary shrimp.

Small, focalized points close to the coast can be seen within the same zone of the bay, where there are extractions that exceed 75,000 kilograms, and in these small areas with huge extraction there is fishing of roñosa clam which is found in a phase of incorporation to the labor market of the SCPP.

Zoning by sale value

In economic issues, it can be stated that the main areas of extraction are located primarily within the bay area, where sale values between 1,001 to 7,500 thousand pesos MX are obtained (Figure 3).

Strictly speaking, fisheries of generosa clam and shrimp are the ones that generate higher yields, since the proportion of volume extracted is lower compared to other fisheries; however, the incomes obtained from the extraction of these products are highly profitable due to their high sale value.

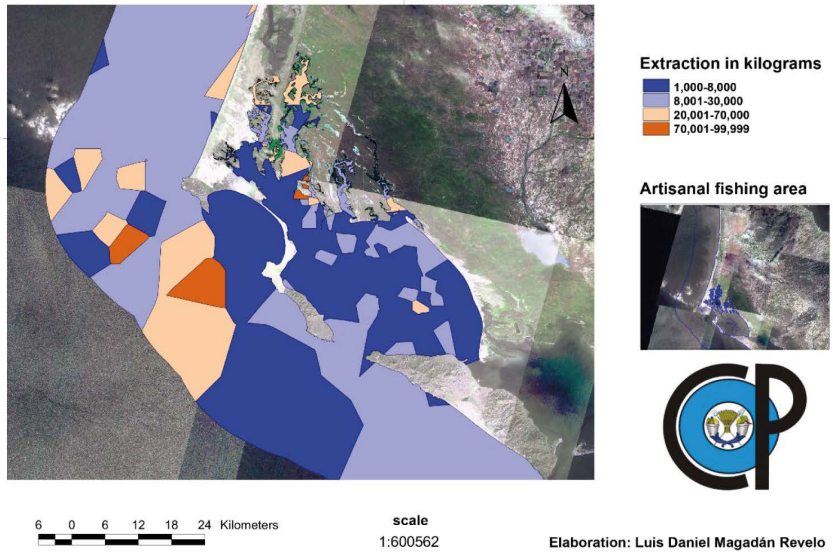

Figure 2. Extraction in kilograms.

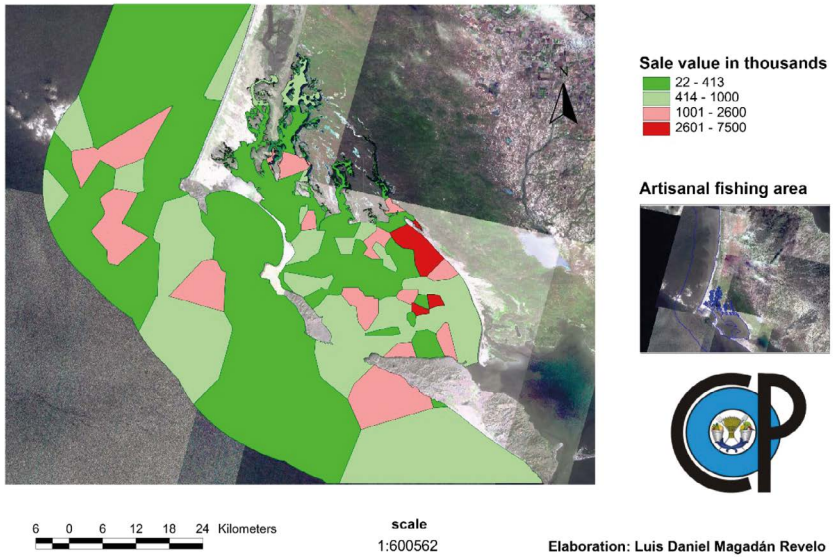

Figure 3. Sale value.

The contrary case can be seen in neighboring estuaries and the Pacific coast, since the proportion of product is much higher than that extracted from the bay zone, since it reports lower sale values. We can affirm that fisheries of crab, marine scale, squid and octopus manifest a directly proportional relation between the extraction volume and the value obtained from the sale of the product.

Zoning by fishing effort

The fishing effort refers to the number of barges or vessels allowed for the extraction of target species (Figure 4). It shows the number of barges there are by cooperative in

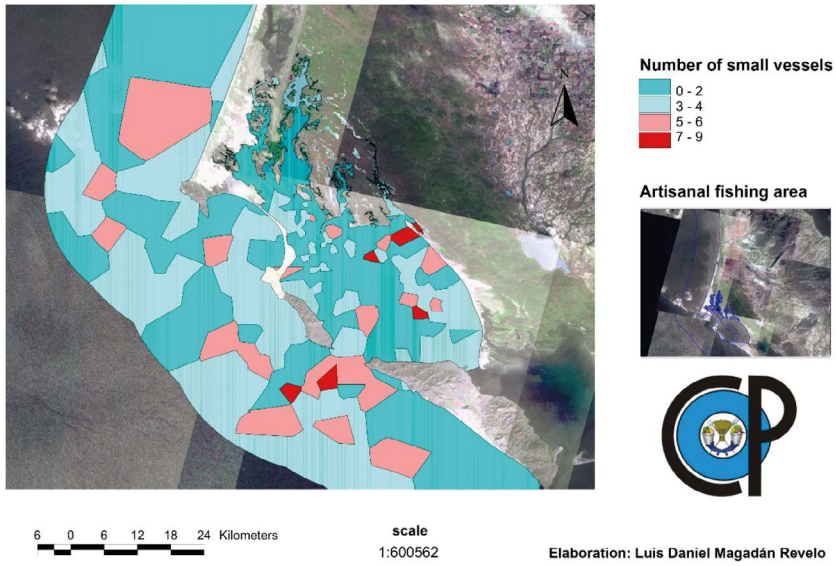

Figure 4. Number of small-scale fishing vessels.

the three different fishing zones (estuary, bay and coast). As can be observed, taking into consideration that the average number of vessels from each cooperative is 4 barges, the zones with the highest number of fishing efforts are located in the Pacific coast, since this is where a greater fishing area is concentrated, with possibilities of 3 to 9 permitted vessels; since this is the zone of migratory species, the institutions in charge of fishing codes offer a higher number of extraction permits.

There is a strip within the bay that shows a number of 3 to 6 barges per cooperative, so it can be inferred that the target species to which this fishing effort is directed is that of estuary shrimp.

On the other hand, a fishing effort of 1 to 2 barges permitted per SCPP can be seen in most of the bay zone and this corresponds to the sedentary nature of the target species that are extracted there, which mostly belong to the group of bivalves.

Meanwhile, in the estuary area we find again a fishing effort of 1 to 2 barges, where the work that is performed does not need greater vessel infrastructure because since it is a zone of crab and minor marine scale, fishing arts such as *chinchorro* and traps are used, which work for prolonged periods of time, allowing 1 to 2 vessels to carry out the work required there.

CONCLUSIONS

The socioeconomic activity of artisanal fishing actively impacts the marine ecosystem but it is important to analyze that the productive-commercial transit of the products obtained is a direct and indirect financial source for a large number of social stakeholders, and this is why in face of management and zoning plans for marine areas, there should be an analysis of the socioeconomic aspects of artisanal fishermen. This interest is a central piece of the study by Rodríguez *et al.* (2015) who describe the need to zone these aspects in order to generate a general action plan jointly, which could have special emphasis in the zoning of the areas of interest presented here.

Applying the interpolation method for the definition and characterization of areas in function of some significant variables or criteria makes it possible to obtain cartographic products of huge interest, not only for the characterization of fishing zones but also for the management, monitoring and planning of actions that can be centered in local needs that can be linked with state and federal initiatives, and thus facilitate the management of marine ecosystems involving the local population in decision making.

The resulting maps from the interpolation process through *kriging* offer the visualization of the working zone in the BMLC, based on socioeconomic perspectives that together with biogeographic data, contribute greatly to the clarification of priority attention areas, future decision making, and for management plans to consider the variables of the fisherman's social life.

This working methodology can be used at the national level and thus generate a delimitation of priority zones of artisanal fishing in Mexico. The reach of future works must consider the universe of SCPPs settled in the localities that are studied, as long as there are the necessary monetary and human resources.

The communication channel of this cartography can be more efficient when it is linked to a web cartographic visor that allows access and free use of the information for people interested in this thematic axis.

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Antifungal potential and chemical composition of *Tagetes lunulata* Ort. essential oil for the control of *Trichophyton rubrum* Malmsten

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ABSTRACT
The essential oils of aromatic and medicinal plants are an important resource used to control several health conditions; however, information about their composition and antimicrobial activity is scarce. This study used a gas chromatography-mass spectrometry (GC-MS) to analyze the composition of the essential oil (EO) of *Tagetes lunulata* Ort., a Mexican endemic plant, known as wild *cempaxúchitl*. The major components of the EO include: verbenone (47.17%), α-pinene (10.93%), 1,1,1-Trifluoro-2-hexanone (9.63%), β-caryophyllene (6.10%), germacrene-D (4.99%), L-verbenone (4.89%), and E-tagetone (4.44%). The disk agar diffusion method was used to evaluate the antimicrobial activity of *T. lunulata* against *Trichophyton rubrum* (athlete's foot). A significant antimicrobial activity was observed with a ≥60% EO concentration. The dilution method was used to determine the minimum inhibitory concentration (MIC): 200 μg ml^{-1}. The *T. lunulata* EO recorded a strong antimicrobial activity against *T. rubrum*; therefore, it is a natural alternative for the control of natural antifungals.

Keywords: Essential oil, *Tagetes*, antimicrobial activity, athlete's foot.

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INTRODUCTION

The use of traditional medicinal and aromatic plants (MAPs) and the isolation of their phytochemical components have allowed the discovery of drugs aimed to control several diseases (Ojah, 2020). Consequently, they are an excellent option to treat some infectious diseases and to control resistant strains (Chouhan *et al.*, 2017). Several studies have proven the antimicrobial efficiency of essential oils (EO) extracted from MAPs against fungi, bacteria, and viruses (Swamy *et al.*, 2016).

Mexico has 23,314 native plant species, 49.8% of which are endemic plants. The Asteraceae family has the highest species diversity (Villaseñor, 2016). Mexico is the center of diversity of the *Tagetes* species, which has shown a biological activity against several

organisms (Barajas-Pérez *et al.*, 2011). However, its chemical composition and its use as a potential antifungal are not fully understood. In addition, information about its effects against dermatophytes that impact human health is scarce. The *Tagetes lunulata* Ort. species belongs to the Asteraceae family and it is endemic to Mexico. It is an annual, wild, and aromatic plant, with bright yellow or orange flowers and a red marking in its base. *Tagetes lunulata* Ort. is commonly known as wild *cempaxúchitl*, *clamol*, *flor de muerto*, or *cinco llagas* and it is found from northern Mexico to Central America, particularly in the central-south region and some states of northern Mexico (Serrato-Cruz, 2009). It is associated with shrubs, pastures, or *Quercus-Juniperus* woodlands (Rzedowski and Rzedowski, 2005), mainly in ruderal vegetation or disturbed fields located at 2,250 and 3,000 m.a.s.l. Serrato-Cruz (2004) has proved its antimicrobial activity against phytopathogen fungi and bacteria, applying aqueous extracts.

The *Trichophyton rubrum* fungus is the most common dermatophyte and it is the causative agent of tinea corporis, tinea pedis (athlete's foot), and onychomycosis. It causes 60% of superficial infections (Graser *et al.*, 2000; Wang *et al.*, 2006). During the last few years, an increase in global infections has been recorded (Arenas, 2002; Hernández-Salazar *et al.*, 2007), as a result of fungi resistance to antifungal medication; in addition, there has been a relapse in the number of cases (Méndez-Tovar *et al.*, 2007). Consequently, seeking new control alternatives to guarantee the elimination of the pathogenic agent and a decrease of the side effects (such as hepatoxicity caused by several drugs) is fundamental. One of these alternative treatments is the use of photodynamic therapy: a combination of photosensitizing agent, an appropriate light wavelength, and molecular oxygen. Although this treatment has been successfully used against several pathogens (Smijs and Pavel, 2011), it is only applied in specialized centers and is therefore unavailable for the general population. Consequently, affordable control alternatives without side effects are required.

Therefore, the objective of this research was to determine the chemical characterization of the essential oil extracted from the flowers of wild *cempaxúchitl* (*Tagetes lunulata*) and to evaluate its antimicrobial activity against *Trichophyton rubrum*.

MATERIALS AND METHODS

EO extraction

The collection of *Tagetes lunulata* was carried out in October, during its flowering stage. The plants were found in agricultural areas, located within the Teuhitli volcano (19° 14' 03.1" N and 99° 01' 02.41" W, at 2,500 m.a.s.l.), in Milpa Alta, Mexico City. The plant material was place in cotton fabric bags and transported to the biological assays with medicinal plants lab of the Colegio de Postgraduados, where they were put on newspaper sheets, in order to divide the inflorescence from the stems and leaves. The botanical identification was carried out at the herbarium-hortorium of the Postgrado en Botánica of the Colegio de Postgraduados. Steam hydro-distillation was used to extract the essential oil from the fresh flowers. A semi-industrial stainless-steel distiller, with a 5 kg capacity, was used to process the plant material for 3 h at 80 °C. The output of the essential oil was determined following the method proposed by Quert *et al.* (2001).

Chemical composition of the cempaxúchitl EO

The chemical composition of the *cempaxúchitl* EO was analyzed through a gas chromatography-mass spectrometry (GC-MS), using a LECO Pegasus® BT 4D (St. Joseph, MI, USA), with a time-of-flight mass spectrometer coupled to an Angilet 6890N network gas chromatograph (Shanghai, China). A 10 m×0.18 mm×0.18 μm HP-5ms (DB5) capillary GC column (phase) (Shanghai, China) was used. Helium was the carrier gas; it had a flux speed of 1 ml min^{-1}. The sample was diluted in methylene chloride. An Agilent 7683B automatic liquid sampler (Wilmington, DE, USA) was used to inject 1 μl of the sample. The mass analyzer was the time-of-flight. Perfluorotributylamine (PFTBA) was used as calibration standard. The C_8, C_9, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, and C_{24} lineal saturated hydrocarbons were used as standards of Kovats retention indexes.

Inoculum preparation

The Mycology Laboratory of the Facultad de Medicina of the Universidad Nacional Autónoma de México (UNAM) provided the *Trichophytum rubrum* dermatophyte. Subsequently, it was cultured in a Sabouraud dextrose agar growing medium and distributed applying the striated technique with an inoculation loop, at 32 °C for 15 d until sporulation. Afterwards, 1 ml of distilled and sterile water was poured into the Petri dish containing the fungus. An inoculation loop was used to scrap the sample. The suspension was then collected using a micropipette and was adjusted with a spectrophotometer, at 0.5 in the McFarland scale, in a saline solution (1×10^6 UFC ml^{-1}).

Antimicrobial activity evaluation

The completely randomized design was made up of 10 treatments (10-100% EO dilutions) and two control treatments (1% terbinafine and distilled water). Each treatment had six repetitions and each repetition was a Petri dish. Dimethyl sulfoxide (DMSO, Sigma Aldrich) was used to dilute the EO. The statistical analysis consisted of an analysis of variance ($\alpha=0.05$ significance level); the SAS statistical package (SAS®, 2013) was used for this purpose. The comparison of means was determined with a Tukey's test.

The antimicrobial activity was evaluated using the disk agar diffusion method, according to modifications made to the method proposed by Khadka (2017) for filamentous fungi. A 6 mm wide filter paper disk was saturated with 10 μL of each treatment. It was then placed in the center of a Petri dish with a Sabouraud medium, which had been previously inoculated with 100 μL of the fungi suspension, adjusted to 0.5 in the McFarland scale, at 32 °C for 15 d. The diameter of the inhibition halo was measured using the ImageJ2 analysis software (Rueden *et al.*, 2017), based on scanning images of the Petri dishes that were calibrated with a scale graduated in millimeters.

