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Socioeconomic and environmental factors that impact vegetable production

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ABSTRACT

Objective: To analyze the factors that contribute to the reduction of vegetable production in the San Vicente Chicoloapan *ejido*.

Design/Methodology/Approach: Sixty out of a total of 150 *ejidatarios* who produced vegetables in 2022 participated in a convenience sampling. Producers who agreed to be surveyed were interviewed in their plots.

Results: The *ejidatarios* have an average of 1.47 agricultural ha (minimum: 1 ha; maximum: 5 ha), considering both irrigated and rainfed lands. The use of the sample studied was divided as follows: 43.3% of the owners sold their land to developers who built housing units and new houses; 38.3% cultivated their own land or those they rented; and 18.3% rented out their lands. The *ejidatarios* suffer from vegetable theft, particularly pumpkin (45.0%), artichoke (31.7%), onion (13.3%), chard (8.3%), and cabbage (1.7%).

Study Limitations/Implications: Designing a random sample was impossible given the lack of access to a register of *ejidatarios*. Estimating the harvested area or estimating yield based on the production volume was impossible, since producers grow several crops in their plots, besides vegetables.

Findings/Conclusions: Vegetable production in the San Vicente Chicoloapan *ejido*, State of Mexico, is at risk of disappearing, as a consequence of economic and social issues and a lack of support. The *ejidatarios* who still grow vegetables have tried to mitigate these problems; however, their future as producers is severely limited and older people have chosen to sell their land, which are used by developers to build houses.

Keywords: Vegetables, polycultures, agricultural land sale, land use change, peri-urban agriculture.

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INTRODUCTION

In 2021, the irrigated area used to grow vegetables in Mexico consisted of 492,690 ha, which represented 8.44% of national agricultural area (5,839,756 ha) and 22.25% of production value (SIAP, 2022). Given their intrinsic characteristics —such as a short shelf life (Anaya *et al.*, 2020)—, vegetables must be marketed immediately after harvest or preserved under controlled atmospheres or cold hours in an adequate infrastructure (Pinto *et al.*, 2016). Regarding marketing, vegetable prices undergo a great variation (market risk) from one day to the next (González, 2013), while small-scale producers face intermediaries, known in Mexico as coyotes (Lugo *et al.*, 2010). For these reasons, not all small producers can produce vegetables; furthermore, vegetables have a higher production cost than basic grains (Echánove and Steffen, 2006).

The San Vicente Chicoloapan *ejido* is part of the peri-urban agriculture area in the eastern Mexico City Metropolitan Area (MCMA). It covers the municipalities of Texcoco, Chimalhuacán, Chicoloapan, Nezahualcóyotl, Ixtapaluca, and La Paz. In the year 2000, the population of the six municipalities of the eastern MCMA was 2,508,689 inhabitants and it increased by 24% in 20 years, reaching 3,107,012 by the year 2020 (INEGI, 2020).

The municipality of Chicoloapan had an area of 41.03 km^2 (4,103 ha) and, in 2020, it had a population of 200,750 inhabitants, resulting in a population density of 4,892.7 inhabitants/km^2 (INEGI, 2020).

The population of the municipality of Chicoloapan has settled on irrigated agricultural lands where vegetables used to be the main production activity. This situation resulted in an annual decrease of 5.84% in the 2006-2020 period (Figure 1).

The Mexico City Metropolitan Area is the result of the fusion of two or more municipalities with more than 10 thousand inhabitants (Sánchez, 2009), exceeding the boundaries of the original municipality. In the east, the growth of the MCMA is putting strong pressure on agricultural lands. The objective of this article is to analyze the factors that contribute to the reduction of vegetable production in the San Vicente Chicoloapan *ejido*.

MATERIALS AND METHODS

The San Vicente Chicoloapan *ejido* borders to the north with the municipality of Texcoco; to the south with the municipalities of La Paz and Ixtapaluca; to the east with the municipalities of Texcoco and Ixtapaluca; and to the west with the municipalities of Chimalhuacán and La Paz (IEEM, 2015) (Figure 2). The extreme coordinates of the municipality are 19° 26' 07" and 19° 20' 54" N and 98° 56' 40" and 98° 48' 05" W (Galeote, 2018).

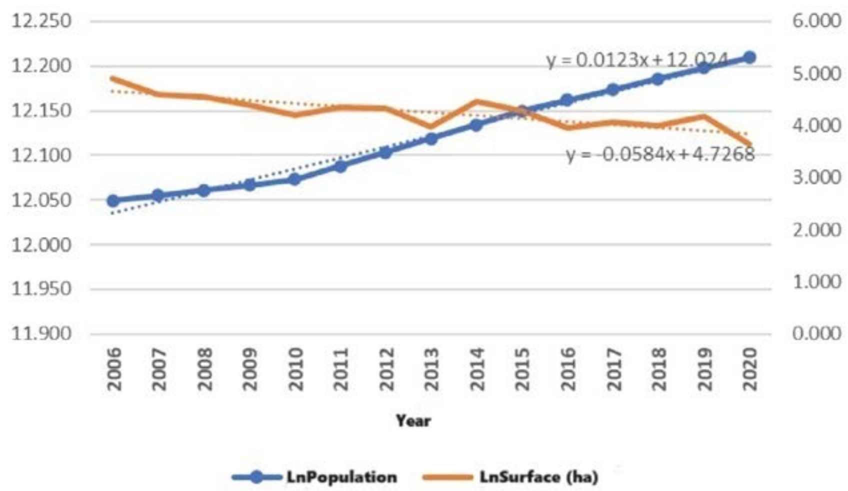

Figure 1. Population growth trends and vegetables crop area (ha) decrease trends in the municipality of Chicoloapan, State of Mexico (2006-2020).
Source: Table developed by the authors based on statistical data from the Agricultural and Fisheries Information Service (SIAP, 2022) and the National Institute of Statistics and Geography (INEGI, 2020).

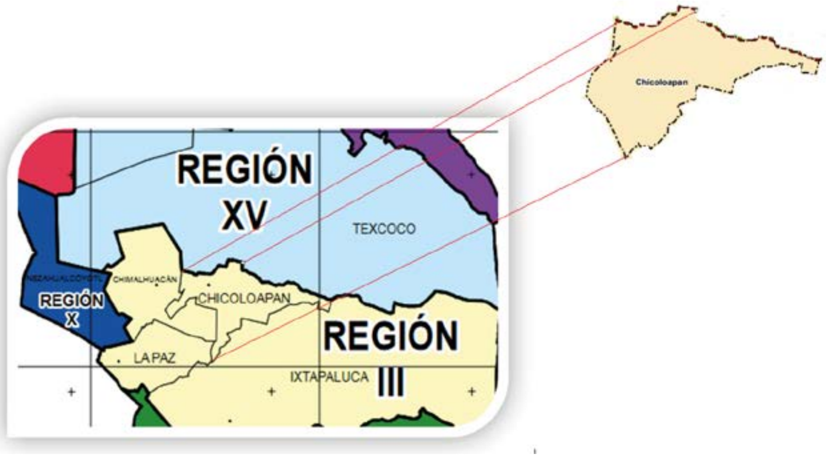

Figure 2. Study area location. Source: Regionalization 2017-2023, Comité de Planeación para el Desarrollo del Estado de México (COPLADEM) (2022).

A survey was used to obtain the data. The field work was carried out from September 12 to November 15, 2022. Sixty *ejidatarios* were surveyed out of a total of 150 producers who grew vegetables in the 2022 agricultural cycle.

The participating *ejidatarios* were working on their plots at the time of the survey and agreed to participate, resulting in a non-probabilistic convenience sampling. In order to identify the *ejidatarios* on their plots, an *ejidatario* accompanied the survey team during the entire process of collecting field information.

Excel and the Statistical Program for the Social Sciences (SPSS) were used for the statistical analysis of data obtained in the field.

RESULTS AND DISCUSSION

The San Vicente Chicoloapan *ejido* core has a register of 635 *ejidatarios* and an area of 1,865.49 hectares which, according to the Padrón e Historial de Núcleos Agrarios (PHINA, 2022), represents 45.45% of the municipal territory. The *ejido* lands are distributed as follows: 41.5% is parceled out (773.71 ha), 56.8% is common lands (1,059.72 ha), 0.7% corresponds to delimited human settlements (13.04 ha), and 1.0% features undelimited human settlements (19.02 ha).

In average, the producers were 61.98 years-old and 48.3% were 65 years or older. This percentage of older people contrasts with that of producers in the overall State of Mexico, where 30.9% were 65 years or older, according to the 2022 Agricultural Census (INEGI, 2022). The average age of the *ejidatarios* of San Vicente Chicoloapan was 61.98 years, with a minimum of 32 and a maximum of 90 years (standard deviation: 13.709). Based on the average age of the producers and the human life cycle, they belong to the elderly population.

Regarding their education level, 25% of the producers did not have the opportunity to attend school. However, all respondents pointed out that "they learned to read and write out of necessity, to take care of their interests as *ejidatarios* and [to prevent] others from taking advantage of their ignorance." Of those who attended school, 91.1% studied until high school and 8.9% studied a bachelor's degree. Regarding marital status, 43% was married and 57% lived alone —either because they were divorced, widowers or widows or because they had never married.

More than three quarters (78.3%) of *ejidatarios* do not have the support of their children for agricultural activities and only a fifth (21.7%) have such support (average: 1.77 children).

Production unit size

Regarding the distribution of agricultural land, the initial *ejido* allocated consisted of one hectare per *ejidatario*; however, as time went by, through the purchase and sale of land, some producers were able to increase their production units. Of the total number of respondents, only 23 (38.3%) *ejidatarios* used their agricultural lands; in average, they cultivated 1.47 ha (minimum: 1 ha; maximum: 5 ha), considering both irrigation and rainfed systems. In the case of irrigated lands, the average was 1.09 ha (minimum: 1 ha; maximum: 2 ha), while rainfed plots recorded in average 1.67 ha (minimum: 1 ha; maximum: 3 ha).

Vegetable production system

The *ejidatarios* use the multiple crop system or polyculture to produce vegetables, combining different crops (Koohafkan and Altieri, 2011). In the same plot, the *ejidatarios* grow rows of vegetables and, at the same time, introduce other crops such as corn (*Zea mays*), oats (*Avena sativa*), beans (*Phaseolus vulgaris*), wheat (*Triticum sativum*), marigold flower (*Tagetes erecta*), and barley (*Hordeum vulgare*), as a strategy to reduce vegetable theft.

The main vegetables produced in the ejido are chard (*Beta vulgaris* var. *cicla*), artichoke (*Cynara scolymus*), pumpkin (*Cucurbita pepo*), onion (*Allium cepa*), cabbage (*Brassica oleracea* var. *capitata*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), red tomato (*Solanum lycopersicum*), green tomato (*Physalis philadelphica*), carrot (*Daucus carota*), and cilantro (*Coriandrum sativum*). The most stolen vegetables are pumpkin (45%), artichoke (31.7%), onion (13.3%), chard (8.3%), and cabbage (1.7%).

All the vegetable producers stated that they suffered vegetable theft. Therefore, they do not plant a complete hectare, since it would easily attract the attention of neighbors, increasing the thefts. In an interview, an *ejidatario* mentioned that:

> "…not long ago, a whole family who live near my plot used to steal my vegetable crops. Even small children participated in this theft, taking advantage of the fact that I was away carrying out other activities. I immediately approached them and told them that I was going to report them if they did not return what they had stolen. At that time, the women told me that they would accuse me of assault if I did not let them go with the harvest. Since I was alone and they were three women, I had no witness and they took the vegetables. This family has already been caught on other occasions stealing vegetables with other companions and it is well known that this family sells vegetables, mainly on the roads near the *ejido*."

Regarding this issue, Espinoza (2020) states that some families make a living by entering plots and orchards to steal significant quantities of products which they later sell. This is one reason why *ejidatarios* do not find it easy to plant entire hectares of vegetables, since they do not recover the investment and their loses are greater than their profits. In addition to the theft of crops, they also suffer the theft of water intended for agricultural irrigation by people who build residential houses for domestic use. Likewise, the plots are impacted by garbage pollution from those houses. The *ejidatarios* have implemented some strategies to reduce the abovementioned problems (Table 1). Some strategies are implemented adequately, but others cause long-term harm to the *ejidatarios*.

Vegetable sale points

The ejidatarios sell their products at different points. Most of them (69.6%) sell at the Central de Abastos of Iztapalapa, México City's supply center. Others (21.7%) sell at their plot and a few also do it directly to the consumer (4.3%) or to intermediaries (4.3%).

The *ejidatarios* who sell their vegetables at the Central de Abastos pointed out that having direct contact with buyers provides them the advantage of having a guaranteed sale for several years. And their disadvantage is the transportation or freight costs (12.5%), in addition to the price haggling with buyers (87.5%).

Ejidatarios who sell at their plot do not incur in transportation costs, but they do not always sell their entire harvest (40%) and they also must participate in price haggling (60%). Finally, those who sell directly to the consumer sell little and every client haggles over the price. The intermediaries also haggle over the price and the *ejidatarios* have no other choice but to lower the price to avoid keeping the harvest. Consequently, many *ejidatarios* have decided to sell their agricultural lands.

Land sale

From 1998 to 2008, urban growth in the municipality of Chicoloapan caused a change in land use and agricultural areas started to be taken over by housing constructions (Sánchez and Ontiveros, 2011). In 2003, the vegetable agricultural area was 164 ha, but it had decreased to 38.41 ha by 2020 (SIAP, 2022). The population of the municipality increased from 124,228 inhabitants (2003) to 200,750 inhabitants (2020). In other words, the population grew at an annual rate of 2.68%. At this growth rate, the population will reach 248,456 by 2027 —double the population that the area had in 2003.

Table 1. Main problems found in agricultural plots and the strategies implemented by ejidatarios.

Problem	Causes	Solution strategy
Vegetable Theft	- Creation of new housing areas (98.3%) - Presence of new colonies (98.3%) - Presence of people from outside the ejido (6.7%)	1.- Planting vegetables with basic grains. 2.- Substitution of vegetables for staple crops such as: oats (31.6%), corn (26.3%), wheat (15.8%), marigold flower (15.8%), beans (5.3%) and barley (5.3%).
Theft of water for irrigation of vegetables	Homes that do not have potable water are supplied by irrigation water, which increases irrigation hours and costs.	Decrease in irrigation hours for the plots, which in some cases is not enough for the crop, which means that the harvest has not a good quality.
Garbage contamination of vegetable plots.	The creation of housing complexes and new housing constructions are the cause for garbage contamination in the plots (98.3%).	The ejidatarios collect garbage near located and on their plots.

Source: Table developed by the authors based on data from field work (2022).

Urbanization —clearly seen in the building of houses— is taking over agricultural lands, consequently decreasing the area used to grow vegetables. Of the total respondents, 43.3% sold agricultural land that is currently occupied by residential houses; these sales occurred mainly in 2020.

Ejidatarios who sold agricultural land gave multiple reasons, mainly of a social nature, followed by economic reasons and, finally, political and market reasons (Figure 3).

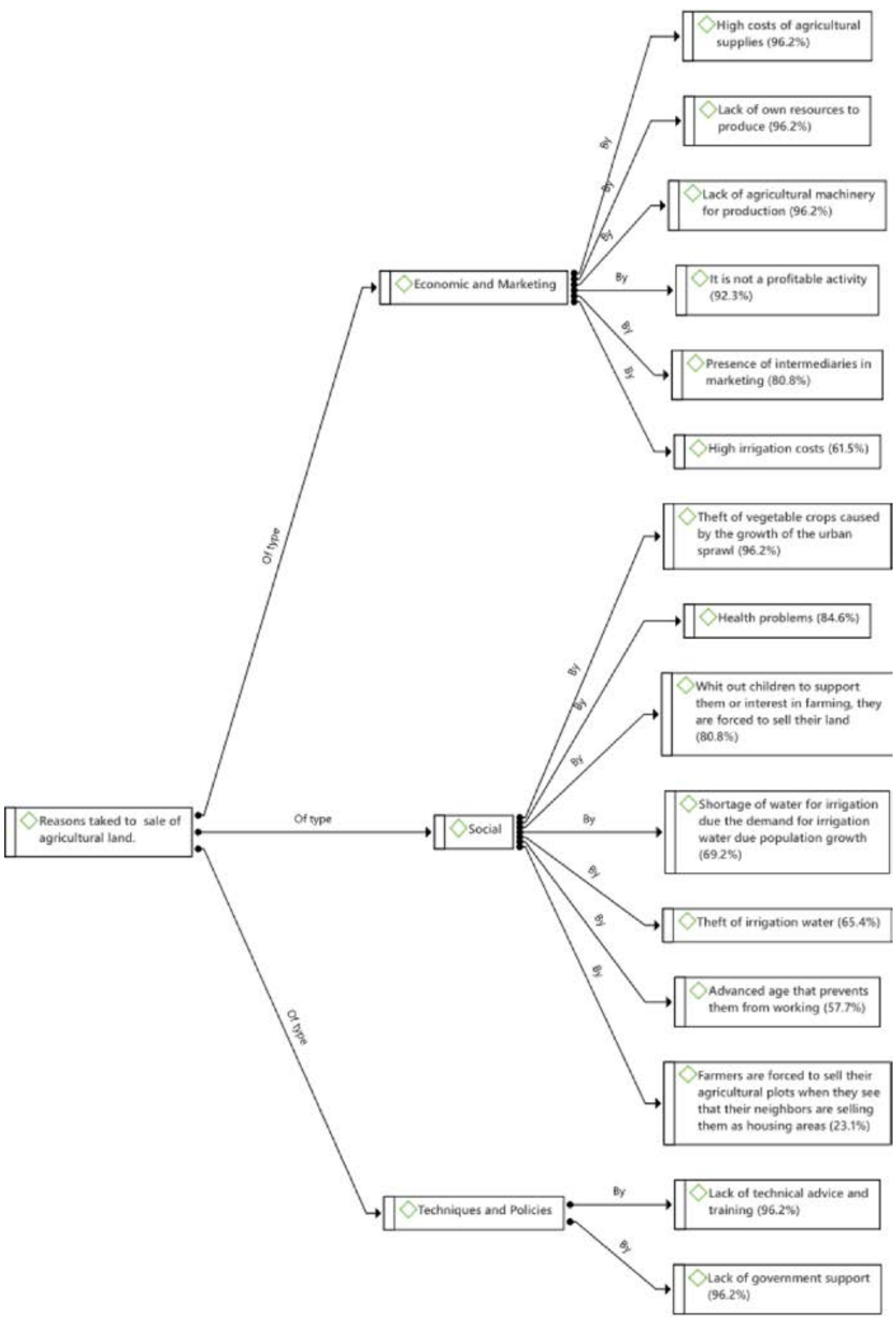

Figure 1. Reasons given by ejitadarios for selling agricultural land.
Source: Table developed by the authors based on data from field work (2022).

CONCLUSIONS

Vegetable production in the San Vicente Chicoloapan *ejido* is at risk of disappearing for multifactorial reasons (economic, social, and technical) and lack of support. The *ejidatarios* who still grow vegetables have tried to mitigate these problems; however, they do not believe that there is much future in the production of vegetables and the elderly have chosen to sell their lands, which are now used to build houses.

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Alternative substrates for the production of container-grown Mexican cempaxóchitl (*Tagetes erecta* L.)

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ABSTRACT

Objective: To evaluate substrate mixtures for the production of Mexican marigolds grown in containers in the community of Santa Cruz Itundujia, Oaxaca, Mexico.

Methods: Four mixtures were tested: 1) 70% ocote pine needles+30% soil, 2) 70% leaf mold+30% soil, 3) 70% river sand+30% soil, and 4) 70% sawdust+30% soil. Mexican marigold (*Tagetes erecta*) var. Inca II Deep Orange plants were established in pots under open field conditions. Height, number of leaves, plant width, branching, leaf area, and number of buds were evaluated in the different phenological stages of the plant (15, 45 and 90 days after transplant). Soil fertility parameters were analyzed, and physical analysis of the substrates was performed.

Results: The treatments produced differential results; ocote pine needles+soil and leaf mold+soil were the best for producing container-grown marigolds. The lowest results in yield variables were found with the river sand+soil mixture.

Implications: Using local and inexpensive substrates will impact production costs for marigold farmers. Currently, a variety of commercial mixes and substrates of foreign origin are available and used for the production of container-grown plants.

Conclusions: The best substrate for the cultivation of pot-grown *Tagetes erecta* var. Inca II Deep Orange under open field conditions were soil+ocote pine needles and soil+leaf mold. These materials are common, cheap and easily acquired in the region, which will facilitate the production of various ornamental species in containers based on the results of this research.

Keywords: Asteraceae, yield, sawdust, needle, Oaxaca.

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INTRODUCTION

Cempaxochitl, also known as the Mexican marigold (*Tagetes erecta*), is a native Mexican plant mainly used as an ornamental due to its beautiful yellow and orange inflorescences and peculiar fragance (Mier *et al.*, 2019). Its use dates back to pre-Hispanic cultures as a medicinal, ceremonial, and gastronomic plant (Hernández, 1960). Currently, potted marigolds are important for gardening and for decorating altars and commemorative spaces (Serrato, 2022). In Mexico for the production of this plant, different mixtures of imported substrates such as peat, vermiculite and perlite are used, which increase production costs

(Kaushal and Kumari, 2020; Ördögh, 2021). Some ornamental farmers prefer mixtures of leaf mold with perlite or volcanic material (tezontle or tepojal) (Gayosso *et al*., 2016). However, in the Mixteca Alta region of the state of Oaxaca, ornamental plants are rarely produced due to altitudes exceeding 1700 meters above sea level and the predominance of pine oak forests (López *et al*., 2022). Therefore, it is necessary to seek local substrates that utilize residues from timber products (sawdust and pine needles) that are low-cost, readily available, and contribute to reducing organic waste in the area. In this work, mixtures of four local substrates from Santa Cruz Itundujia, Oaxaca, a community characterized by timber activity, were evaluated in order to choose the most suitable for the production of Mexican marigold.

MATERIALS AND METHODS

The present study was carried out in Laguna area, municipality of Santa Cruz Itundijia, Putla district, of the State of Oaxaca in the Mixteca region, coordinates 97° 39' west longitude and 16° 52' north latitude (INEGI, 1991). Four readily available and inexpensive substrates in the area were selected: 1) ocote pine needles, 2) leaf mold, 3) river sand, and 4) sawdust. The ocote pine needles and sawdust were not fresh since they presented 50% degradation. The experiment was established in an open field, using the following proportions of substrates (Table 1) and regional soil, which was classified as Eutric Cambisol.

Inca II Deep Orange seeds were obtained from Freesias S. de R. L. de C. V. and black flexible plastic pots with a six-inch diameter were used.

Development and fertilization

Fertilization was performed through the irrigation water with a soluble commercial formula of N-20 P-20 K-20 Harvest More® at a dose of 0.5 g L^{-1} in the vegetative phase, gradually increasing to a dose of 1.0 g L^{-1} in the flowering stage. Fertilization was carried out weekly, alternating with water irrigation. Foliar fertilizer was also applied for all treatments, using 0.3 ml L^{-1} of Bayfolan forte® with 1 ml L^{-1} of adherent per liter of water in the vegetative stage and reaching 1.0 ml L^{-1} in the flowering stage.

For disease prevention (in the case of fungi), benomilo® (diluted with water to a concentration of 0.5 ml L^{-1}) was applied to the leaves every 15 days. No plagues were present during the experiment.

Experimental design

The experiment used a randomized complete-block design with four treatments (ocote pine needles + soil, leaf mold + soil, river sand + soil and sawdust + soil) and 20 replicates per treatment. A total of 80 pots were established.

Table 1. Treatments used in the experiment

Treatment 1	Treatment 2	Treatment 3	Treatment 4
Ocote pine needles (70%) + soil (30%)	Leaf mold (70%) + soil (30%)	River sand (70%) + soil (30%)	Sawdust (70%) + soil (30%)

Data analysis

The response to the treatments was evaluated by measuring the following variables: plant height, number of leaves, number of branches, width or breadth of the plant, leaf area, and number of buds. These variables were measured at different plant phenological stages (15 and 45 dat), finalizing with the flowering stage (90 dat).

The data were recorded using IMAGE-J software to determine the leaf area. The data were analyzed using the ANOVA analysis of variance and comparison of means (Tukey's test) in the Statistical Analysis System (SAS) software.

Analysis of physical properties of substrates and soil fertility

Physical tests were performed on the substrates and mixtures used in the experiment according to NOM 021-RECNAT 2000 to determine aeration and moisture retention. Moreover, the fertility analysis of the soil used for the mixtures was carried out according to NOM 021-RECNAT 2000.

RESULTS AND DISCUSSION

Height. Significant differences were found in this variable on the three sampling dates (Table 2). Fifteen days after transplanting (dat), treatment 1 (needles + soil) and 2 (leaf mold + soil) were statistically higher than treatment 3 (sand + soil) and 4 (sawdust + soil). At 45 dat, the treatments containing needles (T1) and leaf mold (T2) had a 38% increase over the treatments containing sand (T3) and sawdust (T4). During the flowering phase (90 dat) the best treatments were T1 (needles + soil) and T4 (sawdust + soil) (Table 3). Leaf mold is inexpensive and easy to obtain in the community. One of the advantages of leaf mold is the contribution of nutrients and the presence of microorganisms such as bacteria and arbuscular fungi (Mantero *et al.*, 2019).

Treatment 3 (sand + soil) had the lowest height values during the three data collection periods (15 dat, 45 dat, and 90 dat), representing a lower development (Table 3). Sand is considered cheap and easy to acquire for ornamental producers; however, its characteristics, such as higher weight, porosity, and lower water retention, lead to lower plant development (*i.e.*, height) compared to substrates with a higher percentage of organic materials (Ördögh, 2021). Ampim *et al.* (2010) indicated that the disadvantages of using sand in the substrate

Table 2. Results of Pr > F in ANOVA.

Variables	Days after transplant (dat)		
	15	45	90
Height	0.0001	0.0001	0.0001
Number of leaves	0.0032	0.0001	0.0001
Branching	*	0.0001	0.0001
Plant width	0.0001	0.0001	0.0001
Number of flower buds	*	*	0.0001
Leaf area	0.0001	0.0001	0.0001

* No data, α=0.05.

are the low retention of nutrients and water, the weight of the mixture, and the limitation of providing good nutrition since it is an inert material.

Number of leaves. For this variable, significant differences were also found in the dates sampled (Table 2), at 15 dat and 45 dat, the two best treatments were needles + soil and leaf mold + soil, while at 90 dat, the treatment containing needles + soil statistically outperformed the other treatments, with an increase of 44% over T2 (leaf mold + soil), 46% over T4 (sawdust + soil), and 73% over T3 (sand + soil) (Table 3). Leaves are an important anatomical part of *Tagetes erecta* and all plants because this is where photosynthesis occurs; this process provides photoassimilates to the plant necessary for cell growth (Azcón and Talón, 2013). However, it is necessary to provide a suitable medium for plant development. In this context, using ocote pine needles + soil favored leaf development. Gupta *et al.* (2023) indicated that pine needles contain 68%-69% holocellulose (cellulose and hemicellulose), 27%-31% lignin, and 4%-4.5% extracts that, when degraded, facilitate the development of different species in that substrate. When mixed with soil, the needles stored moisture and reduce water evaporation (Sahin and Yalcin, 2017).

Branching. These were measured at 45 dat and 90 dat due to the pruning and development of the plant (Table 3). Like the other variables, the analysis of variance showed significant differences between the means of the values obtained (Table 2). At 45 dat, ramifications were similar in treatments T1 (needles + soil) and T2 (leaf mold + soil); in contrast, treatments T3 (sand + soil) and T4 (sawdust + soil) had the lowest values. At 90 dat, branching was statistically lower in T3 (sand + soil) than the other treatments.

Table 3. Yield parameters of *Tagetes erecta* (Mexican cempaxóchitl) in four substrates.

Treatment	Height (cm)			Number of leaves			Branching		
	15	45	90	15	45	90	15	45	90
	dat								
T1. Ocote pine needles+soil	7.9a	10.2a	15.3a	6.4a	27.2a	51.1a	0	5.7a	6.5a
T2. Leaf mold+soil	7.4a	10.2a	10.6b	6.5a	28.3a	28.3b	0	5.4a	6.2a
T3. River sand+soil	4.2b	6.3b	9.4b	5.0b	11.6b	13.5c	0	2.9b	3.3b
T4. Sawdust+soil	4.6b	6.3b	13.4a	5.4ab	16.5b	27.2b	0	3.4b	5.3a
MSD	0.60	1.63	2.83	1.29	7.65	6.04		1.57	1.40
Treatment	**Width (cm)**			**Number of flower buds**			**Leaf area (cm^2)**		
	15	**45**	**90**	**15**	**45**	**90**	**15**	**45**	**90**
	dat								
T1. Ocote pine needles+soil	8.10a	14.55a	18.45 ab	0	0	5.45a	19.68a	90.67a	62.49a
T2. Leaf mold+soil	7.64a	11.90b	18.65 a	0	0	5.90a	9.85b	43.68b	52.04b
T3. River sand+soil	5.45b	8.58c	8.90 c	0	0	3.35b	23.62a	23.20c	23.41c
T4. Sawdust+soil	5.37b	9.23c	14.40 b	0	0	4.70ab	20.57a	15.53c	49.28b
MSD	1.11	2.04	4.10			1.31	4.53	10.15	7.73

MSD=Minimum significant difference, dat=days after transplant. Different letters between dates and treatments show significant differences (Tukey α=0.05) Mean ± standard error, n=20.

The lateral development of a plant is due to the growth of axillary buds when apical dominance is inhibited or diminished. Auxins participate in this process and promote the formation of leaf primordia (Azcón and Talón, 2013). Additionally, Mann *et al.* (2023) indicated that the physicochemical properties of the growth medium and water quality influence the development and quality of *Tagetes erecta*. If a plant acquires essential nutrients from the medium (substrate or soil), apical and axillary bud development will increase; otherwise, growth will decrease, as occurred in the sand + soil mixture, which showed less branching development.

Plant width. ANOVA showed significant differences between the means of the treatments (Table 2).

At 15 dat, plant width in T1 (needles + soil) was 32% higher than T3 (sand + soil) and 33% higher than T4 (sawdust + soil). Moreover, at 45 dat, plant width increased in all treatments, with T1 (needles + soil) as the highest, and T3 (sand + soil) and T4 (sawdust + soil) as the lowest. At the flowering stage, the best widths were found in T1 (leaf mold + soil) and T2 (needles + soil), while the lowest was T3 (sand + soil). These values represent a 100% increase in width in the needle treatment compared to the sand treatment (Table 3). Plant yield and growth depend on the fertility of the medium and fertilizer supply (Souri *et al.*, 2018). As a substrate, leaf mold provides enough nutrients such as N, P, K, Ca, and S due to the decomposition of organic litter. *Tagetes erecta* is susceptible to nutrient deficiency since supplying it with only 100 mg kg^{-1} of nitrogen reduced growth and flower production (Mantero *et al.*, 2019). Because of this, marigold plants in this study were fertilized with optime nitrogen additions. According to Kaushal and Kumari (2020), leaf mold and pine bark provide nitrogen and good moisture retention to the substrate, which could explain the results of the present experiment.

Number of flower buds. The emission of flower buds is a response to the change of activity and the differentiation of the vegetative meristem to a reproductive meristem, originating the flower components (Taiz and Zeiger, 2006). Therefore, flower buds were only registered at 90 dat, when more than 50% of flowering occurred (Table 3). The results showed that plants in T3 (river sand + soil) presented fewer flower buds than the other treatments (Figure 1). The best treatments were T2 (leaf mold + soil) and T1 (needles + soil). These data agree with Ördögh (2021), who studied different proportions of sand mixed with peat moss in the development of *Tagetes patula*; their findings indicate that substrates with a higher percentage of peat moss were more effective in the species' development and flower bud production. In contrast, the substrates with sand did not promote the adequate development of *Tagetes patula*.

Leaf area. Leaf area is the measure of the total leaf surface of a plant, where photosynthesis takes place (Fang *et al.*, 2019). At 15 dat, the plants presented differential leaf areas, and T2 (leaf mold + soil) had the lowest (Table 3). In all treatments, leaf area increased at 45 dat and 90 dat; however, T1 (needles + soil) presented the best values. The overall analysis of the evaluated variables in *Tagetes erecta* suggests that plant development was lower and deficient in T3 (sand + soil), resulting in small plants. From a commercial point of view, these plants are not viable due to their inferior quality (Figure 1).

Figure 1. Marigold (cempaxóchitl) development in (a) ocote pine needles + soil and (b) sand + soil.

Physical characteristics of substrates. When using substrates, aeration capacity, and moisture retention capacity are important because they are closely linked to bulk density and total porosity (Bashir *et al.*, 2021).

Moisture retention capacity was obtained by saturating samples of the substrates and applying water column tensions (10 cm, 50 cm, and 100 cm). The amount of water retained at each tension was measured, and the corresponding curves were constructed. The results are shown in Table 4.

Since moisture retention with a water column can only be applied in substrates with organic materials, moisture retention in the river sand and the river sand + soil mixture was determined by Field Capacity (FC) and Permanent Wilting Point (PWP) (pressure between 0.3 bar - 15 bar) (Table 5).

Table 4. Physical characteristics and moisture retention of the substrates.

Materials and mixtures	BD (g cm^{-3})	Total porosity	Aeration	Moisture retention	UW	EAW	RW	HAW
Ocote pine needles	0.14	80	21	59	27	12	31	25
Ocote pine needles + soil	0.32	69	15	54	21	16	18	41
Sawdust	0.05	80	63	18	41	4	6	13
Sawdust + soil	0.15	83	52	31	45	20	28	13
Leaf mold	0.13	83	28	55	8	4	5	60
Leaf mold + soil	0.24	73	24	49	34	20	20	24
River sand + soil	0.88	52	14	38	*	*	*	*

BD=Bulk density, UW=Unavailable water, EAW=easily available water, RW=Reserve water, HAW=Hardly available water, *=Data in Table 5.

Table 5. Moisture retention in sand.

Material and mixture	Field Capacity (FC) (%)	Permanent Wilting Point (PMP) (%)
River sand	11	7
River sand + soil	19	10

The bulk density of sawdust was low due to the size and organic nature of the particles and the porous space between them. In both sawdust and soil + sawdust mixture, unavailable water (UW) was high because it was lost by draining (Table 4).

River sand is a heavy material, so mixing it with the soil produced a high bulk density compared to the other materials and mixtures. The total porosity of this mixture was low, resulting in low aeration and moisture retention (Table 4). Table 5 shows the low interval between the Field Capacity (FC) and the Permanent Wilting Point (PWP) of the sand and the soil + sand mixture, which implies a low margin of water availability for the plant. The treatment with sand showed the lowest results in plant response. According to Barbaro *et al.* (2017), the bulk density values should be considered when choosing a suitable substrate. This value should allow easy handling, transport, and anchoring of the plant. Moreover, the total void space must be greater than 85% to facilitate air and water flow. According to the void size and the nature of the material, total porosity can be divided into aeration porosity and moisture retention. These two parameters are higher in needles and leaf mold and in their mixtures with soil (Table 4), which is reflected in the distribution of unavailable water (UW), easily available water (EAW), reserve water (RW), and hardly available water (HAW). According to Taiz and Zeiger (2006), the root system is directly affected by the hydric state of the soil (or substrate). The absence of easily available water (EAW) between the field capacity and permanent wilting percentage (Azcón and Talón, 2013) causes stomata closure. This results in a reduction in the photosynthetic rate, which determines plant growth. In the case of the sand treatment, this development was limited due to its low water retention. However, clay particles are smaller than sand particles, thus providing a greater surface area to retain water and smaller channels between particles. Regarding field capacity, sand only contains one-third of the amount of water that clay does (Azcón and Talón, 2013).

Of note, the characteristics of the materials (ocote pine needles, leaf mold, sawdust, river sand, and soil) may vary between these and their mixture with soil. Therefore, it is also important to consider the soil's characteristics and properties.

Soil analysis. The soil used in the experiment was a silty loam soil (9.7% Sand, 65% Silt, and 25.3% Clay) with high organic matter content (11.47) and nitrogen (0.45%) content, pH=6.3, and high cation exchange capacity (CEC) CEC=28 cmol(+)Kg^{-1}. The wet color was 7.5R 3/3 dark brown, the dry color was 7.5YR 4/3 brown, and its bulk density was 1.25 g cm^3. The soil was classified as an Eutric Cambisol and presented favorable characteristics for the development of Mexican marigolds. According to Upadhya *et al.* (2022), *Tagetes erecta* requires high nutrient concentrations for its growth, especially N. The high cation exchange capacity (CEC) allows good retention of nutrients, and the slightly acidic pH favors their availability. In addition, the silty loam texture allows good root development.

CONCLUSIONS

Based on the results, the best substrate for the cultivation of pot-grown *Tagetes erecta* var. Inca II Deep Orange under open field conditions were soil + ocote pine needles and soil + leaf mold.

The use of substrates with materials generated from the timber industry in the community of Santa Cruz Itundujia, Oaxaca, will reduce the production costs of mexican marigold by taking advantage of waste materials that are common in the region. Similarly, this substrate can be used as a base for the production of various ornamental species produced in containers.

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Biological importance and environmental quality of the Laguna Santa Ana in Zacatecas, Mexico

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ABSTRACT

Objective: To determine the environmental quality and diversity of the waterfowl species of the Laguna Santa Ana, located in the municipality of Fresnillo, Zacatecas.

Design/Methodology/Approach: A physicochemical analysis of the water was carried out to determine its organic matter pollution. In addition, the Bradford method was applied to establish protein levels, and an optical emission spectrometry was conducted to detect heavy metals. The Shannon-Weiner diversity index (H') was carried out to identify and count the populations of the lagoon.

Results: The physicochemical analysis of water recorded 55 and 230.6 mg/L BOD5 and COD, respectively. High protein levels (0.27-2.95 mL/mL) indicated organic pollution and high arsenic levels. The Shannon-Weiner index (2.7) recorded a high waterfowl diversity.

Study Limitations/Implications: Biological abundance was not determined, as a result of the sample size. The changes regarding waterfowl diversity may be the consequence of the seasonal conditions.

Findings/Conclusions: The Laguna de Santa Ana has a high waterfowl diversity, including species of international interest; however, organic pollution has caused a a significant environmental deterioration.

Keywords: wetlands, organic pollution, waterfowl, Zacatecas.

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INTRODUCTION

As a result of their environmental characteristics, wetlands are the most productive ecosystems of the planet. They perform different ecological functions during hydrological and chemical cycles (Carrera, 1999). Mexico is located between the Nearctic and Neotropical biogeographic regions. This situation provides the country with a wide soil, climate, and ecosystem diversity, enabling the development of a wide variety of wetlands. Mexico has 11.4 million hectares of wetlands. Out of this total, 56% are located in the mainland (DUMAC, 2020) and are the habitat of different Nearctic resident and migratory waterfowl species (Baldassare and Bolen, 1994; Wilson and Ryan, 1997). The biological diversity of the wetlands is related to their biota and geographical location within the Mexican territory

(Cervantes, 2007). Five physiographic regions converge in the Mexican plateau: Sierra Madre Occidental, Central Plateau, Sierra Madre Oriental, Bajío, and the Trans-Mexican Volcanic Belt (Leopold, 1959). The three first areas make up the Northern plateau, where the State of Zacatecas is located. The wetlands of this area include permanent and seasonal swamps, and they are located in enclosed basins or endorheic wetlands. Wetlands have been substantially altered or destroyed by agriculture, overgrazing, pollution, salinization, and industrial use (Carrera and de la Fuente 2003, Clemente *et al.*, 2014). The Laguna Santa Ana is a lacustrine system with a permanently flooded subsystem and saline brackish (Semeniuk and Semeniuk, 1997). This wetland is vital for the recharge of the basins of the hydrological area of El Salado, the Fresnillo-Yesca basin, and the Fresnillo sub-basin (CONAGUA, 2015). It is part of the Central Migration Route of the American waterfowls and, consequently, it is their habitat during winter (Roberts *et al.*, 2023). The sandhill crane (*Antigone canadensis* Linnaeus, 1758) and the Mexican duck (*Anas diazi* Ridgway 1886) are the most important waterfowl species of this area. Mainly as a consequence of the destruction of their habitat, sandhill cranes are considered vulnerable and are included in the threatened species list for this area (Clemente-Sánchez *et al.*, 2014). In addition, due to its population dynamics, the Mexican duck is also included in the NOM-059-2010, as a threatened species. Other species of national and international interest in the area include the northern shoveler (*Spatula clypeata* Linnaeus, 1758) and the greater white fronted goose (*Anser albifrons* Scopoli, 1769). However, the wetland faces major problems that cause great damages to the area. Some of these problems include approximately 172,000 ha close to the wetland that are mainly used for irrigated agriculture (CONAGUA, 2015) and livestock raising (mainly cattle and horses). In early 2000, a brewery started to discharge treated water into the wetland. In addition, wastewater from the nearby Santa Ana community, garbage, and poaching have damaged the environmental quality of the wetland. Therefore, the hypothesis of this research was that the Laguna Santa Ana has a low environmental quality, as a consequence of the damage caused to the ecosystem, reducing the occurrence of waterfowls in the area. Consequently, the objective of this research was to determine the environmental quality and waterfowl diversity of the Laguna Santa Ana, in Zacatecas, Mexico.

MATERIALS AND METHODS

Study area

The Laguna Santa Ana is in northern Zacatecas, in the municipality of Fresnillo (23° 13' 19.4" N, 102° 44' 01" W). According to the Koppen Climate Classification, modified by García (2004), the climate is BS1kw (semidry), with summer rains and a lower proportion of winter rains.

Water sampling collection and waterfowl diversity analysis

In order to determine the water quality of the area where migratory and resident waterfowls inhabit, a manual punctual water sampling was carried out in 12 strategic points, following the guidelines for monitoring discharges and superficial and underground water (Contreras *et al.*, 2004). The samplings were divided as follows: the samples from points 1 to 8 were taken from the inner lagoon, near the discharge and the waterfowl areas, while the samples from points 9 to 12 were taken from the course of the discharge of the brewery; the aim of this process was to determine protein content. The samples were observed under a microscope to identify water microhabitats and microorganisms.

In order to determine water quality, samples were taken from 4 strategic points, taking care to prevent air bubbles, at a 15-30 cm depth. The samples were stored in a cooler until the lab analysis was carried out, following the guidelines of the Norma Mexicana de Aguas Residuales (DOF, 1980).

An intensive monitoring was carried out to determine biological diversity, following the fixed radius point count method (Hernández *et al.*, 2019). The procedure was carried out during winter (January and February 2019) when waterfowls are present in the wetlands. The monitoring points were established at less than 50 m from the birds. Four collaborators counted the specimens. They were distributed around the area where the waterfowls were located, near the discharge of the treated water released by the brewery. Optical equipment, including 30×50 binoculars and telescopes, were used, following the guidelines of the Manual para monitores comunitarios de aves (Ortega *et al.*, 2012). A reference bibliography specialized on Mexican waterfowl was used to identify the species (Peterson and Chalif, 1989; DUMAC, 2009). Subsequently, the data were subjected to a Simpson's diversity index (DSi) and the Shannon-Weiner diversity index (H') to determine the waterfowl species diversity (Roswell *et al.*, 2021).

Quality analysis of the water from the Laguna Santa Ana

The field water samples were subjected to a quality evaluation, using different types of analyses. The Bradford method or Coomassie brilliant blue (Bradford, 1976) determined the protein levels of the samples, while the physicochemical characteristics of wastewater were established using the method proposed by the Norma Mexicana de Aguas Residuales (DOF, 1980). An optical emission spectrometry with an induced coupled plasma was used to detect heavy metals in the water and soil of the reservoir, following the recommendations of the NOM-147-SEMARNAT/SSA1-2004 (DOF, 2004).

RESULTS AND DISCUSSION

Protein analysis

The Bradford method indicated a 0.27-2.95 μL/mL range of protein concentration, recording higher values in spring (Table 1). The protein content in the samples was associated with the presence of bacteria. According to Saker *et al.* (2022) microorganisms in polluted water increase during the warmest seasons of the year, because high temperatures are ideal for the reproduction of microorganisms.

Previous studies suggest that proteins can be found in water polluted by industrial discharges. This situation allows the formation of decomposers, which are microhabitats for different invertebrates, including arthropods that are a great part of the waterfowls' diet, including the following families: Chironomidae, Cyprididae, Corixidae, and Hyalellidae. These species are deeply related to polluted environments, because they are resistant to environmental conditions altered by organic matter and heavy metals (Tapía *et al.*, 2018).

Table 1. Protein levels during the three sampling periods (2019).

1	2	3
15/03/2019	**29/04/2019**	**11/09/2019**
1.7 μL mL^{-1}	2.95 μL mL^{-1}	0.27 μL mL^{-1}

The analysis identified the presence of the Spirulina (*Arthrospira*) micro-algae (Figure 1). Gutierrez-Salmeán *et al.* (2015) have described the high protein, fatty acids, carbohydrates, minerals, and vitamin content of these micro-algae.

Physicochemical analysis of water from the Laguna Santa Ana

Regarding the physicochemical analysis, water recorded a 9.16±0.56 average between the sampled areas. Castro-González *et al.* (2019) studied the water from the Río Grande in Texas, USA, and established that an alkaline pH indicated that metals could precipitate in the sediments or be absorbed by other suspended particles.

The physicochemical studies carried out in this research found heavy metals in the water, with high arsenic (As) content (59 ppb) in the area of occurrence of the birds and a higher As content (434 ppb) in the innermost sample collection point of the lagoon. Del Águila-Juárez *et al.* (2005) reported >1 Cr and Cd values, proving a moderate pollution level in the Lerma River basin. These results match the Cd findings of this research; the

Figure 1. A: Aquatic microhabitat; B: Paramecium with Spirulina parts inside it; C: Spirulina and Protist; D: Arthropods.

>1 values obtained suggest that the Laguna Santa Ana has a moderate-to-minimum pollution level.

CONAGUA (2013) has established chemical oxygen demand (COD) and biological oxygen demand (BOD5) as organic matter pollution indexes (Table 2). Consequently, high levels of both indexes indicate a high pollution level, taking into account that CONAGUA (2013) has established that the parameters for BOD5 are >30-<120 (polluted) and >120 (very polluted) and for COD are >40-<200 (polluted) and >200 (highly polluted).

Migratory waterfowl diversity analysis

The monitoring identified 22 waterfowl resident and migratory species in the area (Figure 2). The Shannon-Weiner Diversity Index and the Simpson's Diversity Index recorded 2.7 and 0.076 values, respectively, suggesting a high diversity level (Roswell, 2021).

The higher number of waterfowls was recorded in the effluent, the reservoir, and the mouth of the treated water discharge of the brewery, which is in the municipality of Calera de Víctor Rosales. The pollution and turbidity levels are lower in this area and the water oxygenation values (up to 9.6 mg/L) are permissible for aquatic life, according to

Table 2. Pollution index results from the Laguna Santa Ana.

Variable	Point 1	Point 2	Point 3	Point 4
Temperature °C	14	15	19	20
pH	8.51	8.88	9.61	9.67
Total dissolved solids mg L^{-1}	2.76	2.32	19	16.9
Conductivity mhos/cm	5.43	4.64	38	33.7
Total hardness as ($CaCo_3$) mg L^{-1}	111	89	322	111
Chlorides (-Cl) mg L^{-1}	273	332	6,986.00	3.72
Nitrates (NO_3) mg L^{-1}	1.5	0	1.9	4.7
Total phosphorus mg L^{-1}	158	130	310	680
Total phosphates mg L^{-1}	154	102.5	257.5	570
Total nitrogen Kjeldahl mg L^{-1}	5	0	6.25	15
Dissolved oxygen mg L^{-1}	9.6	5.9	6.1	6.1
Turbidity NTU	38	369	102	216
Free residual chlorine mg L^{-1}L	0	0	0	0
COD mg L^{-1}	65	137	719	1.195
BOD_5 mg L^{-1}	98	31	27	64
Total Coliforms NMP mL^{-1}	7	15	<3	<3
Coliformes fecales MPN mL^{-1}	<3	4	<3	<3
E. coli units	Positivo	Positivo	Negativo	Negativo
Mercury mg kg^{-1}	<1	<1	<1	<1
Chromium mg kg^{-1}	0.03	0.02	0.05	0.08
Lead mg kg^{-1}	0.16	0	1.36	0.11
Arsenic mg kg^{-1}	59	49	43	434

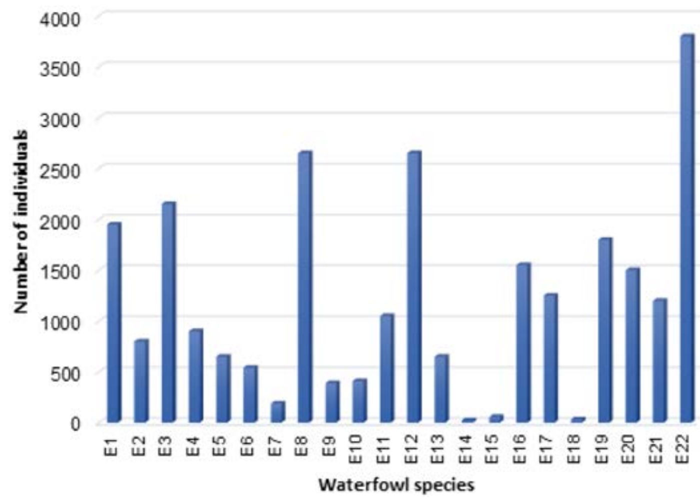

Figure 2. Number of individuals per species. E1: *Anas acuta*; E2: *Anas americana*; E3: *Anas clypeata*; E4: *Anas cynaptera*; E5: *Anas discords*; E6: *Anas creccacarolinensis*; E7: *Anas platyrhinchos*; E8: *Anas steptera*; E9: *Aythya affinis*; E10: *Bucephala albeola*; E11: *Oxyura jamacinensis*; E12: *Anser albifroms*; E13: *Chen caerulescens*; E14: *Ardea herodias*; E15: *Bubulcus ibis*; E16: *Fulica americana*; E17: *Himantopus mexicanus*; E18: *Pelecanus erythrorhynchos*; E19: *Recurvirostra americana*; E20: *Actitis macularius*; E21: *Calidris melanotos*; E22: *Antigone canadensis*.

CONAGUA (SEMARNAT, 2013). A higher number of waterfowls have been reported in the treated water discharge areas of the wetlands, possibly as a consequence of its increase of nutrients and the formation of artificial wetlands. The high pollution levels of these areas can be an important source of diseases (Jarma *et al.*, 2021).

The counting identified 22 waterfowl species, including the sandhill crane, which recorded the highest number of individual (3,800) in the lagoon. This result is extremely important, because this species is included in the special protection category of the NOM-059-SEMARNAT-2010 (DOF, 2010). Consequently, this wetland is very important for waterfowl diversity and as habitat for migratory species. Clemente (2014) pointed out that the state of Zacatecas is in the southern limit for the migration of this species and that the wetland of the Laguna Santa Ana is part of their winter habitat.

The diversity of waterfowl species in the Laguna Santa Ana suggests its biological importance as a winter habitat for migratory species. Ayala *et al.* (2013) reported similar biodiversity indexes in other regions, including the Tláhuac swamp and the Chalco and Mexico lagoons, which they describe as "important areas for bird preservation." The Laguna de Santa Ana has a high diversity index. On the one hand, the presence of internationally important migratory birds and threatened bird species, such as the sandhill crane, shows its importance as habitat for priority species. On the other hand, the wetland is moderately polluted, which damages its environmental quality. However, organic matter pollution can alter the food habits of some duck species, as a consequence of the increase of the invertebrate populations that are part of their diet (Szalay and Resh, 2000). Meanwhile, the environmental degradation of the wetlands could cause a long-term damage to the waterfowl population (Cotín, 2012). Therefore, determining the anthropogenic vulnerability of the wetland is fundamental, as well as the pollution to which it is exposed. In addition, restoration and preservation actions should be carried out, since this research shows the biological importance of the area as a habitat for internationally important waterfowl species.

CONCLUSIONS

The water quality analysis recorded low-moderate to high pollution values, depending on the area of the lagoon where the sample was taken from. The results highlight the arsenic content of the whole lagoon. The diversity indexes recorded high values regarding migratory waterfowl species, including ecologically important species listed under the preservation status of the NOM-059-SEMARNAT-2010.

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Seasonal anestrus of sheep flocks

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ABSTRACT

Objective: To determine seasonal anestrus in relation to the season of the year, feeding type, breed, and social environment variables of sheep production systems in Singuilucan, Hidalgo, Mexico.

Design/Methodology/Approach: Based on n=41 semi-structured surveys applied to the producers of the study area, a logistic regression analysis was carried out to determine the influence of the study variables on the seasonal anestrus of sheep production systems.

Results: Thirty-nine production units with seasonal anestrus periods were identified. Seasonal anestrus was influenced by the season of the year, feeding type, breed, and social environment variables ($P<0.05$). The anestrus periods were influenced ($P<0.05$) by spring (season of the year), range and shed system with concentrate feeding (feeding type), wool sheep (breeds), and the presence or absence of rams in the flock (social environment).

Study Limitations/Implications: Determining which variables influence seasonal anestrus will help to develop sheep reproductive programs.

Findings/Conclusions: Wool sheep have a seasonal anestrus in spring. The season of the year, feeding type, breed, and social environment variables influenced the seasonal anestrus of sheep production systems in Singuilucan, Hidalgo, Mexico.

Keywords: sheep reproduction, interviews, photoperiod.

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INTRODUCTION

Sheep production is the main livestock activity in Mexican rural communities; this activity allows the inhabitants to overcome poverty, complementing their income (Galaviz *et al.*, 2011; Herrera *et al.*, 2019). Mexico has 8,805,206 sheep. Out of that total, 1,105,275 sheep are located in Hidalgo, which is the second sheep producer in the country (SIAP, 2022). The sheep from this region are mainly crossed livestock (Suffolk or Hampshire × hair sheep) and are located in marginal areas, pastures, and areas with agricultural waste (Hernández *et al.*, 2017). It is of minor importance because, in some cases, it is a subsystem of agricultural or livestock production systems (Pérez *et al.*, 2011). Although they are a major source of livelihood for rural populations, there is a lack of information about sheep production systems (Vázquez *et al.*, 2018).

Seasonality of reproduction is one of the main limitations of sheep productivity (Ramírez-Ramírez *et al.*, 2021) and poses a main challenge for the development of sheep reproduction management strategies (Vera *et al.*, 2013). This phenomenon is the result of the incomplete knowledge about the mechanisms that rule seasonal reproduction (Zahoor *et al.*, 2018). In this type of reproduction, ewes have a reproductive cycle and an anestrus cycle during different times of the year (Ungerfeld, 2016). This behavior is one of the adaptation mechanisms developed by mammals as part of their survival strategies and is synchronized by external environmental signs that, in turn, drive the internal circuit of the seasonal breeding cycle (Wood and Loudon, 2018). The photoperiod is one of the major environmental factors that regulate the reproductive cycle of sheep (Leyva *et al.*, 2023; Ramírez-Ramírez *et al.*, 2021) and it is mainly based on the daily increase or reduction of sunlight. Melatonin secretion takes place at night; this hormone synchronizes the physiological processes with the environmental conditions (Ungerfeld, 2016). Meanwhile, nutrition is an important regulator of reproduction and it may stimulate the hypothalamic-pituitary-gonadal axis (Urviola and Fernández, 2017). In addition, other factors, such as breed and social environment, impact reproductive processes and determine the duration of the seasonal anestrus (Arroyo, 2011; Urviola and Fernández, 2017). However, there is not enough knowledge about all the variables involved in this process and the degree to which they influence the seasonal anestrus of sheep. Consequently, further research should be carried out to understand sheep reproduction (Arroyo, 2011; Abecia *et al.*, 2024). Therefore, the objective of this study was to determine the relation of seasonal anestrus with the season of the year, feeding type, breed, and social environmental variables of the sheep production systems in Singuilucan, Hidalgo, Mexico. The hypothesis was that seasonal anestrus depends on the evaluated variables.

MATERIALS AND METHODS

Study Area

The study was carried out in the municipality of Singuilucan, Hidalgo, Mexico, located between 19° 52' and 20° 08' N at 2,645.88 m.a.s.l. The area has a temperate subhumid climate with summer rains. The mean annual temperature ranges from 10 °C to 16 °C, while precipitation reaches 400-1,100 mm (INEGI, 2009).

Data Collection

Data were obtained from primary sources, through semi-structured interviews with producers, relatives, and employees. The interviews included some specific questions. Qualitative and quantitative research methods were used to analyze data. In addition, a simple random sampling method was used, considering each production unit as a sample (Bustamante, 2011).

Determining sample size and pilot test

Sample size was determined using the sample size determination method for a finite population, with a 95% confidence level, a 1.96 coefficient ($Z\alpha$), and 6% accuracy (Bustamante, 2011).

For this study, a pilot study (García, 2013) was carried out to determine a real (p) value (5%), which resulted in a sample size (n) of 40 sheep production units that should be surveyed out of a population of 191 registered production units (N) (INEGI, 2007). However, 41 surveys were conducted.

Evaluated variables

The seasonal anestrus dependent variable was obtained classifying the reproduction inactivity period according to natural anestrus types, starting on the months when producers reported births and estrus periods for their flocks. These classifications consider a 5-month gestation period and a 3-month anestrus period due to lactation.

There were four independent variables: season of the year, feeding type, breed, and social environment. The first variable recorded the variations of the hours of light per day during the different seasons of the year (spring, summer, autumn, and winter). This factor influences the reproductive behavior of sheep (Bittman and Karsch, 1984). The second variable included three feeding types: extensive system (range sheep grazing in pastures and fed on stubble from cereal crops); a shed system with concentrate feeding and forage systems (based on agricultural by-products); and mixed range, combined with grain and agricultural by-products (Partida *et al.*, 2013). Two groups were established for the third variable: 1) Hampshire and Suffolk crossed breed wool sheep; and 2) Dorper-Kathadin crossed breed hair sheep. The fourth variable depends on the presence or absence of a ram in the flock. Pregnant, lactating, or seasonal anestrus ewes are separated from rams.

Statistical Analysis

A bivariate and multivariate logistic regression was carried out. The percentage of significance was obtained with the R-square, based on the ratio between each independent variable and the dependent variables ($P<0.05$). The IBM SPSS Statistics software was used for this purpose (Rivadeneira *et al.*, 2020).

RESULTS AND DISCUSSION

Season of the year

Out of the 41 production units surveyed, two did not record seasonal anestrus, while the seasonal anestrus in the other 39 (95.1%) was dependent on the season of the year. Thirty-seven production units included in the latter group reported seasonal anestrus in spring ($P<0.05$) and two in summer (Table 1).

Feeding type

Thirty-nine production units with seasonal anestrus were identified for this variable. Thirty-three production units have a range feeding system. One of the two production units that used a shed-based feeding system had wool sheep, while the other had hair sheep; the former recorded seasonal anestrus, while the latter did not report this situation ($P<0.05$). Additionally, five out of the six production units that had a mixed feeding system recorded seasonal anestrus.

Table 1. Sheep production units that recorded seasonal anestrus in Singuilucan, Hidalgo, Mexico.

Variables	Production units with seasonal anestrus	p-value***
Seasons of the year*	39/41	0.05
Spring (April-June)**	37/37	0.05
Summer (July-September)**	2/2	0.743
Types of feed*	39/41	0.05
Free grazing**	33/33	0.05
Stabled**	1/2	0.05
Mixed**	5/6	0.147
Breed type**	39/41	0.05
Wool sheep	39/39	0.05
Hair sheep	0/2	0.05
Social environment**	39/41	0.05
Presence of males	31/31	0.05
Absence of males	8/10	0.05

* Polytomous independent variables: season of the year, feeding type.
** Dichotomous independent variables: breed, social environment.
*** *p* value of the production units that recorded seasonal anestrus, in relation with their production units per variable.