Minimum inhibitory concentration (MIC)

MIC was determined using the dilution method, based on the CLSI standard for filamentous fungi (Cantón-Lacasa *et al.*, 2007). Sterile test tubes (11×70 mm) with screw caps and 1 ml of culture medium were used. Different concentrations of EO diluted with DMSO (Sigma Aldrich) were added to the test tubes. Control treatments consisted

of the inoculating medium, terbinafine, and 1% of DMSO. Each test tube contained 9 ml of growing medium, inoculated with 5×10^3 UFC mL^{-1}. One-hundred μL of the EO concentration solutions under evaluation (1600-3.12 μg mL^{-1}) were poured into the said test tubes. Afterwards, the test tubes were incubated at 37 °C for 48 h. Subsequently, a 100 μL aliquot from each tube was taken and read with a spectrophotometer at 530 nm. The MIC was the lowest EO concentration that inhibited fungal growth.

RESULTS AND DISCUSSION

The output of *Tagetes lunulata* EO was 0.11% higher than fresh weight. This result is 10 times higher than the findings of Zarate-Escobedo *et al.* (2018), who reported 0.008-0.01% fresh weight for the *T. lucida* populations. Consequently, this research obtained a good output, considering that it involved a wild species, to which it would provide an added value.

The GC-MS analysis identified 15 chemical components (Table 1), mainly: verbenone (47.17%), α-pinene (10.93%), 1,1,1-Trifluoro-2-hexanone (9.63%), β-caryophyllene (6.10%), germacrene-D (4.99%), L-verbenone (4.89%), and E-tagetone (4.44%). The main chemical component of the *T. lunulata* EO is monoterpene verbenone, which is also the main chemical component (22% concentration) of *T. lacera* (Díaz-Cedillo *et al.*, 2012). Several studies about this terpene recorded antimicrobial activity against gram-positive and gram-negative bacteria, as well as fungi and yeasts (Santoyo *et al.*, 2005; Scollard *et al.*, 2016; Petrovic *et al.*, 2022). Consequently, the recorded verbenone concentration would seem to be the chemical component with the biological properties needed for antifungal activities.

Table 1. Chemical components of the essential oil of the flowers of *Tagetes lunulata* Ort. identified by the GC-MS.

Chemical compound	Retention time (s)	Kovats Retention Index	Relative Peak Area (%)
Verbenone	489.7	1239.4	47.17
α-Pinene	385.9	1035.2	10.93
1,1,1-Trifluoro-2-hexanone	394.4	1051.3	9.63
β-Caryophyllene	568.3	1425	6.10
Germacrene D	591.7	1487.2	4.99
L-Verbenone	484.5	1227.2	4.89
E-Tagetone	447.3	1151.7	4.44
Binapacryl	818.1	2199.9	1.82
allo-Ocimene	434.8	1127.9	1.56
Isopiperitonone	503.4	1271.1	1.54
α-Phellandrene	365.6	997.48	1.54
Phytol	709.2	1826.9	1.52
Cyclobutane, 1,2-bis(1-methylethenyl)-, trans-	380.6	1025	1.47
3,3-Dimethylacryloyl chloride	427	1113.2	1.25
6-Methyl-6-hepten-4-yn-3-ol	442.4	1142.4	1.15

Additionally, the α-pinene monoterpene has shown a strong antimicrobial action against fungi (Rivas da Silva *et al.* 2012). Verbenone is generated by an auto-oxidation process of α-pinene (Lajunen and Koskinen, 1994), which could explain the high concentration of both components in the *T. lunulata* EO. The main components found in *T. lucida* were sesquiterpene germacrene D and β-caryophyllene (Zárate-Escobedo *et al.* 2018), while E-tagetone and allo-ocimene were found in other *Tagetes* species (Muthee *et al.*, 2016; Álvarez *et al.*, 2016; Lizárraga *et al.*, 2017).

Regarding antimicrobial activity, high concentrations (90% and 100%) of the *T. lunulata* EO recorded a total growth inhibition of *T. rubrum*. The 1% terbinafine antifungal control has a similar effect; terbinafine is the conventional drug used to control *T. rubrum*. However, an important antifungal activity was detected with a ≥60% concentration, when >4 cm *in vitro* inhibition diameters were recorded (Figure 1). According to the Duraffourd *et al.* (1986) scale, this result falls within the very sensitive category regarding the antifungal agent. Based on the analysis of variance and the Tukey's mean comparison test (α=0.05), significant differences were recorded between the treatments 60, 70, 80, 90, 100, and control (1% terbinafine). Some of the treatments recorded a zero-standard error, given the total inhibition caused by the antifungal agent. Meanwhile, a total growth within the Petri dish was recorded in the distilled water treatment.

The MIC concentration of *T. lunulata* EO was 200 μg mL^{-1}, the lowest concentration at which the *T. rubrum* dermatophyte did not record any growth. This concentration was lower than the one reported by Lima *et al.* (2009), who recorded 500 μg ml^{-1} for the *T. mendocina* EO used against *T. rubrum*. This result could also be consequence of a high verbenone concentration in the EO. Several studies have proven that the antifungal action mode of essential oils is a result of their capacity to penetrate and break cell walls and cytoplasmatic membranes, which leads to the disintegration of the mitochondrial membranes (Swamy *et al.*, 2016). Consequently, the *Tagetes lunulata* EO has antifungal activity because it breaks the three-layered cell wall of *T. rubrum*, which is made up of

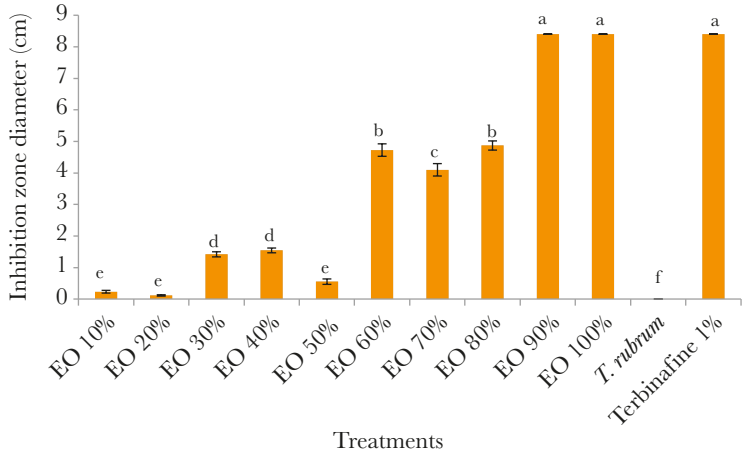

Figure 1. Average diameter of the inhibition halo of *T. rubrum*, recorded at 15 d of exposure to the *T. lunulata* essential oil. Different letters are statistically different (p>0.0001, α=0.05, Tukey). The vertical bars show the ± ES (SE).

β-glucan, galactomannan, and chitin. Additionally, its cell membrane contains ergosterol. New antifungal control alternatives should be focused on the destruction of growing cells and the conidia, which are responsible for the spreading of fungi. Consequently, the antifungal treatment will be shorter and more successful, while the relapse of the infection will be null (Smijs and Pavel, 2011).

CONCLUSIONS

The results suggest that the *T. lunulata* EO is an efficient natural antifungal against *T. rubrum*, proving that it can be used as an alternative to conventional antifungals. Verbenone —the main phytochemical component that provides the EO with its antifungal properties— is the main component of the essential oil extracted from the flowers of *T. lunulata*; therefore, further studies about its capacity to control other type of microorganisms, such as viruses and bacteria, should be carried out. The combination of the main components of *T. lunulata* (verbenone, α-pinene, 1,1,1-Trifluoro-2-hexanone, β-caryophyllene, germacrene-D, L-verbenone, and E-tagetone, which have proven to have antifungal activity) provide the resulting EO with outstanding antimicrobial properties against *T. rubrum*. Further clinical evaluations should be carried out to determine its behavior and its application in the human health sector. In addition, organs of the plant should be studied to determine the chemical composition of their EO. Furthermore, the constitution of the EO extracted from plants from other areas must be established, particularly to determine verbenone concentration, which plays a key role in their antifungal activity.

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Colegio de Postgraduados

Biochemical and functional characterization of milk from alpina and toggenburg goat breeds

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ABSTRACT

Objective: This work aimed to evaluate the biochemical and functional properties of milk from two goat breeds (Alpina and Toggenburg) which could give goat's milk a higher added value and market, making it an attractive option for milk producers.

Design/methodology/approach: Several biochemical analyses were performed to both breed milks: Total Fat (Gerber); Total Protein (Lowry); Lactose (reducing sugars); Fatty acid composition (Mass-mass coupled gas chromatography) and antihypertensive activity (angiotensin-converting enzyme inhibition). To determine possible applications, functional characteristics of yogurt and cheese were also evaluated. Statistical analyses were performed using NCSS software.

Results: Fat content of Alpina breed was higher than Toggenburg's (4.76% *vs.* 2.96%, α=0.00013), as was lactose (8.26% *vs.* 5.37%, α=0.003), while Toggenburg presented higher protein content (5.53% *vs.* 4.77%, α=0.00016). Potential biologically active fatty acids were found in both milks in similar concentrations. Toggenburg milk sowed higher antihypertensive activity than Alpina (100% *vs.* 77.27%), which was maintained and, in some cases, increased, when fermented to obtain different derivatives such as cheese and yogurt.

Limitations on study/implications: Further study is still needed to determine the entire biofunctionality of goat's milk and provide milk producers with options to increase market and added value of their products.

Findings/conclusions: Both, Alpina and Toggenburg goat's milk showed a high Biofunctional potential due to their fat and protein fractions; The fact that biofunctionality can be transferred to derivatives such as yogurt or cheese may increase producers' interest in producing and commercializing it, since the products can be marketed as "functional foods".

Keywords: goat milk, antihypertensive activity, fatty acids, oligosaccharides, functional foods.

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INTRODUCTION

Human beings consume milk from birth to adulthood due to the fact that it is a nutritionally complete food containing water (85-87%), carbohydrates (4.8-5%), and fats (3.8-5%); 5.5% of which comprises short-chain fatty acids, high biological value proteins (2.9-3.5%) as well as vitamins A, D, K, B6 and B12, and mineral salts such as K and Ca (Foroutan *et al.*, 2019). Dairy products emerged over 8,000 years ago and are currently

consumed by more than 6 billion people worldwide (Guha *et al.*, 2021). The increase in world milk consumption has forced consumers and researchers to look for different milk sources to help satisfy the demand. In this respect, goat milk in addition to having similar nutritional characteristics as cow's milk, can provide more essential health benefits production and has shown several bioclimatic and economic advantages (Wadhwani *et al.*, 2023). Currently in Mexico, goat milk (GM) is the second most popular milk with a share of only 1.28% of national production (SIAP 2020). Likewise, some components of goat milk have been attributed to promoting health benefits, as shown in Figure 1. This work aimed to evaluate the biochemical and functional properties of milk from two goat breeds (Alpina and Toggenburg) that could give goat's milk a higher added value and market, making it an attractive option for milk producers.

MATERIALS AND METHODS

Materials: Milks were collected from two different goat farms: Alpine from the herd of La Cabrita ranch, located at Rancho "El Arenal" SN, Tecoyuca, 73306 Chignahuapan, Puebla; and Toggenburg from Santa Irene, Ranch Ejidos, San Luis Huexotla, Texcoco, Méx. After collection milks were separated in sterile jars into 125 mL batches; They were labeled, frozen and stored for further analysis.

Physical-chemical analysis

Protein determination: Protein content was determined according to Lowry (1951).

Lactose determination: Lactose content was determined according to Müller reducing sugars test (DNS, Miller, 1959).

Fat determination: The Gerber method was used. Ten mL of 80% sulfuric acid was added to the Gerber butyrometer, then 1 mL of isoamyl acid was added. Finally, the 10 mL of milk to be analyzed was added and mixed by inversion until the casein was dissolved. It was centrifuged at 1000 rpm for 10 minutes and placed in a 65 °C hot water bath for 5 minutes. Finally, the reading was made on the butyrometer scale.

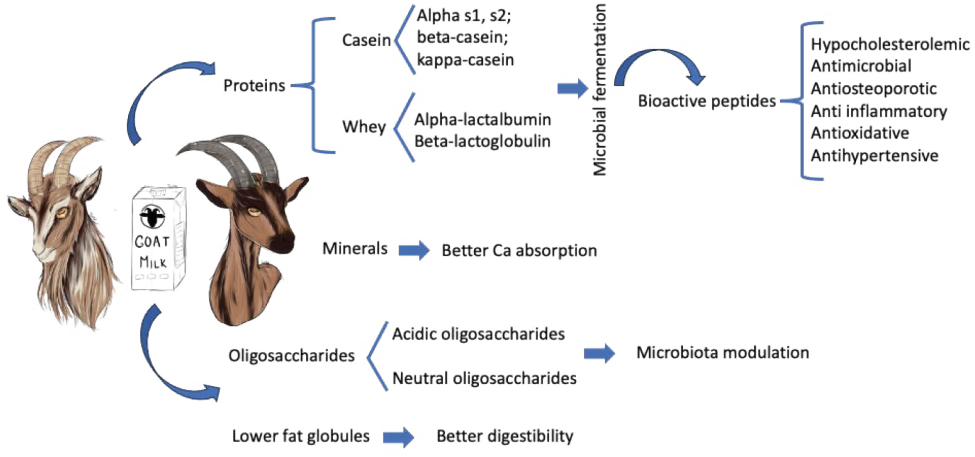


Figure 1. Molecules with beneficial effects of goat milk.

Fatty acid analysis: Fat was extracted by Moubry Technique according to Frank *et al.* (1975). Esterification was performed as established by Piccioli *et al.*, 2019, mixing a 400 mg fat sample sample with 4 mL of hexane; followed by the addition of 200 μL of saturated 2M KOH solution (in methanol). The mixture was allowed to stand for 30 min at 4 °C. It was then centrifuged at 5000 rpm for 5 min, and the upper phase was recovered. It was then injected into a gas chromatograph equipped with self-contained capillary ionization detector (Agilent GC SYSTEM Model 78900B, Santa Clara, U.S.A). An HP-88 column, 100 m×0.250 mm×0.20 μm film thickness (Agilent, Santa Clara, U.S.A) and a temperature ramp (50 °C initial temperature, 1 °C/min to 160 °C, 20 min at 198 °C, 1 °C/min to 230 °C, 15 °C/min, with 250 °C interface) were used. Helium was used as carrier gas and the injection was done in split mode (1:50 ratio). A standard fatty acid mix (Supelco 37 component FAME Mix, Inc., Bellefonte, PA, USA) was used to identify fatty acid methyl esters.

Dairy derivatives processing: Panela Cheese and yogurt were made from Alpina and Toggenburg goat milk. Panela cheese was made inoculating milk with 2% mesophilic lactic starter culture (freeze-dried cultures, DEM3, Centro Sperimentale del Latte) and fermented for 3 hours at 42 °C. Milk was then clotted with natural rennet (1:10000 strength); the curd was cut into 1 cm^3 cubes. It was then drained and molded in a 20 cm diameter circular container. The product was stored at 4 °C until analysis. Yogurt was prepared, pasteurizing milk at 90 °C for 5 minutes, then 5% Yogurt starter culture (freeze-dried cultures, YSC, Centro Sperimentale delLatte) was added as inoculum and fermentation was carried out at 42 °C until a pH of 4.5 was reached. Yogurt was then stored at 4 °C until analysis.

Antihypertensive activity: Alpine and Toggenburg goat milk and cheese and yogurt samples were assayed for antihypertensive activity with a slight modification of the method proposed by Cushman and Cheung (1971). A reaction tube was prepared mixing 80 μL product extract sample with 200 μL borate buffer (0.1 M, pH 8.3); 5 mM Hypuryl-Histidyl-Leucine (HHL, SIGMA Aldrich, USA) solution diluted in 0.3 mM NaCl (JT Baker, Xalostoc, Mexico) was used as substrate. Then 20 μL of rabbit Angiotensin Converting Enzyme (ACE, SIGMA Aldrich, USA) were added to the substrate mixture and incubated for 60 minutes at 37 °C. Reaction was stopped with 250 μL of HACL and 1.7 mL of ethyl acetate (JT Baker, Xalostoc, Mexico). Solution was centrifuged at 4500 rpm for 5 minutes. After which 800 μL of supernatant were evaporated at 95 °C for 30 minutes and resuspended in distilled water. Absorbance was recorded at 230 nm. To determine the degree of ACE inhibition, two standards were prepared: one with ACE enzyme without inhibitor (A, 100% ACE activity) and the other without ACE (C, 0% Activity). The assay was performed in triplicate for each sample. The degree of inhibition was calculated using the following equation:

$$\%\,inhibition\ ACE = \frac{A-B}{A-C} * 100$$

Where: A is the absorbance of the standard with 100% *ACE* activity; B is the absorbance obtained with the hydrolysates; C is the absorbance with 0% *ACE* activity.

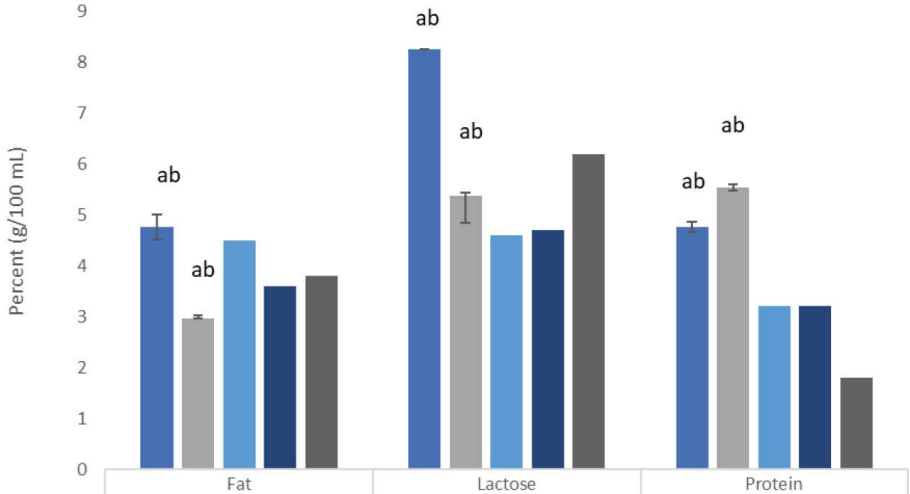
Statistical analysis: All tests were performed in triplicate and statistical analyses were done using NCSS software.

RESULTS AND DISCUSSION

Physico-Chemical composition

Physico-chemical composition of Alpine (AGM) and Toggenburg (TGM) goat milk are shown in Figure 2. AGM contained approximately twice the fat concentration of TGM (4.76±0.25% *vs.* 2.96±0.057%, respectively) and similar to goat milk from stall cattle (SCGM) reported by Kumar *et al.*, (2016) while containing approximately 20% more fat than cow (CM) and human (HM) milk (Wadhwani *et al.*, 2023). Nayik *et al.*, (2021) indicates that a diet rich in natural pastures generates fat and micronutrients rich milk, further suggesting that goats fed and grazed in the mountains with access to green pastures can produce milk in smaller quantities but with high protein and fat content. The fat content may also vary according to the mammal and the lactation cycle since, as the process progresses, the amount of fat decreases, which will have repercussions on the fatty acid content of each species.

Concerning lactose content (Figure 2), it was observed that AGM content is 35% higher compared to TGM (8.26±0.25% *vs.* 5.27±0.52%, respectively) and twice as much compared to SCGM and CM. In comparison, it is only 25% higher than HG; this can be attributed to the fact that AGM milk was obtained from recently parturient goats, and lactose is always higher at the beginning of lactation as, among other functions, it is important to modulate intestinal microbiota during the first stages of life.



	Fat	Lactose	Protein
AGM **	4.76	8.26	4.77
TGM**	2.96	5.37	5.53
SCGM (Kumar et al., 2016)	4.5	4.6	3.2
CM (Wadhwani et al., 2023).	3.6	4.7	3.2
HM (Wadhwani et al., 2023).	3.8	6.2	1.8

Figure 1. Physico-Chemical composition of milk in different mammals. Different letters indicate statistically significant differences at $p<0.05$.

Protein content (Figure 2) in TGM reaches up to 5.53±0.06%; significantly higher (α=0.00) than that observed for AGM SCGM and CM (4.77%, 3.2% and 3.2%) Due to the fact that goats milk undergo a high range of postransductional modifications and rearrangements, and to the high protein concentration, TGM and AGM would be expected to contain a higher concentration of bioactive peptides, increasing their biofunctional potential. The increase in protein concentration in TGM and AGM with respect to SCGM implies an improvement in the nutritional value of milk obtained through free grazing with night supplementation.

Marletta *et al.* (2007) reported that casein fraction of GM undergoes post-translational modifications that derive into changes at the structural level of these fractions, facilitating the release of peptides and their corresponding bioactivity as opposed to CM. This finding increases the added value of the milk produced by this method and opens the door to determining bioactivities such as antihypertensive activity.

Fatty acids profile

GM is a high source of medium-chain fatty acids, highlighted for their functionality because they are present in small fat globules, making them more digestible than the fat globules present in MC (Wadhwani *et al.*, 2023). Table 1 shows the results obtained for fatty acid content in AGM and TGM. When comparing with the data obtained by Verruck *et al.*, (2019), a slight decrease is observed in some short chain fatty acids for example, 33%-↓ butyric (C4:0), 23%-↓ caproic (C6:0), and 19.7%-↓ caprylic (C8:0), which are responsible for some sensory properties of goat milk such as odour and taste; on the other hand, long chain fatty acids show an increase of 17-18.15%-↑ fatty acids in both breeds studied such as stearic (C18:0), Oleic (C18:1) with respect to CM. Some isomers of Conjugated Linoleic

Table 1. Fatty acid content in the milk of different mammals.

Fatty acid	AGM (%)	TGM (%)	GM* (%)	CM* (%)
Butyric ($C_{4:0}$)	1.45	1.38	2.18	3.70
Caproic ($C_{6:0}$)	1.83	1.74	2.39	2.40
Caprylic ($C_{8:0}$)	2.19	2.19	2.73	1.50
Capric ($C_{10:0}$)	6.57	6.57	9.97	3.20
Laurico ($C_{12:0}$)	4.0	4.00	4.99	3.60
Myristic ($C_{14:0}$)	9.25	9.25	9.81	11.10
Myristoleic ($C_{14:1}$)	0.14	0.14	0.18	0.90
Pentadecanoic ($C_{15:0}$)	0.85	0.85	0.71	1.20
Palmitic ($C_{16:0}$)	28.80	28.80	28.0	28.30
Palmitoleic ($C_{16:1}$)	0.85	0.85	1.59	160
Stearic ($C_{18:0}$)	10.83	10.83	8.88	11.80
Oleic ($C_{18:1}$)	23.25	23.25	19.3	18.80
Linoleic ($C_{18:2}$)	2.83	2.83	3.19	1.40
Linolenic ($C_{18:3}$)	0.19	0.04	0.42	0.90
Conjugated Linoleic ($C_{18:2}$)	0.04	0.08	0.70	1.10

* Source: Verruck *et al.*, 2019.

Acid (CLA, C18:2) were observed in AGM and TGM in concentrations higher than those of CM, which would increase milk functionality since CLA has has been reported to decrease risks for cardiovascular diseases and atherosclerosis or inhibit some types of cancer (Verruck *et al.*, 2019).

Likewise, in the AGM and TGM milks analyzed, the presence of vaccenic, α-linoleic and arachidonic, which are considered functional compounds, was observed. Fatty acid composition of milk depends both on the microorganisms found in the rumen and the feeding, so if both goats herds are managed under the same conditions and nearby, it is possible to explain the similarity in fatty acids by these factors.

Antihypertensive activity

Since antihypertensive activity is mostly related to peptides rather than native proteins, milk was hydrolyzed with porcine chymotrypsin emulating the digestive process that would naturally occur in the human digestive tract. ACE-Inhibiting activity was then determined in the hydrolysates using porcine kidney angiotensin converting enzyme (ACE) and hypuryl-histidyl-leucine (HHL) as substrate. In order to give milk producers several options for milk and dairy products commercialization, ACE-Inhibiting activity was also determined in dairy products such as cheese and yogurt to study if the antihypertensive effect could be affected by the fermentation process.

ACE-Inhibition results are shown in Figure 3. Antihipertensive activity observed in both AGM and TGM was high (77.27%±6.74 y 100.00%±3.47 y 69.05, for AGM, TGM and CM respectively. Statistical analysis showed a significant difference between both breeds and species (α=0.0001).

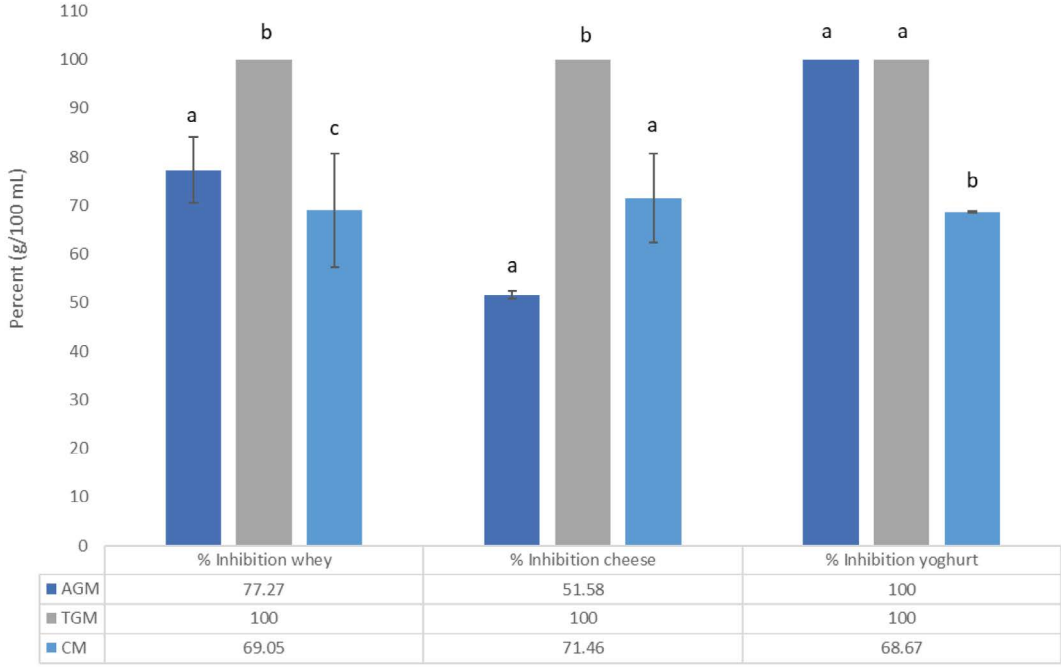


Figure 3. Antihypertensive activity of AGM, TGM and CM derived products. Different letters indicate statistically significant differences at $p<0.05$. Bars with no common letters are significantly different ($p<0.05$).

A slight, but significant diminution in ACE-I activity was observed when analyzing cheeses made with Alpina breed milk, which diminished to reach 51.58%±0.75 (α=0.00). Toggenburg milk cheese maintained a 100% ACE-I activity, suggesting that it was not significantly affected by fermentation with the mesophilic mixed culture (α=0.12). On the other hand, ACE-I of cows milk cheese was determined to be 71.46%±9.1, which is not significantly different from that found for cows milk (α=0.395). Duarte (2021) reported that ACE-I activity of cheese depends on the culture used for fermentation as well as its ripening stage,this is due to the fact that bioactive peptides are produced by enzymatic hydrolysis of proteins during fermentation and/or digestion. The results obtained could be due to the fact that during fermentation the proteolytic system of the microorganisms contained in the culture may have hydrolysed some of the peptidic chains ranging from 300 to 1000 Da, which contain residues of Tyr, Pro y Phe, which have been identified to be of substantial importance for ACE-I activity (Parmar *et al*., 2020).

Lactococcus lactis proteases are highly specific in their cutting sites; hence, slight genetic variations (as between breeds) or post-transductional changes in the primary protein structure may change the affinity of the enzyme for the protein, explaining the different ACE-I activity observed in the cheeses made from both breeds in which proteolysis may have been affected by the primary structure of the proteins (genetic variations) or the steric hindrance given by rearrangements caused by postransductional modifications.

On the other hand, yogurts made with both Alpina and Toggenburg milk showed an ACE-I activity close to 100%, while cows milk yogurt showed am ACE-I activity of 68%±0.29, significantly lower than both goat milks (α=0.0000) but not statistically different from that of cows milk or cows milk cheese, and also comparable to that reported by Vera (2017) who reported a 70% ACE-I activity for cows milk yogurt. Comparing the results obtained for goats milk vs cows milk it is observed that both species have a high potential for commercialization as functional dairy products.

CONCLUSIONS

Biofunctionality of fatty acids, as well as antihypertensive activity in goat milk has been little studied, therefore, these kinds of analyses are of great interest and a beginning for exploration, especially for goats bred in Mexico.

The bio-functional potential of goat's milk is clear considering the functionality of the most abundant milk biomolecules such as fat, and protein fractions. Its high functional fatty acid content, as well as the antihypertensive activity observed which is higher than that of cow's milk, may increase the added value of milk, mostly in the case of Toggenburg breed. The fact that this potential may be transferred to derivative products such as yogurt or cheese may also increase the interest of producers to use welfare care breeding conditions in their herds, since the products may be commercialized as "functional foods."

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Innovation and development of a new snack based on blue corn and grasshopper

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ABSTRACT

Objective: Develop a similar snack to marzipan, with higher nutritional quality by adding protein to provide a healthy consumption alternative.

Design/methodology/approach: A snack made from blue corn flour, grasshopper, agave honey and cocoa was elaborated. The proportions of the aforementioned ingredients were modified in four different formulations. Acceptance tests were conducted with 140 individuals to determine the formulation with the highest degree of approval and laboratory tests were carried out to determine its nutritional quality.

Results: In this research it was possible to develop an innovative nutritional product such as marzipan containing protein, which had a 72% acceptance rate by the participants in the sensorial test. Furthermore, it was managed to use a carbohydrate source from blue corn, which is low on the glycemic index. On the other hand, an attractive packaging design was proposed to capture the consumer's attention, so not only a nutritious snack was developed, but also a marketing package was proposed for its commercialization.

Limitations on study/implications: During the development of this product, no preservatives were used, therefore the shelf life for this product is shorter if no preservatives are added.

Findings/conclusions: A considered functional snack was successfully developed which also has great potential for becoming a distinguished quality product since it is made with local ingredients from the region or country. The product was well accepted; besides it was suggested a packaging for its preservation and distribution.