Breed

Out of the 41 evaluated production units, 39 reported seasonal anestrus. These productions units have wool sheep, while the two production units without seasonal anestrus have hair sheep ($P<0.05$).

Social environment

Out of the 39 production units that reported seasonal anestrus, 31 have a ram, while 8 did not have a ram in the flock ($P<0.05$).

Regarding the season of the year variable, the reports indicate that anestrus takes place during spring. Several factors regulate the seasonality of reproduction of wild and domestic ungulates, including the photoperiod, which changes depending on the season of the year. Photoperiod is the primary environmental factor that regulates annual reproduction cycles, followed by the environmental factors that influence food availability (Urviola and Fernández, 2017). Sheep secrete melatonin —a key hormone indicator of the changes in the length of a given day. This indicator changes depending on the season of the year (Li *et al.*, 2021). Melatonin is secreted at night, with a circannual rhythm stimulated mainly by the photoperiod. In its turn, it stimulates the pulsatile activity of the gonadotropin-releasing hormone neurons (Chemineau *et al.*, 2010). On the one hand, the seasonal anestrus stage (long days) takes place during spring and summer, with low levels of melatonin secretions. On the other hand, the reproduction stage (short days) takes place in autumn and winter, with high levels of melatonin secretion (Bittman and Karsch, 1984; Arroyo, 2011). In this regard, Arroyo *et al.* (2007) determined that Suffolk sheep, located in central Mexico (19° N) have a clear and defined seasonal anestrus, during winter and spring.

Partida *et al.* (2013) reported different feeding systems: range, concentrate, and mixed. This study found that production units with range sheep have seasonal anestrus mainly during spring. The reproductive activities of most animal species are inhibited during certain periods of the year. This is a common process that guarantees the survival of the litter, because it prevents births during unfavorable periods. Consequently, births take place at the end of winter or the beginning of spring, when the weather is more favorable for the development of the litters (Ramírez-Ramírez *et al.*, 2021). In this regard, González *et al.* (2014) pointed out that wool sheep give birth during spring, when the native grasses from which they graze reappear thanks to the rains of the season. This study did not analyze the estrus and births data to determine the seasonal anestrus period; however, the data about estrus and birth seasons reported by the producers were used to infer that the reproduction season took place in summer. Meanwhile, regarding the two production units that use concentrate feedings under a shed-based system, one bred wool sheep with a seasonal anestrus, while the other had hair sheep without seasonal anestrus. In this regard, Arroyo *et al.* (2007) found that, under a constant nutritional plan, Suffolk sheep have ovarian inactivity periods from February to June. These findings match the results obtained in this study with wool sheep fed with concentrates under a shed-based system. In addition, these authors mentioned that, under a control feeding regime, Pelibuey sheep have a constant ovulatory activity throughout the year. These results also match the findings of this research regarding hair sheep. Consequently, the breed variable influenced the surveyed production units.

Regarding breed, all the wool sheep production units of this study reported seasonal anestrus during spring. In this sense, ewes from most breeds are seasonal breeders, with ovulatory cycles in autumn and winter and anovulatory periods (seasonal anestrus) in spring and summer (Kopycinska *et al.*, 2022). Interestingly, Mediterranean wool sheep breeds have seasonal breeding patterns, mainly regulated by the changes in the photoperiod (Bittman and Karsch, 1984). Sheep from high latitudes (>35°) have a two-period seasonality of reproduction during a year: one mating season and one reproductive inactivity season, which is mainly ruled by the photoperiod (Bittman and Karsch, 1984; Arroyo, 2011).

Meanwhile, the Katahdin and Dorper hair sheep production units recorded no seasonal anestrus in this study. In this regard, González *et al.* (2014) mentioned that, at 23° 53' N, hair sheep such as Dorper and Katahdin can reproduce in spring. Meanwhile, Arroyo *et al.* (2007) reported that Pelibuey hair sheep can ovulate throughout the year at 19° N. For their part, Juárez *et al.* (2018) pointed out that, at 21° N (subhumid tropics), Pelibuey sheep do not have seasonality of reproduction; however, they have a higher follicular atresia during spring. In this regard, Macías *et al.* (2015) mentioned that, at 32° N, Pelibuey sheep may not have estrus periods in winter and spring, under Mexican arid conditions. Gastelum *et al.* (2015) studied the circannual estrus of Pelibuey sheep at 32° N and found that the estrus activity diminishes from January to June, under Mexican arid conditions. This reduction can be related to the individual sensitivity of some Pelibuey sheep to the photoperiod.

The social environment of the sheep in this study was closely related to the seasonal anestrus, with and without a ram in the flock. The social interaction between ewes and rams modifies the reproductive cycle of ewes. This phenomenon is a consequence of the pheromone-driven "male effect." Exposing an ewe during its seasonal anestrus to a sexually active ram can usually result in a fast increase of its LH pulse frequency (Delgadillo *et al.*, 2008). This stimulus triggers the ovulatory activity and the estrus signs in ewes (Abecia *et al.*, 2024). Meanwhile, Arroyo (2011) reported that the social signs largely influence ewes with a marked seasonality, because the separation and subsequent incorporation of the ram in an ewe flock can synchronize the reproductive cycle of ewes. On the contrary, the year-long presence of a ram within a flock result in larger anestrus periods. According to Delgadillo *et al.* (2008), the "male effect" requires certain conditions, such as separating the rams from the flock, in order to prevent any chemical, visual, audio, and tactile interaction. This study did not include the evaluation of these conditions; however, De St Jorre *et al.* (2012) have proven that separating the ram from the flock is unnecessary, because the introduction of a new sexually active male in the flock promotes a good response from the ewes. Both the presence and the absence of a ram in a flock makes seasonal anestrus possible, because factors such as the ram stimulus quality, age, body condition, and nutritional and genetic conditions of the ewes significantly impacts the response to the "male effect" (Abecia *et al.*, 2024). Although no differences were recorded regarding the presence of the estrus with or without rams in the flock, this practice —separating and subsequently introducing a ram to the flock— can prevent the pharmacological manipulation used to control the reproductive activities of ewes, because the socio-sexual factors that drive the introduction of a ram in the flock can stimulate ovulation in ewes (Hawken and Martin, 2012) and speed up puberty in female lambs. Finally, it is an efficient and sustainable system to increase the productive lives of ewes, simultaneously preventing the use of hormonal treatments (Abecia *et al.*, 2016).

CONCLUSIONS

Seasonal anestrus appears in spring and is influenced by the season of the year, feeding type, breed, and social environment of wool sheep production systems in Singuilucan, Hidalgo. In conclusion, determining the factors that influence seasonal anestrus is fundamental to developing recommendations aimed to improve productivity in sheep production systems.

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Colegio de Postgraduados

Catiknifap, new native variety of Xcat ik pepper (*Capsicum annuum* L.)

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ABSTRACT

Objective: To describe the morphological and agronomic characteristics of Catiknifap, a native variety of the Xcat ik pepper.

Design/Methodology/Approach: Catiknifap was formed by mass selection method from a P108 accession collected in 2013 in Muna, Yucatan. Characterization and yield trails were carried out in greenhouse during three cycles from 2021 to 2022. Forty-five characteristics were evaluated based on UPOV guidelines.

Results: Catiknifap is an early-ripening variety. Its fruits ripen between 77 and 81 days after transplanting. Its main morphological characteristic is the rounded shape at the junction with the peduncle. The yield depends on the growing season and ranges from 32.3 to 42.3 t ha^{-1}.

Study Limitations/Implications: Assays were carried out in greenhouses. Pests and diseases may cause differences in open field yield.

Findings/Conclusions: Catiknifap is the first morphologically characterized variety of Xcat ik pepper. The fruits of this homogenous and stable variety have a high yield potential in Yucatan.

Keywords: *Capsicum annuum* L., vegetable, yield.

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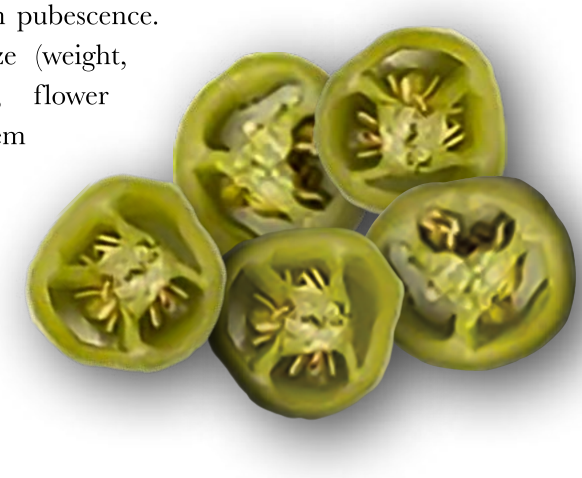
INTRODUCTION

Xcat ik (*Capsicum annuum* L.) is a highly valued native chili pepper in the Yucatan Peninsula. The fruit has an elongated, conical, and pointed shape. Unripe fruit is greenish-yellow, gradually turning orange and red as it ripens, until it becomes fully red (Santamaría and Zavala, 2021). Consumers prefer a yellowish-green, slightly orange fruit with firm texture (Santamaría *et al.*, 2022a). The production is carried out by small-scale farmers. As a result of the limited financial resources they can allocate to cultivation, they employ low technology, particularly in mineral nutrition (Gamboa-Angulo *et al.*, 2020).

There is little variation in the vegetative characteristics of the Xcat ik chili pepper genotypes, such as the purple coloration caused by intermediate to dense anthocyanin at the nodes and stem pubescence. However, they show variation in fruit size (weight, diameter, and length-to-diameter ratio), flower size (anther and corolla length), and stem characteristics (Vera-Sánchez *et al.*, 2016). The fruit shape at the junction with the peduncle can be obtuse, truncated, or cordate, while the apex can be pointed, blunt, or sunken (Aguilar *et al.*, 2010).



The fruits of Xcat ik chili accessions from the Banco de Germoplasma of the Campo Experimental Mocochá have a narrow triangular shape with a non-enveloping calyx and an acute apex. However, the shape of its the junction can be rounded or flat or have a peduncular cavity (Santamaría *et al.*, 2022a; 2022b).

Gamboa-Angulo *et al.* (2020) and López *et al.* (2019) worked with Xcat ik chili accessions in their studies on the use of microbial biofertilizers and on genetic diversity, respectively, but both failed to describe the morphological characteristics of the said accessions. For their part, Castillo-Aguilar *et al.* (2019) used the descriptors for *Capsicum* from IPGRI, CATIE, and AVRDC (1995) for the morphological characterization of two ecotypes from the state of Campeche. As has been documented for several years, Xcat ik chili remains under-characterized and no varieties are available (González *et al.*, 2010; Santamaría *et al.*, 2022a). Therefore, open-pollinated varieties with outstanding agronomic characteristics should be developed and the seeds should be available for producers. Consequently, the objective of this study was to describe the morphological and agronomic characteristics of Catiknifap, based on the hypothesis that the morphological description of its plant, stem, leaf, flower, and fruit characteristics will establish it as a native variety of Xcat ik chili.

MATERIALS AND METHODS

Origin

Catiknifap originates from accession P108, collected in 2013 in Muna, Yucatán, and introduced into the Banco de Germoplasma of the Campo Experimental Mocochá (21° 06' 15" N, 89° 26' 15" W).

It was developed using a mass selection breeding method, which involves selecting individuals with similar phenotypic characteristics to improve a population by increasing the prevalence of desired types (López, 1995; Camarena *et al.*, 2014). The seeds from individuals with similar characteristics are mixed to form the next generation and this process is repeated as many times as necessary, until the population becomes homogeneous (Márquez, 1988; Ramírez and Méndez, 2018). Stable characteristics lead to a quick selection process (Camarena *et al.*, 2014). For example, a triple-cycle mass selection of sweet chili pepper (*Capsicum annuum* L.) led to significant improvements in the development of two agronomically outstanding varieties (Chi-Kantún *et al.*, 2017).

Growing Conditions

The yield and homogeneity evaluation trials were conducted in a greenhouse with plastic roof and anti-aphid net walls. The plants were spaced 1.5 m between rows and 0.5 m between plants on ≈15-cm tall, raised beds. Drip tape with emitters spaced every 10 cm was used for pressurized irrigation.

Characteristics Evaluation

Accession P108 was collected based on fruit characteristics; however, the varietal description also considered other characteristics. In total, 45 characteristics were evaluated: 3 related to the plant, 2 to the stem, 8 to the leaf, 5 to the flower, and 27 to the fruit. Depending on the characteristic, 10 or 20 readings were conducted based on the test guidelines proposed by UPOV (2018, 2020) for distinctness, uniformity, and stability of *Capsicum annuum* L.

Yield

Three yield assays were conducted. The first was made on August 1, 2021 (at the beginning of the fall-winter cycle), the second on January 5, 2022 (at the end of the fall-winter cycle), and the third on November 18, 2022. Fruits were classified according to the length code established in the NMX-FF-025-SCFI-2014 Mexican standard (Secretaría de Economía, 2015): large fruits >16 cm (size 5), medium fruits from 12.1 to 16 cm (size 4), and small fruits from 8.1 to 12 cm (size 3).

Statistical Analysis

The yield data were subjected to an analysis of variance, considering the cycle (sowing date) as the treatment in a completely randomized design with three replications. The Shapiro-Wilk and Bartlett tests were used to verify the normality and homogeneity of variances, respectively. Means were compared using Tukey's test ($P \leq 0.05$) with the R statistical software version 4.2.3 (R Core Team, 2023).

RESULTS AND DISCUSSION

Morphological Characteristics

Plant. Anthocyanins can be found in the hypocotyl of seedlings during the cotyledon leaf stage. The plant is tall with a semi-erect growth habit. The height range of the Xcat ik chili plant depends on its environmental conditions: from 30 to 70 cm in open field conditions (González *et al.*, 2010) and from 66 to 85 cm in greenhouse conditions (Castillo-Aguilar *et al.*, 2019). The average height of the Catiknifap plant is 120 cm at 85 days after transplanting (DAT) and 160 cm at 160 DAT.

Stem. The stem show medium intensity anthocyanin pigmentation at the nodes and the pubescence is absent or very weak. Anthocyanins at the nodes are a typical characteristic of the Xcat ik chili (Aguilar *et al.*, 2010; Castillo-Aguilar *et al.*, 2019).

Leaves. The lanceloate-shaped leaves have a long blade (13.5 cm average) and medium width (6.5 cm average). Although their size is similar, the lanceolate shape of the leaf differs from the oval shape of the leaves in ecotypes 1 and 2 of Xcat ik chili (Castillo-Aguilar *et al.*, 2019). Young leaves are greener than mature leaves. The margin undulation is weak, blistering is very weak, and the cross-sectional profile is moderately convex. The central vein of the leaf has anthocyanins.

Flowers. Each plant has 1 or 2 flowers per axil with a pendent peduncle, 5 to 6 white petals, and anthers with anthocyanin pigmentation.

Fruit. Xcat ik chili has elongated fruits (Aguilar *et al.*, 2010; Castillo-Aguilar *et al.*, 2019). The fruits of Catiknifap are long (14.0-18.5 cm), but have a narrow triangular shape, with a very sharp apex and medium diameter (2.5-3.5 cm at the widest point), a high length-to-diameter ratio, and an angular cross-sectional shape. They have a slight C-shaped curvature, without twisting. The fruits hang downwards. The calyx has a non-enveloping aspect, except when the fruits have not completed their development. It has no peduncular

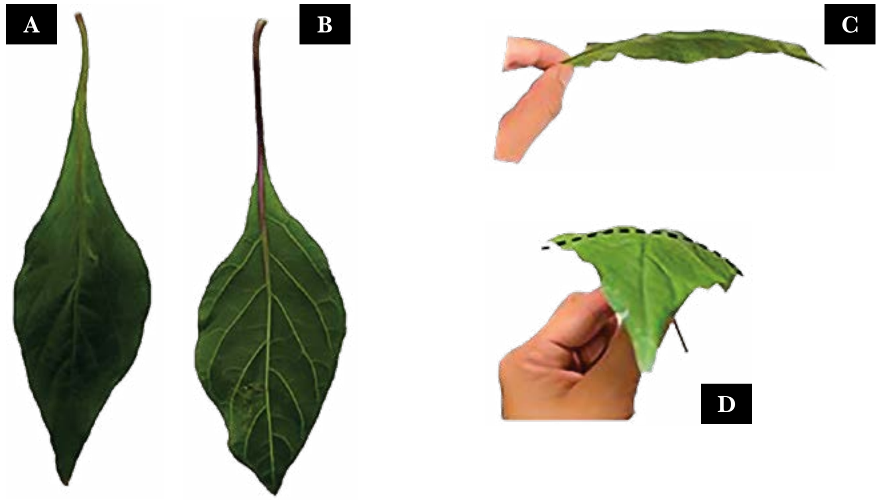


Figure 1. Leaves of Xcat ik chili var. Catiknifap. (A) Lanceolate shape with weak margin undulation. (B) Anthocyanins in the central vein. (C) Very weak blistering. (D) Moderately convex cross-sectional profile.

cavity. The main distinguishing characteristic of the Catiknifap fruit is the rounded shape at the junction with the peduncle. In other plant materials, the fruit shape at the junction with the peduncle can be obtuse, truncated, cordate (Aguilar *et al.*, 2010), rounded, or flat; they could even have a peduncular cavity (Santamaría *et al.*, 2022a; 2022b).

Unripe fruits do not have anthocyanin pigmentation; however, when plants are subjected to stress from high temperatures and/or pest attacks, the fruits may display pigmentation. Upon ripening, the yellowish-green fruit acquires a medium-intensity and very bright red hue. It has no sinuousness of the pericarp at the basal part, or it is very weak, while the non-basal pericarp is weak. The surface texture is smooth. Most of the fruits have 3 locules, although 2 and 4 locules have also been reported. It has no depth in the interlocular grooves. Capsaicin content was recorded in the fruits. The fruits have a thin pericarp (3 mm), a medium-length peduncle (3.5 cm), and a thin diameter. The cream-colored seeds are circular, slightly longer than wide. The average length, width, and weight of the seed of unripen fruits are 4.40 mm, 3.80 mm, and 6.6 g per 1,000 seeds, respectively. The seeds of ripe fruits are 4.25 mm long and 3.70 mm wide and have weight of 7.2 g per 1,000 seeds.

Some of the morphological characteristics of Catiknifap recorded in this study are the same as in other reports, such as anthocyanin pigmentation at the nodes, petal color, anthocyanin pigmentation of the anthers, fruit brightness, absence of anthocyanin pigmentation in the fruit, cream color of the seeds, and weight of 1,000 seeds. However, differences were recorded in other characteristics (plant height, leaf shape, and fruit). The Catiknifap plant is taller (up to 160 cm) than ecotypes 1 and 2 (85 cm), under similar greenhouse conditions. Catiknifap has lanceolate leaves, while ecotypes 1 and 2 have oval leaves. The fruits of Catiknifap hang like those of ecotype 1, but they are different from the erect posture of ecotype 2 fruits reported by Castillo-Aguilar *et al.* (2019).

Three characteristics distinguish Catiknifap fruits: the absence of a peduncular cavity, the rounded shape at the junction with the peduncle, and the fruit shape itself. The first two characteristics are closely related due to their location on the fruit. The third

Figure 2. Fruits of Xcat ik chili var. Catiknifap. (A) Green fruits at commercial ripening. (B) Ripe fruits. (C) Angular cross-section with 2, 3, and 4 locules.

characteristic is the fruit shape; the difference between the elongated shape reported by Aguilar *et al.* (2010) and Castillo-Aguilar *et al.* (2019) and the narrow triangular shape with a very sharp apex reported in this study may be a morphological difference resulting from the choice of descriptor. Castillo-Aguilar *et al.* (2019) used the descriptors proposed by IPGRI, CATIE, and AVRDC (1995), whereas this study employed the descriptors from UPOV (2018). As has been discussed before, Xcat ik chili is poorly characterized and the lack of descriptive studies prevents comparisons between the morphology of its accessions or ecotypes. This work provides the first morphological characterization of Xcat ik chili using UPOV descriptors, which is the methodology accepted by SNICS for the registration of varieties in Mexico.

Yield

Catiknifap is an early-ripening variety, whose fruits reach maturity between 77 and 81 days after transplanting (103 to 107 days after sowing in the seedbed). The harvest lasted for 3.1 to 3.4 months (Table 1). Statistical differences were found regarding yield in the analysis of variance (Table 2). The values for the Shapiro-Wilk and Bartlett tests were 0.4198 and 0.6244, respectively. The yield of the plot established in August 2021 was 35.4 t ha^{-1}, a value that was statistically equivalent to the yield (32.3 t ha^{-1}) obtained in the plot established on November 18, 2022. In both cases, most of the production consisted of large fruits. The yield of the plot established in January 2022 was 42.3 t ha^{-1}. The productive stage of this plot took place during the hottest months, promoting faster growth and greater production. However, this situation impacted the fruit size.

The yields of the Catiknifap variety are higher (32.2 to 42.3 t ha^{-1}) than the yields reported by SIAP (2023) in protected agriculture in Yucatán (17.0 to 21.1 t ha^{-1}). Moreover, they are like the yield obtained from a local variety (37.4 t ha^{-1}), under the same greenhouse conditions during the 2020-2021 cycle (Santamaría *et al.*, 2022b). Gamboa-

Table 1. Fresh fruit production of Xcat ik chili var. Catiknifap on 3 sowing dates.

Date of planting	1 aug 2021	5 jan 2022	18 nov 2022
Planting to harvest (days)	81	79	77
Harvest (months)	3.4	3.1	3.2
Yield (t ha^{-1})	35.4 b	42.3 a	32.3 b
Small size fruits (%)	6.4	6.6	12.3
Medium size fruits (%)	29.6	52.1	31.8
Large size fruits (%)	64.0	41.3	55.9

Means with the same letter in the row are statistically equal (Tukey, $p \leq 0.05$).

Table 2. Analysis of variance for the fresh fruit production of Xcat ik chili var. Catiknifap on 3 sowing dates.

Source of variation	Degree of freedom	Sum of squares	Mean square	F	p
Date of planting	2	156.0017	78.0008	16.9409	0.0034
Error	6	27.6257	4.6043		

Angulo *et al.* (2020) reported 1.69-1.85 kg per plant yields of Xcat ik chili. Considering a density of 13,320 plants per hectare, the yields should be 22.5 to 24.6 t ha^{-1}. However, these results are lower than the yields obtained in this work.

The lack of Xcat ik chili varieties led to the development of Catiknifap; the aim was to uniformly preserve the native characteristics and to provide producers with an open-pollinated variety with outstanding agronomic characteristics.

CONCLUSIONS

Catiknifap is the first morphologically characterized variety of Xcat ik chili. Its rounded shape at the junction with the peduncle is its main distinguishing characteristic compared with other Xcat ik materials. Given its homogenous and stable fruit production, this variety has high yield potential in Yucatán.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of annatto and alfalfa on egg yolk pigmentation in Creole hens

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ABSTRACT

Objective: To evaluate the effect of an alfalfa- and annatto-enriched diet on the external and internal characteristics of eggs laid by Creole hens.

Design/Methodology: A set of 22 Creole hens was divided into two treatment groups. The first group (n=11 females and one male) was fed alfalfa, while the second (n=11 females and one male) consumed alfalfa and annatto. For the study, 116 eggs (alfalfa=58 and annatto=58) were collected and stored in refrigeration until further analysis. The data encompassed external physical characteristics (egg weight, length, width, and shell thickness) and internal physical characteristics (yolk weight, white weight, and yolk pigmentation). Yolk color was evaluated as per the DSM color spectrum.

Study Limitations/implications: Backyard poultry farming does not use artificial pigments to color egg yolks due to their high cost and low availability. Annatto and alfalfa are low-cost, handy alternatives.

Results: Adding fresh alfalfa or annatto to the Creole hens' diet did not significantly affect ($P>0.05$) egg weight, length, or width, nor ($P>0.05$) eggshell thickness. Yolk and white weight were not affected ($P>0.05$) either. However, supplementing the hens' diet with annatto intensified yolk pigmentation.

Conclusion: Adding annatto to the Creole hens' diet intensifies yolk color without affecting the external or internal physical characteristics of eggs. Annatto (known in Mexico as achiote) is a natural pigment with no harmful effects on human health compared to synthetic alternatives. It is also inexpensive and easily accessible.

Keywords: Egg, Yolk, Color, Natural pigment.

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INTRODUCTION

Chicken eggs are considered one of the most complete foods for humans, since they offer a well-balanced source of protein. The most abundant proteins in egg whites are ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%) (Nurliyani *et al.*, 2023). The yolk —separated from the white by the vitelline membrane— contains primarily lipids (Benedé and Molina, 2020). Due to their high nutrient digestibility and low cost, eggs are one of the most consumed animal products in Mexico (López-Sobaler *et al.*, 2017; Mendoza *et al.*, 2016).

The first physical characteristic of eggs noticed by consumers is eggshell color, with shades ranging from white and brown to olive green and blue, depending on the chicken breed (Rodríguez-Ortega *et al.*, 2021). Yolk color is another key feature that influences consumer preferences and approval. Consumers favor eggs with deep yellow yolks, while pale- or light-yellow yolks are perceived as lower in quality and tend to be rejected. Yolk color is directly linked to the carotenoid content in the hens' diet (Shevchenko *et al.*, 2021).

Carotenoids —natural pigments found in seeds, fruits, plants, and fungi— are divided into two groups: carotenes and xanthophylls. The most prevalent carotenes are α-carotene, β-carotene, and lycopene. Xanthophylls include β-cryptoxanthin, lutein, zeaxanthin, astaxanthin, fucoxanthin, and peridinin (Maoka, 2019). Birds and mammals cannot synthesize carotenoids —only plant tissues can (Jaswir *et al.*, 2011). Therefore, hens must obtain carotenoids through their diet. In hen egg yolks, the main carotenoids are lutein and zeaxanthin (Zaheer, 2017), along with small amounts of β-cryptoxanthin, while β-carotene is found but not as a major pigment.

The carotenoids in yolks are highly bioavailable due to their lipid solubility, rendering eggs an excellent source of carotenoids for human dietary enrichment (Kavtarashvili *et al.*, 2019). Eggs enriched with xanthophylls can serve as a functional source of carotenoids in human diets. Annatto or *Bixa orellana* L. (family Bixaceae), known as achiote in Mexico, is a carotenoid-rich plant, particularly high in bixin and norbixin, which provide its orange to red color (Rivera-Madrid *et al.*, 2016). In Mexico, annatto is used as a natural colorant in foods like bread, beverages, regional sweets, and marinated meats such as enchilada or al pastor. Annatto is non-toxic (Scotter, 2009) and has antioxidant and anti-inflammatory properties (Giridhar *et al.*, 2014).

Alfalfa is a highly digestible forage with low cellulose content, making it a suitable alternative feed for backyard poultry. Vera-Vázquez *et al.* (2021) note that this legume is rich in carotenoids, with a total carotenoid content of 257 mg/g MS^{-1}. In small-scale poultry farming, the use of artificial pigments to enhance yolk color is scarce because of high costs and limited availability. However, annatto offers a cost-effective and accessible alternative.

This study aims to evaluate the effect of fresh alfalfa and annatto supplementation in Creole hens' diet on the external and internal physical properties of eggs.

MATERIALS AND METHODS

The study was conducted at the Poultry Unit of the Universidad Politécnica de Francisco I. Madero, located in Tepatepec, Hidalgo, Mexico, at an altitude of 1,900 masl. The site has a temperate-cold climate with an average annual temperature of 17 °C and an average annual precipitation of 540 mm (Rodríguez-Ortega *et al.*, 2020).

Experimental Design

A completely randomized design was applied in this study, using 116 eggs —58 from the alfalfa group and 58 from the annatto group. The eggs were collected from 22 hens divided into two treatment groups. One group was fed commercial feed supplemented with minced fresh alfalfa (n=11 females plus one male), while the other group received the same feed supplemented with both alfalfa and annatto (n=11 females plus one male).

Bird Feeding

The commercial feed met the physiological requirements established by the National Research Council (1994). Each hen received 20 g of fresh alfalfa daily. For the second group, a commercial annatto paste was used to provide 10.41 g of annatto per hen per day. A solution was prepared by dissolving 25 g of annatto paste in 100 ml of water and mixing it with 240 g of fresh alfalfa (20 g of alfalfa/hen/day for 12 hens per treatment). The mixture was provided in free-access feeders measuring 1 m in length, 30 cm in width, and 15 cm in height.

External Physical Characteristics

The collected eggs were stored under refrigeration until further analysis. Egg weight, length, width, and eggshell thickness were measured using a digital caliper (HER-411 model, STEREN, Mexico) with a measuring range of 0 to 150 mm and a resolution of 0.1 mm.

Internal Physical Characteristics and Yolk Color

Yolk, egg white, and eggshell weights were measured using a digital scale with a 200 g capacity and 0.01 g resolution (MH-200 model, MKS Tools, China). Yolk pigmentation was visually assessed by comparing it with the DSM YolkFan (Figure 1). Three evaluators reached a consensus on the closest matching color. The color scale ranged from 1 (light yellow) to 15 (closer to orange).

Statistical Analysis

The Proc Univariate Normal procedure was employed to assess the normality of the data through the Shapiro-Wilk test (Alonso and Montenegro, 2015) for the following variables:

Figure 1. DSM YolkFan. The DSM color fan provides a practical and cost-effective method for evaluating yolk coloration. Color intensity can be considered an indicator of good health, performance, and well-being in hens.

egg weight, yolk weight, egg white weight, egg length, egg width, eggshell thickness, and yolk color. Statistical analyses were conducted using the SAS software (version 2011). The data were analyzed using the PROC GLM (General Linear Model) procedure, and the means were compared using Tukey's test. Yolk color was further examined using GLM and the PROC FREQ procedure. The correlations between egg weight and both egg white weight and eggshell weight were determined using the PROC CORR procedure.

RESULTS AND DISCUSSION

Egg weight is an important characteristic for consumers, who tend to prefer larger eggs. In this study, egg weight was not affected (P>0.05) by adding annatto or alfalfa to the hens' diet (62.32 g *vs*. 63.19 g; Table 1). External morphological characteristics such as egg weight, length, width, color, and eggshell thickness can be influenced by the age of the hens, their genetics, and their nutritional management. Kocevski *et al*. (2011) reported that egg weight increases with the age of hens, while Juárez-Cataracheа *et al*. (2010) found that Creole hens tend to lay smaller eggs compared to commercial breeds. Segura *et al*. (2007) noted that the weight of the first egg laid by Creole hens was 45.3 g, with the weight increasing as hens aged. Segura (2021) reported annatto in drinking water did not affect egg weight in Issa Brown hens. Rodríguez-Molano *et al*. (2023) observed that feeding fresh alfalfa did not affect egg weight in Babcock Brown hens (56.63 g *vs*. 55.62 g).

In this study, egg length and width were not affected by adding annatto or alfalfa to the hens' diet (P<0.05; Table 1). Illescas-Cobos *et al*. (2022) report the following morphometric standard for eggs laid by Creole hens: average weight=55.96 g, length=5.72 cm, and width=4.18 cm. The results in this study are similar to the aforementioned standard.

Egg color and weight, along with eggshell thickness are crucial for consumers and the poultry industry. Due to their hardness and thickness, eggshells protect the egg whites and yolks inside them, so a reduction in eggshell weight leads to breaking or cracking which, in turn, increase losses. In this study, eggshell weight was not affected by feeding hens annatto or alfalfa (Figure 2). The eggshell weights observed (Figure 2) align with the findings of Kibala *et al*. (2018), who reported an average eggshell weight of 7.30±0.6 g in Rhode Island Red hens.

Egg white and yolk weights were not affected (P>0.05) by the dietary treatments (Table 2). The egg white contains highly digestible proteins such as ovalbumin, ovotransferrin,

Table 1. Weight, length, and width of eggs of Creole hens fed with annatto or alfalfa.

Treatment	Weight (g)	Length (cm)	Width (cm)
Annatto	62.32[a]	5.92[a]	4.41[a]
Alfalfa	63.19[a]	5.92[a]	4.39[a]
Standard error	0.7326	0.038	0.032
P-valor			
Shapiro-Wilk	0.0222	<0.0001	<0.0001
ANOVA	0.4059	0.9557	0.7150

Matching letters in each column indicate that there are no significant differences between treatments, according to Tukey's test (P>0.05).

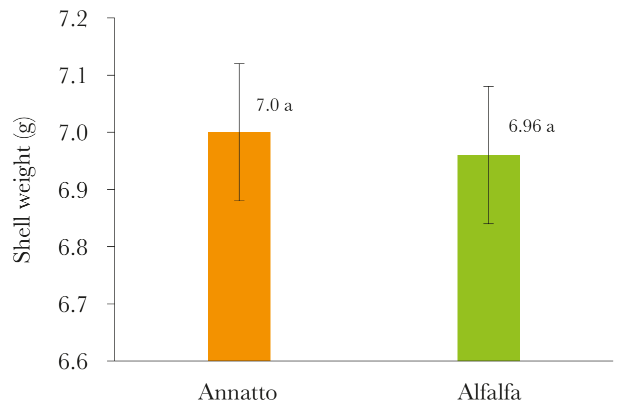

Figure 2. Eggshell weight in Creole hens fed with annatto or alfalfa. The Shapiro-Wilk test was used to determine data normality (P≤0.0001). Matching letters indicate that there are no significant differences between treatments (ANOVA; P=0.0001), according to Tukey's test (P<0.05).

Table 2. Egg white and yolk weight in Creole hens fed with annatto or alfalfa.

Treatment	Egg white weigth	Yolk weigth
Annatto	35.16 [a]	20.15 [a]
Alfalfa	35.39 [a]	20.83 [a]
Standard error	0.62	0.24
P-valor		
Shapiro-Wilk	0.0009	0.0007
ANOVA	0.8024	0.0576

The Shapiro-Wilk test was used to determine data normality. Matching letters in each column indicate that there are no significant differences between treatments, according to Tukey's test (P>0.05).

ovomucoid, ovomucin, and lysozyme (Lomakina and Míková, 2006), with various applications in the pharmaceutical industry. The yolk is made of water, lipids, proteins, minerals, and vitamins (Abeyrathne *et al.*, 2022).

Yolk pigmentation was evaluated using the DSM YolkFan, a practical, reliable, and economical tool. This method is particularly useful in backyard poultry farming, where financial resources may be limited for purchasing advanced equipment like the Konica Minolta colorimeter. Annatto supplementation resulted in significantly heightened (P<0.05) yolk color (Figure 3) in eggs laid by Creole hens. Rojas *et al.* (2015) also observed a deeper yolk pigmentation in laying hens fed with canthaxanthin and annatto extract.

Yolk color is a key factor for consumer preference. A deeper yolk color indicates higher amounts of carotenoids, which is beneficial for human health. Segura (2021) found that adding 200 g of annatto to 4000 ml of drinking water for specialized Issa Brown laying hens did not affect yolk color. According to the Roche color fan, the treatments reached a scale of four, indicating low pigmentation. Hansen *et al.* (2015) assessed the addition of up to 2000 ppm of annatto to the diet of Hy-Line W36 hens and reported no significant changes in yolk coloration. However, in the present study, the addition of annatto resulted in an intensified yolk color, reaching high pigmentation levels. The highest percentages were

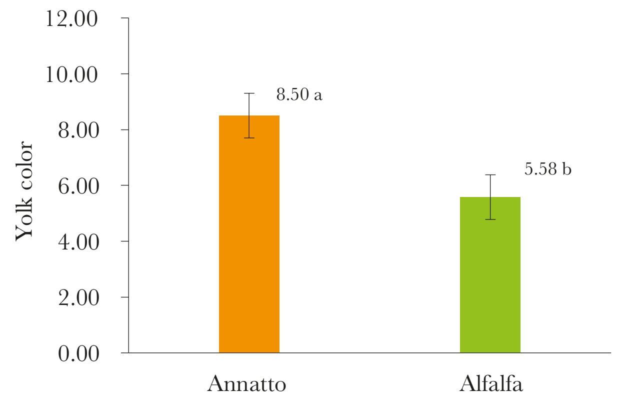

Figure 3. Yolk color in eggs laid by Creole hens fed with annatto or alfalfa.
The Shapiro-Wilk test was used to determine data normality (P=0.0016). Different letters indicate significant differences between treatments (P=0.0001), according to Tukey's test (P<0.05).

found on scales 9 and 10 (intense yellow, Figure 1), a much deeper color compared to the yolk of eggs laid by alfalfa-fed hens (Table 3). Some yolks reached tones on scales 12 and 15, showing orange hues (Figure 1). In comparison, fresh-alfalfa-supplemented feed did not significantly enhance yolk color (Figure 3 and Table 3), though it remains an accessible option for backyard poultry producers.

In this study, the main carotenoids responsible for yolk pigmentation were bixine and norbixine, which are found in annatto. Annatto or achiote is a natural pigment with no harmful effects on human health. Ofosu *et al*. (2010) reported that bixine (chemical formula C25H30O4) is oil-soluble, while norbixine (chemical formula C24H28O4) is water-soluble.

Table 3. Yolk color percentages in eggs of Creole hens fed with annatto or alfalfa, according to the scales of the DSM Yolk Fan.

Treatment	Color scale										
	3	4	5	6	7	8	9	10	11	12	15
Alfalfa	8.9	30.4	16.1	19.6	7.1	5.4	7.1	3.6	1.8		
Annatto			5.0	6.7	21.7	15.0	23.3	16.7	5.0	5.0	1.7

CONCLUSION

Including annatto in the diet of Creole hens intensifies yolk color without affecting the external or internal physical characteristics of eggs, such as weight, width, and length, weight of the egg white or yolk. Using annatto to feed Creole hens is an effective alternative for yolk pigmentation. Unlike synthetic alternatives, this natural pigment does not have harmful effects on human health. Moreover, it is cost-effective and accessible to small-scale producers.

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Colegio de Postgraduados

Biological Activity of Essential Oil from Two Aromatic Species on the *in vitro* Control of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

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ABSTRACT
Objective: Essential oils (EO) from aromatic and medicinal plants (AMP) are considered a viable alternative for controlling phytopathogenic fungi of agronomic importance. This study evaluated the antifungal activity of thyme (*Thymus vulgaris* L.) and rue (*Ruta graveolens* L.) essential oils against *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.
Design/Methodology/Approach: The antifungal activity was assessed using the agar disk diffusion method, with concentrations ranging from 10% to 100% of each EO. The phytochemical composition was analyzed using GC-MS. Morphological observations were conducted with a scanning electron microscope (SEM).
Results: Significant antifungal activity was observed at concentrations of 50-100% of thyme EO. Rue EO exhibited a fungistatic effect for up to six days. Phytochemical analysis identified carvacrol (35.95%) and p-cymene (41.18%) as the major components in thyme EO, and 2-nonanone (24.24%) and 2-undecanone (68.69%) in rue EO.
Findings/conclusions: The evaluated EOs significantly impacted fungal morphology at 60% concentration. The strong antifungal activity of thyme EO against *C. gloeosporioides* suggests its potential as an eco-friendly control alternative.

Keywords: essential oil, antifungal activity, anthracnose, secondary metabolites.

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INTRODUCTION

There are over 8,000 species of phytopathogenic fungi, responsible for more than 70% of plant diseases, causing severe damage and even total loss of agricultural crops (Urbina, 2011). Their control has primarily relied on the use of commercial fungicides (Granados, 2018). However, pathogenic organisms have developed resistance to many fungicidal active ingredients (Gang *et al.*, 2019), in addition to causing harm to the health of producers (Silveira *et al.*, 2018). *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is a facultative fungus that causes anthracnose in various agriculturally significant crops, leading to severe

economic losses due to its pathogenicity, as it infects more than 1,000 plant species (Pérez *et al.*, 2017; Gang *et al.*, 2019). In search of safer alternatives for producers, more effective disease control methods, and environmentally sustainable solutions, various natural products have been evaluated, including essential oils (EOs), which, depending on the species and their chemical composition, possess properties capable of combating pests and diseases (Granados, 2018; Calvo, 2016).

In this context, the present research aimed to find a sustainable solution to the phytosanitary issues faced in the agricultural sector by evaluating the antifungal activity of *Thymus vulgaris* and *Ruta graveolens* EOs against *C. gloeosporioides*.

MATERIALS AND METHODS

Essential Oil Extraction

Two species of aromatic and medicinal plants (AMP) were used: thyme (*Thymus vulgaris* L.) and rue (*Ruta graveolens* L.), both collected from the greenhouse area at the Colegio de Postgraduados, Montecillo Campus. The essential oils (EO) were extracted from the fresh biomass of leaves and stems. The steam distillation method was employed (Rodríguez-Álvarez *et al.*, 2012), where the plant material (PM) was placed in a 5 kg-capacity distiller and distilled for 3 hours. Table 1 shows the biomass and EO yield obtained from each plant species.

Inoculum Preparation

The COLTOR1 strain of *C. gloeosporioides* (Cruz-Lagunas *et al.*, 2020) was provided by the Faculty of Agricultural and Environmental Sciences at the Autonomous University of Guerrero. The phytopathogen was cultured on PDA (Potato Dextrose Agar) medium, using the dehydrated medium from BD Bioxon®. The PDA was prepared according to the product label instructions (39 g per liter of distilled water, sterilized at 120 °C for 15 minutes). A 5 mm diameter punch was used to cut circular pieces of fungal mycelium, which were then transferred to the Petri dishes with the prepared PDA medium. The Petri dishes were incubated at 28 °C for 15 days until the pathogen sporulated, producing master culture plates (Cruz-Lagunas *et al.*, 2020).

Antifungal Activity Evaluation

The antifungal activity was evaluated using the agar disk diffusion method, with slight modifications (Arce-Araya *et al.*, 2019), to determine the inhibition of mycelial growth. Two 5 mm diameter disks were taken from the fungal master culture using a hole punch and placed on Petri dishes containing PDA as the culture medium. Each disk was positioned at opposite ends of the dish, leaving the central part free, and incubated at 28 °C for 24 hours.

Table 1. Biomass quantity and EO yield of each distilled plant species.

Specie	Biomas (kg)	Yield essential oil (%)
Thymus vulgaris	5	0.2
Ruta graveolens	4	0.16

Subsequently, 10 μL of EO from each treatment was applied to a sterilized Whatman® No. 1 filter paper disk (5 mm diameter). The dishes were incubated at 28 °C for 20 days, and daily observations were made. Dimethyl sulfoxide (DMSO) was used to dilute the EOs. The variable evaluated was the inhibition zone, which was scanned on days 6 and 14 after EO application. The scans were processed using two image analysis programs: GIMP and ImageJ.

A completely randomized experimental design was employed, with each Petri dish as the experimental unit. Thirteen treatments were tested (EO dilutions from 10% to 100%, benomyl fungicide, distilled water, and DMSO solvent), with three repetitions per treatment. An analysis of variance and means comparison using Tukey's test ($p \leq 0.05$) was conducted using the SAS statistical package.

Chemical Composition of EOs

The chemical composition of the EOs was analyzed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph (HP-6890) coupled with a mass selective detector (HP-5973) (Ricaldi & Martínez, 2014). A 30 m × 0.25 mm × 0.25 μm HP5-MS column was used for the chromatographic separation. The injection volume for each EO was 1 μL, injected in Split mode (10:1) at a temperature of 280 °C. The oven temperature was initially set at 60 °C and then increased by 5 °C/min until reaching 200 °C, where it was maintained for 1 minute. The column flow was 1 mL/min of ultrapure helium (99.999%). Data acquisition was performed in electron impact mode within a range of m/z 50 to m/z 550 in SCAN mode. The compounds were identified by comparing their mass spectra with the NIST 2011 library and/or with standard compounds.

Scanning Electron Microscopy

The fungi used in the bioassays were observed using a JEOL JSM-6390 scanning electron microscope (SEM) (Gutiérrez-Iparraguirre, 2019). The process began with the fixation of samples by placing thin sections of the fungal mycelium into vials containing 3% glutaraldehyde in 0.1 M Sorensen phosphate buffer at pH 7.2 for 24 hours. The samples were then dehydrated through two rinses with deionized water for 30 minutes, followed by sequential ethanol rinses from 30% to 100% for 20 minutes each. The samples were brought to critical point drying for 2 hours at 1071 psi and 31°C using a Sandri®-780A dryer. The dried samples were mounted on sample holders and coated with gold-palladium.

RESULTS AND DISCUSSION

Biological Activity of *Thymus vulgaris*

The EO of *T. vulgaris* exhibited inhibition at concentrations ranging from 50% to 100% in both recorded evaluations (days 6 and 14), reaching inhibition zones of 1907.4 mm^2 and 1908 mm^2, respectively (Figure 1), relative to the area of the Petri dish (1963.5 mm^2). Similar findings were reported in a study that evaluated the biological activity of thyme EO against *Fusarium* spp. *in vitro*, where complete mycelial growth inhibition was observed at concentrations of 500 and 1000 mg kg^{-1} (Caballero *et al.*, 2018). It has been reported that

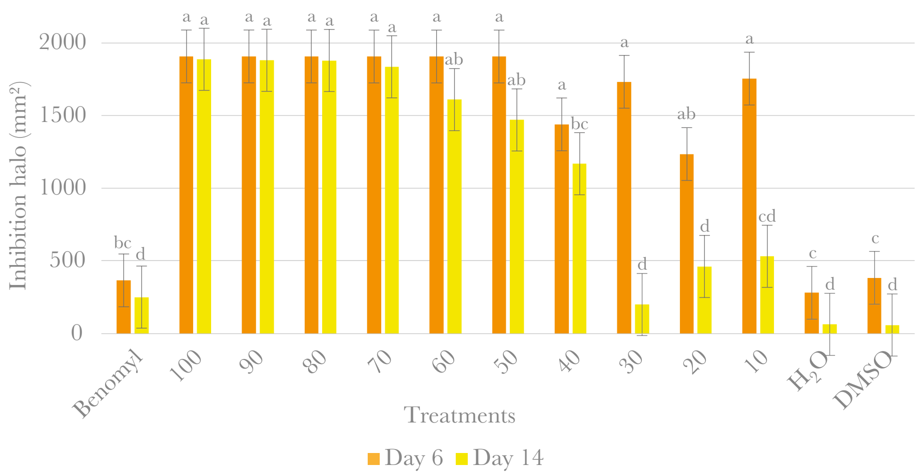

Figure 1. Inhibitory effect of *T. vulgaris* EO on the growth of *C. gloeosporioides*. Each point represents the mean of three replicates taken on days 6 and 14 ± standard error. Different letters in the graphs indicate significance (Tukey, $p \leq 0.05$).

thyme EO possesses fungicidal properties primarily due to its terpene content, particularly thymol and carvacrol (Hernández *et al.*, 2018; Morkeliūnė *et al.*, 2021).

Biological Activity of Ruta graveolens

The results of the in vitro test of *R. graveolens* EO showed an inhibitory effect during the first evaluation (day 6) at concentrations of 50%, 60%, 70%, 80%, 90%, and 100%, with inhibition zones of 970.6 mm^2, 924.1 mm^2, 1665.4 mm^2, 1536.4 mm^2, 1572.3 mm^2, and 1649.6 mm^2, respectively. However, by the second evaluation (day 14), the EO's effect diminished, resulting in inhibition zones of 538.3 mm^2, 400.2 mm^2, 1277.8 mm^2, 816.4 mm^2, 158.6 mm^2, and 618.6 mm^2, respectively (Figure 2). This suggests a fungistatic effect where the pathogen's growth was inhibited during the first six days of EO application.

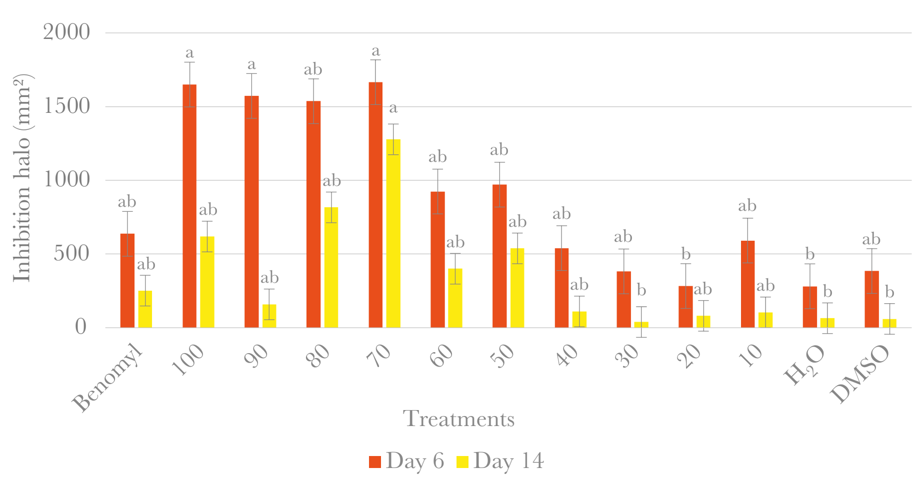

Figure 2. Inhibitory effect of *R. graveolens* EO on the growth of *C. gloeosporioides*. Each point represents the mean of three replicates taken on days 6 and 14 ± standard error. Different letters in the graphs indicate significance (Tukey, $p \leq 0.05$).

In contrast, other recent studies have reported fungicidal activity of rue EO against *Coniothyrium phyllachorae*, where the authors observed complete mycelial growth inhibition with the application of 3 μL of EO (Ceja *et al.*, 2023). The differences in results between this study and the previous one may be due to the different pathogens tested, as well as the potential higher resistance of *C. gloeosporioides* due to its high pathogenicity.

An important observation is that the commercial fungicide typically used to control *C. gloeosporioides* showed no effect, allowing the pathogen to continue growing. The inhibition zone measurements were 367 mm^2 and 251.8 mm^2 during both evaluations. On the other hand, the DMSO control did not exhibit any inhibitory effect on growth in the evaluations performed, with inhibition zones of 384.7 mm^2 and 59.9 mm^2, respectively. In contrast, pathogen growth in the H_2O control developed successfully, yielding inhibition zones of 281.3 mm^2 and 64.5 mm^2 during both evaluations.

Chemical Composition of Essential Oils

In the phytochemical analysis using GC-MS, 15 volatile compounds were identified in *T. vulgaris*, with carvacrol (35.95%) and p-cymene (41.18%) being the main components, while thymol was present in smaller amounts (0.19%) (Table 2). The primary compounds characterizing *T. vulgaris* EO were the monoterpenes thymol, carvacrol, and p-cymene. Thymol, the most extensively studied component, has demonstrated antimicrobial, antitumor, antiparasitic, antifungal, antioxidant, and anti-inflammatory activities (Vassiliou *et al.*, 2023). p-Cymene, found in relatively high concentrations, exhibits strong antiviral, antioxidant, and antitumor properties. Carvacrol, known for its potent antimicrobial and

Table 2. Volatile compounds identified in *Thymus vulgaris* essential oil by gas chromatography-mass spectrometry (GC-MS).

Compounds	Formula	Rt (min)	Score match (%)	Composition (%)
1-hexen-3-ol	$C_6H_{12}O$	5.32	79	0.46
β-myrcene*	$C_{10}H_{16}$	5.60	82	1.20
isoterpinolene	$C_{10}H_{16}$	6.19	87	0.72
p-cymene*	$C_{10}H_{14}$	6.38	88	41.18
eucalyptol	$C_{10}H_{18}O$	6.53	81	0.40
γ-terpinene	$C_{10}H_{16}$	7.17	83	8.72
(Z)-sabinene hydrate	$C_{10}H_{18}O$	7.36	79	0.77
linalool*	$C_{10}H_{18}O$	8.13	85	2.76
(+)-2-bornanone	$C_{10}H_{16}O$	9.32	86	0.84
borneol	$C_{10}H_{18}O$	9.87	79	1.37
thymol methyl ether	$C_{11}H_{16}O$	11.89	85	0.89
carvacrol	$C_{10}H_{14}O$	13.18	83	35.95
thymol	$C_{10}H_{14}O$	13.25	80	0.19
6-ethyl-3,4-xylenol	$C_{10}H_{14}O$	13.40	80	1.13
α-farnesene*	$C_{15}H_{24}$	16.48	82	3.40

*Compound compared with a standard.

antifungal effects, is likely the compound responsible for the fungicidal activity observed in thyme EO. However, it is important to note that the antifungal activity of an EO is not dependent solely on a single compound but rather on the synergy among its components (López, 2006; Vassiliou *et al.*, 2023).

In *R. graveolens*, 9 chemical compounds were identified, with 2-nonanone (24.24%) and 2-undecanone (68.69%) as the major components, and butyl propyl oxalate ester (0.06%) present in smaller amounts (Table 3). Previous studies have reported aliphatic ketones (2-nonanone and 2-undecanone) as the primary components of *R. graveolens* EO, responsible for its antimicrobial properties (Ceja *et al.*, 2023). Although the antifungal activity of rue EO has been attributed to fatty acids such as linoleic, palmitic, and retinoic acids, these compounds were not identified in the phytochemical analysis of this study, which may explain the absence of fungicidal activity (Grande *et al.*, 2019; Ceja *et al.*, 2023).

Morphological Damage Caused by the Essential Oils

Observations made through SEM indicated that the presence of *T. vulgaris* EO at a 60% concentration significantly affected the morphology of *C. gloeosporioides*. The hyphae exhibited swelling, wrinkling, rupture, and deformations, while the conidia showed deformations, perforations, and collapse.

In contrast, *R. graveolens* EO did not cause morphological damage to the conidia; however, slight damage, such as wrinkling and rupture, was observed in the hyphae (Figure 3). These results suggest that *C. gloeosporioides* conidia are resistant to the phytochemical components of rue EO. The study of morphological alterations caused by EOs in phytopathogenic fungi is limited; however, in the few studies conducted, similar damage has been reported in the hyphae and conidia of *C. gloeosporioides* when treated with chitosan and salicylic acid (Ramos *et al.*, 2018).

It has been confirmed that EOs affect the cell membrane, altering its permeability, nutrient uptake, and the mycelial development of the pathogen as part of their fungicidal mechanism (Chávez *et al.*, 2020; He *et al.*, 2018).

Table 3. Volatile compounds identified in *Ruta graveolens* essential oil by gas chromatography-mass spectrometry (GC-MS).

Compounds	Formula	Rt (min)	Score match (%)	Composition (%)
2-nonanone	$C_9H_{18}O$	7.96	78	24.24
oxalic acid butyl propyl ester	$C_9H_{16}O_4$	8.24	78	0.06
1,2-dimethyl-1,3-cyclopentadiene	C_7H_{10}	9.26	83	0.58
2-decanone	$C_{10}H_{20}O$	10.52	81	1.61
6-methyl-2-heptanol acetate	$C_{10}H_{20}O_2$	11.67	75	0.90
2-undecanone	$C_{11}H_{22}O$	13.25	82	68.69
2-dodecanone	$C_{12}H_{24}O$	15.02	76	1.42
butanimidamide	$C_4H_{10}N_2$	15.77	77	0.78
2-octanone	$C_8H_{16}O$	18.27	78	1.29

*Compound compared with a standard.

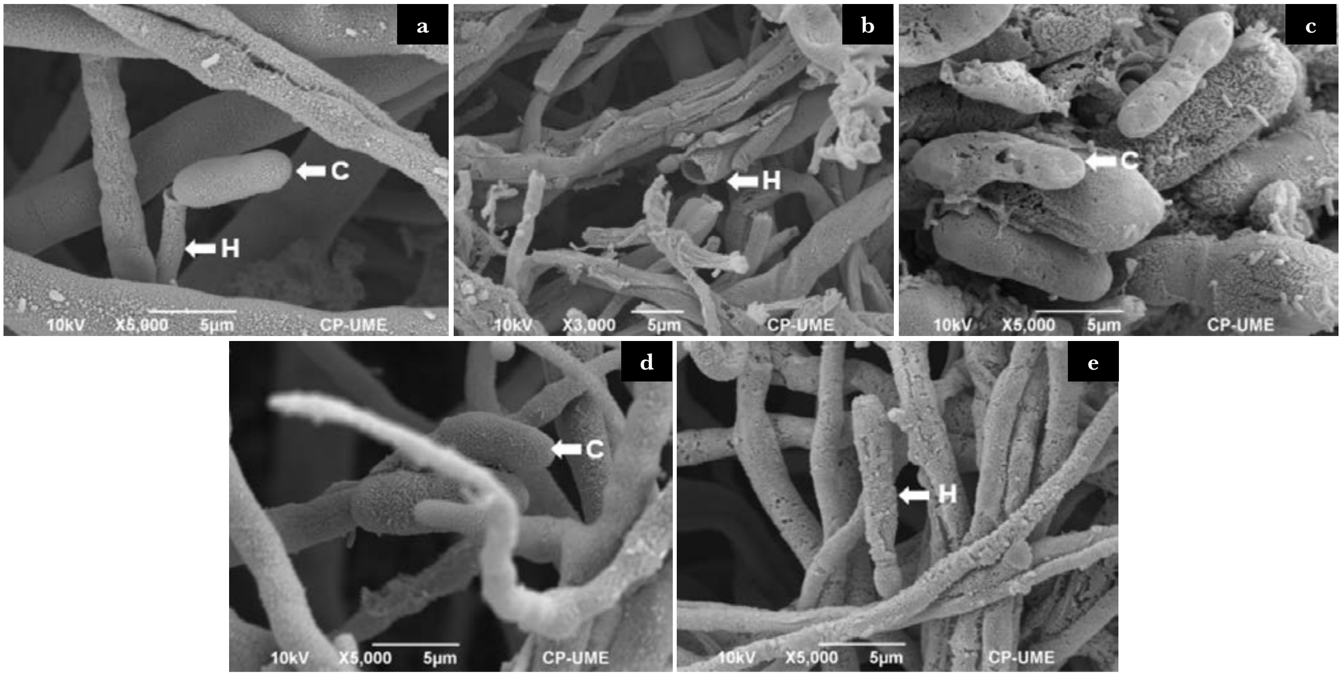

Figure 3. Microphotographs of *C. gloeosporioides* at 5000X, 3000X, 5000X, 5000X, and 5000X magnification, obtained through SEM. a) H_2O control; b) 6 days after applying *T. vulgaris* EO at 60%; c) 14 days after applying *T. vulgaris* EO at 60%; d) 6 days after applying *R. graveolens* EO at 60%; e) 14 days after applying *R. graveolens* EO at 60%. *C: Conidia*; H: Hyphae.

CONCLUSIONS

The main chemical components of *T. vulgaris* EO were carvacrol and p-cymene, while for *R. graveolens* EO, the primary components were 2-nonanone and 2-undecanone. The essential oils of *T. vulgaris* and *R. graveolens* present an alternative for controlling the fungus *C. gloeosporioides*. *T. vulgaris* EO can be used as a fungicidal agent, while *R. graveolens* EO is recommended as a fungistatic agent. For optimal results, periodic applications, at least every six days, are necessary to achieve proper control of *C. gloeosporioides*. The chemical composition of *T. vulgaris* and *R. graveolens* EOs has a morphological impact on *C. gloeosporioides*, even at low concentrations. However, it is recommended to conduct *in vivo* research to compare the results and assess whether the behavior of each EO is consistent under real-world conditions.

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Colegio de Postgraduados

Relationship of season with milk characteristics, yield, and shelf life of cheese in a hot climate

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ABSTRACT

Objective: the objective of the study was to identify whether there is a relationship between the environmental conditions of high temperature and humidity with the parameters of cheese yield and shelf life of Oaxaca-type cheese, made throughout two different times of the year, delimited by environmental temperature, in the Mexicali Valley, Baja California, Mexico.

Design/Methodology/Approach: from January to November, ambient temperature and relative humidity, characteristics of the milk used at the time of reception (temperature, acidity, density, somatic cell count and microorganism count), yield, and shelf life of the cheese were recorded. These variables were related to season (summer and winter) and to different Temperature and Humidity Index– THI intervals. An analysis of variance was performed, also, the relationship among sets of variables by Pearson correlation, and the multiple mean difference with the Tukey test ($p \leq 0.05$).

Results: significant differences were observed in the density, acidity, temperature, somatic cell count and cheese yield of milk due to the effect of the time of year. In addition, an 11% decrease in cheese yield was found as the maximum THI exceeded 77 units. The shelf life showed positive significant difference ($p \leq 0.05$) when milk was pasteurized, but it was not affected by the THI.

Limitations/Implications of the study: this study was limited to a single period of analysis; so, it is necessary to monitor, and include additional milk quality variables.

Conclusions: heat environmental conditions negatively impact the characteristics of the milk, therefore reducing the yield of Oaxaca-type cheese. The microbiological characteristics are unfavorable during the hot season; the pasteurization of milk is the main factor that increases the shelf life of the cheese.

Keywords: Temperature and Humidity Index-THI, performance, heat stress, shelf life.

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INTRODUCTION

The state of Baja California ranks as the fourteenth state with the highest milk production in the Mexican Republic, producing up to 192 thousand liters of milk per year (SIAP, 2019), while the Mexicali Valley is the main producer (Pérez Soto and Godínez Montoya, 2018). In this area there are climatic characteristics that hinder the production and industrialization of milk, since it has a hot desert climate with average maximum temperatures above 40 °C in summer, whereas in winter the environmental temperature is significantly lower (García, 2004).

Milk production is an activity that is influenced by multiple factors, and the alteration of any of these affects the quantity or quality of the milk produced (Ferreira *et al.*, 2018). High temperatures coupled with relative humidity are factors that can cause heat stress to cattle (De Rensis & Scaramuzzi, 2003). It causes an increase in respiratory and heart rate, sweating and vasodilation, resulting in a decreased dry matter intake, therefore decreasing milk production and quality (Ferreira *et al.*, 2018). Such variability in milk quality limits industrialization, livestock management should then focus on reducing heat stress (Pragna *et al.*, 2017).

Also, during the season of high temperatures, bovine mastitis is another recurring problem that occurs more frequently during the months of higher temperature and humidity. Therefore, it is important to focus on an adequate milking routine, good management of the cow and its environment, as well as the milking equipment and tools (Ferreira *et al.*, 2018). Otherwise, the composition, characteristics and properties of the milk will be altered, affecting the industrialization of the product (Souto *et al.*, 2008). Contamination of milk occurs at the time of extraction and contact with the environment, especially in milking facilities with poor hygienic conditions and exposure to high temperatures (Vitali *et al.*, 2016). In fact, a high content of microorganisms reduces the shelf life of milk and modifies its organoleptic characteristics (Larionov *et al.*, 2019).

The real danger of bacterial contamination lies in the presence of toxigenic (toxin-producing) bacteria such as *Staphylococcus aureus* or pathogens such as *Salmonella* sp., *Brucella* spp. or *Mycobacterium tuberculosis* (Ferreira *et al.*, 2018). It is for all of the above that the objective of the study was to identify whether there is a relationship among, the environmental conditions of high temperature and humidity, and the parameters of cheese yield and shelf life of Oaxaca-type cheese (a type of cheese manufactured with pasta filata process) made throughout two different seasons, delimited by the environmental temperature.

MATERIALS AND METHODS

The study was conducted at a production unit for Oaxaca-type cheese, located in the Mexicali Valley, Mexico, between January and November 2021. Climate classification of the region is hot desert climate (BWh) with 72 mm of average annual precipitation, and two distinctive (hot and cold) climate seasons (García, 2004).

Environmental Data

The daily environmental data during the study were obtained with a temperature and humidity recorder (TESTO 174H), used to determine maximum temperature, minimum temperature, maximum relative humidity and minimum relative humidity. Based on the environmental data obtained, the Temperature and Humidity Index (THI), the maximum THI (THI*max*) and the minimum THI (THI*min*) were calculated according to the formula described by Vitali *et al.* (2009):

$$THI = (1.8 \times AT + 32) - (0.55 - 0.55 \times RH) \times [(1.8 \times AT + 32) - 58]$$

where AT is the ambient temperature (°C) and RH is the relative humidity.

Milk Parameters evaluated

Samplings for the determination of parameters were implemented in accordance with the Mexican Standard NOM-110-SSA1-1994. The milk used to make this Oaxaca-type cheese comes from a single producer of milk from Holstein cattle. Upon receiving the milk, temperature, pH (HI1271, Hanna Instruments), and density (Quevenne, Nahita) were recorded. Subsequently, the Wisconsin test (WMT) (Thompson and Postle, 1964) was performed to estimate the number of somatic cells (CCS) in milk. Milk yield (%) was determined by the amount of milk needed to produce 1 kg of Oaxaca-type cheese (Oliszewski *et al.*, 2002).

Microbiological determinations were carried out monthly before cheese production, in accordance with NOM-243-SSA1. The counting of aerial mesophiles was carried out by serial decimal dilutions and plate counting; for *Escherichia coli* by the most probable number (MPN) technique (NOM-112-SSA1-1994) and the presence of *Salmonella* sp. was determined in 25 g of sample according to NOM-243-SSA1-2010. The calculation of the shelf life of the cheese was made according to the method described by Carrillo-Inungaray and Mondragón-Hernández (2011), taking monthly samples of cheese that were stored at refrigeration temperature (4 °C) until unfavorable organoleptic changes were observed.

Statistical Analyses

Variables under study were related to season (summer and winter) and also to different THI intervals. Each interval amplitude represents different environmental heat stress conditions, where THI lower than 72 indicates no heat stress, 72-77 moderate heat stress, 78-87 high heat stress, and greater than 88 means extreme heat stress.

Variable records were analyzed by analysis of variance, the relationship between variables by Pearson correlation and the multiple means difference with the Tukey test ($p \leq 0.05$); this is an alpha-type error (α) probability less than or equal to 5%. The statistical software used was SAS® 9.4.

RESULTS AND DISCUSSION

The average environmental conditions recorded during the study are shown in Table 1. These values were typical of the geographical area. Relative humidity was similar during most of the study period; whereas two seasons could be highlighted, delimited by the ambient temperature. Hot season occurred from April to early November, in which the maximum ambient temperature was around 50 °C. In July and August average THI_{max} were recorded above 88 units, evenly reaching THI_{max} greater than 92 which represents extreme heat stress.

The observed properties of milk are shown in Table 2. Significant differences were found when the THI_{max} exceeded 77 units. The temperature of the milk at the time of reception showed significant differences when the ambient temperature was higher ($p \leq 0.05$). At the ranch, the milk is stored in a cooling tank throughout milking, until it is sent to the cheese plant; however, during transport the cold chain is not maintained, which causes the milk to be subjected to a higher temperature. During days with a

Table 1. Monthly averages of ambient temperature, relative humidity and Temperature and Humidity Index (THI) in the area of the production unit.

Month	Relative humidity (%)			Temperature (°C)			THI		
	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
January	39.2	18.6	63.9	15.4	7.2	20.9	58.9	49.8	64.8
February	28.9	19.5	56.2	17.9	8.5	24.0	61.9	51.3	67.8
March	27.5	16.2	53.3	19.2	9.9	24.7	62.8	52.8	68.1
April	22.2	13.5	44.6	25.6	15.5	32.2	69.1	58.6	75.3
May	23.2	11.3	50.7	27.6	17.5	35.2	71.7	61.2	79.1
June	24.7	9.2	49.1	33.3	23.5	41.2	77.8	67.5	85.3
July	36.6	16.2	59.5	34.9	28.4	41.1	81.8	74.2	88.5
August	37.1	13.1	65.1	34.6	27.3	41.0	81.5	72.6	88.2
September	34.8	15.6	62.0	32.2	23.8	38.7	78.2	69.2	85.3
October	27.5	15.4	49.7	24.1	15.5	30.7	68.3	59.0	75.2
November	36.4	14.8	60.8	22.0	14.2	29.2	66.5	56.8	75.7

maximum THI*max* above 78, the minimum average ambient temperature was 19 °C, which influenced the recorded temperature.

The pH was significantly lower ($p \leq 0.05$) when the ambient temperature was higher. Likewise, both milk density and cheese yield showed significant differences ($p \leq 0.05$) at the highest THI*max*. Cheese yield was reduced by about 10% when THI*max* was above 88 (Table 2).

The microbiological count of milk, and the shelf life of cheese are shown in Table 3. The presence of *Salmonella* sp. was not detected. In the aerobic mesophile count, a significant difference was found between the two seasons; it was lower during the cold season; while there were no differences in *E. coli* content between seasons ($p \leq 0.05$). On the other hand, the count of aerobic mesophiles and *E. coli* exceeded the maximum limit established in the Mexican standard NOM-243-SSA1-2010.

The SCC (somatic cells count) was significantly higher ($p \leq 0.05$) in the hot season; which placed the milk of the hot season within the quality class 2, but the cold season milk was placed within quality class 1, in accordance with the standard proposal PROY-NMX-F-700-COFOCALEC-2012. In addition, it is indicated that the milk with the best quality

Table 2. Average temperature, density, and pH of the milk at time of reception in the cheese production unit; and cheese yield of the milk used according to environmental conditions.

THI*max*	Temperature (°C)	Density (g/cm^3)	pH	Cheese yield (L Kg^{-1})
<72	10.2 ± 0.2^{b}	1.0311 ± 0.0014^{a}	6.63 ± 0.02^{a}	10.2515 ± 0.3292^{c}
72-77	10.8 ± 0.3^{b}	1.0308 ± 0.0019^{a}	6.32 ± 0.03^{a}	10.3280 ± 0.4893^{c}
78-87	15.6 ± 0.6^{a}	1.0282 ± 0.0034^{b}	5.52 ± 0.06^{b}	10.9110 ± 0.7351^{b}
>88	16.2 ± 0.8^{a}	1.0248 ± 0.0035^{c}	5.42 ± 0.08^{b}	11.3758 ± 0.6888^{a}

[a,b,c] Means in columns with different literals indicate significant difference, Tukey ($p < 0.01$)
THI*max*: maximum temperature and humidity index.

Table 3. Average microbiological characteristics and shelf life of milk used for cheese production, and shelf life of Oaxaca-type cheese.

	Time of year	
	Heat	**Cold**
Total aerobic mesophiles[1], (log UFC g^{-1})	6.46[a]	5.98[b]
E. coli[2], (MPN g^{-1})	3.5[a]	3.2[a]
Salmonella sp[3]	Absent	Absent
Somatic cell count, WMT (SCC×1000)[4]	479[a]	233[b]
Shelf life, pasteurized milk, (days)	18.3±0.3[a]	18.6±0.4[a]
Shelf life, unpasteurized milk, (days)	6.8±0.7[b]	7.1±0.6[b]

[a,b,c] Means within rows with different lettering indicate significant difference. Tukey ($p<0.05$)
[1] Maximum limit: 5 log UFC g^{-1}, NOM-243-SSA1-2010
[2] Maximum limit: 3 NMP g^{-1}, NOM-243-SSA1-2010
[3] Maximum limit: Absent, NOM-243-SSA1-2010.
[4] SCC×1000: Class 1: <400, Class 2: 401-500, PROY-NMX-F-700-COFOCALEC-2012

is that of the cold season. In regard to the shelf life of cheese, no difference was observed by season, but there was statistical difference in terms of the milk used, whether it was pasteurized or not.

A positive correlation was observed for milk density in regard to THI; and negative correlations for THI to cheese yield, and for cheese yield to milk density (Table 4).

Milk yield in order to make the pasta filata cheese of Oaxaca-type is one of the main problems in the Mexicali Valley region, as it varies throughout the year. This yield is reduced when the temperature and relative humidity are high; in this study, an average reduction of 11% was determined. Cheese yield was mainly affected by milk quality, which in turn is affected by the heat stress that cows suffer. In addition, high temperatures promote bacterial growth that can lead to bovine mastitis and chemical deterioration of milk (Vitali *et al.*, 2016).

The high count of aerial mesophiles indicates that there are hygienic deficiencies in the execution of milking (Souto *et al.*, 2008). Likewise, Larionov *et al.* (2019) indicated that subclinical bovine mastitis or poor milking increases aerobic mesophilic counts. On the other hand, greater *E. coli* contamination was expected during the hot season, but in this study no differences were found between seasons. In this sense, Medved'ová *et al.* (2020) observed that within the natural microbiota of milk there are several bacterial species that have an antagonistic effect, which could limit the growth of *E. coli*. However, according to

Table 4. Pearson correlation coefficients of the association of the Temperature and Humidity Index (THI) with milk density and cheese yield, in the manufacture of Oaxaca-type cheese.

	ITH	**Density**	**Cheese yield**
ITH	1	0.6185[a]	−0.59439[a]
Density	0.6185[a]	1	−0.64267[a]
Cheese yield	−0.59439[a]	−0.64267[a]	1

[a] $p<0.0001$

what was found in both periods, the limit indicated by the Mexican standard NOM-243-SSA1-2010 was exceeded.

One of the reasons why microbiological counts were higher in the hot season may be due to the fact that the temperature of the milk was higher, as a result of high ambient temperature that favored the proliferation of microorganisms. According to Ndraha *et al.* (2018), the temperature of milk should remain below 4 °C to prevent the proliferation of microorganisms. It is important to consider that the microorganisms composing the natural microbiota of milk have the ability to modify milk characteristics (Ferreira *et al.*, 2018).

Regarding yield, it can be considered that it was lower during the periods when there was higher THI. Pragna *et al.* (2017) mentioned that the physicochemical characteristics of milk can modify cheese yield, especially due to the acidification, flocculation and degradation of caseins and microbiological activity. However, heat stress also affects these parameters due to the physiological changes in the cattle, which is reflected in the quality of the milk, evaluated through milk density.

In addition, according to Vitali *et al.* (2016) and Fusco *et al.* (2020), the incidence of bovine mastitis is higher when there are conditions of high temperature and humidity, which also affect yield. In this study, the incidence that condition was not evaluated, since the milk producer does not perform periodic tests for bovine mastitis. However, indirectly it can be inferred in the storage tank via the CSS. In this study it was found that CSS was higher in the hot season, which could also justify the reduction in yield.

It may be thought that a high somatic cell count (SCC) has a negative influence on cheese yield; however, Moradi *et al.* (2021) reported that the simple fact of obtaining a high SCC does not indicate low yield. But there are various factors that can affect yield, mainly microbial activity and diseases such as bovine mastitis. Pragna *et al.* (2017) mentioned that SCC has a direct influence on cheese yield; during the hot season, it was observed that SCC was higher, although due to the type of test performed, since it was implemented in samples from several milk batches, it could not be directly related to the incidence of bovine mastitis.

In certain months, the lower milk density directly affected cheese yield because of the lower count of total solids (Stankov *et al.*, 2022). Although THI, milk density and cheese yield are correlated, Pragna *et al.* (2017) indicated that although high THIs affect cows in regard to milk production and reduce its quality characteristics, the full process is much more complex. Therefore, studies focusing on each parameter are required to better determine how those parameters are related.

CONCLUSIONS

Hot environmental conditions negatively impact the characteristics of milk, thus reducing yield of Oaxaca-type cheese. Higher ambient temperatures and relative humidity records are climatic factors that can modify the microbiological characteristics of milk and cheese, impacting cheese yield and shelf life.

In order to obtain better yield and longer shelf life in regions where the Temperature and Humidity Index is high, heat stress mitigation measures are required. For example, by implementing a hygienic milking method, pasteurizing milk, and establishing an efficient cold chain system.

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AGRO PRODUCTIVIDAD

Analysing Determinants of Household Broiler Chicken Meat Purchases amidst Social-Media Misinformation: A Tobit Study

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ABSTRACT
Indian poultry sector is a significant contributor to GDP. It is growing at 8-10% annually, reaching $22.97 billion in 2022 and is expected to reach $41.94 billion (10.18% CAGR) between 2023 and 2028. Misinformation on social media negatively impacted the broiler sector, driving down prices and consumption.
Objective: Using Tobit model, broiler purchases by Indian households during misinformation were analyzed.
Methodology: Data on demographics, socioeconomics and monthly chicken meat consumption were collected and analyzed from n=503 respondents.
Results: On average, males preferred broiler chicken, while females preferred native chicken. The potential impact of social media misinformation on women's choices and the influence on households with older people, who consumed significantly less compared to their counterparts, remains intriguing. Unexpectedly, households with better income and higher education purchased less broiler meat. Marital status, place of residence, cohabitation, and presence of children did not significantly affect the outcome. Muslim families purchased more broiler meat, and larger households consumed more. Frequency of consumption was important, with daily and alternate customers making larger purchases. Broiler meat purchases were negatively impacted by country chicken consumption. Amid social media misinformation, while a slight adverse impact on household broiler consumption may have occurred, it is notable that a significant portion of households (97.20%) continued to purchase chicken meat. broiler chicken, demonstrating the potential effectiveness of media-driven interventions in mitigating the impact of misinformation and reiterated the persistent preference for broiler chicken as a dietary protein option within the broader consumer demographic.
Conclusions: The Indian poultry industry is vital for food security and economic growth, so it is imperative to address social media-induced panic. Transparency, trust and accurate transmission of information are essential. To successfully address market challenges, stakeholders need to consider factors such as demographics and dietary preferences that influence consumer behavior.

Keywords: Chicken Meat; Social-media Misinformation; Household Consumption; Tobit; Censored model.

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INTRODUCTION

The poultry sector in India has experienced remarkable growth compared to other segments within agriculture and allied industries. While the production of agricultural

crops has been increasing at a moderate rate of 1.5 to 2% per year, the production of eggs and broilers has been surging at a significantly higher rate of 8 to 10% annually. The poultry industry contributes approximately 1% to the total Gross Domestic Product (GDP) and 11.70% to the livestock GDP in India. The Indian poultry market is worth $22.97 billion by 2022 (DAHD, 2023). Contributions from the chicken meat and egg industries were 75.32% and 24.67%, respectively, and it is projected that the poultry industry market in India will increase at a compound annual growth rate (CAGR) of 10.18% from 2023 to 2028, bringing the total market value to $41.94 billion (IMARC-2023).

In fiscal year 2021, the gross value added (GVA) from poultry meat exceeded INR 1.44 trillion within India's livestock sector, marking a substantial increase compared to previous years. Consequently, the total GVA for meat products in 2021 surpassed INR three trillion. India ranks fifth in global broiler production and a half of the meat produced in India is poultry. Because it is affordable and widely available, chicken meat is preferred by the majority of the population as the main meat option. Over 85% of India's poultry meat is sourced from organized commercial farms, with the remaining percentage originating from backyard poultry, primarily in rural areas (DAHD, 2022). Within the commercial broiler production sector, major poultry companies employing vertically integrated operations contribute to approximately 60-65% of the total production output. Notably, the poultry sector presently provides employment opportunities to approximately 6 million small and medium farmers, along with approximately three million farmers engaged in backyard poultry.

According to the OECD-FAO Agricultural Outlook 2030, poultry meat is projected to account for 41% of the global meat protein supply by the end of this decade. The growth in income levels and urbanization has driven increased demand for poultry products, resulting in consistent consumption growth. Poultry meat is the fastest-growing segment in global meat demand, and India, as the world's second-largest developing country, is witnessing rapid expansion in its poultry sector. The presence of vertically integrated poultry producers has helped lower consumer prices by reducing production and marketing costs. Notably, 62% of all poultry meat consumption in India is concentrated in 15 major cities.

Chicken meat serves as a vital source of protein and essential vitamins and minerals, including iron, selenium, zinc, and various B vitamins, notably B12 (Ahmad *et al.*, 2018; Marangoni *et al.*, 2015; Sharma *et al.*, 2013). Notably, chicken meat presents numerous nutritional advantages; approximately half of its fat content comprises desirable monounsaturated fats, with less healthy saturated fats constituting only about one-third (Kralik *et al.*, 2018; Mir *et al.*, 2017). Furthermore, poultry meat is notably enriched with omega-3 fatty acids, prominently contributing to the provision of essential polyunsaturated fatty acids (PUFAs), particularly the omega-3 (n-3) fatty acids (Alagawany *et al.*, 2019; Kris-Etherton & Fleming, 2015; Pérez *et al.*, 2021; Thanabalan & Kiarie, 2021). Consequently, chicken meat enjoys widespread recognition as a healthy dietary choice, notably due to its absence of trans-fats, recognized contributors to coronary heart disease (Astrup *et al.*, 2020; Zeinab, 2021).

In the face of false social media-induced panic (targeting women, children and elderly), the Indian broiler industry confronted challenges, resulting in reduced chicken consumption and prices, alongside diminished maize production and the influence of COVID-19 and lockdowns (Hafez & Attia, 2020; Kolluri *et al.*, 2021; Thaper, 2023). The poultry sector, which had enjoyed consistent growth driven by increasing consumer demand for protein-rich foods in the preceding two decades, suffered significant setbacks primarily emanated from a marked reduction in demand, attributable to a confluence of factors, including supply chain disruptions and apprehensions propagated through the dissemination of misinformation. During the period of 2019-20, various negative notions or misinformation about broiler chicken emerged, including claims that Covid-19 spreads through its consumption, that it triggers early puberty in girls, leads to cystic ovaries and irregular menstrual cycles in women, and reports of Coronavirus-infected chickens in Bangalore. In the above light, this study aims to analyse the determinants of household broiler chicken meat purchases amidst misinformation in India, employing the Tobit Model.

MATERIALS AND METHODS

Consumers' household monthly purchases of broiler chicken in India were studied using a structured and pretested online questionnaire. The questionnaire was exclusively administered to individuals residing in India who confirmed their consumption of chicken meat. This Google form survey was conducted through social media from April to May 2023. Upon meticulous examination, the final dataset incorporated responses from 503 individuals, among which 494 had purchased and consumed broiler chicken meat. In this study, a snowball sampling technique was utilized, with researchers and stakeholders serving as social media gatekeepers to promote the survey. Participants were required to meet specific inclusion criteria: residing in India, actively consuming chicken meat, being over 18 years old, and willingly engaging in and completing the online survey. Additionally, participants, regardless of gender, should either primarily or jointly share responsibility for food consumption decisions within the household, including food shopping and preparation, while also meeting the criterion of being an earning member (for males) or primary shopper (for females, if not earning).

Participants were prompted to indicate if they encountered any misinformation or misconceptions, particularly through social media or any other media channels, regarding broiler chicken. Various false claims, including the spread of Covid-19 through chicken consumption, its alleged role in triggering early puberty in girls, the association with cystic ovaries and irregular menstrual cycles in women, and reports of Coronavirus-infected chickens in India. Additionally, participants were encouraged to mention any other misconceptions they observed through these media platforms. Following this question, respondents were prompted to share their immediate reactions. Options included stopping consumption of chicken, reducing chicken purchases, switching to alternatives like quails or country chicken, continuing to consume broiler chicken without concern, adopting a vegetarian diet, or specifying other responses. Regarding the reduction in broiler chicken consumption, participants were asked to indicate the monthly change in quantity for their entire household before and after the incidents, ranging from no change to specific weight categories. In order to combat these misleading beliefs, participants were invited to outline the strategies they employed. They were encouraged to choose from various methods they utilized, including seeking information from friends, government agencies, veterinarians, newspapers, television, advertisements, poultry federations, or any other approaches they employed to address the misinformation surrounding broiler chicken consumption. Extensive data encompassing demographic, socioeconomic attributes and the monthly quantities procured were collected online.

The statistical and econometric analyses of the data were performed using Stata® 16.0. In the data analysis of broiler chicken meat purchase, both descriptive and inferential statistics were employed. Socio-economic characteristics of the respondents were reported using simple descriptive statistics and a hybrid model, the Tobit model, based on the cumulative normal distribution function (McDonald & Moffit, 1980; Tobin, 1958), was used to examine the determinants of household monthly purchase of broiler chicken meat. In contrast to ordinary least squares (OLS) regression, the Tobit model permits the handling of a continuous dependent variable subject to censoring (Yakubu *et al.*, 2009). It provides the flexibility to define lower (or upper) thresholds for censoring the regression while retaining the linear assumptions required by linear regression. The Tobit model with robust standard errors was employed to obtain more accurate parameter estimates, enhance model fit, and ensure more reliable statistical inferences, given that the residuals did not conform to a normal distribution under default standard errors (Amore & Murtinu, 2021; Wang & Griswold, 2017; Wilson *et al.*, 2020).

The Tobit model was used to analyse the relationship between the non-negative household monthly meat purchase as dependent variable (Yi) and demographic, socio-economic and other attitudes as independent variables (Xi). This model was employed as the dependent variable was censored at 0.5 kg. The model assumes the presence of an unobservable latent variable (Y*), linearly related to the Xi variables through -i coefficients, with an added normally distributed error term (Ui) capturing random influences on this relationship. The observed variable (Yi) equals Y* if Y*>0 and is assumed to be zero otherwise. Maximum Likelihood Estimation (MLE) was used to estimate βi, as OLS would produce biased and inconsistent results (Gujarati and Porter, 2009).

The model can be expressed as,

$$Y = X\beta + u, \; if \;\; \beta' X + u > 0;$$
$$= 0, \text{ Otherwise. Such that the residual, } u \sim N\left(0, \sigma^2\right).$$

Where Y, $(n \times 1)$ is vector of dependant variable; $b\,(k \times 1)$ is vector of unknown parameters; and X is vector of exogenous variables.

RESULTS AND DISCUSSION

Household broiler chicken meat purchase pattern

Monthly household purchases of broiler chicken meat along with native chicken meat (for reference), measured in kg, within different demographic categories are presented in Table 2. Broiler chicken meat purchasing patterns by gender were analysed. Men purchased an average of 3.34 kg of broiler chicken meat per month, while women purchased an average of 3.37 kg. However, these differences were not statistically significant, supporting

Table 1. List and levels of variables used in the Tobit model.

Explanatory variable	Category	Specification	Variable in model
Gender	Male; Female	1 - If Male; 0 - If otherwise	X_1
Age Group	26 years; 26 - 35 years; 36 - 45 years; 46 - 60 years; 60 years	1 - If - 26 years; 2 - If 26–35 years; 3 - If 36–45 years; 4 - If 46–60 years; 5 - If - 60 years	X_2
Monthly Income Brackets (INR)	≤ 10000; 10,001 - 30,000; 30,001 - 60,000; 60,001 - 90,000; > 90,000	1 - If ≤ 10000; 2 - If 10,001 - 30,000; 3 - If 30,001 - 60,000; 4 - If 60,001 - 90,000; 5 - If > 90,000	X_3
Educational levels	Primary School; Secondary School; Collegiate	1 - If Primary; 2 - If Secondary; 3 - If Collegiate	X_4
Marital status	Married; Unmarried (single)	1 - If Married; 0 - If otherwise	X_5
Habitat (living area)	Urban; Rural	1 - If Urban; 0 - If otherwise	X_6
Cohabiting with family	Yes; No	1 - If Yes; 0 - if otherwise	X_7
Senior citizens at home	Yes; No	1 - If Yes; 0 - If otherwise	X_8
Children at home	Yes; No	1 - If Yes; 0 - If otherwise	X_9
Household size	Count	Count	X_{10}
Religion[a]	Christian; Hindu; Muslim; Others	1 - If Hindu; 0 - If otherwise	X_{11}
		1 - If Muslim; 0 - If otherwise	X_{12}
		1 - If Others; 0 - If otherwise	X_{13}
Chicken consumption[b] (frequency)	Daily; Alternate Days; Twice Weekly; Weekly; Fortnightly; Occasionally (randomly)	1 - If Daily; 0 - If otherwise	X_{14}
		1 - If Alternate Days; 0 - If otherwise	X_{15}
		1 - If Twice Weekly; 0 - If otherwise	X_{16}
		1 - If Weekly; 0 - If otherwise	X_{17}
		1 - If Fortnightly; 0 - If otherwise	X_{18}
Quantity of Native Chicken Purchased (kg/ month)	Continuous in kg	Continuous	X_{19}

Reference categories: [a] - Christian; [b] - Occasionally (randomly).

Table 2: Household monthly purchase of broiler and native chicken meat (kg).

Characters	Category	N	Broiler Chicken Quantity (kg./ month)				Native Chicken (kg/month)
			Mean	SD (σ)	Test Statistic	p	
Gender	Male	376	3.34	1.428	t=0.185	0.854	1.50
	Female	127	3.37	1.413			1.57
Age Groups (years)	- 26	61	3.48	1.523	F=1.426	0.224	1.71
	26-35	103	3.33	1.562			1.49
	36-45	116	3.43	1.416			1.54
	46-60	178	3.38	1.289			1.45
	- 60	45	2.89	1.449			1.50
Income Brackets (INR)	≤10000	44	2.89	1.385	F=3.322	0.11	1.79
	10,001-30,000	75	3.77	1.713			1.70
	30,001-60,000	96	3.43	1.513			1.54
	60,001-90,000	103	3.39	1.330			1.38
	> 90,000	185	3.23	1.261			1.47
Education	Primary	13	4.00	2.000	F=1.453	0.235	1.75
	Secondary	18	3.44	1.617			1.56
	Collegiate	472	3.33	1.396			1.51
Marital status	Married	412	3.35	1.443	t=0.094	0.925	1.53
	Unmarried	91	3.36	1.338			1.46
Habitat (living area)	Urban	421	3.40	1.420	t=1.673	0.095	1.48
	Rural	82	3.11	1.423			1.74
Cohabiting with family?	Yes	437	3.33	1.405	t=0.733	0.464	1.52
	No	66	3.47	1.541			1.51
Senior citizens at home?	Yes	251	3.55	1.523	t=3.237	0.001	1.60
	No	252	3.15	1.287			1.43
Children at home?	Yes	251	3.49	1.460	t=2.149	0.032	1.54
	No	252	3.21	1.375			1.49
Household size (count)	≤2	29	2.14	1.481	F=29.745	0.000	1.50
	3	117	2.83	1.177			1.52
	4	220	3.25	1.158			1.44
	5	77	3.87	1.361			1.66
	≥5	60	4.63	1.667			1.65
Religion	Christian	35	3.94	1.162	F=23.100	0.000	1.36
	Hindu	420	3.14	1.310			1.51
	Muslim	41	4.85	1.711			1.69
	Others	7	3.70	1.337			2.30
Chicken meat consumption frequency	Daily	34	5.43	1.547	F=55.838	0.000	1.67
	Alternate Days	63	4.21	1.152			1.47
	Twice Weekly	171	3.63	1.089			1.52
	Weekly	134	3.11	1.148			1.44
	Fortnightly	24	2.17	1.049			1.71
	Occasionally (randomly)	77	2.06	1.099			1.61

Test statistics (t or F) are to compare the categories in Broiler Chicken Meat alone.

Table 3. Determinants of Monthly Household Broiler Chicken Meat Purchase (in kg).
Tobit regression: Dependant variable=Purchase in kg.

Explanatory variables		Coefficient	Rob. SE	t	P value	Mean (X)
Gender	X_1	0.211	0.102	2.070	0.039	0.748
Age Groups (years)	X_2	0.078	0.058	1.330	0.183	3.085
Income Brackets (INR)	X_3	−0.077	0.037	−2.100	0.036	3.616
Educational levels	X_4	−0.341	0.170	−2.010	0.045	2.913
Marital status	X_5	0.062	0.194	0.320	0.748	0.819
Habitat (living area)	X_6	0.252	0.126	1.990	0.047	0.837
Cohabiting with family?	X_7	−0.372	0.165	−2.250	0.025	0.869
Household size (count)	X_8	0.520	0.044	11.720	0.000	4.093
Senior citizens at home?	X_9	−0.174	0.089	−1.960	0.050	0.499
Children at home?	X_{10}	−0.123	0.102	−1.210	0.228	0.499
Religion-Hindu	X_{11}	−0.175	0.152	−1.150	0.252	0.829
Religion-Muslim	X_{12}	0.470	0.228	2.060	0.040	0.082
Religion-Others	X_{13}	0.209	0.240	0.870	0.383	0.020
Daily	X_{14}	3.083	0.240	12.820	0.000	0.060
Alternate Days	X_{15}	2.135	0.149	14.340	0.000	0.125
Twice Weekly	X_{16}	1.491	0.111	13.400	0.000	0.340
Weekly	X_{17}	1.117	0.119	9.380	0.000	0.266
Fortnightly	X_{18}	0.341	0.216	1.580	0.115	0.048
Country Chicken Quantity	X_{19}	−0.069	0.034	−2.040	0.042	0.888
Constant		1.194	0.621	1.920	0.055	
Var (e. Broiler Quantity)		0.826	0.060			

N=503; Uncensored=494; Left-Censored=9

F (19, 484)=35.53; p=0.000

Log pseudo likelihood=−663.430; Pseudo R^2=0.249

y=Linear prediction (predict)=3.345 kg

similar research by Charlebois *et al.* (2016) and Kennedy *et al.* (2004) that also found that gender has little to no impact on the amount of meat purchased. In terms of the monthly purchases of broiler chicken meat, there were no significant disparities between the age groups. Individuals under the age of 26 showed the highest average monthly purchase, 3.48 kg, while those over the age of 60 tended to make the lowest purchases, 2.89 kg on average. These results are consistent with other studies, such as the work of Rolls (1999), which constantly emphasises the disparities in nutritional preferences and choices between age groups.

The results indicated that individuals with a monthly income of less than or equal to 10,000 INR had the lowest mean broiler chicken purchase at 2.89 kg per month. As income levels increased, there was an upward trend in broiler chicken purchase. Those in the income range of 10,001 to 30,000 INR had the highest average purchase at 3.77 kg per month, accompanied by a higher SD of 1.713, suggesting greater purchase variability within this group. While there were differences in mean purchase across income categories, the results of one-way ANOVA (F=3.322) exhibited that the differences in broiler chicken purchase among income groups were not statistically significant. As individuals and households have more disposable income, they tend to allocate a larger portion of it to meat and protein-rich foods, such as broiler chicken. This behavior is consistent with the idea that as people's economic well-being improves, they may choose to consume more meat products (Aral *et al.*, 2013; Zhang *et al.*, 2018).

Consumers with a primary educational level, on average, purchased the highest quantity of broiler chicken at 4.00 kg per month. In contrast, individuals with a secondary education level had a slightly lower mean purchase of 3.44 kg per month, while those with a collegiate-level education exhibited the lowest average purchase at 3.33 kg per month. The statistical analysis (F=1.453, p=0.235) suggests that although there are differences in mean purchase quantities across educational levels, these variations were not statistically significant, which is consistent with reports from Escriba-Perez *et al.* (2017) in Spain where they found no specific behaviour pattern in chicken purchase. Similarly, Marital status had no significant impact on broiler chicken purchases, with both married (3.35 kg/month) and unmarried individuals (3.36 kg/month) displaying comparable purchase quantities. This was supported by a non-significant statistical study (t=0.094, p=0.925), which showed that buying habits for broiler chicken were not significantly influenced by marital status.

Results of chicken purchases by residents of urban and rural areas revealed that urban residents purchased an average of 3.40 kilogrammes of chicken per month, while residents of rural areas purchased somewhat less at 3.11 kilogrammes per month. These differences, according to the statistical analysis (t=1.673, p=0.095), were not statistically significant. This shows that living conditions or habitat had no impact on broiler chicken purchase trends. Similar to this, family cohabitation status had little effect on consumers' buying habits for broiler chicken. The average monthly purchase was 3.33 kilogrammes for individuals living with family members, and 3.47 kilogrammes for those who did not. These differences, according to the statistical analysis (t=0.733, p=0.464), were not statistically significant.

The findings showed that households with elderly people at home purchased more broiler chicken on average each month (3.55 kilogrammes compared to 3.15 kilogrammes in households without senior citizens). The presence of older individuals in a family may affect the buying habits of broiler chickens, according to this statistically significant difference (t=3.237, p=0.001). It's reasonable that dietary preferences or nutritional needs of senior citizens contribute to this variation (Edfors & Westergren, 2012; Zaragoza-Martí *et al.*, 2020). Similar to this, households with children bought 3.49 kilogrammes of broiler chicken on average per month, whereas households without children bought 3.21 kilogrammes. This difference was also statistically significant (t=2.149, p=0.032), suggesting that a family's decision to eat broiler chicken may be influenced by the presence of youngsters. Children meal planning and dietary preferences may be influencing variables (Caswell *et al.*, 2013; Kostecka *et al.*, 2021). Depending on the size of the household, broiler chicken purchased differ significantly. The average monthly purchase for smaller families with two or fewer people was 2.14 kilogrammes, whereas the average buy for larger households with five or more members was 4.63 kilogrammes. Given that this variation was highly statistically significant (F=29.745, p=0.000), it is likely that household size has a considerable impact on the intake of broiler chicken. Larger households may require more substantial quantities for family meals (Berman, 2020; Cornelsen *et al.*, 2016). Additionally, buying habits for broiler chicken varied significantly according to religion. Muslim households had a higher average monthly purchase of broiler chicken at 4.85 kg compared to Christian households at 3.94 kg and Hindu households at 3.14 kg. This variation was statistically significant (F=23.100, p=0.000), indicating that religious beliefs or dietary restrictions associated with specific religions may impact broiler chicken consumption (Ayman *et al.*, 2020).

Households that consumed chicken daily had the highest average purchase at 5.43 kg, while those consuming it occasionally (randomly) had the lowest average purchase at 2.06 kg. This difference was highly statistically significant (F=55.838, p=0.000), demonstrating that broiler chicken purchase volumes are significantly influenced by the frequency of chicken consumption (Escobedo del Bosque *et al.*, 2021; Memon *et al.*, 2009).

Factors influencing the household broiler chicken meat purchase pattern

The study, using Tobit regression, examined the various demographic and lifestyle factors influencing the monthly household broiler meat purchase and the results are presented in Table 3. The study identified a significant relationship between gender and the volume of monthly household broiler meat purchases. Being male was associated with a significant increase in monthly household broiler meat purchase of about 0.211 kilogrammes compared to being female. These results indicated that, on average, men had a larger propensity to consume broiler meat than women, while at the same time, women had more native chicken. Although not vividly accepted, this could have been the possible effect of social media misinformation. This gender-based disparity in consumption could have also been attributed to a variety of factors, including dietary preferences and distribution of cooking responsibilities (Rosenfeld & Tomiyama, 2021). However, age groups, as categorized in this study, did not exhibit a statistically significant effect on monthly broiler consumption. The coefficient of 0.078 is not statistically significant at the 0.05 level (p=0.183).

Consistent with the findings of Tan *et al.* (2018), the results indicated that income had a significant negative impact on the monthly quantity of broiler chicken meat households purchased. Monthly household purchases of broiler meat decreased by 0.077 kilogrammes for every incremental rise in the household's income category. However, contrary to the findings of Ani and Antriyandarti (2019) and Siburian *et al.* (2021), the results revealed a striking tendency wherein households with greater incomes typically consumed less broiler meat, possibly reflecting dietary preferences, purchasing patterns, or a preference for other meat types. Similarly, educational levels significantly negatively influenced monthly household broiler meat purchases, with each stage increase in the decision-making respondent's education linked to a 0.341 kg reduction in the quantity of broiler chicken meat purchased by the household. This suggests a potential connection between higher education levels and preferences for alternative protein sources with a reduced broiler meat consumption (Hajiis *et al.*, 2018).

The analysis investigated the impact of marital status, habitat, cohabitation with family, household size, the presence of senior citizens, and children in the household on monthly broiler meat purchases. It revealed that marital status and the presence of children did not exert a statistically significant influence on purchase quantity, while the other variables demonstrated significant effects. Specifically, habitat, representing participants' living areas, exhibited a statistically significant increase in monthly household broiler meat purchases. Urban consumers bought, on average, 0.252 kg more broiler meat per month than their rural counterparts. Conversely, residents in rural areas had a higher average consumption of native chicken meat. This suggests that place of residence may play a pivotal role in shaping dietary choices, potentially resulting in distinct consumption patterns between urban and rural dwellers (Sahin *et al.*, 2013; Stamatopoulou & Tzimitra-Kalogianni, 2022). Conversely, consumers who did not cohabit with family members displayed a statistically significant increase in broiler meat purchases, consuming approximately 0.372 kg more per month. This underscores the substantial impact of living arrangements on broiler meat consumption, with individuals living alone or without family members exhibiting higher broiler meat consumption on average. Furthermore, household size demonstrated a highly significant positive effect on monthly broiler meat purchases. With each unit increase in household size, monthly broiler consumption increased by 0.520 kg. These findings indicate that larger households tend to consume more broiler meat on average, likely attributable to increased meal preparation requirements (Devi *et al.*, 2014). In contrast, households with senior citizens exhibited a slightly significant negative impact on monthly broiler meat purchases, but they purchased more native chicken meat. When other variables were held constant, these households consumed around 0.174 kg less broiler meat per month, with this effect being marginally significant ($p=0.050$). This reduction could be attributed to a combination of factors, including the influence of social media misinformation and the dietary preferences and habits of senior citizens within the household.

Tobit analysis unveiled distinct patterns in broiler chicken meat purchases among households of various religious affiliations. Specifically, when compared to Christian households, Hindu households and households of other religious faith did not demonstrate statistically significant differences in their broiler chicken meat purchases. In contrast, Muslim households exhibited a notable and statistically significant preference for broiler meat, purchasing approximately 0.470 kg more compared to their Christian counterparts. This could be indicative of varying dietary preferences and consumption patterns influenced by religious beliefs or cultural factors among different religious groups (Sathyamala, 2019; Usama *et al.*, 2022).

Various consumption frequencies demonstrate distinct impacts on monthly broiler meat purchases. Daily consumers significantly increased their broiler meat purchases by 3.083 kg compared to "occasional consumers." Similarly, households consuming broiler meat on alternate days purchased an additional 2.135 kg, a highly statistically significant difference. Those households consuming broiler meat twice a week increased their purchases by about 1.491 kg ($p=0.000$), while weekly consumers added 1.117 kg more to their purchases than "occasional consumers," also with statistical significance (p=0.000). However, households consuming broiler meat fortnightly experienced a modest increase of approximately 0.341 kg compared to "occasional consumers," which did not reach statistical significance at the 0.05 level (p=0.115). Overall, the frequency of broiler meat consumption significantly impacts monthly purchases, with daily and alternate-day consumers showing substantial increases, indicating varying consumption patterns among these groups (Schmid *et al.*, 2017).

The quantity of country chicken consumed exhibits a statistically significant negative effect on monthly broiler meat purchases. Specifically, for each one-kg increase in country chicken quantity, monthly broiler meat purchases decreased by 0.069 kg. This implies that households that consume larger quantities of country chicken tend to have lower average broiler meat purchases, potentially influenced by dietary preferences or substitution effects.

It is imperative to emphasize that within the cohort of 503 household respondents, a noteworthy proportion, 494 (98.21%), purchased broiler chicken meat, while a mere 9 households (1.79%) exclusively favoured to buy native chicken. A subsegment of 296 households bought both broiler chicken meat and native chicken meat, among the 494 households that purchased broiler chicken meat. The Tobit analysis predicted that the average monthly household purchase of broiler chicken meat was 3.345 kg at the average level of the explanatory variables. Furthermore, households concurrently purchased about 1.50 kg of country (native) chicken meat in addition to broiler chicken meat. Among the 503 survey participants, a significant majority of 489 (97.20%) individuals acknowledged encountering negative misinformation concerning broiler chicken via social media platforms, notably through WhatsApp. This misinformation was either directly received by them or shared within their family circles. Specifically, 343 (68.19%) respondents were aware of the misleading claim that "Covid-19 (corona) virus spreads through broiler chicken." Additionally, statements suggesting that "Broiler chicken consumption triggers early puberty in girls" and "Broiler chicken consumption may lead to cystic ovaries and irregular menstrual cycles in women" were noticed by 201 (39.96%) respondents. Moreover, the assertion of "Chicken infected with Coronavirus found in Bangalore" was received by 96 (19.09%) participants. Nevertheless, it's crucial to note that despite these false claims, a substantial majority of participants continued to purchase and consume broiler chicken even after the dissemination of such misinformation. This persistence occurred because health authorities promptly clarified the situation within a couple of days of the news spreading. Social media misinformation about broiler chicken meat have been mitigated by the government, poultry farmers, and health authorities' efforts through media coverages.

CONCLUSIONS

Broiler chicken meat is a popular choice among the public due to its accessibility and affordability. It also plays a vital role in supplying consumers with protein and other essential nutrients, despite facing challenges such as social media-induced panic, supply chain disruptions, and the impact of Covid-19. This study explored household broiler chicken meat purchasing patterns, uncovering gender-based consumption differences. On average, men favored broiler chicken, while women preferred native chicken. The potential impact of social media misinformation on women's choices and the influence on households with senior citizens, which consumed significantly less quantity compared to their counterparts, remained intriguing. In contrast, the presence of children within households did not have a statistically significant impact on purchasing habits.

The study also revealed that as incomes rose, households tended to buy less broiler chicken meat, influenced by factors like dietary preferences and alternative protein sources. Higher education levels were linked to reduced broiler chicken meat purchases. Household size had a significant impact, with larger families purchasing more. Frequency of consumption also played a key role in the monthly purchase quantity. In the context of the prevalence of social media misinformation, although there might have been a marginal adverse effect on household broiler consumption, it is noteworthy that a substantial portion of households persisted in their purchases of broiler chicken. This observation underscored the potential efficacy of media-based interventions in mitigating the influence of misinformation and reaffirmed the enduring preference for broiler chicken as a dietary protein choice among the wider consumer demographic. Moreover, to mitigate the effects of such challenges, it is crucial to enhance consumer confidence and trust in the reliability and quality of chicken products.

Informed Consent and Voluntary Survey Participation Statement:

In this research study, all survey participants provided informed consent and participated voluntarily. They were provided with clear information about the study's objectives and procedures. Participants were assured that their participation was entirely voluntary, with the right to refrain from submitting the survey form at any time without consequences. Strict ethical guidelines were followed to protect their rights and privacy.

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Colegio de Postgraduados

Bibliometric analysis of scientific research on cocoa (*Theobroma cacao* L.) in Mexico

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ABSTRACT

Objective: to analyze the scientific contributions made in Mexico on Cocoa tree cultivation during the period 1999-2022 and to quantify those contributions by gender.

Design/Methodology/Approach: scientific articles were collected from major publishers (Elsevier, MDPI, Wiley and Springer), database of articles from Open Access Journals (CONRICYT, Scielo, Redalyc, Latindex, Claryvate Analytics, Periodica and DOAJ), and the open access web search engine Google Scholar. The Gephi software was used to build networks of the institutions, the researchers' affiliation and co-authorship networks.

Results: scientific production showed an exponential growth of scientific texts at the national scale where Cocoa tree was the subject of research. In the last 22 years, scientific productivity was concentrated in three main topics: biotechnology (18.51%), economy (15.26%), and diseases, (13.31%). The research was focused on the Mexican southeast, and was led by Colegio de Postgraduados (COLPOS), the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), and the Juarez Autonomous University of Tabasco. The male gender presented a higher percentage of articles published as first author and as author for correspondence.

Limitations/Implications of the study: the documents analyzed were exclusively scientific articles.

Findings/Conclusions: There is a research gap in species propagation techniques, ethnobotany, irrigation, plant physiology, and the influence of indigenous cultures and groups on the transfer of knowledge.

Keywords: *Theobroma cacao*, bibliometric analysis, co-authorship networks, gender.

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INTRODUCTION

Cocoa tree (*Theobroma cacao* L.) originated in the Neotropics of South America, in the Amazonian region. Mexican cocoa tree has developed across cultural groups, in different geographical environments where it has thrived and evolved with intense gene flows among populations (Lanaud *et al.*, 2024).

In Mexico, cocoa tree is economically important because it sustains the income of many families. On a social scale, it represents a source of employment, and in a cultural aspect, the annual consumption of chocolate is very important (0.5 kg of processed cocoa nut per capita). Mexico's domestic production satisfies 18% (28 thousand tons, Megagrams –Mg) of the domestic demand for cocoa, so about 120 thousand Mg are needed to satisfy it (CEDRSSA, 2020).

Cocoa yields in the South-Southeast region, where more than 97% cocoa is produced, are very low (0.49 Mg ha^{-1}) (SIAP, 2024). Cocoa is subject to many threats in Mexico, including its susceptibility to disease and climate change (Lahive *et al.*, 2019).

Bibliometric studies allow retrospective analysis of how the scientific advances generated specifically in this crop have been achieved and made known. The results of this type of study can be used for the development of scientific policy and its management, to the extent that scientific knowledge is perceived as a strategic value for the generation of useful results (Sanz-Valero *et al.*, 2014). For this reason, the objective of this research was to analyze the scientific contributions accomplished in Mexico on cocoa tree cultivation during the period 1999-2022, and to quantify those contributions by gender.

MATERIALS AND METHODS

Origin of Data

In this study, we considered the set of scientific articles where cocoa was the object of research and carried out by Mexican researchers or with an area of study in Mexico. Keywords used in the search for the scientific articles were Cacao and its English equivalent, cocoa. The scientific articles were collected from the main publishers (Elsevier, MDPI, Wiley and Springer); databases of articles from Open Access Journals (CONRICYT, Scielo, Redalyc, Latindex, Claryvate Analytics, Periodica and DOAJ), and the open access web search engine Google Scholar.

The scientific articles were collected from January to June 2023, and the number of texts available until December 2022 was considered. Through content analysis, the studies that were not developed in Mexico or by Mexican researchers were discarded. In addition, the "snowball" technique was used to expand the search for reports, based on the list of references of articles found in the initial search (Leipold, 2014).

Bibliometric indicators

Variables analyzed for each of the scientific articles collected were authors, year of publication, title, journal, and bibliographic citations. Then, through content analysis, the authors' affiliation institutions, the country of origin of the first author, and the topic of each research were identified: 1) Biotechnology (included studies associated with food and beverage production processes); 2) Economics (studies where added value is given to direct and indirect cocoa products); 3) Diseases (pathogens associated with cocoa production); 4) Pests (parasites associated with cocoa production); 5) Fertilization (compounds added to the cocoa tree to improve yields in the field); 6) Agroforestry (association of cocoa with other crops); 7) Plant breeding (studies related to propagation techniques of the cocoa tree); 8) Ecology (research on the influence of cocoa on the environment where it grows); and 9) Botany (studies where the cocoa species is described).

Data was captured in a spreadsheet and the original language of each of the texts was kept. During the capture of data, records that presented variants, but with the same significance, were standardized. In addition, special characters were changed such as: š (for s) ç (for c), ã (for a), or eliminated, accents, superscripts, subscripts, ® or © among others, in order to facilitate the analysis.

Data Analysis

The methodology described by Santillán-Fernández *et al.* (2023) was followed. A graph of temporality of scientific production was created with the variables year of publication and number of citations. For the variable frequency of scientific articles per year, an ordinary least squares regression model was estimated to determine the trend in the frequency of publications. A graph was also generated with the main research topics, with the intention of determining potential areas for the development of new research about cocoa.

Bibliometric indicators were generated for journals that published scientific articles in which cocoa was studied; more frequently, and of greater relevance; which is measured by the number of times they appear in bibliographic citations. Through the Gephi program (Bastian *et al.*, 2009), networks of the institutions of affiliation of the researchers were identified. This in order to determine those institutions that have generated most of the knowledge about cocoa, and the co-authorship networks which identify the main researchers. Once the articles were selected, the number of researchers was reclassified by gender. In those articles where the full name of the author and co-authors does not appear, an email was sent to the author for correspondence, asking for the full names of the authors.

RESULTS AND DISCUSSION

During the period analyzed (1999-2022), 199 scientific articles have been published on cocoa cultivation. A linear increase in the number of articles published was observed, which denotes the importance of cocoa as a local crop to contribute to food sovereignty and to the generation of self-employment of families living in rural areas where cocoa is grown. During the period 2015-2020, more articles were published per year; the increase was due to the greater number of research carried out on the control of cocoa moniliasis, a disease that causes necrotic damage to the internal tissue of the fruit, causes its fall and therefore decreases the yield of cocoa almonds (Hipólito-Romero *et al.*, 2020).

Most of the research in Mexico has focused on topics such as how to generate cocoa-based food and beverages (biotechnology 18.51%); adding value (economics 15.26%); and disease control (13.31%) (Figure 2). But, there are research gaps in agronomic management such as fertilization, irrigation, shade levels, physiology, uses of GRAS substances (substances generally recognized as safe), that could improve cocoa almond yields, as well as the understanding of the potential effects of climate change on this crop.

Mexican researchers are used to publish their findings in Mexican journals (7 out of 10), which limits the scope of research at the international scale. These journals are mostly published in Spanish without impact factor- JIF (Table 1).

The journal Agro Productividad ranks first with 28 (14.07%) articles and REMEXCA the second, with 16 (8.04%). The preference for these magazines may be due to a few reasons. Agro Productividad in its beginnings was classified as a scientific communication journal by CONACYT (today CONAHCYT). But now it is a multidisciplinary scientific journal, which allows the publication of a large number of diverse topics. In addition, it has color illustrations and publishes images of field and lab experiments. This features make this journal more attractive.

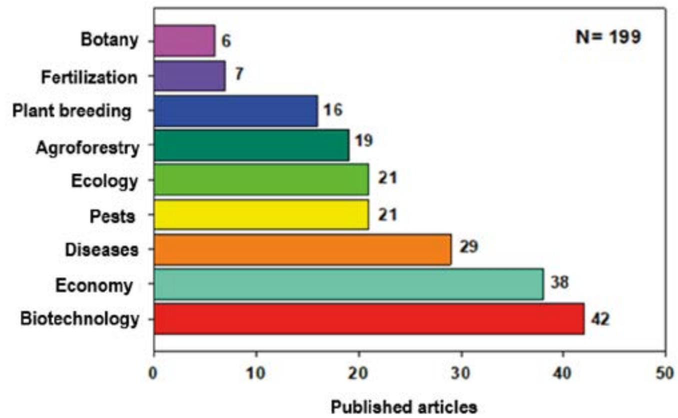

Figure 1. Temporal evolution of scientific production and bibliographic citations on the species *Theobroma cacao* in Mexico.

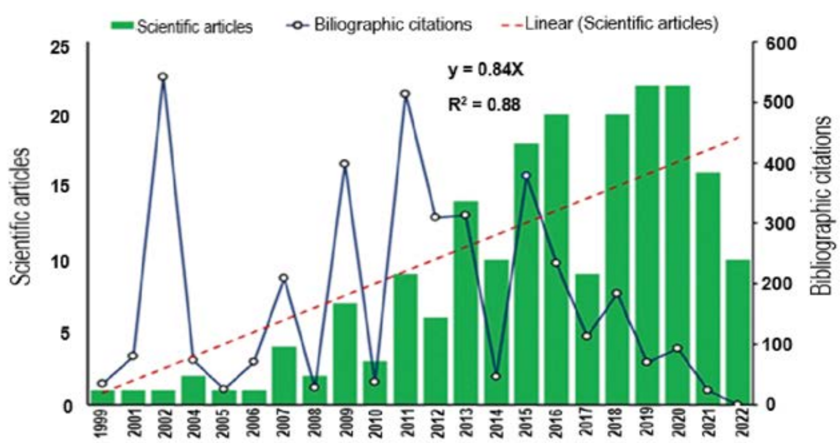

Figure 2. Main research topics where cocoa (*Theobroma cacao*) was the object of research in Mexico from 1999 to 2022.

Table 1. Bibliometric indicators of the main journals that published scientific articles where cocoa (*Theobroma cacao*) was the object of research in Mexico from 1999 to 2022, ranked according to the number of published articles.

Journal	Country	Publisher	Language	Topic	Articles		Citation	
					Number	%	Number	%
AgroProductividad	México	ColPos	Spanish	Agriculture	28	14.07	126	3.34
REMEXCA	México	INIFAP	Spanish	Agriculture	16	8.04	204	5.40
RFM	México	SM_Fitogenetica A. C.	Spanish/English	Agriculture	6	3.02	74	1.96
TSA	México	UADY	Spanish	Agroforestry	6	3.02	38	1.01
Universidad y Ciencia	México	UJAT	Spanish	Biotechnology	6	3.02	296	7.84
Botanical Sciences	México	SBM A.C.	English	Botany	4	2.01	5	0.13
Agroforest Syst	USA	Springer	English	Agroforestry	3	1.51	20	0.53
Interciencia	Venezuela	Asociación Interciencia	Spanish/English	Biotechnology	3	1.51	165	4.37
RMB	México	UNAM	Spanish/English	Ecology	3	1.51	91	2.41
Tecnología en Marcha	Costa Rica	ETCR	Spanish	Biotechnology	3	1.51	30	0.79
Otras (106)					121	60.80	2728	72.23
Total (116)					199	100.00	3777	100.00

In contrast, REMEXCA is published by the Institute of Forestry, Agricultural and Livestock Research-INIFAP, which is the institution where much of the cocoa research on various topics is carried out in Mexico. In fact, INIFAP is a national leader in the genetic improvement of *Theobroma cacao*.

Tabasco is the state where the most studies on cocoa are developed. This is because it is the state with the highest production and planted area of Cocoa tree. It should be noted that the most cited studies were published mostly in high-impact journals, under world-class publishers which publish only in English. Interestingly, although there is little research on the influence of pre-Hispanic cultures and current ethnicities on cacao (Ethnobotany), they turn out to be the reports with the highest number of citations. In addition, those journals show the tendency of indigenous groups of people to consume local resources and their influence on food sovereignty, which are interesting topics to be developed.

The analysis of networks of research institutions revealed that in the 199 scientific articles published, there was co-participation of different research institutions on 114 instances (nodes) and 204 links are apparent (Figure 3).

Table 2. Bibliometric indicators of the main scientific articles where cocoa (*Theobroma cacao*) was the object of research in Mexico from 1999 to 2022, ranked according to the number of bibliographic citations obtained.

First author			Scientific article						
Name	**Institución**	**Country**	**Área of stady**	**Journal**	**ILanguage**	**Publisher**	**WoS (2021)**	**Subject**	**Citations**
Motamayor *et al.* (2002)	CIRAD	France	Tabasco	Heredity	English	Springer	3.8	Ethnobotany	542
Rodriguez-Campos *et al.* (2012)	IPN	Mexico	Tabasco	Food Chemistry	English	Elsevier	8.8	Biotechnology	262
Rodriguez-Campos *et al.* (2011)	IPN	Mexico	Tabasco	Food_RI	English	Elsevier	8.1	Biotechnology	259
Crown y Hurst (2009)	UN_Méx	USA	Sureste Mexico	PNAS	English	PNAS	11.1	Ethnobotany	195
Powis *et al.* (2011)	KSU	USA	Sureste Mexico	PNAS	English	PNAS	11.1	Ethnobotany	123
Salgado-Mora *et al.* (2007)	ECOSUR_ Chiapas	Mexico	Chiapas	Interciencia	Spanish/ English	Interciencia	0.4	Ecology	112
Cordova_Avalos (2001)	ColPos_ Puebla	Mexico	Tabasco	Universidad y Ciencia	Spanish	UJAT	Sin factor	Agroforestry	80
Muñoz *et al.* (2006)	ECOSUR_ Chiapas	Mexico	Tabasco	AJ_ Primatology	English	Wiley	2.4	Ecology	71
Romero-Cortes *et al.* (2013)	TecNM_ Veracruz	Mexico	Tabasco	J Sci Food Agric	English	Wiley	4.1	Biotechnology	59
Priego-Castillo *et al.* (2009)	ColPos_ Tabasco	Mexico	Tabasco	Universidad y Ciencia	Spanish	UJAT	Sin factor	Agroforestry	56

CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Développement; IPN: Instituto Politécnico Nacional; UN_Méx: Univerity if new Mexico; KSU: Kennesaw State University; ECOSUR: El Colegio de la Frontera Sur; ColPos: Colegio de Postgraduados; TecNM: Tecnológico Nacional de México; PNAS: Proceedings of the National Academy of Sciences; Food_RI: Food Research International; AJ_Primatology: American Journal of Primatology; Interciencia: Asociación Interciencia; UJAT: Universidad Juárez Autónoma de Tabasco.