Keywords: Corn, grasshopper, agave honey, cocoa

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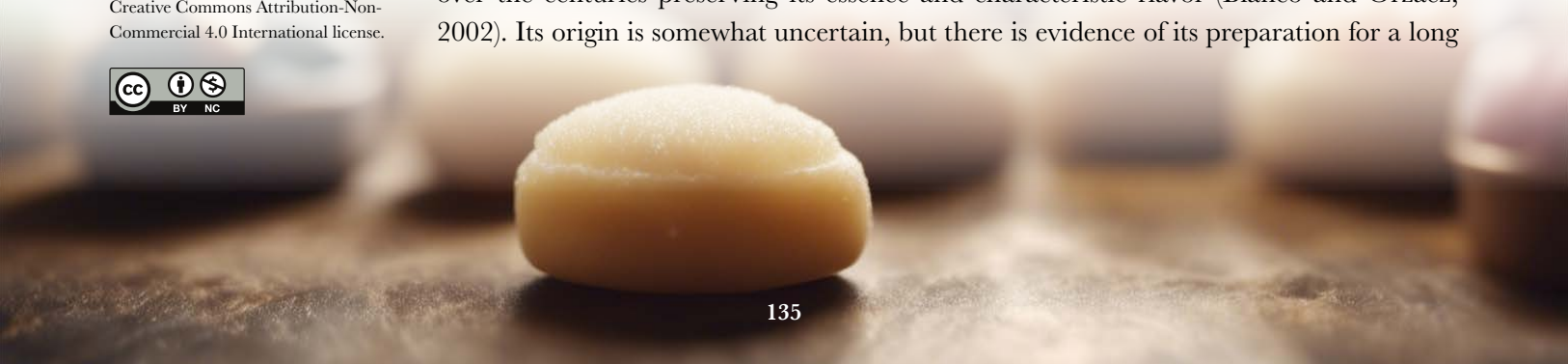
INTRODUCTION

The marzipan is a sweet food product mainly for its high sugar content, it is considered as a dessert. This product is traditionally made from a mixture of almonds, peanuts or nuts and sugars, in the market there are different derivatives of this product that has endured over the centuries preserving its essence and characteristic flavor (Blanco and Orzáez, 2002). Its origin is somewhat uncertain, but there is evidence of its preparation for a long



time, for example, in medieval times, specifically in Venice, it was known as "marzipane", although older records describe it as a preparation based on almonds, sugar and oil carried out by the Arabs in the 10th century. The current term "marzipan" possibly has a Spanish origin, since it is described as a dessert in the festivities of Toledo, where in the absence of wheat, the nuns devised this sweet taking advantage of the sugar and almonds abundance, which were peeled and crushed together with the sugar using wooden mallets in stone mortars, obtaining a mixture that once baked would give rise to the marzipan (Blanco and Orzáez, 2002). The name has been kept in Spain until present, deriving from the word *maza*, in reference to the tool used to crush the almonds, sugar, and bread, which is the product aimed to obtain (Blanco and Orzáez, 2002). Nowadays and due to the different alterations that the product underwent over the years since its arrival to Mexico, such as the substitution of almond flour for peanut flour in order to make the product more economical, marzipan has become a typical Mexican sweet because of its great recognition and acceptance among the population at large.

Mexico is the second country in Latin America with the highest consumption of sweets, where *per capita* consumption was estimated at 4.5 kg from January to August 2018. Marzipan is one of the highest consumed products with a 15.9% increase (Higuera-Albarrán, 2021). Excessive intake of this sweet can result in consumer disorders such as: dental damage, increased appetite and weight, but sugars can also cause the pancreas to produce more insulin, responsible for transforming food into energy, which can lead to obesity, diabetes, among many other negative health aspects (Carbajal-Azcona, 2013). This over-consumption is related to unhealthy weight gain, development of diabetes and hypertension, as well as cardiovascular diseases. Therefore, the purpose of this project was to design, produce and analyze a similar product to marzipan with healthier components. Almonds were replaced by blue corn flour and grasshopper flour, which are the basis of the product, providing a source of carbohydrates and protein. Sugar was replaced by agave honey, which has been reported to have a prebiotic capacity and a low glycemic index compared to other natural syrups and honeys (Mellado and López, 2013), mainly due to the fructans present in agaves, especially insulin. In addition, it was decided to incorporate a touch of cocoa, in order to balance the flavor that could be influenced by the presence of grasshoppers and also to add sources of fatty acids. The idea was to create a product that would be a nutritious food, rather than a candy or a sweet, so that it could be consumed by the population as a healthy product, including those people with conditions related to glycemic control, diabetes, overweight, among other factors, as long as it is not consumed in excess.

To design this product, blue corn (*Zea mays* L.) was incorporated in its formula, offering some interesting nutritional features such as: a lower amount of starch, a lower glycemic index than conventional corn and a protein content up to 20% higher than that of white corn. On the other hand, this type of corn has nutraceutical properties related to its high content of anthocyanins which have a beneficial effect on health, due to their antioxidant activity, reduce mutagenesis and the proliferation of cancer cell growth, and anti-inflammatory, in addition, corn grain anthocyanins have a protective action against nephropathies that develop in patients with type 2 diabetes (Agama-Acevedo *et al.*, 2011).

Regarding the incorporation of grasshopper meal (*Sphenarium purpurascens* Charpentier) this edible insect is a devastating pest in central and southern Mexico (Guzman, 2018), however, if it is collected and used as food it can provide between 55 to 77%, of protein, given that these insects consume plants that have 4 to 14% of protein on a dry basis, it is obtained from 5. 5 to 17.5 times more than the protein content of animal origin combined an excellent quality, contains essential amino acids such as lysine, valine, threonine and isoleucine, it is rich in vitamin B and minerals such as sodium, potassium, phosphorus and calcium, (Ramos-Elorduy *et al.*, 2012; Van Huis *et al.*, 2013; Melo-Ruiz *et al.*, 2015; Aragón-García *et al.*, 2018). This protein is environmentally friendly as the production is less polluting than meat form cattle. Agave honey, from the *Agave tequilana*, provides a high content of fructo oligosaccharides, components that facilitate the proper functioning of the intestinal system, and can provide higher quality energy than refined sugars or industrial sweeteners. The cocoa (*Theobroma cacao*), is a tree that grows in many countries in the Americas, as Mexico. Chocolate is obtained from its seeds and it has been documented that it has more than 300 compounds with diverse beneficial activities for human health, including some with antioxidant activity, hypoglycemic, anti-inflammatory, with potential applications to mitigate diabetes (Kababie-Ameo *et al.*, 2022) was added to avoid the predominance of the grasshopper flavor, besides the fact that it is a source of fatty acids. Due to the characteristics of the raw material used in this product, this development can be considered a distinguished quality product because its manufacture uses raw material obtained in a sustainable and natural way, resulting in high quality products, and it can also be classified as a functional food because it contains anthocyanins that inhibit free radicals, which are known to be involved in the development of certain diseases.

MATERIALS AND METHODS

Formulation desing: This project was conducted in the Department of Food Science laboratories of the Universidad Autónoma Metropolitana-Unidad Lerma. The grasshoppers were recollected in Hidalgo State, México, which were dried in an oven, then crushed and ground in a porcelain mortar. The blue corn was purchased in San Miguel Totocuitlapilco, a town located in Metepec, Edo. de México and it was shelled, toasted, milled and crushed in a porcelain mortar. The agave honey used was of the brand "Tía Ofilia" marketed as organic agave syrup and the cocoa used was purchased at the central supply center, roasted and milled. With these four basic ingredients, four different formulations were developed, varying the proportions of each one of them.

Sensory evaluation: For the sensory analysis of the four proposed formulations, the effective method with a 9-point hedonic scale was applied. In this analysis, the following attributes were measured: flavor, colour, odor, sweetness, texture in the mouth and global acceptance.

Nutritional evaluation: To determine the nutritional quality of the developed product, tests were carried out to determine: carbohydrates, proteins, crude fiber, ashes and humidity content using the methods described in Association of Official Analytical Chemists (AOAC, 1984).

Product name and packaging proposal: In order to have an attractive name to identify this product, the Namelix website was used, which suggests names based on the combination of words that are entered on the website. The circular-shaped snack, silicone molds were used, which were filled with 30 grams of the formulation, then manual pressure was exerted and the blocks obtained were covered with food grade aluminum foil, labeled and stored in boxes.

RESULTS AND DISCUSSION

This project was carried out with the purpose of developing a product that has the necessary features to be considered a nutritious snack, rather than a candy when compared to the traditional marzipan. Since our country occupies the fifth place worldwide in obesity and has the seventh place in diabetes in the world and the second place in Latin America, also on international ranking, it is of great significance that in national government policies a goal is to ensure the population health, by showing the food products labeled information that shows the amount of sugars, fats, sodium and other food components that are above the parameters considered to be healthy and necessary for a wholesome growth and lifestyle, and that are needed to be consumed in the daily diet.

In this project, a similar product to marzipan was designed and produced, using ingredients that make it healthier. Figure 1 shows the general diagram for snack production.

The idea behind the product was to reduce the sugar levels and to increase the amount of protein in order to elaborate a product that had the potential to be considered functional and of differentiated quality. For this reason, local ingredients were used, and because of this, blue corn (*Zea mays* L.) was selected. This type of corn is nutritionally of better quality than white corn due to its nutritional quality, which is up to 20% higher in proteins. It also has anthocyanins, which are key molecules since they are antioxidants that help to degrade free radicals, which are highly reactive and in

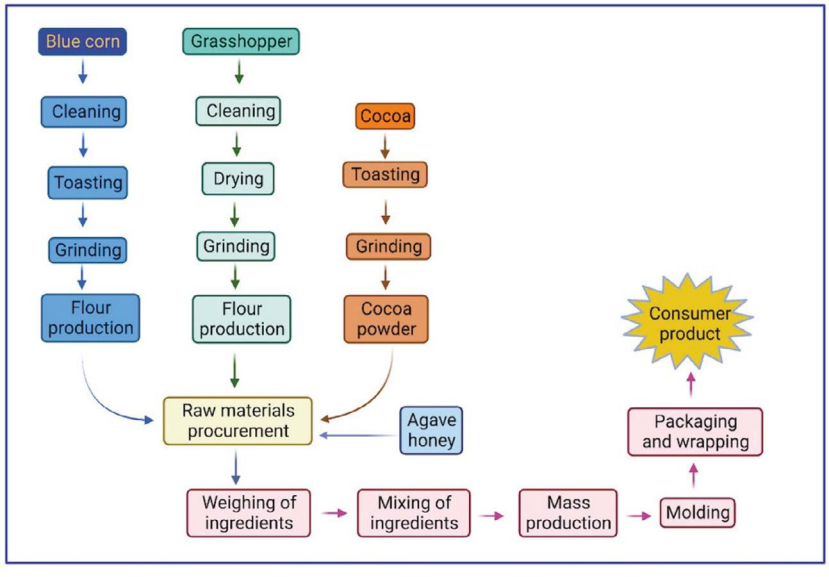


Figure 1. Flowchart used for the production of Mazalín.

extreme situations promote the appearance of cancer cells, therefore, this product can be considered as functional, as it not only fulfills a nutritional role, but it can also help in health protection. Furthermore, the grasshopper (*Sphenarium purpurascens* Charpentier) was chosen as a source of protein, because it was necessary to substitute the excess of carbohydrates in traditional marzipan with a protein source, besides the fact that this insect is deeply rooted in Mexican gastronomy and although its consumption remains popular, it is clear that younger generations in our country do not integrate it into their diet regularly, either by cultural issues, habit or aberration towards insects. In this product, the insect is milled, which is why it is not perceptible to the naked eye. To provide an attractive taste for the consumer, it was decided to use honey from the blue agave (*Agave tequilana* Weber var. azul), which is known to have a sweetening capacity of 1.4 times greater than common sugar, used in sweetening processes, and has a low glycemic index, which prevents increases in blood glucose. Lastly, the fourth ingredient was cocoa (*Teobroma cacao*), employed to balance the product's flavor, as well as for being a source of vegetable fats.

Table 1 shows the following four different formulations that we proposed.

To determine which of the four proposed compositions had the greatest acceptance among consumers, 140 acceptability tests were conducted, used a nine-point hedonic test was carried out allowing a sensory evaluation of the snacks in question; the evaluated categories and the scale used to measure each attribute are shown in Table 2.

From the results it can be seen that the most accepted formula was number 2 with 72.37% acceptance, followed very closely by formula number 1. Formulations number 3 and 4 were in third and fourth position with an acceptance of 67.83% and 67.64%, being

Table 1. Composition of the four different formulations.

Ingredients (%)	Formulations			
	1	2	3	4
Corn flour	63	60	62	69
Grasshopper flour	2	3	4	4
Agave honey	2	3	2	2
Cocoa	33	34	32	25

Table 2. Attributes evaluated using a 9 point hedonic scale.

Attribute	Level of acceptance
Flavor Colour Odor Sweetness Mouth texture General acceptance	1.- I extremely dislike 2.- I dislike it a lot 3.- I dislike moderately 4.- I slightly dislike 5.- I do not dislike or like it 6.- I slightly like 7.- I moderately like 8.- I really like it 9.- I extremely like

almost equal (Figure 2A). Various parameters were determined in the acceptability test such as odor, color, flavor, sweetness, mouthfeel and overall acceptability are presented in a radial plot, Figure 2B. It is observed that the most balanced formulation is formulation two (green). From the six parameters analyzed, five are balanced, odor being one of the weakest, for which it is proposed to use more cocoa or to use another fifth ingredient to improve this sensory characteristic perceived by the sense of smell.

For this reason, the nutritional analyses were conducted on formulation number 2, determining the carbohydrates, ether extract, proteins, crude fiber, ashes and humidity content. The results of the nutritional parameters are shown in Table 3 and reveal that proteins are in higher proportion than in commercial marzipan, these results suggest that the snack created in this project is healthier than traditional marzipan, as it has a higher carbohydrates content. The high protein value present in the product developed in this project is due to the use of grasshoppers, as well as the use of blue corn, which also contributes to increase the protein content. A commercial peanut-based marzipan was used as a control to determine these parameters.

To ensure this product does not only remain in a development stage, it was decided to find a name for its possible commercialization. On the Internet are different websites

A

Acceptance of formulations

Formulation	Percentage of acceptance	SD
1	70.28	0.87
2	72.37	0.41
3	67.83	0.32
4	67.74	0.29

B

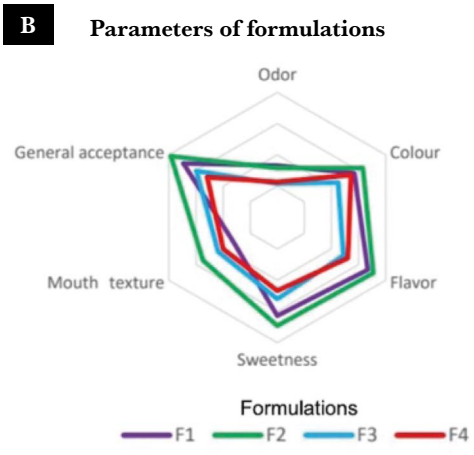


Figure 2. Statistical analysis of sensory tests. Panel A, shows the percentage of acceptance with the standard deviation of the four formulations. Panel B, is a radial plot showing the six parameters evaluated (odor, colour, flavor, sweetness, mouth texture and general acceptance) in the four formulations.

Table 3. Mazalin nutritional content.