Figure 3 shows that six institutions lead cocoa research in Mexico; all of them located in the producing areas within the Mexican southeast. Institutions located in the state of Tabasco tend to a greater degree of association with each other. On the other hand, the institutions located in the state of Chiapas have broader collaboration networks with other research centers in the country.

The institutions that develop research in cocoa cultivation are located in the cocoa production areas. This helps to improve technology transfer, because the information was generated in the cocoa area, in field conditions with cooperating producers. In other words, the spatial validation of knowledge is not an impediment to the transfer.

In the 199 scientific articles analyzed, 485 different authors (nodes) and 661 links were found (Figure 4). A network is composed of nodes interconnected by edges, where the

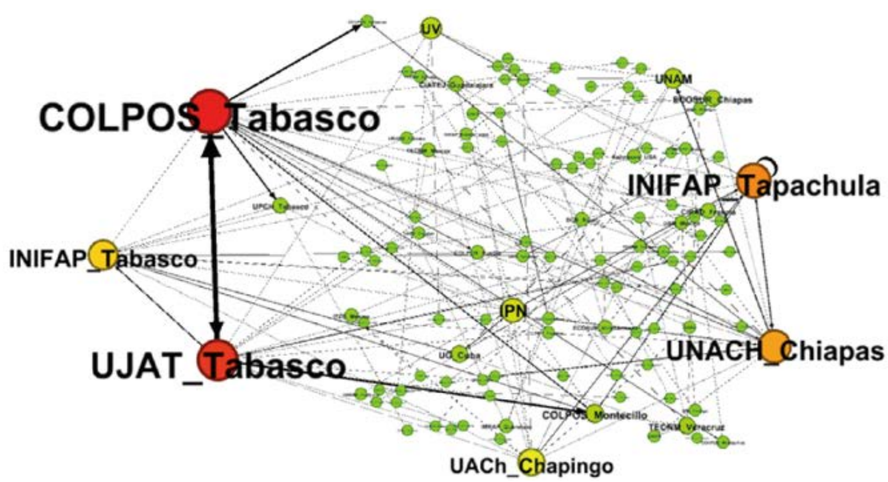

Figure 3. Network of research institutions that published scientific articles on cacao (*Theobroma cacao*) in Mexico from 1999 to 2022. The size of the node corresponds to their productivity.

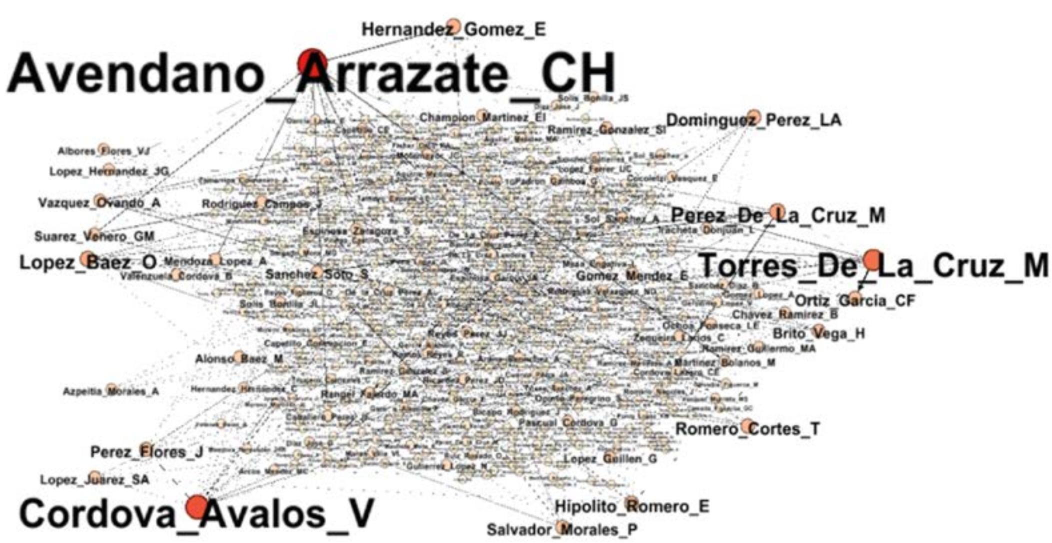

Figure 4. Network of authors who published scientific articles on cacao (*Theobroma cacao*) in Mexico from 1999 to 2022. Node size corresponds to their productivity.

nodes represent the individuals and the edges represent the links that unite them (Aguilar-Gallegos *et al.*, 2016). Links in an analysis of co-authorship networks are important because, through them, an author can access ideas, knowledge, and sources of information that are socially distant (Granovetter, 1973).

Main authors who developed research where cocoa was the object of study from 1999 to 2022 were: Avendano_Azarrate_CH of INIFAP-Tapachula at the Rosario Izapa experimental station; Torres de la Cruz_M de la UJAT_Tabasco, and Cordova_Avalos_V of CP_Tabasco. However, unlike CP_Tabasco, UJAT_Tabasco, and INIFAP_Tapachula, where one researcher is apparently full specialist in cocoa issues, in UNACH_Chiapas a network of researchers was presented, composed mainly of Lopez_Baez_O, Suarez_Venero_GM, Vázquez_Ovando_A, and López_Hernández_JG who are linked to other specialists in different institutions in the country.

Silva *et al.* (2014) found that authors from the same institution tend to associate with each other. This restricts constructive criticism and reduces feedback on the relevance of research. In addition, institutional research groups tend to replicate the same methodologies in different study sites, which limits innovation in research and allows circularity (redundancy in the object of study) of publications (Santillán-Fernández *et al.*, 2023). Therefore, synergies with authors from other institutions can be a good strategy to improve the quantity and quality of research.

The total number of researchers who conducted research in the last 22 years is 485 people. Of this total, 332 (68.45%) are males (men) and 153 (31.55%) are females (women) (Figure 5A). Figure 5B shows that the males published 147 (73.86%) articles as first author, and females 52 (26.13%). The same trend is observed when analyzing the number of articles published as author for correspondence (Figure 5C). Men published 151 (75.87%) and women, 48 (24.12%).

Our findings showed a gender inequality in the results of research conducted on cocoa in Mexico. Thus, this study provides empirical evidence on that imbalance in scientific

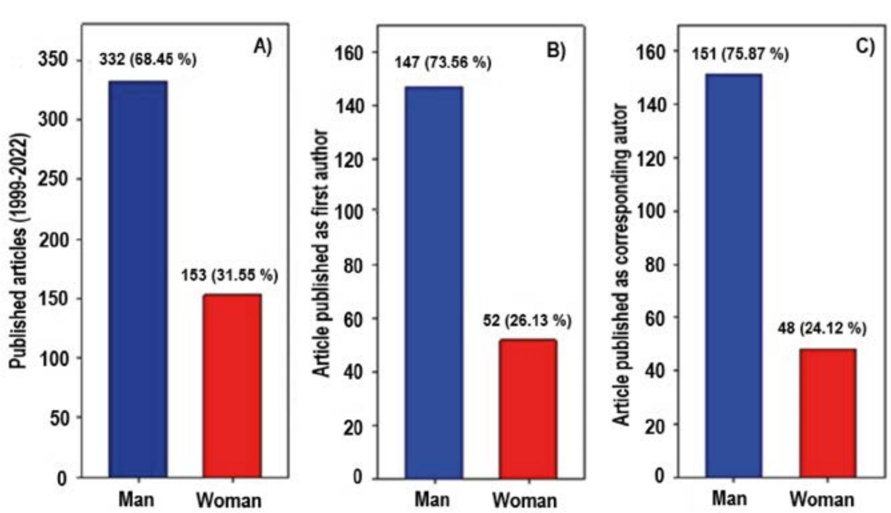

Figure 5. Number of articles on cocoa (*Theobroma cacao*) published by A) gender (only female or male); B) as first author and C) as author for correspondence.

production. This imbalance is in line with global results in which women represent less than 30% of shared authorships, while men represent over 70% (Larivière *et al.*, 2013).

CONCLUSIONS

Scientific production showed an exponential growth of papers at the national scale about the cultivation of cocoa (*Theobroma cacao*) in the recent 22 years past. The greatest scientific productivity was concentrated in topics such as biotechnology, economics and disease control. Research is focused on the Mexican southeast, and was led by Colegio de Postgraduados, the National Institute of Agricultural and Livestock Forestry Research-INIFAP, and the Juárez Autonomous University of Tabasco-UJAT. The male gender is more represented as publisher of the most articles. The highest percentage of publications as first author and corresponding author are also represented by males.

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Colegio de Postgraduados

Value networks, consolidation, and risks of an agricultural territory

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ABSTRACT

Objective: Producer and marketer value networks in an agricultural land in Veracruz, Mexico were identified and characterized, to determine its consolidation level (primary, emergent, potential, or combined), as well as to detect risks and to establish an improvement proposal.

Design/Methodology/Approach: An area of the Altas Montañas Veracruz, Mexico was used for the experiment. Surveys were conducted with producers (n=131) and marketers (n=42) of agricultural produce. Socioeconomic, environmental, technical, commercial, and chain link variables were analyzed to detect risks, opportunities, and revaluations. The study was carried out with the RStudio v. 4.3.3 software.

Results: Six producer value networks and three marketer value networks were identified. Socioeconomic, environmental, commercial, and technological risks were detected. However, public health risks were different depending on the application of raw manure. Chayote and avocado have displaced other crops. The revaluation proposals for emergent and potential producer and marketer networks were focused on technical support (*i.e.*, commercialization and generational change).

Keywords: supply chain, value networks, risks, reevaluation, and local resources.

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INTRODUCTION

The increase of the world population is a major issue. In 1950, the population reached 2.5 billion; however, by 2022, this figure had grown to 8 billion. The world population is projected to reach 8.5 billion in 2030 and to increase further to 9.7 billion in 2050 and 10.4 billion by 2100 (ONU, 2019, 2023). Food demand has grown as a result of this situation and, consequently, the areas used for agriculture have also increased (Monjardín-Armenta *et al.*, 2017). Agricultural activities are related to environmental, economic, and social parameters (Blobel and Meyer-Ohlendorf, 2006). In Mexico, agriculture is part of the primary sector (Cardona Reséndiz *et al.*, 2018), contributing 4% of the gross domestic product (GDP) (INEGI, 2022). The GDP helps to understand the opportunities to produce

goods and services, benefiting the income, welfare, and life quality of the producers (INEGI, 2022; Rincón-Valdéz *et al.*, 2004). However, the biophysical effects of climate change impact food production (Blobel and Meyer-Ohlendorf, 2006). In addition, several socioeconomic factors, such as price and consumption change, influence crop productivity. These changes provide a comparative advantage to certain crops. Consequently, the decision to grow them depends on the adaptation strategies implemented in each region and agricultural system. Veracruz is characterized by its agricultural lands, where producers grow annual and perennial crops (INEGI, 2023; SIAP, 2022). Forty-eight percent of the Región Altas Montañas (RAM), one of ten strategic regions in Veracruz, is used for agricultural activities (INEGI, 2023; Rivera-Hernández, 2018; SIAP, 2022). Some local producers obtain their income from crops that are commercialized in the domestic and international markets. They supply raw materials for the agroindustry, including chayote, coffee, sugarcane, and banana leaf (Bada Carbajal *et al.*, 2010). Other local producers grow and sell food for rural populations (Beltran-Morales, 2022). The value networks identified in the region are links to the agrifood supply chain: producers and marketers carry out activities and use facilities and means of distribution, to guarantee the production of raw materials. In addition, they strengthen the development of crops, add value (cleaning, purification, etc.), and manufacture agricultural products for the final consumer. Producers and marketers carry out one or more activities within the value chain, including field production and packaging (Sánchez-Galván *et al.*, 2020), as well as technical support, phytosanitary and nutritional management, crop residue composting, shelf life, food safety, biosecurity, business transfer, marketing, and sales (Rosa and de Paredes, 2017). However, not all crops have the same structural maturity and operational level (Vargas-Canales *et al.*, 2020). The objective of this study was to identify and characterize producer and marketer value networks in an agricultural land in Veracruz, Mexico. The aim was to establish its consolidation level (primary, emergent, potential, or combined), as well as to detect risks and to develop an improvement proposal.

MATERIALS AND METHODS

The study area included the following municipalities of Veracruz: Alpatlahuac, Calcahualco, Coscomatepec, Fortín, Huatusco, and Ixhuatlán del Café (Figure 1). The region is located from 19° 07” to 18° 58’ 39” N and from 97° 06” to 96° 57’ 25” W, at 987 to 1,860 m.a.s.l. The climate is mainly temperate-semi-warm-humid (INEGI, 2000, 2024).

The crops grown in the area are: avocado (*Persea americana*), coffee (*Coffea arabica*), pumpkin (*Cucurbita pepo* L.), sugarcane (*Saccharum* spp.), chayote (*Sechium edule*), chilacayote (*Cucurbita ficifolia*), rocoto chili (*Capsicum pubescens*), plum (*Spondias* spp.), peach (*Prunus persica* L.), tomato (*Solanum lycopersicum* L.), prickly pear (*Opuntia ficus-indica*), potato (*Solanum tuberosum* L.), pear (*Pyrus communis*), Mexican husk tomato (*Physalis philadelphica*), and banana leaf (*Musa* sp.).

Research method

The study was based on the value network focus proposed by Porter (1985), which includes inbound and outbound logistics, operations, marketing and sales, services,

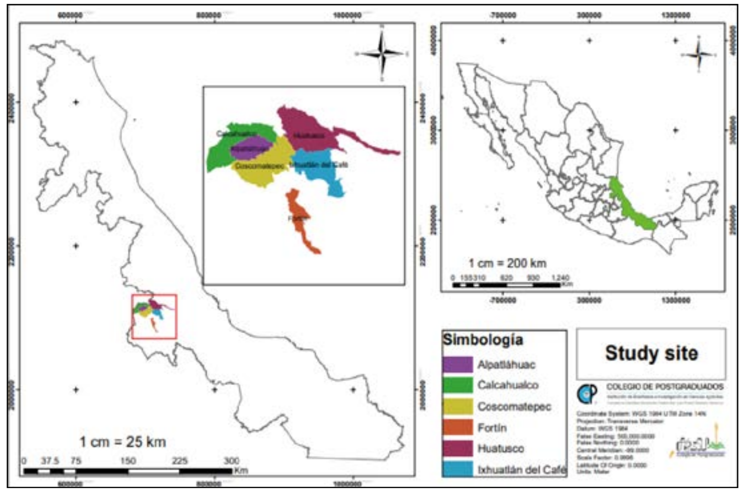

Figure 1. Location of the study area in the Región Altas Monañas, Veracruz, Mexico.

support, human resource management, procurement, technological development, and financial infrastructure. Sánchez-Sánchez *et al.* (2023) defined the primary, emergent, and potential value networks based on Porter (1985). Their aim was to integrate all the production-commercialization activities, determining their presence in the territory and their consolidation level (Table 1).

The ranges and acceptance criteria of value networks were developed following Sánchez-Sánchez *et al.* (2023). In addition, the consolidation level (primary, emergent, and potential) of the region was determined (Table 2), based on the assumption that value networks can include combined characteristics, depending on their structure and operational level.

Variables

Socioeconomic, environmental, commercial, technological, and link-type variables were analyzed. The main characteristics included gender, age, education, main activity, generation of local employment, land ownership, season and cause of manpower scarcity,

Table 1. Concepts and characteristics of value networks in an agricultural land.

Concept	Primary network	Emergent network	Potential network
Definition	The main source of income or business of a family.	An alternative and developing source of income, without a guaranteed or secure income for the families.	An experiment developed as future source of family income and whose main objective is to find out the future feasibility of this alternative.
Operations Post-harvest practices	High production volume, with an extended shelf life.	Low production volume, without an extended shelf life.	Small production volume, without an extended shelf life.
Marketing and sales Sale requirements	Food safety certificate.	No food safety certificate, no selection, no packaging according to product quality.	No food safety certificate. Product selected and packaged according to quality.

Table 2. Ranges and acceptance criteria of value networks, based on Sánchez- Sánchez *et al.* (2023).

Value network	Definition criteria	Acceptance range
Combined primary network	40% Primary-33% Emergent-27% Potential	45%-35-% Primary-38%-28% Emergent-32%-22% Potential
Combined emergent network	28% Primary-39% Emergent-33% Potential	28-26% Primary-39% Emergent-33% Potential
Combined potential-emergent network	21% Primary-45% Emergent-34% Potential	25%-16% Primary-50%-40% Emergent-39%-19% Potential
Primary network prevalence	50% Primary-30% Emergent-20% Potential	55%-45% Primary-35%-24% Emergent-25%-15% Potential
Transition to a consolidated primary network	65% Primary-25% Emergent-10 Potential	69%-59% Primary-26%-23% Emergent-15%-12% Potential
Consolidated primary network	75% Primary-18% Emergent-7% Potential	80%-70% Primary-22%-13% Emergent-11%-1% Potential

generational change, innovation adoption, type of target market (local, regional, domestic, international), post-harvest practices, seed use, and crop yield. Another major characteristic is the partnership advantages to find market niches that would add value to the product and sale. In addition, biosafety, food safety (certifications), technical support, and the presence of new pests were considered. The variables structured the producer and marketer surveys (Table 3).

Agricultural and commercialization risk variables that can impact the value networks were considered (Table 4).

Sample size

Agricultural producers and marketers were surveyed as links of the agrifood chain. Agricultural producers from RAM were identified to determine sample size, based on the

Table 3. Study variables used to identify and characterize value networks.

Variables	Description
Social	Land ownership, crop, area, irrigation, rainfed agriculture, technical support. Age, gender, education, economic activity.
Economic	Local employment (number, week, month, year, and gender). New pests that impact the crop.
Environmental	Agrochemical, chemical, organic, and biological fertilizers. Biosafety: use of manure, raw manure, composts, manure origin.
Commercial	Markets (local, regional, domestic, and international). Direct sale, local intermediary, domestic central market, or supermarkets. Product presentation (bulk and box packaging, with or without refrigeration). Local, domestic, or international destination of the product. Domestic and international certifications (field and packaging).
Technological	Agronomical, nutrimental, health, harvest, and post-harvest management. Associated, extensive ($\geq$1 ha), and backyard monocrop.
Links	Producers, suppliers, collectors, distributors (local, domestic, and international), consumers, manufacturers.

Table 4. Description of the potential risks faced by value networks.

Risks	Description
Public health	Food safety and biosafety
Socioeconomic	Oversupply: crop area increase, low quality, food safety, low prices, crop abandonment, lack of employment, and migration. Economic loss caused by pests.
Environmental	Soil, water, and air pollution caused by agrochemicals, fertilizers, and non-composted manure.
Technological	Lack of training, equipment, and infrastructure.
Economic	Commercialization (ban on exportation due to microbiological charges, residues, untreated water).
Preservation	Genotype displacement and loss of genetic abundance due to the use of uniform areas for monocrops. Higher phytosanitary risks.

Programa para el Bienestar, "Corte a junio 2022, beneficiarios del Programa Producción para el Bienestar 2022" (SADER, 2022) and the 2019, 2021, and 2022 Anuario Estadístico de la Producción Agrícola (SIAP, 2022). The Brenlla-Martínez (1997) formula was applied to the sample of n=1,131 agricultural producers. These authors suggest that a ≥107 ≥145 sample size of producers can obtain a 97% confidence level. The final sample consisted of n=131 producers. The marketer sample size was based on the economic census (INEGI, 2019), which was used to identify the wholesale traders and retailers (economic units) within the RAM (Figure 1). A sample of n=72 economic units resulted in a n=42 sample (Brenlla-Martínez, 1997), obtaining a 95% confidence level.

Statistical analysis

Google Forms was used to collect the data from the surveys applied to producers and marketers. The data were encoded with Excel. The encoding was established based on dichotomous-qualitative, polytomous-qualitative, ordinal-qualitative, and continuous-qualitative variables. After the encoding, the RStudio v. 4.3.3 statistical package was used to perform the cluster analysis. Clusters were developed using the similarity matrix, based on Gower's distance (Palacio *et al.*, 2020), and the fviz_nbclust function of the factoextra package was used to determine the optimal cluster number. Once the cluster number was established, the Ward rostering method was used to develop the scatter plot and to obtain the clusters based on value networks. Subsequently, Excel was used to identify value networks, considering range and acceptance criteria (Table 2). The risks of the value networks were determined using the distinctive characteristics of each network (Table 1). Finally, the Statistic package was used to analyze the main components, in order to reevaluate the emergent and potential networks.

RESULTS AND DISCUSSION

Based on the network characteristics described in Tables 1 and 2, as well as on the ranges and acceptance criteria, six agricultural value networks (Figure 2a) and three marketer value networks were identified (Figure 2b). Agricultural value networks were divided into primary (67%), emergent (17%), and potential-emergent (17%) networks; however, based on

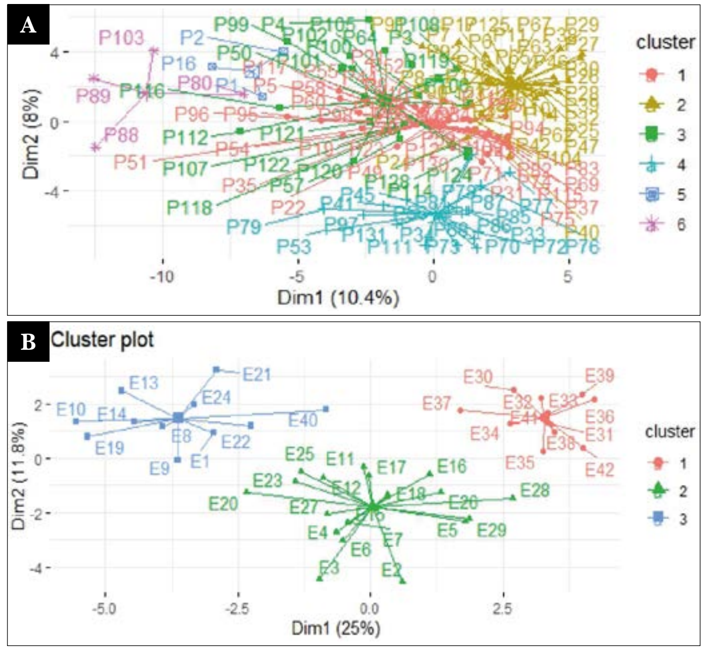

Figure 2. Value networks identified in the territory: A) agricultural value networks; B) marketer value networks.

their characteristics, no network has been fully (100%) identified as a consolidated primary value network (Figure 3a). Likewise, the marketer networks had various consolidation levels: primary (33%), emergent (34%), and potential (33%) (Figure 3b).

The diversity of crops of the agricultural networks can be linked with sustainability and diversification: they generate local employment and strengthen their presence in the territory. The networks are composed of the following crops: chayote (39%), avocado (14%), coffee (13%), peach (6%), plum (5%) and banana leaf (5%) (Figure 4a). Marketer networks receive their raw materials from the following crops: chayote (24%), avocado (12%), coffee (12%), banana leaf (16%), potato (10%), and rocoto chili (6%) (Figure 4b). Figure 4 shows that the crops identified in the value networks are connected with the area sown on the state: chayote (57%), avocado (50%), and banana leaf (47%) (SIAP, 2023).

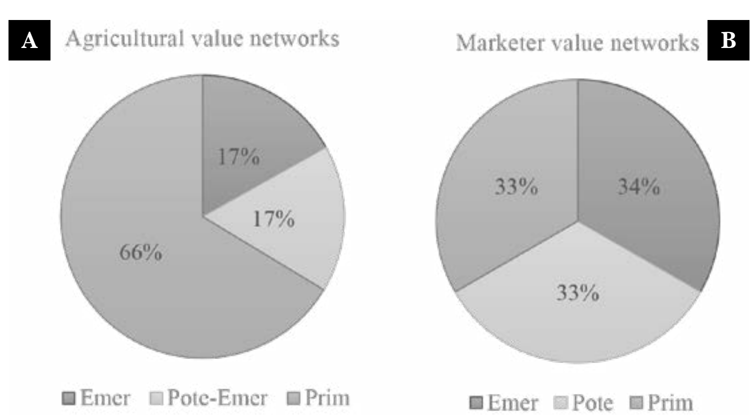

Figure 3. Percentage of the characteristics of the value networks in a part of the Altas Montañas territory, Veracruz, Mexico: A) agricultural value networks; B) marketer value networks.

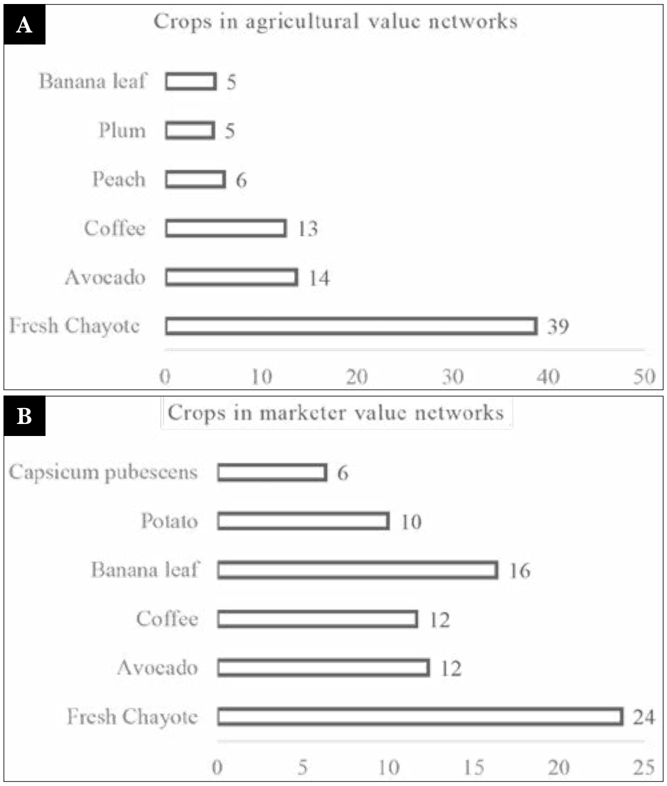

Figure 4. Representative crops of the agricultural value and marketer networks, highlighting their economic importance.

Production and commercialization are links of the internal logistics chain of the value networks. Agricultural networks are mainly composed of raw material supply (67%) and internal raw material supply and procurement, added value, and sale of the produce (33%). The networks of marketers that buy and sell produce are deficient in raw material supply (66%), while the purchase and sale of raw material supply and agricultural produce accounted for the remaining deficiencies (34%). The agricultural and marketer networks face socioeconomic, environmental, technological, and commercialization risks, although they pose different public health and conservation risks (Figure 3). Agricultural networks involve public health and commercialization risks, as a consequence of the application of raw manure to the crops. Meanwhile, marketers are impacted by natural disasters (hail and drought) which ultimately hinder commercialization (low availability of raw material), increasing the produce preparation costs and limiting the fulfilments of agreements with domestic and foreign markets.

Regarding the strengthening of territorial development, base characteristics are used to develop proposals for the revaluation of local or endogenous produce in both value networks. In addition, they provide solutions for the scale and adaptation limitations for quality standards, following international commercialization standards, without losing their value for consumers. The risks identified the measures that must be taken as part of the proposal to reevaluate the value networks (Figure 3). The analysis of the main components (MC) revealed that the agricultural network variables were clustered in four MCs (Figure

4a) and the marketer networks were clustered in three MCs (Figure 4b). The (cumulative) explanatory variable for each link recorded 73.95% and 72.82% values. The analysis revealed that agricultural networks must improve their technical assistance, postharvest management, and sale requirements (Figure 5A). The agricultural value networks involved generational change for the proper establishment of the future of the crop and the network, through business succession and transference. Meanwhile, marketers must be trained in requirement compliance, postharvest practices, produce review, and professional capacity for the improvement of business operations (Figure 5B).

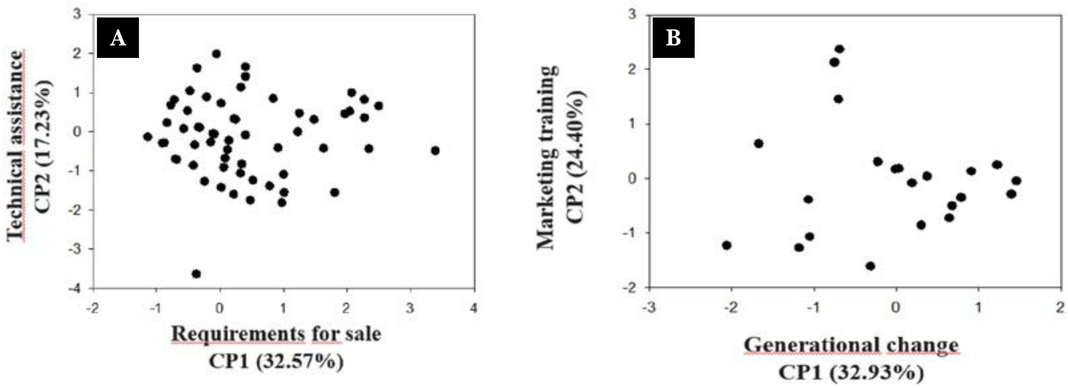

Figure 5. Principal component plots (A: plot of PC1 and PC2 of agricultural value networks; B: plot of PC1 and PC2 of value networks of agricultural marketers).

The six agricultural value networks and the three marketer networks have primary, emergent, and potential characteristics, with different consolidation percentages. According to Sánchez-Sánchez *et al.* (2023), a given territory can have value networks with various consolidation levels, generating a combination of distinctive characteristics. Agricultural value networks face public health risks related to the application of raw chicken manure to the crops that make up combined emergent, combined emergent potential, and consolidated primary value networks. This type of manure can expose the produce and the consumer to *Escherichia colli* and *Salmonella* sp. These findings match the results of Rosas-Martínez and Aguilar-Rivera (2022), who identified *Escherichia colli* and *Salmonella* sp. in pollinaza (chicken manure). Likewise, Nataren-Velazquez *et al.* (2020) reported a similar problem with the application of raw manure and fallen tree leaves in avocado (*Persea americana*) plantations. The marketer value networks (including the CommercializationPrimaryConsolidation network) face the risk of climate-related disasters, which impact the production and availability of raw material, as pointed out by Acevedo-Suárez *et al.* (2012), who detected the adverse effect of weather on production levels.

CONCLUSIONS

Six agricultural value networks and three marketer networks (with various consolidation levels) were identified in the territory. Neither type showed a single classification of consolidated networks (*e.g.*, primary, emergent, or potential). Based on the percentage of distinguishing characteristics, the networks are dynamic and share features that classify them as combined and evolving networks. According to their consolidation level, the networks fulfill the following principles: input supply and product purchase and sales. Diverse risks were identified, but a social segment classification could facilitate their management.

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Colegio de Postgraduados

Agrobiodiversity and food security: challenges and sustainable solutions

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ABSTRACT

Objective: Agricultural biodiversity, also known as agrobiodiversity, encompasses the variety of plants, animals, and microorganisms that are directly or indirectly involved in agriculture. This diversity is the result of millennia of selection, management, and domestication of species, which has allowed societies to adapt to different environmental and cultural conditions. However, in recent decades, the loss of agrobiodiversity, accelerated by industrial agriculture, the expansion of monocultures, and the reduction of varieties, has put global food security at risk. This diversity is crucial to ensuring the resilience of agricultural systems in the face of challenges such as climate change, emerging pests, and the depletion of natural resources. This paper examines the importance of conserving agricultural biodiversity from the perspective of food security. It emphasizes how agrobiodiversity not only contributes to the stability of food production but also improves human diets by diversifying crops and providing essential micronutrients.

Design/Methodology/Approach: A search was conducted on the following scientific information platforms: Web of Science database and Google Scholar. A systematic search for publications related to agrobiodiversity systems was carried out in the WoS database and Google Scholar over the last 49 years (1975-2024).

Results: Genetic erosion is particularly concerning because genetic diversity is essential for crops to face environmental challenges such as climate change, pests, and diseases. The loss of traditional varieties, which are selected by local farmers to adapt to specific conditions, increases agriculture's vulnerability to external disruptions. These landraces, having been cultivated in genetically diverse mosaics, offer protection against catastrophic losses in the event of crop failures due to extreme conditions or diseases.

Findings/Conclusions: Genetic diversity allows for the development of sustainable solutions to pests and diseases, reducing dependence on pesticides and promoting more environmentally friendly farming practices. However, challenges related to biodiversity conservation persist, making it essential to implement public policies that promote agrobiodiversity and address the socioeconomic issues that limit its adoption.

Keywords: Agrobiodiversity, agri-food security, genetic resources, biodiversity and conservation.

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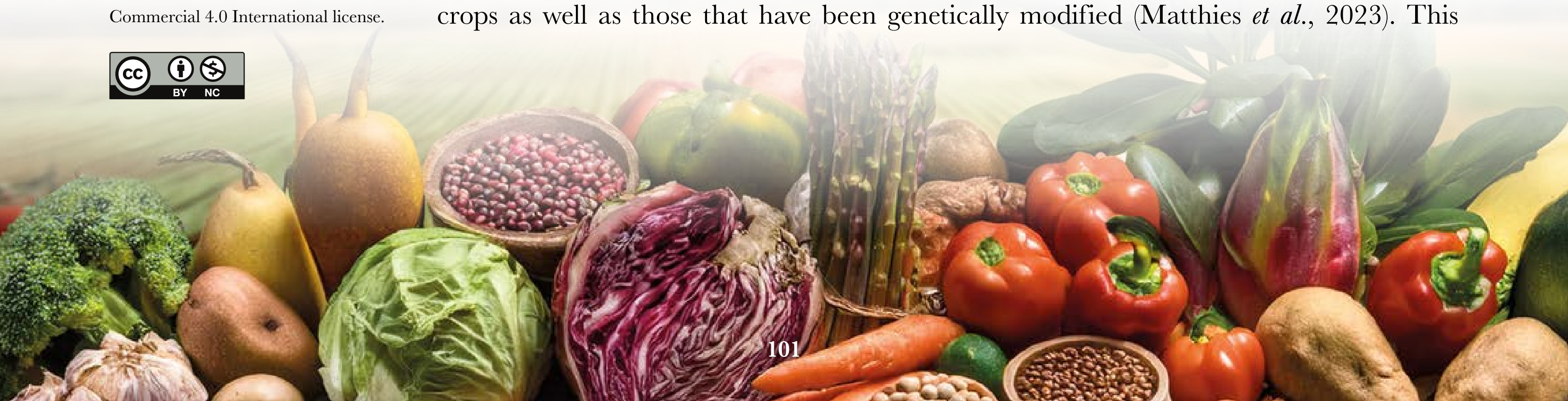
INTRODUCTION

Agricultural biodiversity, also known as agrobiodiversity, refers to the variety of plants, animals, and microorganisms used directly or indirectly in agriculture, including wild-type crops as well as those that have been genetically modified (Matthies *et al.*, 2023). This



concept encompasses the different crop varieties and livestock breeds, as well as the natural systems that sustain them (Maxted *et al.*, 2015). Agrobiodiversity is the result of thousands of years of selection, management, and domestication of species by humans, which has allowed societies to adapt to different environmental, climatic, and cultural conditions (Figure 1) (Agnoletti & Santoro, 2022).

In recent decades, there has been growing concern about the loss of agricultural biodiversity (Agnoletti & Santoro, 2022). Industrial agricultural practices, the expansion of monocultures, and the reduction in the number of crop varieties in production systems have accelerated this loss, putting global food security at risk (FAO, 1999). This biodiversity is key to maintaining a sustainable food system, capable of responding to future challenges such as climate change, the emergence of new pests and diseases, and fluctuations in the availability of natural resources (Jackson *et al.*, 2007; Zimmerer, 2014).

For this reason, this paper explores the importance of conserving agricultural biodiversity from the perspective of food security, emphasizing how this diversity is essential for ensuring food production, addressing environmental challenges, and promoting the resilience of agricultural systems.

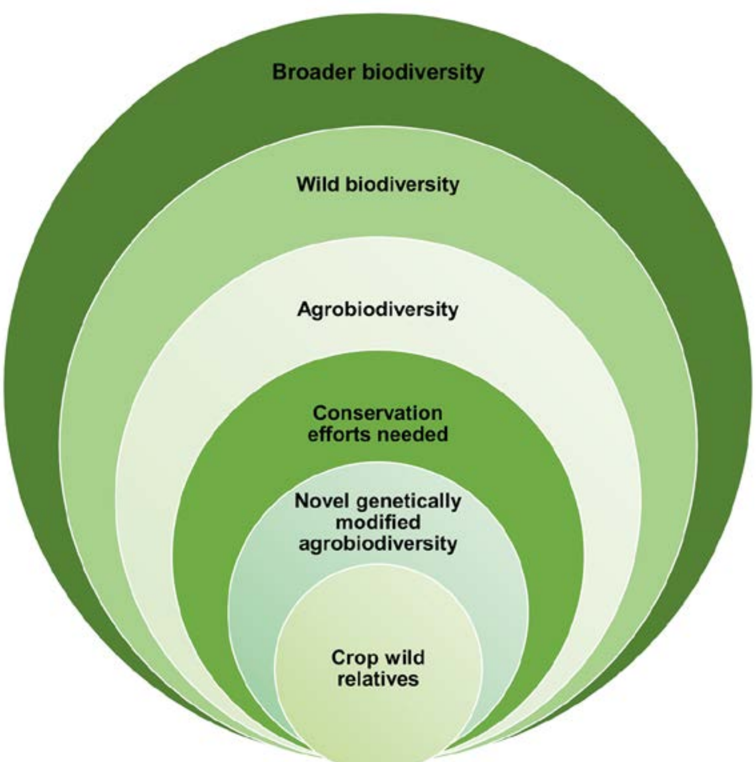


Figure 1. Agrobiodiversity is defined as the subset of broader biodiversity that is used for food and agriculture. Defining Broader biodiversity as: The variety of living organisms from different sources including wild and agricultural landscapes. Agrobiodiversity: as the variety of domesticated animals, plants and microorganisms used for food and agriculture. Novel genetically modified agrobiodiversity: as non-naturally occurring genotypes. Wild biodiversity: as Naturally occurring living species, not subject to human-mediated selection. Crop wild relatives: as wild relatives of domesticated species; and conservation efforts needed: as wild relative plants who need conservation efforts to avoid their extinction.

MATERIALS AND METHODS

The research was conducted using a bibliometric and data mining-based approach, which included the selection of the database as well as the identification of search terms and filters. The titles and abstracts of the articles were reviewed to identify and exclude those that were not relevant. Subsequently, the selected dataset was analyzed.

Bibliographic base

A search was conducted on the following scientific information platforms: Web of Science database (https://www.webofknowledge.com) and Google Scholar (https://scholar.google.com) (Pranckutė, 2021). A systematic search for publications related to agrobiodiversity systems was carried out in the WoS database and Google Scholar over the last 49 years (1975-2024). The most general logical operators were used in this search (agrobiodiversity, agri-food security, genetic resources, biodiversity, and conservation) to extract all possible publications related to the topic. Subsequently, search filters were applied (scientific articles, review articles, systematic reviews, meta-analyses, and international conservation guidelines/protocols) to meet the corresponding inclusion criteria. The exclusion criteria were articles that exceeded the time limit, as well as opinion pieces, case reports, or essays.

RESULTS AND DISCUSSION

Relationship between agricultural biodiversity and food security

Agricultural biodiversity is a fundamental component of global food security, as it enables agricultural systems to be more productive, resilient, and capable of facing various threats arising from climate change, pests, and diseases (Engels *et al.*, 2021; Agnoletti & Santoro, 2022). Throughout history, farmers have selected and cultivated thousands of plant varieties and animal breeds, resulting in an enormous wealth of genetic diversity, which has helped ensure the availability of food in both quantity and quality (Jago *et al.*, 2024).

One of the main benefits of agricultural biodiversity is the possibility of diversifying crops, which not only enriches human diets but also contributes to the stability of food production (Ceccarelli & Grando, 2022; Jago *et al.*, 2024). Instead of relying solely on a few staple crops such as maize, wheat, or rice, which make up the bulk of global food production, genetic diversity in agriculture allows farmers to choose from a wider range of crops (Zimmerer, 2014; Zimmerer & De Haan, 2017). This is crucial for ensuring balanced diets that provide all the necessary nutrients for human health. For example, incorporating a greater variety of legumes, fruits, vegetables, and cereals into farming systems allows rural and urban populations to access a wider range of micronutrients, vitamins, and minerals, thereby preventing nutritional deficiencies, such as iron or vitamin A deficiencies, which are common in regions where diets rely on a few staple foods (Zimmerer *et al.*, 2021; Ceccarelli & Grando, 2022; Jago *et al.*, 2024). This has been demonstrated in rural areas of Kenya, where an increase in agricultural biodiversity has been associated with improved dietary diversity, which in turn reduces malnutrition and growth problems in children (Kahane *et al.*, 2013; Jones *et al.*, 2021). However, this relationship is not always direct, as food security is also influenced by socioeconomic factors such as household income levels, food distribution within the household, and access to food (Chappell & LaValle, 2011; M'Kaibi *et al.*, 2017). The above allows us to conclude that agricultural biodiversity is intrinsically linked to food security, providing a foundation for dietary diversity and improving access to nutritious foods. However, its impact on nutrition depends on various interrelated factors, such as household economic stability and cultural practices. To achieve comprehensive food security, it is crucial to promote policies that not only encourage agricultural biodiversity but also address the social and economic challenges affecting nutrition in rural communities.

Resilience to pests and diseases in agriculture

Resilience in agricultural systems is essential to ensuring food security in a global context increasingly affected by climate change, emerging pests, and diseases (Chappell & LaValle, 2011). The ability of crops to withstand and recover from these stressors is crucial for maintaining sustained yields and protecting the genetic resources that are fundamental to agriculture (Frison *et al.*, 2011; Murrell, 2017). Pests and diseases pose a significant threat to the stability of agricultural systems, and climate change has exacerbated these challenges by altering the geographic distribution of many pest and pathogen species, as well as their life cycles and virulence. This has created a need to develop new strategies that strengthen the adaptive capacity of crops, leveraging both technological advancements and traditional agronomic management practices (Lin, 2011; Shroff *et al.*, 2020; Chauhan *et al.*, 2023).

One of the main strategies to enhance crop resilience against pests and diseases is proper soil management (Dardonville *et al.*, 2022). The use of cover crops and the incorporation of organic amendments not only improve soil structure and increase its water retention capacity but also promote microbial biodiversity, which in turn strengthens the natural defenses of crops. In this way, the addition of organic matter increases biological activity in the soil, enhancing crop resistance to pathogen attacks and creating a less favorable environment for pest development (Nciizah *et al.*, 2021; Dardonville *et al.*, 2022). Another essential strategy is crop diversification, which can reduce pest and disease pressure (Huss *et al.*, 2022). By increasing biodiversity in agricultural systems, the life cycles of many pests are disrupted, and disease spread is reduced, contributing to the creation of more robust agricultural systems (Huang & Zhao, 2017; Yu *et al.*, 2022). Including different species in crop rotations has shown positive effects not only on soil health improvement but also on the capacity of agricultural systems to withstand external disturbances (Wu & Wang, 2017; Wang *et al.*, 2021). In fact, diversification at the field and landscape levels has been associated with lower pest incidence and greater stability of agroecosystems in general. Conversely, when farmers cultivate large areas of monocultures (plantations dominated by a single crop variety), they become more vulnerable to disease spread (Yin *et al.*, 2010; Liu *et al.*, 2022). A pest adapted to a particular species can spread quickly and devastate entire crops, jeopardizing food production (Wang *et al.*, 2021; Liu *et al.*, 2022). In addition to the aforementioned positive effects, agricultural biodiversity promotes healthy agricultural ecosystems by enhancing soil fertility and conserving beneficial insect biodiversity, such as pollinators and natural pest predators (Sarwar *et al.*, 2008; N'Dayegamiye *et al.*, 2017).

Additionally, biotechnology has emerged as a powerful tool to improve crop resilience against pests and diseases. Through advanced techniques such as gene editing and genetic engineering, scientists have developed crop varieties resistant to specific pests and diseases, reducing the need for pesticides and other chemicals (Pathirana *et al.*, 2024). In particular, genetic engineering has enabled the creation of crops with intrinsic resistance to insects or viruses that would otherwise cause significant agricultural yield losses. Moreover, advances have been made in developing crops that are more resistant to extreme environmental conditions, such as drought or high temperatures, which is crucial in the context of climate change (Lindberg *et al.*, 2021; Henderson *et al.*, 2024; Pathirana *et al.*, 2024).

On the other hand, the effects of climate change are not limited solely to the increase in the severity of pests and diseases; they also alter the dynamics of agricultural ecosystems (Nciizah *et al.*, 2021). Higher temperatures and variations in precipitation patterns affect both plants and the organisms that depend on them. Some studies have shown, for example, that certain pest insects, such as aphids, can increase their populations in warmer climates, while the natural enemies of these insects are negatively impacted, reducing their ability to effectively control pests (Lobell *et al.*, 2008; Aukema *et al.*, 2017).

In conclusion, resilience against pests and diseases is fundamental to agri-food security. Strategies that combine adapted agricultural practices, such as climate-smart agriculture and agroforestry, along with the use of advanced biotechnology and sustainable resource management, offer the best opportunities to protect agricultural systems and ensure stability in food production. However, it is crucial to consider the ethical and environmental challenges posed by biotechnology, as well as the inequalities in access to these innovations, especially for small-scale farmers. In this regard, continued investment in research and public policies that promote both the conservation of genetic resources and the development of sustainable technologies will be key to addressing future challenges in an equitable and effective manner.

Impacts of genetic erosion on agriculture

Genetic erosion in agriculture is closely linked to the loss of genetic diversity in crops, which negatively impacts the productivity, resilience, and adaptability of agricultural systems (Sirami *et al.*, 2019; Khoury *et al.*, 2022). This process involves a reduction in variability among species, varieties, and within the crops themselves. Such loss affects both wild relatives of crops and traditional varieties, which have been managed by farmers for generations, limiting their ability to adapt (Harlan, 1975; Egli *et al.*, 2020). Currently, various factors such as habitat fragmentation, climate change, the introduction of non-native species, pollution, and overexploitation have intensified the rate of extinction (Hammer & Teklu, 2008; Pathirana & Carimi, 2022). This has triggered a phenomenon known as the "extinction vortex," where declining populations experience a reduction in genetic variability, diminishing their ability to adapt and survive (Díez-del-Molino *et al.*, 2018; Bosse & van Loon, 2022).

Genetic erosion is particularly concerning because genetic diversity is essential for crops to face environmental challenges such as climate change, pests, and diseases (Bosse & van Loon, 2022; Khoury *et al.*, 2022). The loss of traditional varieties, which are selected by local farmers to adapt to specific conditions, increases agriculture's vulnerability to external disruptions. These landraces, having been cultivated in genetically diverse mosaics, offer protection against catastrophic losses in the event of crop failures due to extreme conditions or diseases (Tsegaye & Berg, 2007; Babay *et al.*, 2020). However, the replacement of these varieties with modern crops, which are generally homogeneous and designed for high yields under controlled conditions, has increased dependence on external inputs such as fertilizers and pesticides (Casañas *et al.*, 2017; Birhanu Abegaz & Hailu Tessema, 2021). Furthermore, genetic erosion has significant implications for global food security. The reduction in crop diversity limits farmers' options, which can result in decreased production, particularly under environmental stress conditions such as droughts or rising temperatures (Fu & Dong, 2015; Hailu, 2017; Legesse, 2020). This point is especially relevant in the context of climate change, where the ability of crops to adapt to new conditions is crucial for ensuring sustainable agricultural production (Dempewolf *et al.*, 2014; Bosse & van Loon, 2022). Another significant impact of genetic erosion is the loss of local adaptation. Traditional varieties have evolved over centuries to adapt to specific environments, making them a vital part of agriculture in regions with complex or changing environmental conditions (Casañas *et al.*, 2017). Replacing these varieties with modern crops can lead to the loss of this valuable adaptive capacity, leaving agricultural systems more exposed to the adverse effects of environmental changes or the emergence of new pests and diseases (Zeven, 1999).

At a cultural level, genetic erosion also has profound consequences. The management and conservation of agricultural diversity are intrinsically linked to traditional knowledge, which forms part of the cultural heritage of many rural communities (Rajeswara, 2016). When traditional varieties disappear, this knowledge is lost along with them, leading to both genetic and cultural erosion (Rogers, 2004). This loss directly impacts the self-sufficiency of farming communities, hindering their ability to effectively manage their agricultural resources (Van de Wouw *et al.*, 2010).

To assess genetic erosion, several methodologies have been proposed, such as genomic heterozygosity analysis and the detection of runs of homozygosity (ROH), which can indicate recent inbreeding (Narasimhan *et al.*, 2016). ROHs reveal regions of the genome where recessive, deleterious mutations may be expressed in a homozygous state, negatively affecting health and reproduction (Bosse *et al.*, 2018; Stoffel *et al.*, 2021). The accumulation of deleterious mutations in small populations, known as genetic load, can also increase rapidly, contributing to population decline and, eventually, extinction (Doekes *et al.*, 2021; Stoffel *et al.*, 2021). One of the main challenges in genetic conservation is the lack of precise tools to quantify genetic erosion. Although whole-genome sequencing has enabled advances in identifying the loss of genetic diversity, there is still no consensus within the scientific community on the optimal methods for accurately measuring it. The difficulty also lies in translating these genomic advances into practical applications for conservation (Silva *et al.*, 2021; Bosse & van Loon, 2022).

In response to these challenges, various conservation strategies have been implemented, such as *ex situ* conservation in gene banks and in situ conservation on farms, allowing varieties to continue evolving in their natural environments (Pathirana *et al.*, 2022).

Additionally, genetic rescue initiatives have been carried out, where genetic variability from other populations is introduced to improve genetic fitness, as seen in cases like the Florida panther and the American bison (Hedrick, 2009). However, these strategies are not without risks, as there is also the possibility of introducing harmful mutations that could increase genetic load in the long term (Salgotra & Chauhan, 2023). Despite international efforts, the scale and implications of genetic diversity loss are still not fully understood, making it difficult to plan more effective conservation strategies (Brush, 1999).

In conclusion, genetic erosion is a critical threat to species survival, especially in the context of accelerated environmental change. Despite advances in genomic technology, it remains urgent to develop and standardize methods that effectively quantify genetic erosion. This effort would facilitate the identification and prioritization of the most vulnerable populations and enable the implementation of appropriate genetic interventions to mitigate the effects of inbreeding and the loss of genetic diversity.

Strategies for the conservation of agricultural biodiversity

The conservation of agricultural biodiversity is a critical challenge that has gained relevance in recent decades due to its importance for the sustainability of global food systems and the ability of crops to adapt to changing environmental conditions (Pe'er *et al.*, 2020; Williams *et al.*, 2021). With the advent of genetic improvement programs in the 20th century, high-yielding varieties resistant to biotic and abiotic factors were promoted, leading to a drastic reduction in genetic diversity in agricultural fields. More than 75% of genetic diversity in plant genetic resources (PGRs) and 90% of crop varieties have disappeared, endangering the sustainability of the global agricultural system (Thrupp, 2000; FAO, 2018; Bélanger & Pilling, 2019). As a result, various international institutions and multilateral agreements have implemented strategies to preserve agricultural genetic resources and promote their sustainable use, with the aim of protecting biodiversity, ensuring food security, and strengthening resilience against threats such as climate change and environmental degradation (Priyanka *et al.*, 2021; Pathirana & Carimi, 2022). In response to this crisis, various conservation strategies have been developed. The first is in situ conservation, which involves maintaining genetic resources in their natural environments or on farms where they continue to evolve (Salgotra & Gupta, 2015; Salgotra & Chauhan, 2023), allowing plant varieties to keep adapting to changing environmental conditions, which is vital in the context of climate change (Ogwu *et al.*, 2014). Additionally, *in situ* conservation promotes the use of landraces and other local varieties by farmers, thereby helping to maintain genetic diversity in the fields (Hammer & Teklu, 2008).

The second approach is *ex situ* conservation, which allows genetic resources to be stored outside their natural environment through seed banks (Pathirana & Carimi, 2022). This has been made possible through the participation of various institutions, such as the Food and Agriculture Organization of the United Nations (FAO), which has played a central role since the 1960s, promoting initiatives like the Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources and collaborating in the adoption of international frameworks such as the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture

(ITPGRFA), which came into force in 2004 and has been a key instrument for coordinating global efforts in the conservation and sustainable use of these resources (Priyanka *et al.*, 2021; Pathirana & Carimi, 2022; Salgotra & Chauhan, 2023). This treaty not only seeks to conserve genetic diversity but also ensures that the benefits derived from its use are shared fairly and equitably among all parties involved. Additionally, the Nagoya Protocol, which came into effect in 2014, sets guidelines for access to genetic resources and the equitable distribution of benefits obtained from their use (Buck & Hamilton, 2011). This protocol creates incentives for the conservation and sustainable use of biodiversity, linking the conservation of genetic resources with economic development and human well-being (Smith *et al.*, 2017). Alongside these agreements, the Cartagena Protocol on Biosafety addresses the importance of ensuring the safe handling of genetically modified organisms (GMOs) that may affect biodiversity, contributing to safety in biotechnology agriculture. This is crucial to promoting crop improvement that can adapt to environmental challenges and meet the food needs of a growing population (FAO, 2019; FAO, 2020). The safeguarding and storage of these genetic resources have been achieved through institutions such as the Svalbard Global Seed Vault and other programs supported by the Global Crop Diversity Trust (Global Crop Trust). This approach provides a secure way to preserve the long-term viability of crops while protecting threatened species (Priyanka *et al.*, 2021). Likewise, organizations like Botanic Gardens Conservation International (BGCI) have played a crucial role in the conservation of live plants through botanical gardens and in-field gene banks, which are particularly useful for protecting species that cannot be stored as seeds or perennial crops (BGCI, 2020) (Acuña *et al.*, 2019; Priyanka *et al.*, 2021; Salgotra & Chauhan, 2023). In Mexico, germplasm banks such as the International Maize and Wheat Improvement Center (CIMMYT) and the National Center for Genetic Resources (CNRG-INIFAP) stand out, with a primary mission of conserving agricultural biodiversity within the context of food security (Ortiz *et al.*, 2008; Vélez-Torres *et al.*, 2023). Similarly, many other conservation centers around the world (Table 1) are dedicated to the conservation of specific genetic resources. These institutions focus on the collection, storage, and preservation of agro-food genetic resources from thousands of crop varieties, ensuring their availability for future generations and their use in plant research and improvement.

However, despite international efforts and technological advances, the conservation of agricultural biodiversity faces significant challenges related to the integration of various stakeholders, particularly farmers and scientists. While both groups recognize the importance of biodiversity, their perceptions of ecosystem services and conservation measures differ considerably; farmers tend to focus on tangible and immediate benefits, such as pest control, whereas scientists emphasize the importance of less visible ecosystem services, such as air quality, water quality, and genetic diversity (Concepción *et al.*, 2020; Maas *et al.*, 2021; Williams *et al.*, 2021). This underscores the importance of improving communication channels between scientists, farmers, and policymakers, with the aim of overcoming this disconnect through the creation of dialogue platforms and educational programs that provide farmers with practical tools for the sustainable management of their lands (Concepción *et al.*, 2020; Maas *et al.*, 2021).

Table 1. Research institutes focused on the conservation and maintenance of genetic resources (Salgotra & Chauhan, 2023; Pathirana & Carimi, 2024).

Institute	Crop	Country
International Rice Research Institute (IRRI)	Rice	Philippines
Centre International de Mejoramiento de Maíz y Trigo (CIMMYT)	Maize and wheat (triticale, barley, sorghum)	Mexico
Center International de Agricultura Tropical (CIAT)	Cassava and beans (also maize and rice), in collaboration with CIMMYT and IRRI	Colombia
International Institute of Tropical Agriculture (IITA)	Grain legumes, roots and tubers, farming systems, cassava, banana, yam	Nigeria
Centre International de la Papa (CIP)	Potato, Andean root, and tubers	Peru
International Crops Research Institute for Semi-Arid Tropics (ICRISAT)	Sorghum, groundnut, pearl millet, Bengal gram, red gram	India
West African Rice Development Association (WARDA)	Regional cooperative rice research in collaboration with IITA and IRRI	Liberia
International Plant Genetic Research Institute (IPGRI)	Genetic conservation	Italy
National Bureau of Plant Genetic Resources	Fruits, tubers, medicinal and aromatic crops, spices, bulbous crops	India
The Asian Vegetable Research and Development Center (AVRDC)	Tomato, onion, peppers, Chinese cabbage	Taiwan
International Center for Tropical Agriculture (CIAT)	Cassava	Colombia
The New Zealand Institute for Plant and Food Research Limited	Kiwi fruit (*Actinidia* spp.)	New Zealand
National Center for Genetic Resources (CNRG)	Plant and animal genetic resources	Mexico

In conclusion, the conservation of agricultural biodiversity cannot rely solely on economic incentives or technological advancements; it requires effective collaboration among the various stakeholders involved. The FAO, CGIAR (Consortium of International Agricultural Research Centres), and other international institutions have established a solid foundation through global frameworks and ex situ and in situ strategies, but it is necessary to better integrate the perceptions and needs of farmers and improve communication between scientists and producers (Noriega *et al.*, 2019). Only through an inclusive and collaborative approach can the adoption of practices that promote agricultural sustainability and the conservation of genetic resources, essential for facing future agricultural challenges, be ensured.

CONCLUSION

Agricultural biodiversity is essential for food security in a world facing unprecedented climatic and environmental challenges. The loss of this biodiversity jeopardizes the ability to produce food sustainably and adapt to a changing environment. Preserving agrobiodiversity not only ensures greater stability in food production but also strengthens rural economies, protects ecosystems, and promotes social equity. For this reason, solutions for its conservation must be comprehensive, involving farmers, governments, national and international institutions, and society as a whole to ensure food security for future generations and build a more resilient and sustainable food system.

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Colegio de Postgraduados

Adaptation of calves in the transition from intensive to dual-purpose system during extreme drought

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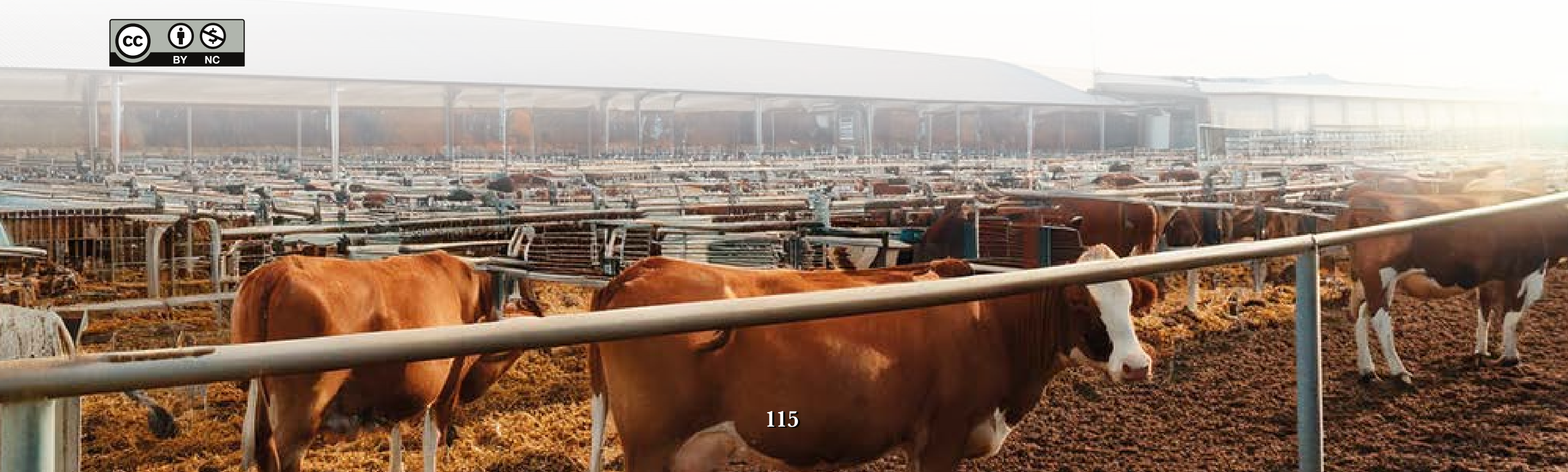
ABSTRACT

Objective: Evaluate the adaptation of calves in the transition from intensive to dual-purpose system during extreme drought.
Design/methodology/approach: The study was carried out in Tamaulipas, Mexico with calves in transition from an Intensive Production System (IPS) in Ciudad Victoria to a Dual-Purpose System (DPS) located in Altamira. Two groups of Swiss×Gyr×Holstein cross calves were analyzed, control group (CG; n=8) born in DPS, and the adapted group coming from IPS (AG; n=6), zoometric measurements were recorded: height at the withers, length of the animal, chest circumference, pelvis length, pelvis width, height at the rump, body condition and weight, the blood concentration of b-hydroxybutyrates (BHB) and glucose were measured. An ANDEVA was carried out with a factorial design and a correlation analysis.
Results: There were differences between AG and CG ($P<0.01$), as well as in the date factor ($P<0.01$). Blood concentrations of BHB and glucose had no difference between groups ($P<0.05$). There was a negative correlation ($R=-0.47$; $P<0.05$) between BHB and body condition.
Limitations on study/implications: Although the dual-purpose system is very versatile, in the study area only 33% of ranchers manage it, due to the accentuation of drought and climate change.
Findings/conclusions: The calves showed adequate development with good adaptation in the transition from IPS to DPS, although there was an effect of the factors, the calves responded favorably to management.

Keywords: cattle, growth, zoometric measurements, betahydroxybutyrates.



INTRODUCTION

In cattle production units, an efficient system of raising calves for breeding is of great importance, which has a direct impact on future income and sustainability (Archbold *et al.*, 2012). The dual-purpose cattle system is characterized by having production units whose purpose is to produce milk and meat (Cuevas-Reyes y Rosales-Nieto, 2018). This production system is carried out by more than 60% of cattle farmers nationwide in Mexico due to its versatility (Noyola *et al.*, 2011). In addition, milk and meat production is not sufficient to meet the population's demand (Martínez-Alba *et al.*, 2021). The calves in these systems represent the future of cattle farms (González-Avalos *et al.*, 2017). In this regard, zoometry allows us to identify productive trends or zootechnical deficiencies, sexual dimorphism and morphometric comparison between breeds (Pardo-Bustamante, 2018). In bovine production systems it is important to look for alternatives, which makes the adaptation process of cattle from one system to another easier, for this reason, it is equally important to analyze the results obtained in body measurements during the adaptation process, for example, observing details such as the presence and maintenance of trees, which reduce high temperatures (Murgueitio *et al.*, 2014). The physiological and behavioral processes adopted by domestic animals in response to environmental changes are crucial for their survival (Neethirajan, 2020). The adaptation of calves from one production system to another is a process during which the animal is subjected to multiple physical and psychological stress factors, which impacts health and productive behavior (Larios-Cueto *et al.*, 2019). On the other hand, the stress level of an animal can be measured by parameters, using current technology sensors, such as: chemical, metabolic, physical and immunological compounds related to physiological adaptation in domestic animals (Neethirajan, 2020), for example, behavioural indicators have been used to assess the effect of stress caused by transporting livestock, pathological and physiological, in research on transport from the cattle finishing corral to the slaughterhouse (Werner *et al.*, 2013). However, the effects of drought stress can affect calf development, which has been little evaluated in dual-purpose systems. There are several factors that can cause stress to livestock in addition to transport, such as drought, high temperatures and lack of forage in the grasslands, some of the main indicators of stress in animals are changes in live weight, as well as the concentrations of glucose and free fatty acids (Larios-Cueto *et al.*, 2019). In this regard, ranchers must have several strategies to survive extreme drought, one of them is that you can sell a percentage of animals to cover the costs of supplementation and prevent high mortality rates (Peña-Cardozo *et al.*, 2023), another is the search for animal breeds more resistant to drought and heat (Chuncho *et al.*, 2013). Thus, the effects of stress caused by drought can affect the development of calves and have been little evaluated in cattle undergoing the transition from an intensive system (semi-dry climate) to dual-purpose (subhumid climate). Therefore the hypothesis of the present study was that the development based on zoometric measurements and blood concentrations of betahydroxybutyrates and glucose would be similar in the control group and the adaptation group. The objective was to evaluate the adaptation of calves in the transition from intensive to dual-purpose systems during extreme drought.

MATERIALS AND METHODS

Experimental design

The study was developed in the state of Tamaulipas during the period from April to September 2022, The growth of two groups of calves was monitored: 1) control group (CG) which were born in the Location 1) "Granja El Paraíso" in the municipality of Altamira and 2) adaptation group (AG) that at the beginning of the experiment were moved from the Location 2) Farm located in the municipality of Ciudad Victoria, to Location 1. The dates coincide with the period in which the region's dry period occurs and this year this drought was more pronounced (NADC, 2022), so the temperatures were high and the grasslands conditions were dry and with little forage.

Location and characteristics of the place

Location 1, which was the target production unit for both groups (CG and AG), it is located in the municipality of Altamira, at the coordinates 22° 45' northern latitude and 98° 05' west latitude, with an altitude of 164 meters above sea level. Its temperature range is from 22 to 26 °C and its annual rainfall is 900 to 1100 mm. It has a warm subhumid climate with summer rains and medium humidity, and its physiography is divided into Coastal Plain from the Northern Gulf of Mexico and Body of Water (INEGI, 2009a).

Location 2, these are where the calves in the AG come from, which is located between the coordinates 24° 00' and 23° 24' northern latitude and 99° 26' and 98° 57' west latitude, with an altitude of 200 y 2200 meters above sea level, with a temperature range of 16 to 24 °C and its annual rainfall is 400 to 1100 mm, whose climate is semi-warm subhumid with rains in summer, with less humidity, and its physiography is divided between the Sierra Madre Oriental and Coastal Plain from the Northern Gulf of Mexico (INEGI, 2009b).

Characteristics of production systems

In Location 1 there is the farm "El Paraíso", a cattle production unit with a Dual-Purpose System (DPS), which has manual milking, in a tandem milking parlor for two cows, drinking troughs supplied with water from prey, diet based on grazing on native and improved pastures, with supplementation of commercial concentrates during milking, ande calves stay with their mothers during milking.

The characteristics of Location 2 are as follows: It is an Intensive Production System (IPS) of cattle with a semi-automatic parallel milking system with four cows, drinking fountains with drinking water available, balanced diets served in a mixer-grinder trailer and artificial calf breeding.

Experimental units

Calves were of the breed Swiss×Gyr×Holstein in the stage of development and body growth, they were organized into two groups: The first group born in the destination DPS of the experiment was the CG (n=9), with aged 8.1 months, an average weight of 92.4 kg and body condition (BC) of 2.3, was measured on a scale of 1 to 5, where 1 is a thin animal and 5 a fat animal (Morales *et al.*, 2012). The second group AG (n=4), with aged 4.5 months, an average weight of 101.7 kg and BC of 2.9.

Management and preventive medicine

The diet of both groups consisted of supplementation with 0.3 kg of commercial starter in calves concentrate (17.5% of Crude Protein) per animal per day; sorghum silage, ground sorghum forage, and minerals *ad libitum*. As a preventive medicine, the 8-way polyvalent vaccine was applied (Pasteurellosis and Clostridiasis). Internal deworming was performed with macrocyclic lactones (ivermectin) and benzimidazoles (fenbendazole), for external deworming and prevention of ectoparasites phenylpyrazolones (fipronil), and the organophosphate group (coumaphos).

Measurement of parameters and zoometry

Nine parameters were taken from the calves, which were: weight (W), calculated weight (CLW), body condition (BC), in addition to zoometric measurements: bovine length (BL), thoracic perimeter (TP), height at dorsal withers (HDW), pelvic length (PL), pelvic width (PW) and height rump (HR). These were measured in cm using a flexible tape measure. Likewise, the W was recorded in kg, using a mechanical livestock scale (capacity of 2,000 kg), CLW was obtained based on the formula TP×TP×BL/10838. The variables were evaluated biweekly. Regarding daily weight gain (DWG), it was obtained by the following formula:

$$DWG = \left(Inicial\ weight - final\ weight\right) / 68\,days$$

Blood sampling

Blood concentration of β-hidroxibutirato (BHB) and glucose were measured monthly, by extracting venous blood from the jugular vein, with syringes for insulin administration. Blood concentrations of BHB and glucose were obtained from each animal two hours before feeding in the manger for both groups AG y CG (Goetz *et al.*, 2023). Blood samples to assess blood concentration of BHB in mmol L^{-1} and glucose in mg dL^{-1} were placed and made into strips (lancets) to obtain the readings of each metabolite electronically on a portable device (FreeStyle Optiurn Neo, Witney UK). All of the above was carried out with the necessary measures of controlled animal welfare (Deelen *et al.*, 2016).

Statistical analysis

The data was recorded and organized in a database. The homogeneity of variance of the data was confirmed and normality was confirmed with the test of Shapiro-Wilk. An ANDEVA with factorial design was performed for each variable, in which the dependent variables were W, CLW, BC, BL, TP, HDW, PL, PW and HR, for each of them there were two independent variables, which were the factors Date and Group. In the case of the Date, there were seven levels: 1) 17 may, 2) 24 may, 3) 05 june, 4) 14 june, 5) 28 june, 6) 08 july and 7) 19 july, and in the case of real weight logistics allowed only five levels: 1) 17 may, 2) 05 june, 3) 21 june, 4) 06 july and 5) 26 july. In the case of the factor Group, were two levels: 1) CG and 2) AG. The initial measurement of each dependent variable was included as a covariate and first-order interactions were considered. Regarding DWG, it was analyzed using the T-Student test, for analysis of blood concentrations of BHB an

Analysis of variance with factorial design was performed in which BHB were the dependent variable and the independent variables were the group factor with two levels 1) CG and 2) AG, and the measurement month factor with five levels: 1) May, 2) June, 3) July, 4) August, 5) September. First order interactions were considered. Regarding blood glucose concentrations, a variance analysis with a factorial design was performed in which the dependent variable was glucose and the factors were the group factor with two levels: 1) CG and 2) AG, and the month factor with three levels 1) May, 2) July and 3) September. First-order interactions were analyzed for this analysis. The correlation between BC and BHB was analyzed with the Pearson method. Statistical analyses were carried out in the statistical package Statgraphics Centurion edition XVI.

RESULTS

The AG dual-purpose calves adapted to the new production system and they had a favorable development to management, which was carried out as a preventive measure and continues to be monitored, zoometric measurements, W, DWG, blood concentrations of BHB and glucose were analyzed with the following results.

Zoometric measurements

For Factor A there were significant differences for W ($P=0.0070$), CG (99.9 kg) was higher than AG (96.2 kg), while for CLW ($P=0.0199$) CG (104.8 kg) was higher than AG (102.2 kg), in addition BL, PL and HDW were significant. For Factor B the W, CLW, LB, TP, HDW, PL, PW and HR were significant (Table 1). It is worth mentioning that for PW there were no differences in the group factor, but for the date factor ($P<0.05$), which may be influenced by the characteristics of the Swiss breed that predominates in the AG, there was no interaction effect between factors.

Daily weight gain

Regarding the DWG of the calves, there were significant differences between AG and CG ($P=0.0045$, Figure 1), considering a time of 68 days after the AG arrived at the farm receiving the animals, an DWG of 207.1 g was obtained in the CG and 18.4 g in the AG, which did not lose weight and managed to adapt to the new system.

Blood concentrations of betahydroxybutyrates (BHB)

Blood BHB concentrations were 0.23 mmol L^{-1} in AG and 0.30 mmol L^{-1} in CG in the month of May, 0.20 mmol L^{-1} in AG and 0.26 mmol L^{-1} in GC in the month of June, 0.33 mmol L^{-1} in AG and 0.40 mmol L^{-1} in CG in the month of July, 0.43 mmol L^{-1} in AG and 0.34 mmol L^{-1} in CG in the month of August, 0.28 mmol L^{-1} in AG and 0.29 mmol L^{-1} in CG in the month of September (Figure 2). The measurement month factor was significant ($P=0.0001$). Although, the group factor and the interaction were not significant ($P=0.2488$ and $P=0.1481$, respectively). However, there was a significant negative correlation ($R=-0.47$; $P<0.05$) between the variable blood concentration of BHB and body condition, which means, higher BHB concentration at lower body condition (Figure 3).

Table 1. Average zoometric measurements, weight and body condition of developing dual-purpose calves.

ANOVA	1°	2°	3°	4°	5°	6°	7°	8°	9°
Response variable	**W (kg)**	**CLW (kg)**	**BC (1-5)**	**LB (cm)**	**TP (cm)**	**HDW (cm)**	**PL (cm)**	**PW (cm)**	**HR (cm)**
CG	92.4	92.1	2.3	92.0	103.3	95.1	30.9	29.4	100.7
AG	101.7	99.8	2.9	95.0	104.5	95.0	30.7	25.5	102.7
CG	94.4	95.9	2.5	94.0	104.3	96.1	31.8	29.6	102.8
AG	101.7	101.9	2.8	95.5	105.5	96.7	31.0	25.7	104.2
CG	96.4	100.0	2.6	95.7	105.6	96.5	32.3	30.1	104.0
AG	104.0	107.6	2.9	97.0	107.7	97.0	31.5	26.7	105.2
CG	96.3	103.3	2.6	97.3	106.5	97.9	33.0	31.1	105.4
AG	101.2	109.2	2.8	98.2	108.0	97.7	32.2	28.0	106.7
CG	106.5	106.9	2.8	99.0	107.4	98.2	33.5	31.2	105.9
AG	103.0	110.8	2.9	98.2	109.0	97.7	32.2	28.5	107.2
CG		108.7	2.6	100.3	107.5	98.8	33.7	31.8	106.3
AG		111.6	2.4	98.2	109.3	98.5	32.5	29.5	107.2
CG		110.2	2.8	100.6	108.2	100.0	34.01	32.0	107.3
AG		112.3	2.2	98.5	109.5	98.5	32.5	30.2	107.7
Factor A Group	** 0.0070	* 0.0199	** 0.0004	** 0.0000	NS 0.2785	NS 0.5477	** 0.0004	NS 0.3869	** 0.0026
CG	99.9a	104.8a	2.7a	97.8a	106.5a	97.5a	32.4a	29.7a	105.3a
AG	96.2b	102.2b	2.4b	95.3b	107.0a	97.3a	31.5b	30.0a	104.3b
Factor B Date (D)	** 0.0033	** 0.0000	NS 0.1046	** 0.0000	** 0.0000	** 0.0000	** 0.0000	** 0.0000	** 0.0000
D1	95.4b	94.4d	2.5a	93.0d	103.7c	95.0c	30.8c	28.1d	101.2d
D2	96.4b	97.6cd	2.6a	94.2cd	104.7bc	96.5bc	31.4c	28.3cd	103.1c
D3	98.4ab	102.3bc	2.7a	95.7bc	106.5ab	96.7b	31.9bc	29.0bcd	104.2bc
D4	97.1b	104.7ab	2.6a	97.2ab	107.0ab	97.8ab	32.7ab	30.2abc	105.6ab
D5	103.1a	107.3ab	2.7a	98.1ab	108.0a	98.0ab	32.8ab	30.5ab	106.2a
D6		108.7.1a	2.4a	98.7a	108.3a	98.7a	33.1ab	31.2a	106.4a
D7		109.6a	2.4a	99.0a	108.6a	99.3a	33.2a	31.8a	107.1a
Average	98.1	103.5	2.5	96.6	106.7	97.4	32.3	29.9	104.8
CVRT	P In ** 0.0000	P Cal In ** 0.0000	CC In ** 0.0000	L In ** 0.0000	PT In ** 0.0000	AC In ** 0.0000	LP In ** 0.0000	AP In ** 0.0000	AG In ** 0.0000
Int AB	* 0.0183	NS 0.7580	** 0.0067	* 0.0159	NS 0.9957	NS 0.4589	NS 0.7634	NS 0.6163	NS 0.8839

CG: control group (n=9); AG: adaptation group (n=4), W: weight, CLW: calculated weight, BC: body condition, BL: bovine length, TP: thoracic perimeter, HDW: height at dorsal withers, PL: pelvic length, PW: pelvic width, HR: height rump; In: initial **: highly significant ($P<0.01$); *: significant ($P<0.05$); NS: not significant. Literal different denotes significant difference Tukey ($P<0.05$) for each factor per column; CVRT: Covariate of the ANOVA; Int: interaction.

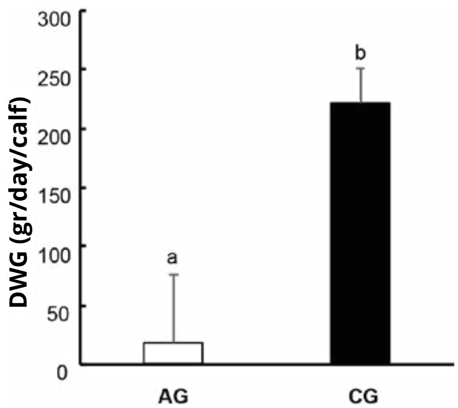


Figure 1. Daily weight gain (DWG) in developing calves of the adaptation group (AG □; n=4) from an intensive system to a dual-purpose system compared to those born in the herd as a control group (CG ■; n=9) during the months of greatest heat stress. Literals indicate significant difference between groups (P<0.05).

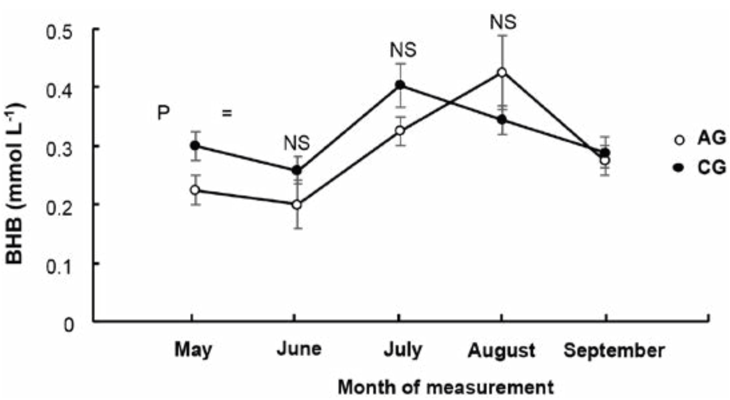


Figure 2. Mean ± SE of blood BHB concentrations over time in developing calves from the adaptation group (AG ○; n=4) from an intensive system to a dual-purpose system compared to those born in the herd as a control group (CG ●; n=9) during the months of greatest heat stress. NS: not significant difference.

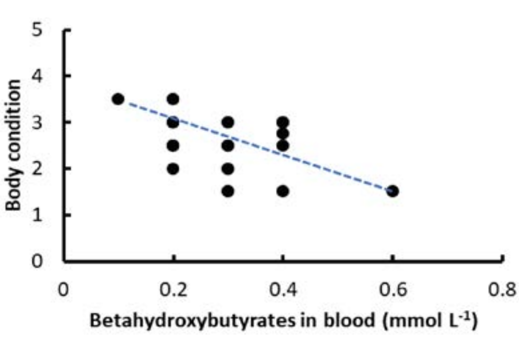


Figure 3. Correlation of body condition with blood concentration of β-hidroxibutirato in dual purpose calves (R=−0.47; P<0.05).

Blood glucose concentrations

Blood glucose concentrations were 49.0 mg dL^{-1} in AG and 58.7 mg dL^{-1} in CG in the month of May, 48.5 mg dL^{-1} in AG and 55.8 mg dL^{-1} in CG in the month of July, 46.3

mg dL^{-1} in AG and 52.3 mg dL^{-1} in CG in the month of August (Figure 4). The group factor was significant (P=0.0070). However, the factor month of measurement and the interaction were not significant (P=0.3866 y P=0.8537, respectively).

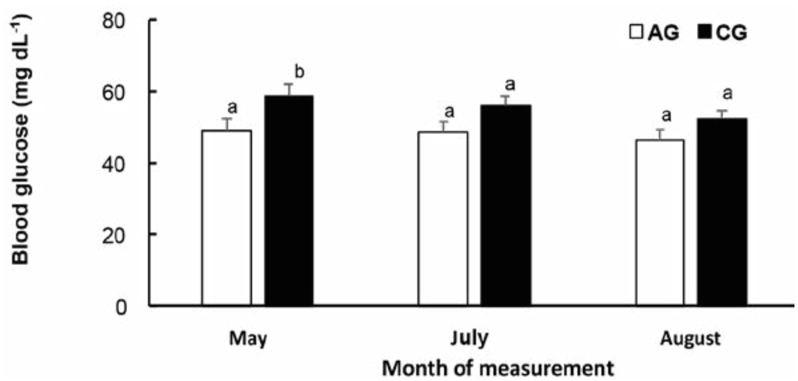


Figure 4. Mean ± SE of blood glucose concentrations over time in developing calves from the adaptation group (AG □; n=4) from an intensive system to a dual-purpose system compared to those born in the herd as a control group (CG ■; n=9) during the months of greatest heat stress. Different literals denote significant difference between groups of the study.

DISCUSSION

Favorable results were obtained in the development of calves from both CG and AG groups, because thanks to the supplementation and management that was given to them. They were able to get through the dry season in the best way, this is of great relevance because any farmer could use similar management and supplementation, since its costs are not high. In this regard, Larios-Cueto *et al.* (2019) mention that the response of animals to stress varies according to different factors, such as: transportation, grouping of unknown animals, new diet, management in the production unit, noise pollution, high population density, type of personnel and their way of caring for them, water conditions and forage quality, among others.

Zoometric measurements

Development based on zoometric measurements was continuous and adequate for the calves, with minimal supplementation of concentrate in addition to minerals *ad libitum* and adequate preventive management in both groups studied. Carrying out measurements on cattle allows monitoring them in order to establish selection processes that manage to maintain their breed condition, since zoometry is an important work element to define a population (Pineda, 2010). Salamanca *et al.* (2013) used 57 Casanare Creole bovine animals (49 cows and eight bulls) for which eight zoometric indices were calculated; the indices that presented significant statistical differences were compared with those of other breeds *Bos taurus* european, Creole and *Bos índicus* to establish some phenotypic differences with the Casanare Creole breed. Their results are similar to those of the present study because they found differences in their measurements in the pelvis and head, although it varies in the development and objectives of the study which compares the zoometric indices between bulls and cows for the purposes of rescue and conservation of the breed. In this regard, Salazar *et al.* (2010) confirmed that the thoracic perimeter is a good indicator of live weight in growing dual-purpose cattle, which can be used on farms that do not have a scale to estimate changes in the weight of animals, especially in animals that have been acquired from other states or climates, as was the case in the present study. Also other authors, Chavan *et al.* (2022) Based on the zoometric measurements obtained, they determined with different treatments (diets) in which the development of the Deoni heifers was superior. Therefore, it has been a useful measure since the beginning of technological advances and in management situations where the live weight of the animal cannot be determined, many other parameters can be measured to estimate it indirectly (Heinrichs *et al.*, 1992).

Daily weight gain

González-Avalos *et al.* (2017) worked with 120 lactating Holstein calves in Francisco I. Madero, Coahuila, Mexico. The calves were separated from their mother at birth and housed individually, fed with a milk replacer with 22% crude protein (CP), and weaned at 50 days of age, when they were already fed with a starter with 21.5% CP, following up for 65 days, in which they reported DWG of 415 to 614 g, results superior to those of the present study, which may be due to differences in the physiological stage in which lactation was included, as well as to the different management due to the intensive production system used in their study. Sandoval *et al.* (2005) studied 177 dual-purpose calves during their first three months of life in Veroes Yaracuy, Venezuela. Management was provided by separating the calves from their mother at birth and in collective housing, suckled by nurse cows, and grazed daily (star grass, *Cynodon plectostachyun*), supplemented with a daily sack of concentrated feed with 16% CP, with an average consumption per animal of 430 g daily. The authors report that the DWG was 190 to 460 g. De Menezes *et al.* (2015) experimented with 60 cows crossbred of ¼ Holstein and ¾ Zebu dual-purpose calves in Felixlandia, Brazil, with the aim of evaluating the effect of feeding strategies on the weaning weight of calves and milk production, consisting of restricted milk supply with corn and soybean concentrate supplementation with 26% CP and Brachiaria grass with six to 11% CP, starting the study at 90 days of age of the calves and evaluating the short milking period strategy of 180 days and normal of 270 days. Their results show DWG from 650 to 1120 g in the calf groups made up of seven males and five females each. Their results are higher than the DWG of this study, mainly due to the physiological stage measured during lactation, as well as the high CP diet, which increases the costs in this type of management.

Blood concentrations of betahydroxybutyrates (BHB)

The BHB levels and their relationship with energy balance have been studied in cattle since they increase as a result of catabolism and fat mobilization (Fenwick *et al.*, 2008). Calves depend on the mobilization of adipose tissue to cope with different environmental conditions, especially during drought, a season in which feed on pasture is scarce, so their dietary sources of energy can be compromised, which can lead to an increase in BHB, resulting in a negative energy balance (Goetz *et al.*, 2023). Deelen *et al.* (2016) conducted a study in Seaforth, Ontario, Canada, with twenty Holstein calves weaned at six and eight weeks of age, with the aim of determining blood BHB levels and their relationship with intake and rumen development, in addition to using the Precision Xtra test for BHB. They reported an association of BHB concentration with rumen volatile fatty acids, ruminal butyrate and with the live weight of the calves, with values ranging from 0 to 0.04 mmol L^{-1}, in addition to the fact that the test was effective and could be used to decide on sufficient intake of starter concentrate and ruminal development. The values found by the authors are similar to those of the present study although the age of the animals varies. This is surely due to the fact that in this case the calves did not have stress or negative energy balance. Goetz *et al*. (2023) worked in Seaforth, Ontario, Canada, with the aim of investigating the effects of transport duration and age at transport on blood parameters in 175 calves after 6, 12 and 16 h of road transport, BHB were higher after transport in calves transported for 12 h of 0.09 mmol L^{-1} BHB, and 16 h of 0.15 mmol L^{-1} BHB compared to calves transported for six hours of 0.05 mmol L^{-1} BHB, in addition, calves transported at older ages (seven to 14 days) had a higher concentration of BHB compared to calves aged two to six days, due to the greater amount of fat available in their body. Their results are greater than those in the present study, probably due to the stress and negative energy balance that the animals face when being transported, in addition to the fact that during this period they generally do not consume food, so it is inferred based on the results that the calves in the present study did not have this kind of stress.

Blood glucose concentrations

Avalos-Rosario *et al*. (2023) worked with the objective of identifying associations in body condition, glucose, BHB, and white blood cells in dual-purpose cows during the transition period in the tropics of Veracruz. They studied 30 multiparous cows, crossbred with *Bos taurus*×*Bos indicus*, which were grazed in an intensive rotation system, with grass, Brizanta (*Brachiaria brizantha*) and African Star (*Cynodon plectostachyus*), and orange pulp silage 10 kg/cow daily. The authors report an average blood glucose of 39.4 mg dL^{-1} in prepartum cows and 37.6 mg dL^{-1} in postpartum cows, in addition to a negative correlation between glucose and BHB levels, the lower the glucose concentration, the higher the BHB concentration ($r=0.51$). The authors conclude that low producing dual-purpose cows experienced fluctuations in body condition, BHB, and immune cell populations during the transition period, suggesting metabolic and immune changes similar to those in high producing dairy cows. The values are slightly lower than those of the present study, probably because adult cows were used, although the behavior of glucose in relation to BHB was negatively correlated, showing that the metabolism of both functioned in a similar way.

CONCLUSIONS

The adaptation of calves when moving between different production systems is of vital importance. The adaptation group of calves had lower body condition, weight and calculated weight; however, their development was adequate and they achieved growth because they were given good preventive medicine management and supplementation, allowing them to develop well during the dry season, reducing the mortality rate during that period. There were no differences in blood concentrations of betahydroxybutyrates and glucose in the groups of developing calves during the transition period from an intensive system to a dual-purpose system during the drought, indicating that they had good adaptation.

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Colegio de Postgraduados

Manures base on *Oreochromis niloticus* L. for agricultural use

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ABSTRACT

Objective: *Oreochromis niloticus* (Tilapia) meal was evaluated as a source of nutrients in agriculture, through a composting process mixed with a zero tillage soil.

Design/methodology/approach: Two manures were formulated, mixing flour and soil at a 30:70 ratio (mass/mass w:w) respectively. Compost A was maintained at 60 days of composting (ddc), compost B at 30 days. The phytotoxicity of the manures was evaluated as percentage of germination in *Solanum lycopersicum* (tomato) seeds.

Results: The N-P-K ratio (% dry mass) was 1.90-2.06-0.30 for manure A (60 ddc), and 2.60-2.24-0.34 for B (30 ddc). On germination, 100% concentration; manure A had 44.9% and B 19.6%, compared to the control. For concentration of 10% it was 93.7 and 111.4%, manure A and B, respectively.

Limitations on study/implications: It is necessary to carry out more phytotoxicity tests. In this experiment, dilutions were made in the manures to increase germination.

Findings/conclusions: The use of manure based on tilapia meal in agriculture is proposed in the future and in this way take advantage of the waste from fish processing.

Keywords: Fish meal, compost, tomato, soil improver

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INTRODUCTION

The fishing industry generates by-products, which will eventually become waste (Caruso *et al.*, 2016). Approximately 60% of them, are constituted by the skeleton, bones, viscera, scales, in recent research it has been proposed to use fishmeal in agriculture and benefits have been obtained in crop yield, nutritional improvement, resistance to salinity, improvement in soil properties, among others (Radziemska *et al.*, 2019; Yusel *et al.*, 2017). As another alternative in addition to its use, it is included in balanced feed. Given the current needs of sustainability, it is pertinent to conduct experiments where the use of fishmeal as organic fertilizer is introduced in crops of comercial and nutritional interest (Ruíz *et al.*, 2018), since these represent an alternative to the dependence on fertilizers of industrial origin in agriculture. The objective of the present research work was to elaborate

two fertilizers based on *Oreochromis niloticus* (tilapia), fishmeal and evaluate their effects on the development and phytotoxicity on *Solanum lycopersicum* L. (tomato) seed, for their potential agricultural use.

MATERIALS AND MÉTHODS

Soil and flour characterization

A no-till soil, without stubble, from the facilities of the Instituto Tecnologico de Tuxtla Gutiérrez (ITTG) (L. N. 16.756; L. W. 93.172) was used *O. niloticus* meal was used. The physicochemical and macronutrient parameters evaluated for the meal and soil, as well as the methods are shown in (Table 1), where the results corresponding to the physicochemical characterization of the soil, meal and fertilizers evaluated in the present work are illutrated. The macronutrient content of *O. niloticus* meal in the present study was similar to that reported for species of commercial interest such as *Salmo salar* (salmon) and *Clupea arengus* (Herring) (Ahuja *et al.*, 2020).

Preparation and characterization of the fertilizers

Two fertilizers were formulated, mixing flour and soil at a 30: 70 ratio (mass/mass w:w) respectively. Compost A was kept at 60 days of composting (ddc) and compost B at 30 (ddc), keeping the mixtures at 60% relative humidity and turning three times a week, temperature and pH were also monitored. The physicochemical parameters determined in the compost and their methods are shown in (Table 1).

Table 1. Physicochemical characteristics: soil flour and fertilizers.

Method	Parameter	Soil	Flour	Fertilizer A	Fertilizer B	Unit
Gravimetric	Humidity	11.4±0.8	8.5±0.7	16±0.8	12±0.4	%
	Cap. de Retention of water (CRA)	69.9±1.16	113.5±5.1	72.5±7.2	88.9±8.3	
Potenciometer	pH	7.9±0.05	5.8±0.02	8.1±0.06	7.9±0.04	(dS/m)
	Electrical conductivity	0.74±0.01	4.2±0.06	3.00±0.01	3.10±0.04	
Combustion	Organic carbon	19.1±0.64	25.3±1.8	8.36±0.58	9.89±0.60	g/kg
Kjeldahl	Total nitrogen	1.8±0.01	80.4±1.5	1.90±0.59	2.60±0.69	
X-ray scattering Spectrometry	Phosphorus	0.02±0.16	2.59±0.21	2.06±0.05	2.24±0.04	% dry weight
	Potassium	0.67±0.03	0.52±0.05	0.30±0.11	0.34±0.09	
	Sodium	0.17±0.02	0.29±0.03	0.16±0.09	0.19±0.05	
	Magnesium	1.62±0.04	0.16±0.05	0.91±0.09	0.94±0.03	
	Calcium	8.29±0.30	4.43±0.38	9.38±0.03	9.39±0.09	
Kononova & Belchikova/ Royal Decree 1110-1990	Humic acids	Not applied	Not applied	2.37	4.39	%/100 g dry weight
	Fuk Fulvic acids	Not applied	Not applied	2.96	4.03	
Ratio basis dry	C/N ratio	10.6	0.31	4.40	3.81	no unit

Fertilizers phytotoxicity

A stock solution was prepared for both fertilizers at a 1:3 (w:v) fertilizer/distilled water ratio. It was mixed vigorously and allowed to stand for 24 h. two concentrations were applied, at 100 and 10%, 5 ml was applied from each treatment to Petri dishes, where 100 tomato seeds were placed on two layers of filter paper to provide moisture (Selim *et al.*, 2012). Seeds of *S. lycopersicum* var. Río Grande, Crown Seeds brandTM, lot 12154-4709 were used. Each treatment and a control (distilled water) were evaluated in triplicate and the toxicity of the fertilizers was determined by the percentage of germination six days after sowing.

With the following equation:

$$\%\ de\ relative\ germination = \frac{No.\ of\ germinated\ seeds\ in\ compost\ extract}{No.\ of\ germinated\ seeds\ in\ the\ control} \times 100$$

A simple ANOVA was applied and a multiple range test (Least significant difference: LSD. $P \leq 0.05$) was performed to determine statical significance, using Statgraphics Centurion XXVI.I software.

RESULTS AND DISCUSSION

In (Figures 1 and 2) the behavior of temperature and pH respectively, of the composting process for the composts are illustrated. Temperature higher than 55 °C were recorded for both composts, this is an indicator of microbial activity when using *O. niloticus* meal as substrate (Radziemska *et al.*, 2019). Acidification of the medium was observed between days 7-15 this is generally due to the presence of short-chain acids such as acetic and lactic. Foul odors were perceived during the composting process, this is an effect of the emission of volatile organic compounds, such as hydrogen sulfide (H_2S) and ammonium (NH_4) (Lin *et al.*, 2021) (Figures 3 y 4) illustrate the color changes in the compost.

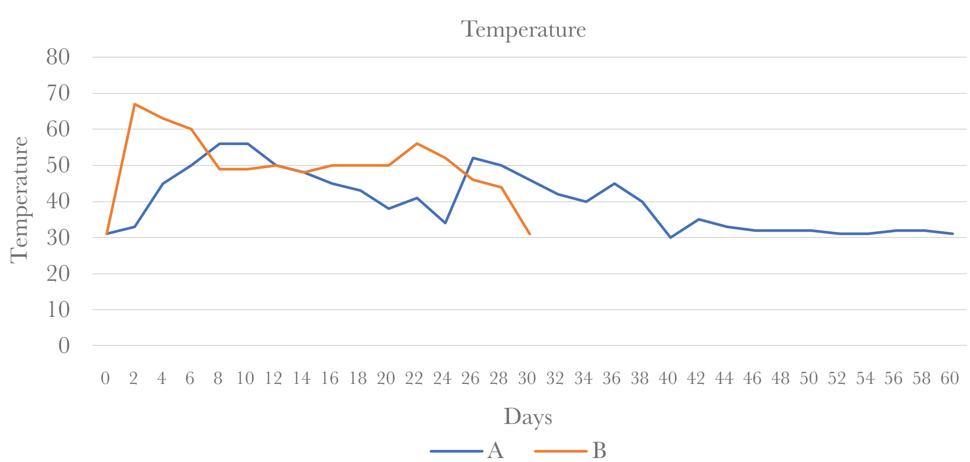

Figure 1. Behavior of temperature during composting.

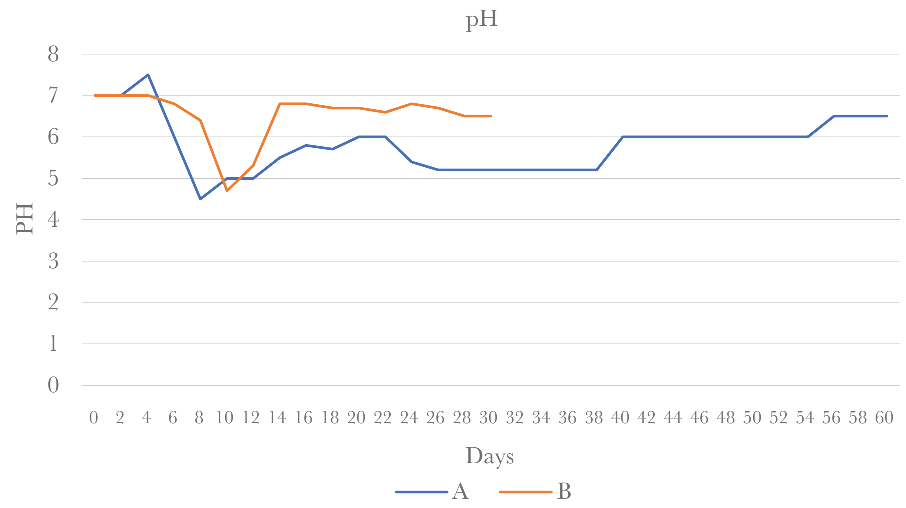

Figure 2. Behavior of pH during composting.

Figura 3. Changes in fertilizer A day 0 to day 60.

Figure 4. Changes in fertilizer B day 0 to day 30.

(Table 1) shows the results corresponding to the physicochemical characterization of the soil, meal and fertilizers evaluated in this study.

The N-P-K ratio (% dry mass) was 1.90-2.06-0.30 Fertilizer A, and 2.60-2.24-0.34 for B. According to (Palaniveloo, 2020), some expected characteristics in stable manures are as follows: 5.5-9.0 (pH), 50-60%. (humedad), 43-65 °C (temperatura) and 25:1-30:1 (C/N ratio), very similar to those obtained by vermicomposting (NMX-FF-109-SCFI-2007), these parameters may vary due to the nature of the materials used in its formulation. (Figure 5) shows photographs of the phytotoxicity test.

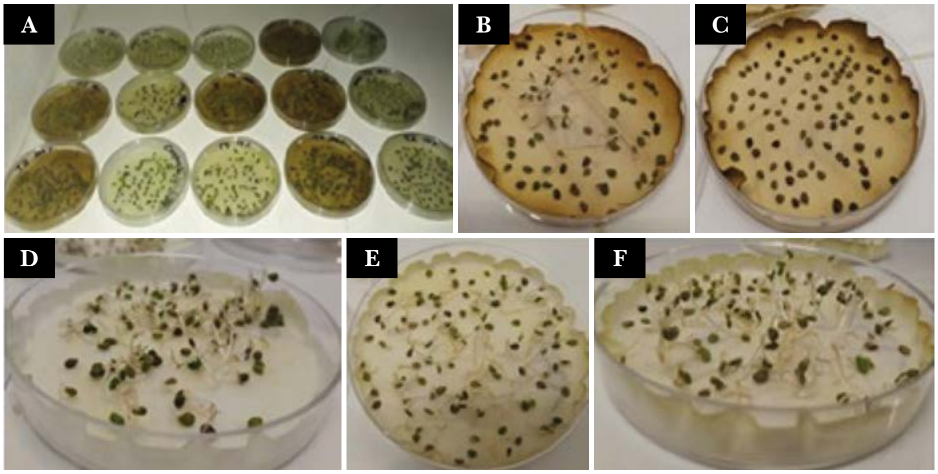

Figura 5. Phytotoxicity test of fertilizers based on O. niloticus meal. A) Treatments. B) Fertilizer A 100%. C) Fertilizer B 100%. D) Control. E) Fertilizae A 10%. F) Fertilizer B 10%.

Phytotoxicity symptoms were greater al 100% concentrations of fertilizer A and B, although there was germination, it was significantly lower than the 10% concentrations and the control. (Table 2 and 3) illustrate the ANOVA results and the effects of fertilizers on tomate seed germination, respectively.

Compost A (100%) exceded the germination percentage of compost B (100%), due to the short composting periodo of the latter. The presence of phenolic compounds, ethylene and ammonium, excess accumulation of salts and organic acids is common in young compost and these substances can delay seed germination and even plant growth (Selim *et al*., 2012). This effect was not the same when decreasing the concentration in Fertilizer B (10%), which presented significant statistical difference compared to fertilizers A and B at 100%, and even surpassing the control in relative germination by 111.4%. The phytotoxicity of

Table 2. ANOVA on the germination *Solanum lycopersicum* seeds.

Source	Sum of squares	Mean square	F-Ratio	P-Value
Entre grupos	5211.60	1303.90	4.09	0.0321
Intra grupos	3185.33	318.53		

Table 3. Effect of fertilizers on germination of *Solanum lycopersicum* seeds.

Fertilizers	Germinated seeds*	Relative germination
Fertilizer A (100%)	23.66±7.50 bc	44.9 %
Fertilizer B (100%)	10.33±9.60 c	19.6 %
Fertilizer A (10%)	49.33±26.08 ab	93.7 %
Fertilizer B (10%)	58.66±20.13 a	111.4 %
Control	52.66±18.92 ab	100 %

* Mean of germinated seeds followed by their standard desviation are indicated. Means with different groups of letters in the same column indicate significant differences, significant difference test (LSD) ($P \leq 0.05$).

the fertilizers according to % germination is reported as follows: <50% highly toxic; 50-80% moderately toxic; >80% shows no toxicity (Muñoz *et al.*, 2015).

CONCLUSIONS

Stability is an important quality parameter in composts, and is referred to the absence of plant or animal pathogens, as well as phytotoxic compounds. Stability is associated with the microbial activity in the compost and the composting time of the compost. The composts were categorized as moderately toxic (compost a 60 ddc) and highly toxic (Compost B 30 ddc) at 100% concentration, but with no phytotoxic effects at 10 % concentrations.

Fertilizer B showed statiscally significant difference over Fertilizer A and B at 100%, and even surpassed the control in germination percentage.

These results indicate that fertilizers based on *O. niloticus* meal can be elaborated and be a prospect for its application in the cultivation of *S. lycopersicum*, and in agriculture in general, at the same time reducing and taking advantage of the residues of the fishing industry.

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Colegio de Postgraduados

Strategy to improve the micropropagation of sugarcane plants using carbon dioxide injection

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ABSTRACT

Objective: To develop a strategy for the improvement of micropropagation of sugarcane plants in the central region of Veracruz, using carbon dioxide injection.

Design/methodology/approach: Intelligent control and micropropagation techniques are used in this development.

Results: Improved micropropagation of sugarcane plants using carbon dioxide injection is demonstrated using intelligent control techniques.

Findings/conclusions: With this controlled carbon dioxide injection system, research can be carried out to obtain the most approximate values to contribute to better growth and adaptability in sugarcane crops. This can guarantee shorter adaptation times and thus generate greater economic gains for the producers.

Keywords: Carbon dioxide, micropropagation, intelligent systems, intelligent control, temporary immersion systems.

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INTRODUCTION

The sugarcane farmland in Mexico presents average yields of 70 tons per hectare (t/ha), with the Central Region of the country being the most productive, with a mean of 117 t/ha (SAGARPA, 2017). The crop is cultivated in 14 states of the Republic, among which Veracruz stands out as the main producer with more than 287,000 ha (Rangel-Estrada S. Eloísa *et al.*, 2016).

Micropropagation of sugarcane plants in the central region of Veracruz is a vital process for the local agricultural industry, given the economic importance of this crop in the zone. Micropropagation or *in vitro* cloning of sugarcane is an important tool for

massive propagation of vigorous plants that are free of pests and diseases (Jose Humberto Caamal Velázquez *et al.*, 2014); however, an integral strategy is required to improve this process in order to ensure the efficiency and quality of the plants produced. Several key strategies have been identified, such as careful selection of genotypes, optimization of culture mediums, rigorous environmental control, and implementation of advanced technologies. In addition, the importance of staff training and collaboration with research institutions is emphasized. These strategies, when applied jointly, have the potential of significantly increasing the quality and the efficiency of micropropagation of sugarcane plants in the region, which would benefit both the producers and the sugar production industry in general.

In the study conducted by Bello-Bello (2022), they use silver nanoparticles for micropropagation (NPsAG) as an alternative to improve the asexual propagation of plants and, with this, to prevent the contamination of explants, the asepsis of the culture medium, and the accumulation of ethylene. In laboratory studies, it has been shown that NPsAG at low concentrations have a dose-response effect on the plant development, known as hormesis. With this study, an alternative is generated for other applications using plant tissue culture (PTC) techniques.

In the study by Joseph Campos Ruiz (2020), *in vitro* culture is presented as an alternative to obtain plants massively in the short term and of high quality. With the aim of providing a mechanism that supplies plants for the establishment of crops, the micropropagation of three sugarcane crops was evaluated (Isidro Elías, 2020). The study conducted by Fabián Contreras-Loera (2021) had the objective of developing an efficient system for the micropropagation of caper bush (*Capparis spinosa* L.), woody shrub of great interest for its products, notable resistance to drought, and tolerance to high temperatures. The research carried out by Reyna Rojas-García (2021) had the objective of establishing a protocol for the in vitro propagation of oregano. The establishment was achieved with nodal segments in MS basal medium; in this stage 57.32% of axenic explants were recovered.

Figure 1 shows the 5 phases of the micropropagation stage; this case study is based on phase (d), where the injection of carbon dioxide is conducted, which is essential for the healthy growth and development of plants (Bello-Bello, 2022). The adequate supply of

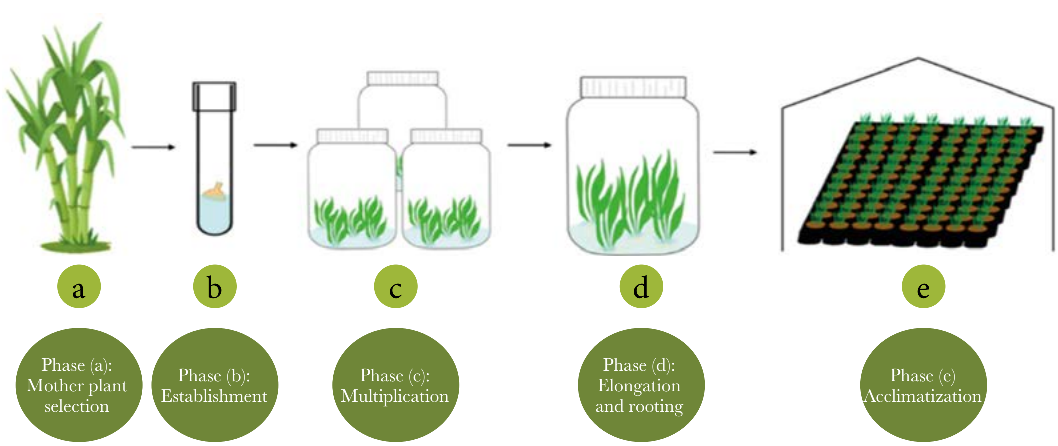

Figure 1. Stages of micropropagation applied in sugarcane crops (Bello-Bello, 2022).

CO_2 allows for plants to grow and develop optimally. A greater availability of CO_2 can increase the photosynthesis rate, which results in a faster growth and increased biomass production. The plants that have access to adequate levels of CO_2 tend to be more resistant to environmental stress, such as drought, high temperatures, or air pollution. This is because a higher photosynthesis rate provides the plant with more resources to combat stress and recover quickly.

Carbon dioxide is essential for the healthy growth and development of plants, since it is a key component in the photosynthesis process. It provides the raw material necessary for the production of carbohydrates, which are the plant's source of energy, and contributes to optimal growth, efficiency in water use, and to the plant's resistance to stress. The study conducted by Hiroki Gonome (2022) suggests an adaptive method to measure the plant's growth in real time by measuring the CO_2 consumption, which is resistant to environmental fluctuations. The study conducted by Xiadong Fan (2020), where the experimentation crop was peppers, examined the effects of a high CO_2 concentration on the growth of plants. The CO_2 provides the raw material for the photosynthesis process, improving the growth and productivity of the plants as consequence (Hesham A. Ahmed, 2020).

In the study performed by Santos (2011), the author mentions that for many intelligent control systems, the design methodology of the controller is essentially heuristic and based on certain principles of Artificial Intelligence or operative research. Among the definitions of Expert Systems, we find the following (Ana Lucía Sandoval Pillajo, 2021): One of the branches of AI is Knowledge-Based Systems (KBS), also called Expert Systems (ES). Within Artificial Intelligence, there are techniques of deep learning, particularly convolutional neural networks (CNN) (Juan C. Olguín-Rojas, 2022).

Advances in the fields of Automatic Control, Artificial Intelligence, Electronics, sensors, signal processing, actors, etc., provide new tools for complex system control (Santos, 2011). The system proposed by Wang (2022) describes in general the directions for the future enrichening in CO_2 for greenhouse production. The lack of CO_2 would not only result in a lower biomass, but rather the plants would also be of lower quality and resistance (Chowdhur, 2021).

MATERIALS AND METODOS

The study was conducted during the months of January to April, 2024, together with 3 institutions: Universidad Tecnológica del Centro de Veracruz (UTCV), Instituto Tecnológico Nacional de México campus Orizaba (ITO), and Colegio de Posgraduados campus Córdoba (COLPOS).

Materials and equipment

For the research project, the following materials and equipment were used:

- MIXER: Plastic container with capacity of 10 liters and maximum pressure of 10psi.
- SENSOR: The sensor used is a carbon dioxide sensor SN-300-LEDFL-CO2-N01-5000P, with measurement ranges from 0 ppm to 5000 ppm, with 24VDC feed and RS485 Modbus RTU exit.

- MICROCONTROLLER: The microcontroller used was an Aduino Mega card.
- ELECTROVALVES: 2-way 12VDC feed.
- OXYGEN TANK: The tank used has a capacity of 680 liters, with a regulator.
- CARBON DIOXIDE TANK: The tank used has a capacity of 680 liters, regulated at a pressure of 10 psi.
- HMI: The HMI used was brand KINCO GL070E, 7" in color, with connection to ethernet.

For the development of the system, a plastic recipient was used (mixer) with capacity of 10 L and pressure of 10 psi, where the carbon dioxide mixture was made. In this mixer, a SN-300-LEDFL-CO2-N01-5000P carbon dioxide sensor was adapted on the top part with measurement ranges of 0 ppm to 5000 ppm with 24VDC feed and RS485 Modbus RTU exit, which was connected directly to our microcontroller (Arduino Mega) in the TX and RX pines. With the processing of the signals in our microcontroller, the carbon dioxide injection was performed according to the parameters established initially; this injection was done through a 2-way 12VDC Electrovalves. An oxygen tank of 680 liters with regulator was used, as well as a carbon dioxide tank with capacity of 680 liters regulated at a pressure of 10 psi; the mixture to perform the injection was generated with these 2 tanks. Finally, an HMI of the brand KINCO GL070E of 7" in color was used, with connection to Ethernet, and this system was programmed with the KINCODTools software, where the RS485 communication was conducted.

Operation and functioning

Figure 2 shows the functioning diagram which is divided into 3 stages.

- Control stage: In the control stage, an HMI is used to establish the carbon dioxide values that the users with to have in each mixer. A microcontroller was used to perform the control and the electrovalves to regulate the flow. Compressed air is used for the mixture.
- Stage of mixture and sampling: In this stage, using the carbon dioxide sensors to measure the parts per million in each mixer, in case the desired parts per million are surpassed, oxygen is injected to decrease them.
- Stage of carbon dioxide injection: In this stage, the injection of carbon dioxide is performed, which is controlled by the electrovalves.

RESULTS AND DISCUSSION

Table 1 shows the results obtained after some time, attaining a total of 24 samples.

The correlation dispersion is proven in Trial 1, presented in Figure 3, where the dependent variable of CO_2 ppm monitored by the sensor is on the ordinate axis, while the seconds elapsed are on the abscissa axis, this being the independent variable.

With the data obtained that are presented in Table 1, calculations to obtain the linear regression equation were carried out.

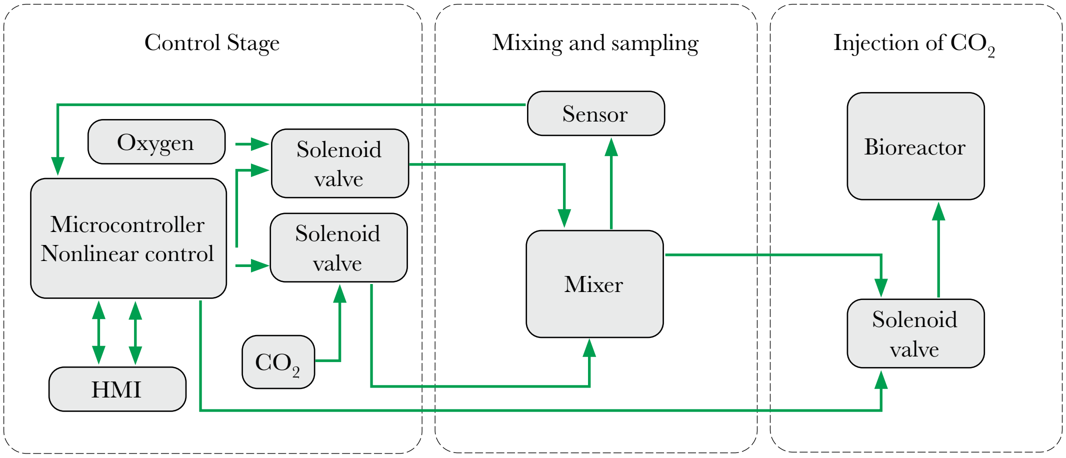

Figure 2. Functioning diagram of the carbon dioxide injection system.
Source: Prepared by the authors.

Table 1. SN-300-LEDFL-CO2-N01-5000P sensor performance data at P-1.

Parts per million (ppm) CO_2	Time (s)	ppm*s	s^2
23	33	2398	11881
22	109	2730	16900
21	130	3020	22801
20	151	3610	36100
19	190	4050	50625
18	225	4386	66564
17	258	4640	84100
16	290	5010	111556
15	334	5306	143641
14	379	5161	157609
13	397	5016	174724
12	418	4972	204304
11	452	4910	241081
10	491	4698	272484
9	522	4488	314721
8	561	4333	383161
7	619	4152	478864
6	692	3700	547600
5	740	3256	662596
4	814	2580	739600
3	860	1820	828100
2	910	958	917764
1	958	958	1006009
0	1003	0	1006009

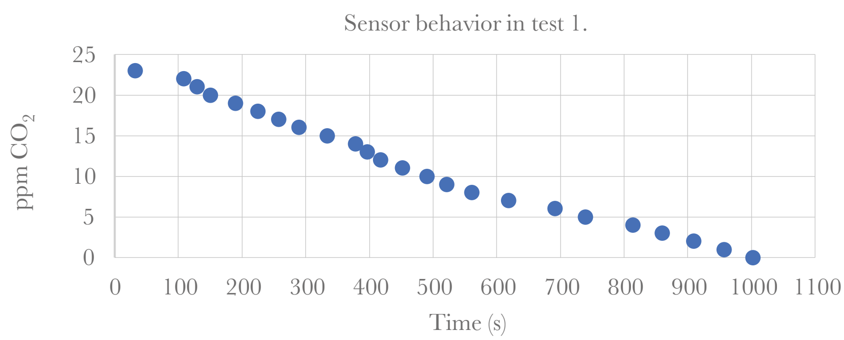

Figure 3. Dispersion of variables in Trial 1.

The simple linear regression is given by a straight line equation:

$$y = mx + b \tag{1}$$

The linear regression equation can be expressed as:

$$y = bx + a \tag{2}$$

Where: x=Seconds (s); y=Parts per million (ppm); n=Number of samples.

$$a = \frac{n\left(\sum xy\right) - \left(\sum x \sum y\right)}{n\sum X^2 - \left(\sum X\right)^2} \tag{3}$$

$$b = \frac{\sum x - a\left(\sum x\right)}{n} \tag{4}$$

Substituting, there is:

$$a = \frac{24(85953) - (11536 * 276)}{24(7473874) - (11536)^2} \tag{5}$$

$$b = \frac{276 - (-0.02421635091 * 11536)}{24} = 23.14 \tag{6}$$

Once the a and b values are available, they are substituted in equation 2, which results in the final correlation equation between x and y.

Substituting 6 in 2, there is:

$$y = -0.2421x + 23.14 \tag{7}$$

Where: -0.241 is the slope and 23.14 the y-intercept.

The correlation coefficient (r) of Trial 1 is obtained with the previous data. The following formulas were used to obtain the data in the table:

$$\sum\left(s - Ms^\wedge 2\right)^2 = 1928903.333 \tag{8}$$

$$\sum\left(ppm - Mppm\right)^2 = 1150 \tag{9}$$

$$\sum(s - Ms)^2 * \left(ppm - Mppm\right)^2 = -46711 \tag{10}$$

We substituted 8, 9 and 10 in 11.

$$r = \frac{\sum(s - Ms)^2 * \left(ppm - Mppm\right)^2}{\left(\sqrt{\sum(s - Ms)^2} * \left(\sqrt{\sum ppm - Mppm}\right)^2\right)} \tag{11}$$

Table 2. Data used to obtain the correlation coefficient in Trial 1.

s−Ms	(s−MS)^2	ppm−Mppm	(ppm−Mppm)^2	s−Ms*ppm−Mppm
−447.67	200405.44	11.50	132.25	−5148.17
−371.67	138136.11	10.50	110.25	−3902.50
−350.67	122967.11	9.50	90.25	−3331.33
−329.67	108680.11	8.50	72.25	−2802.17
−290.67	84487.11	7.50	56.25	−2180.00
−255.67	65365.44	6.50	42.25	−1661.83
−222.67	49580.44	5.50	30.25	−1224.67
−190.67	36353.78	4.50	20.25	−858.00
−146.67	21511.11	3.50	12.25	−513.33
−101.67	10336.11	2.50	6.25	−254.17
−83.67	7000.11	1.50	2.25	−125.50
−62.67	3927.11	0.50	0.25	−31.33
−28.67	821.78	−0.50	0.25	14.33
10.33	106.78	−1.50	2.25	−15.50
41.33	1708.44	−2.50	6.25	−103.33
80.33	6453.44	−3.50	12.25	−281.17
138.33	19136.11	−4.50	20.25	−622.50
211.33	44661.78	−5.50	30.25	−1162.33
259.33	67253.78	−6.50	42.25	−1685.67
333.33	111111.11	−7.50	56.25	−2500.00
379.33	143893.78	−8.50	72.25	−3224.33
429.33	184327.11	−9.50	90.25	−4078.67
477.33	227847.11	−10.50	110.25	−5012.00
522.33	272832.11	−11.50	132.25	−6006.83

Substituting, there is:

$$r = \frac{-46711}{(1388.849)*(33.9116)} = -0.9917811 \quad (12)$$

Trial 1 shows that the type of correlation is negative, since as the independent variable increases, the dependent variable will decrease; that is, as more time elapses, the ppm will be approaching zero. In obtaining the correlation coefficient, we can also see that the result is negative.