Composition %	Control (commercial)	Foremulation 2
Carbohydrates	73.50±0.62	55.3±0.77
Proteins	5.47±0.23	17.03±0.47
Crude fiber	0.19±0.02	3.58±0.03
Ashes	1.02±0.03	2.65±0.41
Humidity	1.56±0.07	1.87±0.09

focused on business, providing names suggestions for newly created products, so it was used the website: http://www.namelix.com and based on the ingredients used, the site provided several alternatives for this product. To select the most suitable name, it was conducted a survey in which 50 people were given the opportunity to taste the product and to choose a name from the five options provided. The 85% chose the name *Mazalín* as they considered it to be the best option, since it is formed with the combination of *mazapán* and *chapulín* (grasshopper). With this progress, it was also considered the possibility of incorporating an attractive design that would give a distinctive feature to the snack, allowing its recognition as well as indicating its composition and nutritional information. It was decided to use food grade aluminum as the packaging to be in contact with the snack to protect it and prevent dehydration. In addition, the product logo label was placed on top and the nutritional information table was added on the reverse side. The snack packaging can be seen in Figure 3. It was also considered to design a box that could transport and contain several snacks while protecting them during transportation and storage, but that would also be eye-catching for the consumer. Different prototypes of boxes were made and it was decided to use the prototype shown in Figure 3.

This box has measures of 15.2 cm long, 11.3 cm wide and 3.4 cm high. The boxes contain 12 pieces. A major feature of this box is that the product can be visualized inside because, although it is mostly made of cardboard, the upper part has a space of 6 cm×10.6 cm cut out and covered with acetate-type plastic, so that the distribution of the snack can be seen inside it. Finally, we worked on a label for the individual pieces in the canva program.

Figure 3. Proposed package for mazalin gold aluminum wrapper with front label. Gold-plated box in which a total of pieces fit.

CONCLUSIONS

In this project a higher nutritional quality product than the traditional marzipan was developed due to the incorporation of blue corn and grasshopper flour, a better source of carbohydrates was obtained because blue corn is of greater quality than other types of corn, besides the fact that it was possible to avoid the use of common sugar, which was replaced by agave honey. The taste of the grasshopper was reduced by adding cocoa, and it also served as a source of vegetable fats, so the product developed is more like a food than a candy. In addition, the ingredients have low glycemic index, such as antioxidant and anti-inflammatory activity, leading the snack to be a functional food. Finally, a packaging and box was designed for its preservation and transportation, which was very attractive to the potential consumer.

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Colegio de Postgraduados

Antioxidant properties of soy-dairy milk blends fermented with probiotics

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ABSTRACT

Objective: Evaluate the effect of the substitution of cow milk with soy beverage on the antioxidant properties, physicochemical parameters, and sensory quality of the probiotic and conventional fermented beverages.

Design/methodology/approach: Different combinations of soy beverage (T1=80%, T2=60%, T3=40%, and T4=20%) with cow milk (20%, 40%, 60%, and 80%, respectively) were fermented with either conventional or probiotic cultures. The antioxidant activity of fermented beverages was evaluated by DPPH method and the samples were also characterized for protein, fat, solids non-fat, density, and acidity. Sensory evaluation was done in order to determine the acceptability of the fermented beverages.

Results: Overall, most treatments fermented with the probiotic culture showed higher ($P<0.05$) antioxidant capacity compared to those fermented with the conventional culture. In contrast, for both starter cultures, it was observed that the T1 treatment displayed the highest ($P<0.05$) antioxidant activity compared with the other treatments (T2, T3, and T4). Similarly, the treatment T1-probiotic culture was the most preferred, being the aroma and appearance, the sensory properties scored with the highest degree of liking.

Study Limitations: Follow-up research is needed to identify the bioactive compounds responsible for antioxidant properties exhibited by fermented soy-dairy milk beverages.

Findings/conclusions: Probiotic cultures can be used to generate soy-dairy milk fermented beverages with noticeable antioxidant and sensory properties.

Keywords: probiotic; fermentation; antioxidant; sensory acceptability; plant-based beverage.

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INTRODUCTION

In recent years, plant-based beverages have gained more attention from the food industry and consumers because they are a good alternative to cow milk, especially for people with lactose intolerance, milk allergies, and prevalence of hypercholesterolemia

(Sethi, Tyagi, & Anurag, 2016). Apart from the foregoing, many consumers are looking for a more sustainable diet with a plant-based lifestyle (*i.e.*, vegetarianism), growing ethical concerns about animal welfare, and the negative environmental impacts associated with dairy production (Mongi & Gomezulu, 2022; Penha, Santos, Speranza, & Kurozawa, 2021).

Among the plant-based beverages available in the market, soy-based beverages are the most consumed because of their nutritive value, especially their higher protein content, and lower price (Sethi *et al.*, 2016; Siddiqui *et al.*, 2022). Despite these advantages, soy-based beverages are characterized for presenting an unpleasant beany off-flavor and grassy aroma, which are generated during their production (B. Wang *et al.*, 2021). Some strategies have been used in order to improve the sensory profile of soy-based beverages including their fermentation and mixing with two or more materials (e.g., plant-based or non-plant-based) (Montemurro, Pontonio, Coda, & Rizzello, 2021; Silva, Silva, & Ribeiro, 2020). These strategies can improve the sensory properties and nutritional composition of the resulting beverage, and also offer the opportunity to develop functional foods with health benefits because the generation of bioactive compounds and the inclusion of probiotics (Marsh, Hill, Ross, & Cotter, 2014). To the best of our knowledge, there are no previous studies reported on the antioxidant activity of fermented soy-dairy milk blends. Thus, the aim of this work was to evaluate the effect of the substitution of cow milk with soy beverage on the antioxidant properties, physicochemical parameters, and sensory quality of the probiotic fermented beverages.

MATERIAL AND METHODS

Materials

Soy-based commercial beverage (Ades, Cola-Cola®) and comercial whole cow milk (Lala®) were obtained at local store in Toluca, state of Mexico, Mexico. Commercial starter cultures (as freeze-dried powders) were obtained from Vivolac Culture Corporation (Greenfield, IN, USA). Chemicals used for the analysis were of analytical grade obtained from Sigma-Aldrich.

Preparation of soy-dairy milk fermented blends

The conventional mixed starter culture for yogurt (Yogurt Dri-Set 442, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*) and probiotic yogurt culture (Bioflora Dri-Set ABY 438, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* ssp.) were activated (0.2% w/v) in commercial pasteurized cow milk under sterile conditions and incubated at 42 °C for 6 h to obtain stock cultures. Different blends of soy-based beverage with cow milk were fermented, which are shown in Table 1. The stock cultures were added (5% v/v) aseptically to 100 mL of pasteurized soy-dairy milk blends in 120-mL sterile bottles. Fermentation was complete when the pH value reached at least 4.2 (5 h), then samples were stored at 4 °C, and all the analyses were performed within 24 h.

Table 1. Experimental combinations of the substitution of cow milk with soy beverage for the production of fermented beverages.

Treatment codes	Soy-based beverage (%)	Dairy (cow) milk (%)
T1	80	20
T2	60	40
T3	40	60
T4	20	80

Physicochemical analysis

The fermented soy-dairy milk blends were analyzed for protein, fat, solids non-fat, and density through milk analyzer device Milkotester Master Eco (Milkotester Ltd, Belovo, Bulgaria). A calibrated digital pH meter was used to determine the pH values of the samples and titratable acidity was measured by titrating the samples with 0.1 N NaOH solution according to the NOM-243-SSA1-2010. All analyses were carried out in triplicate.

Determination of antioxidant activity

Assessment of the antioxidant activity of fermented soy-dairy milk blends was carried out using the DPPH method (Centenaro *et al.*, 2014). A working solution of DPPH (0.1 mM) in 80% ethanol was prepared. A volume of 1000 μL of DPPH in ethanol was added to 2000 μL of diluted soy-dairy milk fermented blends samples (2% v/v), well vortexed and incubated for 30 min in the dark at room temperature. Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, St. Louis, MO, USA) was used as a standard to prepare a reference curve (20 to 250 μM). The results were expressed as micromoles of Trolox equivalents.

Sensory analysis

Two sensory analysis tests were performed on soy-dairy milk blends fermented with conventional and probiotic cultures, 2 days after the production of the samples. Regular consumers of fermented dairy products were recruited at the Metropolitan Autonomous University - Campus Lerma. Panelist pool consisted of university students, lecturers, and employees, aged between 18 and 50 years, 54% female and 46% male. For the sensory tests, first, a preference ranking test with the participation of 22 panelists was applied in order to select the best beverage. Next, the most preferred beverage was evaluated by panelists (N=71) for overall acceptance using a 9-point hedonic scale (from 1="disliked extremely" to 9="liked extremely") evaluating aroma, appearance, flavor, sweetness, and overall liking. Purchase intent, using a 5-point scale (from 5="definitely would buy" to 1="definitely would not buy") was also asked. Panelists were given water and unsalted crackers to cleanse their palate in between samples.

Statistical analysis

The statistical analysis of experimental data was made using ANOVA followed by Tukey's test to compare the results among treatments or Student's.

unpaired t-test to compare the results between the type of starter culture used. Differences were considered to be significant when $P<0.05$. Ranking data of the sensory analysis was analyzed using the Friedman test. All analyses were performed using the Minitab software version 19.1 (Minitab Inc., Pennsylvania, USA). Each experiment was repeated three times.

RESULTS AND DISCUSSION

Physicochemical characterization

The fat, solids non-fat, density, and protein content of the samples were not significantly different ($P>0.05$) among treatments for each culture. However, particularly the acidity showed statistical difference ($P<0.05$) among treatments for each culture, being higher with the increase of the proportion of dairy milk in the treatments. This indicates that lactose, which is converted into organic acids, mainly lactic acid, influences the gel formation (consistency) in fermented milks (Moreno-Montoro *et al.*, 2018). In contrast, in most parameters, there was no difference ($P>0.05$) between the type of starter culture for each treatment, except for the treatment T4, in which the content of solids non-fat,

Table 2. Physicochemical characterization of fermented soy-dairy milk blends.

Parameter	Treatments	Type of starter culture	
		Conventional	Probiotic
Fat	T1	2.40±0.1 a, A	2.53±0.92 a, A
	T2	2.40±0.1 a, A	2.90±1.45 a, A
	T3	2.63±0.66 a, A	2.06±0.75 a, A
	T4	2.80±0.87 a, A	2.46±0.11 a, A
Solids non-fat	T1	7.83±1.80 a, A	6.83±2.15 a, A
	T2	7.70±1.70 a, A	6.80±1.74 a, A
	T3	7.50±1.12 a, A	7.33±1.97 a, A
	T4	7.43±1.05 a, A	8.9±0.81 a, B
Density	T1	27.63±2.83 a, A	30.37±2.80 a, A
	T2	33.70±6.70 a, A	33.03±7.10 a, A
	T3	31.90±7.75 a, A	31.47±6.21 a, A
	T4	26.10±3.75 a, A	36.63±4.51 a, B
Protein	T1	3.96±1.15 a, A	3.20±0.40 a, A
	T2	3.76±0.56 a, A	3.33±0.83 a, A
	T3	3.50±0.43 a, A	3.6±0.40 a, A
	T4	3.16±0.75 a, A	3.8±0.60 a, B
Acidity	T1	0.23±0.004 a, A	0.21±0.012 a, A
	T2	0.31±0.011 b, A	0.30±0.012 b, A
	T3	0.33±0.005 c, A	0.32±0.004 c, A
	T4	0.35±0.009 d, A	0.33±0.005 c, A

Values are mean±standard deviation of three replicates. Different lowercase letters (a-d) indicate statistical difference ($P<0.05$) among treatments for each culture (per column). Different uppercase letters (A,B) indicate statistical difference between type of starter culture for each treatment (per row). Treatments codes are defined in Table 1.

density, and protein were higher ($P<0.05$) in the probiotic culture sample compared to the conventional culture. This could be explained by the microbial activity of the probiotic culture, which is more active than conventional cultures; thus, more production/release of metabolites is expected during their growth in the fermented food matrix (Conway, 1996; Salminen *et al.*, 1998). However, further studies using more sensitive and robust methods (*e.g.*, official and reference methods) such as Kjeldahl (protein content) and Soxhlet (fat content) for physicochemical characterization of soy-dairy milk blends should be done.

Antioxidant activity

Dietary antioxidants are crucial in the prevention of the production of reactive oxygen species and help to reinforce the organism protection mechanism against oxidative stress (Ponnampalam *et al.*, 2022). Our Results (Table 3) show that all fermented soy-dairy milk blends were able to exhibited antioxidant activity ranging from 102.55 to 192.75 μM of Trolox for those fermented with the conventional culture and from 107.45 to 270.20 μM of Trolox for those fermented with the probiotic culture. Overall, most treatments fermented with the probiotic culture showed higher ($P<0.05$) antioxidant capacity compared to those fermented with conventional culture. In contrast, for both starter cultures it was observed that the treatments T1 was those that displayed the highest ($P<0.05$) antioxidant activity followed by T2 compared with the other treatments (T3 and T4). For example, in probiotic fermented soy-dairy milk blends, treatment T1 and T2 showed up to 2.5 times more antioxidant activity than the other two treatments; where the greater the substitution of cow's milk for soy, the greater the antioxidant capacity.

It has been reported that different bioactive components are responsible for the antioxidant activity of both fermented dairy milk and fermented soy-based beverages. For example, fermented dairy milk has bioactive compounds occurring naturally or as a result of microbial activities during the fermentation such as bioactive peptides, exopolysaccharides, fatty acids, organic acids, vitamins, and γ-aminobutyric acid (GABA), which have demonstrated to exhibit antioxidant properties (Fardet & Rock, 2018; Stobiecka, Król, & Brodziak, 2022). In contrast, some studies have shown that fermented soy-based beverages had bioactive compounds with antioxidant properties, mainly phytochemicals such as

Table 3. Antioxidant activity (μM of Trolox) of soy-dairy milk blends fermented with conventional and probiotic cultures.

Treatments	Type of starter culture	
	Conventional	Probiotic
T1	192.75±4.49 a, A	270.20±3.40 a, B
T2	153.53±16.38 b, A	210.39±8.49 b, B
T3	151.57±26.36 b, A	114.31±28.87 c, A
T4	102.55±16.98 c, A	107.45±16.98 c, A

Values are mean±standard deviation of triplicate determinations. Different lowercase letters (a-d) indicate statistical difference ($P<0.05$) among treatments for each culture (per column). Different uppercase letters (A,B) indicate statistical difference between type of starter culture for each treatment (per row). Treatments codes are defined in Table 1.

polyphenols, isoflavone aglycones (*e.g.*, daidzein, genistein, and glycitein), and flavonoids. Moreover, others studies have found that during fermentation, other compounds are present and/or produced including bioactive peptides, and GABA, that exhibit antioxidant properties (de Queirós *et al.*, 2020; Sanjukta, Rai, Muhammed, Jeyaram, & Talukdar, 2015). In addition, it has been found that whole cells of lactic acid bacteria and probiotics possess antioxidant properties (Feng & Wang, 2020; Y. Wang *et al.*, 2017). Thus, with this in mind it is plausible that some of these compounds are present in the fermented soy-dairy milk blends used in this study. According to our results, the antioxidant activity of the samples was higher with the increase of the proportion of soy-based beverage in the fermented blends, which suggested that mainly the compounds produced during the fermentation are derived from soy and are the main ones responsible for the antioxidant properties. However, further studies aimed at identifying of these bioactive compounds responsible for the antioxidant activity exhibited by the samples are necessary.

In similar studies, it has been reported the antioxidant activity of either soy-based beverage or fermented milks, but not on fermented beverages consisting of soy-dairy blends. For example, Tonolo *et al.* (2019) and Marazza, Nazareno, de Giori, and Garro (2012) reported DPPH radical scavenging around 30% of fermented soy-based beverages. On the other hand, Csatlos *et al.* (2023) found values of antioxidant activity of fermented soy-based beverage added with *Chlorella vulgaris* ranging from 301 to 497 μM of Trolox, which are higher that our results. However, these could be due to the addition of Chlorella vulgaris powder.

The comparison of our results from different studies is difficult as the different methods were used or the way the results are presented; our results indicate that fermented soy-dairy milk blends are source of dietary antioxidants with potential health benefits related with this bioactivity. Nevertheless, further work is necessary to carry out *in vivo* studies to test its antioxidant effect in order to identify novel bioactive compounds responsible for such benefit.

Sensory analysis

Overall, it was observed that with increased proportion of soy-based beverage in the treatments, the preference was also increased (Figure 1). The results of total rank sum obtained by Friedman's test show that the treatment T1 (80% soy, 20% dairy milk), for both types of starter cultures, was the most preferred ($P<0.05$) by consumers according to preference-ranking test. Thus, T1-probiotic culture was selected for further sensory evaluation in the acceptance test.

The acceptance test (degree of liking, DOL) indicated that the aroma and appearance were the sensory properties scored the highest (DOL>6.4), whereas flavor and sweetness were the sensory properties scored the lowest (DOL<5.0). In contrast, the overall liking was >5.0. However, it could be noted that the fermented samples were produced without the addition of additives (*e.g.*, sweeteners, flavoring agents, etc.), which could improve their sensory characteristics and improve their overall acceptability. In a similar study, Otolowo, Omosebi, Araoye, Ernest, and Osundahunsi (2022) reported that yoghurt samples prepared with soy-dairy milk blends showed scores ranging from 6 to 8 for color, consistency, aroma,

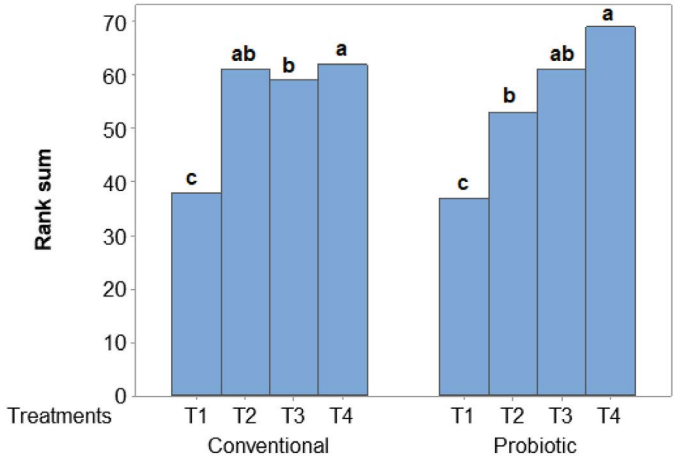

Figure 1. The ranking score of soy-based beverages (N=22). Lower rank sum indicated higher overall acceptance and higher rank sum indicated lower acceptance. Different letters on top of the bars mean significant differences according to Friedman's test. Treatments codes are defined in Table 1.

taste, and overall acceptability indicating 'like slightly' to 'like very much" on the 9-point hedonic scale. These sensory attributes were rated higher in comparison with our study, possibly as a result of flavoring agents being added to the fermented products in that study. However, Jimoh (2020) indicates that the addition of flavoring agents (*i.e.*, banana

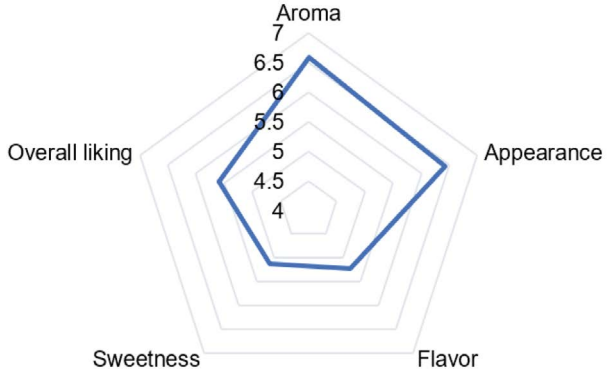

Figure 2. Sensory acceptance results for the fermented soy-dairy milk blend T1 (80% soy and 20% dairy milk blend) obtained from 9-point hedonic scales. N=71.

Table 4. Purchase intention of selected fermented soy-based beverage T1.

Scale	Score	%
Definitely would buy	9	13
Probably would buy	17	24
May or may not buy	28	39
Probably would not buy	6	8
Definitely would not buy	11	15
Total	71	100

puree) to dairy yoghurt or soy-based yoghurt decreased significantly their scores on color, appearance, taste, aroma, mouthfeel, and overall acceptability compared with those samples without the banana puree.

Regarding purchase intent, 37% of the panelists indicated that they would buy, whereas 39% said that they may or may not buy the selected soy-based beverage T1. Only 23% of the panelists indicated they would not buy the fermented beverage.

These results indicate that the development of soy-dairy milk blend fermented with probiotics may be an interesting product for consumers because it has some desirable sensory characteristics, but it is necessary to improve the formulation of this beverage.

CONCLUSIONS

To the best of our knowledge, this is the first study that reports the antioxidant activity of fermented soy-dairy milk blends. Overall, fermentation with the probiotic culture improved the antioxidant capacity of the soy-dairy milk blends compared to those fermented with the conventional yogurt culture, which is interesting and important because of the additional health benefits offered by the consumption of probiotics. Besides, it was observed that the antioxidant activity of samples was related with the proportion of soy-based beverage in the fermented blends, which represents an opportunity to develop plant-based functional foods. However, further studies are needed to determine the bioactive compounds present in soy-dairy milk blends responsible for the antioxidant properties.

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Nutritional characteristics of different types of eggs

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ABSTRACT

Objective: To analyze 5 types of poultry eggs (chicken, turkey, ostrich, duck and quail) to compare their nutritional characteristics.

Design/Methodology/Approach: A physical analysis was performed: weight of the entire egg (weight and proportion of the albumin, yolk and shell) length and width of the entire egg, shape index, shell color, and yolk color, nutritional (determination of raw fat, protein, dry matter and ashes). Different types of egg used: chicken, turkey, ostrich, duck and quail.

Results: The egg containing the most amount of protein was that of the duck (13.02±0.46%), while the sample containing the lowest result was that of the ostrich (9.47±0.27%). The type of egg that contained the fattest level was the duck (10.31±0.75%); on the other hand, the type of egg that demonstrated the least amount of fat was that of the chicken egg (8.28±0.39%).

Results/Findings/Conclusion: Even though some physical differences exist in all types of eggs, they are similar and there is minimal variation in terms of their nutritional value. Therefore, these different types of eggs can be applied for consumption as substitutes for chicken eggs and as an alternative source of protein.

Limitations of the study/Implications: Lack of previous research in regard to comparisons of the types of analyzed eggs.

Keywords: egg, chicken, turkey, quail, ostrich, duck, protein, albumin, yolk.

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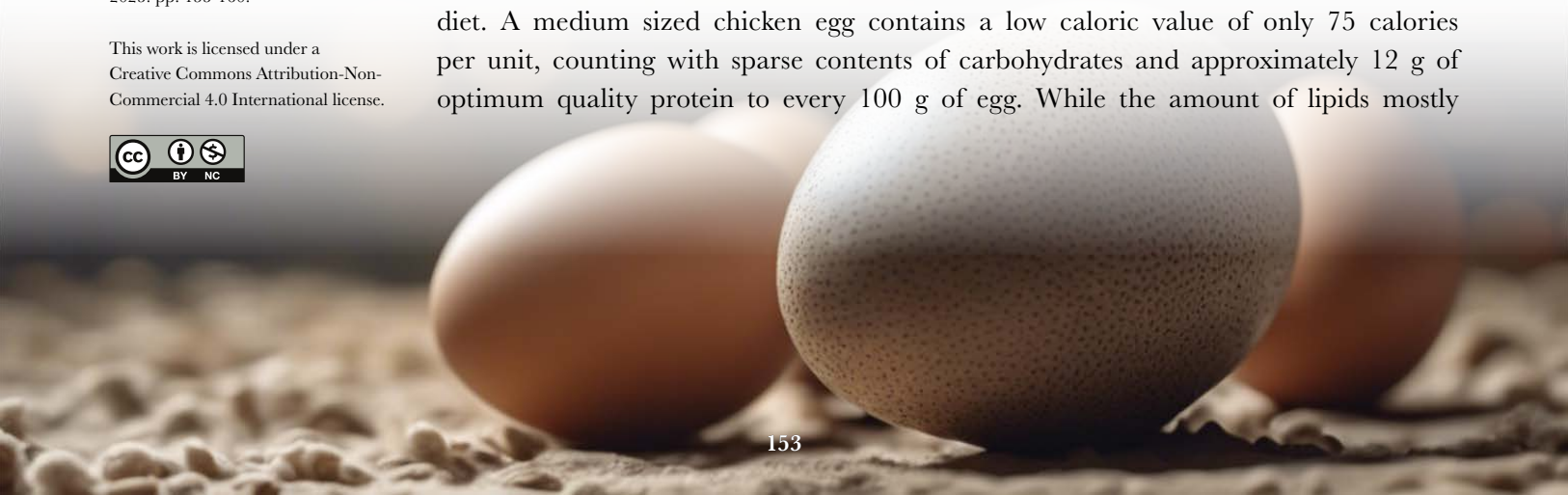
INTRODUCTION

According to FAO (2023), poultry eggs are the most consumed types of food in the world. Demonstrating a vast increase in consumption over the past years due to their demographic growth, urbanization, and increase in income within developing countries. The egg is a nutritious type of food capable of contributing to a balanced diet. A medium sized chicken egg contains a low caloric value of only 75 calories per unit, counting with sparse contents of carbohydrates and approximately 12 g of optimum quality protein to every 100 g of egg. While the amount of lipids mostly



contains monounsaturated fatty acids that contain minimal quantities of saturated fats. At the same time, it constitutes as one of the main sources of cholesterol in a diet, with approximately 220 mg of cholesterol per unit of a medium sized egg (Distillate *et al.*, 2017). Years ago, the egg was categorized as a common type of food that contributed to the increase of serum cholesterol. However, cholesterol has important functions in the human body, such as the development of hormones (testosterone and oestradiol) and at the same time is a precursor of vitamin D and useful bialy salts for digestion and absorption of fats (Hernández *et al.*, 2021). There is also evidence that egg consumption has little to no influence over serum cholesterol levels (López-Sobaler *et al.*, 2017). The egg is made up of three main components as shell, albumin and yolk (González *et al.*, 2018). The egg white is where you will find the highest biological amount of protein, its richness in essential amino acids makes the egg albumin the main protein. Then there are the ova transferrin and ovomucoid, the lysozyme and the ovomucin (Ramírez-Crespo *et al.*, 2022).

On a different note, the yolk is a dispersion of fatty particles in aqueous matter. This is where you will find the highest amount of lipids in the egg, most abundant in triglycerides (66%). Followed by phospholipids (28%, mainly phosphatidylcholine), and lastly cholesterol and cholesterol esters (6%), here you will also find a high carotenoids level, which give coloration (Gonzalez *et al.*, 2018). The most consumed type of egg is chicken, and however, other poultry eggs are also apt for human consumption and function as an alternative source of mainly proteins (these include quail, duck, turkey, and ostrich eggs). Nonetheless, most testing in relation to eggs is centered around chicken eggs, leaving aside the other provident eggs that come from different types of poultry such as the ones mentioned. This is why the objective of this study is to analyze five types of poultry eggs: chicken, turkey, ostrich, duck, and quail to compare their physical characteristics, and their nutritional and sensory properties.

MATERIALS AND METHODS

Material

Different types of eggs were used: chicken, quail, turkey, duck and ostrich. The samples were gathered from the municipal towns of Metepec, Toluca and Amecameca. Parts from each type of egg were freeze-dried to conduct nutritional testing. While the remaining eggs were used for physical testing.

Physical Characteristics

In order to conduct the physical characteristics, 8 eggs of each type were numbered. All with the exception of the ostrich egg which was not evaluated due to the quantity of samples being only two. The evaluated variables were as follows: Weight of the egg (W, g), Length (L, cm), Width (Wid, cm), Shape Index (SI), Volume (Vol, cm^3), Shell Color (SC), Yolk Color (YC), Egg White Weight (EWl, g), Yolk Weight (YW, g), Shell Weight (SW, g), Egg White Proportions (EWPl), Yolk Proportions (YPP) and Shell Proportions (SPP). The variable weight was determined by using an electric scale with a capacity of 65 g ± 0.01 in precision (Model PA64, Brand OHAUS). The L and Wid of each egg were

measured by using a Vernier (Model VER-6PX, PRETUL) with a range of measurement of 0 to 150 mm and 1.0 mm of resolution. The length was determined by the longitude axle of the egg and the width of the transversal axle at mid height of the longitude axle. The SI was calculated using the following Duman *et al.* (2016): $SI=(Wid/L)\times 100$. The variable volume was calculated using the following expression, according to Etches (1996): $Vol=0.913\times$weight of the egg. The color of the yolk and shell were calculated by using the portable equipment MINOLTA (Chroma Meter CR-200), with which the color coordinates were determined as colors L*(luminosity), a* (± red-green) and b* (± yellow-blue). Components EWI, YW and SW were obtained by using an electric scale of 2200 g ± 0.01 in precision (Model H-7294, OHAUD Scout). The weight of each egg was documented, then divided by using an egg white divider which separated each egg component to document weight. Components EWPI, YPP and SPP were calculated according to the weight of the entire egg.

Nutritional Characteristics

The following techniques were applied for the bromatological/nutrimental testing:

- Fat determination: Goldfish Method
- Protein determination: Micro Kjeldahl Method
- Absolute humidity determination: Gravimetric Method
- Ash determination: Gravimetric Method

Statistical Anslysis

A variant analysis was conducted (ANOVA) to observe the differences in nutritional and physical aspects of different types of eggs via the statistical program SPSS (2016).

RESULTS AND DISCUSSION

Table 1 shows the data from the physical analysis. As expected, the weight parameters, length, width, albumin weight, yolk weight, shell weight and volume variate, however no significant differences ($P>0.05$) between types. But, total eggs weights had differences between groups ($P<0.05$), this value was due to the size of the egg. In terms of the length variable, the sample that obtained the least values was that of the quail. Resulting in 3.38±0.23 cm. on opposing ends, the highest value belonged to the turkey egg with a result of 6.45±0.13 cm. Both had significant differences amongst other types, all while finding no significant differences ($P>0.05$) between the chicken and duck eggs. the quail eggs was 2.63±0.1 cm, containing significant differences ($P<0.05$) in comparison to the other eggs, while the chicken, duck and turkey eggs had values of 4.38±0.083 cm, 4.48±0.14 cm and 4.47±0.11 cm, respectively. In terms of the index formula values, the quail and turkey types are significantly different ($P<0.05$) from all samples with results of 78.03±3.46 y 69.39±1.70, respectively. On the other hand, in terms of the variable albumin weight, the quail sample was the only one that demonstrated a significant difference ($P<0.05$), in comparison to the other samples with a result of 6.94±1.25 g also being the lowest result. In terms of yolk weight, all samples were significantly different ($P<0.05$). Shell weight in

quail demonstrated the lowest data (1.41±0.25 g) with a significant difference (P<0.05) in comparison to the other samples. In terms of the albumin proportion, the highest result was 56.98±1.78 %, belonging to the chicken sample. The lowest result belonged to the duck sample with a value of 49.20±3.20%. Samples chicken and quail, and quail, duck and turkey were not significant (P>0.05) amongst themselves. While samples duck and turkey demonstrated a significant difference (P<0.05) in comparison with the chicken sample. The yolk proportions had the highest result was that of the duck egg, which was 37.76±3.34%, while the sample with the lowest value was 29.42±1.46%, obtained from the chicken egg. The chicken and quail, quail and turkey, and duck and turkey samples did not had significant differences (P>0.05) amongst themselves. The shell proportion had the highest data in the turkey eggs with 12.73±1.66%. Nonetheless, this sample did not have significant differences (P>0.05) with the chicken and duck samples. Lastly, in terms of volume variables, the duck sample had the highest result which was 61.89±7.12 cm^3. However, it did not have significant differences (P>0.05) with the chicken and turkey samples. The quail sample had significant differences (P<0.05) with all other types, being the one that had the lowest data of 11.70±1.61 cm^3.