A second trial was conducted using 24 samples, which is presented in Figure 4, where we can see that there is a negative correlation, since as the independent variable increases, the dependent variable will decrease.

Through the results obtained from Trials 1 and 2, the coefficient of determination (R^2) can be calculated.

Coefficient of determination of Trial 1:

$$R^2 = 0.9836 \quad (13)$$

This value obtained is 98.36%, which is due to the dependent variable, that is, the gas monitored by the sensor, while the remaining 1.64% is because of other factors.

Coefficient of determination of Trial 2:

$$R^2 = 0.9953 \quad (14)$$

This value obtained is 99.53% of the dependent variable, that is, the gas flow monitored, while the remaining 0.47% is because of other factors such as the different day of the trial, the room temperature, the number of people within the room, or the sensor itself.

Figures 3 and 4 show the behavior of the sensor in face of the response to constant oxygen injections. As can be seen, the similarities between both graphs do not differ greatly one from the other. This indicates that when the sensor is subjected to different oxygen concentrations, as long as the flow is constant, it will continue to have coherent marking

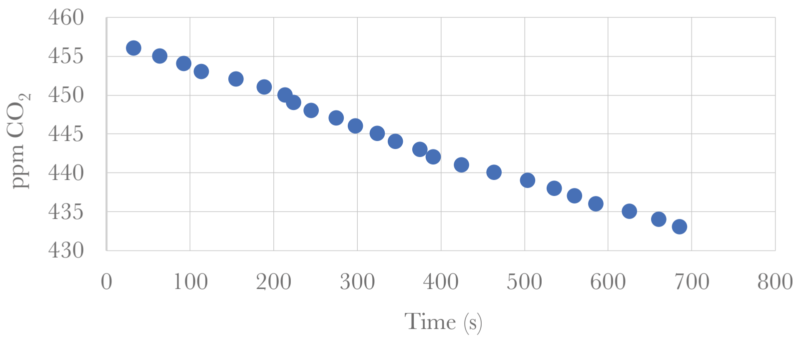

Figure 4. Dispersion of Trial 2 variables.

without considerable critical alterations for the system. With this, we can conclude that as long as the oxygen flow is kept constant, the parts per million of carbon dioxide will decrease depending on the time.

CONCLUSIONS

With the controlled carbon dioxide injection system, studies can be conducted to obtain the closest values to contribute to a better growth and adaptability in the sugarcane crops. This could guarantee shorter adaptation times and, with this, generate greater economic profits for the farmers.

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Colegio de Postgraduados

Effectiveness study of learning algorithms in supply chains of a dairy business

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ABSTRACT

Objective: To develop a control system to prevent over-response of the supply chain of a dairy business.

Methodology: The following methods were used: DQN, Double DQN, Dueling DQN, and Dueling Double DQN to determine the distribution of demand: normal and uniform.

Results: Results were calculated based on stability in learning (the last 10,000 episodes). It was observed that the means of DQN and DDQN were very similar. To validate whether the performance of the Dueling DQN algorithm is better than that of the DQN algorithm, a non-parametric test was performed to compare the mean rank of two related samples and to determine if there are differences between them. The p values were 5.83e−38 and 0.000 for the Normal and Uniform distributions, respectively.

Conclusions: The algorithm with the best results is Dueling DQN, with an average total cost of 151.27 units for the demand with a normal distribution and an average of 155.3 units for the demand with a uniform distribution. This method has less variability once convergence is achieved.

Keywords: Reinforcement learning; Deep learning; supply chain; inventory control.

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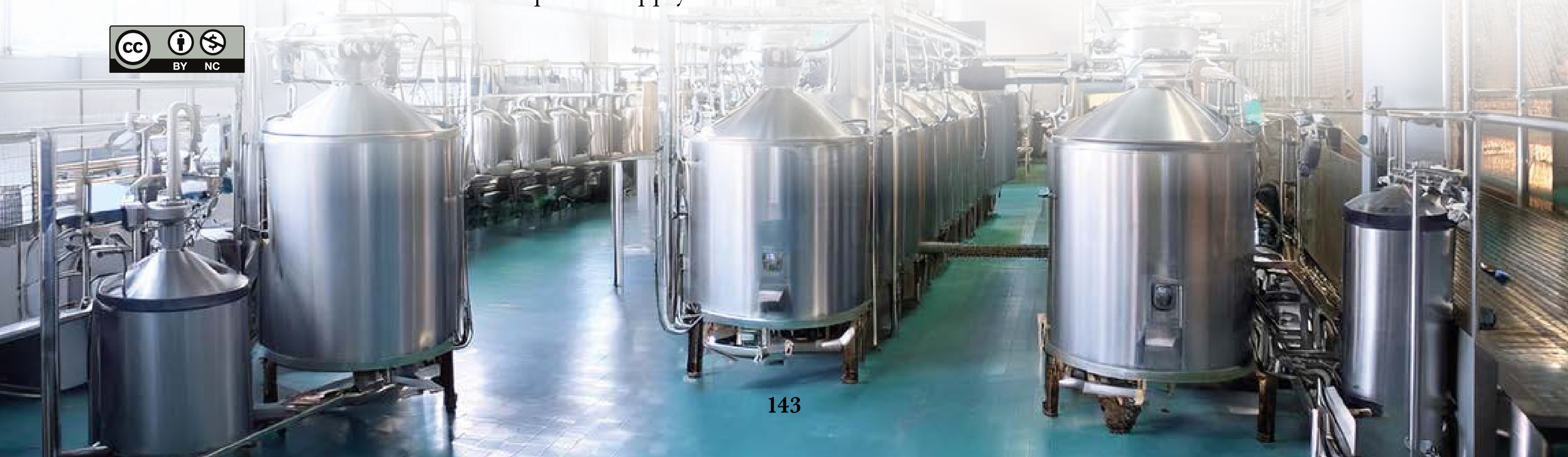
INTRODUCTION

A policy for inventory control to integrally manage the decisions taken in every stage of the supply chain is presented in [9]. The inventory problem is modelled as a Markov Decision Problem (MDP) and is resolved using an algorithm of Reinforcement Learning (RL) to determine a nearly optimal balance policy under a criterion of average reward.

In a dynamic environment, traditional policies for placing orders, based on time and events, can become inefficient, leading to an excess or shortage of inventory. A learning algorithm by case reinforcement learning (CRL) is presented in [9], for dynamic inventory control in a multiple-agent supply chain. A simulation with multiple agents from a two-level simplified supply chain was executed.



An approach is presented in [2] to minimize the inventory costs through the determination of integral purchase order policies for the members of the supply chain. The management of orders is considered as a system of multiple agents which generates a RL model. Therefore, a Q-learning algorithm is proposed to solve the RL model. The results show that the Reinforcement Learning Order Mechanism (RLOM) obtained good results compared to other algorithms, such as the algorithm based on GA.

Optimal base inventory levels are presented in [3], [4] and [5] when there are no costs from lack of stock in the non-retail stages. However, no algorithm was found in the literature to find the optimal levels of base inventory for general cost structures of stock shortage. Recently, an algorithm based on Deep Q Networks (DQN) with experience repetition is proposed in [16] to solve the base inventory policy outlining the use of a RL algorithm.

This article presents a study of a dairy business that has the objective of minimizing the increase in inventory management costs. The business sends the product to a central distributor, which supplies different retailers. In this case, the farmer acts as the agent. There is no feedback between the farmer, the distributor or the retailer. The information available for the agent is the orders made by the distributor to the business. The objective of the agent is to determine the size of the production order to minimize the total level of inventory of the supply chain.

This study is divided into six sections: section two presents a review of the materials and methods used. Section three describes the application of the RL algorithm to the CS, and the configuration of the hyperparameters to achieve convergence. Section four describes the results obtained. Section five proposes the discussion, where the points proposed are defended, and finally, section six presents a conclusion of this study.

MATERIALS AND METHODS

The algorithm used to simulate the supply chain was adapted from the one proposed in the article, "A Deep Q-Network for the Beer Game: Deep Reinforcement Learning for Inventory Optimization". However, another algorithm may be used to represent the environment of the supply chain. The codes used to perform the experiments are available under request to the author through email.

Deep Q-Network (DQN) in the supply chain

In each t period, the agent observes the current state of the environment, where is the set of every state possible. In this case, the agent will be the farmer and the environment contains all the information about the levels of inventory and the costs. In function of the data from the state, the agent selects an action $a_t \in A(s_t)$, where $A(s_t)$ is the set of possible actions when the system is in state s_t. The agent (the farmer) performs an action, that is, issues a production order based on the information of the levels of inventory and the costs. The agent receives a reward $R_t \in \mathbb{R}$. The reward consists in the total accumulated cost until period t. Then the system makes the transition to the state $s_{t+1} \in S$.

The base of this experiment is Q-learning, a temporal difference learning algorithm (TD learning). The algorithm updates the estimation of the agent of the value function in each step of time during the episode.

$$V\left(s_t\right)=V\left(s_t\right)+\alpha\left[R_{t+1}+\gamma V\left(s_{t+1}\right)+V\left(s_t\right)\right] \quad (1)$$

Given that the equation has terms that are proportional to the estimation of time t and time $t+1$, that is, an estimation to update another estimation, it means that Q-Learning is what is known as a booting algorithm. In this case, $V\left(s_t\right)$ represents the prior state. R_{t+1} is the reward in $t+1$, $\gamma V\left(s_{t+1}\right)$ is the updated value in the next step, and the sum of both terms represents the objective of temporal difference (Temporal Difference TD).

Case study

The study was conducted in a condensed milk manufacturing business that is facing challenges related to the excess of inventories. Currently, the business maintains high inventory levels, which results in high costs of storage and risk of obsolescence. In addition, the lack of effective planning of the demand and a limited capacity to predict the sales patterns, due to the scarce information, has led to lost sales when the inventory does not match the demand.

The intention is to find an optimal policy, known as π*, which could lead to the best expected cumulative reward (the least total cost). There are two main types of RL methods used to find this semi-optimal policy:

The methods based on policies directly train the policy to determine the appropriate action to take given a specific state. In this case, the action is calculated in function of factors such as the size of the shipment to the distributor, the level of inventory, the demand, and the order placed by the distributor.

Methods based on the value: These methods train a value function to determine the relative value of different states and to use this information to make decisions. In our case, the most valuable state is the level of inventory that guarantees an efficient delivery and minimizes the storage costs. The optimal order size (action) to maintain this level of inventory is calculated.

There are two different methods to find the value function.

$$v_\pi(s)=\mathbb{E}\left[R_{t+1}+\gamma R_{t+2}+\gamma^2 R_{t+3}+\ldots \mid S_t=s\right] \quad (2)$$

Functions based on policies: The policy is trained directly to select an action given certain state. In this case, we do not have a value function defined by hand that determines the behavior of our policy. The training will define it.

Functions based on value: The value function is trained indirectly to map the value of a state or a state-action pair. With this value function, our policy can take actions. However, given that we do not train directly our policy, we need to specify its behavior. In our case, for example, given that our objective is to minimize the total cost, we use a policy that selects actions that consistently result in the lowest value, given the value function (Equation 2, greedy policy).

$$\pi^*(S)=\frac{\arg\ min}{a} Q^*(s,a) \quad (3)$$

Given a state, our function of action value (which we trained) calculates the size of the order in this state. Then, our greedy policy (which we defined) selects the size of the order that incurs in the lowest storage cost and minimizes lost sales.

We have two types of functions based on the value:

State-Value Function: The state-value function (Equation 3) generates the expected yield for each state, that is, for each level of inventory, if the agent begins in the state and the policy continues until the game ends. However, this does not make sense in a supply chain (SC) since the states are repetitive. This situation could lead to divergence in the learning process of the RL algorithm.

$$v_{\pi}(s) = \mathbb{E}_{\pi}\left[G_{\pi} \middle| S_t = s\right] \tag{4}$$

Value-Action Function: The value-action function (Equation 4) returns the expected yield for each state-action pair when the agent begins in this state, performs this action, and then follows the policy. In the case of the supply chain (SC), this type of function is more appropriate because the agent observes its level of inventory, executes several actions (placing different orders with different order sizes), and then evaluates the cost of each action.

$$Q(s,a) = \mathbb{E}_{\pi}\left[G_{\pi} \middle| S_t = s, A_t = a\right] \tag{5}$$

The difference lies in that, in the value-state function, we calculated the value of a state, while in the value-action function we calculated the value of the state-action pair. This means that we determined the value of taking a particular action in a specific state.

Demand distribution

The demand data has two types of behavior depending on the sales season. The parameters for the normal distribution are N(5,1), while the parameters for the uniform distribution are U(0,10). The action space for the agent considers values between 0 and 10 units. The experiments were conducted with both distributions to evaluate the robustness of the model.

Configuration of hyperparameters

Each experiment in this study uses the same hyperparameters to achieve a valid comparison (see Table 1). The design of the neural network has three layers: 128, 64 and 32 neurons. The training of each method lasted 40,000 episodes.

A greedy strategy ε was used to balance the exploration and the exploitation of the agents. This strategy allows the agent to explore the environment before deciding on an exploitation strategy. This process of exploration and exploitation helps the agent to refine its model of the environment and to gradually approach a value function close to optimal every time the agent tries the state and receives a reward. The maximum value of epsilon, εmax=1, decreases linearly to its minimum value, εmin=0.1, during the first 10,000 episodes of training.

The initial values of the hyperparameters (Table 1) are proposed in [7] and [16]. Initially, the learning rate was 0.00025 and the discount factor was 0.99, although these configurations did not allow for the algorithm to reach convergence. The algorithm could not learn because a discount factor of 0.99 prioritizes the future reward, which does not make sense in an infinite game as in this case. In addition, when there is a delay between the action and the effect of the environment, a high learning rate confounds the learning process of the value function.

Initially, a neural network was configured with a single hidden layer of 10 neurons. However, because of the high non-linearity of the system, it did not produce significant results.

RESULTS AND DISCUSSION

DQN with experience repetition

The results of the DQN method with experience repetition can be seen in Figure 1c (the results from the DQN method with experience repetition show the normal distribution with the blue line, and the uniform distribution with the red line). The average cost is higher with a uniform distribution compared to a normal distribution. However, the agent's actions were similar for both types of demand distribution. It is important to highlight that, due to the nature of the uniform distribution, the cost of lost sales increases because of variability in the demand, which results in an average negative inventory. The behavior of the state variables is presented in Figure 1a, 1b, 1c, and 1d.

In the uniform distribution, the agent learned a policy of not having inventory to minimize the total cost. This policy is similar to the current policy established by several businesses. They prefer to lose sales instead of maintaining a high level of inventory because of the uncertainty in the demand. On the other hand, in the normal distribution, the agent learned a policy based on maintaining a minimum level of inventory to minimize lost sales, since the behavior of sales in this type of distribution is predictable. In the normal distribution, the total cost is made up mainly by the storage cost, while in the uniform distribution the total cost is composed mainly by the cost of lost sales.

Table 1. Configuration of hyperparameters.

Hyperparameters	Values
Gamma	0.9
Learning rate	0.00001
Agent history (m)	3
Number of neurons per layer	[128, 64, 32]
Activation function	[RELU, RELU, RELU, LogSigmoide]
Loss function	MSE
mini batch size	64
Optimization algorithm	Adam

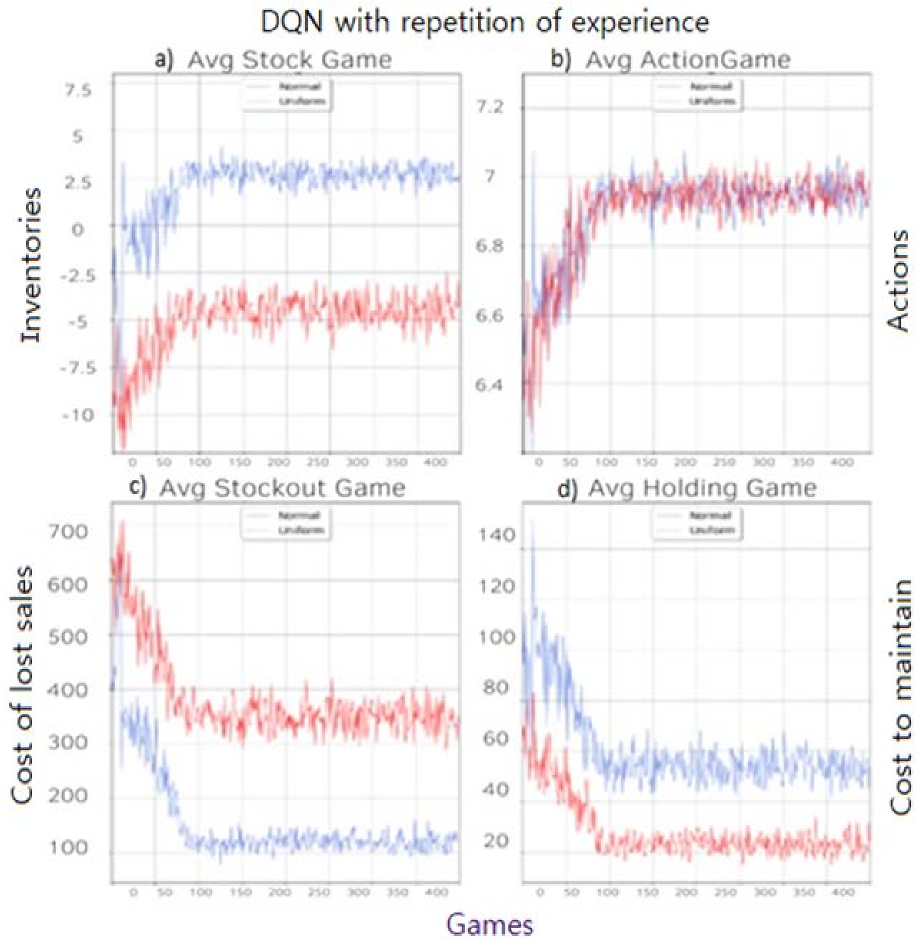


Figure 1. Evolution of the state variables during training.

Double DQN

Figure 2 shows the results of the training used in the DDQN method. Due to the similar behavior between the DQN and Double DQN algorithms, as presented in Figure 3, a Wilcoxon test is carried out to determine whether there is a significant difference between the methods. In the first place, a test is conducted to compare the scores of the algorithms under a normal distribution of the demand, resulting in a p value of 0.741039. Therefore, the differences in the results are not statistically significant. In other words, the yield of both algorithms under a demand with normal distribution is similar. The second Wilcoxon test examines the yield of both algorithms under a demand with uniform distribution, resulting in a p value of 0.8664. Therefore, the differences in the results are also not statistically significant in this case.

Given that the results of the DQN and DDQN methods do not show statistical significance, the DQN algorithm was selected to compare its yield with the Dueling DQN method.

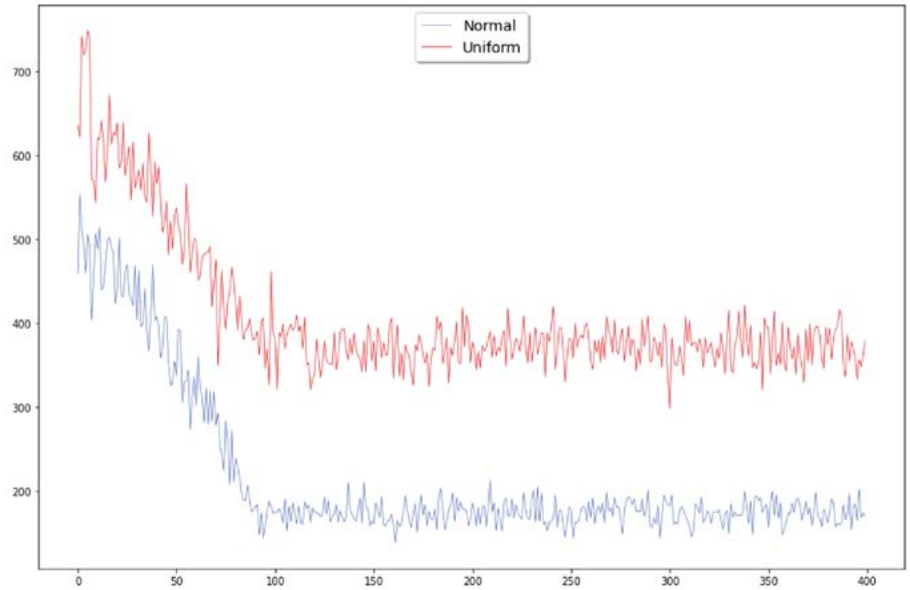


Figure 2. Results from the Double DQN method. Blue line: normal distribution; red line: uniform distribution.

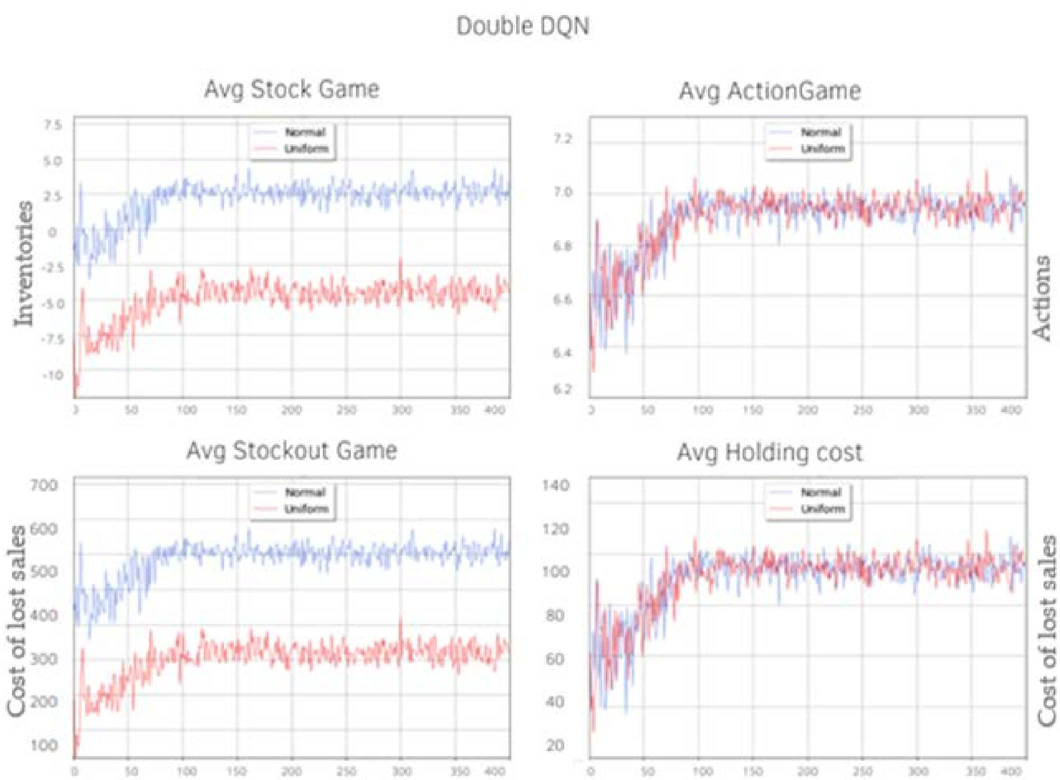


Figure 3. Evolution of the state variables during the training.

Dueling DQN

Figure 4c shows the results of the Dueling DQN method. With blue line: normal distribution; with red line: uniform distribution; and Figure 4a, 4b, 4c, and 4d shows the training results using the Dueling DQN method. Figure 5 shows how, after 30,000

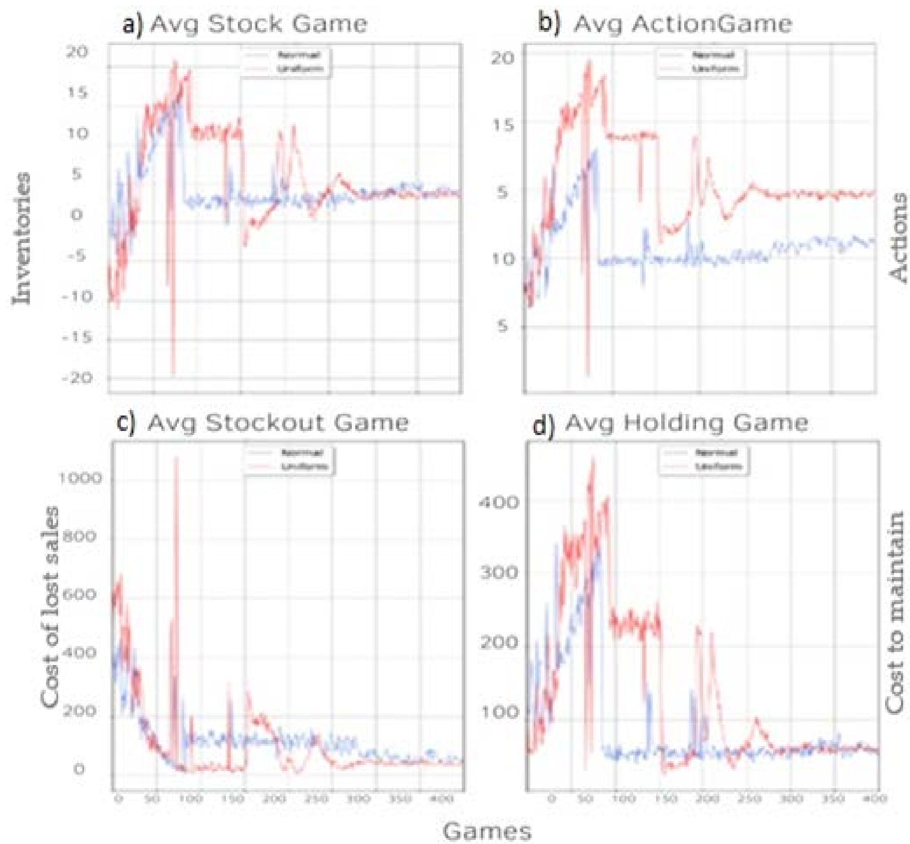


Figure 4. Evolution of the state variables during training.

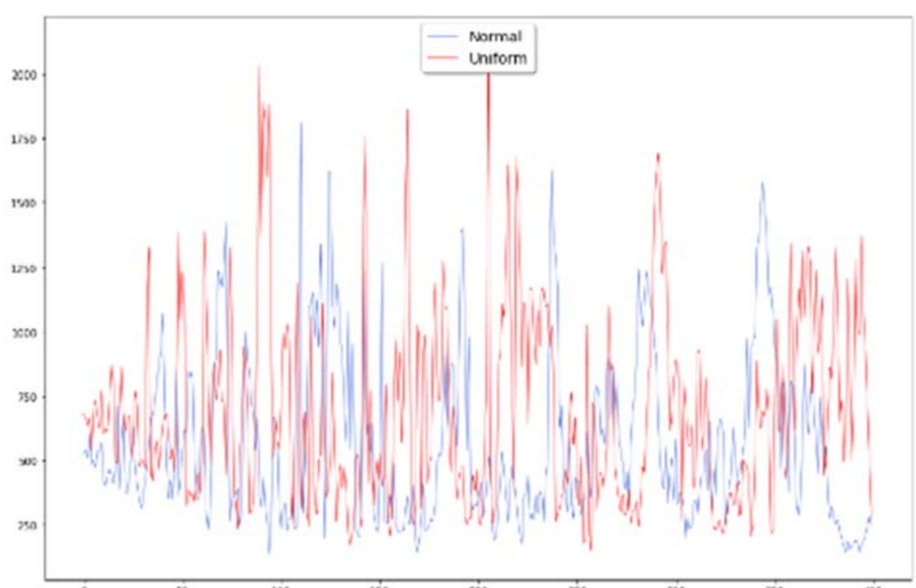


Figure 5. Results from the Dueling Double DQN method. Blue line: normal distribution; red line: uniform distribution.

episodes, the algorithm attains stability in learning and reaches the minimum values of the total cost. It is important to mention that this method achieved a lower total cost than the DQN method for both types of demand distribution. Although this method took more episodes to converge, after completing the 40,000 training episodes, the agent learned a better policy for both types of demand. The policy implies maintaining a minimum level of inventory for both behaviors of demand, to minimize the cost from lost sales. However, if the distribution is uniform, the average level of actions is higher than in a normal distribution to compensate the peaks.

Dueling Double DQN

The Dueling Double DQN method did not attain convergence in any type of demand, as can be seen in Figure 5. The divergence takes place during the training process because it avoids excessive responses, and the separate evaluation of state-action pairs.

The summary of the results obtained from the training of each method is presented in Table 2. These results were calculated based on the stability in learning (the last 10,000 episodes). As can be seen, the means of DQN and DDQN were very similar, which agrees with the previously applied Wilcoxon test [27]. To validate whether the yield of the Dueling DQN algorithm is better than the DQN algorithm, a non-parametric test was conducted to compare the mean range of two related samples and to determine if there are differences between them. The p values were 5.83e−38 and 0.000 for the Normal and Uniform distributions, respectively. Therefore, the conclusion can be reached that the Dueling DQN is statistically better than DQN.

Although the convergence of the DQN algorithm is more stable than the Dueling DQN algorithm, the final policy obtained by Dueling DQN attained a better total cost. In practice, a more efficient method has the objective of reducing storage costs by minimizing the level of inventory without incurring in a high level of lost sales [12].

Among all the hyperparameters, it is interesting to mention that the discount factor had a significant impact on the learning process, since it is not possible to evaluate the long-term reward in a supply chain. The results reached by the Dueling DQN algorithm were lower than those of other methods. In addition, the behavior of the demand was not significant due to the robustness of the method. Therefore, in practice, the use of the Dueling DQN algorithm to determine the size of the order allows reducing inventory costs and lost sales.

Table 2. Scores of reinforcements learning methods.

Method	Score with DN (mean)	Score with DU (mean)
DQN with repetition of experience	173.96	371.53
Double DQN	173.94	371.61
Dueling	151.27	155.30
Double Dueling DQN	571.39	682.39

CONCLUSIONS

Four reinforcement learning (RL) methods were compared to solve the problem of determining the optimal size of the purchase order that a farmer must meet to minimize the total cost of the supply chain. The algorithm with the best results is the Dueling DQN, with an average total cost of 151.27 units for the demand with a normal distribution and an average of 155.3 units for the demand with a uniform distribution. This method presents lower variability once convergence is reached. The policy proposed by the Dueling DQN is applied to the case study, since a perishable product must maintain a minimal inventory to avoid the risk of expired products. As future work, this method will be applied to the entire supply chain, including the retailer, in an environment of multiple products.

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AGRO
PRODUCTIVIDAD

Colegio de Postgraduados

Characterization and evaluation of a tray dryer with change of position: kinetics and thermodynamic properties of the process, preserving the nutraceutical quality in Golden Delicious apple slices

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ABSTRACT

The drying kinetics in apple slices between 45-55 °C was studied until reach a moisture ratio of 10±2%. 8 thin layer drying models were statistically fitted where the Midilli-Kucuk model presented the best performance, moreover its drying constant increased with the temperature, effective diffusivity was ranged from 68.16 E^{-10}-2.28 E^{-08} m^2 s^{-1}, the Arrhenius factor and activation energy were 3.74 E^{-02} m^2 s^{-1} and 28.11 kJ mol^{-1}, the enthalpy decreased from 25.46-25.38 kJ mol^{-1}, the entropy was ranged between (−0.2728-−0.2730) kJ mol^{-1} K^{-1}, and the Gibbs free energy increased from 112.25-114.98 kJ mol^{-1}. The 45 °C treatment preserved the highest phenolic compounds (51.1±1.54 mg EAG 100 g^{-1}), while the initial activity of elimination of radicals was 766.9±91.4 and 973.3±103.4 μg mg^{-1} according to the DPPH and ABTS methods, the antioxidant activity shows an inverse behavior to the drying temperature. This work will allow to establish the temperature, time, moisture ratio and energy to drying apple slices according to local weather or under controlled conditions.

Keywords: Apple drying, mathematical modeling, effective diffusivity, phenolic compounds, antioxidant activity.

INTRODUCTION

The apple is a tree fruit cultivated in various parts of the world, its global production ranks third compared to other fruits, preceded by bananas and watermelons (Ghinea, Prisacaru, and Leahu, 2022). It is a natural food that possesses numerous nutritional properties, such as vitamins, minerals, and fiber (Noori *et al.*, 2021).

Like other fruits, apples provide nutrients and can prevent some diseases due to chemicals that modify the physiology of the organism.

These substances are compounds classified as secondary metabolites or phytochemicals, which contain bioactive properties. Additionally, this product has been characterized regarding its color and according to its antioxidant properties. Apples are consumed in various forms, whole, as dehydrated slices, as juice, in extract, pulverized, and in compote, as they possess diverse nutritional properties to prevent or control various diseases as obesity, cholesterol-related issues, and those related to the immune system.

In Mexico, apple crop yield fluctuates between 11-12 t ha^{-1}. By 2021, global production was estimated to exceed 93 000 000 tons with an average yield of 19-20 ton ha^{-1} (FAOSTAT, 2023). Apple dehydration is carried out either outdoors or in greenhouses with varying temperatures and times, meaning that these parameters are not controlled, relying solely on local environmental conditions, which results in reduced product quality. Dehydration technologies are useful for improving the quality of active ingredients and increasing post-harvest yields, reducing losses due to contamination or product deterioration, and minimizing energy consumption, as demonstrated by Martynenko and Janaszek (2014), who dehydrated and analyzed the texture of apple slices in a convective dehydrator at temperatures between 40-80 °C, or as stated by Solano and Rodríguez (2010), who agree that dehydrators are safe, efficient, and economically viable mechanisms in the post-harvest management of food products.

Several authors have studied the kinetics and mathematical modeling of thin-layer dehydration of food products, such as Inyang *et al.* (2018)for shelf life elongation, to minimize packaging and improving storage for easy transportation. Thin-layer drying of materials is necessary to understand the fundamental transport mechanism and a prerequisite to successfully simulate or scale up the whole process for optimization or control of the operating conditions. Researchers have shown that to rely solely on experimental drying practices without mathematical considerations for the drying kinetics, can significantly affect the efficiency of dryers, increase the cost of production, and reduce the quality of the dried product. An effective model is necessary for the process design, optimization, energy integration and control; hence, the use of mathematical models in finding the drying kinetics of agricultural products is very important. The statistical criteria in use for the evaluation of the best model(s who agree that dehydration modeling is highly useful for determining process behavior, maintaining product quality, and aiding in reducing product losses. Regarding the dehydration of fruits, the semi-empirical Midilli-K. model has shown the best fit, as demonstrated by Mugodo and Workneh (2021) in mango slices. Additionally, thermodynamic properties were determined, as conducted by Nadi and Tzempelikos (2018) in Golden Delicious slices.

Recent studies on apple slice dehydration exist, such as the one conducted by Royen *et al.* (2020)air humidity, air velocity and slice thickness on process kinetics, product water activity and parameters of empirical models has been investigated. Drying characteristics of apple slices were monitored at temperatures of 40, 45 and 50 °C, air velocities of 0.6, 0.85 and 1.1 m/s., slice thicknesses of 4, 6, 8, 10 and 12 mm, and in relative air humidity ranges of 25-28, 35-38 and 40-45%. During the process, samples were dried from an initial moisture content of 86.7% to that of 20% (w.b, based on five thin-layer models, using a convective tray dehydrator. Additionally, Noori *et al.* (2021) contrasted 11 models within a temperature range of 33 to 55 °C. Both studies mentioned that the Midilli-K model represented dehydration best, although they did not delve into the study of its thermodynamic properties.

The aim of this work was to study the kinetics of thin-layer dehydration of Golden Delicious apple slices in a tray dryer with position change, through mathematical modeling and the fitting of dehydration curves. This aimed to evaluate, with a general expression, the effect of temperature on moisture content ratio, total phenolic content, and antioxidant activity.

MATERIAL AND METHODS

Dehydrator features

Dehydration is an operation involving heat and mass transfer. This process extends the shelf life of various food products by reducing the water activity to a sufficiently low level to inhibit microbial, enzymatic, and other deteriorating reactions (Sabarez, 2015).

The experimentation of the thin-layer dehydration process of Golden Delicious apple slices was carried out in a tray dryer with position change, constructed of stainless steel 304-L, with 6 trays measuring 0.27×0.74 m. Figure 1 illustrates the placement of sensors and actuators. This equipment is operated through a graphical interface created in Visual C Sharp software, allowing real-time monitoring of process variables (internal humidity, internal temperature, and airflow velocity at the dryer outlet).

The system consists of a 12 VDC. axial fan that generates air flow velocities of 3 m s^{-1}, located at the entrance of the equipment. Heat is generated by a 1600 W, 60 Hz electric resistance at 127 VA.C, controlled by a digital Autonics TCH-4 controller, with temperature ranges from 30-85 °C, measured by a type J thermocouple with a hysteresis of ±0.5 °C, as shown in Figure 2.

The tray position change during the dehydration process occurs every minute and lasts for 3 seconds, activated by an actuator coupled to the shaft (Figure 3a), which is a 20 Vc.d., 8 A geared motor with a variable rotation frequency between 0-280 rpm sufficient to provide freedom of movement (Figure 3b). This action is carried out in order to maintain heat homogeneity on the product surface and inside the dehydrator.

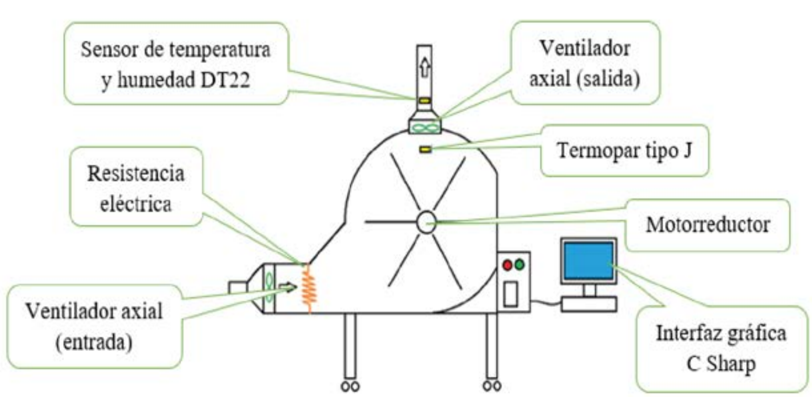

Figure 1. Scheme of the tray dehydrator.

Figure 2. Temperature controller and actuators.

Figure 3. Rotation system. (a) Coupled geared motor. (b) Tray distribution.

A 12 $V_{D.C.}$ axial fan, at the equipment's output, operates as an air extractor. When the percentage of moisture released by the product increases, the fan increases the airflow at the output.

In Figure 4, (a) the graphical interface, and (b) the communication with the physical system are observed, whose design allows the dehydrator to be autonomous under any external alteration or disturbance during its operation.

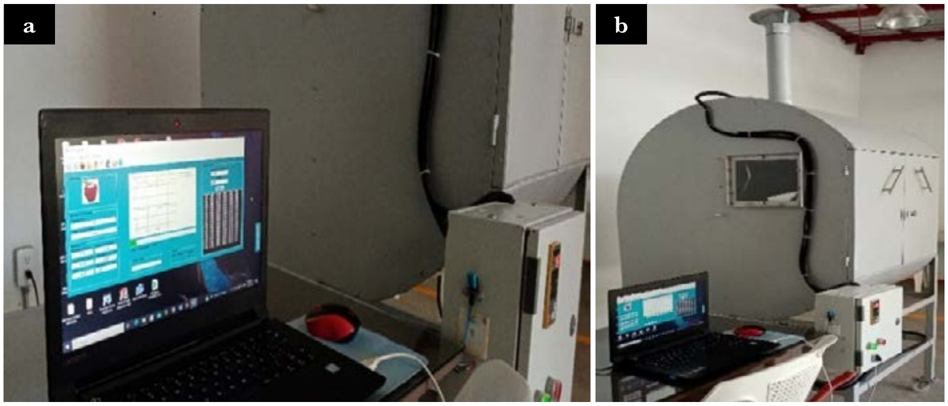

Figure 4. Rotary tray dryer. (a). Interface (b). Actuator connection.

Dehydration process

This research was conducted in Tehuacán, Puebla, located at 19° 29' N, 98° 53' W, at an altitude of 2240 meters. The experiment was based on a completely randomized design with a one-factor arrangement, considering temperature, T, as the sole factor, with 3 treatments ranging from (45-55) °C and ΔT=5 °C, carried out in triplicate, resulting in 9 experimental units. The response variable was the moisture ratio, MR, as described in Equation 1.

$$MR = \frac{M_t - M_e}{M_o - M_e} \approx \frac{M_t}{M_o} \quad (1)$$

Where MR is the moisture ratio (dimensionless); M_t is the moisture ratio at time $t\left(g_{H_2O}\ g_{MS^{-1}}\right)$, M_o is the initial moisture ratio of the product $\left(g_{H_2O}\ g_{MS^{-1}}\right)$ and M_e is the equilibrium moisture ratio $\left(g_{H_2O}\ g_{MS^{-1}}\right)$.

The values of M_e were relatively small compared to M_t and M_o, and it should be noted that $M_e=0$ (Celma *et al.*, 2007).

The apples were purchased from a commercial center during the 2022 season and were conditioned for the dehydration process immediately. To minimize measurement errors, the samples were homogenized with respect to size, color, and ripeness level.

To measure the mass of the samples, *m*, an MA 50/1. R moisture analyzer (Radwag, Poly) was used (precision ±0.001 kg). Temperature was measured using a type J class 1 thermocouple with compacted mineral protection of two terminals and ceramic insulation with a range of −40 °C to +125 °C (precision ±1 °C). The airflow velocity, v, was measured using a TMA-10A anemometer (Amprobe, Netherlands) (precision ±0.01 m s^{-1}).

During the experiments, the temperature and airflow velocity remained constant at the inlet and outlet of the equipment, with some variations inside of ±3 °C and v=3 m s^{-1}. The initial mass of the product was *m*=1 kg per treatment. The fresh apple slices were placed inside the dryer, distributed among the 6 stainless steel trays, each measuring 0.1998 m^2. The diameter of the slices ranged from 0.065-0.075 m with a material thickness (L) of 0.0014 m. During the tests, the mass m was recorded every 3 minutes, ending when the product reached $MR=10\pm2\%$.

Drying rate. It is the change in *MR* per unit of time, Equation 2:

$$DR = \frac{MR_{t+\Delta t} - MR_t}{\Delta_t} \quad (2)$$

Where DR is the dehydration rate $\left(kg_{H_2O}\ kg_{MS^{-1}\ \mathrm{min}^{-1}}\right)$ and Δt is the time period (min).

Dehydration kinetics

Mathematical Modeling

To predict the behavior of the process in apple slices, a statistical analysis of the data was conducted with respect to 8 thin-layer dehydration models (Table 1). The analysis and model fitting were performed using the Curve Fitting Tool included in Matlab v 9.3.

Using Equation 1, *MR* was determined to construct the *MR vs. t* curves. Additionally, the statistical selection criterion to calculate the goodness of fit of the 8 models to *MR* and determine which one best describes the dehydration process was: locating the highest value of the coefficient of determination, R^2, as well as the minimum values of the root mean square error, RMSE, and the sum of squares due to error, SSE (Inyang *et al.*, 2018).

Adjustment in the constant and coefficients of the model

The coefficients were estimated according to T, where *k* should be associated with the ease of removing moisture from the apple slices.

Generalization of the MR curve

The possibility of fitting the curve to the data by correlating *MR vs.* τ for the temperature range was verified (Menezes *et al.*, 2013), *i.e.*, to determine the behavior of the process at any time or moisture ratio within the study temperature range T. Equation 3:

$$\tau = \frac{N_c t}{MR_o} \tag{3}$$

Where τ is time (dimensionless), MR_o is the initial *MR* (dimensionless), and N_c is the dehydration rate constant (1 min^{-1}).

Table 1. Models evaluated for dehydrating apple slices.

Model	MR
Kaleta (Kaleta *et al.*, 2013)	$a \cdot e^{\left(-kt^n\right)} + (1-a) \cdot e^{\left(-bt^n\right)}$
Two term (Olawoye *et al.*, 2017)	$a \cdot e^{\left(-k_0 t\right)} + b \cdot e^{\left(-k_1 t\right)}$
Henderson and Pabis (Olawoye *et al.*, 2017)	$a \cdot e^{(-kt)}$
Logarithmic (Mghazli *et al.*, 2017)	$a \cdot e^{(-kt)} + c$
Midilli-K. (Ekka y Palanisamy, 2020)	$a \cdot e^{\left(-kt^n\right)} + b \cdot t$
Page (Tunckal *et al.*, 2018)	$e^{\left(-kt^n\right)}$
Diffusion Approximation (Şi-mşek *et al.*, 2021)	$a \cdot e^{(-bt)} + (1-a) \cdot e^{(-bct)}$
Verma (Olawoye *et al.*, 2017)	$a \cdot e^{(-kt)} + (1-a) \cdot e^{(-gt)}$

Model Validation

The validation criterion was to plot adjusted *MR*, MR_{aj}, against experimental *MR*, MR_{ex}, for the applied T range (Benseddik *et al.*, 2018; Beye *et al.*, 2019).

Effective Diffusivity and Activation Energy

Effective Diffusivity

The mass transfer in the transient state of thin-layer dehydration of food products is studied using the expression of Fick's second law of diffusion. To establish the value of effective diffusivity, Deff, apple slices were considered as a body of planar geometry (Crank, 1979). Equation 4:

$$MR = \frac{8}{\pi^2} \sum_{i=0}^{\infty} \frac{1}{(2n+1)^2} e^{\left[-\frac{(2n+1)^2 \pi^2 D_{eff} t}{4L^2}\right]} \quad (4)$$

Where D_{eff} is the effective diffusivity ($m^2\ s^{-1}$); L is the material thickness (m), and n is the number of terms (dimensionless).

Equation 4 assumes a uniform distribution of initial moisture content, simplifying diffusion movement and neglecting deformation. For extended dehydration periods, where $MR<0.6$ and $n=0$, Equation 5 is obtained (Tunçkal, 2020):

$$MR = \frac{8}{\pi^2} e^{\left[\frac{\pi^2 D_{eff} t}{4L^2}\right]} \quad (5)$$

Applying the law of logarithms to both sides of the previous equation yielded Equation 6, and by isolating ko, Equation 7, the value of D_{eff}: was determined:

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff} t}{4L^2}\right) \quad (6)$$

$$k_o = \left(\frac{\pi^2 D_{eff}}{4L^2}\right) \quad (7)$$

where k_o (dimensionless) is the slope of the ln(*MR*) *vs.* *t* line (Mugodo y Workneh, 2021).

To estimate D_{eff}, Equation 5 was evaluated with the Fourier number, F_o (Mohammadi *et al.*, 2019), Equation 8:

$$F_o = \left(\frac{D_{eff}\, t}{L^2} \right) \tag{8}$$

where F_o is the Fourier number (dimensionless).

Rewriting Equation 5 and transforming it into its linear form resulted in Equations 9 and 10. By isolating Fo from Equation 10, Equation 11 was derived. Finally, in Equation 8, Deff was isolated and Fo was replaced with Equation 11.

$$MR = \frac{8}{\pi^2} e^{\left(\frac{\pi^2 F_o}{4} \right)} \tag{9}$$

$$\ln(MR) = -0.21 - 2.4674\ F_o \tag{10}$$

$$F_o = -0.4052 \ln(MR) - 0.0851 \tag{11}$$

Activation energy

Expresses the dependency of D_{eff} on temperature, representing the energy required to initiate the moisture diffusion reaction in the product (Ghasemkhani *et al.*, 2021). This dependency is evaluated using Equation 12.

$$D_{eff} = D_o e^{\left(-\frac{E_a}{RT} \right)} \tag{12}$$

where D_o is the frequency or pre-exponential factor of Arrhenius ($m^2\ s^{-1}$); *Ea* is the activation energy ($J\ mol^{-1}$); *R* is the universal gas constant ($8.3143\ J\ mol^{-1}\ K^{-1}$) and *T* is the absolute temperature (K). Linearizing Equation 12 yields:

$$\ln\left(D_{eff}\right) = \ln\left(D_o\right) - \frac{E_a}{RT} \tag{13}$$

The values of E_a are obtained by plotting $\ln(D_{eff})$ *vs.* $1\ T^{-1}$, where $E_a\ R^{-1}$ y $\ln(D_o)$ are the slope and intersection of the obtained line, confirming the correlation between D_{eff} and T, where E_a increases with lower L (Petković *et al.*, 2021). This should be considered when designing dehydration systems and calculating the energy required to remove the moisture content from the product.

Thermodynamic Properties

E Enthalpy, H, is the energy required to remove moisture from a product during dehydration and decreases with increasing temperature (Nadi and Tzempelikos, 2018).

Negative values indicate the absence of endergonic reactions, which require an external energy source for the product to transform.

Entropy, S, is the degree of disorder in the water-product system (Oliveira *et al.*, 2010). As temperature increases, the entropy of the system decreases (Costa *et al.*, 2016), increasing the diffusion rate of water from inside the product to the drying air (Araujo *et al.*, 2017).

Gibbs free energy, G, is the work done by the system during adsorption or desorption, and it also indicates the amount of water contained in the product, evaluating the loss of liquid from the material (Araujo *et al.*, 2017). Positive values of G indicate that there is no spontaneous variation in ΔG, meaning that dehydration is a non-spontaneous process, demonstrating that external energy contributions are required for this phenomenon to occur.

The above parameters were determined using the method of Jideani and Mpotokwana (2009), obtaining Equations (14-16):

$$\Delta \mathrm{H} = E_a - RT \tag{14}$$

$$\Delta \mathrm{S} = R\left[\ln\left(D_o\right) - \ln\left(\frac{k_B}{h_P}\right) - \ln\left(T\right)\right] \tag{15}$$

$$\Delta \mathrm{G} = \Delta \mathrm{H} - T\Delta \mathrm{S} \tag{16}$$

where ΔH represents changes in H (J mol^{-1}), ΔS represents changes in S (J mol^{-1} K^{-1}), ΔG represents changes in G (J mol^{-1}), k_B is the Boltzmann constant ($1.38 \cdot E^{-23}$ J K^{-1}) and h_P is the Planck constant ($6.626 \cdot E^{-34}$ J s^{-1}).

Total Phenolic Compounds and Antioxidant Activity

Preparation of Extracts

A sample of 10 g of finely chopped fruit was homogenized with 20 mL of 80% (v v^{-1}) methanol and sonicated at 25±2 °C and 40 kHz for 20 min (Cole-Parmer, 08895, Vernon Hills, IL, USA) in the presence of inert gas (^{18}Ar) to prevent oxidative degradation of polyphenols. The extract was centrifuged at 5500×*g* for 20 min using a tabletop centrifuge (Eppendorf, 5804, Hamburg, Germany). The supernatant was collected for analysis of phenolic compounds and antioxidant capacity.

Total Phenolic Content

The total phenolic content (CFT) was determined using the colorimetric method of Folin-Ciocalteu (Corona-Leo *et al.*, 2021), which involves the oxidation of the phenolate ion along with the reduction of the phosphotungstic-phosphomolybdic reagent. The chromophore produced is a blue phosphotungstic-phosphomolybdic complex that has a maximum absorption in the region of 750 nm. For the determination, 1 mL of the prepared methanolic extract, 60 mL of distilled water, and 5 mL of Folin-Ciocalteu reagent were poured and mixed in a 100 mL volumetric flask. After 4 minutes, 15 mL of 7% (p v^{-1}) sodium carbonate was added, adjusting the volume to 100 mL using distilled water. The mixture was covered with aluminum foil and allowed to react for 30 minutes at room temperature (25±2 °C). Absorbance measurements were carried out at a wavelength of 750 nm using a single-beam UV-Visible spectrophotometer (LX510SS, Labdex, London, UK). The calibration curve was constructed using gallic acid as the standard with n=6 concentrations in the range of 0-500 mg L^{-1} and adjusted by linear regression (R^2=0.995). The results were expressed as mg gallic acid equivalents (EAG) per 100 g of sample (mg EAG 100 g^{-1} of dry weight of the sample).

Antioxidant Activity

The antioxidant activity exhibited by a metabolite is observed when it neutralizes any free radicals (Frei, 1994).

Free radical scavenging assays of DPPH and ABTS radical

Antioxidant activity was measured using the DPPH reagent method (2.2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich; St. Louis, Missouri, USA) according to Bao *et al.* (2023). 1.2 mL of sample extract (Section 2.3.1) was mixed with 3.6 mL of methanol solution at a concentration of 5 mmol mL^{-1} DPPH. The mixture was kept in the dark at room temperature (25±2 °C) for one hour, and then the absorbance was measured at 517 nm. The DPPH scavenging velocity of the extract was calculated according to Equation 17 (Iordănescu *et al.*, 2021):

$$AER = \frac{A_0 - A_s}{A_s} 100 \tag{17}$$

where AER is the radical scavenging activity, A_0 is the absorbance of the blank or control sample, and A_s is the absorbance of the sample extract.

The ABTS assay (2.2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Sigma-Aldrich; St. Louis, Missouri, USA) was conducted based on the methodology adapted from López-Vidaña *et al.*, (2019). The $ABTS^{+\cdot}$ radical cation was produced by reacting a mother solution of ABTS at a concentration of 7 mM with an oxidizing solution of potassium persulfate ($K_2S_2O_8$) at a concentration of 2.45 mM. The mixture was stored in the dark at room temperature (25±2 °C) for 24 hours prior to the experiments. Subsequently, the $ABTS^{+\cdot}$ solution was diluted with a 5 mM phosphate-buffered saline solution (pH 7.4) to obtain an absorbance of. 0.7±0.02 at λ=730 nm (t_0). Then, 0.1 mL of the sample extract was added to 3.9 mL of the diluted $ABTS^{+\cdot}$ solution. Additionally, absorbance readings were taken every 20 s using a spectrophotometer (LX510SS, Labdex, London, United Kingdom). The reduction of the radical was monitored for 6 minutes (t_6). The inhibition of absorbance vs time was plotted, and the area under the curve was calculated for the interval between 0 and 6 minutes. The degree of inhibition was calculated according to

Equation 18, and the antioxidant activity of the samples was expressed as μmoles ET 100 g^{-1} of dry weight of the sample.

$$\%_{inh} = \left(\frac{A_{t_0} - A_{t_6}}{A_{t_0}} \right) 100 \tag{18}$$

Where *inh* is the inhibition of absorbance (%), A_{t_0}, A_{t_6} are the absorbances during the time intervals t=0.6 min.

Statistical Analysis

For each sample, the results are presented as the mean ± standard deviation of three replicates. Differences between mean values were evaluated using Tukey's honestly significant difference (DHS) de Tukey, p≤0.05. Statistical calculations were performed using OriginPro 8.1 software (OriginLab; Northampton, MA, USA).

RESULTS AND DISCUSSION

Dehydration Process

Kinetics of Dehydration

According to Equation 1, MR_{ex} was obtained for different time intervals and dehydration temperature, T. Figure 5a shows $MR_{ex}-MR$ estimates, the MR_{est} curves, *vs. t* for different T values. As T increased, the values of t decreased from 2.9-2.1 h to achieve MR=10±2%, demonstrating that T was the parameter with the greatest influence on improving the duration of the process, similar to Beigi (2016), who reduced this parameter by 60% at 70 °C and 2 m s^{-1}. Figure 5b shows the fresh and processed apple slices according to Figure 5a.

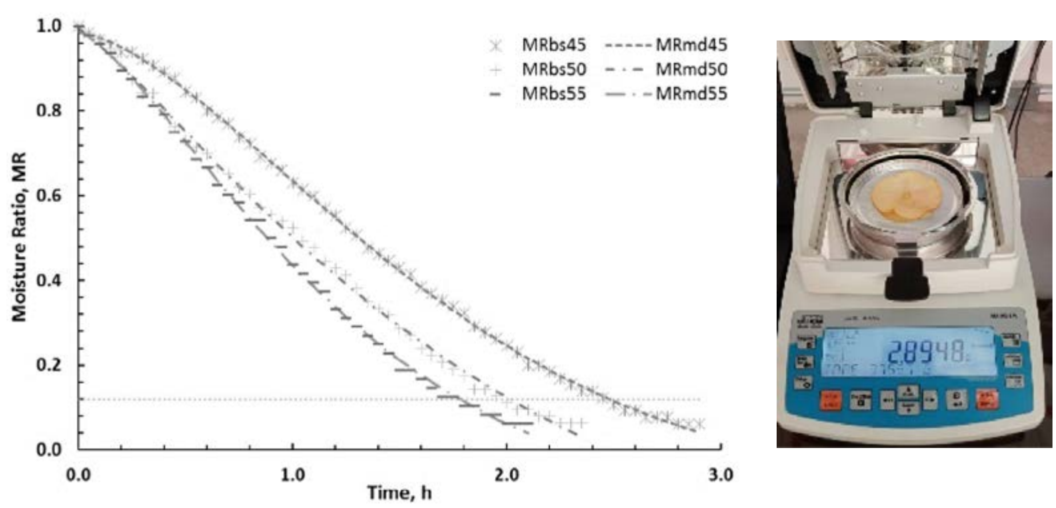

Figure 5. Deshydration of apple slices: (a) MR_{ex} y MR_{est}, *vs t*. (b) during the dehydration process. Source: Authors.

Plotting t *vs.* T yielded the expression to define the time at which MR is reduced to 10±2% with R^2=0.95, Equation 19:

$$t = -0.08\mathrm{T} + 6.45 \tag{19}$$

According to Nadi and Tzempelikos (2018), the values of t for a similar T ranged between (5-4) h, which was lower by (50-60) % compared to the results obtained here, demonstrating that T was the main factor influencing the duration of dehydration.

Dehydration Rate

The primary factor influencing the dehydration of apple slices was temperature; that is, at higher T, the values of D_{eff} and vapor pressure deficit increased (Dufera *et al.*, 2021). The values of DR ranged between (0.005-0.016) $kg_{\mathrm{H_2O}}\ kg_{MS^{-1}}\ \mathrm{min}^{-1}$ for T=(45-55) °C, similar to (0.001-0.05) $kg_{\mathrm{H_2O}}\ kg_{MS^{-1}}\ \mathrm{min}^{-1}$ obtained by Nadi and Tzempelikos (2018). These values were consistent for all temperatures, where stability in DR was more evident. Plotting DR *vs.* T generated Equation 20 with R^2=0.97.

$$\mathrm{DR} = 0.0813 \cdot e^{(0.0312 \cdot \mathrm{T})} \tag{20}$$

As T increased, the values of DR also increased due to the increase in water vapor concentration on the saturated surface of the slices; however, higher T values could cause greater shrinkage and product deterioration.

Mathematical modeling

Table 2 presents the mathematical modeling for dehydrating apple slices at different temperatures, where the Midilli-K model showed the best fit of the curves MR_{ex} *vs.* MR_{est}, with R^2=0.9987, RMSE=0.0108 and SSE=0.0054, followed by the Logarithmic and Kaleta models with R^2=(0.9963 and 0.9957). This is consistent with Noori *et al.* (2021) where this model showed a better fit to the data.