Table 2, demonstrates color data (L*, a* and b*correspond to yolk color from all five test samples. Value L* was highest (P<0.05) in the chicken sample, with 73.71±4.90, where no significant differences (P>0.05) were found between that and the ostrich and duck samples. On the other hand, the lowest result was that of 52.63±1.18, belonging to the quail sample. The values to a*, the highest (P<0.05) data was that belonging to the yolk from the duck eggs and the lowest was from the ostrich sample, with 14.69±5.01 y 2.47±0.34, respectively, Significant differences (P<0.05) were observed between the chicken samples, quail, and ostrich. Even then, no significant differences (P>0.05) between the quail and ostrich samples. In the values of b*, the sample with the lowest value (P<0.05) was that of the quail and the highest was that of the duck resulting in 40.28±1.56 and 73.26±4.95,

Table 1. Chicken, quail, duck, and turkey eggs physical analysis.

	Chicken	Quail	Duck	Turkey
Weight (g)	62.42 ± 2.45^{a}	12.81 ± 1.76^{b}	67.79 ± 7.81^{a}	63.88 ± 8.63^{a}
Length (cm)	5.9 ± 0.11^{b}	3.38 ± 0.23^{c}	6.12 ± 0.34^{b}	6.45 ± 0.13^{d}
Width (cm)	4.38 ± 0.083^{b}	2.63 ± 0.1^{a}	4.48 ± 0.14^{b}	4.47 ± 0.11^{b}
Shape Index	74.37 ± 1.43^{c}	78.03 ± 3.46^{a}	73.35 ± 1.87^{c}	69.39 ± 1.70^{b}
Albumin Weight (g)	35.56 ± 1.70^{b}	6.94 ± 1.25^{a}	33.41 ± 4.91^{b}	32.51 ± 9.28^{b}
Yolk Weight (g)	18.37 ± 1.29^{a}	4.03 ± 0.56^{b}	25.60 ± 3.85^{c}	22.45 ± 1.32^{d}
Shell Weight (g)	7.83 ± 0.37^{b}	1.41 ± 0.25^{a}	7.59 ± 0.66^{b}	8.02 ± 0.55^{b}
Albumin Proportion (%)	6.98 ± 1.78^{a}	53.98 ± 4.70^{ab}	49.20 ± 3.20^{b}	49.97 ± 8.06^{b}
Yolk Proportion(%)	29.42 ± 1.46^{a}	31.76 ± 4.24^{ac}	37.76 ± 3.34^{b}	35.90 ± 6.51^{bc}
Shell Proportion (%)	12.54 ± 0.33^{a}	11.00 ± 1.07^{b}	11.25 ± 0.70^{ab}	12.73 ± 1.66^{a}
Volume (cm^3)	56.98 ± 2.23^{ac}	11.70 ± 1.61^{b}	61.89 ± 7.12^{ac}	58.32 ± 7.87^{c}

[a, b, c, d] Different letters in the same row indicate significant differences throughout (P<0.05) ± means standard deviation.

respectively. The samples belonging to the turkey and ostrich were found to be the only one where no significant differences ($P>0.05$) between them.

Table 3 includes data obtained from the color of the shell from the egg samples that were tested. It is important to note that the values of the quail and duck samples may not be entirely accurate, due to the fact that they do not have a uniform appearance in color with the spots that are presented on both shells.

The L* had the highest value in the ostrich eggs with significant differences ($P<0.05$) from all samples. The a* had a highest value to duck sample and it was similar ($P>0.05$) with the quail and chicken eggs. But the smallest value was that of -0.056 ± 0.20, belonging to the ostrich. The b* value of duck, turkey and ostrich samples had significant differences ($P<0.05$) from all samples, while the chicken and quail had no significant differences ($P>0.05$) between them.

Table 4 shows the data of nutritional analysis. Eggs duck had the highest protein level ($13.02\pm0.46\%$), and the lowest protein level was the ostrich egg ($9.47\pm0.27\%$, $P<0.05$). On the other hand, the quail, chicken and turkey samples had $11.93\pm0.41\%$, $11.73\pm0.39\%$ and $11.41\pm0.35\%$, respectively, without significant differences ($P>0.05$) between them. Meanwhile, the amount of fat in duck was $10.31\pm0.75\%$ and the turkey eggs was $10.17\pm0.18\%$, did not present significant differences ($P>0.05$). On the other hand, the type of egg that demonstrated the least amount of fat was that of the chicken egg ($8.28\pm0.39\%$) without significant differences ($P>0.05$) with the ostrich egg. The ash level was the highest percentage in quail with $4.22\pm0.11\%$, on the contrary, duck ash had $3.74\pm0.13\%$.

The data values for albumin proportion were ~52% for the chicken, duck and turkey. While the yolk proportions were ~34.3 for the chicken, duck and turkey. Both variables, in comparison with the study conducted by Sadaf *et al.* (2022) are similar in terms of the values. However, within this same study, but in the variable shell proportion, the values coming from the duck are higher than the values reported in this research. While the values demonstrated in the chicken and turkey eggs are similar. On the other hand, the shape index of the chicken and turkey eggs obtained in this research were ~71.8, according to Camacho *et al.* (2019), the data from the creole chicken and native turkeys were similar in this study.

The weight, albumin weight, yolk weight, shell weight, albumin proportion, yolk proportion and shell proportion of the eggs coming from the turkey found in this study

Table 2. Color metrics of chicken, quail, duck, turkey, and ostrich eggs.

Type of egg	L*	a*	b*
Chicken	73.71 ± 4.90^{ac}	12.07 ± 4.39^{bc}	62.92 ± 4.73^{a}
Quail	52.63 ± 1.18^{d}	3.90 ± 1.60^{a}	40.28 ± 1.56^{b}
Duck	75.29 ± 4.48^{bc}	14.69 ± 5.01^{cd}	73.26 ± 4.95^{c}
Turkey	64.57 ± 5.25^{e}	12.58 ± 3.08^{bd}	55.51 ± 4.34^{d}
Ostrich	70.28 ± 0.70^{ab}	2.47 ± 0.34^{a}	57.51 ± 0.75^{d}

[a, b, c, d, e] Different letters in the same row indicate significant differences throughout ($P<0.05$)
± means standard deviation.

Table 3. Color metrics of the eggshell belonging to the chicken, quail, duck, turkey and ostrich eggs.

Type of egg	L*	a*	b*
Chicken	55.49 ± 2.07^{ab}	1.65 ± 0.20^{ac}	3.70 ± 0.39^{a}
Quail	18.95 ± 2.07^{d}	2.35 ± 0.20^{ab}	4.74 ± 0.39^{a}
Duck	52.92 ± 2.07^{bc}	1.84 ± 0.20^{bc}	7.64 ± 0.39^{b}
Turkey	55.96 ± 2.07^{ac}	3.27 ± 0.20^{d}	12.56 ± 0.39^{c}
Ostrich	83.14 ± 2.07^{e}	-0.056 ± 0.20^{e}	21.50 ± 0.39^{d}

$^{a, b, c, d, e}$ Different letters in the same row indicate significant differences throughout ($P<0.05$)
± means standard deviation.

Table 4. Protein, fat and ashes in ostrich, quail, chicken, turkey and duck eggs.

Type of egg	% Raw protein	% Raw fat	% Ashes
Ostrich	9.47 ± 0.27^{b}	8.84 ± 0.50^{ab}	4.05 ± 0.7^{ab}
Quail	11.93 ± 0.41^{a}	9.99 ± 0.43^{ac}	4.22 ± 0.11^{a}
Chicken	11.73 ± 0.39^{a}	8.28 ± 0.39^{b}	3.86 ± 0.13^{bc}
Turkey	11.41 ± 0.35^{a}	10.17 ± 0.18^{c}	3.88 ± 0.12^{bc}
Duck	13.02 ± 0.46^{c}	10.31 ± 0.75^{c}	3.74 ± 0.13^{c}

$^{a, b, c, d}$ Different letters in the same row indicate significant differences throughout ($P<0.05$)
± means standard deviation.

were variable. Compared with the results obtain by Galic *et al.* (2018), the weight and weight of the yolk were similar to all data reported in this study. In terms of volume, the albumin weight, shell weight and albumin percentage demonstrated higher values within both mentioned research cycles; while the yolk percentage was less in the study and only the shell percentages were similar within both cycles. These variations may have been due to the conditions and exposure type in which the turkeys were raised (Galic *et al.*, 2018). According to González *et al.* (2018), the reported date for protein and fat in the quail eggs were ~13.11 g/100 g. These data were slightly higher in protein and fat according to this study, while the data by Congjiao Sun *et al.* (2019) had lower protein and ash levels from the chicken, quail, turkey and duck eggs. Few data were found in regard to the ostrich egg, however, research conducted by Al-Obaidi *et al.* (2015), detected 29% lipids and 10.8% in albumin in the yolk of the ostrich eggs. Nonetheless, it is not possible to compare with this research since Al-Obaidi *et al.* (2015) reported separate data in terms of albumin and yolk.

In general, the quality of the egg is found through various factors as the weight, weight of the albumin and yolk, flavor, color of shell, sensory attributes, etc. These characteristics can be affected by age, genetic differences amongst breeds (Hocking *et al.*, 2003; Hussain *et al.*, 2018), feeding (Cortes Cuevas *et al.*, 2016), stress factors within the production system (Ortiz *et al.*, 2013). Because of this, not all types of eggs from tested birds have the same quantity or proportion of certain parameters or do not match other studies.

CONCLUSION

The different types of egg belonging to analyzed birds have some physical differences. However, they have similarities in terms of nutritional value, with few differences. Due to this, the substitution of traditional consumption of the chicken egg for any of the tested types can be applied to diets that require protein and/or fats levels, and this data can also be applied to the food industry. Aside from its nutritional properties, the egg has different technological properties thanks to its foaming, emulsifying and jellifying abilities to name a few.

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Colegio de Postgraduados

Evaluation of protein sources in snail (*Helix aspersa* Müller) diets on the antioxidant bioactivity of peptides in meat and slime

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ABSTRACT

Objective: This work evaluates the effect of a dietary supply of amaranth, oats and lentils as a protein source on anthropometric measurements, the chemical composition in meat, as well as antioxidant activity in meat peptides and secretion of the snail (*Helix aspersa* Múller).

Design/methodology/approach: We worked with three groups of snails of 36 individuals and a control group fed with the same diet varying the protein source: amaranth, oats and lentils. A sample was taken every seven days and the shell's weight, width and length were measured. Five individuals from each group were sacrificed and the meat from which they were sacrificed was extracted: weight, moisture and protein. The hydrolysis soluble proteins in meat and slime were obtained and the antioxidant activity was measured using the reducing radicals DPPH• and ABTS•.

Results: Snail meat was obtained with an increase of more than double in weight when 10% of Am was supplied as a protein source.

Likewise, the dimensions of the shell will increase by 5%-11%. In FSM, it was obtained up to 79.8% moisture, 11.2% protein, 1.2% fat and 2.5% collagen. When obtaining snail meat flour, it was reduced to 12±1.9% humidity with up to 24.53 μg/g of soluble protein. When hydrolyzing the proteins, it was observed that the peptides obtained presented the IC_{50} of DPPH scavenging activity of 21.58±2.7, 5.45±1.8, 12.69±1.7 and IC_{50} of ABTS removal activity 8.86±0.9, 1.62±0.04, 10.84±1.0, for HFSM, HSMF and SS samples, respectively.

Limitations on study/implications: It is necessary to carry out other studies on the functionality of snail meat proteins and thus propose their implementation in food formulations to maximize their commercialization.

Findings/conclusions: Feeding snails with amaranth helps to increase the quality of protein in fresh meat and flour. Likewise, requests for soluble proteins from beef, flour and secretion are alternatives for preparing functional foods.

Keywords: snail meat, snail slime, antioxidant peptides, functional foods.

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INTRODUCTION

Mollusks represent an exceptionally diverse group, giving rise to the gastropod class (Aouji *et al.*, 2023), which includes, slugs and snails, encompassing approximately 35,000 terrestrial gastropods. At present, the commercially significant species belong to the genera *Achatina* and *Helix* (Corzas & Silvia, 2021). Heliciculture is an activity dedicated to the extensive and intensive production of snails, common in countries such as France, Italy, Spain and the United States (Colmenares-Flores & Alcántara-Gutierrez, 2021). Although snail farming, or heliciculture, has limited participation in Mexico, it is gradually expanding due to increased demand from the pharmaceutical, cosmetology and food sectors seeking bioactive compounds for the development of innovative formulations (Dhiman & Pant, 2021. The consumption of snails in Mexico has historical roots dating back to pre-Hispanic times, and their preparation methods have evolved, ranging from roasting and steaming to inclusion in traditional dishes like tamales (Cruz & Gómez, 2021). In contemporary times, snails are highly sought after in gourmet cuisine, commanding a market price of $14.00 MXN/kg (Romero-Díaz *et al.*, 2022). The nutritional composition of the snail meat positions it as an excellent alternative for human consumption, given its high protein content, low fat content and contributions of vitamins and minerals (Figure 1). On the other hand, the secretion produced by snails, known as "snail slime" contains proteins, polypeptides, glycans, and phenolic compounds. This diverse array of bioactive components adds to the potential applications of snails in various industries.

Recent studies have revealed that protein peptides in meat exhibit various functionalities, including antioxidant activity, antioxidant activity, ACE-Inhibitory activity, α-Amylase inhibitory activity and α-glucosidase inhibitory activity. Additionally, Aouji *et al.*, (2023) reported the presence of antimycobacterial, antioxidant, antitumor, antiinflammatory and anticancer compounds in snail slime. The occurrence of these functional compounds is directly influenced by the diet and living conditions of the snails. In the realm of farm animal production, switching protein sources is a common practice employed to enhance the nutritional value of the final product (Rygaÿo-Galewska *et al.*, 2022). The primary

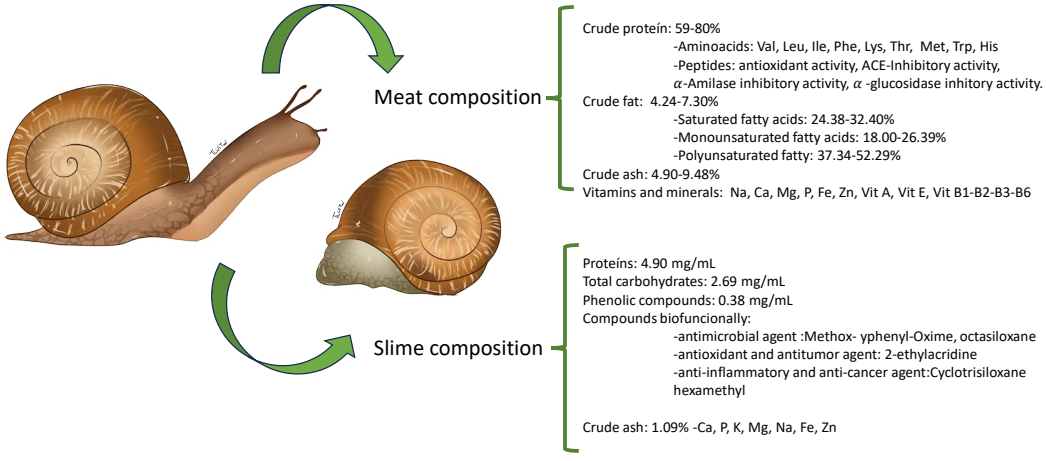


Figure 1. Molecules in meat and slime of nails *Helix aspersa*.

objective of the current study is to investigate the impact of diets incorporating amaranth, lentil and oat proteins on snail weight and size. Additionally, the study aims to assess the antioxidant activity of meat protein hydrolysates and snail slime.