Adjustment in the Constant and Coefficients of the Model

The effect of temperature (T) on the dehydration constant, k, and the coefficients of the Midilli-K model was studied, along with their adjustment and validation.

The statistics for these parameters ranged from (0.9980-0.9994) for R^2, from 0.0076-0.0138 for RMSE, and (0.0032-0.0084) for SSE (Table 2). To fit the model to the data, Equation 21 expressed MR as a function of k, a, n, and b.

$$MR(k,a,n,b) = \frac{MR}{MR_0} = ae^{(-kt^n)} + bt \tag{21}$$

The values of k were directly proportional to T and were modified based on the properties of the product. From the graph of k *vs.* T, Figure 6, Equation 22 was obtained with an R^2=0.99

Table 2. Coefficients and statistics of the dehydration models, where: i) Kaleta, ii) Two-T., iii) Henderson and P., iv) Logarithmic, v) Midilli-K., vi) Page, vii) Diffusion Approximation and viii) Verma.

Model	T (°C)	Coeffs,	Values				R^2	RMSE	SSE
i	45	*k*,*a*	8.46	0.36	0.37	1.32	0.998	0.0105	0.0138
	50		−15.32	0.68	0.68	1.44	0.994	0.0240	0.0234
	55		3.30	0.86	0.86	1.49	0.996	0.0136	0.0187
ii	45	k_0,*a* k_1,*b*	−13.32	0.68	14.47	0.68	0.957	0.2343	0.0653
	50		11.10	0.23	−10.07	0.19	0.997	0.0990	0.0454
	55		4.62	0.29	−3.58	0.17	0.969	0.1200	0.0555
iii	45	*k*,*a*	1.14	0.71			0.955	0.2485	0.0660
	50		1.10	0.89			0.966	0.1400	0.0552
	55		1.12	1.02			0.967	0.1255	0.0553
iv	45	*k*,*a* *c*	2.33	0.20	−1.28		0.994	0.0286	0.0226
	50		1.89	0.32	−0.87		0.997	0.0106	0.0154
	55		1.75	0.42	−0.71		0.996	0.0131	0.0181
v	45	*k*,*a* *n*,*b*	0.98	0.40	1.60	−0.02	0.999	0.0032	0.0076
	50		0.99	0.54	1.24	−0.08	0.998	0.084	0.0138
	55		0.99	0.73	1.39	−0.04	0.998	0.0046	0.0109
vi	45	*k*,*n*	0.46	1.65			0.997	0.0129	0.0150
	50		0.72	1.46			0.992	0.0296	0.0254
	55		0.86	1.49			0.996	0.0136	0.0182
vii	45	*k*,*n*	8.74	0.09	0.54		0.958	0.2313	0.0643
	50		11.05	0.17	0.77		0.996	0.0410	0.0302
	55		0.69	0.90	1.00		0.949	0.1983	0.0704
viii	45	*k*,*a* *g*	−10.86	0.05	0.08		0.993	0.0383	0.0261
	50		−37.78	0.28	0.28		0.996	0.0130	0.0170
	55		175.20	1.36	1.36		0.969	0.1172	0.0541

Source: Authors.

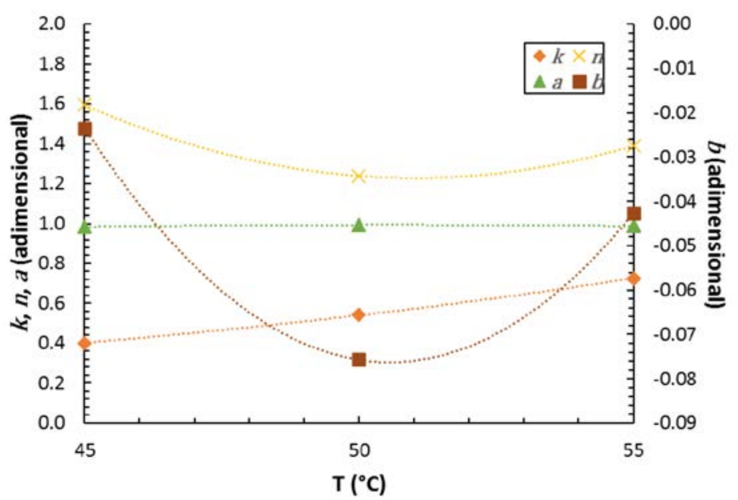

Figure 6. Midilli model constant, *k*, and *a*, *n*, *b*, *vs*. T. Source: Authors.

$$k = 0.0283 \cdot e^{(0.0590 \cdot \mathrm{T})} \tag{22}$$

The values of *a* and *n* maintained a positive relationship with respect to T. The graph of *a vs.* T showed a dispersion of R^2=0.925, with an average of *a*=0.9888. Plotting *n vs.* T resulted in a dispersion of R^2=0.9909, with *n*=1.408. Meanwhile, *b* maintained a negative relationship with T. Through the *b vs.* T curve with R^2=0.9925, an average of *b*=−0.0472 was obtained. It is worth mentioning that for fitting the model, it was not necessary for all parameters to show a similar dispersion among them.

Generalization of the MR Curve

The possibility of adjusting a general expression to the apple slice dehydration data based on the Midilli-K model was verified to determine the *MR* values *vs.* τ for each T. For this purpose, the value of *k* from Equation 22 and the average of the parameters *a*, *n*, and *b* were substituted into Equation 21. Thus, the general expression to model the process was derived as Equation 23.

$$MR_{aj} = 0.99e^{\left[-(k)\tau^{1.41}\right]} - 0.0473\tau \tag{23}$$

Model Validation

MR_{aj} *vs.* MR_{ex} was contrasted, showing an average dispersion of R^2=0.99 around the fit line (Table 3), thus demonstrating the effectiveness of the adjusted model for dehydrating apple slices.

Effective Diffusivity and Activation Energy

Effective Diffusivity

To correlate ln(*MR*) *vs.* *t*, linear regression was applied, with an average goodness of fit of R^2=0.98, Equation 24 (Table 3).

$$\ln(MR) = At + B \tag{24}$$

where *A* and *B* (dimensionless) are temperature-dependent constants.

D_{eff} was obtained according to k_o and *L*, Equation 7. Increasing T led to increased D_{eff} values ranging between, (89.83 E^{-08}-1.24 E^{-06}) $m^2\ s^{-1}$, with R^2=0.96 (Table 4). These

Table 3. Correlation of MR_{ex} *vs.* MR_{aj} and coefficients of ln(*MR*), with k_o *t*, for different temperature values.

T (°C)	MR_{aj} - MR_{aj}	R^2	Coeficientes		R^2
			A=$-k_o$	B	
45	0.98·MR−0.01	0.99	−0.99	0.39	0.94
50	1.00·MR+0.02	0.99	−1.20	0.34	0.95
55	1.02·MR−0.01	0.99	−1.36	0.33	0.96

Source: Authors.

values fall within the permissible range for dehydrating food products, $(10^{-11}\text{-}10^{-6})$ $m^2\ s^{-1}$ (Olanipekun *et al*, 2015). This differs from Beigi (2016), who reported D_{eff} values of (6.75 E^{-10}-1.28 E^{-09}) $m^2\ s^{-1}$, in a range of T=(50-70) °C, with v=(1-2) m s^{-1}.

When contrasting D_{eff} *vs.* MR at different temperatures, D_{eff} values were directly proportional to T. The maximum values were obtained when MR_{ex} decreased, *i.e.*, at the end of dehydration. This allowed verifying that, at higher T, D_{eff} values increased, resulting in a shorter duration of the process. In other words, the internal mass transfer of the product was achieved by diffusion rather than evaporation on the surface. Consequently, the moisture content was extracted in a shorter period of time.

Activation Energy

With the slope and intercept of the ln(D_{eff}) *vs.* 1 T^{-1} line, Equation 25, R^2=0.98, the values of the pre-exponential Arrhenius factor, D_o, and activation energy, E_a, in apple slices were 3.74 E^{-02} $m^2\ s^{-1}$ y 28.11 kJ mol^{-1}, similar to the 23.42 kJ mol^{-1} reported by (Martynenko and Janaszek, 2014), where E_a falls within the permissible range for processing foods and agricultural products.

$$\ln\left(D_{eff}\right) = -\frac{3380.46}{T} - 3.29 \tag{25}$$

The above result was 2.8 kJ mol^{-1} lower than the E_a obtained by Meisami-asl *et al.* (2010) in the Golab variety, meaning in this case, the energy requirements and process costs for dehydrating the product should be 9% lower.

Thermodynamic Properties

During the dehydration of apple slices, the influence of enthalpy, entropy, and Gibbs free energy was observed (Table 4), obtaining positive values of ΔH, which, as T increased, decreased from 25.46-25.38 kJ mol^{-1}, meaning that higher T requires less energy to remove moisture content from the product (Oliveira *et al.*, 2010).

Meanwhile, the values of ΔS decreased from −0.2728-−0.2730 kJ mol^{-1} K^{-1}; these negative values indicate chemical adsorption or structural modifications in the product (Almeida *et al.*, 2021), *i.e.*, on its surface due to water vapor.

Finally, ΔG increased from (112.25-114.98) kJ mol^{-1}, as observed by Nadi and Tzempelikos (2018) when dehydrating apple slices, who obtained ΔG=(118.27-123.39) kJ

Table 4. D_{eff} and Thermodynamic Properties in the Dehydration of Apple Slices.

T (°C)	D_{eff} ($m^2\ s^{-1}$)	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
45	89.83 E^{-08}	25.46	−0.2727	112.25
50	01.09 E^{-06}	25.42	−0.2729	113.61
55	01.24 E^{-06}	25.38	−0.2730	114.98

Source: Authors.

mol^{-1} for T=(50-70) °C. The positive values indicate that external energy input is required for the process to occur, as it does not happen spontaneously (Nadi and Tzempelikos, 2018). Accordingly, for the studied range of T, the values of ΔH, ΔS, and ΔG can be determined using Equations (26-28), with

R^2 values of 0.9999, 0.9997 y 0.9998.

$$\Delta H = -8.31T + 25835.40 \quad (26)$$

$$\Delta S = -0.0257T - 271.63 \quad (27)$$

$$\Delta G = 272.92T + 99967.81 \quad (28)$$

Total Phenolic Compounds and Antioxidant Activity

Total Phenolic Content

This parameter, determined in the dehydrated apple samples, can be observed in Figure 7, where the initial total phenolic content was 168.67±2.16 mg EAG 100 g^{-1} of the fresh sample. Among the evaluated dehydration temperatures, the treatment at 45 °C presented the highest amount of phenolic compounds with 51.1±1.54 mg EAG 100 g^{-1}. For the dehydration treatments of the apple slices at 50 and 55 °C, the analysis of variance showed that there was no statistically significant difference ($p \leq 0.05$). It was observed that the increase in dehydration temperature caused a degradation of the total phenols with respect to the content present in the sample.

Several studies have demonstrated that different factors such as variety, geographical region of cultivation, and even the botanical fraction used for determination (skin, pulp, and whole fruit) have an effect on the total phenolic content (CFT) (Biedrzycka *et al.*, 2008). In a study by Corona-Leo *et al.* (2020), seven apple varieties were evaluated, showing a statistically significant difference ($p \leq 0.05$) in CFT present in the skin, pulp,

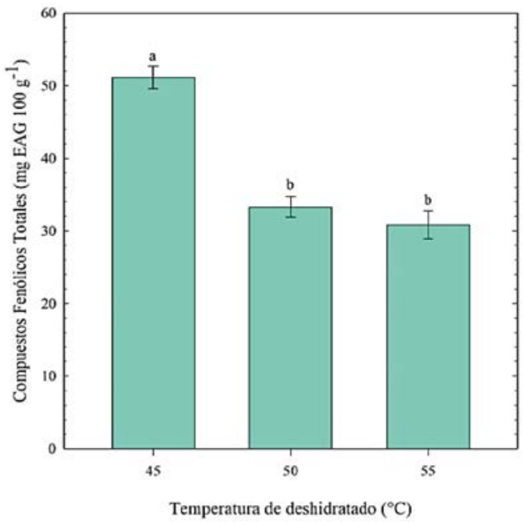

Figure 7. Total phenolic content in dehydrated Golden Delicious apple slices at three different temperatures. The columns represent the mean of n=3 replicates ± standard error.

and whole fruit. The authors also reported that the highest CFT was found in the skin of all apple varieties, ranging from 109.20-257.20 mg EAG 100 g^{-1} in the Golden Delicious imported and Granny Smith varieties, respectively. Regarding the whole fruit, TPC values ranged from 137.2 mg EAG 100 g^{-1} in Golden Delicious to 315.87 mg EAG 100 g^{-1} in Granny Smith apples. These values are consistent with the results obtained in this research (168.67±2.16 mg EAG 100 g^{-1}) for fresh apple samples. In another study by Rana *et al.* (2021), the CFT determined by the Folin-Ciocalteu assay varied between 219±0.09 and 459±0.47 mg EAG 100 g^{-1} in a comparative evaluation of five apple varieties grown in the Western Himalayas, including the Golden Delicious variety, whose value is higher than that reported in this work. Pires *et al.* (2018) quantified the CFT in the 'Bravo de Esmolfe' variety using high-performance liquid chromatography with a diode array detector coupled to mass spectrometry (HPLC-DAD-ESI-MS), obtaining 237±1 mg EAG 100 g^{-1} for the hydro-methanolic extracts of their samples, confirming that CFT in fresh apple fruits is strongly associated with fruit variety.

Furthermore, various studies have shown that multiple factors affect CFT due to the dehydration process; however, the most significant effect corresponds to temperature. In this regard, efforts have been made to determine the optimal conditions to ensure product quality, particularly in minimizing changes in bioactive compounds such as phenolic compounds. In the study by Kahraman *et al.* (2021), three methods were evaluated to characterize dehydration and quality attributes in slices of *Malus*×*domestica* Borkh var. Gala apples, where the authors reported the highest CFT by the freeze-drying method (68.82 mg EAG 100 g^{-1}) and the lowest value (16.12 mg EAG 100 g^{-1}) by the hot air dehydration method at 60±1 °C. These values were approximately consistent with the experimental results obtained in the present study; however, it should be noted that the evaluated fruit variety was different.

CFT is associated with the antioxidant capacity of biological products; however, it is known that phenols are sensitive to temperature. Therefore, the main focus of this study was to find process conditions that would allow the product to be dehydrated while retaining the highest amount of bioactive compounds, as phenolic compounds are functional food nutrients with excellent antioxidant and anti-inflammatory capabilities (Wang *et al.*, 2023).

Antioxidant Activity

DPPH and ABTS Radical Scavenging Assays

The initial radical scavenging activity in the fresh sample was 766.9±91.4 and 973.3±103.4 μg mg^{-1} according to the DPPH and ABTS methods, respectively (Figure 8), where it was confirmed that the highest values of antioxidant activity showed an inverse behavior with respect to the dehydration temperature; that is, the highest antioxidant activity was observed at the lowest dehydration temperature (45 °C). In this regard, Demiray *et al.* (2023) evaluated the effect of three temperatures (45, 55, and 65 °C) on two thicknesses of Granny Smith apple slices (1.5 and 5 mm), where the highest antioxidant capacity values were observed, unlike this study, at 55 °C with 34.44 and 31.45 μmoles equivalents of Trolox for 1.5 and 5 mm thicknesses.

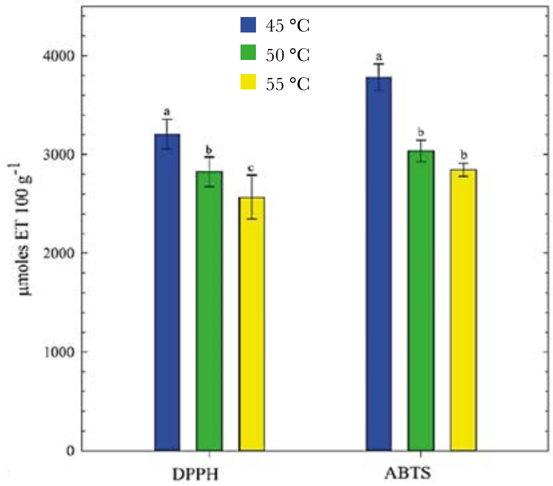

Figure 8. Effect of dehydration temperature and the method of antioxidant activity evaluation using DPPH and ABTS assays, where each column represents the mean of n=3 replicates ± standard error.

Additionally, the results showed that the ABTS assay produced higher values of antioxidant activity compared to those determined by DPPH for all three temperatures. In this regard, it has been mentioned that most methods are limited by the antioxidant extraction technique, as some compounds may remain forming complexes in the extraction residues (Serrano *et al.*, 2007).

On the other hand, the differences between the antioxidant capacity of the fresh sample and that after the dehydration process could be attributed to the generation of Maillard reaction-derived compounds by pyrolysis, which can enhance antioxidant capacity and FRAP during dehydration at moderately high temperatures (López-Vidaña *et al.*, 2019). Antioxidant compounds could be promising agents for managing diseases related to oxidative stress. The results demonstrated that, for the case study, the temperature of 45 °C could prevent the destruction of total phenolics and compounds with antioxidant activity in dehydrated apple slices.

CONCLUSIONS

The kinetics of thin-layer dehydration of apple slices was studied through mathematical modeling to evaluate the effect of temperature on the moisture ratio of the product. As the temperature increased, the processing time for the product between 45 and 55 °C decreased from 174-126 minutes. It was demonstrated that the Midilli-K. model better described the dehydration process, showing the best goodness of fit to the data. The effects of temperature on the constant and coefficients of the model were studied, with this constant being directly proportional to temperature. The dehydration rate, effective diffusivity, and activation energy in apple slices increased with increasing temperature. The thermodynamic properties of the process were determined under the established dehydration conditions. As the temperature increased, the enthalpy and entropy decreased, with the latter values being negative, while the Gibbs free energy increased. With the information obtained, it could be possible to establish the temperature and time at which

Symbols and Abbreviations.

Symbol	Description
AER	Radical scavenging activity (%)
A, *B*	Temperature-dependent constants, dimensionless
ABTS	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
aj	Adjusted
A_o	Absorbance of blank or control sample
A_s	A of sample extract
A_{t_0} , A_{t_6}	A during time intervals t=0.6
a, *e*, *n*, *b*, *c*, *g*	Time-dependent constants, dimensionless
DR	Dehydration rate $\left(kg_{\mathrm{H_2O}}\ kg_{MS^{-1}}\ \mathrm{min}^{-1}\right)$
D_o	Frequency or pre-exponential factor of Arrhenius ($\mathrm{m^2\ s^{-1}}$)
D_{eff}	Effective diffusivity ($\mathrm{m^2\ s^{-1}}$)
DHS	Significant honest difference
DPPH	2.2-diphenyl-1-picrylhydrazyl
Δ	Increment
EAG	Gallic acid equivalents
E_a	Activation energy ($\mathrm{J\ mol^{-1}}$)
est	Estimated
ex	Experimental
F_o	Fourier number (dimensionless)
G	Gibbs free energy ($\mathrm{J\ mol^{-1}}$)
H	Enthalpy ($\mathrm{J\ mol^{-1}}$)
h_P	Planck's constant ($6.626{\cdot}\mathrm{E}^{-34}\ \mathrm{J\ s^{-1}}$)
inh	Inhibition
k_B	Boltzmann constant ($1.38{\cdot}\mathrm{E}^{-23}\ \mathrm{J\ K^{-1}}$)
k_o	Slope of ln(*MR*) *vs.* *t* line
L	Material thickness (m)
MR	Moisture ratio (dimensionless)
M_t	Moisture ratio at time t $\left(g_{\mathrm{H_2O}}\ g_{MS^{-1}}\right)$
M_o	Initial moisture ratio of product $\left(g_{\mathrm{H_2O}}\ g_{MS^{-1}}\right)$
M_e	Equilibrium moisture ratio $\left(g_{\mathrm{H_2O}}\ g_{MS^{-1}}\right)$
MR_o	*MR* initial (dimensionless)
m	Mass, kg
N_c	Dehydration rate constant ($1\ \mathrm{min^{-1}}$)
n	Number of terms (dimensionless)
R	Universal gas constant ($8.3143\ \mathrm{J\ mol^{-1}\ K^{-1}}$)
R^2	Coefficient of determination
RMSE	Root mean square error
SSE	Sum of squares due to error
S	Entropy ($\mathrm{J\ mol^{-1}\ K^{-1}}$)
T	Absolute temperature (K)
T	Temperature, °C
t	Time, min
τ	Time (dimensionless)
ν	Air velocity, $\mathrm{ms^{-1}}$

apple slices could be processed according to local climatology, whether dehydrating the product outdoors or under controlled systems.

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AUTHORS' CONTRIBUTIONS

For this work, the authors performed the following activities:

Conceptualization: Laureano-López, B.; Daza-Merino, C. A.; Santos-Hernández, A. M. Data acquisition: Laureano-López, B.; Santos-Hernández, A. M.; Riviello-Flores, M. L. Data analysis: Laureano-López, B.; Daza-Merino, C. A.; Santos-Hernández, A. M.; Daza-Merino, R.

Methodology design: Laureano-López, B.; Daza-Merino, C. A.; Santos-Hernández, A. M.

Writing and editing: Laureano-López, B.; Santos-Hernández, A. M.; Riviello-Flores, M. L.; Daza-Merino, R.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Shrimp aquaculture, absorption capacity and competitiveness: an analysis based on the case of Ahome, Mexico

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ABSTRACT

Objective: to present empirical evidence of the effect that the Knowledge Absorption Capacity (AC) and its dimensions have on the competitiveness of shrimp farming companies.

Design/Methodology/Approach: a multiple linear regression analysis was performed to determine the degree of influence of the independent variables (AC dimensions) on the dependent variable (competitiveness).

Results: There was a positive effect between AC dimensions and competitive performance. The capacity for transformation of knowledge was the strongest effect.

Study limitations/Implications: due to time and resource constraints, this study was based on a survey limited to a small number of businesses in the municipality of Ahome (Sinaloa), Mexico. Therefore, these results could be specific to the activity evaluated and to that region.

Findings/Conclusions: studies such as this represent a turning point in exposing the importance of conceiving AC as a complex and multidimensional construct that contemplates the processes of acquisition, assimilation, transformation and exploitation of knowledge.

Keywords: knowledge absorption capacity, competitiveness, shrimp farmers, Ahome.

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INTRODUCTION

Globally, efficient and productive agrifood systems are key to addressing food security challenges; and involve the adoption of production practices that are economically viable, socially equitable and environmentally responsible. With a view to guaranteeing the availability of quality food for present and future generations without compromising the quality of the environment (Varela and Moraga, 2020). Aquaculture has established itself as a relevant economic activity that has tripled its production globally in recent decades and has directly favoured employment and social welfare in rural areas. In particular, shrimp farming has become an important pillar of global food security and one of the most dynamic agri-food activities. Sinaloa is the state with the highest contribution to national shrimp production.

However, the activity faces great environmental, innovation, biosecurity and mortality challenges. In particular, producers have had to face recurrent epidemics that attack shrimp in situations of stress or environmental disturbances, and cause substantial economic losses (Tang and Bondad-Reantaso, 2020). Therefore, it is crucial that producers are aware of technological advances in areas such as genetic resources, probiotics, production systems with low environmental impact, food, vaccines and treatments, in order to mitigate the economic, social and environmental impacts of the production crises that this activity has presented.

As smallholders with limited resources to invest in their own research and development activities, shrimp farmers in countries with emerging economies seek to access, acquire, and adopt innovative technologies for aquaculture through informal relationships and social networks (León *et al.*, 2020). Producers can obtain information, ideas, and knowledge about technologies from external sources, such as universities and technology institutes, research centers, government agencies, suppliers, competitors, customers, private consultants, among others. But it is one thing to have knowledge providers that allow knowledge to be acquired about new and emerging technologies, and quite another for that knowledge to be learned, internalized, and effectively absorbed by producers.

The ability of shrimp farmers to efficiently obtain and absorb knowledge about new and emerging technologies has been little studied in the regional context. This capacity has been called Knowledge Absorption Capacity (AC) in the administrative sciences (Cohen and Levinthal, 1990). Studies on AC agree that AC is a key factor in the competitive success of companies in the current context of the knowledge economy (De Zubielqui *et al.*, 2016).

There is a significant increase in the literature focused on AC, but important knowledge gaps persist. For example, although several academics have broken down the AC process into particular dimensions (Camisón and Forés, 2010; Flatten *et al.*, 2011), this fragmentation and the importance that each dimension has in business performance are issues that are not completely elucidated, since few studies have focused on the relationships between multiple dimensions of knowledge, AC and company performance.

Therefore, this study aimed to analyze how the particular capabilities that compose AC and the competitive performance of the company are associated, through presenting empirical evidence of the effect that AC and its dimensions have on the competitiveness of shrimp companies in Ahome, Sinaloa.

MATERIALS AND METHODS

The study was quantitative and correlational, to visualize how the dimensions of AC were related to the competitiveness of shrimp farms. The empirical research is limited to the case of aquaculture developed in a productive region located in the north of Sinaloa (the municipality of Ahome), which stands out for its production levels.

Modelo conceptual

Zahra and George (2002) proposed a reconceptualization of AC that is widely accepted and used; they define it as a set of routines and processes through which organizations acquire, assimilate, transform, and exploit knowledge. According to those authors, acquisition capacity refers to a company's ability to recognize and obtain externally generated critical knowledge. Assimilation refers to the routine operations of the company that allow it to analyze and understand the information acquired. The capacity for transformation allows the development and improvement of procedures that facilitate the combination of existing and new knowledge. Knowledge exploitation capacity is based on activities that help companies to improve and build on existing skills and to establish new ones (Zahra and George, 2002).

Location and characteristics of the study region

Ahome is one of the 18 municipalities of the state of Sinaloa, Mexico. It is the third most important municipality in the state of Sinaloa and a commercial bridge to the northwest of the country. It is located on the Pacific coastal plain, at the entrance to the Gulf of California, and in the heart of a rich agricultural region, the El Fuerte Valley. Ahome is the municipality with the greatest contribution to shrimp production in the state and is one of the most suitable for aquaculture at the national level since it has 120 km of coastline that allow the formation of bays, islands, estuaries and lagoons (Figure 1). The municipality has 11 000 ha available for the development of aquaculture farms, of which approximately 8700 ha are used, with 85 aquaculture production units.

Data

Data was obtained from a survey applied to a sample of 64 aquaculture production units dedicated to shrimp farming in the study region. The survey was applied to farm administrative personnel, such as owners or managers, as it was considered that they should be directly responsible for planning, organizing, directing and controlling aquaculture operations.

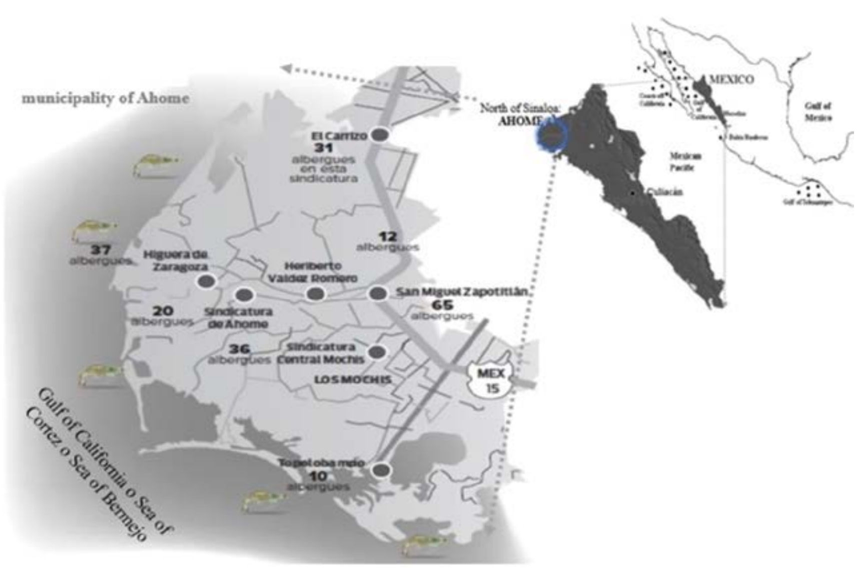

Figure 1. Location of the aquaculture region of Ahome (Sinaloa), Mexico.

The questionnaire consisted of three sections, the first aimed at obtaining information from companies and managers. The second contained the items of the scale designed to measure the different dimensions of the AC, and the last section contained the items to measure the competitive performance of the shrimp farms. Table 1 shows both the variables and the items used in the scale to measure these variables, as well as the sources of previous studies that were taken as a reference for the items used.

Regarding the reliability analysis of the scale, the consistency of the items was evaluated with the Cronbach's Alpha test; values greater than 0.80 were obtained; the measurement of reliability with Cronbach's Alpha assumes that the items (on a Likert-type scale) estimate the same construct and that they are highly correlated.

Table 1. Scale items to measure the dimensions of knowledge absorption capacity (AC) and competitiveness.

Dimensions of the Knowledge Absorptive Capacity
ACQUISITION CAPACITY (Flatten *et al.*, 2011; Tepic *et al.*, 2012; Pradana *et al.*, 2019). 1=completely disagree and 5=completely agree. The farm: - Obtains information on aquaculture technologies through contacts with actors in the sector - Participates in seminars and conferences to update and enrich its technical knowledge. - Allocates enough time for the establishment of contacts with agents that provide information on innovations - Has the skills to establish contacts with agents that provide knowledge and information about innovations
ASIMILATION CAPACITY. (Pradana *et al.*, 2019; Flatten *et al.*, 2011; Müller *et al.*, 2021). 1: completely disagree and 5: completely agree The farm: - Records and stores newly acquired knowledge for future reference. - Has enough skills to recognize changes in technical possibilities. - Allocates enough time to deliberate with advisors in order to recognize technological advances. - Has enough skills to deliberate with advisors on how new technologies can be used in farm
TRANSFORMATION CAPACITY (Pradana *et al.*, 2019; Müller *et al.*, 2021) 1: completely disagree and 5: completely agree The farm: - Recognizes in a timely manner the usefulness of new external knowledge to expand its own internal knowledge. - Allocates enough time for the translation of external information into adaptations to the company's own business. - Has enough skills to translate external information into adaptations to our business.
EXPLOTATION CAPACITY (Tepic *et al.*, 2012) 1: completely disagree and 5: completely agree The farm: - Can translate external information directly into new business applications. - Applies external information to our business to contribute to our productivity. - Has enough skills to convert external information into productive results.
Farm competitiveness
COMPETITIVENESS (Tepic *et al.*, 2012) 1: much smaller and 5: much larger Regarding your farm, how is ….?: - Profitability compared to its competitors. - Market share compared to its competitors. - Product quality compared to its competitors. - Level of exports compared to its competitors. - Cost-benefit efficiency compared to its competitors.

Statistical analyses

First, the data were subjected to a correlation analysis (Pearson and Spearman's Rho), in order to obtain a statistical measure that expressed the magnitude to which the dimensions of the AC had a linear relationship with competitive performance and with each other. Afterwards, a multiple linear regression analysis was performed to determine the degree of influence of the independent variables (the AC dimensions) on the dependent variable (Company competitiveness).

RESULTS AND DISCUSSION

Sample Features

Regarding the size of the companies participating in this study, the vast majority (93.7%) are small and medium-sized enterprises (in Mexico, Pymes). In regard to the age of the companies, 84.4% were founded within the last 15 years. This means that shrimp farming is an activity that has expanded and developed very recently. More than 80% of the companies participating in the survey belong to the private sector, while only 15.6% belong to corporate property, so called social, in particular to the Communal Land System (Table 2).

Correlation Analysis

The results of the correlation analysis indicated that the dimensions of AC were positively correlated with the competitive performance of the company. This means that companies with higher ACs are more likely to perform better competitively. In particular, the variables ASIMIL (assimilation capacity) and TRANS (transformation capacity) showed a positive and significant correlation ($p \leq 0.01$) with competitive performance, with Pearson correlation values of 0.354 and 0.357, respectively.

Table 2. Characteristics of the companies participating in the study.

Size	Frequency	Percentage
1 to 50 Employees	37	57.8
51 to 250 Employees	23	35.9
251 Employees or more	4	6.3
Total	64	100.0
Age	Frequency	Percentage
5 to 10 years old	29	45.3
11 to 15 years old	25	39.1
16 to 20 years old	5	7.8
21 years and older	5	7.8
Total	64	100.0
Sector	Frequency	Percentage
Private	54	84.4
Social	10	15.6
Total	64	100.0

The results of the non-parametric correlation analysis using Spearman's Rho correlation coefficient indicated a positive and significant correlation ($p \leq 0.05$) with competitive performance (COMPE), the variables ASIMIL (0.308), TRANS (0.337) and EXPLO (0.259). The ADQ dimension (ability to acquire knowledge) was the only one that did not present a significant correlation in both implemented tests.

It is important to consider that correlation analysis does not prove causation. However, the results of these analyses suggest that AC is an important factor associated with the competitive performance of aquaculture farms in the region of the municipality of Ahome. Overall, the results of these analyses suggest that those aquaculture farms in the region that wish to improve their competitive performance should focus on improving AC.

Regression Model

The multiple regression model demonstrated the ability to explain 18.1% of the variability in the competitive performance of Ahome's aquaculture companies ($R^2=0.181$, $p<0.05$), as shown in Table 3. It is essential to highlight that the adjusted R^2 value, which considers the number of variables in the model, was 0.126; This means that the model's predictor variables contributed ($p \leq 0.05$) with 12.6% of the variation in competitive performance. This result highlights the relevance of the variables in the model, and supports the idea that they play a crucial role in understanding the competitive performance of aquaculture companies. The probability of error was less than 5% ($p \leq 0.05$), which confirms the validity of the model. In summary, the multiple regression model, when considering the adjusted and significant R^2, provided a solid and relevant view of how variables influence the competitive performance of these companies in the region.

The analysis of variance of the multiple regression model indicated that, for shrimp farms in the municipality of Ahome, the model is better ($p \leq 0.05$) to predict the competitive performance than using only the average performance ($F=3.268$, $p \leq 0.05$; Table 3). The analysis compared variation between groups (in this case, groups of companies with different levels of knowledge and technology absorption capacity) with variation within groups. The F-value is a measure of the proportion of total variation that is attributable to variation among groups. In this case, the value 3.268 indicates that the variation among groups is significantly greater than the variation within the group. This suggests that the dimensions of the AC explain an important part of the variation in the competitive performance of Ahome's aquaculture farms. In other words, companies with higher AC have significantly better competitive performance than companies with lower capacity.

According to the analysis of the coefficients of the model (Table 3), it is observed that the competitive performance of shrimp farms increases to the extent that their capacity to assimilate and transform knowledge increases. However, when examining the individual regression coefficients for each independent variable, only the capacity for knowledge transformation (TRANS) was found to be significant ($p \leq 0.05$). The model includes a constant of 13.029 with a standard error of 4.7 and $t=2.772$ ($p \leq 0.01$). In summary, results indicated that, although the model as a whole is statistically significant, only the individual coefficient of knowledge transformation capacity (TRANS) has effects on the competitiveness of the companies studied.

Table 3. Results of the regression analysis to Competitiveness (COMPE)[a].

COEFFICIENTS							
Model	**Unstandardized coefficients**		**Standarized coefficient**	**t**	**Sig.**		
	ββ	**Std.error**	**ββ**				
1 (Constant)	13.029	4.700		2.772	0.007		
ADQ	−0.015	0.256	−0.008	−0.06	0.952		
ASIMIL	0.278	0.175	0.279	1.59	0.117		
TRANS	0.369	0.186	0.481	1.99	0.050		
EXPLO	−0.636	0.424	−0.371	−1.501	0.139		
MODEL SUMMARY							
Model	**R**	**R^2**	**R^2 adjusted**	**Change statistics**			
				Change of square of R	**Change in F**	**df1**	**df2**
1	.426[a]	.181	.126	.181	3.268	4	59
VARIANCE							
Model			**Sum of squares**	**gl**	**Root mean square**	**F**	**Sig.**
1	Regression		168.228	4	42.057	3.268	.017[b]
	Residual		759.257	59	12.869		
	Total		927.484	63			

[a] Dependent variable: COMPE. Predictors: (Constant), ADQ, ASIMIL, TRANS, EXPLO.

Model evaluation

Evaluation tests of the multiple linear regression model were also performed and the model met the validity criteria for nonparametric tests. It was concluded that the model met these criteria A) normal distribution of residuals (Shapiro-Wilk 0.971; $p=0.139$); B) homoscedasticity (Breusch-Pagan, 1.135; $p=0.286$); C) no autocorrelation among residuals (Durbin-Watson 0.307; $p=0.128$); and D) there was no multicollinearity, the variance inflation factor (FIV) yielded values <10 for each predictor of the model.

The results of the regression model are consistent with those of other studies in which a positive effect of the ability to absorb knowledge has also been found. Either in their capabilities or in particular dimensions of business performance, especially competitiveness and innovation (Xie *et al.*, 2018). They also coincide with those other studies on the matter, arguing that aquaculture companies should consider AC as a driver that helps them achieve a stronger competitive advantage and superior business performance (Lichtenthaler, 2016). AC not only plays a critical role in competitiveness (Camisón & Forés, 2010), but also allows companies to reach leading positions (Tzokas *et al.*, 2015) and to develop dynamic organizational capabilities (Zahra & George, 2002; Camisón & Forés, 2010).

Results also coincide with those of some authors who have only very recently worked in greater depth on research consisting of determining the differentiated role played by AC dimensions in business performance (Zobel, 2017; Kafouros *et al.*, 2020; Knoppen *et al.*, 2022). This research shows that the positive effects of knowledge provided by external actors to improve performance are not the same for all companies, but depend on certain dimensions of absorption capacity. The particularity of our results is that the capacity for knowledge transformation was the only one with a proven and significant positive effect on competitiveness. It is important to broaden the topic of study to the national context in future research. That is, to move towards determining the type of relationship that exists among different dimensions of AC, as some analysts are already doing in China (Xie *et al.*, 2018).

CONCLUSIONS

This study focused on identifying the effect of the different dimensions of the knowledge absorbtion capacity on competitiveness and highlighted the relevance of conceiving it as a complex and multidimensional construct. It also revealed the importance of the ability to assimilate external knowledge in competitiveness. The reinforcement of this capacity can be achieved through the development of an organizational environment that favors technological learning in all areas of the company to enhance the impact of external knowledge on the acquisition of new technologies.

Shrimp farmers should intensify the links that strengthen their capacities to access external knowledge, to maximize the quantity and diversity of technical information obtained; to link and interact with organizations as varied as possible, such as suppliers, customers, government, universities, research centers and civil associations. The study had some limitations, such as the limited number of farms in a single location. Also, that it presents the different dimensions of knowledge absorbtion capacity as determinants of competitiveness, without considering whether some of them play a role in mediating the effect of others.

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Morphological characterization of cacao (*Theobroma cacao* L.) criollo type in Mexico

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ABSTRACT

Objective: Achieve the morphological characterization of Criollo-type cocoa from Mexico.
Design/methodology/approach: For morphological characterization, 17 Criollo-type cocoa accessions and 30 varietal descriptors were proposed by Avendaño *et al*., (2014), and this was carried out on five-year-old trees in the Rosario Izapa Experimental Field of INIFAP during two production cycles.
Results: With the first three principal components, 47.3% of the variation was explained and the variables that most explained this variation were the color of the young leaf, anthocyanin pigmentation of the pedicel, basal constriction of the fruit, shape of the fruit apex and length/diameter ratio of the fruit; sepal length, sepal width length, fruit length, fruit exocarp thickness, seed width, seed length/diameter ratio and cotyledon color. The cluster analysis allowed us to differentiate two groups where the color of the unripe fruit was one of the descriptors that contributed the most to forming the groups.
Limitations on study/implications: Knowledge of the diversity of Mexican Criollo-type cocoas allows for establishing strategies for conserving and using this type of cocoa.
Findings/conclusions: In Mexico, the Criollo cacao genetic group presents a wide morphological variation in the descriptors of leaf, flower, fruit and seed. The shape of the apex of the leaf, the anthocyanin pigmentation in the flower, the color in the mature and immature state, and the shape and basal constriction of the fruit, as well as the color of the cotyledon, are the descriptors that allowed us to differentiate the Criollo cocoas studied.

Keywords: Criollo cocoa, diversity, characterization.

INTRODUCTION

In Mexico, the cultivation of cocoa (*Theobroma cacao* L.) is of great cultural, economic, social and environmental importance since most of the area planted in Mexico is under the agroforestry system (Barómetro del cacao, 2022). Mexico ranks 12th worldwide in

production, accounting for approximately 0.8% (ICCO, 2024) and currently, around 52,449 ha are cultivated, with a production of 28,119 tons and a production value of $1,158,661 (SIAP, 2024). In Mexico, three genetic types of cocoa are recognized: forastero (*T. cacao*, subs. *sphaerocarpum*), criollo (*T. cacao* subs. *cacao*) and the cross between these, trinitario (Lachenaud & Motamayor, 2017); each with its particular morphological characteristics (Cuatrecasas, 1964) and with different varieties within each genetic group.

For their part, Avendaño *et al.*, (2011) mention that in Mexico, the three genetic groups of cocoa are cultivated with different varieties, with Forastero and Trinitario predominating and, to a lesser extent, Criollo type cocoas, even though the international demand for Criollo and Fine Aroma types has grown (Barómetro del cacao, 2022). In addition, Avendaño *et al.* (2018) disseminate Criollo-type cocoas and describe the morphology of the fruits of some Criollo cocoas from Mexico. The fruits of the Forastero-type cocoa are melon-shaped, have a hard and woody shell, are smooth to medium roughness, are flattened beans, and are purple. In Criollo types, the fruits are elongated and green and reddish, with roughness, absent or light basal constriction and rounded white seed color, and these have a higher quality than Forastero and Trinitario cocoa (Avendaño *et al.*, 2018). On the other hand, Trinitario cocoas have fruits with variable roughness, thin to medium shell, the seed is pink to purple, and the flavor and aroma are delicate to medium. In the different cocoa-growing areas of the world and Mexico, different types of characterization have been carried out, highlighting the morphological, biochemical and molecular to know the genetic diversity of the different varieties of the cocoa present, focusing mainly on the fruit and the seed; however, it is necessary to know the morphological diversity of the plant, leaf, flower, fruit and seed (Avendaño *et al.*, 2014), to correlate specific morphological markers of the leaves, with the flower and finally with the seed, the morphological characterization of Criollo cocoas was carried out, to differentiate the Criollo type varieties, where Mexico is characterized by presenting a wide genetic diversity.

MATERIALS AND METHODS

Plant material: 17 criollo cocoa genotypes were used, which are kept in the Criollo Cocoa Germplasm Bank of the Rosario Izapa Experimental Field of INIFAP (Table 1).

Morphological characterization: For the morphological characterization of the plant, leaf, flower, fruit and seed of the 17 Criollo cocoa genotypes, 30 varietal descriptors proposed by Avendaño *et al.*, (2014) were used.

Statistical analysis: The data for each descriptor were captured and systematized in an Excel spreadsheet, and then a principal component analysis was applied to them in order to detect through the new variables (principal components) which descriptors are differentiating the criollo cocoas, and finally, a hierarchical cluster analysis was applied to observe the grouping of the different genotypes; for this purpose, the SAS statistical package ver. 9.0 was used.The morphological characterization was conducted in the unique setting of the Rosario Izapa Experimental Field, a location known for its conducive conditions for cocoa cultivation. The study spanned two production cycles, focusing on five-year-old trees, with the aim of verifying the stability of the evaluated characters.

Table 1. Criollo cocoa genotypes used in the morphological characterization.

Number	Genotype	Acronym	Origin
1	Verde Gustavo	VerGus	Chiapas
2	Rojo Gustavo	RojGus	Chiapas
3	Rojo Samuel	RojSam	Chiapas
4	Baalam	Baa	Chiapas
5	Loxicha	Loxicha	Oaxaca
6	Real Soconusco	RealSoc	Chiapas
7	Real Soconusco 2	RealSoc2	Chiapas
8	Tazantán 1	Tuz01	Chiapas
9	Tuzantán 2	Tuz02	Chiapas
10	Tuzantán 3	Tuz03	Chiapas
11	Cuyul	Cuyul	Yucatán
12	Ces-6	Ces06	Centro América
13	Real Soconusco 3	RealSoc3	Chiapas
14	Lagartito Rojo	Ltorojo	Chiapas
15	Carmelo	Carmel	Tabasco
16	Lacandón	Lacando	Chiapas
17	Sak- Balam	Sakbal	Chiapas

RESULTS AND DISCUSSION

According to the principal component analysis (PCA), the first four principal components (PC) explained 56.2% of the variation; PC1 explained 21.2%; PC2 14.8%; PC3 10.3% and PC4 9.9% (Table 2). This is a significant finding. It is interesting to note that Osorio-Guarin *et al.* (2017), when characterizing 141 cacao accessions with 18 qualitative characteristics, found that the first five principal components explained 60.6% of the variation. In our study, the first five principal components explained an even higher 63.4% of the variation, providing a valuable comparison point for our research community.

The variables that contributed the most in each PC were: for PC1, Sepal Length (SEL); Fruit Shape (FS), Fruit Basal Constriction (FBC), Fruit Apex Shape (FAS), Fruit Length (FL), Fruit Length/Diameter Ratio (FLDR), Green Fruit Color (GFC), =Exocarp Thickness (ET); Fruit Pulp Sweetness (FPS), Cotyledon Color (CC); PC2, Anthocyanin

Table 2. Eigenvalues of the correlation matrix for 30 varietal descriptors of 17 Criollo-type cocoa genotypes.

Eigenvalues of the correlation matrix				
PC	Eigenvalues	Difference	Proportion	Cumulative
1	6.582	1.985	0.212	0.212
2	4.597	1.411	0.148	0.361
3	3.186	0.118	0.103	0.463
4	3.067	0.739	0.099	0.562
5	2.328	0.143	0.075	0.637

PC=Principal component.

Pigmentation of the Pedicel (APP), =Sepal width Length (SLA), Ligule color (LC), Seed Length/Diameter ratio (SLD), =Seed Thickness (ST); for PC3, Anthocyanin Pigmentation of the Sepal (APS), Fruit Pulp Color (FPC), Seed Length (SL); =Seed Width (SW) (Table 3). Osorio-Guarin *et al.* (2017) report that the variables that contributed most to differences in cocoa accessions were leaf bud color, shape and basal constriction of the fruit, thickness of the exocarp, and anthocyanin pigmentation of the pedicel. In addition, Bidot *et al.* (2017) reported that, when using 33 descriptors to characterize cacao varieties in Cuba, the ones that contributed the most in the seed were flat cross section and the intensity of the violet color; in the fruit: color, depth of the grooves, roughness and hardness of the mesocarp; and in flower: anthocyanin pigmentation of the staminodes and the peduncle and the color of the ligule.

Principal component analysis showed a wide dispersion in Criollo-type cocoa from Mexico. Varietal descriptors of flower, fruit and seed mainly give this dispersion. Vásquez-García *et al.* (2022), when characterizing different varieties of cocoa using 20 descriptors, found that leaf descriptors explained 36.07% of the variation, flower descriptors 20.54%, fruit 19.84% and seed 30.74%. On the other hand, Rangel-Fajardo *et al.* (2012) mention the importance of the embryonic sac and seed development based on the size of the fruit in the Criollo cocoa variety "Carmelo." Even though the environment highly influences the fruit size descriptor, it does allow differentiation of the genetic types of cocoa.

When graphing components 1 and 2, it can be observed that the dispersion is not random; this is because the Criollo type cocoas with red fruits in an immature state and wrinkled such as Baalam, red lizard, real soconusco 3, red Gustavo, real soconusco, real soconusco 2 and red Samuel; showed a tendency to come together and share other descriptors such as redbud color, red sepal color and larger flower size, without the basal constriction of the fruit and the shape of the fruit; in addition, they showed a more significant influence in the separation of red fruits with those of green color in an immature state (Figure 1). On the other hand, Marcano *et al.* (2008) report that red pigmentation in the different organs of cocoa (leaf, flower, and fruit) allows the differentiation of the varieties. They also found a high association with QTL markers. On the other hand, for Criollo-type cocoas with green color in an immature state, such as Lacandón, Carmelo, CES-06, Tuzantan 02 and Sak Balam, the descriptors that had the most influence were those of the seed (thickness, width and length) and in the fruit the shape, diameter and shape of the apex (Figure 1).

Figure 2 shows the dispersion of Criollo-type cocoas based on CP1 and CP3; there is a similar trend to Figure 1, where the seed descriptors (length and width) and shape of the fruit apex unite the green-colored cocoas in an immature state and the flower and fruit descriptors for the cocoas with red-colored fruits in an immature state (Figure 2).

According to the cluster analysis, two groups were formed, where the color of the immature fruit was the one that separated the groups; in Group I, the green ones were grouped, and in Group 2, the red ones mainly were grouped, except the green Gustavo. In addition, the GI is characterized by having criollo cocoas where the cream cotyledon color predominates, thus suggesting fine aroma cocoas (Figure 3).

Table 3. Eigenvalues and Pearson correlation coefficient for 30 varietal descriptors of 17 Criollo-type cacao genotypes.

Descriptor	PC1	PC2	PC3	Pearson Correlation Coefficient		
				PC1	PC2	PC3
SLB	−0.11	0.02	0.02	−0.28	0.03	0.04
SBLB	−0.11	−0.10	0.01	−0.27	−0.21	0.02
IGCLB	0.15	0.07	0.10	0.39	0.16	0.19
SALB	0.08	−0.08	0.18	0.21	−0.18	0.33
CYL	0.30	0.21	0.09	**0.76***	0.46	0.17
APP	0.17	0.28	−0.09	0.44	**0.60***	−0.16
SEL	0.23	−0.22	0.14	**0.58***	−0.47	0.25
SLA	0.19	−0.31	0.17	0.48	**−0.65***	0.31
APS	0.02	0.04	−0.40	0.04	0.09	**−0.72***
LC	0.01	0.24	0.13	0.03	**0.51***	0.22
APS	0.15	0.03	−0.11	0.37	0.07	−0.19
FS	0.27	0.08	0.01	**0.68***	0.17	0.03
FBC	0.29	0.06	0.16	**0.74***	0.12	0.28
FAS	−0.21	−0.26	−0.11	**−0.55***	−0.55	−0.19
FL	0.26	−0.11	0.03	**0.68***	−0.23	0.05
DF	−0.17	−0.19	−0.03	−0.44	−0.40	−0.05
FLDR	−0.32	−0.07	−0.02	**−0.83***	−0.16	−0.04
SF	0.09	0.21	0.14	0.24	0.46	0.25
DGF	0.02	0.02	0.20	0.06	0.04	0.37
CF	0.08	0.11	−0.27	0.21	0.24	−0.48
GFC	0.21	0.19	−0.20	**0.53**	0.41	−0.37
ET	0.21	−0.16	−0.04	**0.53**	−0.34	−0.08
FPC	0.02	0.10	−0.36	0.05	0.21	**−0.63***
FPS	0.20	−0.07	0.15	**0.51**	−0.14	0.26
TNS	0.04	−0.20	0.06	0.09	−0.44	0.10
FS	0.05	0.21	0.01	0.14	0.46	0.01
SL	−0.20	0.06	0.38	−0.51	0.13	**0.69***
SW	−0.20	0.25	0.32	−0.52	0.53	**0.56***
SLD	0.07	−0.35	0.10	0.19	**−0.74***	0.17
ST	−0.12	0.28	0.26	−0.31	**0.60***	0.47
CC	0.27	−0.20	0.09	**0.69***	−0.42	0.15

PC=Principal Component; SLB=Size of the Leaf Blade; SBLB=Shape of the Base of the Leaf Blade; IGCLB=Intensity of the Green Color of the Leaf Blade; SALB=Shape of the Apex of the Leaf Blade; CYL=Color of the Young Leaf; APP=Anthocyanin Pigmentation of the Pedicel SEL=Length of the sepal; LAS=Length of the width of the sepal; PAS=Anthocyanin pigmentation of the sepal; CL=Color of the ligule; APS=Anthocyanin Pigmentation of the Staminode; FF=Shape of the fruit; CBF=Basal constriction of the fruit; FAF=Shape of the apex of the fruit; LF=Length of the fruit; DF=Diameter of the Fruit; FRLD=Length/diameter ratio of the fruit; SF=Surface of the fruit; DGF=Depth of the Groove of the Fruit; CF=Color of the fruit; CFV=Color of the green fruit; GEF=Exocarp thickness; CPF=Fruit pulp color; DPF=Fruit pulp sweetness; TNS=Total number of seeds; FS=Seed shape; LS=Seed length; AS=Seed width; RLD=Seed length/diameter ratio; GS=Seed thickness; CC=Cotyledon color.

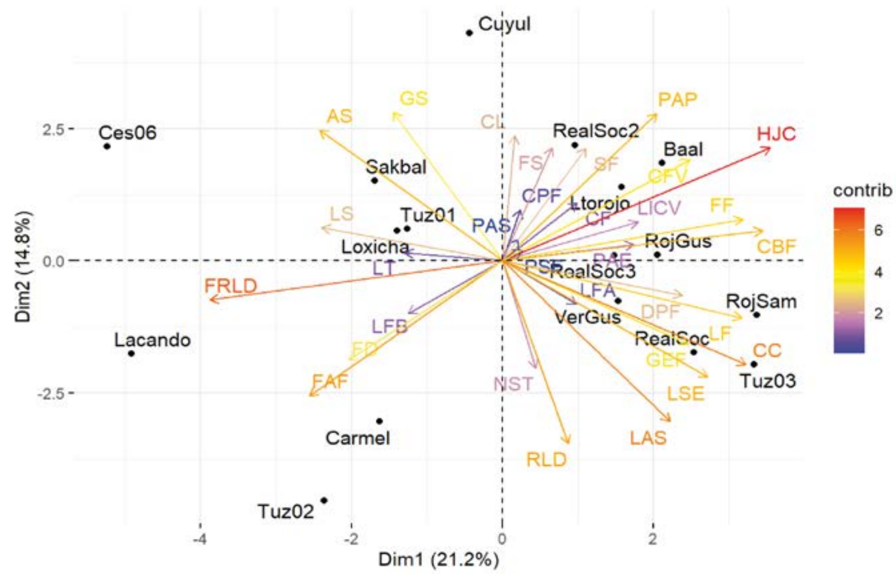

Figure 1. Dispersion of 17 Criollo-type cocoa as a function of principal components 1 and 2.

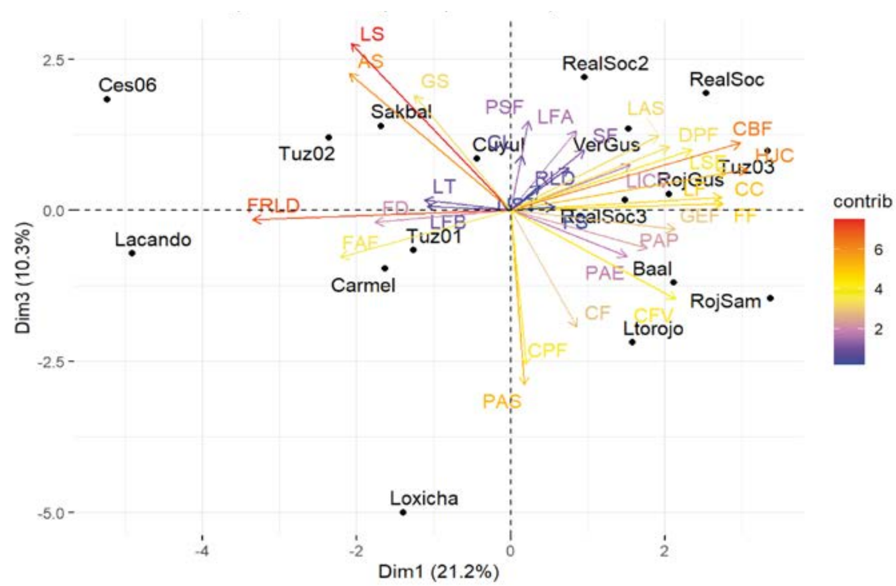

Figure 2. Dispersion of 17 Criollo-type cocoa as a function of principal components 1 and 3.

The morphological characterization of the leaf, flower, fruit and seed allowed us to differentiate the two groups formed in the hierarchical cluster analysis. In bud color, the brown color predominated in GI, and in GII, the colors ranged from medium red to dark red (Figure 4). In the flower, the groups were differentiated by the anthocyanin pigmentation of the pedicel and the sepal; in GII, the pedicels presented more excellent anthocyanin pigmentation (Figure 4). In fruit, the groups presented marked differences; in GI, the ovate shape predominated, yellow color, the apex was obtuse to acute and weak

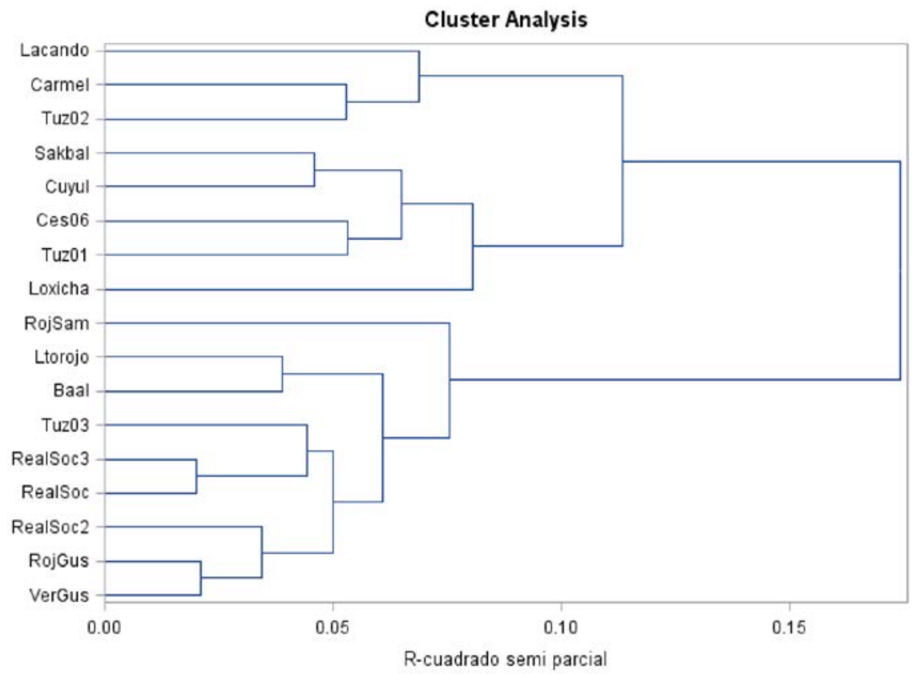

Figure 3. Dendrogram of 17 Criollo-type cocoa based on 30 morphological varietal descriptors.

basal constriction, and in GII, the shape was obovate, red color, notched apex and strong basal constriction (Figure 5). The seed also presented differences between groups; in GI, the cream color and ovate shape predominated (Figure 5). Finally, in the leaf descriptors, the shape of the apex allowed the differentiation of the groups; in the GI, the shape of the acute apex predominated (Figure 5). The diversity of bud colors, color and length of sepal, ligule and pedicel suggests that during the domestication process and given that this is dynamic, natural crossings continue to occur, which favors the appearance of new forms and colors of bud and flower (Figure 4).

The same occurs with the shapes, colors and sizes of leaves, fruits and seeds (Figure 5). Natural crossbreeding, environmental pressure and the selection that producers have been carrying out for many years have allowed the emergence of new varieties of Criollo cocoa, which can be a fundamental source for genetic improvement programs or for the selection of new aromas and flavors that the national and international market is demanding. The development of the chocolate flavor is highly dependent on multiple factors, including the conditions where the cocoa is grown, for example, the soil, climate and the genetics of the cocoa (Engeseth & Ac Pangan, 2018; Vázquez-Ovando *et al.*, 2015). In addition to the morphological differences between the genetic groups of cocoa, there are also marked differences in volatiles, as reported by Xiao-Wei *et al.* (2017). The highest concentration of volatiles is reported in the Trinitario group (79.7%) and the lowest in the Forastero group (70.39%). Also, Vera *et al.* (2014) report that when characterizing cocoa clones from Ecuador, the physical and chemical variables are associated, as well as the sensory attributes of cocoa, such as fruity, sweet, and floral.

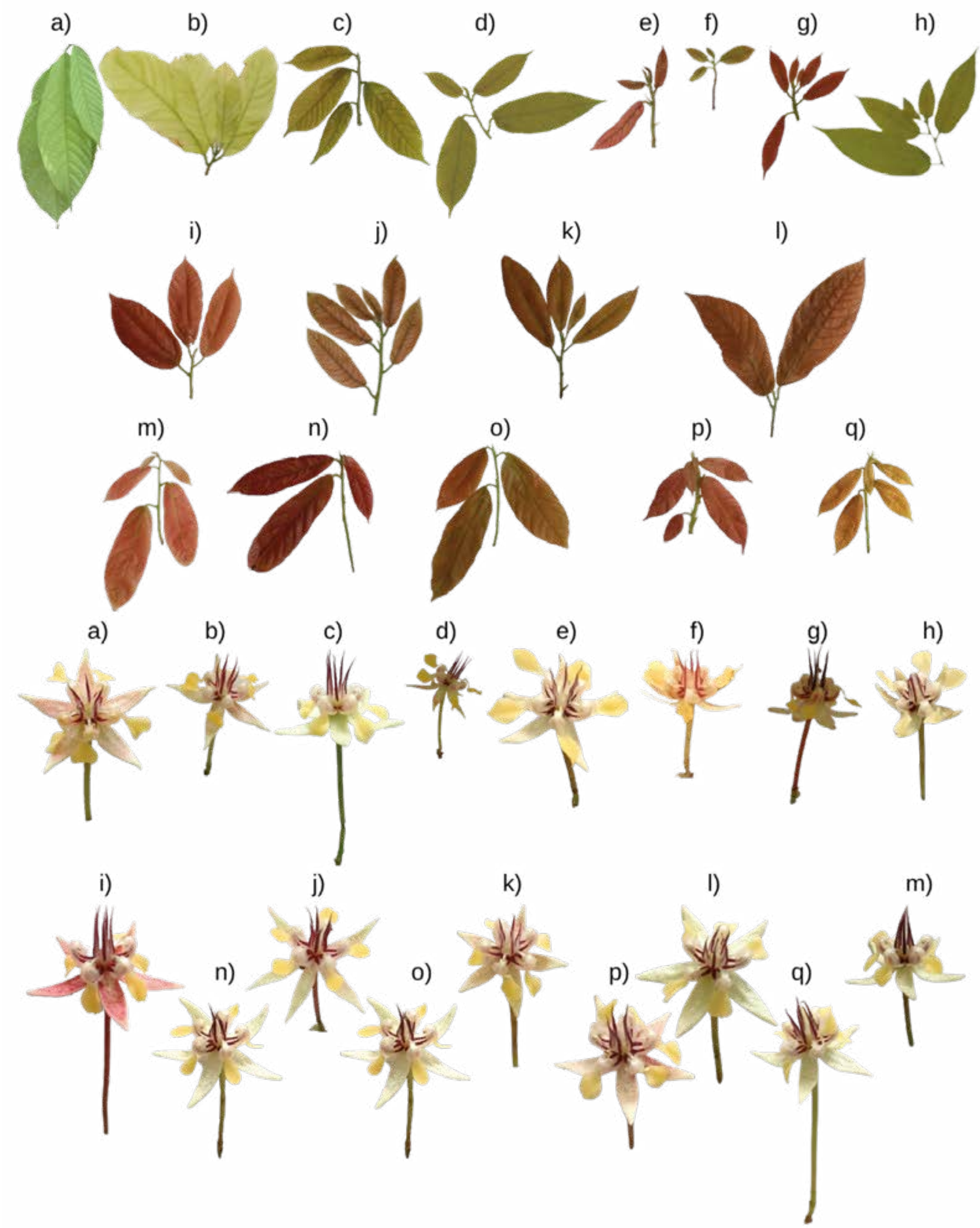

Figure 4. a) Lacando, b) Carmel, c) Tuz02, d) Sakbal, e) Cuyul, f) Ces06, g) Tuz01, h) Loxicha, i) RojSam, j) Ltorojo, k) Baal, l) Tuz03, m) RealSoc3, n) RealSoc, o) RealSoc2, p) RojGus, q) SeeGus.

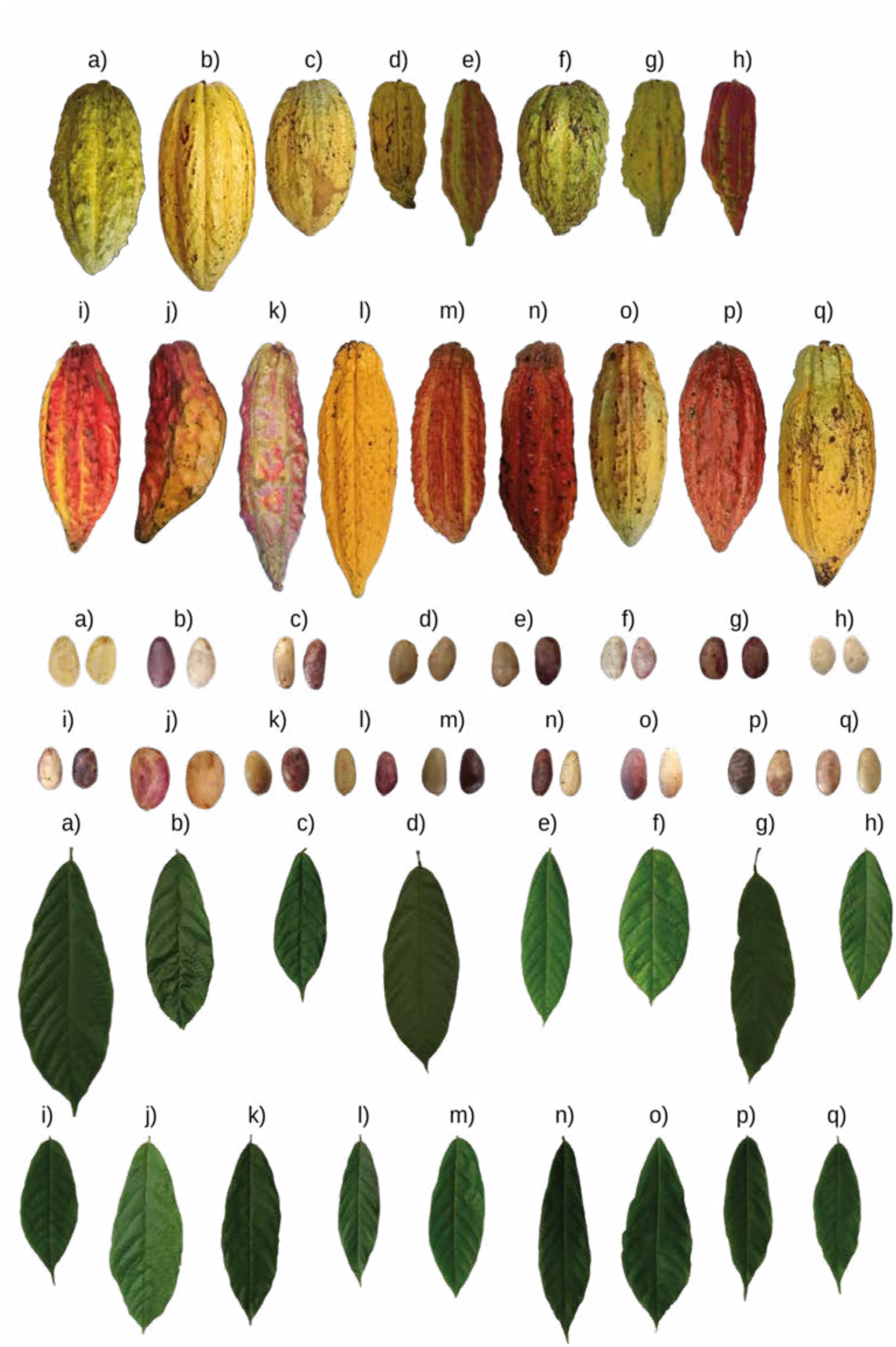

Figure 5. a) Lacando, b) Carmel, c) Tuz02, d) Sakbal, e) Cuyul, f) Ces06, g) Tuz01, h) Loxicha, i) RojSam, j) Ltorojo, k) Baal, l) Tuz03 , m) RealSoc3, n) RealSoc, o) RealSoc2, p) RojGus, q) SeeGus.

CONCLUSIONS

In Mexico, the genetic group of Criollo cacao presents a wide morphological variation in the descriptors of leaf, flower, fruit and seed. The shape of the apex of the leaf and fruit, the anthocyanin pigmentation in the flower, the color in the mature and immature state, the shape and basal constriction of the fruit, as well as the color of the cotyledon, are the descriptors that allowed the Criollo cacaos studied to be differentiated.

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Colegio de Postgraduados

Cluster Analysis as a Strategy to Contribute to the Competitive Advantage of Mangos from Guerrero, Mexico

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ABSTRACT

Objective: To analyze mango production and industrialization in Guerrero, Mexico, in order to identify its components, dynamics, and areas of opportunity, stimulating the development of a mango cluster.

Design/Methodology/Approach: This research is based on Porter's Five Forces methodology, adapted to the agri-food industry. Data were collected through semi-structured surveys in Técpan de Galeana and Cuajinicuilapa, Guerrero. In addition, a systematic literature review (SLR) was conducted along with database analysis.

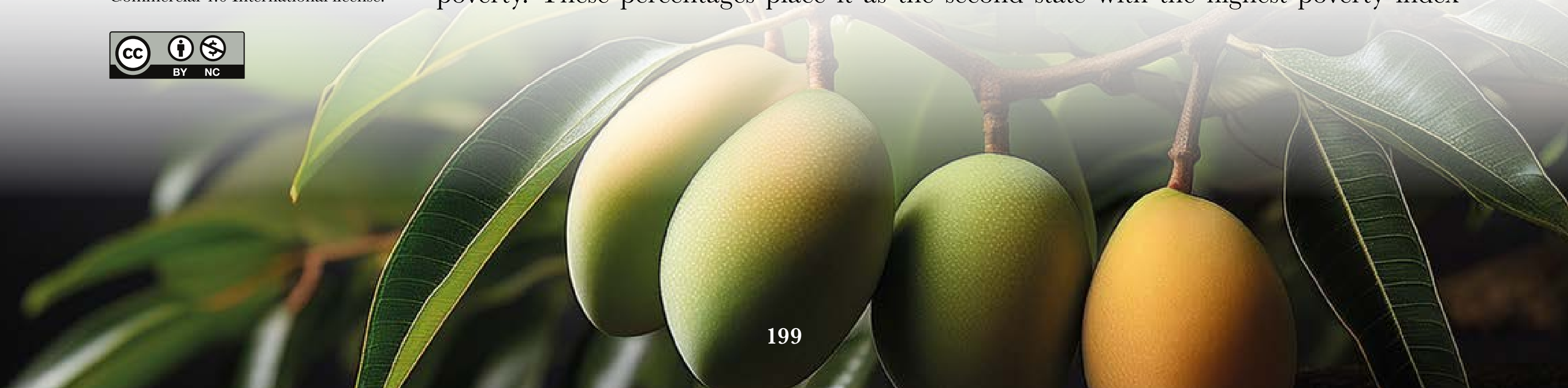
Results: The Guerrero mango supply chain (SC) was mapped, identifying three main challenges: high input costs, lack of price regularization, and the lack of interest shown by young people in agricultural work. Sinaloa and Chiapas were also identified as the main competitors, both in terms of production volume and competitive advantages.

Findings/Conclusions: Enhancing the coordination within the supply chain and implementing strategies are important to mitigate high costs and power asymmetries in negotiations with suppliers and buyers. Furthermore, the significance of developing a cluster to enhance the competitiveness of the Guerrero mango against its competitors is emphasized.

Keywords: cluster, competitiveness, mango industry, agricultural producers.

INTRODUCTION

The state of Guerrero is located in southwestern Mexico. It covers an area of 64,281 km^2 (3.2% of the national territory). In 2020, the state had 3,540,685 inhabitants, making it the thirteenth most populated state in the country. Nevertheless, 66.7% of the population was living in poverty, with 41.1% experiencing moderate poverty and 25.6% facing extreme poverty. These percentages place it as the second state with the highest poverty index



in Mexico, only below Chiapas (INEGI, 2020). In 2023, the primary sector, especially agriculture, was the main source of employment, with 24.6% of the state's total population engaged in this sector (Data Mexico, 2022). The average monthly income from this activity is estimated at 1.14 units of measure and update (UMA) per day ($3,551 MXN), which fails to cover the cost of the basic food basket and services, whose value amounts to 1.39 UMA per day ($4,332 MXN) (Observatorio Laboral, 2023; Statista, 2023). In 2022, mango was the most produced fruit in Guerrero, with a production value of $3,347,381,089 MXN (SIAP, 2022).

The association of rural issues with agriculture has led to the prioritization of agricultural strategies to tackle poverty and rural development. However, the Inter-American Institute for Cooperation on Agriculture (IICA, 2023) proposes a new focus on competitiveness, based on the relationships between the links in the value chain (Tapia, Aramendiz, Pacheco, & Montalvo, 2015). One of the strategies that has been shown to promote development in regions with high levels of marginalization and unemployment is the formation of clusters (Yekimov *et al.*, 2024). A "cluster" is a geographic concentration of interconnected companies and institutions in a particular field that complement each other (Porter, 1998). The strength of clusters lies in the improvement of the coordination and synchronization of its elements (López Jiménez *et al.*, 2016), reducing the probability of interruptions in the supply chain (SC) (Lis and Lis, 2023; Bao and Blanco, 2014). Similarly, it fosters competition among members, encouraging them to consider research, development, and innovation (R+D+I) as a strategy to differentiate themselves and be more competitive (Lehene *et al.*, 2024).

In light of the benefits of clusters and the circumstances of the population of Guerrero, this research analyzes the production and industrialization of mangos in Guerrero, Mexico. The aim is to identify the key components, dynamics, and areas of opportunity that could kickstart the development of a mango cluster.

MATERIAL AND METHODS

The project began with an analysis of the production and industrialization of mango cultivation in the state of Guerrero. This stage involved the collection and subsequent analysis of primary information obtained through semi-structured surveys conducted in two municipalities of the state in 2023. The said municipalities were selected due to the willingness of producers to participate and their representativeness in mango production. The first municipality and largest producer in the state was Técpan de Galeana, where five plantations located in the town of Luis San Pedro were visited. The second municipality and third largest producer was Cuajinicuilapa, where two workshops were held with the participation of 25 small-scale producers, who owned plantations between 1 and 10 ha.

Furthermore, a systematic literature review (SLR) was conducted using key databases, including Scopus, Web of Science, EBSCO and Google Scholar. At the same time, studies and databases from the official websites of the following agencies were analyzed: the Agrifood and Fisheries Information Service (SIAP), the Agricultural Consultation Information System (SIACON), the Ministry of Agriculture and Rural Development (SADER), the National Institute of Statistics and Geography (INEGI), Empacadoras de

Mango de Exportación A.C. (EMEX), the National Population Council (CONAPO), the National Employment Service (SNE), the Food and Agriculture Organization of the United Nations (FAOSTAT).

The information obtained was used in the application of Porter's Five Forces methodology (2017), with adaptations made to align it with the specific characteristics of the agri-food industry (Figure 1).

RESULTS

The favorable soil and climatic conditions for mango production, combined with its organoleptic properties, have contributed to the expansion of its cultivation to 23 of the 32 Mexican states. Its national production grew 112.91% from 2012 to 2022 (SIAP, 2023), while mango consumption per capita reached 13.8 kilograms (Secretaría de Agricultura y Desarrollo Rural, 2024).

Overall, small-scale production can be divided into five levels (Figure 2). The first level comprises suppliers, primarily of machinery, equipment, inputs, and services. The second

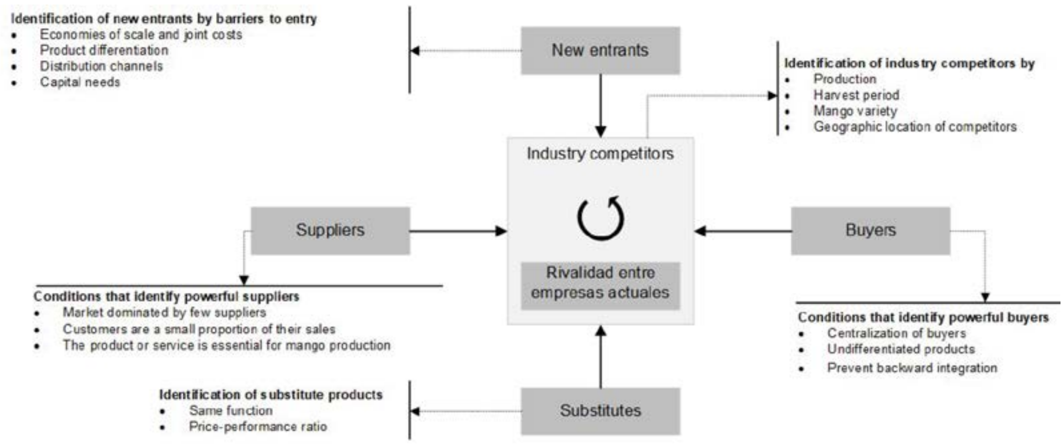


Figure 1. Porter's Five Forces (Porter, 2017).

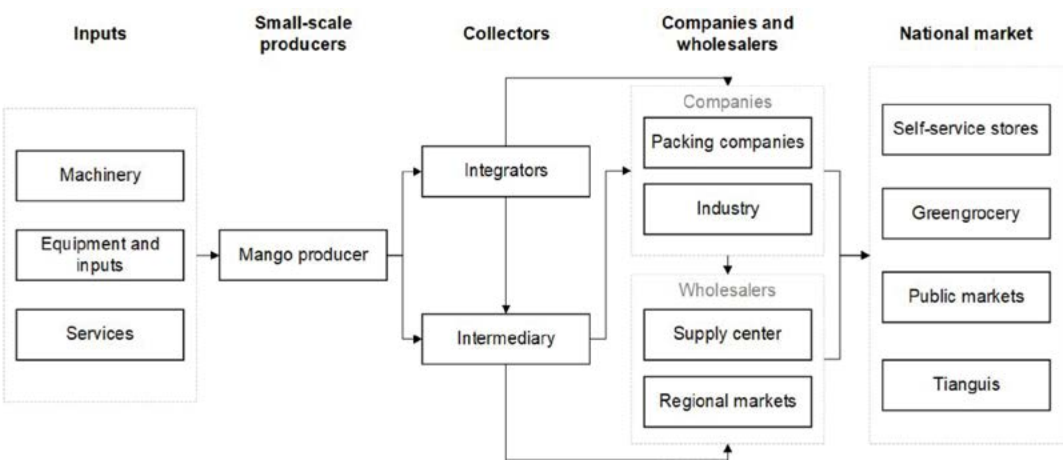


Figure 2. Supply chain of mango in Guerrero.

level consists of producers. The third level includes middlemen, who consolidate production through integrators or formally constituted companies, as well as intermediaries, who are mostly individuals who neither own or belong to an incorporated company. The fourth level encompasses companies that prepare or process mango, along with wholesalers who seek to distribute it in other markets. The final level is made up of the national market, which is accessed through supermarkets, markets, and grocery stores (SIAP, 2023).

Mango growers face several significant challenges, including high input and service costs, the impact of climate change, and the price at which their product is purchased (Figure 3).

Competition between existing companies

The following section summarizes the production, harvest season, variety, and geographic location of the main competitors of mangos from Guerrero.

Production

The states of Sinaloa, Guerrero, Nayarit, Chiapas, Oaxaca, and Michoacán are responsible for 84.7% of the Mexican mango production. Sinaloa is main producer, with 517,119.2 tons of mango in 2023 and a 128.8% growth rate in sown area (GRSA), reaching 49,133 ha at the end of the study period. It is also the leader in organic mango production and has the largest irrigated area (64.1%).

The increase in SA and production is attributed to the diversification of markets and the exploitation of key competitive advantages (Junaidi *et al.*, 2024; Egbadzor *et al.*, 2023). The 6,286 orchards registered in Sinaloa cover 46,710 ha whose produce is exported to the European Union, United States, and Japan, among others (SENASICA, 2024).

Chiapas recorded a 46.1% growth rate in sown area (GRSA), the second highest SA used to grow mango in the country (38,781.8 ha). This increase has driven the production growth rate (PGR) to 68.0%, resulting in the production of 272,174.5 tons of mango.

Although Guerrero is the second largest producer, it has the lowest PGR (22.1%) among the main producing states. Its production reached 415,688.8 tons, due to a yield of 15.72

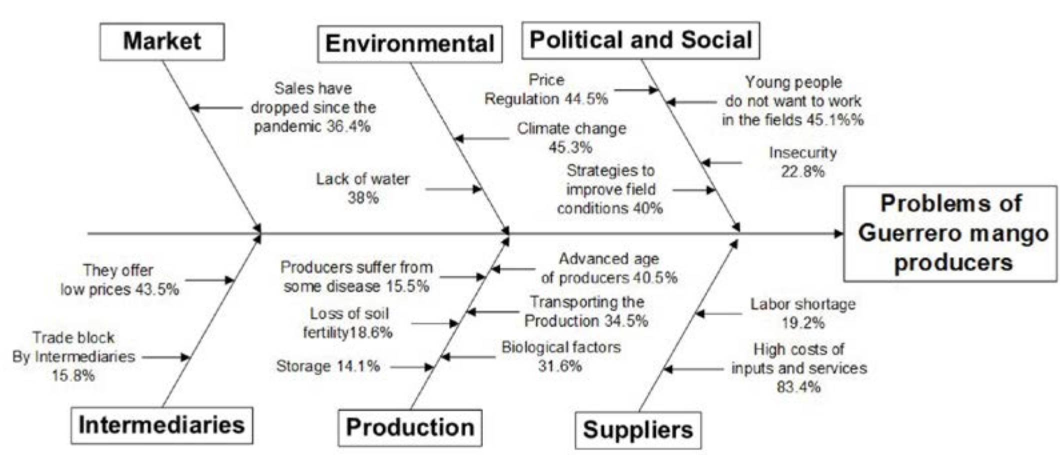


Figure 3. Problems of mango growers in Guerrero.

Table 1. States with the largest mango production in Mexico (2012-2022).

State [a]	TCSS [b]	TCP [c]	SS [d]	P [e]	MPR [f]	MPT [g]	TPC [h]	TPO [i]
Sinaloa	20.7%	128.8%	49,133.0	517,119.2	31,494.3	17,638.7	90.3%	9.7%
Guerrero	11.4%	22.1%	27,490.8	415,688.8	18,913.7	8,577.1	100.0%	0.0%
Nayarit	9.9%	35.6%	29,441.6	329,623.8	5,358.4	24,083.2	99.5%	0.5%
Chiapas	46.1%	68.0%	38,781.8	272,174.5	4,304.8	34,477.0	99.6%	0.4%
Oaxaca	9.7%	56.8%	19,344.3	214,478.6	2,418.0	16,926.3	99.8%	0.2%
Michoacán	15.8%	49.9%	26,110.9	200,119.3	25,510.3	600.6	89.2%	10.8%

SIAP (2023).
[a] Producing state. -[b] Growth rate of sown area. -[c] Production growth rate. -[d] Sown area in hectares. -[e] Production in tons. -[f] Irrigated production in hectares. -[g] Rainfed production in hectares. -[h] Type of conventional production. [i] Type of organic production.

t/ha —the second highest yield after Sonora (17.57 t/ha). Guerrero has 123 plantations (431.19 ha) whose produce is exported to the United States and the European Union (SENASICA, 2024). Since the mango industry of Guerrero has been surpassed by the production of Sinaloa and the GRSA of Chiapas, these two states are the main competitors of Guerrero, in terms of mango production.

Harvest season

In this heading, Guerrero competes with Nayarit, Chiapas, Oaxaca, Michoacán, Colima, and Veracruz (Figure 4). Sinaloa and Colima have gained competitive advantages with late production. According to Siller-Cepeda *et al.* (2009), mangos harvested at this stage lose less weight, are firmer, and have a tempting flesh color, making them more attractive and tastier. In addition, harvesting mangos at the end of the production cycles of other states reduces competition and facilitates their positioning in the market at better prices.

Mango variety and geographical location

Eight varieties of mango are grown in Guerrero, three out of which (Manila, Ataulfo and Haden) account for 85.0% of the state production. Veracruz, Chiapas, Oaxaca and Michoacan grow the same varieties and are therefore Guerrero's main direct competitors (Figure 5).

Figure 4. Months of mango production per state (Imagen del Golfo, 2019; Villanueva, 2016).

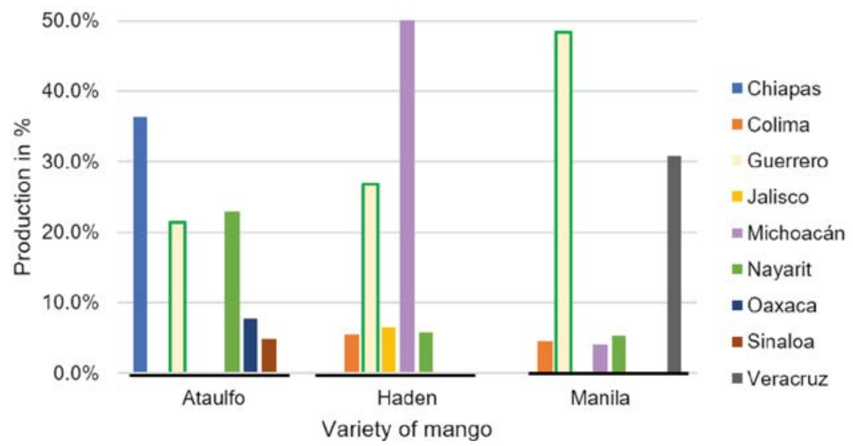


Figure 5. Main producers of Ataulfo, Haden, and Manila mangos.

Guerrero and Veracruz are the major producers of Manila mango, accounting for 79.1% of the domestic production. In the case of the Ataulfo mango, 85.4% of the domestic production comes from Chiapas, Nayarit, Guerrero, and Oaxaca. Finally, 77.2% of the Haden mangos are produced in Michoacán and Guerrero.

The joint production of these varieties, as well as the geographical closeness, promotes the competition for the main markets between Chiapas, Guerrero, Michoacán, Nayarit, Oaxaca, and Veracruz.

Suppliers

Identifying the existing bargaining power between suppliers and customers is important, because the profitability of the less powerful actor can be compromised, as can be seen in the case of small-scale mango producers from Guerrero. According to Michael Porter (2017), when the bargaining power is held by suppliers, the market is dominated by a few of them, customers are not a significant proportion of their sales, and the product or service offered by the supplier is essential for the customer.

In a market dominated by a limited number of suppliers, customers are forced to accept the prices, quality, and conditions of the transaction (Porter, 2017). In the mango industry of Guerrero, the availability of suppliers is not constrained by offer, but rather by the purchasing power of mango producers.

Astudillo-Miller *et al.* (2020) found that 36.5% of growers have their own machinery, while 52.7% use leased machinery. Meanwhile, producers with greater economic capacity tend to acquire equipment and inputs in the local informal market (INEGI, 2019). For their part, 63.6% of growers lack this capacity and consequently often rely on credit from packing companies or intermediaries, which frequently result in higher prices than those in the market. This financial dependence forces producers to sell their harvest to the lender, who also sets the purchase price, further limiting the bargaining power of the producer.

Another indication that suppliers are the primary decision-makers is that independent small-scale growers in Guerrero do not represent a significant proportion of the sales of the suppliers. In light of these circumstances, several authors, including Ahoda *et al.*

(2024), Guyalo and Ifa (2023), Ordóñez-Trujillo *et al.* (2023), and Zhu and Wang (2024) have proposed establishing or strengthening agricultural cooperatives as a strategic organizational approach. This organizational structure will enable them to make bulk purchases, share joint costs, be jointly represented, and open new markets, among other activities. As a result, suppliers will view the organization as a more important client, offering reasonable prices and other services (such as technical assistance).

When the product or service provided by the supplier is essential to the customer, the bargaining power of the supplier increases. According to SIAP statistics (2023), 100% of the mango production in Guerrero is conventional. Therefore, the most common method to control pests, weeds, and diseases and to fertilize the soil is the use of synthetic chemicals. Such practices have several disadvantages, including soil and water pollution, health risks to farmers and consumers, adverse effects on plants, and high input costs (Şener *et al.*, 2020). To reduce reliance on chemical products, Espinoza *et al.* (2022) suggest exploring agroecological alternatives for agricultural production. These measures could enhance the management capacity of the producers, reduce input purchase costs, and introduce them to the organic food production trend.

Buyers

Buyers have the power to set product prices, negotiate for better quality or additional services, and, in this case, confront producers with each other. Buyer empowerment is facilitated by the following conditions: centralization of buyers, undifferentiated products, and avoidance of backward integration.

In Guerrero, 70.3% of mango purchases are made through local middlemen, 21.6% through local markets, fruit processors, or sale points, and 8.1% through packers (Astudillo-Miller *et al.*, 2020). An alternative proposed by Balcom *et al.* (2023) and Connolly *et al.* (2022) is the use of short supply chains (SSC), which allow farmers to sell directly to consumers. This model is designed to promote family farming, improve their economic income, and avoid the monopoly of intermediaries.

Adding value along the supply chain is a strategic aspect that improves trading conditions, quality, variety, safety, and consumer access. However, most agricultural growers sell their harvest outside their own orchard, which results in a lack of differentiation in their production (IICA, 2014). This standard purchase option allows customers to have a wide range of choices, as the characteristics of the mango remain consistent.

Backward or forward integration in the agri-food supply chain reduces costs while also improving competitiveness. Furthermore, it reduces uncertainty and also fosters long-term relationships among participants, eliminates information barriers, and ensures equitable risk and profit sharing (Fusacchia *et al.*, 2022; Wang *et al.*, 2023).

However, some buyers engage in commercial blocking and abuse, and promote information asymmetries to achieve "significant appropriation," which is a term associated with an income that is proportionally higher than the service provided or the contribution to the value generated by the chain as a whole (Gaudin and Padilla, 2020). The supply chains of mangos produced in Guerrero frequently face this situation (Astudillo-Miller *et al.*, 2020).

Alternatives

Mango is a sweet, juicy, and fleshy fruit that is mainly consumed fresh. As a result, it is more expensive than other fruits and it becomes vulnerable to competition from other cheaper fresh and processed products. Mango is one of the most highly valued fruits in the market. In July 2024, the average price of Ataulfo and Manila mangso in the Central de Abasto (central market) of Iztapalapa, Mexico City, reached $26.8 MXN per kg, while Haden mango cost $14.29 MXN per kg (SNIIM, 2024). The high cost of this fruit leads consumers to choose cheaper alternatives, such as other fresh fruits or processed foods. Mexico has other popular fresh fruits that consumer can afford: orange (with a *per capita* consumption of 36.9 kg and an average price of $14.0 MXN per kg), lemon (with a *per capita* consumption of 18.6 kg and an average price of $13.2 MXN per kg), and banana (with a *per capita* consumption of 15.9 kg and an average price of $18.9 MXN per kg) (SNIIM, 2024; Statista, 2022).

The high demand for processed and ready-to-eat mango products can be attributed to their extended shelf life and their convenient storage and transportation. Furthermore, they are available out of season in a wide variety of presentations, including porridges, conserves, jams, ice cream, dried fruit, juices, and more. These products have innovative flavors and reduce waste (The Food Tech, 2024).

Since prices are subject to fluctuation in the Mexican market, considering the impact of competition is fundamental to enhance processes and diversify commercial strategies that contribute to the ongoing competitiveness of mangos produced in Guerrero.

New participants

In order to prevent the entry of new participants in the industry, Porter (2017) proposes the creation or reinforcement of six barriers to entry. In the case of the mango industry of Guerrero, four barriers were considered: economies of scale and joint costs, product differentiation, distribution channels, and capital needs.

Eighty-percent of small-scale mango producers have neither achieved economies of scale nor identified joint costs that can reduce the cost of mango production. The cost of production varies among plantations, between $3,520.9 to $8,689.2 MXN per ton (Cabañas, 2016). The price per ton in 2022 was reported at $8,141.0 MXN (Government of Guerrero, 2024).

Product differentiation

Three types of differentiators were considered. 1) Price differentiation. Growers in Guerrero have reported that, in order to sell their production, they often have to sell it cheaper than the competition, which results in minimal or no profit at all. However, cost differentiation does not necessarily entail lower prices. Instead, it involves providing added value to the customer (*e.g.*, service, quality, and experience), for the same price. 2) Differentiation through advertising and marketing. The mango from Guerrero has been advertised in various media, including television, radio, and internet websites. These advertisements are primarily the responsibility of the state government. However, in addition to the advertisement itself, an effective advertising and marketing strategy requires a comprehensive plan, market research, segmentation, positioning, analysis, and adjustment. Therefore, no differentiation can be found between the advertising and the marketing. 3) Differentiation by quality and traceability. This step includes the monitoring of mangos throughout the supply chain, to guarantee the quality and safety of the product and consumer health. Small-scale mango growers are unable to achieve this level of differentiation, as they often lack the ability to identify their position within the supply chain.

Distribution channels

More exclusive distribution channels and a stronger trust (linkage) reduces the risk of new participants. However, in the mango industry of Guerrero, exclusivity between producers and middlemen is not reciprocal. The commercial blockade suffered by the mango growers and the imposition of prices by the middlemen cause distrust. Additionally, middlemen allow any products that give them a higher yield to go through the channels.

Capital needs

The capital required to join a supply chain is a significant barrier for mango producers in Guerrero, limiting their ability to participate in new markets, both domestic and foreign. Mexico is the sixth largest producer of mangos in the world, contributing 4.3% of the total production (FAOSTAT, 2022). It is the leading exporter-producer, with 14 countries as its main clients. However, the United States and Canada purchase 98.7% of Mexico's exports (EMEX, 2022), highlighting the significance of these markets for the Mexican mango industry. The Republic of India, the world's largest mango producer, poses a significant risk. Its production accounts for 43.2% of the global production (FAOSTAT, 2022). India exports mangos to 104 countries, mainly to Saudi Arabia, the Netherlands, the United Arab Emirates, and Nepal. An increased international competition could arise, if India seeks to expand its presence in the markets where Mexican mangos are currently positioned.

CONCLUSIONS

The mango industry of Guerrero must undergo a strategic restructuration, integrating key players into a cluster that will strengthen relationships within the supply chain. This proposal involves the use of alternative inputs to reduce dependence on suppliers, as proposed by Espinoza *et al.* (2022) and the creation of direct markets through short supply chains (Balcom *et al.*, 2023; Connolly *et al.*, 2022). In order to address the challenges of high costs and significant losses, both tactical and operational solutions must be implemented. If mangos from Guerrero are to compete on a global scale, losses in the chain, which currently reach 54% (Romero-Romero *et al.*, 2024), must be minimized and market diversification and quality certification mechanisms should be explored. The implementation of public policies that facilitate the access of small growers to financial and technological resources is essential to stimulate the growth of the industry. These initiatives will not only enhance the competitiveness of mangos from Guerrero, but will also boost economic and social development in the region, creating employment opportunities and reducing poverty in rural areas.

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Colegio de Postgraduados

Morphological and agronomic evaluation of short-cycle native maize varieties (*Zea mays* L.)

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ABSTRACT

Objective: The objective of this study was to analyze the morphological composition and agronomic behavior of five varieties of *Zea mays* L. two Zapalote Chico, two Zapalote Grande and an improved variety (V-424) which are characterized by having a short vegetative cycle.

Desing/methodology/approach: The maize varieties evaluated were sourced from various locations within the municipality, with explicit consent from the producers, who provided the seeds for the study. Four native cultivars (treatments) were selected from the Zapalote Chico and Zapalote Grande races and one improved and open-pollinated maize variety (V-424 or Tuxpeño Precoz) as a control, released by INIFAP. The local farmers assigned the common names to the evaluated maize varieties, except for V-424. The varieties were distributed in a randomized block design with four replications, resulting in a total of 20 experimental units. Each unit consisted of four furrows, each measuring 4 meters in length.

Results: The findings of this study on native short-cycle maize varieties in Chiapas have significant implications at the genetic, social, cultural, economic, and environmental levels. Each of these aspects provides crucial guidance for decision-making related to the utilization, management, and conservation of local maize varieties. A key outcome of this study is the recognition of native maize as a repository of essential genes for food security in rural areas. Short-cycle landraces exhibit unique genetic adaptations that enable them to thrive under the specific agroclimatic conditions of the Central Valleys of Chiapas.

Limitations on study/implications: none

Findings/conclusions: The native maize varieties exhibited significant genetic variability across the variables under consideration. The Zapalote Chico race is characterized by its favourable characteristics, as recognized by farmers, including early maturity, drought resistance, lodging resistance, and resistance to the fall armyworm. These qualities make it a promising candidate for future research and for inclusion in genetic improvement programs, whether conventional or participatory. It is crucial to continue promoting agroecological inputs with the goal of fostering the development of resilient agroecosystems and, consequently, enhancing the well-being of farmers and the conservation of native maize reservoirs.

Keywords: *Zea mays* L., Native varieties, Endogenous biodiversity, Traditional agriculture, Mexico.

INTRODUCTION

In Mexico, maize (*Zea mays* L.) holds significant cultural, economic, and political importance. It is estimated that 70-80% of the cultivated area is planted with native varieties, which play a critical role in the social reproduction of many peasant communities. Native maize also serves as a vital biotic resource, preserving the identity and history of rural families (Pérez-García, 2023).

In 2022, Chiapas, a state in southern Mexico, led the country in cultivated area with 686,943 hectares, ranking eighth in production, yielding 1,379,252.44 tons (SIAP, 2023). The region is home to nearly 34% (20 races) of Mexico's native maize races, including: Tehua, Comiteco, Cubano Amarillo, Nal-Tel, Olotillo, Olotón, Tepecintle, Tuxpeño, Vandeño, Zapalote Chico and Zapalote Grande (Caballero-Salinas *et al.*, 2023; Guevara-Hernández *et al.*, 2020). As a result, Chiapas is recognized as a region of significant genetic diversity (Hernández-Ramos *et al.*, 2020; Guevara-Hernández *et al.*, 2022).

In maize research, the term "race" is used to categorize individuals or populations that share common morphological, ecological, genetic, and historical characteristics, facilitating their identification as distinct groups. These races are structured into racial complexes, which are associated with their geographical and climatic distribution as well as their shared evolutionary history. In Mexico, maize races are grouped into seven categories: Chapalote, Conical (Cónico), Late Maturing (Maduración tardía), Eight-Row (Ocho hileras), Sierra Chihuahua, Early Tropical (Tropicales precoces), and Tropical Dentate (Dentados tropicales) (Estrada-Contreras *et al.*, 2022). The latter two categories include the distinctive races Zapalote Chico (Xhuba Huiini) and Zapalote Grande (Barrera-Guzmán *et al.*, 2020).

These races are distinguished by their short physiological cycles and high photosynthetic efficiency, requiring less water for vegetative development. These traits make them particularly well-suited for drought conditions (Barrera-Guzmán *et al.*, 2020). The primary use of this crop is in the production of traditional Mexican dishes and by-products such as tostadas, tamales, atole, and tortillas, contributing to the concept of food sovereignty (García-Franco and Gómez-Galindo, 2023). Given their origins, historical development, and unique characteristics, these varieties have been integrated into maize breeding programs (Guevara-Hernández and Mariaca-Méndez, 2023).

In Chiapas, the morphological and agronomic diversity of native maize populations has been studied to develop utilization and conservation strategies (Caballero-Salinas *et al.*, 2023). However, some maize races commonly cultivated by farmers in the municipality of Cintalapa, such as Zapalote Chico and Zapalote Grande, remain under-researched. Therefore, this study aims to characterize the morphological diversity and agronomic behavior of these races in the Central Valleys of Chiapas.

MATERIALS AND METHODS

The research was conducted in the experimental area at the Chiapas Regional Academic Centre, part of the Antonio Narro Agrarian Autonomous University, located in the municipality of Cintalapa, Chiapas, Mexico. The Centre is situated at 16° 39' North latitude and 93° 44' West longitude, at an altitude of 540 m. The mean annual temperature ranges between 24 °C and 26 °C, with annual precipitation levels between 800 and 1,200 mm.

The maize varieties evaluated were sourced from various locations within the municipality (Table 1), with explicit consent from the producers, who provided the seeds for the study. Four native cultivars (treatments) were selected from the Zapalote Chico and Zapalote Grande races and one improved and open-pollinated maize variety (V-424 or Tuxpeño Precoz) as a control, released by INIFAP (Coutiño-Estrada, 2014). The local farmers assigned the common names to the evaluated maize varieties, except for V-424. The varieties were distributed in a randomized block design with four replications, resulting in a total of 20 experimental units. Each unit consisted of four furrows, each measuring 4 meters in length.

The sowing process was conducted manually during the spring-summer 2022 agricultural cycle. Plants were arranged in rows with 60 cm spacing between rows and 30 cm between plants, resulting in a population density of 110,556 plants per hectare. At each sowing point, three seeds were planted, followed by manual thinning to leave two plants per clump.

Table 1. Treatments with the maize varieties evaluated and their communities of origin in the municipality of Cintalapa, Chiapas.

Treatment	Local name /(Race)	Origin	Ear characteristic	Altitude (masl*)
T1	V-424 (Tuxpeño)	Villamorelos		704
T2	Cuarentano blanco (white) (Zapalote Chico)	Cintalapa		540
T3	Cuarentano amarillo (yellow) (Zapalote Chico)	Tuxtlita		540
T4	Opamil (Zopilote Grande)	Villamorelos		704
T5	Tapanero (Zapalote Grande)	Villamorelos		704

* masl: meters above sea level.

Vegetative variables were measured at various stages of the crop's growth and development. To ensure representativeness, 20 plants were randomly selected from each experimental unit. The evaluated characteristics included vegetative type, ear length (measured during the milky stage of the grain), and ear size, following the descriptors for maize established by the International Board for Plant Genetic Resources (IBPGR) in 1991 (CTA, 1992).

At 85 days after sowing (DDS), vegetative development variables were measured, including plant height (AP), stalk diameter (DT), number of leaves below the ear (NHDM) and above the ear (NHAM), total number of leaves (TH), and leaf width (AH) and length (LH) associated to the main ear.

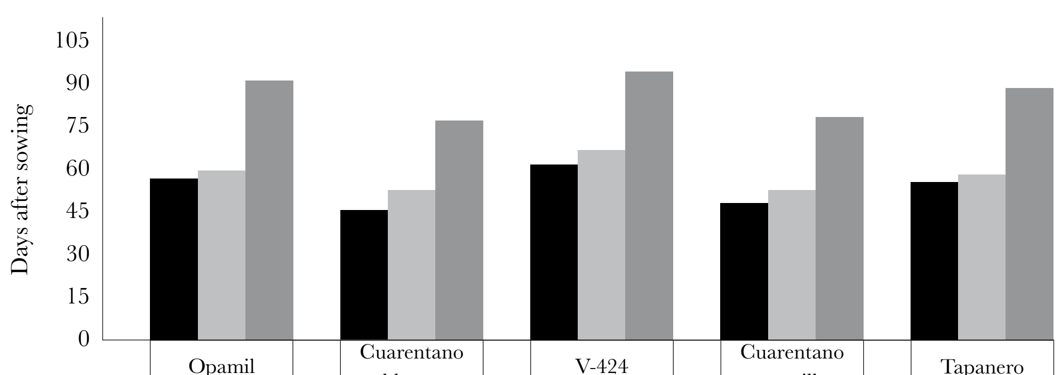
Phenological variables, such as days to male flowering (DFM) and days to female flowering (DFF), were recorded when 50% of the plants had released pollen and exhibited receptive stigmas. After the flowering period, additional characteristics were evaluated: spike length (LE), number of branches per spike (RE), stalk length (LP), branch length (LTR), and panicle length (LPA). Physiological maturity was determined when the ears reached senescence (SEN), indicated by the presence of dry bracts in 50% of the plants. Ear-related variables measured included diameter (MD), length (LM), number of rows (NH), number of grains per row (NGH), and corncob diameter (DO). Ear harvesting was carried out manually during the first two weeks of September, coinciding with the physiological maturity of the genotypes under evaluation.

For Statistical Analysis, SAS Studio® was used. Data were initially analyzed using the Shapiro-Wilk test and Bartlett's test to ensure they met the criteria for normality and homogeneity of variance. A two-way analysis of variance and Tukey's multiple comparison test were then conducted to analyse the results.

RESULTS AND DISCUSSION

Days to male and female flowering and Senescence

The observed data for DFM ranged from 45 to 62 DDS, while for DFF, it ranged from 49 to 66 days (Figure 1). The maize varieties exhibiting above-average values for



	Opamil	Cuarentano blanco	V-424	Cuarentano amarillo	Tapanero
DFM	56	45	62	47	53
DFF	60	49	66	50	57
SEN	91	76	94	77	88

Figure 1. Days to male flowering (DFM), female flowering (DFF), and senescence (SEN) of the maize varieties studied in Cintalapa, Chiapas.

DFM were Tapanero, Opamil, and V-424. The two Cuarentano maize varieties (white and yellow) showed the lowest DFM and DFF values, indicating early crop maturation. The female flowering values for Cuarentano maize varieties were like those reported by López-Romero *et al.* (2005), who characterized 14 populations of Zapalote Chico. Many studies found that the evaluation of 18 Zapalote Chico populations yielded results ranging from 45 to 51 days for DFM and 48 to 53 days for DFF.

The results regarding days to flowering indicate that the four native maize varieties (T2-T4) evaluated are considered short-cycle. However, the V-424 (T1) variety exhibited the highest values for both variables and is classified as an early to intermediate cycle cultivar among improved open-pollinated maize varieties. Regarding flower synchronization, the yellow Cuarentano maize showed the shortest period.

The early maturation of the Zapalote Chico and Zapalote Grande cultivars suggests that short-cycle maize may serve as a viable alternative in regions with limited precipitation or prolonged drought periods, as they can complete their developmental cycle with reduced water requirements. Additionally, these varieties are well-adapted to the spring-summer rainy season.

In this context, strategic actions can be developed to address the challenges posed by drought, water scarcity, and irregular rainfall patterns due to climate change. In their study, like the days to flowering, the two Cuarentano maize evaluated exhibited the shortest ear senescence periods. The highest values were observed in the Opamil and V-424 varieties, with durations of 91 and 94 days, respectively, after sowing. This highlights one of the most notable attributes of the Zapalote Chico maize variety: its early maturation. This characteristic allows for the possibility of two production cycles per year on the same plot, provided that the planting schedule is aligned with the onset of the regional rainy season.

Vegetative variables

Although no significant differences in plant height (AP) were observed, as expected, the V-424 variety showed a tendency for increased plant height, followed by the varieties of Zapalote Grande and Zapalote Chico, respectively (Table 2). On average, the ears were

Table 2. Response of vegetative variables in the maize varieties studied in Cintalapa, Chiapas.

Variety	AP (cm)	AM (cm)	DT (cm)	NHDM	NHAM	TH	AH (cm)	LH (cm)
Opamil	124.00	74.25^{a}	1.51^{a}	5.80^{b}	5.62^{b}	11.41^{b}	6.00^{ab}	67.49^{a}
White Cuarentano	122.40	66.70^{ab}	1.33^{b}	4.89^{cd}	4.65^{c}	9.57^{d}	4.83^{c}	52.14^{c}
V-424	131.80	68.67^{ab}	1.52^{a}	6.34^{a}	6.19^{a}	12.52^{a}	6.36^{a}	67.96^{a}
Yellow Cuarentano	121.70	58.00^{c}	1.29^{b}	4.59^{d}	4.66^{c}	9.25^{d}	4.48^{c}	54.72^{c}
Tapanero	123.30	63.20^{bc}	1.46^{b}	5.17^{c}	5.46^{b}	10.65^{c}	5.57^{b}	60.17^{b}
CV (%)	26.30^{NS}	27.40	21.40	20.70	17.60	14.60	21.30	19.90

Note: Different letters within the same column indicate a statistically significant difference between treatments. NS: Not significant. **Highly significant difference ($p<0.01$). The following variables were measured: AP (plant height), AM (ear height), DT (stem diameter), NHDM (number of leaves below the ear), NHAM (number of leaves above the ear), TH (total number of leaves), AH (leaf width), LH (leaf length), and CV (coefficient of variation).

observed at a mean height of 66.09 cm. However, Opamil maize exhibited a significantly greater ear height (74 cm) compared to yellow Cuarentano and Tapanero varieties (Table 2), although it was statistically like V-424 and white Cuarentano maize. The greatest stem diameter (DT) was observed in the V-424 and Opamil maize varieties. While the remaining cultivars exhibited lower values for this variable, they did not experience lodging due to their shorter height. Additionally, the V-424 variety outperformed others in several traits, including the total number of leaves (TH), number of leaves below the ear (NHDM), number of leaves above the ear (NHAM), leaf height (AH), and leaf length (LH) (Table 2). In contrast, the two Cuarentano varieties exhibited the lowest values for these variables, leading to reduced leaf development.

The variability in the number of leaves above and below the ear represents a crucial variable for study, as it contributes to understanding genetic and phenotypic diversity among maize populations. Numerous studies have shown that these traits influence not only maize yield and adaptation to diverse environmental conditions but also crop efficiency and disease resistance (Hernández and Avellaneda, 2021).

In this context, the specific number of leaves significantly influences sunlight capture, nutrient distribution, and pollination-factors that are fundamental for optimal plant development and grain production (Bisetti-Rivera, 2023).

Ear variables

Significant differences were observed across all ear-related variables (see Table 3). The largest ear diameter (DM) was observed in the V-424 maize variety, followed by Opamil and Tapanero. Maize with shorter plant height (AP) exhibited reduced ear length (LM) and ear diameter (DM), as seen in the white and yellow Cuarentano varieties (Table 3). This result may be associated with a shorter vegetative phase, which could lead to smaller ears (Sánchez-Hernández *et al.*, 2013). In their agronomic evaluation of two Zapalote Chico populations, others researchers reported mean ear lengths of 8.4 and 8.8 cm, and mean ear diameters of 3.6 and 3.5 cm, respectively.

In this study, Tapanero maize exhibited the highest length-to-maturity (LM) value (10.75 cm) and the highest number of grains per row (NGH) (24.02). Furthermore, the

Table 3. Ear variables of the maize varieties studied in Cintalapa, Chiapas.

Variety	DM (cm)	LM (cm)	NH	NGH	DO (cm)
Opamil	4.33^{b}	10.18^{a}	11.25^{b}	20.43^{b}	2.56^{b}
White Cuarentano	3.97^{b}	8.25^{b}	9.62^{d}	19.07^{b}	2.09^{d}
V-424	4.58^{a}	10.57^{a}	13.38^{a}	18.72^{b}	2.88^{a}
Yellow Cuarentano	3.86^{c}	6.89^{c}	9.92^{cd}	13.53^{c}	2.41^{bc}
Tapanero	4.19^{b}	10.75^{a}	10.57^{bc}	24.02^{a}	2.41^{c}
CV (%)	8.80	20.00	12.60	25.10	10.70

Note: Different letters within the same column indicate a statistically significant difference between treatments. A highly significant difference was observed ($p<0.01$). The variables measured include DM (ear diameter), LM (ear length), NH (number of rows), NGH (number of grains per row), DO (corncob diameter), and CV (coefficient of variation).

maize varieties with the highest LM values (Tapanero, V-424, and Opamil) also exhibited the highest values for days to male flowering (DFM), days to female flowering (DFF), and plant height (AP). Regarding the number of rows (NH), V-424 maize had the highest value, a characteristic feature of Tuxpeño ears (Caballero-Salinas *et al.*, 2023). Conversely, the lowest NH was observed in both Cuarentano maize varieties. Wellhausen *et al.* (1951) described the Zapalote Chico race as typically having ears with 10 to 12 rows. Ultimately, the largest corncob diameter (DO) was observed in V-424 maize, with significant differences compared to the other varieties. The smallest DO was found in the white Cuarentano maize.

Spike Variables

Notably, stalk length (LP) and branch length (LTR) exhibited the highest coefficients of variation, at 36.1% and 29.5% respectively (Table 4), indicating a high degree of relative variability in these traits within the studied population. The variables branches per spike (RE) and branch length (LTR) are particularly noteworthy in the Zapalote Grande maize, specifically in both Opamil and Tapanero varieties (Table 4). The plants with the longest peduncles (LP) were observed in the Zapalote Chico maize, including both yellow and white Cuarentano varieties. Similar findings were reported by Cabrera-Toledo *et al.* (2019). The LP in Zapalote Chico maize ranged from 9.45 to 9.96 cm, differing somewhat from the values reported by López-Romero *et al.* (2005). Ultimately, the highest values for panicle length (LPA) were observed in the Opamil and V-424 maize varieties, while the Cuarentano populations exhibited the lowest values (Table 4). These findings are consistent with those studies evaluated 18 native maize varieties of the Zapalote Chico race from Oaxaca, Mexico.

It is important to note that ear traits are phenotypic characteristics commonly used to describe and analyse genetic diversity in native maize varieties. These traits not only serve as visual indicators of variation between different races and varieties of maize but also reflect specific adaptations to local agroecological environments and traditional cultivation practices (Guevara-Hernández *et al.*, 2019). Numerous researchers have employed these traits to examine the genetic composition of maize and to facilitate the genetic improvement of existing varieties. Their importance in the selection and development of new varieties

Table 4. Spike variables of the maize varieties studied in Cintalapa, Chiapas.

Variety	LE (cm)	RE	LP (cm)	LTR (cm)	LPA (cm)
Opamil	35.70	11.19^{a}	7.00^{b}	9.28^{a}	29.61^{a}
White Cuarentano	33.30	9.11^{bc}	9.45^{a}	7.54^{b}	25.38^{bc}
V-424	35.30	10.14^{ab}	5.60^{c}	6.70^{b}	29.60^{a}
Yellow Cuarentano	33.50	8.56^{c}	9.96^{a}	7.08^{b}	23.29^{c}
Tapanero	33.30	10.44^{a}	6.44^{bc}	8.85^{a}	27.13^{ab}
CV (%)	22.20^{NS}	27.00	36.10	29.50	22.20

Note: Different letters within the same column indicate a statistically significant difference between the treatments in question. NS: Not significant. **Highly significant difference ($p<0.01$). LE: spike length; RE: number of branches per spike; LP: length of the peduncle; LTR: length of the branched section; LPA: length of the panicle; CV: coefficient of variation.

has been underscored by several studies (Wellhausen *et al.*, 1951; Estrada-Contreras *et al.*, 2022; Vizcarra Manríquez, 2023).

The results reported here confirm that native maize varieties represent a dynamic and resilient agricultural system. Open pollination facilitates genetic flow, which, together with the continuous exchange of seeds among farmers, generates a high diversity of genetic combinations (Hernández *et al.*, 2020). This process enables the preservation and adaptation of native varieties with valuable agronomic traits that are specifically sought after by farmers. These varieties are well-adapted to regional agroecological niches, ensuring their sustainability and relevance in diverse local environments (Fonseca-Flores *et al.*, 2023).

In the case of maize varieties belonging to the Zapalote Chico race, the phenotypic values observed are consistent with those reported in previous research conducted in the Isthmus of Tehuantepec, the region of origin for this race. This consistency demonstrates that, despite being cultivated outside of its original habitat, Zapalote Chico has shown a satisfactory adaptive response to the agroenvironmental conditions of the Central Valleys of Chiapas.

The attributes exhibited by the Zapalote Chico maize varieties —including early flowering, drought resistance, tolerance to armyworm, and wind resistance— highlight its valuable genetic characteristics for mass selection processes based on agromorphological criteria that are important to farmers, particularly in the context of climate change. Although it is often regarded as a low-yielding variety, with an average yield of approximately 1.25 t ha^{-1} under rainfed conditions and 2.0 to 3.0 t ha^{-1} under irrigation, its ability to produce two or even three harvests per year makes it a highly attractive option. In this context, it would be a mistake to focus exclusively on yield-based productivity criteria, overlooking other unique attributes of the race, such as its adaptation to local conditions, resistance to diseases, and cultural or nutritional value. These attributes are equally important for the long-term sustainability and utility of the race.

The findings of this study on native short-cycle maize varieties in Chiapas have significant genetic, social, cultural, economic, and environmental implications. These conclusions are crucial for decision-making regarding the utilization, management, and conservation of these local varieties.

A key outcome of this research is the recognition that native maize represents a vital repository of essential genes for food security in rural areas. Short-cycle landraces have developed unique genetic adaptations that allow them to thrive under the specific agroclimatic conditions of the Central Valleys of Chiapas. These adaptations, shaped over centuries by natural selection and traditional management practices, are critical for addressing contemporary challenges such as climate change, soil degradation, and emerging pests. The data generated from this study can strengthen germplasm banks and inform both *in situ* and *ex situ* conservation strategies, ensuring the preservation of seeds not only in laboratories but also in the fields of indigenous and peasant communities, thus safeguarding these varieties for future use and contributing to Mexico's agricultural and genetic heritage (Guevara *et al.*, 2019).

Maize holds deep cultural significance beyond being a staple food; it is a cornerstone of cultural identity for many rural communities in Chiapas and throughout Mexico. Preserving native varieties is crucial not only for food security but also for maintaining the diversity of traditional dishes, which are a vital part of these communities' cultural heritage. This study offers valuable insights that can help promote the cultivation of native maize, particularly varieties adapted to local conditions, enhancing the resilience of agricultural production in the face of current challenges (Guevara *et al.*, 2022).

Additionally, the cultivation of these varieties supports sustainable agricultural practices by leveraging the traditional knowledge of local farmers, who have managed these varieties without relying on external inputs like chemical fertilizers and pesticides. This approach strengthens food self-sufficiency in rural communities and reduces reliance on agricultural technologies that may not be sustainable in the long term (Guevara *et al.*, 2019; Pérez-García, 2023).

Finally, the study highlights the need for public policies that recognize the inherent value of native maize and promote its conservation and use. The increasing homogenization of agricultural biodiversity, driven by the commercial interests of large agribusinesses, poses a significant threat. However, preserving native varieties provides a crucial countermeasure, as these landraces retain invaluable genetic traits, such as resistance to diseases and extreme environmental conditions, which may prove indispensable for future breeding programs (Guevara-Hernández *et al.*, 2023).

CONCLUSIONS

The native maize varieties exhibited significant genetic variability across the variables under consideration. The Zapalote Chico race is characterized by its favourable characteristics, as recognized by farmers, including early maturity, drought resistance, lodging resistance, and resistance to the fall armyworm. These qualities make it a promising candidate for future research and for inclusion in genetic improvement programs, whether conventional or participatory. It is crucial to continue promoting agroecological inputs with the goal of fostering the development of resilient agroecosystems and, consequently, enhancing the well-being of farmers and the conservation of native maize reservoirs.

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