MATERIALS AND METHODS

Helix aspersa Müller snails, sourced from heliciculturists in the municipality of Lerma, State of Mexico, were utilized in this study. All reagents were of analytical grade.

Organism rearing: Three groups and a control of *Helix aspersa* Müller snails of 3-4 g comprising 36 individuals, were acclimated one week prior to the study. They were housed in separate mesh containers with a pre-sterilized 1.5 cm soil bed and a container for water and food. Daily cleaning involved the removal of excreta and leftover food. Water was sprayed twice a day, and environmental conditions were maintained at a constant temperature (20±1 °C), humidity (80±5% RH) and a photoperiod (14:10 light-dark) were kept constant (Çelik *et al*., 2022).

Experimental design: The study was conducted in the Food Science laboratory of the Universidad Autónoma Metropolitana Lerma campus, held from March 7 to April 11, 2023. Three protein sources were tested: Amaranth (Am), Oatmeal (Oa), Lentil (Le). Diets were formulated following the guidelines of Ademolu *et al.* (2004), with slight modifications. Each group's diet included lettuce (35%), nopal (35%), calcium carbonate (16%), vitamins (4%), and the respective protein source 10% Am, Os and Le. Weekly samples were collected from six individuals over a span of five weeks. Snail weight and morphometry were documented by measuring the length and width of the shell with a Vernier caliper. Slaughter was carried out according to NOM-033-ZOO-1995, for the humane slaughter of domestic and wild animals. The meat was dissected, deveined, washed, and its weight recorded. On the other hand, the volume of snail slime (SS) obtained was measured using a pipette and stored at −4 °C.

Snail meat meal: Fresh snail meat (FSM) samples were arranged in a tray with a 2 cm separation subjected to a drying oven at 100 °C for 8 hours, cooled, and milled using a MOLINOX mil. The resulting snail meat flour (SMF) particles were sieved until achieving a particle size of 240 μ. Chemical analysis: The protein, fat, moisture and collagen content of SMF were determined using a FoodScanTM-lab meat analyzer. The equipment was calibrated prior to use, and 180 g of the sample was placed in the holder to prevent the formation of bubbles. Moisture in SMF samples from each week was determined using the oven method (method No. 14004). Soluble protein was determined using the Bradford method (1976) with Bovine Serum Albumin standard (10-100 μL in 10mM Tris-HCL buffer pH 7.3), and absorbance was measured at 595 nm.

Protein hydrolysis: Hydrolysis in FSM, SMF and SS was performed following the method of Hamid (2015) with some modifications. A 50 g sample (FSM, SMF) was mixed with 130 mL of 0.5 M phosphate buffer, pH 7.5. SS was taken directly after extraction. In both, native enzymes were inactivated by heating at 90 °C for 10 min, followed by an ice bath for 10 minutes. The samples were placed in a shaking incubator at 50 °C, and 85 μL of Alcalase® enzyme (Sigma Aldrich, 165 mUA) was added. The reaction was stopped by adding 120 μL of phenylmethylsulfonyl fluoride in ethanol (2 mg/ml).

Antioxidant activity of snail protein peptides: The antioxidant capacity of snail meat protein hy-drolysates (H-SMF) and snail slime hydrolysates (H-SS) was determined by their ability to reduce DPPH- and ABTS- radicals, according to Brand-Williams (1995) and Re-Pellegrin (1988), respectively. The IC_{50}, representing the amount of sample needed to inhibit 50% of each radical, was recorded.

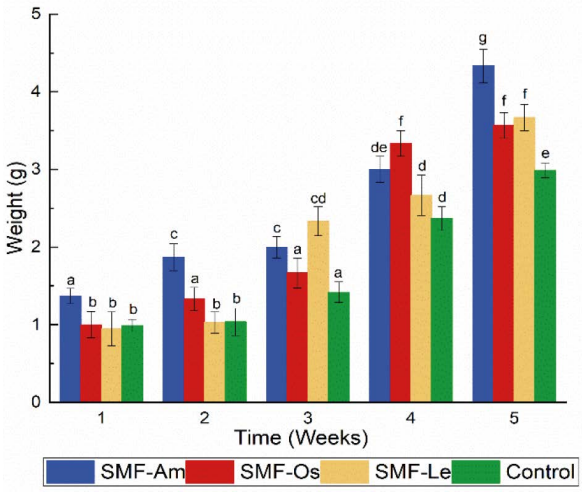
Statistical Analyses: Multiple assays were performed in triplicate, and the statistical analysis for sample comparison was conducted using NCSS software.

RESULTS AND DISCUSSION

Snail growth

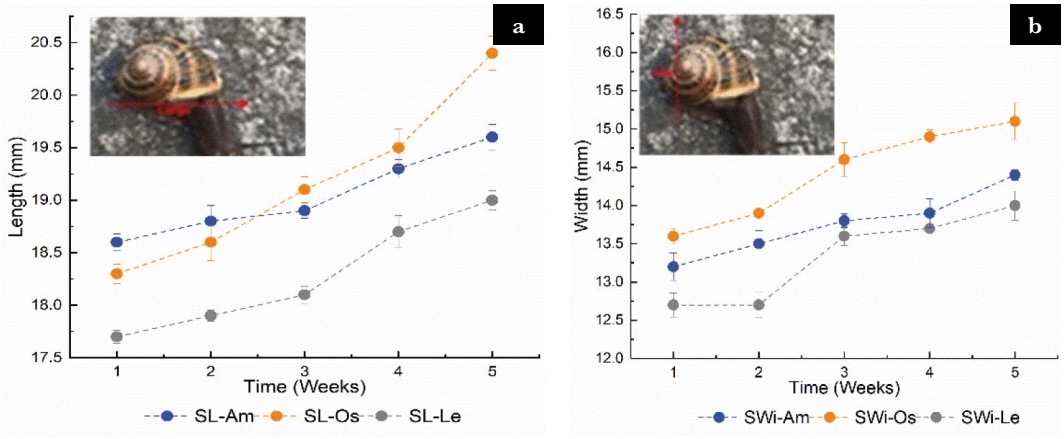
The weight gain in SMF is illustrated in Figure 2, indicating a 48% increase in SMF-Am, 28% in SMF-Os and 36% in SMF-Le compared to the control. SMF-Am samples are notably higher than others, while there is no significant difference between SMF-Os and SMF-Le, In comparison to the control, SMF-Am shows a 20% higher weight.

The growth effect in SMF is similar observed for snails fed with compound feed to accelerate growth, as reported by García *et al*., (2005). When compared to a vegetable diet, a 25% growth inhibition is noted, attributed to a dwarfing phenomenon related to feeding. Numerous studies have indicated that the nutritional value can vary between species base don the supplied diet, as well as factors such as the place of collection, season, and sexual condition. Diets enriched with amaranth, oat and lentil flour are found to be favorable nutritional supplements for weight gain in SMF. Morphometric records are presented in Figure 3, revealing a growth of 5.37% in length and 9.09% in width for SL-Am, 11% in both length and width for SL-Os, and 7.34% in length and 10.23% in width for SL-Le, compared to the control. This growth shows no significant differences regardless of the diets fed.



SMF-Am: amaranth-fed snail meat flour, SMF-Os: oat-fed snail meat flour, SMF-Le: lentil-fed snail meat flour.

Figure 2. Weight control of fresh snail meat fed with Amaranth, Oats and Lentils. Different letters indicate statistically significant differences at $p<0.05$.



SL-Am: Amaranth-fed snail shell length, SL-Os: Oat-fed snail shell length, SL-Le: Lentil-fed snail shell length
SWi-Am: Amaranth-fed snail shell width, SWi-Os: Oat-fed snail shell width, SWi-Le: Lentil-fed snail shell width

Figure 3. Record of carapace measurements a) carapace length, b) carapace width, in snails with. Amaranth, Oat and lentil diets.

The shell width data align closely with findings by Kathyuska *et al.*, (2016) in snails fed with soy-bean, pea and wheat. For shell length, the values are slightly below those reported by the same author. These results suggest that the calcium supplied during this growth phase is adequate, covering the nutritional requirements for shell deposition. A deficiency in calcium colud otherwise lead to reduced growth and eventual mortality, as noted by Mayoral *et al.*, (2004).

The shell is a by-product of this process, can serve as a calcium source, containing over 80% calcium carbonate with 25-35% fractional absorption bioavailability (García *et al.*, 2011).

Partial characterization of FSM

Observations indicate a protein content of 11.2±0.17%, twice that of *Limicolaria* FSM and comparable to *Achatina* FSM as reported by Babalola and Akinsoyinu (2009). Snail meat emerges as a non-conventional source of high-quality protein, complementary to cereal sources due to its lysine content. The determined fat content is 1.2±0.02%, similar to that reported for *Limicolaria* and half fat content of *Achatina*, a commercial species in Nigeria. Cholesterol levels are comparable to those found in *Achatina fulica* and *A. Limicolaria* snails (Zarai, 2012), offering consumers the Benefit of reducing the risk of cardiovascular diseases, such as heart attacks, hypertension and cerebrovascular events. Moisture was 78.8±3.52%, falling within the reported range for various species between 84.91% and 73.67%. Moisture content is indicative of the freshness of meat. Values above 70% typically indicate fresh meat, while below 40% may suggest that the snail meat is the process of deterioration. Collagen recorded for FSM is 2.5±0.26%, twice that reported in soy-fed *Lupinus* snails (Gogas *et al.*, 2021). Collagen, a high molecular weight protein, holds significance in the cosmetological and pharmaceutical industry.

Soluble protein and moisture content in SMF

The moisture content of the SMF samples ranged from 12-13.9%, irrespective of the diet fed. Soluble protein (P) content is depicted in Figure 4. P-Am samples exhibited the highest increase in soluble protein in their SMF (62%), while P-Le and control samples maintained the increase of 44.2-43.6% and P-Os had the lowest increase (29.6%).

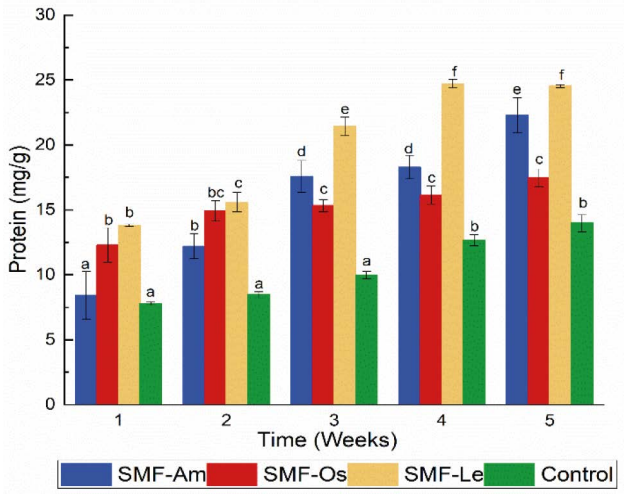
P-Am, P-Os and P-Le simples were significantly higher at the end of the treatments compared to week 1. Notably, P-Le achieved approximately twice the soluble protein content as the control, followed by P-Am with 37.35%, and P-Os with only a 20% increase over the control.

The results suggest a direct correlation between soluble protein and the supplied dietindicating a notable protein efficiency rate for all cases. This could be attributed to modifications in the oral mass and, primarily, in the randula, allowing snails to adapt various available food sources (Aguilera, 1996). Hydrolyzed SMF proteins may contain peptides of 3, 5 and 10 Kda (Saallah *et al.*, 2020).

Antioxidant activity of fresh meat and snail slime protein hydrolysates

Antioxidant activity, assessed by the reduction of DPPH• and ABTS• radicals was determined in hydrolysates (H) of FSM, SMF and SS, as presented in Table 1. HFSM-Am, HSMF-Am and HSS-Am exhibited the lowest inhibition IC_{50} for both radicals, indicating superior antioxidant activity compared to the other samples from the Os and Le groups. Evaluation of the antioxidant activity is complex and system-dependent, hence the preference for assessment in multiple systems.

In HFSM samples, significant differences in antioxidant activity were observed, irrespective of the radical used, with HFSM-Am and HFSM-Os requiring 3 times less for inhibition. Lower IC_{50} values were obtained in HFSF compared to HFSM simples



P-Am: SMF Soluble Protein Fed with amaranth, P-Os: SMF Soluble Protein Fed with oat, P-Le: SMF Soluble Protein Fed with lentils.

Figure 4. Soluble protein in snail meat meal fed with alternate protein sources. Different letters indicate statistically significant differences at $p<0.05$.

Table 1. Evaluation of the DPPH• and ABTS• radical reduction capacity of snail protein hydrolysates.

Sample	IC_{50} radical inhibition DPPH• (g/mL)	IC_{50} radical inhibition ABTS• (g/mL)
HFSM-Control	61.261 ± 3.3^{a}	25.088 ± 1.5^{a}
HFSM-Am	21.587 ± 2.7^{b}	8.866 ± 0.9^{b}
HFSM-Os	27.077 ± 1.9^{c}	14.491 ± 1.1^{c}
HFSM-Le	45.005 ± 1.9^{d}	14.088 ± 1.7^{d}
HSMF-Control	20.058 ± 2.6^{b}	11.686 ± 0.1^{e}
HSMF-Am	5.451 ± 1.8^{e}	1.629 ± 0.04^{a}
HSMF-Os	12.571 ± 2.5^{f}	1.244 ± 0.07^{e}
HSMF-Le	16.804 ± 3.6^{g}	5.608 ± 0.4^{f}
HSS-Control	45.483 ± 4.1^{d}	15.796 ± 0.8^{ag}
HSS-Am	12.692 ± 1.7^{f}	10.846 ± 1.0^{ag}
HSS-Os	10.866 ± 1.9^{f}	10.552 ± 1.3^{a}
HSS-Le	17.592 ± 2.2^{g}	11.473 ± 0.8^{g}

Equal letters are not statistically different ($p \leq 0.05$).
Different letters are statistically different ($p \leq 0.05$).

due to concentration, and HSMF-Am exhibited the lowest IC_{50} in inhibiting both radicals. The volume of slime extracted was 3.6-4.4 mL/1 snail. SS samples displayed antioxidant activity, especifially in HSS-Am and HSS-Os with 3 times less IC_{50} of DPPH• radical and approximately 50% less IC_{50} for ABTS• radical compared to the control. These findings align with Aouji *et al.*, 2023, where hydrolyzed pep-tides with antioxidant activity on the DPPH• radical were obtained, particularly when featuring amino acid sequences HTYHEVTKH and WPVLAYHFT. The ability to reduce the ABTS• radical may be atributed to amino acids containing hydrophobic groups (Petsantad *et al.*, 2020).

SS peptides exhibit various bioactivities. Hayes and Mora (2021) derived peptides from *Phylum mollusca*, showing functionality on angiotensin converting enzyme ACE-1 up to 95% *in silico* assays, identifying peptides from 628 to 2343 Da.

Peptides from mollusks, l have been increasingly utilized in pharmaceuticals, cosmeceuticals and nutraceuticals, and functional foods, due to their diverse biological properties (Ovchinnikova, 2019).

CONCLUSIONS

The protein sources Am, Os and Le supplied to *Helix aspersa* Müller snails have a positive effect on the growth of the individual as well as in obtaining soluble proteins and slime with a functional potential due to their antioxidant activity. Am the protein source that presented the greatest effect on snail weight and size as well as the highest antioxidant activity in protein hydrolysates. Due to its composition, fresh snail meat is an alternative

that competes nutritionally in the meat market. Additionally, snail protein hydrolysates hold promise as a potential raw material for the development of functional foods.

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